

Progress in Drug Research 74

Series Editor: K. D. Rainsford

A. N. M. Alamgir

Therapeutic Use of Medicinal Plants and their Extracts: Volume 2

Phytochemistry and Bioactive
Compounds



Springer

Progress in Drug Research

Volume 74

Series editor

K. D. Rainsford, Sheffield Hallam University, Sheffield, UK

Progress in Drug Research is a prestigious book series which provides extensive expert-written reviews on highly topical areas in current pharmaceutical and pharmacological research.

Founded in 1959 by Ernst Jucker, the series moved from its initial focus on medicinal chemistry to a much wider scope. Today it encompasses all fields concerned with the development of new therapeutic drugs and the elucidation of their mechanisms of action, reflecting the increasingly complex nature of modern drug research. Invited authors present their biological, chemical, biochemical, physiological, immunological, pharmaceutical, toxicological, pharmacological and clinical expertise in carefully written reviews and provide the newcomer and the specialist alike with an up-to-date list of prime references.

Starting with volume 61, Progress in Drug Research is continued as a series of monographs.

More information about this series at <http://www.springer.com/series/4857>

A. N. M. Alamgir

Therapeutic Use of Medicinal Plants and their Extracts: Volume 2

Phytochemistry and Bioactive Compounds



Springer

A. N. M. Alamgir
Department of Botany
Chittagong University
Chittagong
Bangladesh

ISSN 0071-786X
Progress in Drug Research
ISBN 978-3-319-92386-4
<https://doi.org/10.1007/978-3-319-92387-1>

ISSN 2297-4555 (electronic)
ISBN 978-3-319-92387-1 (eBook)

Library of Congress Control Number: 2018942181

© Springer International Publishing AG, part of Springer Nature 2018

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

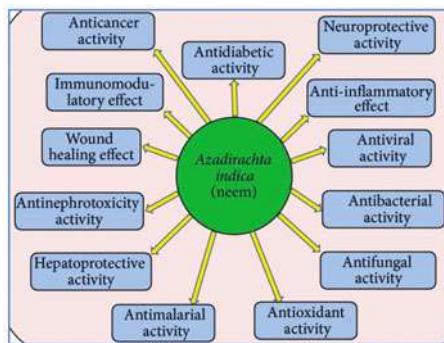
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Printed on acid-free paper

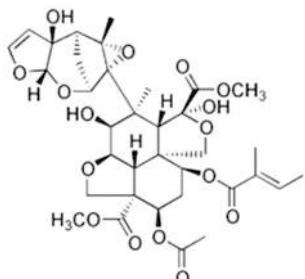
This Springer imprint is published by the registered company Springer International Publishing AG
part of Springer Nature
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland



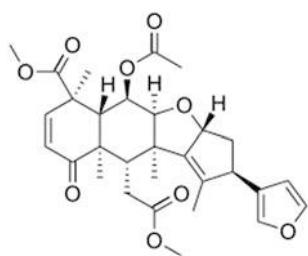
Neem: the village pharmacy (*Azadirachta indica* A. Juss.)



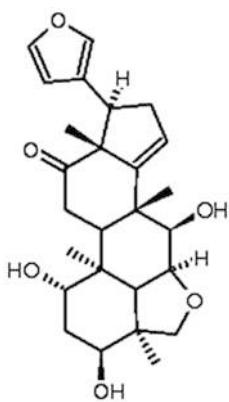
Potential pharmacological activities of Neem (*A. indica*) in diseases management



Azadirachtin (tetranortriterpenoid)



Nimbin (triterpenoid)



Nimbidinin (tetranortriterpene)

*Dedicated to the memory of my beloved
parents, who always inspired me to learn
from nature*

Preface

The textbook of “Phytochemistry and bioactive compounds”, Volume 2 of the series—Therapeutic use of Medicinal Plants and their Extracts, has been written to meet the needs of undergraduate and postgraduate students, who are pursuing education in the Department of Pharmacognosy and Pharmacy, Botany, and Plant Biochemistry of different universities as well as Ayurvedic and Unani colleges. Natural drugs including botanicals are gaining increasing momentum in popularity and use all over the world for their diversity and less toxic side effects. Complementary and alternative medicine botanicals are equally important in the developing and developed countries, and the uses of more and more herbs are now found as monographs in the national and international pharmacopeia. The knowledge about the nature and properties of phytochemicals and bioactive compounds present in the crude drugs as fundamental constituents and their mechanism of work is important for their optimum utilization. The present book “Phytochemistry and bioactive compounds” may fulfill the purpose of the knowledge hunters in natural resource arena, who take care about the quality, standard, and efficacy of herbal drugs. This book contains up-to-date information of the subject and may help the scientific community (students, teachers, and researchers) to keep themselves updated with the research and development work being carried out for a particular medicinal plant; and persons interested in herbal medicine bioactive compounds also may find its usefulness. It contains 9 chapters and covers many aspects of phytochemistry and molecular pharmacognosy including the source, classification, properties, molecular structure, and use of natural drugs, excipients, nutraceuticals, cosmeceuticals, lead compounds, precursors, etc., of primary and secondary metabolic origin. Medicinal herbs having a tremendous biosynthetic capabilities are yet considered as a potential source for precursors and lead compounds for the development of life-saving drugs against cancer, hepatitis, asthma, HIV, etc.

The herbal biosynthetic potentialities can be explored further under controlled scientific method of in vitro cultivation of cells, tissues, and organs of medicinal herbs for the smooth production of useful secondary metabolites. The role of these botanicals in national economy and world trade has been proved to be significant.

I hope, this book will be helpful for the students, researchers, and general readers who have an interest in herbal drugs. I am grateful to the publishers for kind assistance.

Chittagong, Bangladesh

A. N. M. Alamgir

Contents

1	Introduction	1
1.1	Phytochemistry: Introduction, a Borderline Discipline Between Natural Product Organic Chemistry and Plant Biochemistry	2
1.2	Medicinal Phytochemistry	8
1.3	Bioactive Compounds of Medicinal Plants	10
1.4	Metabolomics and Phytoconstituents	14
1.4.1	Metabolomics of Medicinal Plants	15
1.4.2	Metabolomics as a Tool for Quality Evaluation of Herbal Products	17
1.5	Methods (Techniques) of Phytochemical Investigation and High-Throughput Screening (HTS) for Active Plant Constituents	18
	References	21
2	Phytoconstituents—Active and Inert Constituents, Metabolic Pathways, Chemistry and Application of Phytoconstituents, Primary Metabolic Products, and Bioactive Compounds of Primary Metabolic Origin	25
2.1	Phytoconstituents	26
2.1.1	Active Drug Constituents	26
2.1.2	Inert Nondrug Constituents	28
2.2	Metabolic Pathways and the Origin of Primary and Secondary Metabolites Chemistry of Plant Constituents and Their Application	29
2.2.1	Primary Metabolic Pathways and Primary Metabolites	30
2.2.2	Secondary Metabolic Pathways and Secondary Metabolites	30
2.2.3	Plant's Defensive or Survival Secondary Metabolites	34

2.2.4	Pollinator Attracting Secondary Metabolites	41
2.2.5	Factors Affecting the Metabolic Pathways of Medicinal Plants	43
2.3	Chemistry of Plant Constituents, Their Classification and Application	47
2.3.1	Primary Metabolic Products Consisting of C & H; C, H & O; N, S & P Elements (Carbohydrates, Lipids, Amino Acids, Proteins, Nucleic Acids, Organic Acids)	48
2.4	Sources, Chemistry, and Health Effects of the Bioactive Compounds of Primary Metabolic Origin	155
	References	157
3	Secondary Metabolites: Secondary Metabolic Products Consisting of C and H; C, H, and O; N, S, and P Elements; and O/N Heterocycles	165
3.1	Secondary Products Consisting of C, H, and O Elements	167
3.1.1	Terpenes and Terpenoids	167
3.1.2	Steroids and Sterols	185
3.2	Volatile Oils	188
3.3	Miscellaneous Isoprenoids	191
3.3.1	Resins	191
3.4	Phenols and Phenylpropanoids	194
3.4.1	Phenol, Polyphenol, Phenolic Acids and Phenylpropanoids	194
3.5	Alkaloids	202
3.6	Glycosides	242
3.7	Bitter Principles	258
3.8	Resins, Saponins, Cardioactive Drugs and Other Steroids	278
3.9	Antibiotics from Higher Plants	285
3.10	Tumor Inhibitors, Antiprotozoal, Antihepatotoxic, Antihyperglycemic, Antihypertensive, etc., Herbal Products	287
3.11	Sources, Chemistry, and Health Effects of the Bioactive Compounds of Secondary Metabolic Origin; Biotechnology of Bioactive Compounds	305
	References	306
4	Bioactive Compounds and Pharmaceutical Excipients Derived from Animals, Marine Organisms, Microorganisms, Minerals, Synthesized Compounds, and Pharmaceutical Drugs	311
4.1	Bioactive Compounds and Excipients from Animal Sources	311
4.1.1	Carmine	319
4.1.2	Gelatin	321
4.1.3	Glycerol	321

4.1.4	Heparin	322
4.1.5	Insulin	323
4.1.6	Lactose	326
4.1.7	Lanolin	327
4.1.8	Magnesium Stearate	328
4.1.9	Premarin	329
4.1.10	Vaccines	330
4.1.11	Chitosan	333
4.2	Bioactive Compounds and Excipients from Marine Organisms	334
4.2.1	Major Marine Invertebrates and Their Bioactive Compounds	335
4.2.2	Major Marine Vertebrates and Their Bioactive Compounds	343
4.2.3	Bioactive Compounds from Seagrass	348
4.2.4	Bioactive Compounds from Seaweeds	353
4.2.5	Bioactive Compounds from Marine Bacteria	356
4.2.6	Bioactive Compounds from Marine Cyanobacteria	357
4.3	Bioactive Compounds and Pharmaceutical Excipients from Microorganisms	364
4.3.1	Bioactive Compounds from Prokaryotes: Bacteria, Cyanobacteri, and Actinomycetes	365
4.3.2	Bioactive Compounds from Protists: Microalgae (Unicellular Algae) and Protozoa	366
4.4	Bioactive Compounds Obtained from Minerals	373
4.4.1	Kaolin	373
4.4.2	Calomel	374
4.4.3	Iodine	374
4.4.4	Iron	374
4.4.5	Gold	375
4.4.6	Sulfur	375
4.4.7	Aluminum Hydroxide	376
4.4.8	Magnesium Hydroxide	376
4.4.9	Magnesium Trisilicate	377
4.4.10	Magnesium Sulfate	377
4.4.11	Mercurial Salts	378
4.4.12	Zinc and Zinc Oxide	378
4.4.13	Flourine	379
4.4.14	Borax	380
4.4.15	Selenium and Selenium Sulfide	381
4.4.16	Petroleum	381

4.5	Bioactive Sythesized Compounds and Pharmaceutical Drugs	382
	References	393
5	Vitamins, Nutraceuticals, Food Additives, Enzymes, Anesthetic Aids, and Cosmetics	407
5.1	Natural Sources, Classification, Chemistry and Therapeutic Use of Vitamins	408
5.2	Natural Sources, Classification, Chemistry, and Therapeutic Use of Nutraceuticals, Food Additives and Excipients (e.g., Coloring, Flavoring, Emulsifying and Suspending Agents, Diluents, Bulking or Filler Agents, Disintegrants, Sweeteners, Binders, Adhesives, Solidifiers, etc.)	469
5.3	Natural Sources, Classification, Chemistry, and Therapeutic Use of Enzymes and Anesthetic Aids	506
5.4	Natural Sources, Classification, Chemistry, and Therapeutic Use of Cosmeceuticals	517
	References	527
6	Poisons, Hallucinogens, Teratogens, Pesticides, and Xenobiotics—Their Sources, Classification, Chemistry, and Metabolism	535
6.1	Poisons—Their Sources, Classification, Chemistry, Mode of Action, Symptoms of Poisoning Application and Application	536
6.2	Hallucinogens and Teratogens—Their Sources, Classification, Chemistry, Mode of Action and Application	558
6.3	Pesticides—Their Sources, Classification, Chemistry Mode of Action and Application	562
6.4	Xenobiotics—Their Sources, Classification, Chemistry and Metabolism	568
	References	582
7	Biotechnology, In Vitro Production of Natural Bioactive Compounds, Herbal Preparation, and Disease Management (Treatment and Prevention)	585
7.1	Biotechnology and Production of Bioactive Compounds and Techniques of Molecular Biotechnology	586
7.1.1	Techniques of Molecular Biotechnology	588
7.2	Advantages of Tissue Cultures in Production of Useful Bioactive Compounds	599
7.3	Herbal Preparations and Disease Management (Prevention and Treatment)	601
7.3.1	Herbal Extracts and Management of Chronic Diseases	602
7.3.2	Viral Disease Management with the Use of Antiviral Bioactive Phytoconstituents	625

7.4	Natural Immunopotentiators, Vaccine and Biotechnology in Health Care	631
7.4.1	Natural Immunopotentiators and Vaccine Adjuvants from Plants and Other Sources	631
7.5	Biotechnology of Disease Prevention	642
	References	663
8	Molecular Pharmacognosy—A New Borderline Discipline Between Molecular Biology and Pharmacognosy	665
8.1	Concept of Molecular Pharmacognosy and Its Development	666
8.2	Pharmacognosy at the Molecular Level	667
8.3	Development of Species Biology and Molecular Systematics	668
8.4	Molecular Identification of Traditional Medicinal Materials	670
8.5	Basic Methods of Systems Biology	689
8.6	Conservation of Medicinal Plant and Animal Biodiversity and Sustainable Utilization of Crude Drugs Resources	695
8.7	Molecular Breeding Marker in Herbal Drug Technology and New Variety Cultivation	696
8.8	Gene Regulation of Metabolic Pathway and Directional Control of the Quality of Herbal Medicines	700
8.9	Biological Process of the Formation of Secondary Metabolites in Medicinal Plants	702
8.10	Application of Systems Biology in Secondary Metabolites Study	703
8.11	Use of Genetic Engineering and Tissue Culture Technique for the Production of Active Ingredients	705
8.12	Genetic Engineering and Green Pollution-Free Medicinal Plant	707
8.13	Metabolomics of Medicinal Plants: Genomics, Proteomics, and Metabolomics	707
8.14	The Goal of Molecular Pharmacognosy	708
	References	709
9	Methods of Qualitative and Quantitative Analysis of Plant Constituents	721
9.1	Extraction of Plant Constituents	723
9.2	Phytochemical Screening of Secondary Metabolites	725
9.3	Separation of Plant Constituents	731
9.3.1	Separation Techniques	731
9.4	Isolation and Characterization of Drug Principles from Plant and Other Natural Sources	743
9.5	Bioassay Techniques	745
9.6	Qualitative and Quantitative Analysis of Secondary Metabolites	785

9.6.1	Qualitative Analysis of Secondary Metabolites	785
9.6.2	Quantification of Phytochemicals in Crude Extract of Medicinal Plants	792
9.6.3	Spectrophotometric Method of Determination of Total Phenolic Content	794
9.6.4	Spectrophotometric Method of Determination of Tannins	795
9.6.5	Spectrophotometric method Determination of total Flavonoids	795
9.7	Molecular Biology: PCR-Based DNA Technology	796
	References	803
Index	805

Abbreviations

1D NMR	One-dimensional NMR spectroscopy
2D NMR	Two-dimensional NMR spectroscopy
AA	Asiatic acid
ABA	Abscisic acid
ACE	Angiotensin-converting enzymes
ADA	Adenosine deaminase
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADH	Antidiuretic hormone
ADHF	Anti-diabetes herbal formulation
AFLP	Amplified fragment length polymorphism
AIDS	Acquired immune deficiency syndrome
ALA	Alpha-linolenic acid
ALL	Acute lymphocytic leukemia
ALS	Amyotrophic lateral sclerosis
AMD	Age-related macular degeneration
AML	Acute myeloid leukemia
AMP	Antimicrobial peptide
APC	Allophycocyanin
API	Active pharmaceutical ingredient
apoA, apoC, and apoE	Apolipoproteins
AP-PCR	Arbitrarily primed PCR
ara-A	9-β-D-arabinofuranosyladenine
ASI	Active specific immunotherapy
AS-ODNs	Antisense oligodeoxynucleotides
AThDP	Adenosine thiamine diphosphate
AThTP	Adenosine thiamine triphosphate
ATP	Adenosine triphosphate
AVP	Arginine vasopressin
BAOEC	Bovine aortic endothelial cell
bFGF	Basic fibroblast growth factor

BITC	Benzyl isothiocyanate
BMAA	β -N-methylamino-L-alanine
BN	Byakko-ka-ninjin-to
BoNTs	<i>Botulinum</i> neurotoxins
B-PE	B-Phycoerythrin
BRs	Brassinosteroids
BST	Brine shrimp toxicity
BTC	Behind-the-counter medication
CaBP	Calcium-binding protein
CAM pathways	Crassulacean acid metabolism pathways
CAPS	Cleaved amplified polymorphic sequences
CAS	Cardioactive steroids
CB	Cannabidiol
CCl ₄	Carbon tetrachloride
CCLs	Continuous cell lines
CDDP	Cis-diamminedichloroplatinum (II)
cDNA	Complementary DNA
(CE)-MS	Capillary electrophoresis
CF	Cystic fibrosis
CGs	Cardiac glycosides
-CH ₂ -COOH	Carboxymethyl group
CHO	Chinese hamster ovary
CLA	Conjugated linoleic acid
CM L	Chronic myelogenous leukemia
CMC	Carboxymethyl cellulose
CML	<i>Chlorophyllum molybdites</i> lectin
CNS	Central nervous system
CoA	Acetyl coenzyme A
CoA	Acetyl-CoA=Acetyl coenzyme A
COCP	Combined oral contraceptive pill
CPC	C-Phycocyanin
CTX or CT	toxin
CTX	Ciguatoxin
CVDs	Cardiovascular diseases
CYN, CYL	Cylindrospermopsin
DAD	Diode array detector
DADS	Diallyl disulfides
DALP	Direct amplification of length polymorphisms
DArT	Diversity arrays technology
DATS	Diallyl trisulfides
DBP	Diastolic blood pressure
dcSTX	Decarbamoylsaxitoxin
DE	Degree of esterification
DE	Dextrose equivalent
DGDG	Digalactosyldiacylglycerol

DHA	Docosahexaenoic acid
DIBOA	2, 4-dihydroxy-1,4-benzoxazin-3-one
DIM	3, 3'-diindolylmethane
DIMBOA	2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one
DIMS	Direct injection mass spectroscopy
DM	Diabetes mellitus
DMAPP	Dimethylallyl pyrophosphate
DMN	Dimethylnitrosamine
DMSO	Dimethylsulfoxide
DMSP	Dimethylsulfoniopropionate
DMT	Dimethyltryptamine
DNA	Deoxyribonucleic acid
DNTI	Drugs from nature targeting inflammation
dNTPs	Deoxyribonucleoside triphosphates
DP	Degree of polymerization
DPA	Docosapentaenoic acid
DPP-4	Dipeptidyl-peptidase-4
dsDNA	Double-stranded DNA
DTC	d-tubocurarine
DXM	Dextromethorphan
EC	(-)-epicatechin
ECG	(-)-epicatechin-3-gallate
ED ⁵⁰	Median effective dose
EFA's	Essential fatty acids
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
ELISA	Enzyme-linked immunosorbent essay
ELSD	Evaporative light scattering detector
EMPs	Endothelial microparticles
EPA	Eicosapentaenoic acid
e-PCR	Electronic PCR
EPS	Extracellular polysaccharides
ERT	Enzyme replacement therapy
ESTs	Expressed sequence tags
EUV	Extreme ultraviolet 10–121 nm
FDA	US Food and Drug Administration
FDCA	Food, Drug, and Cosmetics Act
FMD	Flow-mediated dilation
FOS	Fructooligosaccharides
FOSHU	Foods for specified health uses
FpyFn	β -D-fructopyranosyl-[D-fructofuranosyl] (n-1)-Dfructofuranosides
FTIR	Fourier-transform infrared spectroscopy
FTMS	Fourier transform mass spectrometry

G	α -L-guluronate(G) residues
GABA precursor	Gamma-aminobutyric acid precursor
GABAA	γ -Aminobutyric acid type A
GABA	Gamma-aminobutyric acid
GAGs	Glycosaminoglycans
GalN	D-Galactosamine
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
G-CSF	Granulocyte colony-stimulating factor
GERD	Gastroesophageal reflux disorder
GFR	Glomerular filtration rate
GGMMGM _n GGM	The repeating unit of D-glucose (G) and D-mannose (M) in a proportion of 5:8 joined by β -1 \rightarrow 4 glycoside linkages in glucomannan
GI	Glycemic index
GLC	Gas-liquid chromatography
GMS	Glycerol monostearate
GnRH	Gonadotropin-releasing hormone
GOS	Galactooligosaccharides
GpyFn	α -D-glucopyranosyl-[β -D-fructofuranosyl] (n-1)-D-fructofuranosides
GSH	Glutathione
GSL	Glucosinolates
GTX-2	Gonyautoxins 2
GTX	Gonyautoxins
HAT	Hypoxanthine aminopterin thymidine
HCN	Hydrogen cyanide
HDL	High-density lipoprotein
HFCS	High fructose corn syrup
HGS	Hydrogenated glucose syrup
HIV	Human immunodeficiency virus
HMG-CoA	Reductase (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase)
HMGR pathway	HMGR-3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
hMG	Human menopausal gonadotropin
hCG	Human chorionic gonadotropin
HNO ₃	Nitric acid
hnRNA	Heterogeneous RNA
HPLC	High-performance liquid chromatography
HPMC	Hydroxypropyl methylcellulose
HPV	Human papilloma virus
HRT	Hormone replacement therapy
HSH	Hydrogenated starch hydrolysates
HSV	Herpes simplex virus

HTN	Hypertension
HTS	High-throughput screening
IDDM	Insulin-dependent diabetes mellitus
IDL	Intermediate-density lipoprotein
IL1 β	Interleukin-1 β
IP6	Inositolhexaphosphate
IPP	Isopentenyl pyrophosphate
ISH	International Society of Hypertension
ISSRs	Inter Simple Sequence Repeats
IUPAC	International Union of Pure and Applied Chemistry
JA	Jasmonic acid
JFM	Japanese Folk Medicine
K $^{+}$ -ATP chanel	An ATP-sensitive potassium channel
KB	Kenacid blue
KDO	Ketodeoxyoctulosonic acid
TTKKS	Collagen pentapeptide (Lys-Thr-Thr-Lys-Ser)
LA	Linoleic acid
LAL	Limulus amebocyte lysate
LBG	Locust bean gum
LCFA	Long-chain fatty acids
LC-HUFA	Long-chained highly unsaturated fatty acids
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
LC-MUFA	Long-chained monounsaturated fatty acids
LC-PUFA	Long-chained polyunsaturated fatty acids
LDL	Low-density lipoprotein
L-DOPA	L-dihydroxyphenylalanine
LHRH	Luteinizing hormone-releasing hormone
LPS	Lipopolysaccharide
LRF	Luteinizing hormone-releasing factor
LSA	d-lysergic acid amide
LSD	Lysergic acid diethylamide
M	β -D-mannuronate (M)
MAbs	Monoclonal antibodies
MAH	Monocyclic aromatic hydrocarbon
MAPs	Medicinal and aromatic plants
MCFA	Medium-chain fatty acids
MDMA	Methylenedioxymethamphetamine
MEP	Methylerythritol phosphate or methylerythritol phosphate pathway
MGDG	Monogalactosyl diacylglycerol
MHRA	Medicines and Healthcare products Regulatory Agency
miRNAs	MicroRNAs
MLSA	Multilocus sequence analysis

MMR	Measles, mumps, rubella
MOS	Mannan oligosaccharides
MPLC	Medium pressure liquid chromatography
MPS I	Mucopolysaccharidosis I
M-residues	(M-blocks)
mRNA	Messenger RNA
MS media	Murashige and Skoog medium=MSO=MS0=MS-zero
MSD	Mass spectrometric detector
MS	Mass spectrometry
MSSM	Mechanically separated seal meat
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NAG	N-acetyl-glucosamine
NADPH	XXXNicotinamide adenine dinucleotide phosphate hydrogen
NBSB	National Biological Standards Board
NCD	Non-communicable disease
NCEs	New chemical entities
NEFA	Nonesterified fatty acids
NGS	Next-generation sequencing
NIDDM	Noninsulin-dependent diabetes mellitus
NMDA receptors	N-methyl-D-aspartate receptors
NMDA	N-methyl-D-aspartate
NMR-MS techniques	Nuclear magnetic resonance spectroscopy-mass spectrometry techniques
NMR	Nuclear magnetic resonance spectroscopy
NOx	Nitrogen oxides
NPAAs	Nonprotein amino acids
NPs	Nanoparticles
NSAIDs	Nonsteroidal anti-inflammatory drugs
NSA	Non-starch polysaccharides
NSTX	Neosaxitoxin
OA	Orotic acid
OCPs	Oral contraceptives
ODAP- β -N	Oxalyl-L- α , β -diaminopropionic acid
-OH	Hydroxyl group
-ONO	2 nitrate groups
OPC	Oligomeric flavonoids
OPCs	Oligomeric proanthocyanidins
OTA	Ochratoxin A
OTB	Ochratoxin B
OTC	Ochratoxin C
OTC	Ornithine transcarbamylase
OTC	Over-the-counter medication

P5P	Pyridoxine 5'-phosphate
PAAs	Proteinogenic amino acids
PABA	p-Aminobenzoic acid
PAH	Polycyclic aromatic hydrocarbon
PAHs	Polycyclic aromatic hydrocarbons
PAR	Photosynthetically active radiation
PBB	Polybrominated biphenyls
PCB	Polychlorinated biphenyls
PCOs	Proanthocyanidin oligomers
PC	Phycocyanin
PCP	Phencyclidine
PCP	Pneumocystis carinii pneumonia
PC	Proanthocyanidins
PCR	Polymerase chain reaction
PCR-RFLP	polymerase chain reaction-restriction fragment length polymorphism
PDC or PDHC	Pyruvate dehydrogenase complex
PD	Parkinson's disease
PEG	Polyethylene glycol
PEITC	Phenethyl isothiocyanate
PE	Phycoerythrin
PET	Positron emission tomography
PFCs	Perfluorocarbons
PGG	1, 2, 3, 4, 6-pentagalloyl glucose
pi	Isoelectric point
PI	Phosphatidylinositol
PIP	Phosphatidylinositol phosphate
PKC	Protein kinase C
PLP	Pyridoxal 5'-phosphate
PL	Pyridoxal
PMP	Pyridoxamine 5'-phosphate
PMPs	Plant-made pharmaceuticals
PM	Pyridoxamine
PN	Pyridoxine
POM	Prescription-only medicine
PQ	Plastoquinone
PQQ	Pyrroloquinoline quinone
PSP	Paralytic shellfish poisoning
PST	Paralytic shellfish toxin
PSTs	Paralytic shellfish toxins
PTP1B	Protein tyrosine phosphatase 1B
PT	Pertussis toxin
PYLIS	Pyrrolysine insertion sequence
qPCR	Quantitative PCR
RACE-PCR	Rapid amplification of cDNA Ends PCR

RAPD	Random amplified polymorphic DNA
RCO	Acyl groups
RFLP	Restriction fragment length polymorphism
RFO	Raffinose family oligosaccharides
Rf	Retention factor
RID	Refractive index detector
RNAi	RNA interference
RNA	Ribonucleic acid
ROS	Reactive oxygen species
R-PE	R-Phycoerythrin
rRNA	Ribosomal RNA
RT-PCR	Real-time PCR
RT-qPCR	Reverse transcription qPCR
Rt	Retention time
SAC	S-Allyl cysteine
SAM	S-Adenosylmethionine
SBP	Systolic blood pressure
SCARs	Sequence characterized regions
SCFA	Short-chain fatty acids
SCID	Severe combined immunodeficiency disease
SC-MUFA, SC-PUFA	Short-chained mono- and polyunsaturated fatty acids
SC-SAFA	Short-chained saturated fatty acids
SECIS	Selenocysteine insertion sequence
SeS	Selenium sulfide
SFN	Sulforaphane
shRNA	Short hairpin RNA
siRNAs	Small interfering RNAs
SLE	Systemic lupus erythematosus
SMM	S-Methylmethionine
SNPs	Single nucleotide polymorphisms
snRNA	Small nuclear RNA
SPCD-1,2-Dibromo-3	Chloropropane- DBCP-5-HTP-5-hydroxytryptophan
SPCD	Systemic primary carnitine deficiency
sPS	Sulphated polysaccharides
SQAG	Sulfo-quinovosyl-acyl-glycerol
ssDNA	Single-stranded DNA
SSRs	Microsatellites or simple sequence repeats
STR	Short tandem repeat
STSs	Sequence tag sites
STX	Saxitoxin
T2DM	Type II diabetes mellitus
TAA	Thioacetamide
TAIL-PCR	Thermal asymmetric-interlaced PCR
Taq polymerase	Polymerase from bacterium <i>Thermus aquaticus</i>
TCA cycle	Tricarboxylic acid cycle

TCAs	Tricyclic antidepressants
TCA	Target compound analysis
TCDD	Tetrachlorodibenzodioxin
TCM	Traditional Chinese Medicine
TDN	1,1,6,-Trimethyl-1,2-dihydronaphthalene
TeCA	Tetracyclic antidepressant
TF	Theaflavin
THC	Tetrahydrocannabinol
ThDP	Thiamine diphosphate
ThMP	Thiamine monophosphate
ThTP	Thiamine triphosphate
TLC	Thin-layer chromatography
TNAs	Therapeutic nucleic acids
TNF α	Tumor necrosis factor- α
TPP	Thiamine pyrophosphate
TQ	Thymoquinone
TRH	Thyrotropin-releasing hormone
tRNA	Transfer RNA
TTS	Transdermal therapeutic system
TTX	Tetradotoxin
UGA	Uracyl, adenine, adenine codon
UV radiation	Ultraviolet radiation 100–400 nm wave length
UVA	Ultraviolet A 400–320 nm
UVB	Ultraviolet B 320–290 nm
VEGF	Vascular endothelial growth factor
VFDF	Very fast death factor
VLCFA	Very-long-chain fatty acids
VLDL	Very-low-density lipoproteins
VLP	Virus-like particle
VOCCs	Voltage-operated calcium channels
VOCs	Volatile organic compounds
X-rays	0.01–10 nm
YGD	Yerbe Mate-Guarana-Damiana
ZEN	Zearalenone
α -GAL	α -Galactosidase

Chapter 1

Introduction



Abstract Phytochemistry or plant chemistry, a borderline discipline between natural product organic chemistry and plant biochemistry, studies different chemicals including drug principles, food additives, and cosmetics of plant origin. It also deals with the structures, synthesis, regulation, and biological properties of secondary metabolites of plants and other bioactive principles as well as their biological functions in plants, human being, and other organisms. Phytochemistry plays significant role in the identification of therapeutically important plant substances and, in association with herbalism, ethnobotany, ethnopharmacology, metabolomics, and bioinformatics, computational biology, plays important role in discovery of new drugs. Phytochemistry initiated to play a significant role in pharmacognosy in the remote past as medicinal phytochemistry (but not until nineteenth century) when people began to isolate different active principles of medicinal plants such as quinine from *Cinchona* bark (1820), morphine, and codeine from the latex of the *Cannabis*, digoxin from *Digitalis* leaves, atropine from *Hyoscyamine*, etc. Thus, medicinal phytochemistry emerged, which includes the study of phytochemicals from medicinal plants including the bioactive phytochemicals, phytonutrients, food additives, cosmeceuticals, etc. These chemicals are of secondary metabolic origin of plants, and they protect plants from damages due to biotic and abiotic stresses. Such metabolites also contribute to the plant's color, aroma, and flavor to assist insect pollination, repel herbivore, or elicit pharmacological or toxicological effects in man and animals. Metabolome and metabolomics include the systematic large-scale study of the small molecules or metabolic products (metabolome) of a biological system. Phytochemicals are non-nutritive but have health curative and disease preventive properties when their dietary intake is significant. They provide health benefits for humans beyond macronutrients and micronutrients. Phytochemicals as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation, and modulation of hormone metabolism and anticancer property. A large diverse group of phytonutrients are found in vegetables, fruit, whole grain products, legumes and sprouts, nuts and seeds, onion, garlic, cumin, anise, basil, bay leaf, sparsely, cilantro, allspice, condiments, tea, coffee, chocolate, algae, etc. They include allyl sulfides, anthocyanins, β -carotene, β -sitosterol, caffeic acid, capsaicin, carnosol, catechins, chlorogenic acid, coumarins,

cryptoxanthin, dietary fiber, 3,3'-diindolylmethane, ellagic acid, epicatechin, essential and fixed oils, ferulic acid, flavonoids (2-phenylchromans), folate, folic acid, hydrolysable tannins puncalagins, indoles, indole-3-carbinol, isoflavones (3-phenylchromans), isothiocyanates, lactones, lignans, limonene, lutein, lycopene, monoterpenes, monounsaturated fat, nasunin, niacin, organosulfures, omega (omega-3, omega-6 fatty acids), oxalic acid, perillyl alcohol, phenols and polyphenols, phytic acid, phytosterols, protease inhibitors, saponins, sesquiterpene, soluble fiber, sulforaphane, quercitin, resins, oleoresins, resveratrol, sulforaphane, tannic acid, thiosulfinate, saponins, silymarin, tartaric acid, vitamins B₁, B₆, E, K, vitamin C, xanthones, zeaxanthin, etc., as well as elements potassium, copper, manganese, selenium, magnesium, zinc, iodine, iron, chromium, etc. Crude drugs, over-the-counter remedies, ethical phytomedicines (standardized toxicologically and clinically defined crude drugs), etc., are promising low-cost alternative medicines used in primary health care of rural people of developing countries. The field herbal medicine also has benefited greatly in recent years from the interaction of the study of traditional ethnobotanical knowledge and the application of modern phytochemical analysis and biological activity studies to medicinal plants. Phytonutrients, vitamins, and minerals contained in plants had been hinted at for millennia. The field herbal medicine also has benefited greatly in recent years with the use of high-throughput robotic screening technique developed by industry, bioassay-guided fractionation of crude extracts aided by chromatographic separation techniques (LC, GC, TLC, MPLC, HPLC), chemical structure elucidation by spectroscopic (HPLC/MS, NMR, DIMS, FTMS), immunoassay, etc. Medicinal phytochemistry is receiving ever greater attention in research, pharmaceutical industry as well as in trade and economy. The estimated value of plant-based crude drugs in world trade is nearly US\$45,000 million per year.

1.1 Phytochemistry: Introduction, a Borderline Discipline Between Natural Product Organic Chemistry and Plant Biochemistry

Phytochemistry (Gk. Phyto-plant) or plant chemistry is the study of phytochemicals or chemicals of plant origin. It is a borderline discipline between natural product organic chemistry and plant biochemistry. It includes chemistry of drugs, food additives, and cosmetics of plant origin. Phytochemistry includes the scientific study of plant chemistry, plant life processes, and plant products, i.e., phytochemicals, chemical composition of plants, chemical compounds produced by plants or phyto-drugs as well as the chemical processes associated with plant life. Phytochemistry describes the structures of a large number of secondary metabolites of plants, their biosynthetic processes, and also their biological functions in plants, human being, and other organisms. Phytochemistry also deals with the structures,

synthesis, regulation, and biological properties of bioactive principles. Plants synthesize chemicals for many reasons, including their protection under biotic and abiotic stresses. Phytochemicals in food of plant origin are often active in human biology and many of them provide health benefits. The subjects botany, chemistry, biochemistry, plant physiology, molecular biology, genetics, ethnobotany, herbal medicine, pharmacognosy, metabolomics, bioinformatics, computational biology, etc., are now the important fields related to phytochemistry and phytochemical investigation. Phytochemistry plays significant role in the identification of therapeutically important plant substances and, in association with herbalism, ethnobotany, ethnopharmacology, plays important role in discovery of new drugs.

Phytochemistry integrates or combines natural product organic chemistry (chemistry of purified organic compounds or substances produced by a living organism and that are found in nature) with plant biochemistry, and lies in between the two subfields. The term natural product has also been extended for commercial purposes to refer to cosmetics, dietary supplements, and foods produced from natural sources without added artificial ingredients. Natural products organic chemistry is usually restricted to mean purified organic compounds isolated from natural sources that are produced by the pathways of primary or secondary metabolism. Organic chemistry includes studies of the structure, properties, and reactions of organic compounds and organic materials. Natural products within the field of organic chemistry are often defined as primary and secondary metabolites while the term medicinal chemistry is often restricted to secondary metabolites. Plant biochemistry explains the chemical processes, molecular function, etc., of plants. Much of biochemistry deals with the structures, functions, and interactions of biological macromolecules such as proteins, nucleic acids, carbohydrates, and lipids, and it contributes to the solution of agricultural and pharmaceutical problems.

Phytochemistry is a significant subject of the pharmacognosy curriculum. The use of plants as medicines goes back to antiquity but chemical analysis of the plant constituents was not initiated until the nineteenth century when people began to isolate the active principles of medicinal plants. Particular landmark was the discovery of quinine from *Cinchona* bark (1820), followed by morphine and codeine from the latex of the *Cannabis*, digoxin from *Digitalis* leaves, atropine from *Hyoscyamine*, etc. (Fig. 1.1).

Quinine is an old antimalarial drug in a modern world; other cinchona alkaloids including quinidine, cinchonine and cinchonidine are all effective against malaria.

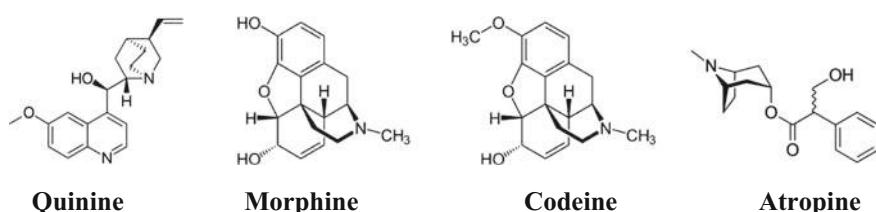


Fig. 1.1 Structure of quinine, morphine, codeine, and atropine

The active opiates are found in the opium poppy are morphine, codeine, thebaine, and papaverine having analgesic or pain-relieving effects. Atropine is commonly classified as an anticholinergic.

Phytochemicals of secondary metabolic origin of plants are biologically active compounds and they protect plants from damages due to biotic (e.g., against prey, predators, herbivores, insect, diseases, pathogens and competing organisms, etc.) and abiotic (e.g., pollution, UV radiation, drought, salinity, temperature, etc.) stresses; and secondary metabolites also contribute to the plant's color, aroma and flavor to assist insect pollination (Gibson et al. 1998; Mathai 2000). Phytochemicals are non-nutritive plant chemicals that have health curative and disease preventive properties when their dietary intake is significant. They provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg 1999). Phytochemicals are plant-derived chemical compounds and phytonutrients refer to phytochemicals that come from edible plants. Phytochemicals have important biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There is abundant evidence from epidemiological and other studies that the phytochemicals in fruits and vegetables can significantly reduce the risk of cancer, probably due to antioxidant and anti-inflammatory effects. Phytonutrients, vitamins and minerals contained in plants had been hinted at for millennia (Seddon et al. 1994; Yoshizawa et al. 1998; Agarwal and Rao 2000; Dalessandri et al. 2004; Letavayova et al. 2006; McGuire et al. 2006; Rao et al. 2006; Ray et al. 2006; Canene-Adams et al. 2007; Hurwitz et al. 2007).

Phytonutrients or plant nutrient are natural compounds found in plant foods, spices, beverages, e.g., vegetables, fruit, whole grain products, legumes and sprouts, nuts and seeds, onion, garlic, cumin, anise, basil, bay leaf, sparsely, cilantro, allspice, condiments, tea, coffee, chocolate, algae, etc. Phytonutrients are often referred to as phytochemicals and there are hundreds of phytonutrients and some common phytonutrients include allyl sulfides, anthocyanins, β -carotene, β -sitosterol, caffeoic acid, capsaicin, carnosol, catechins, chlorogenic acid, coumarins, cryptoxanthin, dietary fiber, 3,3'-Diindolylmethane, ellagic acid, epicatechin, essential and fixed oils, ferulic acid, flavonoids (2-phenylchromans), folate, folic acid, hydrolysable tannins punicalagins, indoles, indole-3-carbinol, isoflavones (3-phenylchromans), isothiocyanates, lactones, lignans, limonene, lutein, lycopene, monoterpenes, monounsaturated fat, nasunin, niacin, organosulfures, omega (omega-3, omega-6 fatty acids), oxalic acid, perillyl alcohol, phenols and polyphenols, phytic acid, phytosterols, protease inhibitors, saponins, sesquiterpene, soluble fiber, sulforaphane, quercitin, resins, oleoresins, resveratrol, sulforaphane, tannic acid, thiosulfinate, saponins, silymarin, tartaric acid, vitamins B₁, B₆, E, K, vitamin C, xanthones, zeaxanthin, etc., as well as elements potassium, copper, manganese, selenium, magnesium, zinc, iodine, iron, chromium, etc. Phytonutrient rich food includes red, orange and yellow vegetables and fruit (e.g., tomatoes, carrots, peppers, squash, sweet potatoes, peaches, mangos, melons, citrus fruits, and berries); dark green leafy vegetables (e.g., spinach, kale, bok choy, broccoli, Swiss

chard, and romaine lettuce); spices (e.g., garlic, onions, chives and leeks); whole grain products (e.g., brown rice, wild rice, quinoa, barley, wheat berries, and whole wheat whole grain breads and whole grain cereals); nuts and seeds (e.g., walnuts, almonds, sunflower, sesame and flax seeds); legumes (e.g., dried beans, peas, lentils, soy beans and soy products); tea, coffee and chocolate (e.g., coffee, green tea, black tea, and other herbal teas and dark chocolate).

Phytochemicals have been used as drugs for millennia, e.g., Hippocrates around fourth century BC used to prescribe willow tree leaves to abate fever and advocated liver as a remedy for night blindness. The active component of willow tree leaves with potent anti-inflammatory and pain-relieving properties was identified as salicin (salicin → saligenin → salicylic acid → aspirin) and that of liver, vitamin A, was not chemically defined until 1827 and 1913, respectively. The 1920s and 30s were ripe for vitamin discovery, accounting for 11 of the 15 vitamins. A diet rich in tomatoes and broccoli was more effective in inhibiting prostate cancer growth than a leading drug for prostate cancer. Lutein and zeaxanthin from spinach have been shown through clinical trials to directly improve human visual performance and help prevent the onset of macular degeneration and cataracts. Health-promoting phytochemicals 3,3'-diindolylmethane (DIM) from Brassica vegetables (broccoli, cauliflower, cabbage, kale, Brussels sprouts) has potent antiviral, antibacterial, and anticancer properties; paclitaxel (taxol) from Pacific yew tree is potentially a very important anticancer phytonutrient. Now, there is a new impetus of discovery around health-enhancing compounds in plant foods known as phytonutrients. Indeed, many well-defined twentieth-century drugs were derived from herbal plants. Drugs including salicylic acid (*Salix* sp.), curare (*d*-tubocurarine) (*Chondrodendron tomentosum*), digitoxin (*Digitalis purpurea*), taxol (*Taxus brevifolia*), and many others, are well-recognized drugs derived from plants with pharmaceutical and clinical potentials (Ackermann 1973; Sumner 2000; Dewick 2002). Salicin (a glycoside, a precursor for aspirin synthesis) from willow (*Salix* sp.) on hydrolysis produces saligenin (salicylic alcohol), which on oxidation produces salicylic acid and on acetylation produces aspirin (acetylsalicylic acid) (Fig. 1.2). Mahdi (2010) described in detail the formation of aspirin from salicin of willow bark.

D-tubocurarine (DTC) is a toxic alkaloid (an arrow poison) (Fig. 1.3). In the mid-1900s, it was used as an adjunct for clinical anesthesia to provide skeletal muscle relaxation during surgery or mechanical ventilation. Now, it is more safer alternative, e.g., cisatracurium and rocuronium are available.

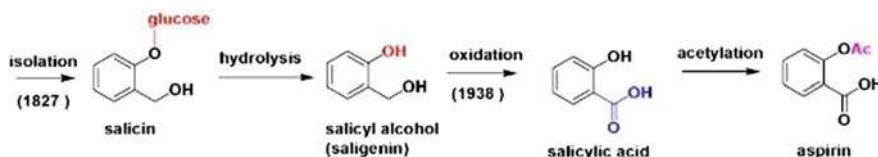
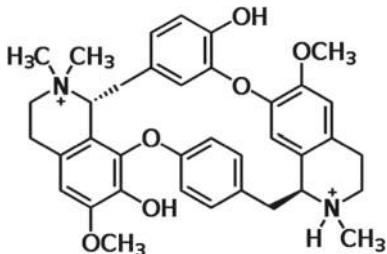


Fig. 1.2 Synthetic pathway of aspirin from salicin, a glycoside precursor from willow (*Salix* sp.)

Fig. 1.3 Structure of d-tubocurarine (DTC), a toxic alkaloid used as arrow poison



D-tubocurarine (DTC)

Paclitaxel (brand name taxol), a natural product derived from the bark of Pacific yew tree (*Taxus brevifolia*), was first isolated in 1971 and approved for medical use in 1993 to treat a number of types of cancer, e.g., ovarian cancer, breast cancer, lung cancer, Kaposi sarcoma, cervical cancer, and pancreatic cancer, an essential medicine in WHO list, is now manufactured by cell culture. DIM is a therapeutic for numerous forms of cancer. Digitoxin is a cardiac glycosidic phytosteroid (Fig. 1.4).

Phytonutrients are secondary metabolites and show biological properties like antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. Phytonutrients with antioxidant properties may prevent damage to cells throughout the body and many phytonutrients have been shown to reduce the risk of cancer, heart disease, stroke, Alzheimer's and Parkinson's disease. Phytonutrients have health-promoting bioactive functions; many exert positive effects on the immune system and hormones and other may act as antibacterial or antiviral agents. Many phytochemicals have anti-inflammatory properties, including Turmeric and Chia. Inflammation is a factor in many diseases of aging including Alzheimer's and Arthritis, and many artificial anti-inflammatories have unfortunate side effects. Turmeric is also reported to be active against skin cancer (melanoma). Phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of low-density lipoprotein

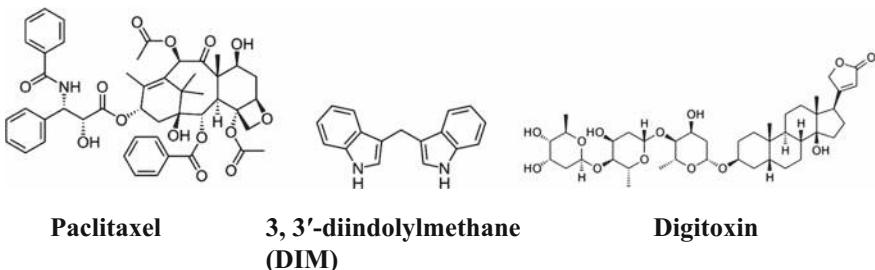


Fig. 1.4 Structure of paclitaxel, DIM, and digitoxin

(LDL) cholesterol, reducing the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity. Phytochemicals may detoxify substances that cause cancer, neutralize free radicals, inhibit enzymes that activate carcinogens, and activate enzymes that detoxify carcinogens. Clinical investigations are ongoing worldwide on thousands of phytochemicals with medicinal properties.

Phytonutrients are not essential by traditional definitions, but they apparently reduce risks of diseases of aging, e.g., the isoflavones in soy products may reduce the risk of heart disease, osteoporosis, and several types of cancer; and certain flavonoids in blueberries may reverse nerve cell aging. And a wide array of compounds including vitamins C or E in fruits and vegetables may protect cell components against oxidative damage. Phytonutrients have provided the impetus for plant and nutrition scientists to work together. Researchers are now deeply engaged to screen germplasm for specific phytonutrients or to find ways to increase or preserve them in cultivated varieties. Breeding will be central to putting produce with enhanced phytonutrients on the table. Lycopene of tomato, the cancer-preventing red pigment, through genetic engineering can be increased up to three times more than normal and a shelf life several weeks longer. Environmental and genetic factors also make a difference in beta-carotene levels in Cantaloupes by 500% depending on the soil, the cultivar, and fruit size. Onions kept in cold storage up to 90 days show more antiplatelet activity, useful for reducing cardiovascular disease risk by interfering with the clumping of blood platelets. Diseases like beriberi and scurvy were long believed to be due to toxins from pathogens in the digestive tract although they were due to vitamins B₁ and C deficiencies, respectively. In 1747, British naval surgeon James Lind cured scurvy with lime juice.

Phytochemicals have been considered as possible drugs for millennia. They include a wide variety of natural chemical compounds of secondary metabolic origin including alkaloids, terpenoids, phenolics, glycosides, etc., but are not established as essential nutrients like carbohydrates, proteins, lipids and similar other compounds. Some phytochemicals with potent medicinal properties may be elements rather than complex organic molecules. For example, selenium found abundantly in Brassica vegetables has shown potent antiviral and anticancer properties and in human clinical trials, selenium supplementation has been shown to reduce the HIV viral load and is currently being recommended worldwide by physicians as an adjuvant nutritional supplement to AIDS treatments. It has also been shown to reduce mortality among prostate cancer patients. Selenium, an essential dietary mineral nutrient, abundant in many fruits and vegetables, is involved with major metabolic pathways, including thyroid hormone metabolism, immune function, and cofactor for the enzymatic synthesis of an endogenous antioxidant glutathione. Chinese were able to described goiter and recommended seaweed and burnt sponge (both good sources of iodine) as early as 5000 years ago. The element iodine, however, was not recognized as dietarily essential until 1850. Iron as an essential element for the treatment of anemia was not known until the start of the nineteenth century. Sodium and potassium were soon followed by the other major minerals—calcium, sulfur, magnesium, and chlorine. Discovery of

other essential trace elements did not happen until the early twentieth century. Four elements, e.g., selenium, chromium, fluorine, and silicon (considered toxic food contaminants) gained essential status when were found to have a function in the body in the 1950s and 1970s.

Phytochemicals in freshly harvested plant foods may be degraded by modern processing techniques, including cooking due to thermal decomposition. However, lycopene may remain stable or increase in content from cooking due to liberation from cellular membranes in the cooked food. Industrially processed foods are likely to contain fewer phytochemicals and may thus be less beneficial than unprocessed foods.

Phytochemicals accumulate in different parts of the plant, e.g., in the roots, stems, woods, barks, leaves, flowers, fruits, seeds, latex and other exudates and the levels vary from plant to plant depending upon the variety, growing conditions, processing, etc. (Costa et al. 1999; King and Young 1999; Mathai 2000). A wide-ranging dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, herbs, spices, and fungi. Broccoli, cabbage, carrots, onions, garlic, whole wheat bread, beans, legumes, soy foods, tomatoes, grapes, cherries, strawberries, raspberries, and other edible grains, vegetables, and fruits are common dietary sources. Phytochemicals have important biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property.

1.2 Medicinal Phytochemistry

Medicinal phytochemistry includes the study of phytochemicals from medicinal plants and they are rich in bioactive phytochemicals or phytonutrients. The classification of phytochemicals may be done in various ways including on the basis of metabolic pathways such as primary or secondary constituents. Primary constituents include the soluble sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll, etc. Secondary constituents are alkaloids, terpenes, flavonoids, lignans, plant steroids, curcuminoids, saponins, phenolics, flavonoids, and glucosides. Of the secondary metabolites, phenolics (45%) are the most numerous and structurally diverse plant phytoconstituents, followed by terpenoids and steroids (27%), alkaloids (18%) and others (10%). Phenolic phytochemicals are the most widely distributed in the plant kingdom and the three most important groups of dietary phenolics are flavonoids, phenolic acids, and polyphenols. Phenolic phytochemicals show antioxidant activity. Almost all the alkaloids have a bitter taste and quinine is one of the bitterest tasting substances known. Among plant secondary metabolites terpenoids are a structurally most diverse and the largest group of natural products. They are widespread in nature, mainly in plants as constituents of essential oils. Many terpenoids are commercially important because of their use as medicine, flavors and fragrances in therapy, foods, and cosmetics.

(e.g., menthol, sclareol) or because they are important for the quality of agricultural products (e.g., flavor of fruits, fragrance of flowers like linalool). Isovaleric acid, prenol, eucalyptol, limonene, citral, camphor, pinene, artemisinin, bisabolol, ernesol, eudesmol, retinol, retinal, lanosterol, squalene, lycopene, gamma-carotene, alpha-and beta-carotenes, etc., are some other common terpene compounds. Terpenoids function as phytoalexins in plant direct defense, or as signals in indirect defense responses. Many plants produce volatile terpenes to attract insect pollinators or to expel certain herbivore animals. Strongly bitter-tasting or toxic terpenes as antifeedants also repel animals. Some terpenes function as signal compounds and growth regulators (phytohormones) of plants. Terpenoids may possess anticarcinogenic (e.g., perilla alcohol), antimalarial (e.g., artemisinin), anti-ulcer, hepatocidal, antimicrobial or diuretic (e.g., glycyrrhizin) activities. The sesquiterpenoid antimalarial drug artemisinin and the diterpenoid anticancer drug taxol are widely used in modern therapy. Alkaloids have many pharmacological activities including antihypertensive effects (many indole alkaloids), antiarrhythmic effect (quinidine, spareien), antimalarial activity (quinine), and anticancer actions (dimeric indoles, vincristine, vinblastine). Some of them have stimulant property, e.g., caffeine and nicotine; morphine is used as the analgesic and quinine as the antimalarial drug. Saponins are widely distributed in the plant kingdom and saponins as a group include compounds that are glycosylated steroids, triterpenoids, and steroid alkaloids. Spirostan and furostan derivatives are two steroid aglycones and derivative of oleanane is the main triterpene aglycone. The carbohydrate part consists of one or more sugar moieties containing glucose, galactose, xylose, arabinose, rhamnose, or glucuronic acid glycosidically linked to a sapogenin (aglycone). The physiological role of saponins in plants is not yet fully understood while many saponins are known to be antimicrobial, to inhibit mold, and to protect plants from insect attack. Saponins possess membrane-permeabilising, immunostimulant, hypocholesterolaemic, and anticarcinogenic properties, and they have also been found to significantly affect growth, feed intake, and reproduction in animals. These compounds have also been observed to kill protozoans and molluscs, to be antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycaemia, and to act as antifungal and antiviral (Takechi et al. 1999; Traore et al. 2000; George et al. 2002).

Crude drugs, over-the-counter remedies, ethical phytomedicines (standardized toxicologically and clinically defined crude drugs), etc., are promising low-cost alternative medicines used in primary health care of rural people of developing countries. The field herbal medicine also has benefited greatly in recent years from the interaction of the study of traditional ethnobotanical knowledge and the application of modern phytochemical analysis and biological activity studies to medicinal plants. For example, with the use of high-throughput robotic screening technique developed by industry, it is possible to carry out thousands of tests per day in search for desired therapeutic compounds; and bioassay-guided fractionation of plant extracts aided by chromatographic separation techniques is suitable for the isolation of biologically active molecules whose chemical structures can be elucidated by spectroscopic method.

Medicinal phytochemistry is receiving ever greater attention in research, pharmaceutical industry as well as in trade and economy. The European herbal market is the largest and accounts for about 50% of the world trade (Anonymous 2000). North American and Asian markets are other two big trade centers. China, India, and Germany are three leading exporting countries for herbs and herbal products (Kate and Laird 1999). The global trade of medicinal plants stands at US\$7592 million in 2011 and may touch US\$5 trillion by 2050. The estimated value of plant-based drugs is nearly US\$45,000 million per year.

1.3 Bioactive Compounds of Medicinal Plants

Bioactive compounds in plants can be defined as secondary plant metabolites eliciting pharmacological or toxicological effects in man and animals. A compound that has an effect on a living organism, tissue or cell is generally known as a bioactive compound. Although nutrients elicit pharmacological or toxicological effects when ingested at high dosages (e.g., vitamins and minerals), nutrients in plants are generally not included in the term bioactive plant compound. The typical bioactive compounds in plants are produced as secondary metabolites. Thus, a definition of bioactive compounds in plants is: secondary plant metabolites eliciting pharmacological or toxicological effects in man and animals. Bioactive compounds differ from essential nutrients; the nutrients are essential to the sustainability of a body, the bioactive compounds are not essential but provide health benefits. A number of bioactive substances have been shown to possess antioxidant and other activities of therapeutic importance. Bioactive compounds are mostly of biological origin (found in plant and animal products). Bioactive compounds (secondary metabolites) are derived from the products of plant primary metabolites, which are associated with the process of photosynthesis, viz., carbohydrates, amino acids, and simple lipids. Bioactive principles are synthesized by two major pathways: the shikimic acid or aromatic amino acid and mevalonic acid pathways (Ramawat et al. 2009). Bioactive compounds include diverse group of secondary metabolites including phenolics, terpenoids and steroids, alkaloids, glycosides, etc., and these are the most important active components of herbal drugs. The use of bioactive compounds from medicinal plants as therapeutic agents has been an important area in biomedical and natural product research (Mgbeahuruike et al. 2017). Plants, both most food and feed plants, produce a broad range of bioactive chemical compounds via secondary metabolism. Bioactive compounds consist of chemicals that are found in small volumes in plants and particular foods such as fruits, vegetables, nuts, oils, and whole grains.

The active components in a drug are the chemicals that create the intended therapeutic effects, and any side effects. The inert ingredients in the medication that do not exert the intended effect for taking it, and do not cause the side effects, known or unknown, associated with that drug. The inert constituents are the cellulose, wood, and other structural parts of the drug, and in some instances starch,

albumen, etc. Inactive ingredients are used in the manufacturing process and/or are present in the final medication product. They fulfill a variety of purposes, from delivering the active ingredient to making the pill look and taste good, along with other tasks such as coatings for easier injection, and timed or targeted release, flavors, and colors to make “swallowing medicine” more pleasant. Different types of nanoparticles are being used to encapsulate the active ingredients of drugs, to reduce required doses and improve organ and tissue specificity.

The medicinal value of a crude drug depends on the presence of one or more active chemical constituents. They may be glycosides, alkaloids, organized resins, enzymes, etc. A vegetable drug is composed of a number of tissues such as cells, fibers, vessels and other structures. The cell walls may consist of cellulose, lignins, tannins or cork cells. The cells of aromatic drugs like Cinnamon and Coriander contain volatile oils occurring in specialized cells or glands. The glycosides and alkaloids may occur in solution in the cell sap and deposit in the cells later on. The total contents of the cells do not carry physiological importance. For example, calcium oxalate occurs as a crystalline deposit and protein may occur as solid aleurone grains. Both these components are rejected in the preparation of a tincture or extract of the drug. The unorganized drugs possess no cellular structure but consist of extracts, exudation, secretions and other products of the plants. The value of gums, gum-resins, oleo-resins, starch, fixed oils, catechu, and opium depends on the whole of the material present. The constituents of drugs of medicinal value generally belong to one of the following group: carbohydrates, gums, acids, glycosides, anthraquinone derivatives, alkaloids, tannins and other phenols, enzymes, proteins, resins, fixed oils, fats, waxes, volatile oils. Anthocyanins, carotenoids, caffeine, carnitine, choline, coenzyme Q, creatine, dithiolthiones, fatty acids, found in milk and fish oil, flavonoids, glucosinolates, polyphenols, phytosterols, polysaccharides, phytoestrogens, polyphenols, prebiotics, taurine, etc., and they are examples of some common bioactive compounds (Srivastava and Kulshreshtha 1989; Golmohamadi et al. 2013; Paul et al. 2015).

Medicinal plants are a rich source of bioactive phytochemicals or bionutrients and bioactive compounds from medicinal plants provide unlimited opportunities for new drug leads because of their unlimited chemical diversity. Studies carried out during the past 2–3 decades have shown that these phytochemicals have an important role in preventing chronic diseases like cancer, diabetes, and coronary heart disease. The major classes of phytochemicals with disease-preventing functions are dietary fiber, antioxidants, anticancer, detoxifying agents, immunity-potentiating agents and neuropharmacological agents. Each class of these functional agents consists of a wide range of chemicals with differing potency. Some of these phytochemicals have more than one function. There is, however, much scope for further systematic research in screening Indian medicinal plants for these phytochemicals and assessing their potential in protecting against different types of diseases.

Medicinal plants have been utilized as a source of phytochemicals for the pharmaceutical, cosmetic and agrochemical industries. Bioactive phytochemicals like anticancer drugs paclitaxel, vincristine and vinblastine, antimalarial agent

Table 1.1 Bioactive phytochemicals in medicinal plants

Classes	Main groups of compounds	Biological function
Non-starch polysaccharides (NSA)	Cellulose, hemicellulose, gums, mucilages, pectins, and lignins	Water holding capacity, delay in nutrient absorption, binding toxins and bile acids
Antibacterial and antifungal	Terpenoids, alkaloids, phenolics	Inhibitors of microorganisms, reduce the risk of fungal infection
Antioxidants	Polyphenolic compounds, flavonoids, carotenoids, tocopherols, ascorbic acid	Oxygen free radical quenching, inhibition of lipid peroxidation
Anticancer	Carotenoids, polyphenols, curcumine, flavonoids	Inhibitors of tumor, inhibited development of lung cancer, antimetastatic activity
Detoxifying agents	Reductive acids, tocopherols, phenols, indoles, aromatic isothiocyanates, coumarins, flavones, carotenoids, retinoids, cyanates, phytosterols, inhibitors of tumourogenesis	Inhibitors of procarcinogen activation, inducers of drug binding of carcinogens
Other	Alkaloids, terpenoids, volatile flavor compounds, biogenic amines	Neuropharmacological agents, antioxidants, cancer chemoprevention

artemisinin; cosmeceuticals like vitamins, polyphenols, amino acids, peptides, etc., and agrochemicals like pyrethrum, rotenone, essential oils, etc., are obtained from medicinal plants. Bioactive phytochemicals present in medicinal plant may be grouped in the following way (Table 1.1).

Bioactive principles are responsible for the therapeutic activities of medicinal plants such as hypoglycemic, anti-diabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anticholinergic, anti-leprosy activities, etc. (Marles and Farnsworth 1995; Negi et al. 2011). Some other compounds (antinutrient compounds) of plant origin are the cyanogenic glycosides, myristicin, phytohaemagglutinins or lectin, neurotoxic amino acids, e.g., β -N-Oxalyl-L- α , β -diaminopropionic acid (ODAP), protease inhibitors, chlorogenic acid, amylase inhibitors, gossypol, goitrogens, veratridines, etc. (Rao et al. 1964; Aregheore and Agunbiade 1991; FSANZ 2004; Lee et al. 2005; Schep et al. 2006; Akande et al. 2010; Tadele 2015).

Bioactive compounds, pure compounds or standardized extracts from medicinal plants, provide unlimited opportunities for new drug leads because of the unmatched availability and chemical diversity of bioactive principles from the plant kingdom (Ramawat et al. 2009; Sasidharan et al. 2011; Cragg et al. 2012; Cragg and Newman 2013). In recent years, a number of new drugs, such as the anticancer chemotherapies-paclitaxel (taxol) from *Taxus brevifolia* and the analog, docetaxel, Vinca alkaloids vinblastine and vincristine from *Catharanthus roseus*, etoposide and teniposide from roots of *Podophyllum* species, antimalarial drug artemisinin

from *Artemisia annua* gave mankind have been developed in addition to other drugs derived from natural products, which account for substantial quantity of new registered drugs (WHO 2001; Gueritte and Fahy 2005; Fadeyi et al. 2013). Bioactive principles from medicinal plants are also used as agrochemicals, flavor and fragrance ingredients, food additives and pesticides (Ramawat et al. 2009; Rahimi et al. 2012). Databases are now available on pharmacophore analysis of active principles possessing anti-diabetic, antimicrobial, anticancerous, and antioxidant properties from medicinal plants (Pitchai et al. 2010).

The plant kingdom includes a high number of species, producing a diversity of bioactive compounds with different chemical scaffolds. Medicinal plants are the source of different bioactive compounds of secondary metabolic origin such as alkaloids, flavonoids, terpenoids, phlobatannins, steroids, glycosides, reducing sugars, polyols, etc. Medicinal plants bioactive compounds have potentials for curing and healing of various ailments; play role in antioxidant, antidiaryentery, anti-inflammatory, antianalgesic, anticancer, antiviral, plasmid curing, antibacterial, antimalarial, and antifungal activities. Medicinal plants also provide therapeutic benefits as pugative anthelmintic, deflatulent, rejuvenating tonic, aphrodisiac, etc., and may also be used for treatment in scrofula, hematological disorders, worm infestations, haemorrhoids, skin disorders, conjunctivitis, leucoderma, dyspepsia, urinary discharges, jaundice, diabetes, asthma, bronchitis, general debility as well as polyurea. The therapeutic as well as beneficial effects of medicinal plants is due to the presence of the bioactive compounds in them. The multicomponent formulation forms the basis of phytotherapy. The polypharmacological effects of plants constitute increased bioavailability, interference with cellular transport processes, activation of pro-drugs or deactivation of active compounds to inactive metabolites and action of synergistic action.

Of the 1073 new chemical entities belonging to the group of small molecules that had been approved between 1981 and 2010, only 36% were purely synthetic, while more than the half were derived or inspired from nature (Newman and Cragg 2012). A substantial number of these compounds have been discovered in higher plants (Kinghorn et al. 2011). Particularly prominent examples of plant-derived natural compounds that have become indispensable for modern pharmacotherapy can be found in the field of anti-cancer agents, e.g., paclitaxel and its derivatives from yew (*Taxus*) species, vincristine and vinblastine from Madagascar periwinkle (*Catharanthus roseus*), and camptothecin and its analogs initially discovered in the Chinese tree *Camptotheca acuminata* Decne (Cragg and Newman 2013; Kinghorn et al. 2011). Further notable examples include the cholinesterase inhibitor galanthamine that has been approved for the treatment of Alzheimer's disease and was initially discovered in *Galanthus nivalis* L. (Mashkovsky and Kruglikova-Lvova 1951), and the important antimalarial and potential anti-cancer agent artemisinin originally derived from the traditional Chinese herb *Artemisia annua* L. (Klayman et al. 1984).

Many potent plant-derived natural products such as paclitaxel, podophyllotoxin, or vinblastine share severe difficulties to meet the market demands, as their natural sources are often slow-growing or even endangered species that tend to accumulate

these compounds at very low quantities over long growth periods (Miralpeix et al. 2013; Staniek et al. 2014). Cultivation of endangered medicinal plants under controlled conditions represents a promising protective approach (Tasheva and Kosturkova 2013).

1.4 Metabolomics and Phytoconstituents

Metabolomics is the systematic large-scale study including the identification and quantification of the small molecules or metabolic products (metabolome) of a biological system (cell, tissue, organ, biological fluid, or organism) under a given set of conditions at a specific point in time. But for simplicity, metabolomics is the study of small molecule metabolites as shown in Fig. 1.5 in the omics schema.

The metabolome represents the collection of all metabolites in a biological cell, tissue, organ, or organism, which are the end products of cellular processes (Jordan et al. 2009). Plant metabolites are small chemical molecules that aid in a cell's survival. They are used by plant cells for growth and development, which are known as primary metabolites. Secondary metabolites serve in specialized

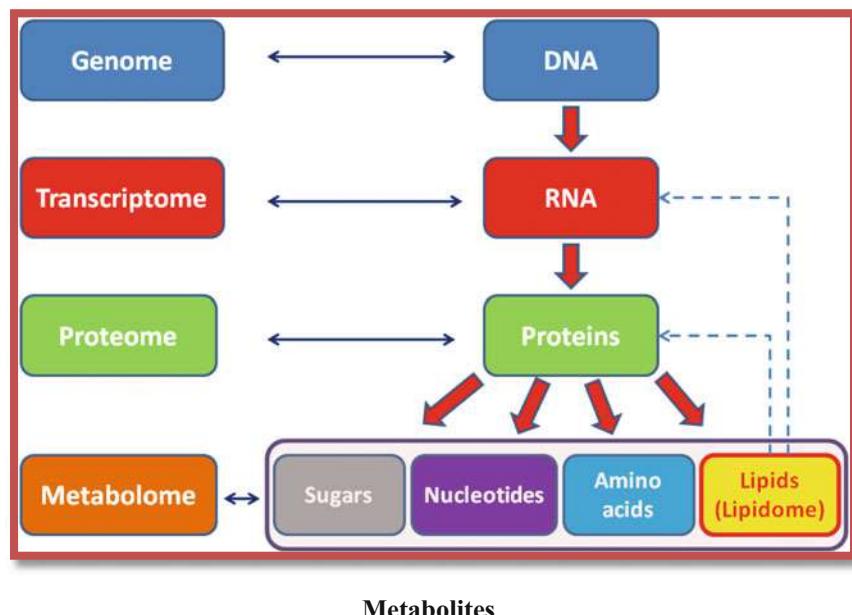


Fig. 1.5 The omics schema showing genomics (studying genes), transcriptomics (studying gene by-products), proteomics (studying proteins), metabolomics (studying metabolites), glycomics (studying simple carbohydrates), lipidomics (studying fats), etc., omics including the study of nucleotides, amino acids, secondary metabolites, and other small molecules

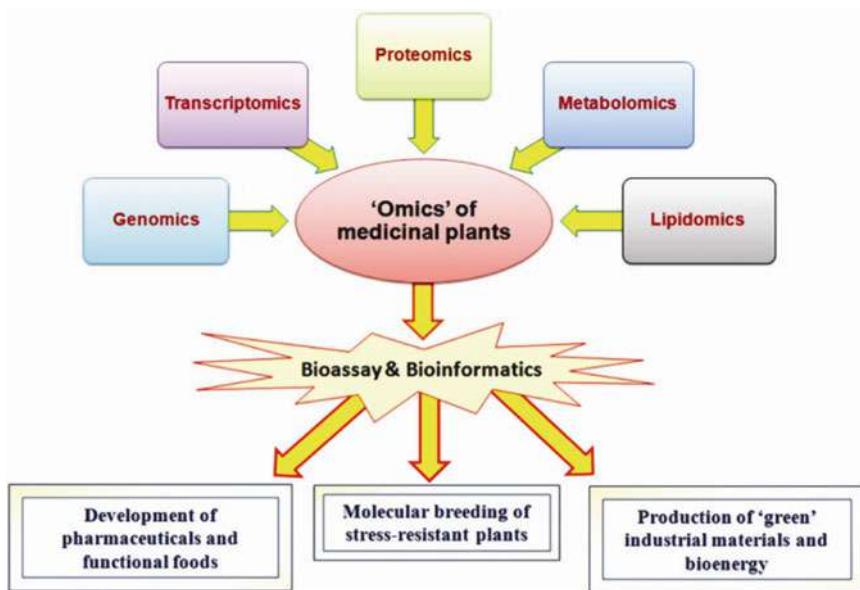
processes and tend to be used in defense mechanisms, hormonal signaling and flower color, etc. Secondary metabolites are not found in all plants and will be found in higher or lower concentrations depending on the current state of the plant and the environment.

Metabolomics, a modern omic-technique for comprehensive analysis of phyto-constituents or chemicals is increasingly playing a vital role in natural products drug discovery drug discovery and development processes by identification and profiling of secondary metabolites in medicinal plants (Gahlaut et al. 2013). It is an important sector of post-genome science era and deals with all cellular metabolites. Metabolomics study of medicinal plants and herbs is very important because they synthesize a large number of primary and secondary metabolites and thousands of secondary metabolites ($\geq 200,000$) from plants have been explored (Trethewey 2004). Metabolomics aims at the comprehensive qualitative and quantitative analysis of the plant metabolome, which consists of both primary and secondary metabolites, and some of them have potential therapeutic importance. Secondary metabolites from plants provide lead molecules for drug development and metabolomics holds promise in comprehensive profiling of secondary metabolites (Ellis et al. 2007).

Metabolomics is a powerful analytical tool in herbal medicine research for assessment of various secondary metabolites (Tweeddale et al. 1998) and provides a systems biology approach for target compound analysis (TCA) in medicinal plants. For metabolite profiling in medicinal herbs and herbal products, different analytical techniques and methods such as sample preparation, instrumental analysis, and data processing are required. It has been employed for quality evaluation, TCA, and metabolite fingerprinting of Ayurvedic herbs (Fukusaki and Kobayashi 2005). Factors like genetic condition, cultivation, collection, storage, milling, processing of final products, etc., affect the quality and standards of food and herbal products. Metabolomics can readily help in characterization of useful metabolites and chemical markers present in herbal products (Wolfender et al. 2013) and exhaustive metabolomic profiling of herbal medicines is necessary for scientific validation. Metabolomics having multidisciplinary facets can be explored in different fields including drug discovery and development, high-throughput screening for evaluation of herbal drugs and many others (Ulrich-Merzenich et al. 2007).

1.4.1 Metabolomics of Medicinal Plants

Plant secondary metabolites like paclitaxel (Taxol), camptothecin (irinotecan, topotecan), aspirin (precursor salicin from the *Salix alba*) and podophyllotoxins (etoposide, teniposide), etc., have been known to possess potential anticancer, anti-inflammatory, and analgesic activities and medicinal plants or their products are now firmly considered as alternative sources of finding new chemical entities (NCEs) for drug discovery and development (Harvey 2007; Newman and Cragg 2007). The ethnic uses of herbs or extracts as traditional medicines are the right



The phytochemical array- a concept for using various omics based systems in plants

Fig. 1.6 Various phytochemical techniques used in the field of medicinal plant research. *Source* Mukherjee et al. (2016)

pathways for finding new molecules like morphine from opium and so on. Metabolomics can be used as an effective platform to understand the phytochemical basis of the synergistic effects of different therapeutically active phytoconstituents present in herbs or their preparations even in Nano quantity (Williamson 2001). Figure 1.6 shows different omics-based “phytochemical arrays” (genomics, transcriptomics, proteomics, metabolomics, etc.) of medicinal plants that have been established for measurement and analysis of several aspects including metabolite profiling in plants (Bino et al. 2004).

Metabolomics of medicinal plants is an important sector of post-genome science era and deals with all cellular metabolites of medicinal plants. It is derived from transcriptomics, genomics, and proteomics in providing systematic approaches to the study of biological systems. Figure 1.2 shows different omics-based phytochemical arrays (genomics, transcriptomics, proteomics, metabolomics, etc.) that have been established for measurement and analysis of several aspects including metabolite profiling in plants (Bino et al. 2004).

Phytochemical genomics is a recently emerging field, which investigates the genomic basis of the synthesis and function of phytochemicals (plant metabolites), particularly based on advanced metabolomics (Saito 2013). Functional genomics analyses include investigations at the level of gene expression (transcriptomics), protein translation (proteomics) and more recently the metabolite network

(metabolomics). Metabolomics is the study of global metabolite profiles in a system (cell, tissue, or organism) under a given set of conditions. The analysis of the metabolome is particularly challenging due to the diverse chemical nature of metabolites in a cell.

Metabolomic fingerprinting can be very helpful in the field of herbal medicine for drug discovery, systems biology, gene-function analysis and various diagnostic techniques through different modern hyphenated technologies. A study involved in the characterization of a set of defined metabolites is known as “targeted” metabolomics and usually combine NMR-MS techniques, which is applied for such types of analysis (Dudley et al. 2010). In case of “untargeted” metabolomics study, the undefined and unknown metabolites from plant may be identified through LC-MS and GC-MS analysis. This can be very useful for characterization and identification of metabolites and can be helpful for evaluation of herbal medicine (Patti et al. 2012). Metabolomics study has diverse fields of application. It includes metabolite fingerprinting, which can be applied in different aspects like qualitative and quantitative analysis of target compound, identification of a set of compounds, quantification of all metabolites and rapid analysis of metabolites (Ulrich-Merzenich et al. 2007).

1.4.2 Metabolomics as a Tool for Quality Evaluation of Herbal Products

Metabolomics approach is based on the unbiased acquisition of MS and NMR data from specially prepared samples. Advances in mass spectrometry (MS) based platforms like GC-MS and LC-MS, helped in separation and identification of several metabolites. Mass spectrometry (MS) analysis can be a valuable tool for identifying potential biomolecules from medicinal plants. Despite the potential use, metabolomics data of medicinal plants, spices, health food, etc., used in the conventional systems of medicine are extremely limited. Metabolomic studies on Ayurvedic formulations, e.g., Triphala are lacking. Metabolomic information or data of medicinal herbs are crucial for quality evaluation and scientific validation of herbal products. Since the aim is to comprehensively profile the largest possible array of low molecular weight metabolites in a given biological sample, techniques such as multiple-phase solvent systems coupled to accelerated extraction processes such as microwave, ultrasonic, pressurized or accelerated liquid can be applied to drastically reduce extraction times. Sample preparation usually involves the use of quality buffer and deuterated solvents that will minimize the chemical shift due to pH of the sample solution and result in good quality spectra.

Plant metabolomics provides a comprehensive understanding of the spectrum of phytochemical constituents of plants and metabolomics approach is now widely applied in medical science, synthetic biology, Ayurvedic medicine and predictive modeling of plant, animal, and microbial systems. The working principle of

metabolomics deals with sample preparation, separation of compounds, identification, data processing and finally analysis. Due to the development of effective technology for separation and identification of metabolites, the technique of metabolomics is fast becoming a versatile tool for exploitation of medicinal plants, for biomarker-driven drug discovery and development. Measurement and analysis of metabolites can be a precise and potentially valuable technology for identifying biomarkers. Metabolomics offers a promising approach to plant metabolite fingerprinting and such studies are urgently needed for better understanding of medicinal plants (Mukherjee et al. 2016).

1.5 Methods (Techniques) of Phytochemical Investigation and High-Throughput Screening (HTS) for Active Plant Constituents

Pharmacological activity in herbal drugs is based on the presence of chemical compounds which are called active principles, drug principles or components. The phytochemistry deals with methods of obtaining these active ingredients, their classification according to the functional organic chemical group to which it belongs, and studies the analytical methods to verify its quality. Active components are found in different parts and organs of plants. A wide range of active components has been discovered, and among them, the most important are alkaloids, terpenoids, phenolics, glycosides or heterosides, mucilage and gums, tannins, etc. Other constituents in plants are vitamins, minerals, amino acids, carbohydrates and fibers, some sugars, organic acids, lipids, and antibiotics. The premier steps to utilize the biologically active compound from plant resources are extraction, pharmacological screening, isolation and characterization of bioactive compound, toxicological evaluation, and clinical evaluation. Phytochemistry is very important for the determination of the bioactive chemical ingredients of medicinal plants, their quantification, and analysis of the beneficial and harmful effects to human health. A brief summary of the general approaches in extraction, isolation, and characterization of bioactive compound from plants extract is shown in Fig. 1.7.

The analysis of bioactive compounds present in the plant extracts involving the applications of common phytochemical screening assays, chromatographic techniques such as TLC, HPLC and HPLC/MS as well as non-chromatographic techniques such as immunoassay and Fourier Transform Infra Red (FTIR) and Fourier Transform Mass Spectrometry (FTMS). Since bioactive compounds occurring in plant material consists of multicomponent mixtures, their separation and determination still create problems. Practically most of them have to be purified by the combination of several chromatographic techniques and various other purification methods to isolate bioactive compound(s).

Phytochemical techniques are widely used in the quality control of Chinese, Ayurvedic and Unani or other herbal medicines for the determination of various

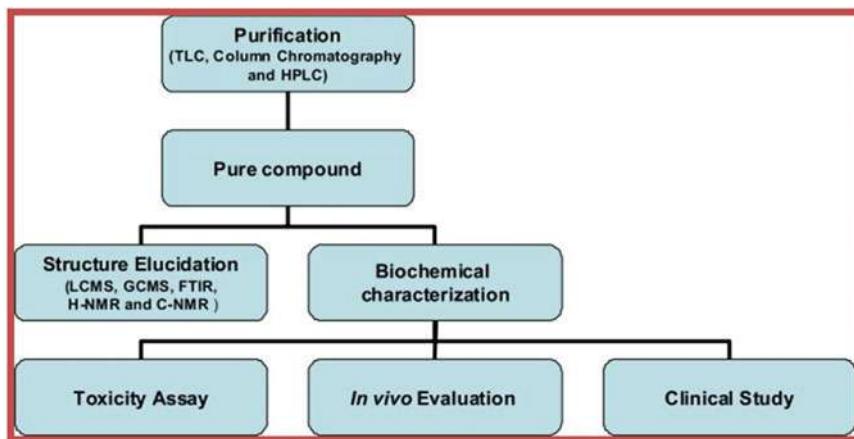


Fig. 1.7 A brief summary of the general approaches in extraction, isolation, and characterization of bioactive compound from plants extract

chemical components, e.g., saponins, alkaloids, volatile oils, flavonoids, anthraquinones, etc. Techniques commonly used in the field of phytochemistry are extraction, isolation, and structural elucidation (MS, 1D and 2D NMR) of natural products, high-throughput screening (HTS) techniques for efficacy and interaction assessment, as well as various chromatography techniques (LC, GC, MPLC, HPLC). There are many other hyphenated chromatographic techniques for data acquisition such as the LC-MS, gas chromatography (GC)-MS and capillary electrophoresis (CE)-MS, as well as direct spectroscopic methods such as NMR and direct injection mass spectroscopy (DIMS). Among others, LC-MS is the most widely used technology in phytotherapeutic drug discovery due to its ability to detect and separate a diverse range of molecules with high sensitivity. When coupled to the NMR, it is even more versatile. The successful combination and application of these analytical tools, data mining, and processing, in combination with bioassay profiling methods serve an important role in metabolomics for the purpose of dereplication in natural products-based drug discovery. The Austrian Drugs from Nature Targeting Inflammation (DNTI) program aimed at identifying and characterizing natural products with anti-inflammatory activity by the combined and synergistic use of computational techniques, ethnopharmacological knowledge, phytochemicals analysis and isolation, organic synthesis, plant biotechnology, and a broad range of *in vitro*, cell-based, and *in vivo* bioactivity models.

For the development of rapid and reproducible analytical techniques in medicinal phytochemistry, phytochemical techniques such as HPLC combined with different detectors (e.g., diode array detector—DAD, refractive index detector—RID, evaporative light scattering detector—ELSD, mass spectrometric detector—MSD, etc.) are applied for determination and standardization of various bioactive

chemical components (e.g., alkaloids, volatile oils, saponins, flavonoids, anthraquinones, etc.) of medicinal plants with a view to upholding the quality matter of herbal medicines used in the traditional systems of like TCM, Ayurvedic medicines, etc. Binding to receptors or ion channels on cell membranes is the first step of some drug actions, and a new method in phytochemistry, biochromatography, has been developed that combines human red cell membrane extraction and HPLC to screen potential active components in traditional medicines. A new method in phytochemistry called biochromatography has been developed. This method combines human red cell membrane extraction and high-performance liquid chromatography to screen potential active components in Chinese medicine.

High-throughput screening (HTS)

High-throughput screening (HTS) is one of the newest methods for scientific experimentation used in drug discovery and may be applied in biological and chemical sciences (Inglese and Auld 2009; Macarron et al. 2011). It has gained widespread popularity over the last two decades and has become a standard method for drug discovery in the pharmaceutical industry. It is basically a process of screening and assaying a large number of biological modulators and effectors against selected and specific targets. HTS consist of several steps such as target identification, reagent preparation, compound management, assay development and high-throughput library screening (Armstrong 1999). Using robotics, data processing-control software, liquid handling devices, and sensitive detectors, HTS allows to conduct a series of analyses of chemical compounds in a short and due to the implementation of automation of HTS technique, it is now possible to perform examine millions of chemical, genetic, or pharmacological tests per day. Through this process, one can rapidly identify active compounds, antibodies, or genes that modulate a particular biomolecular pathway. The results of these experiments provide starting points for drug design and for understanding the interaction or role of a particular biochemical process in biology. The HTS method is more frequently utilized in conjunction with analytical techniques such as NMR or coupled methods, e.g., LC-MS/MS.

The key labware (testing vessel) of HTS is the microtiter plate (microplate), a small disposable and made of plastic container having many small, open divots (wells), e.g., 384, 1536, or 3456 wells (multiples of 96), with spaced wells of 8×12.9 mm. Most of the wells contain test items, depending on the nature of the experiment. A screening facility typically holds a library of stock plates, whose contents are carefully catalogued. Assay plates (copy of a stock plates) are created from the stock plates by pipetting a small amount of liquid (nanoliters) from the wells of a stock plate to the corresponding wells of a completely empty plate.

Natural products continue to have great potential in screening for new drug leads. High-throughput screening (HTS) has been used for drug discovery for over a decade, it is an important and effective tool used in Western pharmaceutical development. This automatic process might also prove useful for Chinese pharmaceutical screening. Besides, to improve drug screening efficacy and minimize animal testing, recent efforts have been dedicated to developing cell-based

high-throughput screening (HTS) platforms that can provide more relevant in vivo biological information than biochemical assays and thus reduce the number of animal tests and accelerate the drug discovery process. HTS assays are used for screening of different types of libraries, including combinatorial chemistry, genomics, protein, and peptide libraries. The main goal of the HTS technique is to accelerate drug discovery by screening large compound libraries at a rate that may exceed a few thousand compounds per day or per week. High-throughput screening methods are also used to characterize metabolic, pharmacokinetic and toxicological data about new drugs. HTS technology can reduce the costs of drug development (Armstrong 1999; Hann and Opera 2004; Fernandes et al. 2009; Clark et al. 2010).

References

- Ackerknecht EH (1973) Therapeutics: from the primitives to the 20th century. Hafner Press, NY
- Agarwal S, Rao AV (2000) Tomato lycopene and its role in human health and chronic diseases. *CMAJ* 163(6):739–744
- Akande KE, Doma UD, Agu HO, Adamu HM (2010) Major antinutrients found in plant protein sources: their effect on nutrition. *Pak J Nutr* 9:827–832
- Anonymous (2000) Report of the task force on conservation and sustainable use of medicinal plants. Planning Commission, New Delhi
- Aregheore EM, Agunbiade OO (1991) The toxic effects of cassava (*Manihot esculenta grantz*) diets on humans: a review. *Vet Hum Toxicol* 33:274–275
- Armstrong JW (1999) A review of high-throughput screening approaches for drug discovery. *Am Biotechnol Lab* 17:26–28
- Bino RJ, Hall RD, Fiehn O, Kopka J, Saito K, Draper J et al (2004) Potential of metabolomics as a functional genomics tool. *Trends Plant Sci* 9(9):418–425
- Canene-Adams K, Lindshield B, Wang S, Jeffery E, Clinton S, Erdman J (2007) Combinations of tomato and broccoli enhance antitumor activity in dunning R3327-H prostate adenocarcinomas. *Cancer Res* 67(2):836–843
- Clark RL, Johnston BF, Mackay SP, Breslin CJ, Robertson M, Harley AL (2010) The drug discovery portal: a resource to enhance drug discovery from Akademia. *Drug Discov Today* 15:679–683
- Costa MA, Zia ZQ, Davin LB, Lewis NG (1999) Toward engineering the metabolic pathways of cancer-preventing lignans in cereal grains and other crops. In: Romeo JT (ed) Recent advances in phytochemistry, vol 33. Phytochemicals in Human Health Protection, Nutrition, and Plant Defense, New York, pp 67–87
- Cragg GM, Katz F, Newman DJ, Rosenthal J (2012) The impact of the United Nations convention on biological diversity on natural products research. *Nat Prod Rep* 29:1407–1423
- Cragg GM, Newman DJ (2013) Natural products: a continuing source of novel drug leads. *Biochem Biophys Acta* 1830(6):3670–3695
- Dalessandri KM, Firestone GL, Fitch MD, Bradlow HL, Bjeldanes LF (2004) Pilot study: effect of 3,3'-diindolylmethane supplements on urinary hormone metabolites in postmenopausal women with a history of early-stage breast cancer. *J Nutr Cancer* 50(2):161–167
- Dewick PM (2002) Medicinal natural products: a biosynthetic approach, 2nd edn. Wiley, Baffins Lane
- Dudley E, Yousef M, Wang Y, Griffiths W (2010) Targeted metabolomics and mass spectrometry. *Adv Protein Chem Struct Biol* 80:45–83
- Ellis DI, Dunn WB, Griffin JL, Allwood JW, Goodacre R (2007) Metabolic fingerprinting as a diagnostic tool. *Pharmacogenomics* 8:1243–1266

- Fadeyi SA, Fadeyi OO, Adejumo AA, Okoro C, Myles EL (2013) In vitro anticancer screening of 24 locally used Nigerian medicinal plants. BMC Complement Altern Med. <https://doi.org/10.1186/1472-6882-13-79>
- Fernandes T, Diogo MM, Clark DS, Dordick JS, Cabral J (2009) High-throughput cellular microarray platforms: Applications in drug discovery, toxicology and stem cell research. Trends Biotechnol 27:342–349
- FSANZ (2004) Cyanogenic glycosides in cassava and bamboo shoots: A human health risk assessment. Technical report series no. 28, Food Standards Australia New Zealand (FSANZ)
- Fukusaki E, Kobayashi A (2005) Plant metabolomics: potential for practical operation. J Biosci Bioeng 100:347–354
- Gahlaut A, Vikas DM, Gothwal A, Kulharia M, Chhillar AK, Hooda V et al (2013) Proteomics and metabolomics: mapping biochemical regulations. Drug Invent Today 5:321–326
- George F, Zohar K, Harinder PS, Makkar Klaus B (2002) The biological action of saponins in animal systems: a review. British J Nutrition 88:587–605
- Gibson EL, Wardel J, Watts CJ (1998) Fruit and vegetable consumption, nutritional knowledge and beliefs in mothers and children. Appetite 31:205–228
- Golmohamadi A, Möller G, Powers J, Nindo C (2013) Effect of ultrasound frequency on antioxidant activity, total phenolic and anthocyanin content of red raspberry puree. Ultrason Sonochem 20(5):1316–1323
- Gueritte F, Fahy J (2005) The vinca alkaloids. In: Cragg GM, Kingston DGI, Newman DJ (eds) Anticancer Agents from natural products. Taylor and Francis Group, Boca Raton, Florida, pp 123–136
- Hann M, Opera TI (2004) Pursuing the leadlikeness concept in pharmaceutical research. Curr Opin Chem Biol 8:255–263
- Harvey AL (2007) Natural products as screening resource. Curr Opin Chem Biol 11:480–484
- Hasler CM, Blumberg JB (1999) Symposium on phytochemicals: biochemistry and physiology. J Nutr 129:756S–757S
- Hurwitz BE, Klaus JR, Llabre MM, Gonzalez A, Lawrence PJ, Maher KJ et al (2007) Suppression of human immunodeficiency virus type 1 viral load with selenium supplementation: a randomized controlled trial. Arch Intern Med 167(2):148–154
- Inglese J, Auld DS (2009) Application of high throughput screening (HTS) techniques: applications in chemical biology in Wiley Encyclopedia of chemical biology, vol 2. Wiley, Hoboken, NJ, pp 260–274
- Jordan KW, Nordenstam J, Lauwers GY, Rothenberger DA, Alavi K, Garwood M et al (2009) Metabolomic characterization of human rectal adenocarcinoma with intact tissue magnetic resonance spectroscopy. Dis Colon Rectum 52(3):520–525
- Kate KT, Laird SA (1999) The commercial use of biodiversity: access to genetic resources and benefit-sharing. Earthscan, London, UK. books.google.com
- King A, Young G (1999) Characteristics and occurrence of phenolic phytochemicals. J Am Dietetic Association 24:213–218
- Kinghorn AD, Pan L, Fletcher JN, Chai H (2011) The relevance of higher plants in lead compound discovery programs. J Nat Prod 74:1539–1555
- Klayman DL, Lin AJ, Acton N, Scovill JP, Hoch JM, Milhous WK et al (1984) Isolation of artemisinin (qinghaosu) from *Artemisia annua* growing in the United States. J Nat Prod 47:715–717
- Lee BK, Kim JH, Jung JW, Choi JW, Han ES et al (2005) Myristicin-induced neurotoxicity in human neuroblastoma SK-N-SH cells. Toxicol Lett 157:49–56
- Letavayova L, Vichova V, Brozmanova J (2006) Selenium: from cancer prevention to DNA damage. J Toxicology 227(1–2):1–14
- Macarron R, Banks MN, Bojanic D, Burns DJ, Cirovic DA, Garyantes T et al (2011) Impact of high-throughput screening in biomedical research. Nat Rev Drug Discov 10:188–195
- Mahdi JG (2010) Medicinal potential of willow: a chemical perspective of aspirin discovery. J Saudi Chem Soc 14:317–322

- Marles RJ, Farnsworth NR (1995) Antidiabetic plants and their active constituents. *Phytomedicine* 2:137–189
- Mashkovsky MD, Kruglikova-Lvova RP (1951) On the pharmacology of the new alkaloid galantamine. *Farmacol Toxicol (Mosk)* 14:27–30 (in Russian)
- Mathai K (2000) Nutrition in the adult years. In: Mahan LK, Escott-Stump S (ed) Krause's food, nutrition, and diet therapy, 10th edn. pp 271, 274–275
- McGuire K, Ngoubilly N, Neavyn M, Lanza-Jacoby S (2006) Diindolylmethane and Paclitaxel Act synergistically to promote apoptosis in HER2/Neu human breast cancer cells. *J Surgical Res* 132(2):208–213
- Mgbeahuruike EE, Yrjönen T, Vuorela H, Holm Y (2017) Bioactive compounds from medicinal plants: focus on *Piper* species Review. *South African J Bot.* 112:54–69
- Miralpeix B, Rischer H, Hakkinen ST, Ritala A, Seppanen-Laakso T, Oksman-Caldentey KM et al (2013) Metabolic engineering of plant secondary products: which way forward? *Curr Pharm Des* 19:5622–5639
- Mukherjee PK, Harwansh RK, Bahadur S, Biswas S, Kuchibhatla LN, Tetali SD et al (2016) Metabolomics of medicinal plants—a versatile tool for standardization of herbal products and quality evaluation of ayurvedic formulations—review article. *Current Sci.* 111(10):1624–1630
- Negi JS, Singh P, Rawat B (2011) Chemical constituents and biological importance of *Swertia*: a review. *Curr Res Chem* 3:1–15
- Newman DJ, Cragg GM (2007) Natural products as sources of new drugs over the last 25 years. *J Nat Prod* 70:461–477
- Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod* 75:311–335
- Patti GJ, Yanes O, Siuzdak G (2012) Innovation: metabolomics: the apogee of the omics trilogy. *Nat Rev Mol Cell Biol* 13:263–269
- Paul CC, Chiedozie OI, Ferdinand NM (2015) Bioactive principles from medicinal plants. *Res J Phytochem* 9:88–115
- Pitchai D, Manikkam R, Rajendran SR, Pitchai G (2010) Database on pharmacophore analysis of active principles, from medicinal plants. *Bioinformation* 5:43–45
- Rahimi M, Farhadi R, Balashahri MS, Racisi AS (2012) Applications of new technologies in medicinal plant. *Int J Agron Plant Prod* 3:128–131
- Ramawat KG, Dass S, Mathur M (2009) The chemical diversity of bioactive molecules and therapeutic potential of medicinal plants. In: Ramawat KG (Ed) Herbal drugs: ethnomedicine to modern medicine. Springer, New York, ISBN: 978-3-540-79116-4, pp 7–32
- Rao SLN, Adiga PR, Sarma PS (1964) The isolation and characterization of β -N-oxalyl-L- α , β -diaminopropionic acid: a neurotoxin from the seeds of *Lathyrus sativus*. *Biochemistry* 47:432–436
- Rao AV, Ray MR, Rao LG (2006) Lycopene. *Adv Food Nutr Res* 51:99–164
- Ray AL, Semba RD, Walston J, Ferrucci L, Cappola AR, Ricks MO et al (2006) Low serum selenium and total carotenoids predict mortality among older women living in the community. *J Nutrition* 136(1):172–176
- Saito K (2013) Phytochemical genomics—a new trend. *Curr Opin Plant Biol* 16:373–380
- Sasidharan S, Chen Y, Saravaran D, Sundram KM, Latha LY (2011) Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr J Tradit Complement Altern Med* 8:1–10
- Schep LJ, Schmierer DM, Fountain JS (2006) Veratrum poisoning. *Toxicol Rev* 25:73–78
- Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC et al (1994) Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye disease case-control study group. *J Am Med Assoc* 272(18):1413–1420
- Srivastava R, Kulshreshtha D (1989) Bioactive polysaccharides from plants. *Phytochemistry* 28(11):2877–2883
- Staniek A, Bouwmeester H, Fraser PD, Kayser O, Martens S, Tissier A et al (2014) Natural products—learning chemistry from plants. *Biotechnol J* 9:326–336
- Sumner J (2000) The natural history of medicinal plants. Timber Press, Portland, Oregon

- Tadele Y (2015) Important anti-nutritional substances and inherent toxicants of feeds. *Food Sci Qual Manage* 36:40–47
- Takechi M, Matsunami S, Nishizawa J, Uno C, Tanaka Y (1999) Haemolytic and antifungal activities of saponins or anti-ATPase and antiviral activities of cardiac glycosides. *Planta Med* 65:585–586
- Tasheva K, Kosturkova G (2013) Role of biotechnology for protection of endangered medicinal plants. In: Petre M (ed) Environmental biotechnology—new approaches and prospective applications. InTech Publisher, pp 235–285
- Traore F, Faure R, Ollivier E, Gasquet M, Azas N, Debrauwer L et al (2000) Structure and antiprotozoal activity of triterpenoid saponins from *Glinus oppositifolius*. *Planta Med* 66:368–371
- Trethewey R (2004) Metabolite profiling as an aid to metabolic engineering in plants. *Curr Opin Plant Biol* 7:196–201
- Tweeddale H, Notley-McRobb L, Ferenci T (1998) Effect of slow growth on metabolism of *Escherichia coli*, as revealed by global metabolite pool ('metabolome') analysis. *J Bacteriol* 180:5109–5116
- Ulrich-Merzenich G, Zeitler H, Jobst D, Panek D, Vetter H, Wagner H (2007) Application of the 'omic' technologies in phytomedicine. *Phytomedicine* 14:70–82
- WHO (2001) Guidelines on the use of Insecticide-treated mosquito net for the prevention of malaria in Africa. CTD/MAL/AFR/97.4, World Health Organization (WHO), Geneva, Switzerland
- Williamson EM (2001) Synergy and other interactions in phytomedicines. *Phytomedicine* 8:401–409
- Wolfender JL, Rudaz S, Choi YH, Kim HK (2013) Plant metabolomics: from holistic data to relevant biomarkers. *Curr Med Chem* 20:1056–1090
- Yoshizawa K, Willett WC, Morris SJ, Stampfer MJ, Spiegelman D, Rimm EB et al (1998) Study of prediagnostic selenium level in toenails and the risk of advanced prostate cancer. *J Natl Cancer Inst* 90(16):1219–1224

Chapter 2

Phytoconstituents—Active and Inert Constituents, Metabolic Pathways, Chemistry and Application of Phytoconstituents, Primary Metabolic Products, and Bioactive Compounds of Primary Metabolic Origin



Abstract Phytoconstituents are non-nutrient active plant chemical compounds or bioactive compounds and are responsible for protecting the plant against infections, infestations, or predation by microbes, pests, pathogens, or predators. Some are responsible for color, aroma, and other organoleptic properties. Phytoconstituents are synthesized in plants through primary and secondary metabolic pathways and many of them may be grouped as active drug constituents and inert nondrug constituents. A wide range of active components has been discovered and they have been divided into 16 main or more groups and the most important of them are alkaloids, terpenoids, phenols and phenolic glycosides, coumarins and their glycosides, anthraquinones and their glycosides, flavones and flavonoid glycosides or heterosides, mucilage and gums, tannins, volatile oils, saponins, cardioactive glycosides, cyanogenic glycosides, etc. Other relevant active constituents in plants, such as vitamins, minerals, amino acids, carbohydrates and fibers, some sugars, organic acids, lipids, and antibiotics, are essential nutrients. In addition to other functions, secondary metabolites produced in plants are used for communication as signal compounds to attract different pollinating agents including insects (honey bees, bumble bees, moths), birds, lizards, bats, etc. Classification of phytochemicals may be made based on their elemental constituents such as C & H; C, H & O; C, H, O, N, S & P containing compounds, O/N containing heterocyclic compounds, and other miscellaneous compounds. Some of these may be grouped as primary and others as secondary metabolites. Primary metabolic products consisting of C & H; C, H & O; N, S & P elements include hydrocarbons, carbohydrates, lipids, amino acids, proteins, nucleic acids, organic acids, etc. Genetic effects and environmental factors exert both qualitative and quantitative alterations of the active constituents in medicinal plants.

2.1 Phytoconstituents

Plant or phytoconstituents are non-nutrient plant chemical compounds or bioactive compounds and are responsible for protecting the plant against infections, infestations or predation by microbes, pests, pathogens, or predators. Phytoconstituents are active chemical compounds that occur naturally in plants but not established as essential nutrients. Some are responsible for color, aroma, and other organoleptic properties. The term is generally referred to as biologically significant chemicals of secondary metabolic origin with therapeutic importance. Phytochemistry determines the biological activities of phytoconstituents including their qualitative and quantitative assessment as well as analysis of their beneficial and harmful effects on human health. Phytoconstituents are synthesized in plants through primary and secondary metabolic pathways and many of them may be grouped as active drug constituents and inert nondrug constituents.

2.1.1 Active Drug Constituents

The active constituents in plants are the chemicals that have a medicinal effect on the body. Pharmacological activity in herbal drugs is due to these chemical compounds which are called active principles, drug principles, or components. A wide range of active components has been discovered. Phytochemistry deals with methods of obtaining these active ingredients and their classification according to the functional organic chemical group which it bears and studies the analytical methods to verify its quality. Active components are found in different parts and organs of plants or plant exudates. They have been divided into 16 main or more groups of which the most important are alkaloids, terpenoids, phenols and phenolic glycosides, coumarins and their glycosides, anthraquinones and their glycosides, flavones and flavonoid glycosides or heterosides, mucilage and gums, tannins, volatile oils, saponins, cardioactive glycosides, cyanogenic glycosides, etc. The main chemical groups of active drug components under broader heads are heterosides (e.g., anthraquinones, cardiac glycosides, cyanogenics, coumarins, phenols, flavonoids, ranunculosides, saponosides, sulfurides), polyphenols (e.g., phenolic acids, cumarins, flavonoids, lignans, tannins, quinones), terpenoids (e.g., essential oils, iridoids, lactones, diterpenes, saponins), and alkaloids (atropine, cocaine, daturin, hiosciamin, lysergic acid, nicotine, quinine). Mucilage and gums are heterogeneous polysaccharides, formed by different sugars, in general, they contain uronic acids. Other relevant active constituents in plants, such as vitamins, minerals, amino acids, carbohydrates and fibers, some sugars, organic acids, lipids, and antibiotics, are essential nutrients.

Alkaloids are nitrogen-bearing molecules that make them particularly effective as medicines (e.g., deadly nightshade, aconite, cinchona, etc.), alkanoid, though poisonous, are valuable as medicines, e.g., curarine is a powerful muscle relaxant,

atropine is used to dilate the pupils of the eyes, and physostigmine is specific for certain muscular diseases; narcotic alkaloids morphine and codeine are used for the relief of pain and cocaine as a local anesthetic; quinine, caffeine, nicotine, strychnine, serotonin, and lysergic acid diethylamide (LSD) are examples of some other common alkaloids; anthocyanins maintain blood vessel health (e.g., blackberries); anthraquinones stimulate the large intestine, causing contractions and bowel movement (e.g., aloe, rhubarb, cascara, senna, frangula); bitter principles are recognized by their disagreeable, astringent, or acrid taste (e.g., gentian, chirata, picrorrhiza, quassia). The active ingredient stimulates the flow of saliva and gastric juices, thereby improving appetite and digestive function (e.g., wormwood and devil's claw); saponins are glycosides with foaming characters (e.g., soapwort, saoproot, soapbark, soapberry; commercial source: *Yucca schidigera* and *Quillaja saponaria*) and many saponins have beneficial effects on blood cholesterol levels, cancer, bone health and stimulation of the immune system, and detergent properties of saponins have led to their use in shampoos, facial cleansers and cosmetic creams; cardiac glycosides (e.g., digitalis, squill, strophanthus, ouabain, thevetia) have a strong direct action on the heart and support and strengthen the rate of contraction. They are significantly diuretic and these plants help lower blood pressure (e.g., foxgloves), aglycone of glycosides may be; phenolics are widely spread throughout the plant kingdom (fruits and vegetables), they are important for their potential protective role against oxidative damage diseases (coronary heart disease, stroke, and cancers); coumarins (e.g., visnaga, ammi, psoralea) as multitasking constituents thin the blood, relax smooth muscle and can act as a sunscreen all at once (e.g., celery); cyanogenic glycosides have a sedative and relaxing effect on the heart and muscles (e.g., elder plants); flavonoids are anti-inflammatory, and also maintain healthy circulation (e.g., lemons); glucosinolates increase the blood flow to the painful joint area and this aids in healing as it helps remove the build-up of waste products (e.g., radish are applied as a soft, moist mass onto painful joints); phenols are antiseptic and can reduce inflammation when taken internally, but if used externally on the skin can have an irritant effect (e.g., arbutin, siccin thyme); saponins (steroidal saponins and triterpenoid saponins) are expectorants, i.e., increase bronchial secretions and facilitate their expulsion through coughing, spitting or sneezing, also aid in nutrient absorption and have a marked effect on hormonal activity (e.g., liquorice); tannins (gallotannins, ellagittannins, complex tannins) are widely distributed in many plant species of plants and protect from predation and pesticides, and function in plant growth regulation. Tannins can contract the skin's tissue and thereby improving the skin's resistance to infection (e.g., oak tree); and volatile oils have many therapeutic effects and are used in perfumes, food flavorings, and aromatherapy (e.g., chamomile). Minerals can act as mineral supplements (e.g., Dandelion); mucilage soothes inflammation and stops irritation and acidity, by lining the mucous membranes of the digestive tract (e.g., slippery elm), vitamins function as structural material as well as coenzymes (dog rose contains enough vitamins to contribute to one's daily intake). More than 600 carotenoids provide yellow, orange, and red colors in fruits and vegetables, act as antioxidants in the body, and scavenge harmful free radicals, alpha-carotene,

beta-carotene, and beta-cryptoxanthin (pumpkins and carrots) which are provitamins necessary to keep immune system active and eye healthy. Lycopene (e.g., tomatoes, watermelon, pink grapefruit) has been linked to a lower risk of prostate cancer. Lutein and zeaxanthin (e.g., spinach, kale, collards) protect from eye problems like cataracts and age-related macular degeneration. Ellagic acid of different berries (e.g., strawberries, raspberries, pomegranates) protects against cancer in several different ways.

Medicinal and aromatic plants (MAPs) are the richest bio-resource of crude drugs for traditional systems of medicine, modern medicines, folk medicines, nutraceuticals, food supplements, fragrances, flavors, cosmeceuticals, health beverages, pharmaceutical intermediates, and chemical entities for synthetic drugs. The medicinal value of a crude drug depends on the presence of one or more active chemical constituents (e.g., alkaloids, glycosides, resins, enzymes, etc.). A vegetable drug is composed of many tissues such as cells, fibers, vessels, and other structures. The cell walls may consist of cellulose, lignins, tannins, etc. The aromatic drugs like cinnamon and coriander contain volatile oils in specialized cells or glands. The glycosides and alkaloids may occur in solution in the cell sap and deposit in the cells later. The total contents of the cells do not carry physiological importance, e.g., calcium oxalate occurs as a crystalline deposit and protein may occur as solid aleurone grains. Both these components are rejected in the preparation of a tincture or extract of the drug.

2.1.2 *Inert Nondrug Constituents*

The chemical constituents present in plant (or animal) kingdom, which do not possess any definite therapeutic values as such but are useful as an adjunct either in the formulation of a drug or in surgery are collectively known as inert constituents. The inert components of a drug product do not increase or affect the therapeutic action of the active ingredient (active drug constituent). The inert nondrug constituents are added during the manufacturing process of pharmaceutical products, e.g., tablets, capsules, suppositories, injections, etc. The inert nondrug constituents or inactive ingredients may also be referred to as excipients including binding materials, dyes, preservatives, and flavoring agents. Agents that combine with active ingredients to facilitate drug transport in the body or palatability are also considered inactive or inert nondrug constituents. The inert ingredients or excipients in the medication do not exert the intended effect for taking it, and do not cause the side effects. The inert constituents are the cellulose, wood, and other structural parts of the drug, and in some instances starch, albumen, etc. Inactive ingredients are used in the manufacturing process and/or are present in the final medication product. They fulfill a variety of purposes, from delivering the active ingredient to making the pill look and taste good, along with other tasks such as coatings for easier ingestion, and timed or targeted release, flavors, and colors to make swallowing medicine more pleasant. Different types of nanoparticles are being used to

encapsulate the active ingredients of drugs, to reduce required doses, and improve organ and tissue specificity.

The FDA approves inactive ingredients that are included in pharmaceutical products. However, not all inactive ingredients are always inactive, e.g., alcohol as an ingredient that may be active or inactive depending on the specific formulation of the medication. Patients may have allergic reactions or other adverse effects to inactive ingredients, but a patient may have an allergic reaction to this inactive ingredient. Inactive ingredients like sulfites, benzoates, aspartame, saccharin, oleic acid, benzyl alcohol, lactose, soya lecithin, propylene glycol, and sorbitan trioleate may cause reactions in some patients as per the previous report. Patients who have allergic or adverse reactions to certain inactive ingredients may be able to use products that are color or preservative free.

The inert constituents, both plant and animal origin, that are invariably present in natural drugs include starch, cellulose, lignin, suberin, cutin, albumin, coloring matters, etc., (plant origin); and keratin, chitin, etc., (animal origin). Microcrystalline forms of cellulose are used as combination binder disintegrants in tabletting. Colloidal cellulose particles aid in stabilization and emulsification of liquid; lignin is used to precipitate proteins and to stabilize asphalt emulsions; suberins are esters of higher monohydric alcohols and fatty acids as cutin does; starch as pharmaceutic aid functions as tablet filler, binder, and disintegrant; albumin from soybean (albumins) functions as emulsifiers; coloring matters like cochineal are used for coloring food products and pharmaceuticals; keratin is used for coating enteric pills that remains unaffected in the stomach but undergoes disintegration (dissolved) by the alkaline into intestinal secretions; chitin as deacetylated chitin (chitosan) is used for treatment of water but sulfated chitin functions as anticoagulant in laboratory animals. It has been observed that the very presence of "Inert Constituents" either act towards modifying or check the absorbance and the therapeutic index of the active constituents.

2.2 Metabolic Pathways and the Origin of Primary and Secondary Metabolites Chemistry of Plant Constituents and Their Application

Metabolites are intermediates and products of metabolism. Physiologically active plant chemical constituents are usually classified in groups according to their metabolic origin, chemical structure, and function. Metabolism in plants may be classified into primary and secondary metabolic pathways.

2.2.1 Primary Metabolic Pathways and Primary Metabolites

Primary metabolic pathway produces primary metabolites, usually referred to as central metabolites, which are directly involved in normal growth, development, and reproduction; produced in generous quantities and can easily be extracted from the plant; part of the basic molecular structure of the cell; perform physiological functions in the organism (i.e., an intrinsic function); distribution is ubiquitous to all organisms or cells (Fig. 2.1).

In microorganisms, primary metabolites are typically formed during the growth phase as a result of energy metabolism, and are deemed essential for proper growth, e.g., alcohol (ethanol), organic acids (acetic acid, lactic acid, citric acid), nucleotides (5'guanylic acid), antioxidants (isoascorbic acid), certain amino acids (aspartic acid, L-glutamate and L-lysine), vitamins (B₂), and polyols (glycerol). Compounds, such as phytosterols, acyl lipids, nucleotides, amino acids, and organic acids, are found in all plants and perform metabolic roles that are essential and evident. Products resulting from primary metabolism include glucides, lipids, amino acid derivations, etc. Many of these metabolites can be used in industrial microbiology to obtain amino acids, develop vaccines and antibiotics, and isolate chemicals necessary for organic synthesis.

2.2.2 Secondary Metabolic Pathways and Secondary Metabolites

Secondary metabolic pathway produces secondary metabolites that are typically organic compounds and are produced through the modification of primary metabolites (Fig. 2.2).

Secondary metabolites are not directly involved in growth and developmental processes, are not part of the basic molecular structure of the cell, are produced in small quantities and their extraction from the plant is difficult and usually performs important ecological function (i.e., a relational function), i.e., important adaptive significance in protection against herbivory and microbial infection, as attractants (pigments or scents) for pollinators and seed-dispersing animals, and as allelopathic agents (allelochemicals that influence competition among plant species); secondary

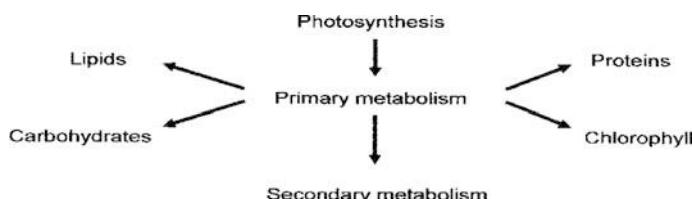


Fig. 2.1 Primary metabolism and primary metabolites in plants

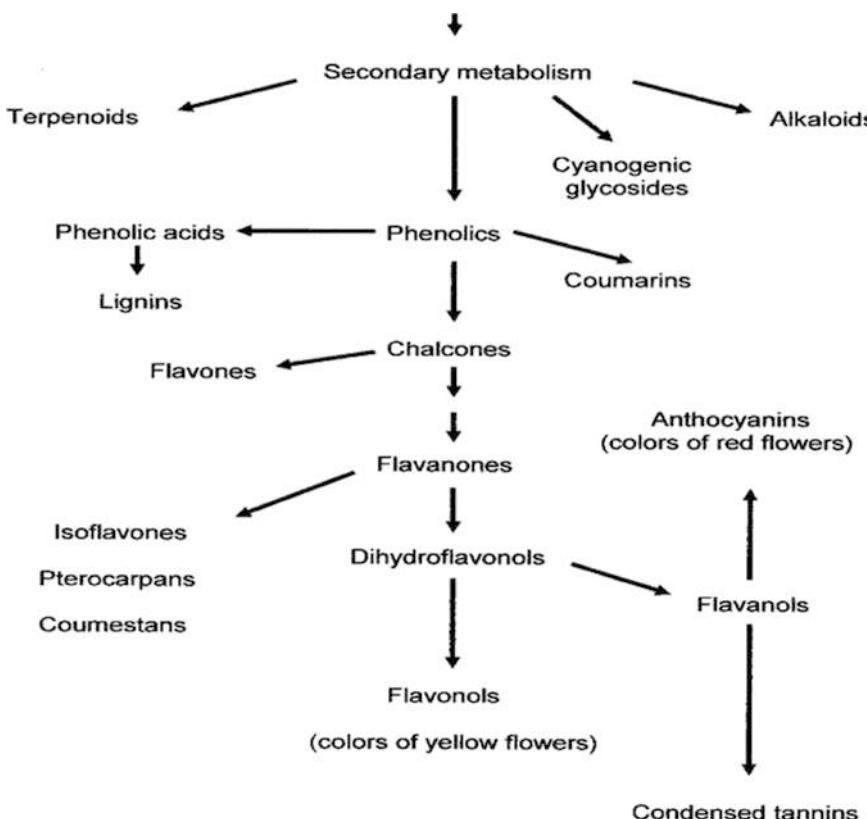


Fig. 2.2 Secondary metabolism and secondary metabolites in plants

metabolites seem to be important primarily in ecological interactions with other species and between the plant and its environment; distribution is not ubiquitous to all organisms or cells, i.e., restricted to a limited taxonomic groups or set of organisms or cells of plants, fungi, bacteria, etc. They include some broad groups of metabolites such as alkaloids (strychnine, nicotine, caffeine, cocaine, capsaicin), phenolics (flavonoids, tannins, lignin, salicylic acid), terpenoids (aromatic oils, resins, waxes, steroids, rubber, carotenoids), and some other miscellaneous products (glycosides, antibiotics, peptides and growth factors). These are the most important active components of herbal drugs. Secondary metabolites are typically formed during the end or near the stationary phase of growth. Many of the identified secondary metabolites have a role in ecological function, including defense mechanism(s), by serving as antibiotics and by producing pigments (carotenoids, anthocyanin). Examples of secondary metabolites with importance in industrial microbiology include atropine and antibiotics such as penicillin, erythromycin, and bacitracin. It is, however, difficult to distinguish between primary and secondary metabolites by either structure, biochemistry, or function. Plant growth regulators

may be classified as both primary and secondary metabolites due to their role in plant growth and development. Some of them are intermediates between primary and secondary metabolism.

Metabolic pathways are a linked series of chemical reactions that take place within a cell, tissue, or organism. The reactants, products, and intermediates of an enzymatic reaction are collectively known as metabolites and in a metabolic pathway, the product of one enzyme acts as the substrate for the next. For convenience, different metabolic pathways function in different cell compartments or organelles. In a eukaryotic cell, the photosynthetic CO₂ fixation Calvin cycle, H₂O photolysis, cyclic and noncyclic electron transport, generation of energy (photophosphorylation) and reductive pool such as nicotinamide adenine dinucleotide phosphate-NADPH, etc., take place within chloroplast; the citric acid cycle, electron transport chain, and oxidative phosphorylation all take place in the mitochondrial membrane. In contrast, glycolysis, pentose phosphate pathway, and fatty acid biosynthesis all occur in the cytosol of a cell. Anabolic (synthetic) and catabolic (degradative) pathways are two basic metabolic pathways which are characterized by their ability to either synthesize molecules with the utilization of energy or break down of complex molecules by releasing energy in the process. The two pathways complement each other in that the energy released from one is used up by the other, i.e., the degradative process or catabolic pathway provides the energy required to conduct a biosynthesis of an anabolic pathway (coupling processes). In addition to these, there exists another pathway, the amphibolic pathway, which can share the products of both catabolic and anabolic processes. Metabolites that are produced in different processes are arbitrarily grouped as primary and secondary metabolites and their pathways are so named as primary (including photosynthesis, glycolysis, TCA cycle, photo- and oxidative phosphorylation, pentose phosphate pathway, fatty acid synthesis, amino acids and protein synthesis, nucleotides and nucleic acid synthetic pathways, etc.) and secondary metabolic pathways (shikimic acid pathway, malonic acid pathway, mevalonate pathway, MEP-methylerythritol phosphate pathway, etc.). Products of the primary metabolic pathways are the source materials for secondary metabolic pathways. Cells have evolved to use feedback inhibition (when a reaction product is used to regulate its own further production, i.e., the products inhibit further enzyme activity) to regulate enzyme activity in metabolism.

The primary metabolism consists of chemical reactions that allow the plant to live. The secondary metabolites almost play no role in growth, photosynthesis, reproduction, solute transport, translocation, nutrient assimilation, and differentiation or other primary functions but secondary metabolism facilitates the primary metabolism in plants and plays a pinnacle role in keeping all the plants' systems working properly. These chemicals are extremely diverse but not ubiquitous like primary metabolites rather they are restricted or limited distribution to few plant families, genus or species. They can sometimes be used as taxonomic characters in classifying plants. Secondary metabolites can be classified in different ways (e.g., based on structure, composition, solubility in various solvents, synthetic pathways, etc.). Based on their biosynthetic origins, plant secondary metabolites can be divided into three major groups such as terpenoids, phenolics (flavonoids and allied

phenolic and polyphenolic compounds), and nitrogen-containing alkaloids and sulfur-containing compounds.

Secondary metabolites play an important role in plant defense against UV radiation, toxicity (as deterrent), herbivory, pests (deterrent), pathogens and pests as well as in interspecies defenses (allelopathy); in metal transportation; in symbiotic interaction between microbes and plants, nematodes, insects, and higher animals; formation of UV nectar guide in flowers (e.g., 4-deoxyaurones of *Bidens ferulifolia*), pollinator attractants and also as sexual hormones and differentiation effectors, etc. Humans use secondary metabolites as therapeutic, flavoring, and recreational agents.

The active components in a drug are the chemicals that create the intended therapeutic effects, and any side effects. The constituents of herbal drugs of medicinal value generally are diverse including carbohydrates, gums, acids, glycosides, anthraquinone derivatives, alkaloids, tannins and other phenols, enzymes, proteins, resins, fixed oils, fats, waxes, volatile oils, etc. These metabolites and their synthetic pathways are shown in Fig. 2.3.

The shikimic acid pathway is a multi step metabolic pathway used by bacteria, fungi, algae, some protozoan parasites and higher plants for the synthesis of folates and aromatic amino acids (phenylalanine, tyrosine and tryptophan). This pathway is

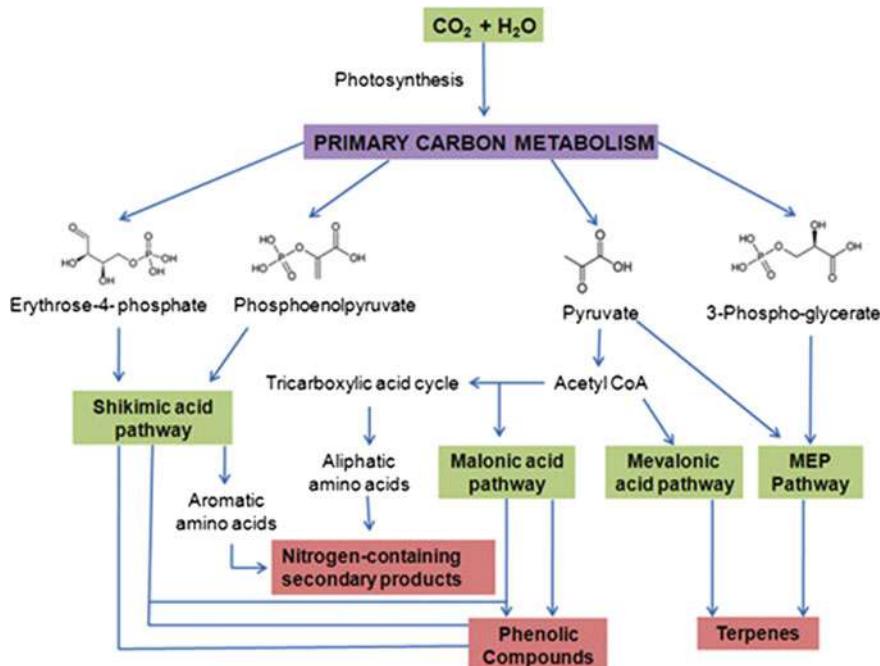


Fig. 2.3 A schematic diagram showing the metabolic pathways of synthesis of primary and secondary metabolites in plants

not found in animals and the amino acids produced in this pathway must be supplied through animal's diet (essential amino acids). The shikimic acid pathway participates in the biosynthesis of most plant phenolics. The malonic acid pathway is an important source of phenolic secondary products in fungi and bacteria, but is of less significance in higher plants. Diethyl ester of malonic acid is used in syntheses of vitamins B₁ and B₆, barbiturates, and numerous other valuable compounds. The mevalonic acid pathway, also known as the isoprenoid pathway or HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, HMGCR) pathway is present in eukaryotes, archaea, and some bacteria. The mevalonate pathway begins with acetyl-CoA and ends with the synthesis of two five-carbon building blocks called isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). These two building blocks are used in the synthesis of a diverse class of isoprenoids including cholesterol, heme, vitamin K, coenzyme Q₁₀, and all steroid hormones. IPP and DMAPP also serve as the basis for the biosynthesis of molecules used in processes as diverse as protein prenylation, cell membrane maintenance, hormone synthesis, protein anchoring, and N-glycosylation. MEP (methylerythritol phosphate) pathway is an alternative or non-mevalonate metabolic pathway that forms IPP and DMAPP used for isoprenoid biosynthesis (including the dimeric isoprenoids, the terpenoids) by bacteria, plants, and apicomplexan protozoa (e.g., malaria parasites).

2.2.3 Plant's Defensive or Survival Secondary Metabolites

Plants produce a diverse group of secondary metabolites with a prominent function in the protection against predators and microbial pathogens due to their toxic nature and repellence to herbivores and microbes; some of them are involved in defense against abiotic stresses, some others are important for the communication of the plants with other organisms and a few are insignificant for growth and developmental processes (Rosenthal 1991; Wink 1999; Schafer and Wink 2009). Secondary metabolites such as terpenes, phenolics and nitrogen (N) and sulfur (S) containing compounds defend plants against a variety of herbivores and pathogenic microorganisms as well as various kinds of abiotic stresses. A clear majority of the different terpenes produced by plants as secondary metabolites are involved in defense as toxins and feeding deterrents to a large number of plant feeding insects and mammal herbivores.

Monoterpenes esters pyrethrroids occur in the leaves and flowers of *Chrysanthemum* species show strong neurotoxin insecticidal responses to insects; and monoterpenes α -pinene, β -pinene, limonene, myrcene, etc., present in resin ducts of pine and fir (Gymnosperms), are toxic to numerous insects. Essential oils (a mixture of volatile monoterpenes and sesquiterpenes) like limonene and menthol (monoterpenes present in glandular trichomes on epidermis) of lemon oil and peppermint oil, respectively with well-known insect resistant properties help in protecting the plant from insect infestation. Sesquiterpenes costunolides (a cyclic

ester characterized by a five-membered lactone ring) of Asteraceae are antiherbivore and feeding repellent agents; abscisic acid (ABA-a sesquiterpene growth regulator) plays regulatory role in plant response to water stress by modifying the membrane properties, minimizes the interference of absorption of 400–700 nm photosynthetically active radiation (PAR) by significantly increasing the level of UV-B absorbing flavonols (quercetin and kaempferol), and reduces damage due to UV-B by increasing the concentration of hydroxycinnamic acids (caffeic and ferulic acids), antioxidant enzymatic activities and sterols. Abietic acid (a diterpene) of pines and leguminous trees are chemical deterrents to continued predation; phorbol (a diterpene ester) of Euphorbiaceae works as skin irritants and internal toxins to mammals.

Terpenoid aldehyde (or polyphenolic aldehyde) gossypol produced by cotton (*Gossypium hirsutum*) has strong antifeedant, antifungal and antibacterial properties. Several triterpene steroid alcohols (sterols) such as better tasting glucosides (sterols) of milkweeds protect them against herbivory insects and cattle; phytoecytosones produced by spinach (*Spinacia oleracea*) disrupt larval development and increase insect mortality; limonoids (a group of bitter triterpenes substances) present in lemon and orange peels (fresh scent) are triterpenoids act as antiherbivore compounds; azadirachtin of neem (*Azadirachta indica*) is a very powerful insect repellent and feeding deterrent limonoid. Insect repellent citronella, an essential oil of lemongrass (*Cymbopogon citratus*), contains high limonoid levels. Triterpenoid cardiac glycosides like digitoxin and digoxin of Foxglove (*Digitalis purpurea*) are highly toxic to vertebrate herbivores including humans if ingested in high quantities.

A clear majority of secondary metabolites play vital functions in plant growth and defenses (Fig. 2.4). For example, gibberellins (diterpenes) and brassinosteroids (BRs), a sixth class of plant hormones, are important plant hormones, and sterols (triterpene) are essential components of cell membranes and carotenoids (tetraterpenes) act as important pigment class in photosynthesis and protect plants from harmful effects of photooxidation. The ecdysones (steroids) of fern *Polypodium vulgare* play defensive role against insects (Heftmann 1975; Slama 1980). Solanine (triterpene steroids/glycoalkaloid poison) acts as a plant defense molecule. Rubber (polyterpene) provides protection to plants as a defense against herbivores and in mechanism for wound healing (Klein 1987; Eisner and Meinwald 1995).

Phenolics, a chemically heterogeneous group of compounds, have several important defensive roles in the plants against pests and diseases (defense-related phenolics include flavonoids, anthocyanins, phytoalexins, tannins, lignin, and furanocoumarins). For example, coumarins (oxygen heterocycle) protect plants against insect herbivores and fungi, and furanocoumarins (abundant in the members of Apiaceae such as celery, parsnip and parsley) become phytotoxic after activation by UV-A. Lignin, a highly complex and branched polymer of phenylpropanoic alcohols (e.g., econiferyl-, sinapyl-, and *p*-caumaryl alcohols), protects plants from herbivorous animals by its physical toughness while its chemical durability makes it relatively indigestible to herbivores and insects pathogens. Flavonoid pigments (invisible to human eye but visible to bees) (flavones—luteolin, apigenin,

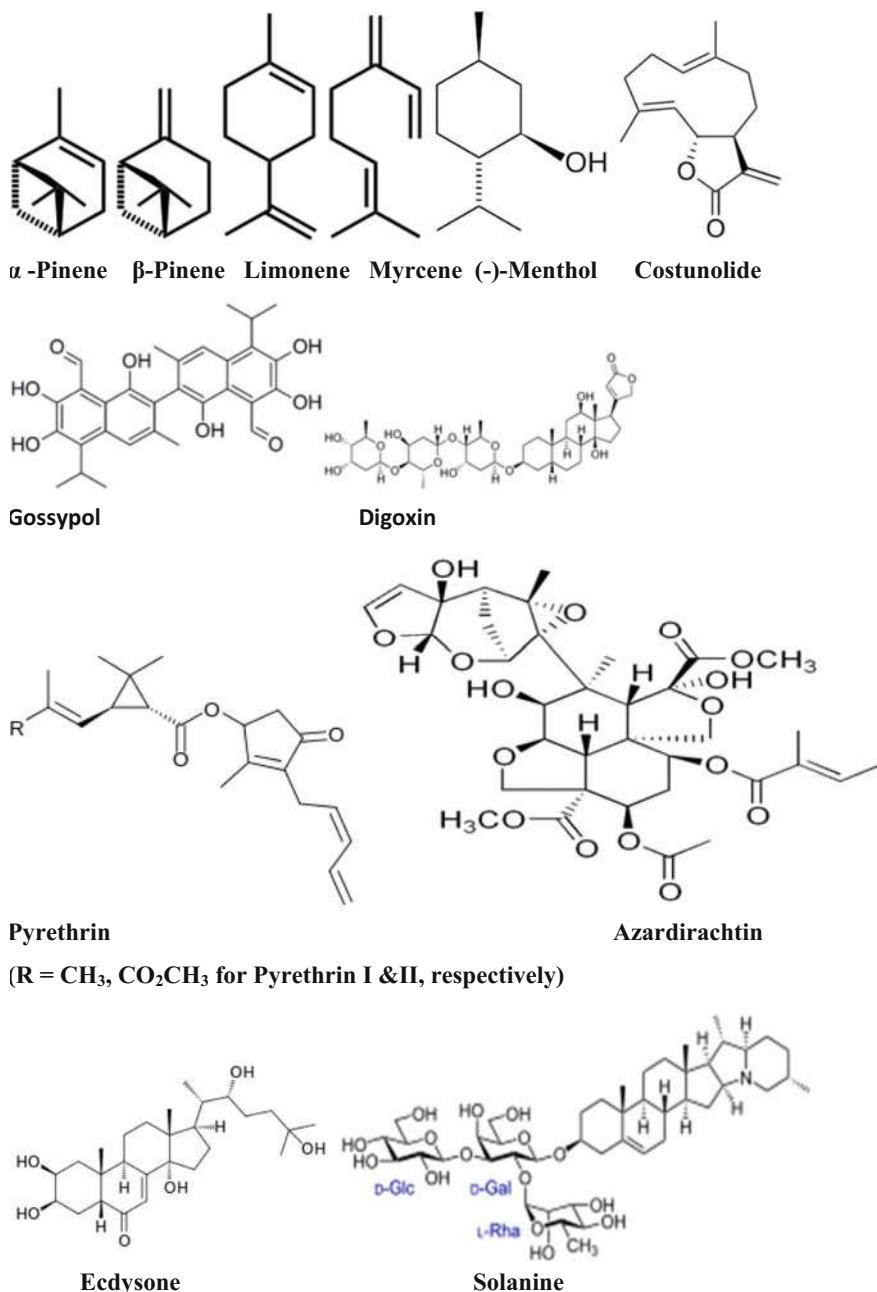
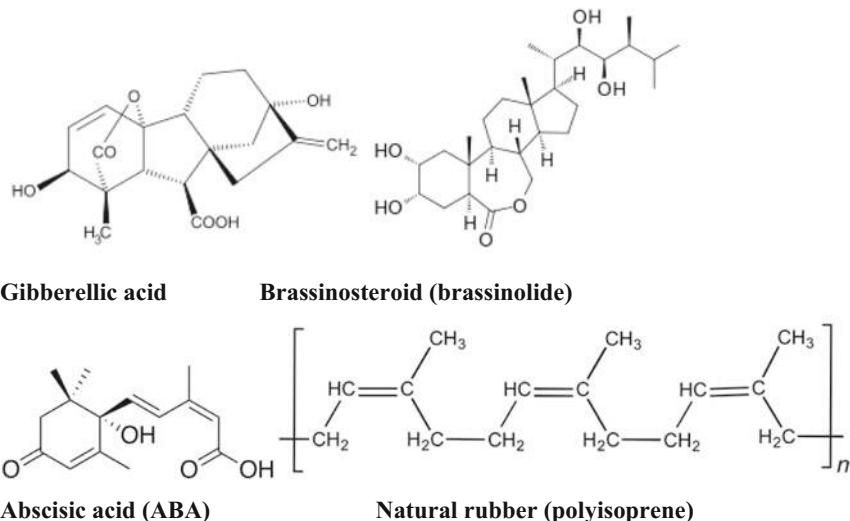
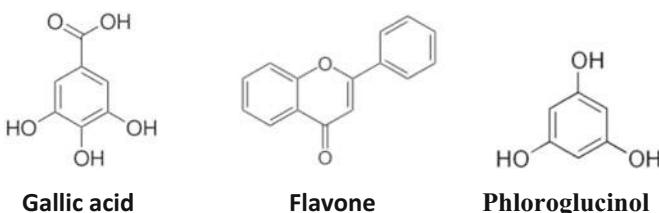


Fig. 2.4 Different terpenes secondary metabolites involved in defense as toxins and feeding deterrents to plant feeding insects and mammal herbivores

**Fig. 2.4** (continued)

tangeritin; and flavonols—quercetin, kaempferol, myricetin, fisetin, galangin, isorhamnetin, pachypodol, rhamnazin, pyranoflavonols, furanoflavonols) function as shields against harmful UV-B radiation. Anthocyanins (flavonoids pigments) protect plant foliage from the damaging effects of ultraviolet radiation (e.g., cyanin glycoside). Phytoalexins (isoflavonoids) with antibiotic and antifungal properties are produced in plants in response to pathogen attack (e.g., medicarpin in alfalfa, rishitin in tomatoes and potatoes, camalexin in *Arabidopsis thaliana*). Miean and Mohamed (2001) analyzed the flavonoids (myricetin, quercetin, kaempferol, luteolin, and apigenin) contents in 62 edible tropical plants and among them, the highest total flavonoids content was in onion leaves (1497.5 mg/kg quercetin, 391.0 mg/kg luteolin, and 832.0 mg/kg kaempferol). Rotenone of *Derris* sp., an isoflavone, can act as an insect feeding deterrent. Tannins are plant phenolic polymers with defensive properties and act as feeding repellents to a great diversity of animals. There are three major classes of tannins (hydrolysable gallic acid polymer, nonhydrolyzable, or condensed flavones polymer and phlorotannins

**Fig. 2.5** The basic units of tannin: gallic acid, flavone and phloroglucinol

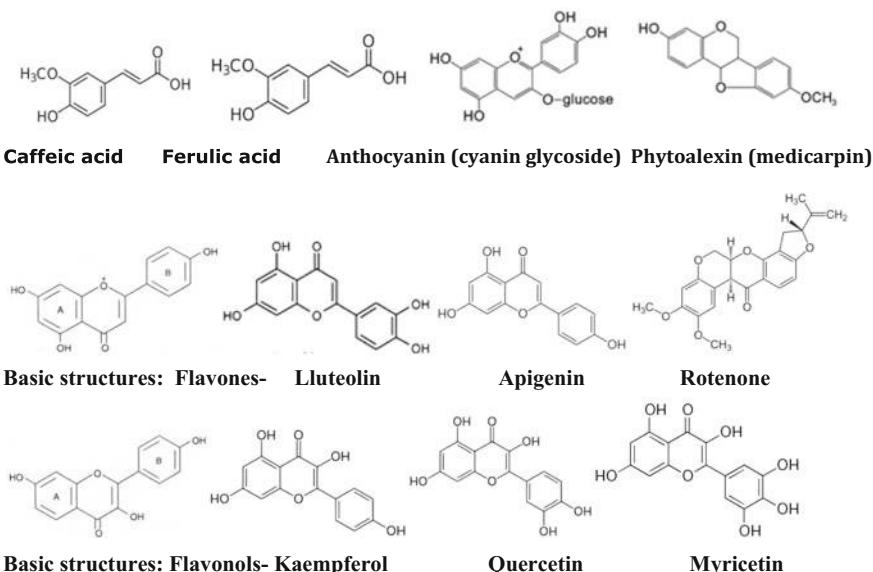


Fig. 2.6 Different phenolic compounds released by some plants show allelopathic activity



Fig. 2.7 Building blocks of lignin: Coniferyl-, sinapyl- and *p*-coumaryl alcohols

phloroglucinol polymer) having three different basic unit or monomer of the tannin. A tannin molecule requires at least 12 hydroxyl groups and at least five phenyl groups to function as protein binders. Basic units of tannin are gallic acid, flavone, and phloroglucinol (Fig. 2.5).

Phenolic compounds like ferulic and caffeic acids when released by some plants show allelopathic activity, i.e., inhibit the germination and growth of their neighboring plants and thus may increase its access to nutrients, light, and water (agents of plant–plant competition). Structure of different phenolic compounds is shown below (Figs. 2.6, 2.7 and 2.8).

Nitrogen-containing secondary metabolites including alkaloids (nicotine, caffeine, threonine, atropine, capsaicin, etc.), cyanogenic glucosides, and nonprotein amino acids are of considerable interest because of their role in the antiherbivore

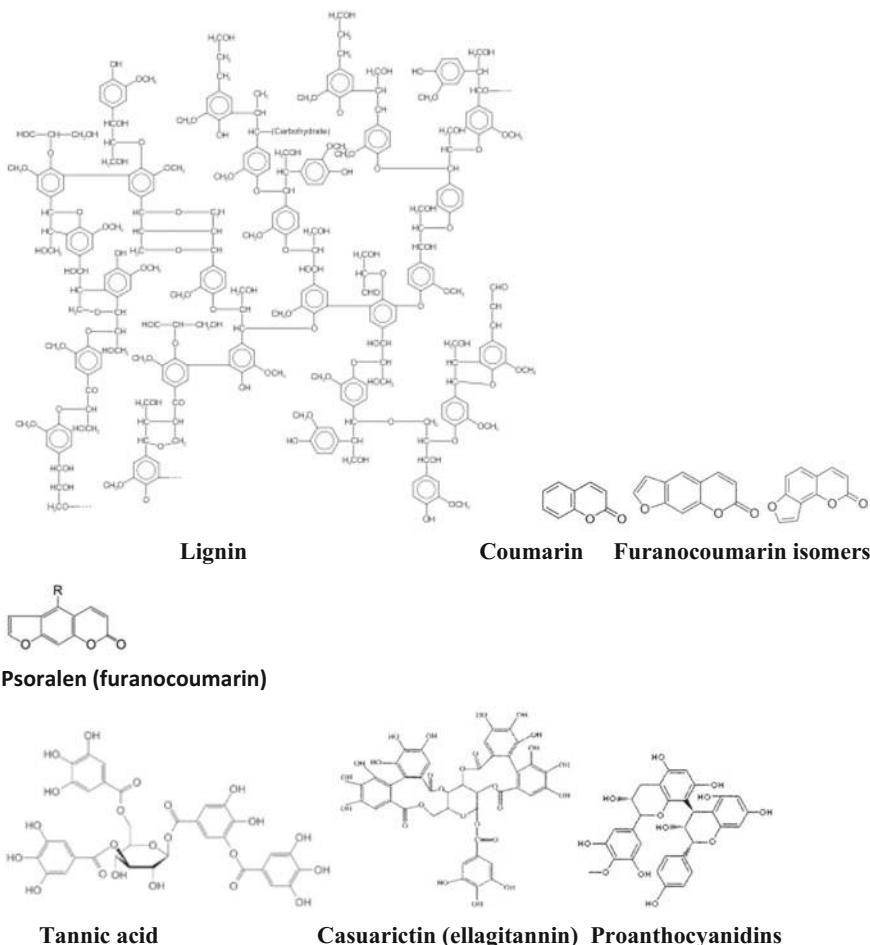


Fig. 2.8 Different plant phenolics: Lignin, Coumarin, Furanocoumarin isomers, Psoralen (furanocoumarin), Tannic acid, Casuarictin (ellagitannin), Proanthocyanidins, etc.

defense and toxicity to humans (Fig. 2.9). Defensive proteins (including defensins, amylase inhibitors, lectins, ricin, proteinase inhibitors, etc.) are cysteine rich small molecules (contained in seeds or other organs of plants) that inhibit pest enzymes activities and/or digestive enzyme activities of herbivores. Alkaloids are believed to function as defenses against herbivores because of their general toxicity and deterrence capability.

Nitrogenous protective compounds other than alkaloids are found in plants, e.g., cyanogenic glycosides and glucosinolates. *Cyanogenic glycosides* release the poisonous gas hydrogen cyanide (HCN). The presence of cyanogenic glycosides deters feeding by insects and other herbivores such as snails and slugs.

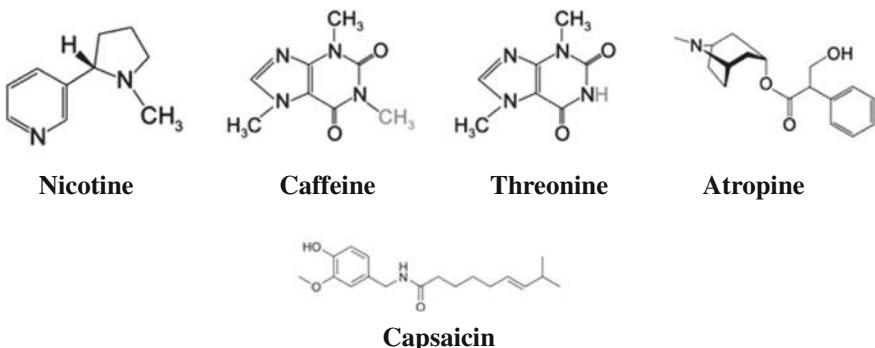


Fig. 2.9 Nitrogen-containing secondary metabolites (alkaloids): nicotine, caffeine, threonine, atropine, capsaicin, etc.

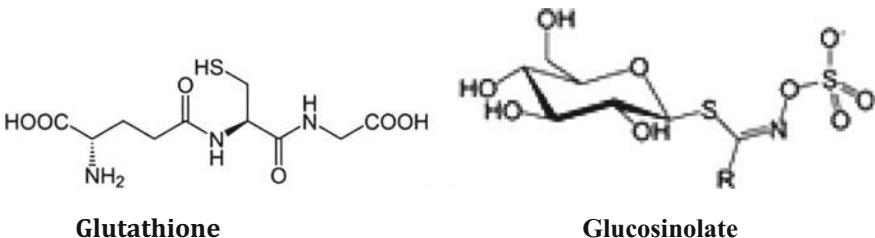


Fig. 2.10 Sulfur containing secondary metabolites: Glutathione and Glucosinolates

Sulfur-containing secondary metabolites include GSH, GSL (glutathione, glucosinolates), phytoalexins, thionins, defensins, allinin, etc., and they have direct or indirect link with the defense mechanism of plants against microbial pathogens (Fig. 2.10). The *glucosinolates*, or mustard oil glycosides, break down by the hydrolytic enzyme thioglucosidase or myrosinase to release defensive substances including S or sulfur. These are found principally in the *Brassicaceae* and related plant families. Like cyanogenic glycosides, glucosinolates are stored in the intact plant separately from the enzymes that hydrolyze them, and they are brought into contact with these enzymes only when the plant is crushed.

Plants defensive strategies against herbivory can be divided into two categories: constitutive and induced defenses. The *constitutive* defensive mechanisms are always present in the plant, often be species-specific and may exist as stored compounds, conjugated compounds (to reduce toxicity), or precursors of active compounds that can easily be activated if the plant is damaged. Most of the defensive secondary compounds are constitutive defenses. *Induced defenses* are initiated only after actual damage occurs, e.g., lectins and protease inhibitors as well as the production of toxic secondary metabolites. In principle, induced defenses require a smaller investment of plant resources than constitutive defenses, but they must be activated quickly to be effective. Plant hormone *Jasmonic acid* (JA) acts as

a major signaling agent in most plant defenses against insect herbivores that triggers the production of many proteins involved in plant defenses. Several other signaling compounds (e.g., ethylene, salicylic acid, methyl salicylate, etc.) are also induced by insect herbivory. In many cases, the concerted action of these signaling compounds is necessary for the full activation of induced defenses.

High concentrations of secondary metabolites might result in a more resistant plant; the production of secondary metabolites is thought to be costly and reduces plant growth and reproduction (Karban and Baldwin 1997; Siemens et al. 2002). Defense metabolites can be divided into constitutive substances (also called prohibitins or phytoanticipins) and induced metabolites formed in response to an infection involving de novo enzyme synthesis (phytoalexins) (Grayer and Harborne 1994; Van Etten et al. 1994). Phytoanticipins are high energy and carbon consuming but recognized as the first line of chemical defense that potential pathogens must overcome (Mauricio 1998) while phytoalexin production may take 2 or 3 days (Grayer and Harborne 1994). The cost of defense has also been invoked to explain why plants have evolved induced defense, where concentrations generally increase only in stress situations (Harvell and Tollrian 1999). Defensive chemicals (including structures) are costly as they require resources that could otherwise be used by plants to maximize growth and reproduction, yet many make this investment in defenses against predators.

Unlike simple chemicals such as terpenoids, phenolics, alkaloids, etc., proteins require a great deal of plant resources and energy to produce; consequently, many defensive proteins are only made in significant quantities after a pathogen or pest has attacked the plant. Once activated, however, defensive proteins and enzymes effectively inhibit fungi, bacteria, nematodes, and insect herbivores.

2.2.4 Pollinator Attracting Secondary Metabolites

In addition to other functions, secondary metabolites produced in plants are used for communication as signal compounds to attract different pollinating agents including insects (honey bees, bumble bees, moths), birds (hummingbirds, honey eaters' sunbirds, flower peckers, honeycreepers, bananaquits), lizard (*Hoplodactylus geckos*), mammals (bats, fruit bats, lemurs in Madagascar, and some Australian marsupials such as sugar gliders, honey possums, and some marsupial mice), etc., to enhance fertilization (Fig. 2.11). Nectar sugars, floral pigments, and headspace volatiles are important in this regard to filter flower visitors. Secondary terpene metabolites volatile oils (floral scents and fragrance) and pigments (carotenes) as well as phenolics and flavonoids are involved primarily to provide either visual or olfactory attraction in terms of flower aroma and color. The secondary metabolites involved in scent production are usually low-molecular weight volatile products with phenylpropanoids, benzenoids, and terpenes; and the major scent compound emitted by *Antirrhinum* flowers is methylbenzoate. Many flavonoids are responsible for color, aroma of flowers, fruit to attract pollinators and consequently fruit

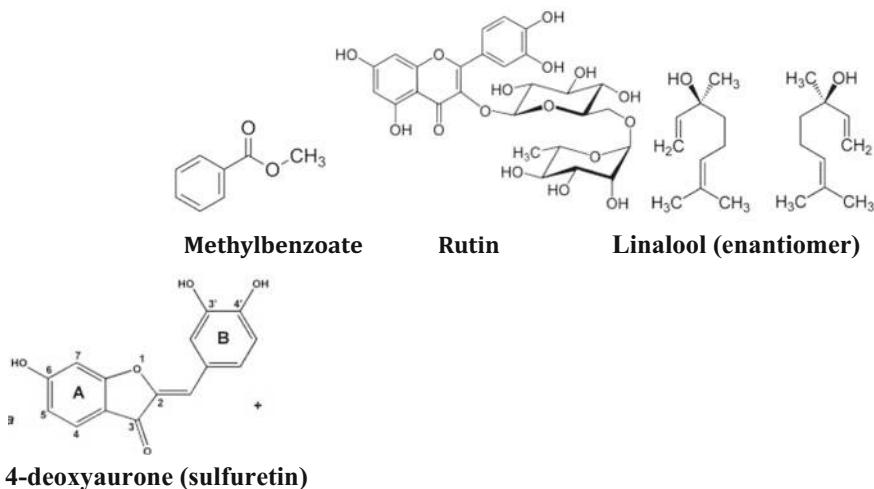


Fig. 2.11 Pollinator attracting secondary metabolites: Methylbenzoate, Rutin, Linalool (enantiomer), 4-deoxyaurone (sulfuretin), etc.

dispersion. Rutin is a bioflavonoid plant pigment (a glycoside combining the flavonol quercetin and the disaccharide rutinose- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranose). It functions as a visual pollinator attractant in many plant species (e.g., *Forsythia intermedia*). Plants emit volatile organic compounds (VOCs) for the attraction of pollinating agents, e.g., linalool, a terpene alcohol (two enantiomers of a terpene alcohol) with pleasant scent found in flowers of many plant species, functions as a pollinator attractant; 4-deoxyaurone (sulfuretin), a flavonoid function as UV nectar guide. Flower nectar and secondary metabolites in it are also important in attracting pollinators. Introduction of secondary metabolites in nectar may introduce specialization in plant–pollinator interactions, protects nectar from larceny, and preserves nutrients in nectar from degradation and reduction in disease levels in pollinators. When insects, bats, birds and other animals visit flowers to feed on the nectar and pollen, they usually pollinate the flowers in the process, so that both partners benefit from this mutualistic association.

Miscellaneous secondary metabolites

Canavanine of *Canavalia ensiformis* (deterrence of mammalian herbivores), alliin of onion and garlic (antioxidant and imparts aroma), and azetidine-2-carboxylic acid of *Convallaria majalis* (deters the growth of predators) are nonprotein amino acids, while sinigrin of Brassicaceae members is a glucosinolate, acts as defensive factor against insects, as well as positive feeding stimulus to cabbage butterflies and aphids. All these are grouped as miscellaneous secondary metabolites.

2.2.5 Factors Affecting the Metabolic Pathways of Medicinal Plants

The secondary plant products or bioactive constituents in plants may be considered as (a) superfluous metabolites, i.e., substances that have no value as such and perhaps their presence is due to the lack of excretory mechanism in them and ultimately results in the “residual lockup” superfluous metabolites, and (b) characteristic survival substances, i.e., substances which exert a positive survival value on the plant wherein they are present. They offer a natural defense mechanism whereby these host plants are survived from destruction owing to their astringent, odorous, and unpalatable features, e.g., poisonous alkaloidal containing plants, astringent containing shrubs, and pungent volatile oil-containing trees, etc.

Genetic effects exert both qualitative and quantitative alterations of the active constituents in medicinal plants, e.g., (i) eugenol is naturally present in two different species in varying quantities as follows: *Eugenia caryophyllus* (70–95%) and *Syzgium aromaticum* (~85%); (ii) Reserpine-rescinnamine group of alkaloids in *Rauvolfia serpentina* (~0.15%) and *Rauvolfia vomitoria* (~0.20%); (iii) Rutin in *Fagopyrum esculatum* (3–8%) and *Sophora japonica* (20%); (iv) menthol in *Mentha piperita* (50–60%) and *Mentha arvensis* (75–90%).

The environment factors also contribute to the quantitative aspect of secondary metabolites or active constituents of medicinal plants, e.g., (i) modified strains of *Claviceps purpurea* can produce ~0.35% of ergotamine in comparison to the normal one producing ~0.15% of total ergot alkaloids; (ii) eucalyptol (cineole, cajeputol) is present in the fresh leaves of *Eucalyptus globulus* to the extent of 70–85%. It has been observed that the chemical races of some species of *Eucalyptus* invariably display significant variations in the content of essential oils. The composition of soil (mineral contents); climate (dry, humid, cold); associated flora (*R. serpentina* and *R. vomitoria*) and lastly the methods of cultivation (using modified strains, manual and mechanical cultivation) also affect the quantitative aspect of secondary metabolites production. For instance, a soil rich-in-nitrogen content gives rise to a relatively higher yield of alkaloids in the medicinal plants; whereas a soil not so abundant in nitrogen content and grown in comparatively dry zones may yield an enhanced quantum of volatile oil; ontogeny (or aging of plant) of a medicinal plant has a direct impact on the concentration of the active constituent (but not always true that older the plant greater would be the active principal), e.g., (i) cannabidiol, present in *Cannabis sativa* (*C. sativa* var. *indica*) and possessing euphoric activity, content attains a maximum level in the growing season and subsequently declines; however, the concentration of dronabinol (or tetrahydrocannabinol) starts to enhance reciprocally till the plants get fully matured; (ii) Morphine, the well-known narcotic-analgesic present in the air-dried milky exudate of *Papaver somniferum* or *P. album* is found to be the highest just 2–3 weeks after flowering. An undue delay in harvesting from this “critical-period” would ultimately result in the decomposition of morphine and similar is the case with the allied alkaloid like codeine and thebaine.

Metabolites of medicinal plants produced in different metabolic pathways have been proved to be the active or potentially active drug components in both traditional and modern medicines. Pharmaceutical as well as many food industries exploit these pools of biogenic resources of medicinal plants. Under outdoor conditions, medicinal plants are exposed to a multitude of environmental stress factors, both abiotic and biotic (herbivores and myriads of pathogenic microbes), during the growing season. The way plants sense stress depends on the duration and magnitude of the stress episode and its sustained effects depend on its severity, timing, duration, and the physiological status of the plant (Niinemets 2010). Stress factor by reducing the net photosynthetic rate reduces carbon and energy allocation for the synthesis of secondary metabolites and bringing alterations in the activity of key enzymes of secondary metabolic pathways also can modify the secondary metabolite pool under different stress regimes (Singsaas and Sharkey 2000). The quality and quantity of production of these chemical metabolites in plants are influenced by a multitude of internal and external environmental factors including circadian rhythm, developmental stage and age, tissue damage, season, altitude, temperature, water availability, soil nutrients, UV radiation, atmospheric composition, etc. Different stress regimes differentially affect quality and quantity of the secondary metabolite pool in plants. Co-occurrence of multiple stress factors and their effects may be additive or diverse and cannot always be extrapolated from responses to individual stress factor.

The secondary metabolites in plants are synthesized by different biochemical pathways and are strongly influenced by both environmental and biotic factors (Pavarini et al. 2012). The secondary metabolism of plants, and the expressed metabolite levels, may change considerably due to the influence of several biotic and abiotic stress signals.

2.2.5.1 Light (Visible and UV Radiation)

The visible spectrum of solar radiation energy (400–700 nm) is an important environmental factor required for photosynthesis, biomass accumulation, growth, and development of plants. Longer light exposure produced higher levels of ginsenosides in *Panax quinquefolius* (Fournier et al. 2003). Continuous solar radiation activated the flavonoid biosynthesis pathway in *Vaccinium myrtillus* (Jaakola et al. 2004). *Ocimum basilicum* on exposure to red light (600–700 nm) accumulates rosmarinic acid accumulation (Shiga et al. 2009). Light effects also have crucial importance on preharvest of *Camellia* sp. (Tea) shoots where synthesis and accumulation of phenolic derivatives (i.e., flavanols and catechin) become upregulated in leaf on overexposure to light and light exclusion of shoots just before harvest enhances the proportion of purine alkaloid (caffeine) level in leaves yielding high quality tea (Anan and Nakagawa 1974; Ashihara et al. 2008).

Plants have developed their ability to sense different light spectra and ultraviolet (10–400 nm, the range covers EUV to UVA) light present in the solar radiation and plants have evolved biochemical protective mechanisms against extreme light

intensities and potentially damaging elevated doses of ultraviolet (UV) radiation (shorter than that of visible light but longer than X-rays). UV-B radiation (280–315 nm) impacts on the levels of a broad range of secondary metabolites, including phenolic compounds, terpenoids, and alkaloids (Rozema et al. 1997; Kazan and Manners 2011). Coumarins such as psoralen, bergapten, and xanthotoxin biosynthesized on exposure to UV radiation in leaves of celery (*Apium graveolens*) vegetables of Apiaceae can damage eukaryotic DNA (Taiz and Zeiger 2006). *Catharanthus roseus* (cell culture) on exposure to UV radiation enhanced the production of catharanthine (Ramani and Chelliah 2007; Jenkins 2009). Phenylpropanoid derivatives selectively absorb in the UV-B spectral region without decreasing penetration of photosynthetic radiation into the leaf. Flavonoids, hydroxycinnamic acids and their esters have also been implicated in this role in a broad range of plant species (Burchard et al. 2000; Kliebenstein 2004). Glycoalkaloids such as α -solanine and α -chaconine reportedly accumulate in potato tubers exposed to mechanical stress or light and the compounds lead to gastrointestinal or neurological disorders in humans.

2.2.5.2 Soil Nutrients

The level of secondary metabolites in plant tissues is reported to vary with resource availability (Coley et al. 1985). For example, proanthocyanidins increase quantitatively following nutritional stress involving phosphate deficiency (Kouki and Manetas 2002); biosynthesis of phenolic compounds increases under iron stress (low iron level) (Dixon and Paiva 1995). High conditions of Cu and Mn nutrition decreased both tannins and flavonoids contents in *Eugenia uniflora* (Santos et al. 2011) while enrichment of carbon dioxide in atmosphere elevated tannin levels in *Quercus* spp. (Stiling and Cornelissen 2007).

2.2.5.3 Moisture

Drought events of relative ranges of magnitudes and durations are commonly experienced in many environments and can drastically impact plant survival and/or stress tolerance. Moisture stress causes reduction in the rate of photosynthesis as well as plant growth and development and one might expect a negative relationship between water shortage and the synthesis of secondary metabolites. Reduced rate of water availability and high temperature influence high phenolic production in plants (Glynn et al. 2004; Alonso-Amelot et al. 2007). Phenolic and saponin levels and the corresponding bioactivity were found to vary seasonally in medicinal bulbs (Ncube et al. 2011). In the same study, high phenolic compounds were recorded in all the species during the winter season, where moisture stress is a typical characteristic. Drought stress lowers monoterpane emissions in *Quercus ilex* (Lavois et al. 2009).

2.2.5.4 Temperature

Temperature stress in plants is generally known to induce or enhance the active oxygen species-scavenging enzymes like superoxide dismutase, catalase, peroxidase, and several antioxidants. Temperature stress may lead to many physiological, biochemical, and molecular changes in plant metabolism (protein denaturation or perturbation of membrane integrity) and many of these changes can alter the secondary metabolite concentrations in the plant tissues (Zobayed et al. 2005). High temperature (35 °C) treatment increased the leaf total peroxidase activity together with an increase in hypericin, pseudohypericin, and hyperforin concentrations in the shoot tissues of St. John's Wort (Zobayed et al. 2005). Also, an exponential increase in a variety of VOCs with a linear increase in temperature has been described in a range of plant species (Parker 1977; Sharkey and Loreto 1993; Sharkey and Yeh 2001). Cold stress has been shown to stimulate an increase in phenolic production and their subsequent incorporation into the cell wall (Christie et al. 1994). Levels of anthocyanins increase following cold stress and are thought to protect plants against this effect (Pennycooke et al. 2005; Huang et al. 2012). Ncube et al. (2011) attributed the high levels of total phenolic compounds obtained during the winter season in their study as being consistent with this fact and support similar findings from previous other studies (Prasad 1996; Pennycooke et al. 2005). Lower temperature was reported to increase the level of artemisin in *Artemisia* spp. (Wallaart et al. 2000; Brown 2010).

2.2.5.5 Altitude

Altitude starting from sea level to timberline and beyond influences plant life as it (with the increase) changes climate, quality and amount of solar radiation, availability of soil water and nutrients, etc., to plants and also metabolism and metabolites content. Altitude affected the flavonoids contents in *Leontodon autumnalis* (Zidorn and Stuppner 2001; Grass et al. 2006). Higher altitudes increased the flavonoids and phenolic acids contents in flowers of *Matricaria chamomilla* (Ganzera et al. 2008) whereas higher altitudes in association with lower temperatures affected phenolics derivatives in *Arnica montana* (Albert et al. 2009).

2.2.5.6 Ozone

Ozone has also been demonstrated to affect secondary metabolism in plants (Eckey-Kaltenbach et al. 1994; Jordan et al. 1991). Elevated O₃ levels increased the concentrations of terpenes, but decreased the concentrations of phenolics in *Ginkgobiloba* leaves grown under greenhouse conditions (He et al. 2009).

2.2.5.7 Salt Stress

Salt stress is a contributing factor to secondary metabolism in plants. Plants adjust metabolism to acclimate to different salt levels in soil and other growth media. High levels of alkaloids were reported for *Achnatherum inebrians* plants cultivated under salt stress (Zhang et al. 2011).

2.2.5.8 Biotic Stress Factors

Herbivore and pathogenic attacks have been repeatedly shown to cause an increased release of inducible secondary compounds in plants (Hagerman and Butler 1991; Bernays and Chapman 2000). Biotic effects include more sophisticated interactions with plant biochemistry and plant physiology (Briskin 2000). In a larger sense, it can be assumed that biotic effects are related either to plant interactions with microorganisms or plant physiological aspects, as phenology and ontogeny (Pavarini et al. 2012). Saponins occur constitutively in many plant species as part of their defense system and saponin content in plants seems to be dynamic, responding to many external factors including various biotic stimuli connected to herbivory attack and pathogenic infection, as well as involved in plant mutualistic symbioses with rhizobial bacteria and mycorrhizal fungi (Szakie et al. 2011).

2.3 Chemistry of Plant Constituents, Their Classification and Application

Classification of phytochemicals based on their elemental constituents (e.g., C & H; C, H & O; C, H, O, N, S & P containing compounds; O/N containing heterocyclic compounds; other miscellaneous compounds)

Living organisms are made of combinations of inanimate inorganic elements and out of the 92 naturally occurring elements, cells of living organisms are basically made of only a small selection of these elements, e.g., C, H, O, N, P, S, Ca, Fe, Cu, Mg, Zn, etc.; the first six of the series (C, H, O, N, P and S) make up bulk of the tissue component, but four of which (C, H, O and N) make up about 95% of the body weight of an organism. Cell sap contains electrolyte like Cl, K and Na as major elements. Atoms of different elements are linked together in groups in many ways to form molecules. Therefore, the chemical bonds (ionic, covalent, polar covalent, etc.) that hold atoms together in molecules in a living cell play the crucial role. Except water (which accounts for about 70% of a cell's weight), almost all the molecules in a cell are based on carbon. It has the unique capacity to form large molecules and because of its nano size, and four vacancy electrons in its outermost shell, it can form highly stable covalent bonds with neighbor carbon as well as other atoms covalent C–C bonds to form chains and rings and hence generate large and

complex organic molecules with no obvious upper limit to their size. A few basic categories of carbon-based molecules, e.g., hydrocarbons, carbohydrates, lipids, proteins, nucleic acids, secondary metabolites, etc., formed from different elements, give rise to all the extraordinary richness of form and behavior shown by living things. Based on the origin and the type of functions they perform in cells, some of these may be grouped as primary and others as secondary metabolites.

2.3.1 Primary Metabolic Products Consisting of C & H; C, H & O; N, S & P Elements (Carbohydrates, Lipids, Amino Acids, Proteins, Nucleic Acids, Organic Acids)

2.3.1.1 Hydrocarbons (C & H) and Derivatives

Hydrocarbons are organic compounds consisting of only carbon and hydrogen, e.g., the simplest form of lipids containing only C and H. Some latex-producing plants (hydrocarbon plants) of families, such as Euphorbiaceae, Apocynaceae, Asclepiadaceae, Sapotaceae, Moraceae, Dipterocarpaceae, etc., convert a substantial amount of photosynthetics (products of Calvin cycle) into latex which contains liquid hydrocarbons of high molecular weight (10,000 da). Hydrocarbons formed of isoprene units belong to the large group of terpenes.

Classification

Hydrocarbons include four classes of compounds such as alkanes, alkenes, alkynes, and aromatic hydrocarbons (Fig. 2.12). Hydrocarbons include linear or branched carbon chain; saturated (e.g., alkanes, C_nH_{2n+2} , ethane- C_2H_6) or unsaturated (e.g., alkenes, C_nH_{2n} , ethylene- C_2H_4 ; alkynes, C_nH_{2n-2} , ethyne- C_2H_2); and cyclic (benzene- C_6H_6 , toluene- C_7H_8), alicyclic (e.g., cyclobutane- C_4H_8) hydrocarbons and carotenoids. Several hydrocarbons may be substituted with oxygen-containing groups (e.g., xanthophylls). Hydrocarbons may be classified as follows:

Alkanes are saturated hydrocarbons with a general formula C_nH_{2n+2} , e.g., methane (CH_4), ethane (C_2H_6), propane (C_3H_8), butane (C_4H_{10}), etc. Alkenes are unsaturated hydrocarbons and contain a carbon–carbon double bond. The number of hydrogen atoms in an alkene is double the number of carbon atoms, e.g., the molecular formulae of ethene (IUPAC name) or ethylene and propene are C_2H_4 and C_3H_6 , respectively. Alkyne is an unsaturated hydrocarbon containing at least one carbon–carbon triple bond with the general chemical formula C_nH_{2n-2} , e.g., acetylene, propyne, butyne, etc. Cycloalkanes contain carbon–hydrogen bonds and carbon–carbon single bonds and the carbon atoms are joined in a ring, e.g., cyclopropane, –butane, –pentane, –hexane, etc., are some common examples. Cycloalkenes or cycloolefin are alkenehydrocarbons with ring structure having at

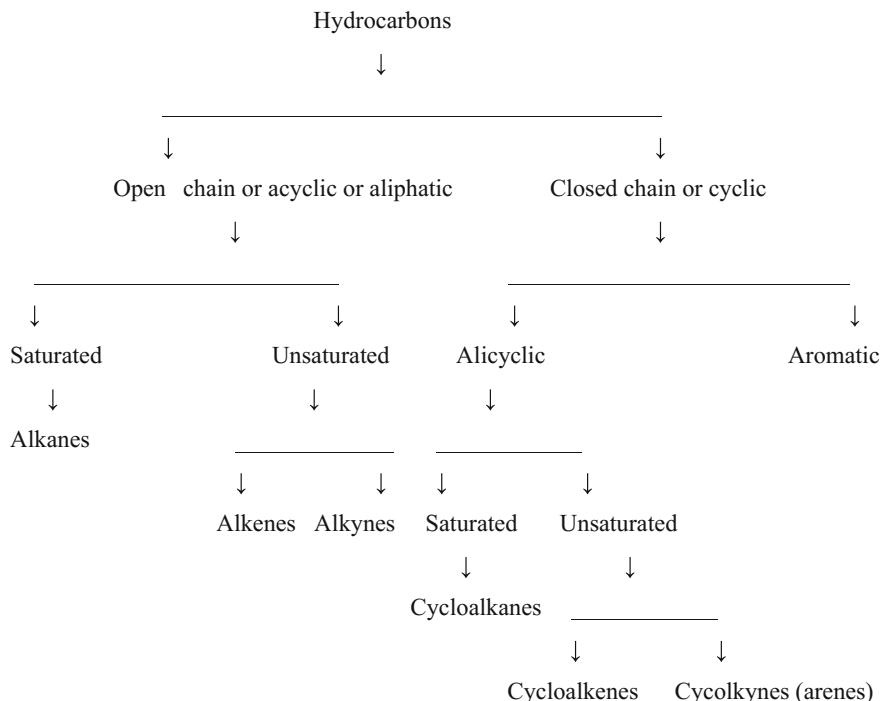
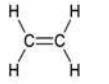
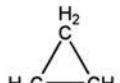
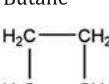
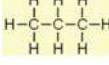
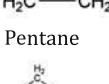
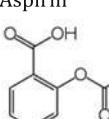
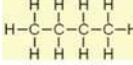
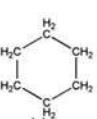
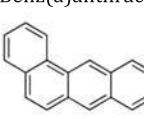
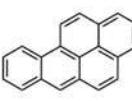
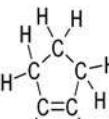
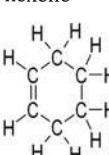


Fig. 2.12 Different classes of hydrocarbons: alkanes, alkenes, alkynes and aromatic hydrocarbons; saturated, unsaturated, cyclic and alicyclic

least one C=C double bond in the ring, but has no aromatic character. Cyclopropene, cyclopentene, cyclohexene, etc. are some of the examples cycloalkenes. Aromatic hydrocarbons (or arene or aryl hydrocarbon) are compounds that contain benzene (a cyclic hydrocarbon with the formula C_6H_6) as a part of their structure. Many of these compounds have a sweet or pleasant odor. Aromatic hydrocarbons can be monocyclic (MAH) or polycyclic (PAH). PAHs consisted of fused aromatic rings and do not contain heteroatoms or carry substituents. They are most widespread organic pollutants produced largely a result of natural emissions as well as anthropogenic activities (e.g., fossil fuel-burning, oil refining, coke and asphalt production, aluminum production, etc.). The most significant endpoint of PAHs (e.g., benz[a]anthracene, benzo[a]pyrene) toxicity is cancer (e.g., increased incidences of lung, skin, and bladder cancers are associated with chronic occupational exposure to PAHs). Some non-benzene-based compounds (heteroarenes) are also called aromatic compounds in which one carbon atom may be replaced by one of the heteroatoms (oxygen, nitrogen, or sulfur). For example, furan and pyridine are five- and six-membered ring heterocyclic compounds containing one O and one N atom, respectively in the heterocycle. Some examples of different classes of hydrocarbons are given in Table 2.1.

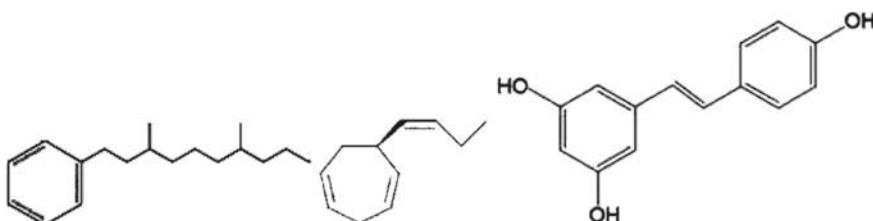
Table 2.1 Examples of different classes of hydrocarbons

Hydrocarbons			
Open chain or acyclic or aliphatic		Closed chain or cyclic	
Saturated	Unsaturated	Alicyclic	Aromatic
Alkanes: C_nH_{2n+2}	Alkenes: C_nH_{2n}	Saturated- Cycloalkanes:	Homocyclic: Benzene
Methane 	Ethene or ethylene 	Cyclo-propane 	
Ethane 	Propene 	Butane 	Phenol 
Propane 		Pentane 	Aspirin 
Butane 		Hexane 	Benz(a)anthracene 
	Alkynes: C_nH_{2n-2}	Unsaturated- Cycloalkenes C_nH_{2n-2}	Benzo(a)pyrene 
	Acetylene $HC\equiv CH$	Cycloalkynes (arenes)	Heterocyclic: Furan 
	Propyne $HC\equiv C-CH_3$	Cyclopentene 	Pyridine with its free electron pair 
	Butyne $HC\equiv C-CH_2CH_3$	hexene 	Pyrimidine 

Several hydrocarbons may be substituted with non-hydrogen atoms and thus produce hydrocarbon derivatives. Hydrocarbon derivatives contain different elements (e.g., oxygen, nitrogen, halogen atoms, etc.) or functional groups (e.g., hydroxyl, carbonyl, carboxyl groups, etc.) instead of hydrogen and in this way almost an innumerable number of carbon compounds are formed (e.g., alcohols, alkyl halides, amines, amides, carboxylic acids, esters, aldehydes, ketones, ethers, etc.).

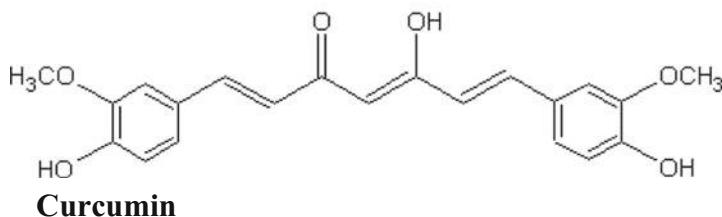
Alkanes–alkenes molecules found in many living organisms are directly derived from fatty acids (e.g., undecane in ants and eicosane in *Bryonia dioica*). They are distinct from the terpenoid hydrocarbons. Terpenoids contained in the latex of *Hevea* spp. of Euphorbiaceae are plant-derived hydrocarbon products (e.g., rubber). Some algae also produce hydrocarbons, e.g., the green form of single cell alga *Botryococcus braunii* produces hydrocarbon (chain length between 34 and 38 carbons containing many double bonds). Hydrocarbons in plants may be formed as products of fatty acid cleavage during peroxidation processes. Alkanes as well as alkenes appear during hydroperoxide decomposition. Plant-derived aliphatic hydrocarbons include vegetable, olive, and other cooking oils.

There may be monocyclic and polycyclic hydrocarbons of biological origin (Fig. 2.13). Monocyclic hydrocarbons include several branched alkylbenzenes of Archaebacteria (*Thermoplasma* and *Sulfolobus*) with two methyl groups branched on a saturated chain of 9–12 carbon atoms, and algal pheromone ectocarpene is an unsaturated heptacyclic hydrocarbon found in the brown algae *Ectocarpus*, *Adenocystis*, and *Sphaerelaria* (Müller et al. 1971), resveratrol



Alkylbenzenes Ectocarpene

Resveratrol



Curcumin

Fig. 2.13 Monocyclic and polycyclic hydrocarbons of biological origin with anticancer, antiviral, neuroprotective, antiaging, anti-inflammatory, strong antioxidant, etc., activities

(3,4',5-trihydroxystilbene) is present in grapes and blueberries (*Vaccinium*). It has numerous pharmacological properties including anticancer, antiviral, neuroprotective, antiaging, and anti-inflammatory, etc. Diarylheptanoids, a group of compounds having phenyl rings at 1,7 positions of n-heptane. Curcumin of turmeric is a diarylheptanoid. Because of its strong antioxidant properties, numerous therapeutic activities have been assigned to turmeric for a wide variety of diseases and conditions (Aggarwal et al. 2007).

Polycyclic hydrocarbons consist of fused rings containing only carbon (naphthalene, perylene, denthryrsinin) or heterocycles including foreign atoms such as O (coumarin, osthole, psoralen), N (2-Heptyl-3-hydroxy-4-quinolone), etc. Naphthalene is a constituent of Magnolia flowers (Azuma et al. 1996); it may protect tissue against insect herbivores, and attracts insects to pollinate by the UV absorption of accumulated naphthalene in the floral parts and floral scent. Several forms of phenanthrenes are present in higher plants of several families like Orchidaceae, Dioscoreaceae, Combretaceae, Euphorbiaceae, Juncaceae, and Hepaticae. Denthryrsinin is reported in the orchid species *Cymbidium pendulum*, *Dendrobium* spp., *Eulophia nuda*, *Nidema boothii*, *Scaphyglottis livida*, *Thunia alba*, etc. It, as others, displayed potent cytotoxic activities (Kovács et al. 2008).

Heterocyclic hydrocarbons include coumarins, and a coumarin is the simplest compound of this group (Fig. 2.14). Several other are coumarin derivatives by various additions. It is found in many plants including tonka bean (*Dipteryx odorata*) of Fabaceae, vanilla grass (*Anthoxanthum odoratum*) and buffalo grass (*Hierochloe odorata*) of Poaceae, woodruff (*Galium odoratum*) of Rubiaceae. All these plants are strongly scented due to the presence of coumarin which has been used in perfumes since 1882 (imitation of vanilla products). Coumarin is used as rodenticide, and extracts from these plants are potential harmful as coumarin is the precursor for several anticoagulants, notably warfarin. Osthole [7-methoxy-8-(3-methylpent-2-enyl) coumarin] is a coumarin derivative found in *Cnidium monnierii*, a plant used in traditional Chinese medicine to treat skin affections. Osthole was shown to exhibit several biological functions, including antiosteoporotic, antiallergic, anti-inflammatory, and antitumor functions. Recently, it was found that osthole might be a potent antidiabetic agent (Lee et al. 2011).

Umbelliferone (or 7-hydroxycoumarin) occurs in many familiar plants from the Umbelliferae family such as carrot or coriander but also from other families such as Asteraceae (*Pilosella officinarum*). Umbelliferone absorbs ultraviolet light strongly but despite possible harmful mutagenic properties, it is used in sunscreens. Psoralen (or psoralene) is a furanocoumarin. It is a derivative from umbelliferone by addition of a furan ring. Psoralen has been described in the seeds of the Fabaceae (*Psoralea corylifolia*). It is also present in many plants of Rutaceae (*Ruta*, *Citrus*), Moraceae, Leguminosae (*Psoralea*, *Coronilla*), Apiaceae, etc. Psoralen-rich plants are used in Chinese and Indian medicines and psoralene, due to its UV absorption properties, is used in treatment of psoriasis, eczema, and vitiligo and in some cutaneous lymphoma.

The core of quinolines is the 1-azanaphthalene nucleus. The simplest one is quinolin. That compound is rarely found in living material but is present, as its

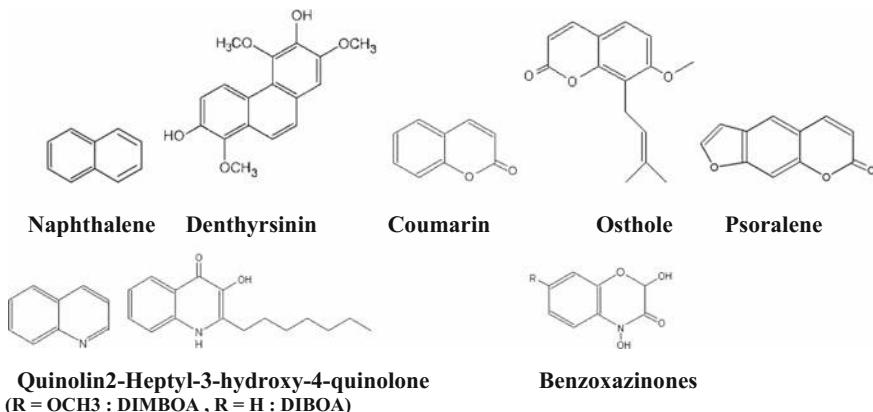


Fig. 2.14 Heterocyclic hydrocarbons coumarin and several coumarin derivatives possessing different biological functions: antiosteoporotic, antiallergic, anti-inflammatory, and antitumor activities

derivatives, in the plant Rutales and even in some insects. Quinolin is also present in some insects, as phasmids, where it plays a role against predators. The bacteria *Pseudomonas aeruginosa* was shown to produce a new cell-to-cell signal molecule, e.g., 4-quinolone base structure with an alkyl chain (2-heptyl-3-hydroxy-4-quinolone) and has been designated as the *Pseudomonas* quinolone signal (Pesci et al. 1999). Compounds related to quinolines, the benzoxazinones, are present as inactive glucosides (phytoanticipins), mainly in Gramineae (rye, wheat, corn). They are sometimes described as cyclic hydroxamic acids. In rye, the principal compound is the glucoside of DIBOA (2, 4-dihydroxy-1,4-benzoxazin-3-one), in wheat and corn, it is the glucoside of the methoxylated form, DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one).

Carotene hydrocarbon compounds consist of C40 chains (tetraterpenes consisting of eight 5C isoprenoid units) with conjugated double bonds (carotenes), while their oxygenated derivatives are known as xanthophylls. They are the predominant class of tetraterpenes (or terpenoids), show strong light absorption and often are brightly colored (red, orange), and are found as pigments in bacteria, algae and higher plants. Carotenoids perform three major functions in plants (e.g., accessory pigments for light harvesting by expanding the absorption spectra of photosynthesis, prevention of photooxidative damage by dissipating excess light, and pigmentation attracting insects). Carotenoids like α -carotene, β -carotene and lycopene are major carotenes while lutein, zeaxanthin, and cryptoxanthin are some major xanthophylls. The human intake of carotenoids may be appreciated using databases such as that established for Swiss vegetables (Reif et al. 2013). In mammals, carotenoids exhibit immunomodulatory actions, likely related to their anticarcinogenic effects. β -carotene was thus shown to enhance cell-mediated immune responses (Hughes 1999). The decrease in prostate cancer risk has been

linked to the consumption of tomatoes (lycopene rich vegetable), but there is yet limited direct evidence in favor of such link (Kavanaugh et al. 2007).

Hydrocarbons are found at the outer surface in higher plant leaves, e.g., C27, C29, and C31 *n*-alkanes are the most abundant (from 11 to 19%) in needle wax of the Serbian spruce *Picea omorika* of Pinaceae. Volatile oils of plant and animal origin are the oxygenated derivatives and hydrocarbons. Ethylene occurs in plants functions as a natural growth regulator that promotes the ripening of fruit. Several alkenes with 8 or 11 carbon atoms and 3 or 4 double bonds play a role in algae gamete attraction (pheromones): cystophorene in *Cystophora* sp., finavarrene in *Ascophyllum* sp. and *Sphaerotrichia* sp., fucoserratene in the brown seaweed *Fucus serratus* and in the freshwater diatom *Asterionella formosa* (Bacillariophyceae). Cyclic hydrocarbons may be monocyclic or polycyclic. Monocyclic hydrocarbons have mostly two methyl groups branched on a saturated chain of 9–12 carbon atoms. Algal pheromone, ectocarpene, is an unsaturated heptacyclic hydrocarbon found in the brown algae Ectocarpus, Adenocystis, and Sphaelaria. Resveratrol (3, 4, 5-trihydroxystilbene), a polycyclic species, is present in grapes and wine and blueberries and shows numerous pharmacological properties such as anticancer, antiviral, neuroprotective, antiaging, anti-inflammatory, etc. Other examples include phenanthrenes (a polycyclic aromatic hydrocarbon composed of three fused benzene rings) that are present mainly in Orchidaceae family as well as in Dioscoreaceae, Combretaceae, Euphorbiaceae, Juncaceae, and Hepaticae.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous, present in the atmosphere, surface water, sediments and soil, food and lipid tissues; and human exposure to PAHs is mainly from food and inhaled air including the occupational exposure (EC 2002). Food can be contaminated from industrial as well as domestic food processing including drying, smoking, roasting, grilling, frying, barbecuing (Harvey 1997; Howsam and Jones 1998). They have been detected in a wide variety of herbs like lime, pansy, mint, lemon balm, panax, Fructus liquidambaris, liquorice root, mulberry twig, cassia seed, eucommia bark, rose flower, indigowoad leaf, fleeceflower root, and in many crude drugs (Kataoka et al. 2010; Krajian and Odeh 2013; Zongyan et al. 2014). Anthraquinone (a PAH) having anthracene nucleus is found in several plant species (Aloes), fungi, and lichens. They are toxic and many of the compounds in this class are both genotoxic and carcinogenic (induce mutations, promote tumor formation). They are actively involved in enzyme induction, immunosuppression, and teratogenicity.

The hydrocarbon carotenoids are known as carotenes (tetra terpenes), while oxygenated derivatives of these hydrocarbons are known as xanthophylls (e.g., lutein, zeaxanthin, neoxanthin, violaxanthin, flavoxanthin, α - and β -cryptoxanthin, etc.) (Fig. 2.15). About 700 carotenoids have been identified, 50 are regularly consumed in the human diet and 24 have been detected in human plasma so far. Carotenoids are important components of photosynthetic pigments in plants and in mammals, carotenoids exhibit immunomodulatory actions, likely related to their anticarcinogenic effects. β -carotene was thus shown to enhance cell-mediated immune responses. The decrease in prostate cancer risk has been linked to the consumption of tomatoes, vegetable rich in lycopene, as prostatic tissues. Lutein

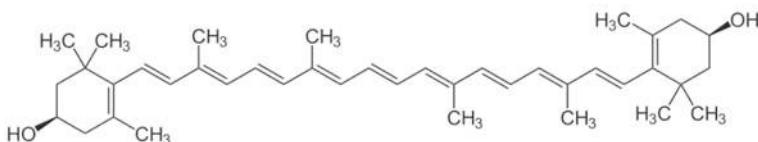
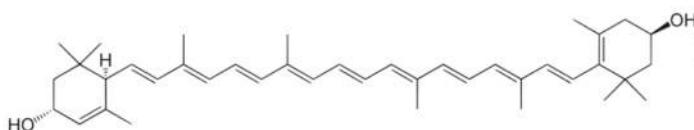
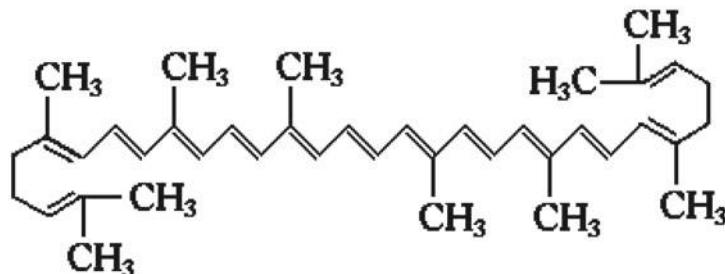
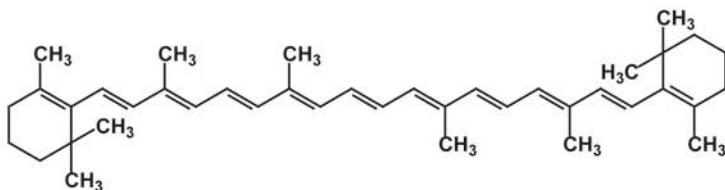


Fig. 2.15 The hydrocarbon carotenes and xanthophylls

and zeaxanthin are isomers (differing only in the placement of one double bond) and represent xanthophylls. They are found in high quantities in green leafy vegetables and fruits such as spinach, kale, yellow carrots, papaya, peaches, prunes, and squash. Animals get lutein from plants (in egg yolks and animal fats). Broiler feed now is fortified by lutein to improve the color of broiler chicken skin and egg yolk.

2.3.1.2 Carbohydrates (C, H & O)

Carbohydrates are polyhydroxy aldehydes or ketones or substances that yield such compounds on hydrolysis. Carbohydrates are plant products and, in most of the cases, hydrogen (H) and oxygen (O) remain as associates with carbon in the ratio (1:2:1) similar to that of water ($\text{C}_2\text{H}_2\text{O}$), e.g., glucose, fructose ($\text{C}_6\text{H}_{12}\text{O}_6$), sucrose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$) and starch ($\text{C}_6\text{H}_{10}\text{O}_5$) $_n$ as well as gum, mucilage and pectin (derived carbohydrates). Carbohydrates are widely distributed in plants, remain both as transitory and stored constituents, and provide energy, carbon skeleton for metabolic synthesis and building blocks of the cell wall materials and other metabolites.

Sugars, starches, gums, and other carbohydrates are of very little pharmacologic action and of little importance as remedies, but of importance in dietetics for energy for the body and brain cells, they are used in pharmacy mainly as pharmaceutic necessities, such as suspending and emulsifying agents (Tragacanth, Acacia, Agar, Alginates, etc.), adhesives and binders (tragacanth, dextrins, acacia, etc.), demulcents (acacia, sterculia, etc.), thickening agents, diluants and tablet disintegrants (alginates, starch, etc.).

Classification

Carbohydrates are classified into following groups:

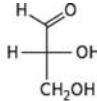
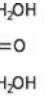
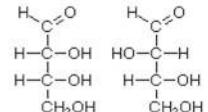
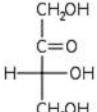
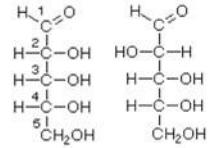
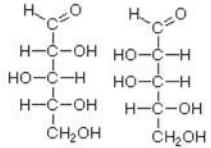
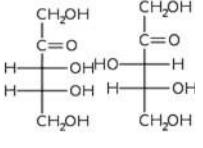
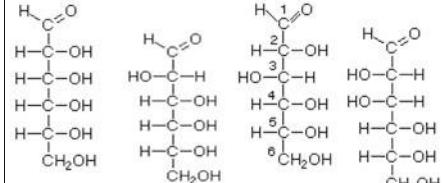
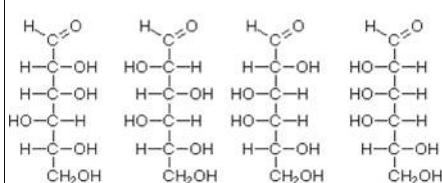
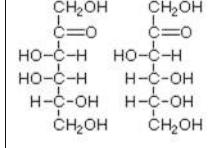
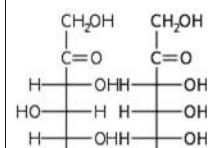
- (i) **Monosaccharides** molecule consisting of only one unit (sugars containing 3–9 carbon atoms—triose to nanos, 5 and 6 being most common), e.g., glucose, fructose;
- (ii) **Oligosaccharides** molecule consisting 2–10 monosaccharide units (disaccharide to decasaccharide), e.g., sucrose, gentianose;
- (iii) **Polysaccharides** molecule consisting 11 to $-n$ number of monosaccharide units, e.g., starch, cellulose.

The simple carbohydrates or sugars are water soluble and sweet in taste, e.g., glucose, fructose, and similar other carbohydrates. Drugs containing sugars include liquorice, which contains free glucose and fructose and gentian, which contains sugars like gentianose (trisaccharide) and gentiobiose (disaccharide).

Carbohydrates may also be grouped as sugars and non-sugars or polysaccharides on the basis of their taste and solubility. Sugars are sweet crystalline substances and soluble in water and further divided into monosaccharides (e.g., glucose, galactose, fructose) and oligosaccharides (e.g., sucrose, raffinose, stachyose etc.) while non-sugars are long chain polymers of monosaccharides units, polysaccharides [$\text{C}_6\text{H}_{10}\text{O}_5$] $_n$, linked to each other by glycosidic linkages. They are tasteless amorphous substances and insoluble in water and further divided into homopolysaccharides (e.g., starch, cellulose, inulin, etc.) and heteropolysaccharides (e.g., pectin, lignin, glycoprotein, etc.).

(i) **Monosaccharides (CH_2O)₃₋₁₀**: Monosaccharides consist of single unit and divided into (a) aldoses (each contains an aldehyde group, $-\text{CHO}$, e.g., aldotrioses—glycerose; aldötetroses—erythrose, threose; aldopentoses—ribose, arabinose, xylose; aldohexoses—glucose, galactose, mannose; aldoheptoses-L-glycero-D-

Table 2.2 Empirical and structural formulae of different classes of monosaccharides

Group and empirical formulae	Structural formulae of different classes of monosaccharides	
	Aldoses	Ketoses
Trioses C₃H₆O₃	 D-Glyceraldehyde	 Glycerone
Tetroses C₄H₈O₄	 D-Erythrose D-Threose	 D-Erythrulose
Pentoses C₅H₁₀O₅	 D-Ribose D-Arabinose  D-Xylose D-Lyxose.	 D-Ribulose D-Xylulose
Hexoses C₆H₁₂O₆	 D-Allose, D-Altrose D-Glucose, D-Mannose  D-Gulose D-Idiose D-Galactose D-Talose	 D-Tagatose, D-Fructose  D-Sorbose,D-Psicose

<p>Heptoses C₇H₁₄O₇</p> <p>D-Glycero-D-gluco-heptose</p> <p>L-glycero-D-manno-heptose</p> <p>Mannoheptulose</p> <p>Sedoheptulose</p>

manno-heptose, etc.); and (b) ketoses (each contains a keto group, >C=O, e.g., ketotrioses—dihydroxyacetone; ketotetroses—erythrulose; ketopentoses—ribulose, xylulose; ketohexoses—fructose; ketoheptoses—sedoheptulose, mannoheptulose; octoses—D-*manno*-octulose2-keto-3-deoxy-manno-octonate; nonoses—D-*glycero-D-galacto*-nonulose, sialose, L-ribo-D-manno-nonose (ketose), etc.), decose-3,6-Dideoxy-L-threo-L-talo-decose (aldose). Empirical and structural formulae of monosaccharides are shown in Table 2.2.

A triose is a monosaccharide containing three carbon atoms, the simplest monosaccharides. There are only two trioses, an aldotriose (glyceraldehyde) and a ketotriose (dihydroxyacetone). In green plants, trioses are formed by the fixation of carbon dioxide in the process of photosynthesis. And they are also important in respiration as lactic acid and pyruvic acids are derived from aldotriose and ketotriose, respectively. Glyceraldehyde is the simplest of all common aldoses. It is a sweet, colorless, crystalline solid and is an intermediate compound in carbohydrate metabolism. Dihydroxyacetone, the simplest ketose, is an isomer of glyceraldehyde. Trioses and triose phosphates are important metabolic intermediates. A monosaccharide with four carbon atoms is a tetrose. They either have an aldehyde functional group in position C₁, aldötetroses, e.g., D-erythrose, D-threose, or a ketone functional group in position C₂, ketotetroses, e.g., D-erythrulose. A pentose is a five-carbon monosaccharide. Pentoses are organized into two groups, e.g., aldopentoses have an aldehyde functional group at position C₁ and ketopentoses have a ketone functional group in position C₂ or C₃. D-arabinose, D-lyxose, D-xylose, D-ribose, L-arabinose, L-lyxose, L-xylose, and L-ribose are eight examples of aldopentose stereoisomers (they aldopentoses have three chiral centers and so $2^3 = 8$ isomers) and D-ribulose, D-xylulose, and L-ribulose, L-xylulose are four examples of ketopentose stereoisomers (these aldopentoses have two chiral centers

and so $2^2 = 4$ isomers). Ribose and deoxyribose are constituents of RNA and DNA, respectively. A polymer of pentose sugars is called a pentosan.

Hexose is a monosaccharide with six carbon atoms. Hexoses are classified by functional group, with aldohexoses having an aldehyde at position C₁ (e.g., glucose), and ketohexoses having a ketone at position C₂ (e.g., fructose). The aldohexoses have four chiral centers for a total of 16 possible aldohexose stereoisomers (2⁴). The D/L configuration is based on the orientation of the hydroxyl group at position 5, and does not refer to the direction of optical activity. The 8 D-aldohexoses are allose, altrose, glucose, mannose, gulose, idose, galactose, talose, etc. Of these D-isomers, all except D-altrose are naturally occurring. The ketohexoses have three chiral centers and therefore eight possible stereoisomers (2³). Of these, only the four D-isomers are known to occur naturally (D-psicose, D-fructose, D-sorbitose, D-tagatose). Only the naturally occurring hexoses are capable of being fermented by yeasts. Hexose sugars can form dihexose sugars with a condensation reaction to form a 1, 6-glycosidic bond. Many of these simple sugars are found in many fruits and vegetables and are the common building blocks for the more complex sugars. Glucose (also known as D-glucose, dextrose, or grape sugar), an important carbohydrate in biology, is the most widely distributed sugar in the plant and animal kingdoms and it is the sugar present in blood as blood sugar. Cells use it as a source of energy and a metabolic intermediate. Only the “right-handed form” of glucose, D-glucose (dextrose) is very common in nature but not the L-glucose. Fructose (levulose or fruit sugar), a ketohexose, is abundant in honey and some fruits. Fructose and glucose are the main carbohydrate constituents of honey. Fructose is more easily appropriated by diabetics than are cane sugar, glucose, and many starchy foods. It has been used by Strauss as a test of the functional power of the liver, the assertion being made that if the levulose is recoverable from the urine unchanged, the liver is seriously impaired. Manna, derived from a tree of the ash family Oleaceae (manna ash—*Fraxinus ornus*), contains the sugar, mannitol (C₆H₁₄O₆), is laxative. A sugary extract from the sap is extracted by making a cut in the bark. The sugar mannose and the sugar alcohol mannitol both are derived from the extract.

Heptulose is a monosaccharide with seven carbon atoms, e.g., mannoheptulose. It is found as D-mannoheptulose in appreciable amount in avocado fruit (*Persea gratissima* Gaertn. of Lauraceae) and trace amount in guava, passion fruit, mango, papaya, fig, alfalfa, and primerose. Mannohexulose is a hexokinase inhibitor. By blocking the enzyme hexokinase, it prevents glucose phosphorylation. As a result, the breakdown of glucose is inhibited. Mannohexulose has been reported to inhibit insulin secretion from pancreas and to induce hyperglycemia. This inhibition occurs because when mannoheptulose is present, the glycolysis is inhibited (because there is no production of glucose-6-P) and therefore no increase in ATP concentration which is required to close the K⁺-ATP channel in the beta cells of the pancreas causing a diminution of calcium entry and insulin secretion. D-alto and D-talo are two other heptuloses.

Volemitol (D-glycero-D-manno-heptitol, α-sedoheptitol) is an unusual seven-carbon sugar alcohol that fulfills several important physiological functions in

certain species of the genus *Primula*, major nonstructural carbohydrate in leaves of all stages of development, followed by sedoheptulose (*D-altr*-2-heptulose, 36 mg/g fresh weight). Volemitol is important in certain *Primula* species as a photosynthetic product, phloem translocate, and storage carbohydrate. The physiological roles of alditols are manifold and largely resemble those of disaccharides and oligosaccharides. They include photosynthetic assimilation, translocation, and storage of carbon, and reducing power, as well as protection against different types of stresses.

Octose is a monosaccharide with eight carbons and includes *D*-glycero-*D*-manno-Octulose, *D*-glycero-*L*-galacto-Octulose.

The aldehyde (-CHO) and ketone (>C=O) functional groups in the pentose as well as in the hexose carbohydrates react with the neighboring hydroxyl functional groups to form intramolecular hemiacetals and hemiketals, respectively resulting ring structures. The pentose (ribose) and hexose (glucose) ring forms are created when the oxygens on C₄ or C₅, links with the carbon comprising the carbonyl group (aldose C₁, ketose C₂) and transfers its hydrogen to the carbonyl oxygen to create a hydroxyl group. The resulting ring structure is related to furan, five-sided ring (furanose) in case of C₁→C₄ and pyran, and six-sided ring (pyranose) in case of C₁→C₅ links. The ring spontaneously opens and closes, allowing mutarotation to occur about the bond between the carbonyl group and the neighboring carbon atom yielding two distinct configurations, e.g., α and β. The rearrangement produces α-ribose or glucose when the hydroxyl group is on the opposite side of the -CH₂OH group, or β-ribose or glucose when the hydroxyl group is on the same side as the -CH₂OH group. Cyclic forms with a 7-atom ring (the same of oxepane), rarely encountered, are called heptoses. Some of the structural formulae of Penta furanoses (Fig. 2.16) and Hexa pyra- and furanoses (Fig. 2.17) are shown below.

The ring forms of β-*D*-ribose and β-*D*-2-deoxyribose (missing oxygen at position 2) are two ribo-pentose sugars and they are the structural components of RNA and DNA, respectively (Fig. 2.17).

Monosaccharides with same empirical formulae may differ in their structural formulae (isomers) leading to changes in their physical and chemical properties. For example, many saccharides differ in their structural formulae only in the orientation of the hydroxyl groups (-OH) and this structural difference makes a big difference in the biochemical, organoleptic, (taste) and physical properties (melting point, optical activity, etc.). These carbohydrates form stereoisomers and the German chemist Emil Fischer received Nobel Prize in 1902 in chemistry as he identified the stereoisomers in aldohexoses in 1894. Structures that have opposite configurations of a hydroxyl group at only one position, such as glucose and mannose, are called *epimers*. Isomers which differ only in their configuration about their carbonyl carbon atom are called *anomers*. The D refers to dextrorotatory (rotates polarized light to the right) character natural glucose, but it now denotes a specific configuration.

Sugars may be modified by natural processes into compounds like sugar alcohols, amino sugars, and uronic acids. Sugar alcohols are a type of carbohydrates called polyols; part of their chemical structure resembles sugar, and part of it

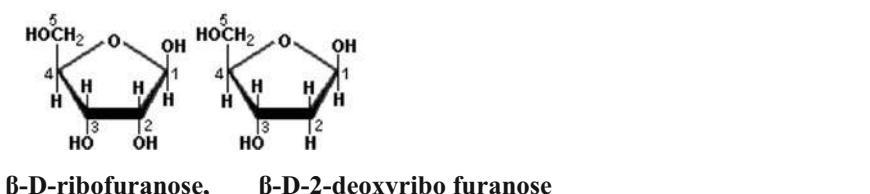
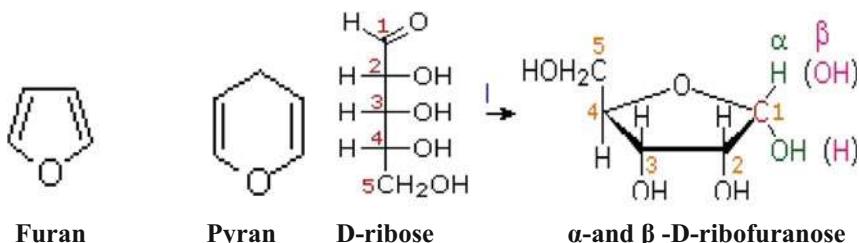


Fig. 2.16 Structures of different penta furanoses—Furan, Pyran, D-ribose, α - and β -D-ribofuranose, β -D-2-deoxyribo furanose

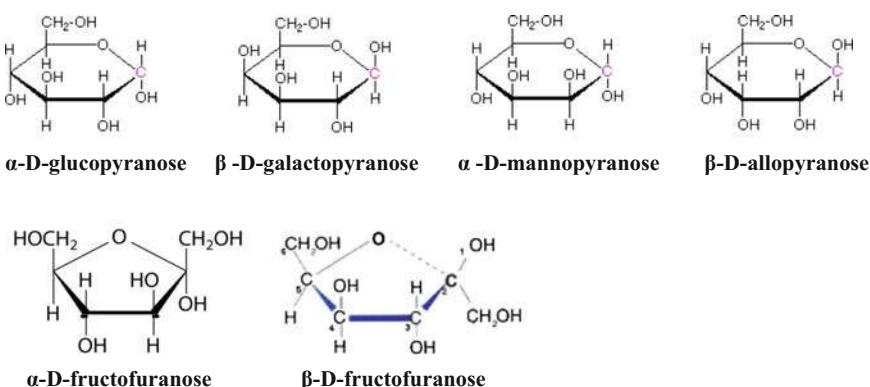


Fig. 2.17 Structure of different hexa pyra- and furanoses

resembles alcohol. Sugar alcohols are one type of reduced calorie sweetener. Both monosaccharides and disaccharides (maltitol and lactitol) can form sugar alcohols. Some of the common sugar alcohols are methanol (1-carbon), ethylene glycol (2-carbon), glycerol (3-carbon), erythritol (4-carbon), threitol (4-carbon), arabitol (5-carbon), xylitol (5-carbon), ribitol (5-carbon), mannitol (6-carbon), sorbitol (6-carbon), galactitol (6-carbon), fucitol (6-carbon), iditol (6-carbon), inositol (6-carbon, a cyclic sugar alcohol); volemitol (7-carbon), isomalt (12-carbon), maltitol (12-carbon), lactitol (12 carbon), maltotriitol (18-carbon), maltotetraitol (24-carbon), polyglycitol, etc. The simplest sugar alcohols, ethylene glycol and methanol, are sweet but highly toxic chemicals used in antifreeze. The more complex sugar alcohols are generally nontoxic. Sugar alcohols occur naturally in

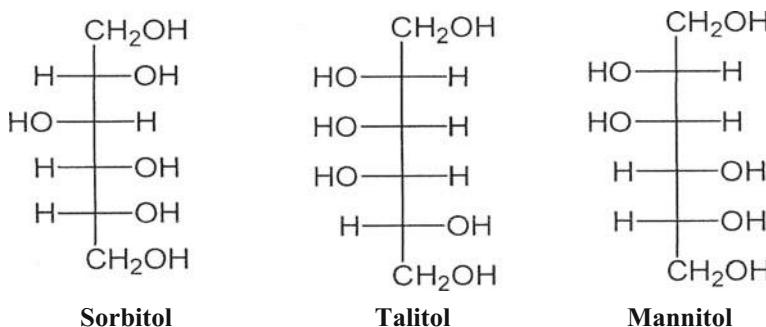


Fig. 2.18 Structure of different sugar alcohols—sorbitol, talitol and mannitol

plants (e.g., sorbitol from corn syrup and mannitol from seaweed) or they are manufactured from sugars and starches. Sugar alcohols (e.g., glycitol, polyols, polyhydric alcohols, or polyalcohols) are the hydrogenated forms of the aldose or ketose sugars, e.g., sorbitol (glucitol), where the aldehyde ($-CHO$) group is replaced with a $-CH_2OH$ group (Fig. 2.18).

The sugar alcohols commonly found in foods are sorbitol, mannitol, xylitol, isomalt, and hydrogenated starch hydrolysates (HSH). Their relative sweetness may be graded as sugar = xylitol > erythritol > maltitol > mannitol > sorbitol > isomalt > HSH > lactitol, etc. and that of their energy content (calories per gram) as sugar (4%) > HSH > sorbitol > xylitol > maltitol > isomalt > lactitol > mannitol > erythritol, etc. These sugar substitutes provide fewer calories (1.5–3 calories per gram) than table sugar (4 calories per gram), mainly because they are not well absorbed and may even have a small laxative effect. In commercial foodstuffs, sugar alcohols are commonly used in place of table sugar (sucrose) as thickeners and sweeteners. Many so-called dietetic foods that are labeled sugar free or no sugar added in fact contain sugar alcohols.

Erythritol, a four-carbon polyol, 60–70% as sweet as table sugar, is only partially absorbed by the body and so it has only 0.2 calories per gram, i.e., 95% less than table sugar. Erythritol is used as a food additive throughout the world. It is used in food for diabetics because it does not affect blood sugar and does not cause dental caries. Although erythritol is well tolerated by humans but appeared toxic to *Drosophila melanogaster* like insecticide. Xylitol, a five-carbon polyol, is a very common ingredient in sugar-free candies and gums because it is approximately as sweet as sucrose, but contains 40% less food energy. This sugar alcohol is safe for humans; however, xylitol in small doses can cause seizures, liver failure, and death in dogs. Sorbitol, a six-carbon polyol, found primarily in stone fruits and also manufactured from corn syrup, is used in diet sodas, sugar-free ice creams and desserts, as well as in mints, cough syrups, and gum. Maltitol, a 12-carbon polyol derived from chicory and roasted malt, is very similar to actual sugar in terms of mouthfeel, sweetness, and cooking (except for browning) but calories and so it is used in copious amounts in sugar-free desserts and other products. Unlike sugars,

sugar alcohols do not contribute to dental caries, blood sugar, and insulin levels, but may cause bloating and diarrhea when consumed in excessive amounts.

An amino sugar contains an amino ($-\text{NH}_2$) group in place of a hydroxyl group of the sugar molecule. More than 60 amino sugars are known, many of them have been isolated and identified in recent times as components of antibiotics. Examples of amino sugars include D-glucosamine, D-mannosamine, D-galactosamine, N-acetyl-D-glucosamine (main component of chitin), α -D-glucosamine α , -D-N-acetylglucosamine, sialic acid, L-daunosamine (a deoxy hexosamine and a component of birch juice), etc. (Fig. 2.19).

Glucosamine is amino sugars or aminosaccharide and is a well-known amino sugar that produces glycoconjugates like glycosylated lipids and proteins. Glucosamine has a structural role in composing the hard exoskeleton of chitins of arachnids, crustaceans, and insects. Wheat, rice, and barley grains as well as bovine and shark meats are the important sources of glucosamine. It can help in the treatment of osteoporosis, or osteoarthritis. Galactosamine is one of eight essential amino acids that functions in cell-to-cell interaction, research has shown that it may help those with joint inflammations, lacking in galactosamine may even be one of the factors related to heart disease, it may also function as a toxin leading to liver failure. Sources of galactosamine include bovine cattle, oxen, and shark meat as well as red algae. Sialic acid is a very important sugar amine for mental and physical health. Growth, development, and hair as well as skin pigmentation are affected due to sialic acid deficiency in children while improved sialic acid concentrations in infants proved to improve their synaptogenesis and neurological development. However, sialic acid allows different viruses to enter cell, e.g., the Influenza virus. N-acetyl-D-glucosamine is the main component of the

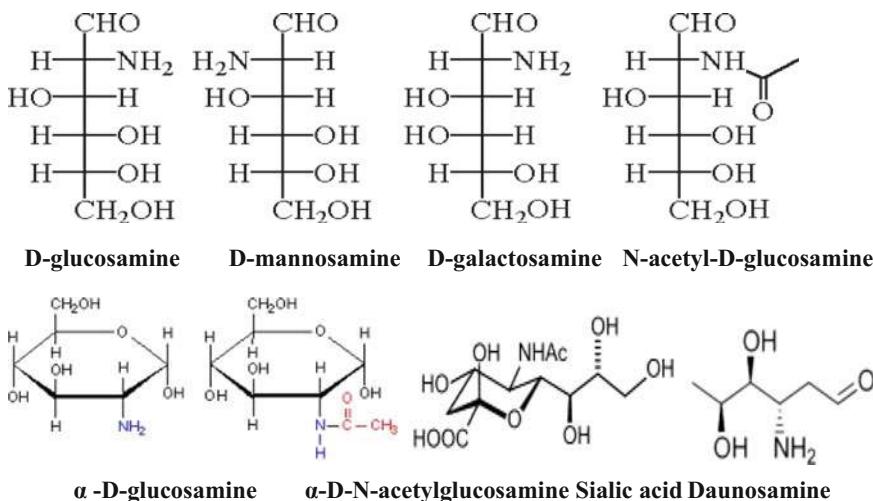
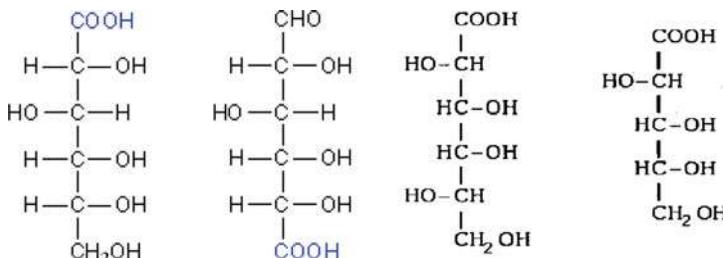


Fig. 2.19 Structure of different sugar amines D-glucosamine, D-mannosamine, D-galactosamine, N-acetyl-D-glucosamine, D-glucosamine, D-N-acetylglucosamine, sialic acid, L-daunosamine

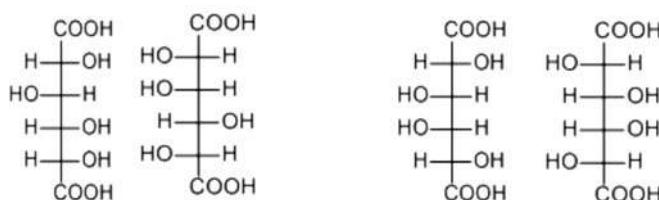
polysaccharide in chitin, the substance that makes up the tough outer skeleton of arthropods and insects. Aminoglycosides are a class of antimicrobial compounds that inhibit bacterial protein synthesis.

Sugar acids are monosaccharides with a carboxyl group (Fig. 2.20). Main classes of sugar acids include: Aldonic acids, in which the aldehyde functional group of an aldose is oxidized (e.g., glyceric acid—3C, xylonic acid—5C, gluconic acid—6C and ascorbic acid—6C, unsaturated lactone); ulosonic acids, in which the first hydroxyl group of a 2-ketose is oxidized creating an α -ketoacid (e.g., neuraminic acid—5-amino-3,5-dideoxy-D-glycero-D-galacto-non-2-ulosonic acid, ketodeoxyoctulosonic acid, KDO or 3-deoxy-D-manno-oct-2-ulosonic acid); uronic acids, in which the terminal hydroxyl group of an aldose or ketose is oxidized (e.g., glucuronic acid—6C, galacturonic acid—6C, iduronic acid—6C) and aldaric acids, in which both ends of an aldose are oxidized (e.g., tartaric acid—4C, meso-galactaric acid, mucic acid—6C, D-glucaric acid-saccharic acid—6C).

(ii) Oligosaccharides [C₆H₁₂O₆]₂₋₁₀: Oligosaccharide molecules consist of 2–10 monosaccharide units and depending upon the number of monosaccharide units formed on hydrolysis they are grouped into: (a) disaccharides—consisted of two molecules of the same or different monosaccharides (e.g., sucrose formed by two molecules of glucose; lactose formed by glucose and galactose; maltose and isomaltose formed by two molecules of glucose; cellobiose formed by two molecules of β -D glucose); trehalose; (b) trisaccharides—consisted of three molecules of the



D-gluconic acid D-glucuronic acid L-galactonic acid D-arabonic acid



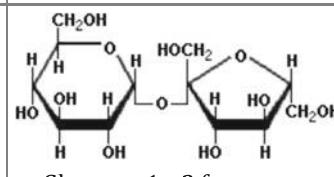
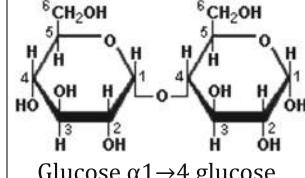
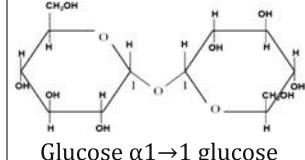
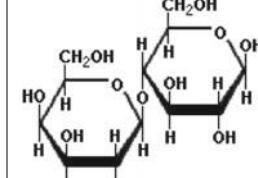
D-glucaric acid L-glucaric acid D-galactaric acid L-galactaric acid

Fig. 2.20 Structure of different sugar acids—D-gluconic acid, D-glucuronic acid, L-galactonic acid, D-arabonic acid, D-glucaric acid, L-glucaric acid, D-galactaric acid and L-galactaric acid

same or different monosaccharides (e.g., raffinose also called melitose formed by galactose, glucose and fructose); (c) tetrasaccharide—consisted of four molecules of the same or different monosaccharides (e.g., stachyose formed by 2 galactose, 1 glucose and 1 fructose molecules) and so on up to decasaccharides (heparin) through penta-, hexa-, hepta-, octa-, and nanosaccharides. Undigestible oligosaccharides are trisaccharide raffinose, the tetrasaccharide stachyose, and the pentasaccharide verbacose.

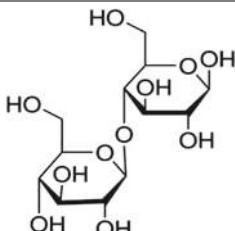
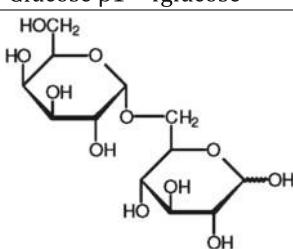
Disaccharides [C₆H₁₂O₆]₂: These molecules consist of two simple sugars, e.g., sucrose, maltose, trehalose, etc. Some of them are shown with structural formulae, structural components, bonds, and sources in Table 2.3.

Table 2.3 Structure of different disaccharides—formulae, structural components, bonds, and sources

Disaccharides		
Name and empirical formulae	Structural formulae, components and glycosidic bond	Sources
Sucrose consisting of glucose and fructose molecules, C ₁₂ H ₂₂ O ₁₁	 Glucose $\alpha 1 \rightarrow 2$ fructose	Common table, cane, beet sugar
Maltose consisting of two α -D-glucose molecules C ₁₂ H ₂₂ O ₁₁	 Glucose $\alpha 1 \rightarrow 4$ glucose	Product of starch hydrolysis
Trehalose consisting of two α -D-glucose molecules C ₁₂ H ₂₂ O ₁₁	 Glucose $\alpha 1 \rightarrow 1$ glucose	Found in fungi
Lactose consisting of galactose and glucose C ₁₂ H ₂₂ O ₁₁	 Galactose $\beta 1 \rightarrow 4$ glucose	Main sugar in milk

(continued)

Table 2.3 (continued)

Disaccharides		
Name and empirical formulae	Structural formulae, components and glycosidic bond	Sources
Cellobiose consisting of two β -D-glucose molecules $C_{12}H_{22}O_{11}$	 Glucose $\beta 1 \rightarrow 4$ glucose	Product of cellulose hydrolysis
Melibiose consisting of α -D-galactose and glucose $C_{12}H_{22}O_{11}$	 Galactose $\alpha 1 \rightarrow 6$ glucose	Found in legumes

Different disaccharides shown in Table 2.3 are described in the following paragraphs.

Sucrose or saccharose is ordinary table sugar refined from sugarcane or sugar beets and consists of glucose and fructose molecules linked by α -1 \rightarrow 2 glycosidic bond. It is found in abundance in the sap of the sugar maple, in sugarcane, in sorghum, and in the root of the sugar beet. It is the main ingredient in turbinado sugar, evaporated or dried cane juice, brown sugar, and confectioner's sugar. It dissolves in half its weight of water and is insoluble in alcohol. It ferments with yeast but does not reduce Fehling's solution.

Lactose or milk sugar has a molecular structure consisting of galactose and glucose linked by β -1 \rightarrow 4 glycosidic bond and requires five times its weight of water. It is not very sweet, and is chiefly used as a nutritive in infant feeding and typhoid fever. In pharmacy, it is employed as a diluent. Lactose intolerance is an intestinal distress caused by a deficiency of intestinal enzyme lactase needed to absorb and digest lactose in milk. Undigested lactose ferments in the colon and causes abdominal pain, bloating, gas, and diarrhea. Yogurt does not cause these problems because lactose is consumed by the bacteria that transform milk into yogurt.

Maltose, maltobiose or malt sugar, is a disaccharide formed from two units of α -D-glucose molecules joined with an α -(1 \rightarrow 4) glycoside bond. Maltose disaccharide is produced when amylase breaks down starch in germinating seeds and

also when glucose is caramelized. Due to O -glycosidic link (free hemiacetal group), maltose can reduce Fehling's reagent. Figure 2.21 shows the hydrolyzing reaction mechanism of amylase leading to producing maltose.

Trehalose (mycose or tremalose) is a natural alpha-linked disaccharide formed by two α -D-glucose molecules connected through $\alpha 1 \rightarrow 1$ glucosidic bond. It is a nonreducing disaccharide, less soluble than sugar, and resistant to acid hydrolysis (except at high temperatures $>80^\circ\text{C}$). Trehalose, widely distributed in nature, can be found in animals (shrimp, grasshoppers, locusts, butterflies, bees, as blood sugar), plants (sunflower seeds, moonwort, *Selaginella*, sea algae), fungi and microorganisms (mushrooms, yeast and it is metabolized by a number of bacteria, including *Streptococcus mutans*). The major dietary source is mushrooms. The trehalose is then broken down into glucose by the catabolic enzyme trehalase for use and so it is more efficient than sugar as energy source. It is implicated in anhydrobiosis and cryptobiosis, i.e., it helps plants and animals to withstand desiccation and freezing, respectively. It forms a gel phase as cells dehydrate that prevents disruption of internal cell organelles. *Selaginella* (the resurrection plant) growing in desert and mountainous areas may revive again after drying out for years following a rain because of the function of trehalose. Trehalose is used as excipient during freeze drying of a variety of products in the pharmaceutical industry and as an ingredient for dried, baked, and processed food, as well as a nontoxic cryoprotectant of vaccines and organs for surgical transplants. It has high water retention capabilities, and is used in food, beverages, and cosmetics. As a

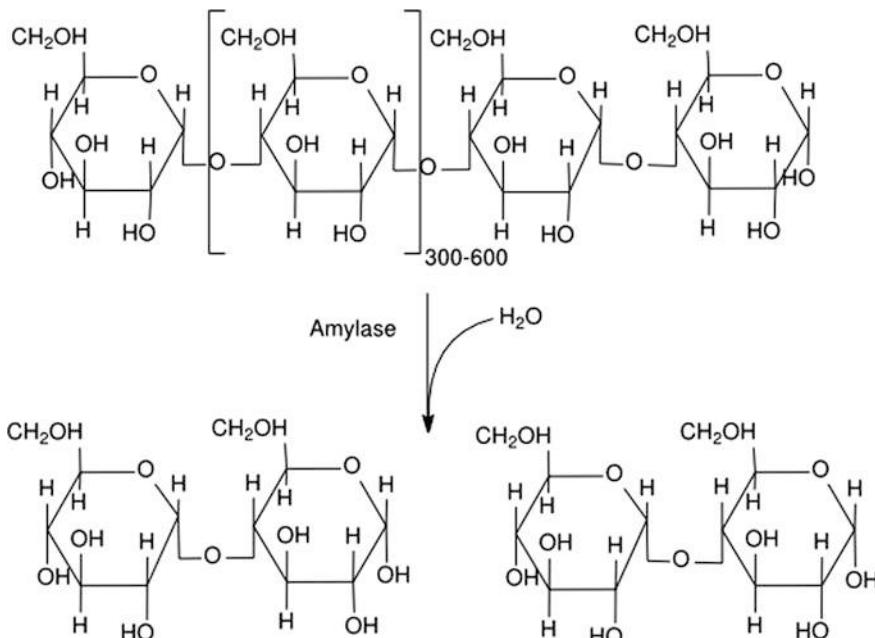


Fig. 2.21 Amylase reaction consisting of hydrolyzing amylose, producing maltose

mulfunctional sugar with nearly half the sweetness of sucrose, high thermostability, and a wide pH-stability range, trehalose strongly improves the taste, texture, and appeal of foods. Trehalose has the added advantage of being an antioxidant.

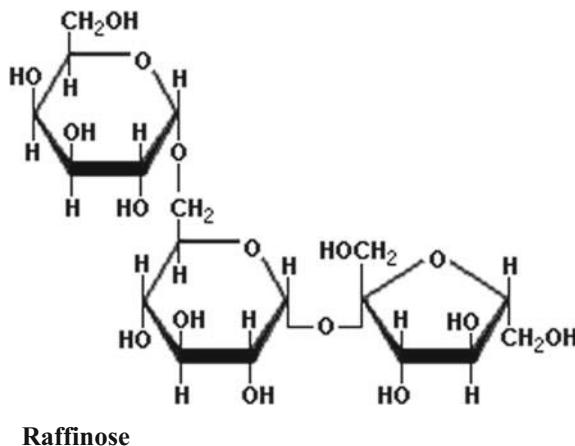
Cellobiose, a repeating unit of cellulose, is a disaccharide consisting of two β -D-glucose molecules that have a β 1 \rightarrow 4 glycosidic bond as in cellulose. The beta glycosidic bond makes D-cellobiose strong, stable and highly crystallizing character. Cellobiose has no taste, whereas maltose and trehalose are about one-third as sweet as sucrose.

Melibiose, hydrolysis of raffinose, is a reducing disaccharide formed by galactose α 1 \rightarrow 6 glucose linkage. It can be formed by invertase-mediated hydrolysis of raffinose, which produces melibiose and fructose. This sugar is produced and metabolized only by enteric and lactic acid bacteria and other microbes. Melibiose, a nondigestible saccharide, enhances the intestinal absorption of quercetin glycosides, an important antioxidant, anti-inflammatory, and anticarcinogenic agent.

Trisaccharides [C₆H₁₂O₆]₃

Raffinose, also called melitose, is a trisaccharide that is widely found in legumes and cruciferous vegetables, including beans, peas, cabbage, brussels sprouts (a cabbage cultivar), broccoli, asparagus, other vegetables, and whole grains. It consists of galactose connected to sucrose by α 1 \rightarrow 6 glycosidic linkage (Fig. 2.22). Humans and other monogastric animals (pigs and poultry) cannot digest saccharides with this linkage as they do not possess the α -galactosidase (α -GAL) enzyme and these oligosaccharides pass undigested through the stomach and upper intestine. In the lower intestine, they are fermented by gas-producing bacteria that do possess the α -GAL enzyme and make carbon dioxide, methane, and/or hydrogen—leading to the flatulence commonly associated with eating beans and other vegetables. Tablets Beano containing the enzyme α -GAL are frequently used as digestive aids to prevent gas and bloating. The enzyme is derived from selected strains of the food grade fungus *Aspergillus niger*.

Fig. 2.22 Structure of trisaccharide—raffinose



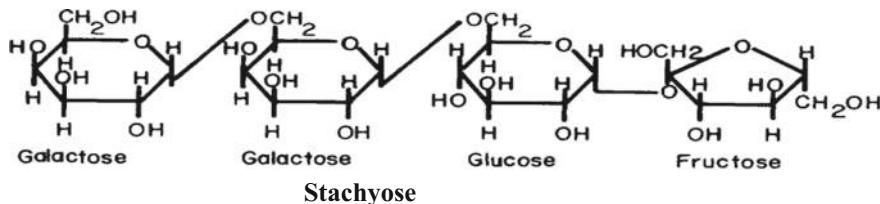


Fig. 2.23 Structure of tetrasaccharide—stachyose

Tetrasaccharides [C₆H₁₂O₆]₄

Stachyose, a tetrasaccharide, on hydrolysis produces four molecules of monosaccharides consisting of two α -D-galactose units, one α -D-glucose unit, and one β -D-fructose unit sequentially linked by 3 glycoside bonds as gal(1 \rightarrow 6)gal(1 \rightarrow 6)glc (1 \rightarrow 2 β) fru (Fig. 2.23). Stachyose is a tetrasaccharide Stachyose together with related oligosaccharides such as raffinose occurs naturally in numerous vegetables, e.g., green beans, soybeans and other beans, artichoke, and also other plants. Stachyose is less sweet than sucrose, at about 28% on a weight basis. It is mainly used as a bulk sweetener. Stachyose is not completely digestible by humans and delivers 1.5–2.4 kcal/g or 6–10 kJ/g.

Stachyose undergoing partial hydrolysis may yield different di- and trisaccharides like galactobiose (galactose + galactose), sucrose (glucose + fructose), melibiose (galactose + glucose) manninotriose (2galactoses + glucose) and raffinose (galactoses + glucose + fructose).

Examples of other tetrasaccharides include Lychnose (1- α -Galactosyl-raffinose = O- α -D-Galp-(1 \rightarrow 6)-O- α -D-Glcp-(1 \rightarrow 2)-O- β -D-Fruf-(1 \rightarrow 1)-O- α -D-Galp), Maltotetraose (O- α -D-Glcp-(1 \rightarrow 4)-O- α -D-Glcp-(1 \rightarrow 4)-O- α -D-Glcp-(1 \rightarrow 4)-D-Glcp), Nigerotetraose (O- α -D-Glcp-(1 \rightarrow 3)-O- α -D-Glcp-(1 \rightarrow 3)-O- α -D-Glcp-(1 \rightarrow 3)-D-Glcp), Nystose (β -D-Fructosyl-1-kestose, O- α -D-Glcp-(1 \rightarrow 2)- β -D-Fruf-(1 \rightarrow 2)- β -D-Fruf-(1 \rightarrow 2)- β -D-Fruf), Sesamoose (O- α -D-Galp-(1 \rightarrow 6)-O- α -D-Galp-(1 \rightarrow 6)-O- β -D-Fruf-(2 \rightarrow 1)-O- α -D-Glcp), etc.

Pentasaccharides [C₆H₁₂O₆]₅

Verbascose (O- α -D-galactopyranosyl-(1 \rightarrow 6)-[O- α -D-galactopyranosyl-(1 \rightarrow 6)-2-O- α -D-glucopyranosyl-(1 \rightarrow 2)- β -D-fructofuranoside], a raffinose family oligosaccharides (RFO), is a non-digestible pentasaccharide, galactose-galactose-galactose-glucose-fructose, isolated from roots of mullein *Verbascum Thapsus* of Scrophulariaceae found in legumes; fermented by intestinal bacteria and causes flatulence (Fig. 2.24).

(iii) Polysaccharides [C₆H₁₀O₅]_{11-n}: Polysaccharides are polymers of monosaccharide units, linked to each other by glycosidic linkages. Many polysaccharides, unlike sugars, are tasteless and insoluble in water. Dietary fiber includes polysaccharides and oligosaccharides that are resistant to digestion and absorption in the

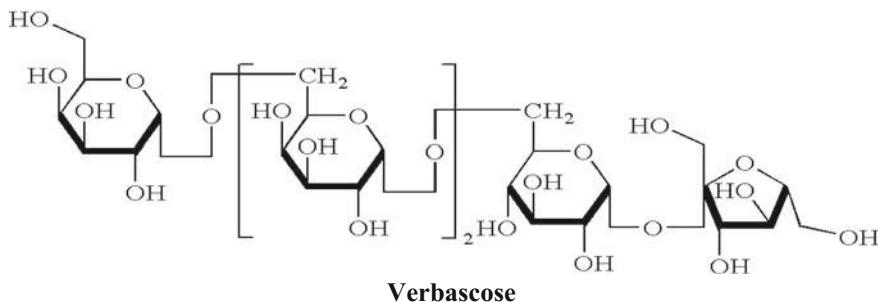


Fig. 2.24 Structure of pentasaccharide verbascose—verbascose

human small intestine but which are completely or partially fermented by microorganisms in the large intestine. Polysaccharides are of two types, e.g., reserve (starch, glycogen, etc.) and structural (cellulose). Reserve polysaccharides provide energy and building material while structural polysaccharides supply dietary fiber. The polysaccharides described below play important roles in nutrition, food preparation, and biology. They are further divided into:

- (a) Homopolysaccharides: polysaccharides consist of only one type of monosaccharide (e.g., pentosans, polymer of pentose and includes arabans, xylans, etc., and glucosans, polymer of glucose: starch, straight chain amylose and branched chain-amylopectin starch are formed by α -D glucose and connected respectively by α -1-4, α -1-4 and α -1-6 linkages, dextrin-intermediate product of starch digestion formed by α -D glucose, cellulose—formed by β -D glucose, glycogen—formed by α -D glucose, highly branched, linked by α -1,4 and α -1,6 glycosidic bonds, chitin—constructed from units of *N*-acetylglucosamine linked together in β -1,4 fashion like cellulose; fructosan: levan, inulin, polymer of fructose known as fructosans, polymer of fructose—storage carbohydrate for energy source in many plant species and also important as dietary fibers commercially extracted from chicory root; galactosan, polymer of galactose: agar—structural carbohydrate of the cell wall of agarophytes algae, consisting of chains of repeating alternate units of β -1,3-linked-D-galactose and α -1,4-linked 3,6-anhydro-L-galactose (a linear polymer of galactose);
- (b) Heteropolysaccharides (heteroglycans): polysaccharides consist of two or more types of monosaccharides or their derivatives and are closely associated with lipid or protein. The major heteropolysaccharides include pectins, a structural heteropolysaccharide in the primary cell walls of terrestrial plants rich in galacturonic acid; hemicelluloses are polysaccharides in plant cell walls that have beta-(1 \rightarrow 4)-linked backbones with an equatorial configuration and include xyloglucans, xylans, mannans, and glucomannans, and beta-(1 \rightarrow 3,1 \rightarrow 4)-glucans, lignin, a phenylpropanoid-derived heteropolymer important for the strength and rigidity of the plant secondary cell wall a complex polymer of aromatic alcohols, the connective tissue polysaccharides, blood group

substances, glycoproteins (gamma globulin), and glycolipids (found in the central nervous system of animals and in a wide variety of plant gums), mucopolysaccharides (glycosaminoglycans GAG)—made of sugar amino sugars and uronic acids, hyaluronic acid formed by thousands of *N*-acetyl glucosamine and glucuronic acid), chondroitin, and sulfated heteropolysaccharides included chondroitin sulfate, dermatan sulfate, keratan sulfate, heparin, etc.

Starch Starch is the major form of reserve polysaccharide carbohydrate in plants and composed of α -D-glucose units. Starch is divided into two groups: amylose, a straight chain or linear polysaccharide linked by α -1 \rightarrow 4 glycosidic bond, and amylopectin, a highly branched chain polysaccharide linked by α -1 \rightarrow 4 and α -1 \rightarrow 6 glycosidic bonds. Natural starches contain 10–20% amylose and 80–90% amylopectin. Amylose forms a colloidal dispersion in hot water whereas amylopectin is completely insoluble. Starch is abundantly present in the roots, rhizomes, and seeds of many plants. Corn starch or amylose is employed as a dusting powder for the skin, or for pills to prevent their sticking together, or in the form of starch water as a soothing injection in irritative conditions of the lower bowel. Cornstarch and arrowroot starch (obtained from the rhizomes or rootstock of *Maranta arundinacea* of Marantaceae) are used as foods.

Amylose molecules consist typically of 200–20,000 α -D-glucose units which form a helix as a result of the bond angles between the glucose units (Fig. 2.25).

Amylopectin is also made up of α -D-glucose but differs from amylose in being highly branched. Short side chains of about 30 glucose units are attached with α 1 \rightarrow 6 linkages approximately every twenty to thirty glucose units along the chain (Fig. 2.26). The side branching chains are clustered together within the amylopectin molecule. Amylopectin molecules may contain up to two million glucose units.

Starches are transformed into many commercial products by hydrolysis using acids or enzymes as catalysts. In hydrolysis, water is added to glycosidic bonds to break the long polysaccharide chains into smaller chains of simple carbohydrates. The resulting products are assigned a dextrose equivalent (DE) value which is related to the degree of hydrolysis. A DE value of 100 corresponds to completely hydrolyzed starch, which is pure glucose (dextrose). Derived from dextrose (glucose), dextrans are a group of low-molecular-weight carbohydrates produced by the hydrolysis of starch. Dextrans are polymers of α -D-glucose units linked by α -1 \rightarrow 4

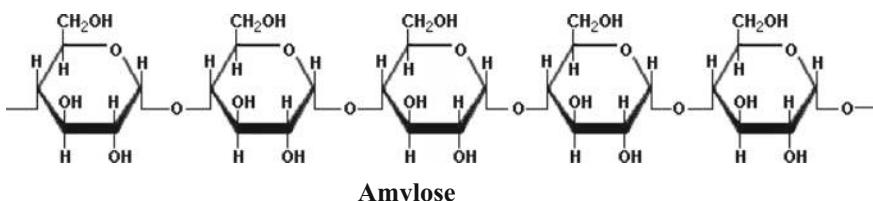
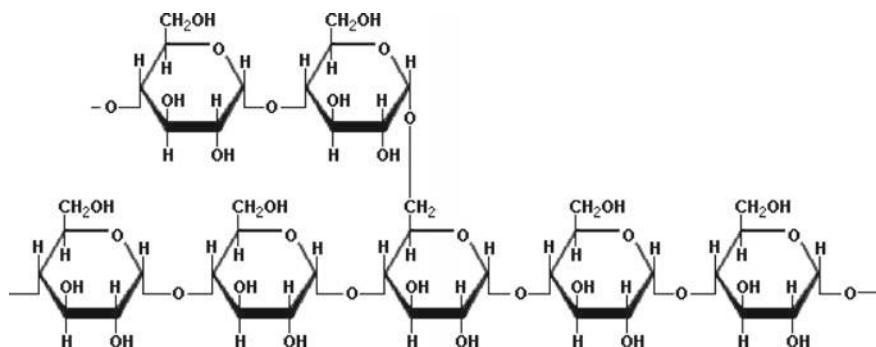


Fig. 2.25 Structure of polysaccharide—amylose

**Amylopectin****Fig. 2.26** Structure of polysaccharide—amylopectin

glycosidic bonds. Dextrans are usually made from corn, potato, arrowroot, rice, wheat, or barley. There are several types of dextrin, e.g., white, yellow, or brown powders, partially or fully soluble in water yielding optically active solutions of low viscosity. Dextrin is used in many glue products due to its adhesive qualities and safety. The indigestible form of dextrin is often used as a fiber supplement. Dextrin fiber offers a lot of health benefits including weight loss, toxin cleansing, etc. Dextrin promotes healthy intestinal flora (probiotics), supports heart health, colon health, healthy cholesterol levels, and relieves constipation.

Maltodextrin is partially hydrolyzed starch that is not sweet and has a DE value less than 20. Syrups, such as corn syrup made from corn starch, have DE values from 20 to 91. Commercial dextrose has DE values from 92 to 99. Corn syrup solids, which may be labeled as soluble corn fiber or resistant maltodextrin, are mildly sweet semicrystalline or powdery amorphous products with DEs from 20 to 36 made by drying corn syrup in a vacuum or in spray driers. Resistant maltodextrin or soluble corn fiber are not broken down in the digestive system, but they are partially fermented by colonic bacteria thus providing only 2 calories per gram instead of the 4 calories per gram in corn syrup. High fructose corn syrup (HFCS), commonly used to sweeten soft drinks, is made by treating corn syrup with enzymes to convert a portion of the glucose into fructose. Commercial HFCS contains from 42 to 55% fructose, with the remaining percentage being mainly glucose. There is an effort underway to rename HFCS as corn sugar because of the negative public perception that HFCS contributes to obesity. Modified starch is starch that has been changed by mechanical processes or chemical treatments to stabilize starch gels made with hot water. Without modification, gelled starch–water mixtures lose viscosity or become rubbery after a few hours. Hydrogenated glucose syrup (HGS) is produced by hydrolyzing starch, and then hydrogenating the resulting syrup to produce sugar alcohols like maltitol and sorbitol, along with hydrogenated oligo- and polysaccharides. Polydextrose (poly-D-glucose) is a synthetic, highly branched polymer with many types of glycosidic linkages created by heating dextrose with an acid catalyst.

and purifying the resulting water-soluble polymer. Polydextrose is used as a bulking agent because it is tasteless and is similar to fiber in terms of its resistance to digestion. The name resistant starch is applied to dietary starch that is not degraded in the stomach and small intestine, but is fermented by microflora in the large intestine. The relative sweetness of various starch hydrolysates and other carbohydrates in relation to sucrose (=100%) may be graded as follows: Fructose (173), invert sugar—a mixture of glucose and fructose found in fruits (120), HFCS (42% fructose) (120), sucrose (100), xylitol (100), tagatose (92), glucose (74), high-DE corn syrup (70), sorbitol (55), mannitol (50), trehalose (45), regular corn syrup (40), galactose (32), maltose (32), and lactose (15).

Glycogen

Glycogen, a multibranched polysaccharide of α -D-glucose units (up to 120,000 glucose residues) linked by $\alpha 1 \rightarrow 4$ and also $\alpha 1 \rightarrow 6$ glycosidic bonds, is a storage polysaccharide of animals, fungi, and cyanobacteria (Fig. 2.27). Glycogen is identical to amylopectin, but the branches in glycogen tend to be shorter (~ 13 glucose units), extensively branched and compact than starch. The glucose chains are organized globularly like branches of a tree originating from a pair of molecules of glycogenin, a protein that acts as a primer at the core of the structure. In humans, glycogen is stored primarily in the hepatocytes of the liver ($\sim 8\%$ of its fresh weight) and the skeletal muscle cells (1–2% of its fresh weight) and functions as the secondary long-term energy storage, the primary energy stores being fats held in adipose tissue. The amount of glycogen stored in the body, especially within the muscles, liver, and red blood cells mostly depend on physical training and basal metabolic rate. Small amounts of glycogen are found in the kidneys, and even smaller amounts in certain glial cells in the brain and white blood cells. The uterus also stores glycogen during pregnancy to nourish the embryo. Muscle glycogen is converted into glucose by muscle cells, and only liver glycogen converts to glucose

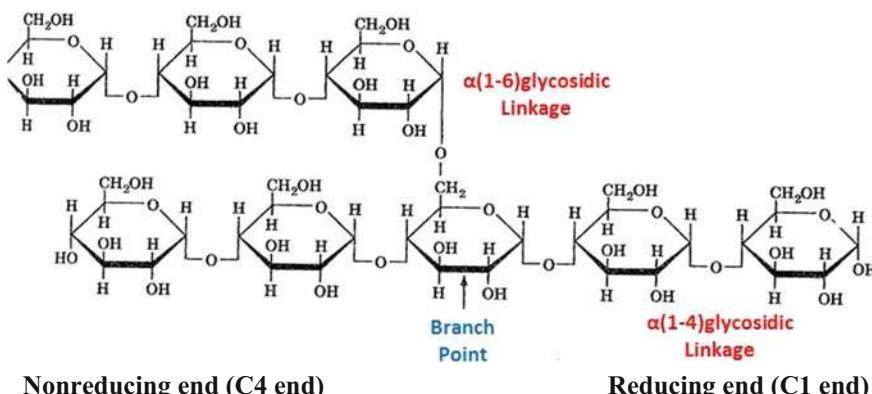


Fig. 2.27 Structure of polysaccharide—glycogen structure showing $\alpha 1 \rightarrow 4$ and also $\alpha 1 \rightarrow 6$ glycosidic linkages

which can be made accessible to other organs including the central nervous system. When glucose cannot be stored as glycogen or used immediately for energy, it is converted to fat.

Dextran

Dextran is a complex branched polysaccharide, similar to amylopectin, consisting of a linear backbone (main chain) of α -1 \rightarrow 6 glycosidic bond linked D-glucopyranosyl repeating units and the dextran may have branches of smaller chains of D-glucose linked to the backbone by α -1 \rightarrow 2, α -1 \rightarrow 3 or α -1 \rightarrow 4 glycosidic bonds. It differs from dextrin, starch (amylose and amylopectin), etc., in main chain formation and chain branching. Dextrin is a hydrolysate polymer product of starch (amylose), composed of α -D-glucose units, linked by α -1 \rightarrow 4 glycosidic bonds and chain length is less than starch (Fig. 2.28). Dextran was first discovered by Louis Pasteur as a microbial product in wine. Historically, dextrans had been long recognized as contaminants in sugar processing and other food production. Dextran is synthesized from sucrose by certain lactic acid bacteria, the best-known being *Leuconostoc mesenteroides* and *S. mutans*.

Dextran is an oral bacterial product that adheres to the teeth, creating a film called dental plaque (plaque rich in dextrans). Dextran is also formed by the lactic acid bacterium *Lactobacillus brevis* to create the crystals of tibicos, a water kefir fermented beverage which supposedly has some health benefits. It is also used commercially in confections, in lacquers, as food additives, and as plasma volume expanders. It is used medicinally as an antithrombotic (antiplatelet) to reduce blood viscosity, and as a volume expander in hypovolaemia. It is used in some eye drops as a lubricant and in certain intravenous fluids to solubilize other factors, e.g., iron

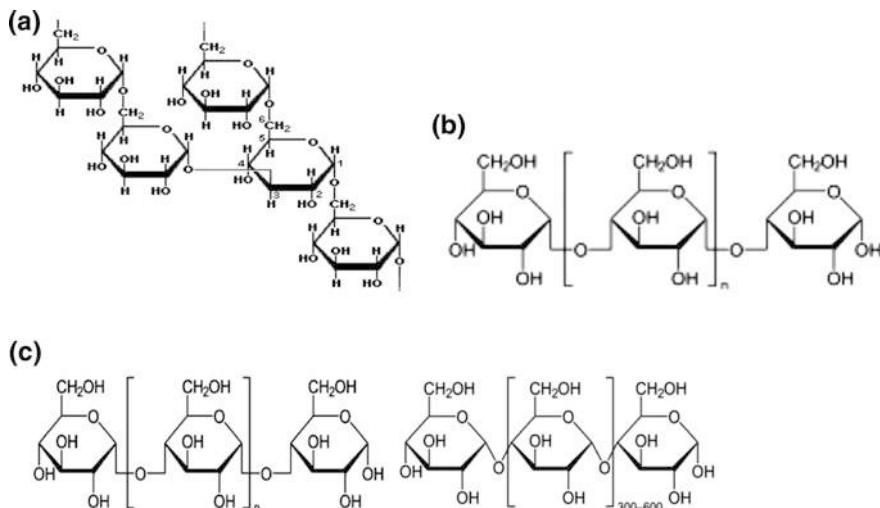


Fig. 2.28 Structure of polysaccharide—**a** Dextran (~ 20 glucose units). **b** Dextrin ($n < 300\text{--}600$ glucose units). **c** Starch (300–600 glucose units)

(= iron dextran such as Cosmofer), or as a derivative in Monofer, intravenous solutions with dextran function both as volume expanders. Dextran is used in the osmotic stress technique for applying osmotic pressure to biological molecules. It is also used in some chromatography matrices (e.g., Sephadex). Dextran is used to make microcarriers for industrial cell culture. Although there are relatively few side effects, the side effects associated with dextran use can be very serious and these include anaphylaxis, volume overload, pulmonary edema, cerebral edema, or platelet dysfunction. An uncommon but significant complication of dextran osmotic effect is acute renal failure.

Inulin

Inulins are a group of naturally occurring storage polysaccharides present in many vegetable and fruit plants including wheat, onion, banana, garlic, asparagus, leeks, chicory, and Jerusalem artichokes. Most often, inulin is extracted from chicory. Inulins are polymers of fructose units, also called fructans, and typically have a terminal glucose (Fig. 2.29). The fructose units in inulins are joined by a β -2 \rightarrow 1 glycosidic bond. In general, plant inulins contain between 20 and several thousand fructose units. Smaller compounds, consisting of 10 or fewer fructose units, are called fructooligosaccharides, and among them, the simplest being 1-kestose, which has 2 fructose units and 1 glucose unit (a trisaccharide found in vegetables consisting of β -D-fructofuranose having β -D-fructofuranosyl and α -D-glucopyranosyl residues attached at the 1- and 2-positions respectively). Oligofructose has the same structure as inulin, but the chains consist of 10 or fewer fructose units. Inulins with a terminal glucose are known as α -D-glucopyranosyl-[β -D-fructofuranosyl](n-1)-D-

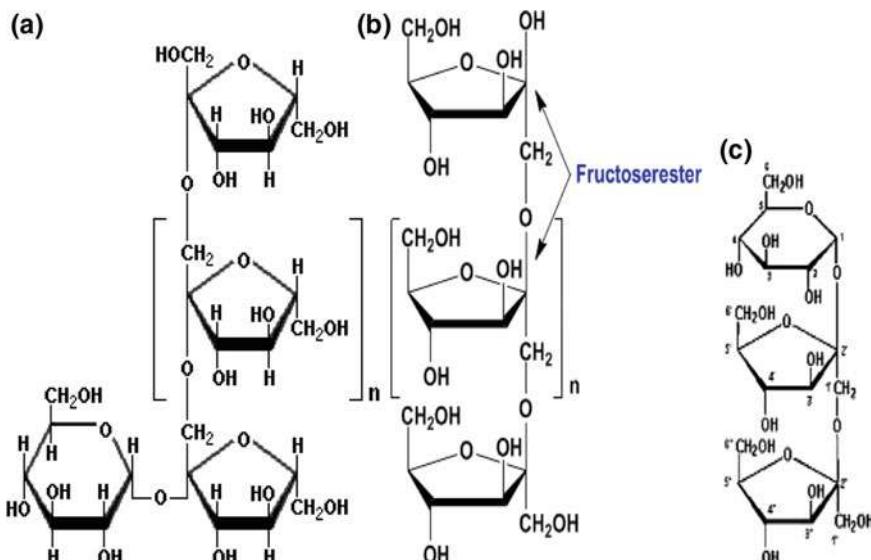


Fig. 2.29 Structure of **a** Inulin ($n = 11-\infty$). **b** Oligofructose ($n = 4-10$). **c** 1-Kestose (2 fructose + 1 glucose)

fructofuranosides, GpyFn. Inulins without glucose are β -D-fructopyranosyl-[D-fructofuranosyl](n-1)-D-fructofuranosides, FpyFn. Hydrolysis of inulins may yield fructooligosaccharides, which are oligomers with a degree of polymerization (DP) of ≤ 10 .

Because of the β -2 \rightarrow 1 linkages, inulins and oligofructose are nondigestible by human intestinal enzymes, and form a class of dietary fibers fructans, contributing to its functional properties, e.g., reduced calorie value, dietary fiber and prebiotic effects. They are nondigestible by human intestinal enzymes, but they are totally fermented by colonic microflora. The short chain fatty acids (SCFA) and lactate produced by fermentation contribute 1.5 kcal per gram of inulin or oligofructose. Without color and odor, inulin has little impact on sensory characteristics of food products. Oligofructose has approximately 30–50% of the sweetness of table sugar. Inulin is less soluble than oligofructose. When thoroughly mixed with liquid, inulin forms a gel and a white creamy texture similar to fat that provides a fat-like mouth feel. It can also improve the stability of foams and emulsions. Inulin and oligofructose are used to replace sugar, fat, and flour and reduce the calories of foods like ice cream, dairy products, confections, and baked goods. In addition to being a versatile ingredient, inulin has many health benefits. Inulin increases calcium absorption and possibly magnesium absorption, while promoting the growth of beneficial intestinal bacteria. Chicory inulin is reported to increase absorption of calcium in girls with lower calcium absorption and in young men. Inulin and its analog sinistrin are used to help measure kidney function by determining the glomerular filtration rate (GFR). Inulin is postulated to benefit the immune system through the direct interaction between the inulin and its metabolites with the gut-associated lymphoid tissues and especially Peyer's patches, though this link has not been established in humans. Inulin is reported to decrease amount of cholesterol and triglycerides, and hence benefits lipidemia and cardiovascular system. It is also used for rehydration and remineralization following important loss of water, like diarrhea and diaphoresis. Due to the body's limited ability to process fructans, inulin has minimal increasing impact on blood sugar. It is considered suitable for diabetics and potentially helpful in managing blood sugar-related illnesses. The consumption of large quantities, however, can lead to gas and bloating due to overgrowth of intestinal methanogenic bacteria.

Most plants that synthesize and store inulin do not store other forms of carbohydrate such as starch. Some plants store carbohydrates in the form of inulin as an alternative, or in addition, to starch. Inulin is used by some plants as a means of storing energy and is typically found in roots or rhizomes. For these plants, inulin is used for reserving energy as well as regulating cold resistance. It is osmotically active for it is soluble in water. The plants can change the osmotic potential of cells by changing the DP of inulin molecules with hydrolysis process. Being able to change osmotic potential without changing the total amount of carbohydrate, plants can withstand cold and drought during winter periods. Nonhydrolyzed inulin can also be directly converted to ethanol in a simultaneous saccharification and fermentation process, which may have great potential for converting crops high in inulin into ethanol for fuel.

Cellulose

Cellulose is a polysaccharide consisting of a linear chain of several hundred to many thousands β -D-glucose units linked by β -1 \rightarrow 4 glycoside bonds (Fig. 2.30). The absence of side chains allows cellulose molecules to lie close together and form rigid structures. Cellulose is an important structural component of the primary cell wall of green plants, many forms of algae and the oomycetes fungi. Some species of bacteria secrete it to form biofilms. The cellulose content of cotton fiber is 90%, that of wood is 40–50% and that of dried hemp is approximately 45%. Cellulose is the most abundant organic polymer on Earth.

Humans cannot digest cellulose due to the lack of the enzyme cellulase and it mainly acts as a dietary fiber. Cellulose provides a lot of volume or bulk in food but because it is indigestible to humans, it has no caloric value and for this reason, cellulose has become a popular bulking agent in diet foods. Consumers who eat foods with high cellulose content feel full physically and psychologically without having consumed many calories. With rising awareness about fiber intake, cellulose has become one of the most popular food additives. Adding cellulose to food allows an increase in bulk and fiber content without a major impact on flavor. Because cellulose binds and mixes easily with water, it is often added to increase the fiber content of drinks and other liquid items when the gritty texture of regular fiber supplements would be undesirable. The gelling action of cellulose when combined with water provides both thickening and stabilizing qualities in the food to which it is added. Cellulose gel acts similarly to an emulsion, suspending ingredients within a solution and preventing water from separating out. Cellulose is often added to sauces for both the thickening and emulsifying action. Cellulose gum or cellulose gel, which are hydrated forms of cellulose, are often used in sauces or other wet items like ice cream and frozen yogurt.

Animals like ruminants and termites can digest cellulose with the help of symbiotic microorganisms that live in their guts, e.g., anaerobic bacteria, protozoa, fungi, etc. in ruminants and Trichonympha (a genus of parabasalian protists) in termite species. Cellulose is mainly used to produce paperboard and paper. Smaller quantities are converted into a wide variety of derivative products such as cellophane and rayon. Conversion of cellulose from energy crops into biofuels such as cellulosic ethanol is under active investigation. Cellulose for industrial use is mainly obtained from wood pulp and cotton. Cellulose may be modified in the laboratory by treating it with nitric acid (HNO_3) to replace all the hydroxyl groups with nitrate groups ($-\text{ONO}_2$) to produce cellulose nitrate (nitrocellulose or

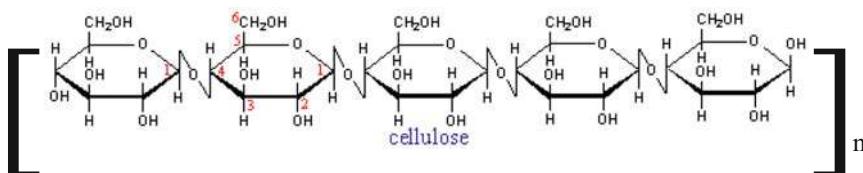


Fig. 2.30 Structure of cellulose showing β -1 \rightarrow 4 glycoside bond

guncotton) which is an explosive component of smokeless powder. Partially nitrated cellulose, known as pyroxylin, is used in the manufacture of collodion, plastics, lacquers, and nail polish.

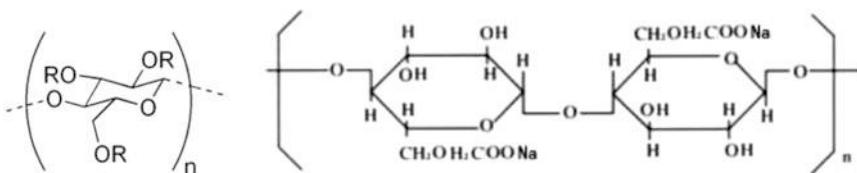
Carboxymethyl cellulose (CMC)

Carboxymethyl cellulose (CMC) or cellulose gum is a chemical derivative of cellulose where some of the hydroxyl groups ($-OH$) are substituted with carboxymethyl groups ($-CH_2-COOH$) (Fig. 2.31). The properties of cellulose gum depend on the degree of substitution and the length of the cellulose chains. Cellulose gum is nontoxic and becomes very viscous when combined with water. Cellulose gum is a versatile, cost-effective and easy-to-use thickening agent that has numerous industrial applications. It is found in a range of products, including tobacco, paper, and yogurt. Cellulose gum stabilizes proteins, adds texture and mouth feel, forms oil-resistant film, and retains moisture in industrial and processed food products. It is used as a thickener for foods and as an emulsion stabilizer in products like ice cream. Cellulose gum is also a constituent of many nonfood products, such as personal lubricants, toothpaste, laxatives, diet pills, water-based paints, detergents, textile sizing, and various paper coatings.

Hemicellulose

Hemicelluloses (also known as polyoses) are polysaccharides (heteropolymer matrix polysaccharide) in plant cell walls that have β -1 \rightarrow 4-linked backbones with an equatorial configuration (Fig. 2.32). Hemicelluloses include heteromannans, xyloglucans, heteroxylans, xylans, mannans, glucomannans, and β -1 \rightarrow 3, 1 \rightarrow 4-glucans. These types of hemicelluloses are present in the cell walls of all terrestrial plants, except for β -1 \rightarrow 3 and 1 \rightarrow 4-glucans, which are restricted to Poales and a few other groups. The chemical structure of hemicelluloses consists of long chains of a variety of pentoses, hexoses, and their corresponding uronic acids.

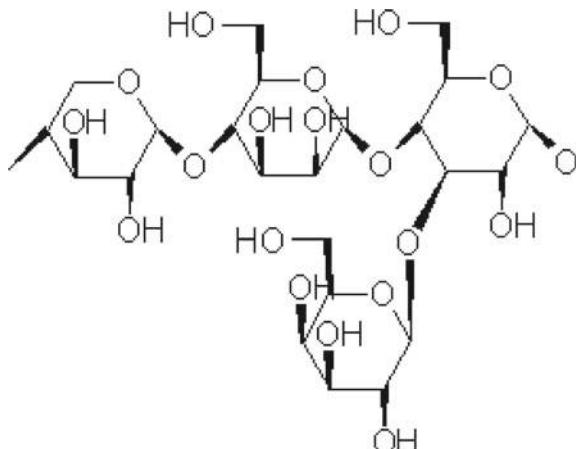
Hemicelluloses constitute roughly one-third of the wall biomass. Hemicelluloses consist of shorter chains of 500–3000 sugar units while cellulose consists of longer chains of 7000–15,000 glucose units; hemicellulose is a branched polymer but cellulose is unbranched; cellulose is crystalline, strong, and resistant to hydrolysis; hemicellulose has a random, amorphous structure with little strength, easily hydrolyzed by dilute acid or base as well as myriad hemicellulase enzymes. In contrast to cellulose, which contains only anhydrous glucose, hemicellulose



R = H or CH_2CO_2H

Fig. 2.31 Structure of carboxymethyl cellulose (CMC)

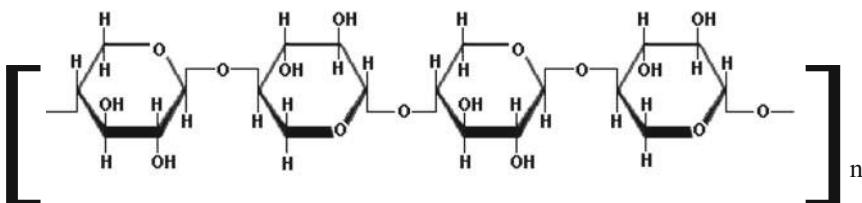
Fig. 2.32 Most common molecular motif of hemicellulose



Hemicellulose: -Xylose- β -1 \rightarrow 4-Mannose- β -1 \rightarrow 4-Glucose- α -1 \rightarrow 3-Galactose

contains many different sugar monomers, e.g., xylose, mannose, galactose, rhamnose, arabinose and glucose as well. Hemicelluloses contain most of the D-pentose sugars, and occasionally small amounts of L-sugars as well. The most common hemicelluloses contain xylans, uronic acid, and arabinose (Fig. 2.33). Xylose is the predominant sugar monomer in most cases, but in softwoods, mannose can be the most abundant monomer unit. Hemicellulose also contains the acidified form of sugars like glucuronic acid and galacturonic. The polysaccharides yielding pentoses on hydrolysis are called pentosans and xylan is an example of a pentosan consisting of D-xylose units with β -1 \rightarrow 4 linkages.

Hemicelluloses comprise almost one-third of the carbohydrates in woody plant tissue. The fine structure of these polysaccharides varies depending on the plant species and tissue type (Fig. 2.34). Hemicellulose found in hardwood trees is predominantly xylan with some glucomannan, while in softwoods, it is mainly rich in galactoglucomannan and contains only a small amount of xylan. Hemicelluloses



Xylan

Fig. 2.33 Structure of xylan

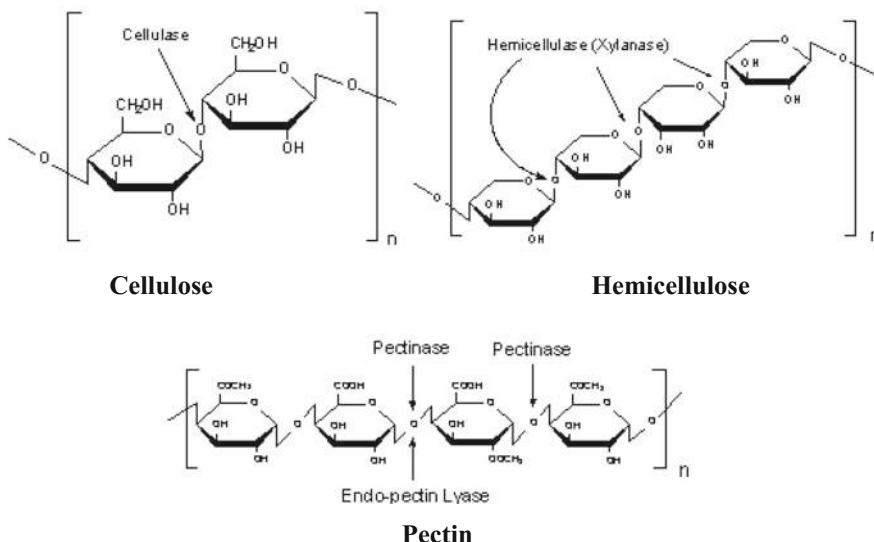


Fig. 2.34 Structure of cellulose, hemicellulose and pectin

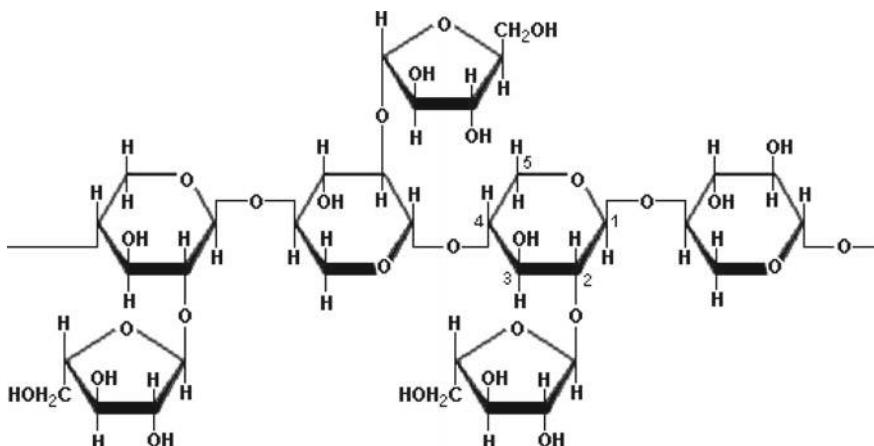
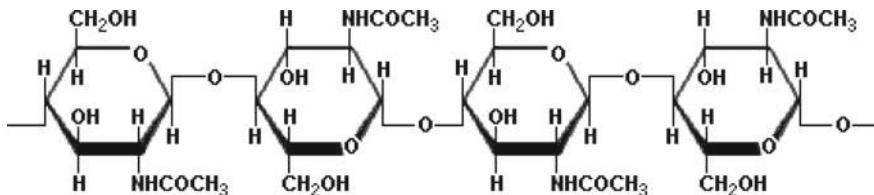
may be found in fruit, plant stems, and grain hulls. The most important biological role of hemicelluloses is their contribution to strengthening the cell wall by interaction with cellulose and, in some walls, with lignin. The hemicelluloses are used in numerous industrial applications such as food additives as well as in medicinal applications. Although hemicelluloses are not digestible, they can be fermented by yeasts and bacteria.

Arabinoxylan

Arabinoxylans are polysaccharides found in the bran of grasses and grains such as wheat, rye, and barley. Arabinoxylans consist of a xylan backbone with L-arabinofuranose (L-arabinose in its 5-atom ring form) attached randomly by α -1→2 and/or α -1→3 linkages to the xylose units throughout the chain (Fig. 2.35). Since xylose and arabinose are both pentoses, arabinoxylans are usually classified as pentosans. Arabinoxylans are important in the baking industry. The arabinose units bind water and produce viscous compounds that affect the consistency of dough, the retention of gas bubbles from fermentation in gluten-starch films, and the final texture of baked products.

Chitin

Chitin is an unbranched polymer of *N*-Acetyl-D-glucosamine (Fig. 2.36). It is found in fungi and is the principal component of arthropod and lower animal exoskeletons, e.g., insect, crab, and shrimp shells. It may be regarded as a derivative of cellulose, in which the hydroxyl groups of the second carbon of each glucose unit have been replaced with acetamido [$-\text{NH}(\text{C=O})\text{CH}_3$] groups.

**Arabinoxylan****Fig. 2.35** Structure of Arabinoxylan**Chitin****Fig. 2.36** Structure of Chitin

β -Glucan

Beta-glucans consist of linear unbranched polysaccharides of β -D-glucose like cellulose, but with one β -1 \rightarrow 3 linkage for every three or four β -1 \rightarrow 4 linkages (Fig. 2.37). β -glucans form long cylindrical molecules containing up to about 250,000 glucose units. β -glucans occur in the bran of grains such as barley and oats, and they are recognized as being beneficial for reducing heart disease by lowering cholesterol and reducing the glycemic response. They are used commercially to modify food texture and as fat substitutes.

Glycosaminoglycans

Glycosaminoglycans (GAGs) or mucopolysaccharides are long unbranched chain of negatively charged polysaccharides containing repeating disaccharide units that contain (except for keratan) either of two amino sugar compounds (*N*-acetylglucosamine or *N*-acetylgalactosamine) and a uronic sugar (glucuronic acid or iduronic acid) (Fig. 2.38). The physiologically most important GAGs are

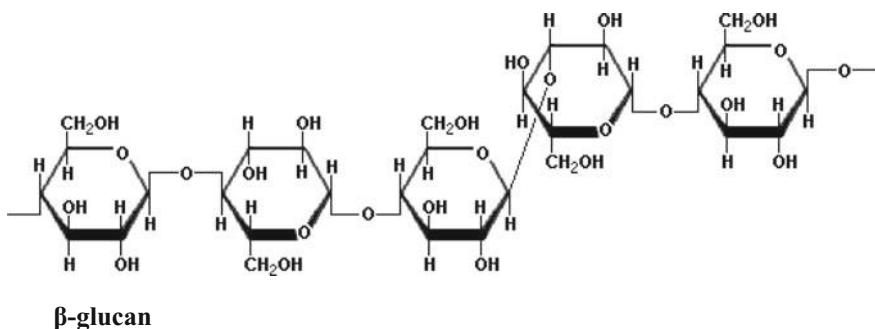


Fig. 2.37 Structure of β -glucan

hyaluronic acid, dermatan sulfate, chondroitin sulfate, heparin, heparan sulfate, and keratan sulfate. Chondroitin sulfate is composed of β -D-glucuronate linked to the third carbon of N-acetylgalactosamine-4-sulfate as illustrated here. Heparin is a complex mixture of linear polysaccharides that have anticoagulant properties and vary in the degree of sulfation of the saccharide units.

GAGs are highly polar, highly viscous molecules, and attract water. They are therefore useful to the body as a lubricant or as a shock absorber. GAGs are found in the lubricating fluid of the joints and as components of cartilage, connective tissues, synovial fluid, vitreous humor, bone, heart valves, skin, and cell membranes. These substances have anticoagulant, anti-lipemic and antithrombogenic properties in addition to facilitating wound healing. They help with the transport of oxygen around the body and play a vital role in all cell growth. Mucopolysaccharides often appear as a thickening agent in shampoos and conditioners because they improve circulation of nutrients to the hair and accelerate the removal of naturally produced waste products. Mucopolysaccharides are marketed as nutritional supplement for conditions that affect human joints and connective tissue such as arthritis and osteoporosis. Mucopolysaccharides are also a natural humectant so hold moisture in the hair thus improving condition. GAGs are available different animal and plant sources including beef cartilage (beef trachea), bone marrow, neck meat, and broths that contain boiled bone and connective part, green-lipped mussels, all crustaceans (lobster, shrimp, crab, shell, etc.), shell, and seaweed. *Aloe vera* is also a plant source for GAGs,

Agar and carrageenan

Agar (agar agar) is a linear polymer chain (galactosan) of agarobiose (Figs. 2.39 and 2.40), a disaccharide consisting of β -(1 \rightarrow 3)-D-galactose and α -(1 \rightarrow 6)-L-galactose. Most of the α -(1 \rightarrow 4) residues are modified by the presence of a 3 \rightarrow 6 anhydro bridge.

Agar is extracted from the cell wall of marine algae agarophytes (*Gelidium* and *Gracilaria*) that belong to the Rhodophyta. Agar is approximately 80% fiber, so it serves as an exceptional intestinal regulator as a laxative, an appetite suppressant, vegetarian gelatin substitute, as a thickener for soups, jellies and ice cream, as a

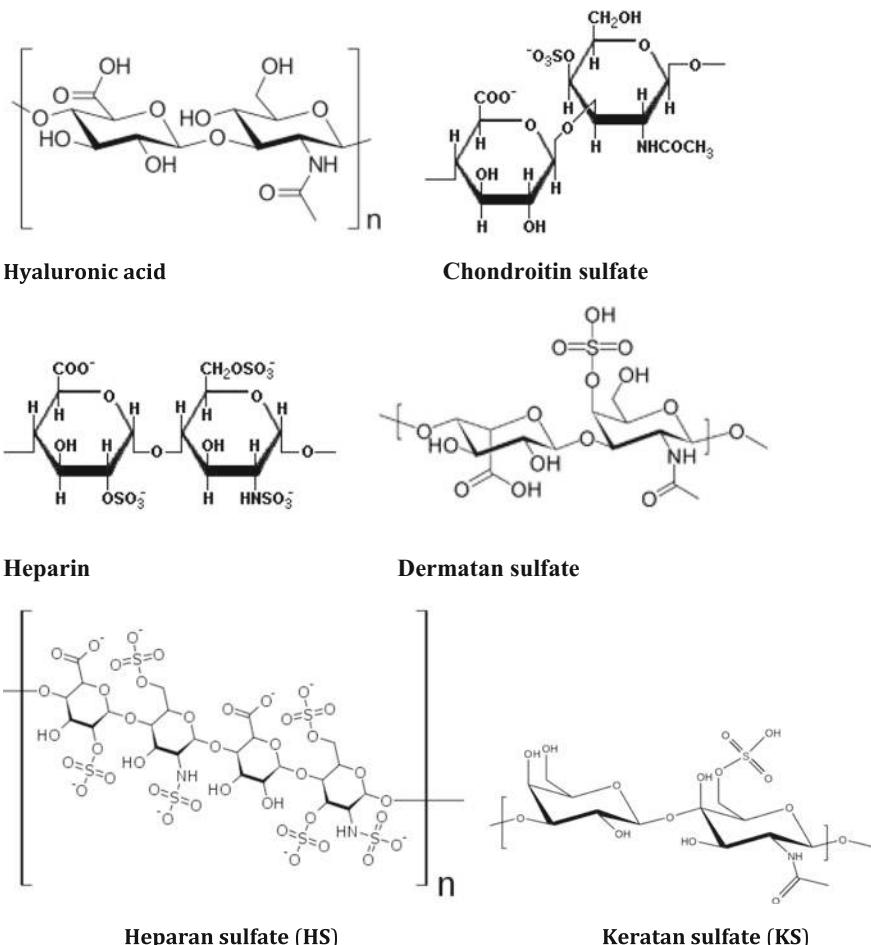
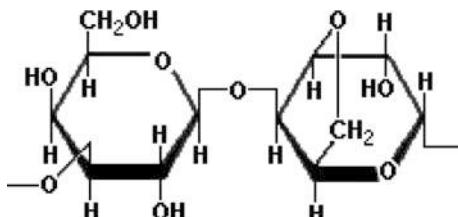


Fig. 2.38 Glycosaminoglycans (GAGs) or mucopolysaccharides

Fig. 2.39 Agarobiose, a disaccharide unit in agar consisting of β -(1 \rightarrow 3)-D and α -(1 \rightarrow 4)-L galactose residues



binder for puddings, custards and other desserts, in fruit preserves, as a clarifying agent in brewing, and as a filler in paper sizing fabrics. Highly refined agar is used in microbiology as a medium for culturing bacteria, fungi, etc., used to measure

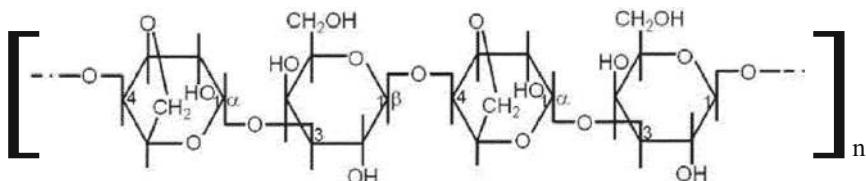


Fig. 2.40 A fragment of agar molecule showing 4 repeating monosaccharide units [α -(1 \rightarrow 4)-Lgalactose- β -(1 \rightarrow 3)-D-galactose- α -(1 \rightarrow 4)-L galactose- β -(1 \rightarrow 3)-D-galactose-]

microorganism motility and mobility; in biotechnology for cellular tissue culture, micropropagule development, and for DNA fingerprinting. Agar is used as an ingredient in desserts in Japan and other Asian countries. The gels produced with agar have a crispier texture than the desserts made with animal gelatin.

Carrageenans or carrageenins are a family of high-molecular-weight linear sulfated polysaccharides that are extracted from red edible seaweeds (e.g., *Chondrus crispus*, *Eucheuma denticulatum*, *Kappaphycus alvarezii*). Carrageenan compounds differ from agar in that they have sulfate groups ($-\text{OSO}_3^-$) in place of some hydroxyl groups. There are three main classes of carrageenan, kappa, iota, and lambda, each of which has different degrees of sulfation (1, 2 and 3 sulfate group per disaccharide, respectively) and gel strengths. All carrageenans are polysaccharides made up of repeating galactose units and 3 \rightarrow 6 anhydrogalactose (3 \rightarrow 6-AG), both sulfated and nonsulfated. The units are joined by alternating α -1 \rightarrow 3 and β -1 \rightarrow 4 glycosidic linkages (Fig. 2.41).

Carrageenans are widely used in the food industry, for their gelling, thickening, suspending, binding and stabilizing properties. It gives foods a smooth texture and accentuates flavor. It is used in dairy-based foods, like ice cream, yogurt, and cottage cheese, because it reacts well with milk proteins. Carrageenan is also found in jelly, pie filling, chocolate, salad dressing, and even as a fat substitute in processed meat. Because it comes from algae, it can be used as a substitute for gelatin

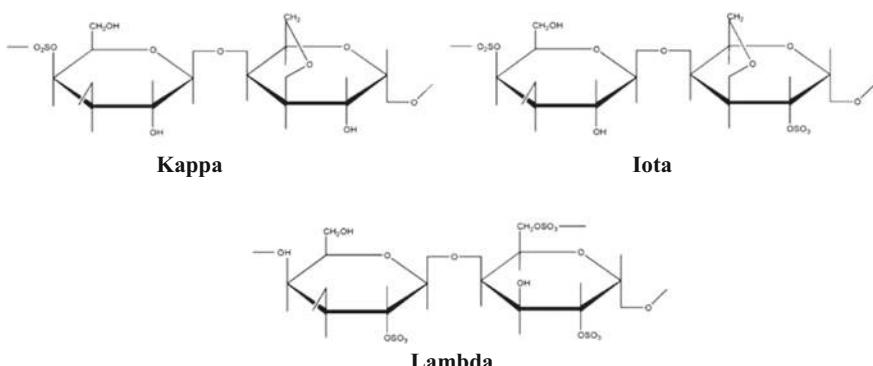


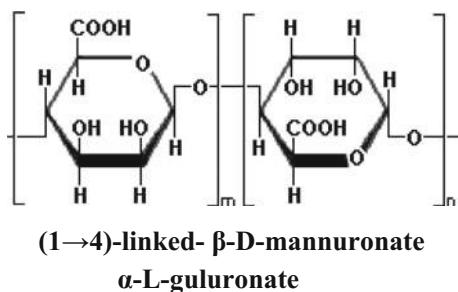
Fig. 2.41 Three main classes of carrageenan—kappa, iota and lambda

for vegetarian and vegan products. However, degraded carrageenan could cause gastrointestinal problems and only the undegraded variety has been deemed safe for human by the Food and Drug Administration (FDA), USA. The use of carrageenan in infant formula, organic or otherwise, is prohibited in the EU, but is permitted in other foodstuffs. Nonfood items like toothpaste, personal lubricants, and air freshener gels may also include carrageenan to thicken and stabilize the product, and make it smoother. Some types of firefighting foam also use carrageenan, which thickens the foam and helps it become sticky and more effective. In chemistry, gels made with it can be used to carry microbes or immobilize cells.

Alginic acid

Alginic acid (algin or alginate) is an anionic polysaccharide distributed widely in the cell walls of brown algae such as giant kelp (*Laminaria* spp., *Macrocystis pyrifera* of Phaeophyceae). It is sold in filamentous, granular, or powdered forms. Alginic acid is a linear copolymer with homopolymeric blocks of (1→4)-linked β -D-mannuronate (M) and its C-5 epimer α -L-guluronate(G) residues (i.e., linear polymer of mannuronic and glucuronic acids), respectively, covalently linked together in different sequences or blocks (Fig. 2.42). The monomers can appear in homopolymeric blocks of consecutive G-residues (G-blocks), consecutive M-residues (M-blocks) or alternating M, and G-residues (MG-blocks). Alginates are insoluble in water, but absorb water readily, capable of absorbing 200–300 times its own weight in water. It is widely used in processed foods and in medicinal, industrial and household products, including swabs, filters, and fire retardants. It is useful as gelling and thickening agents. Alginates are used as foam, clotting agents, and gauze in absorbable surgical dressings and packing. Alginate dressings are derived from seaweed made of soft nonwoven fibers, and are available as pads, ropes or ribbons. Alginate dressings are extremely lightweight, absorb many times their own weight, form a gel-like covering over the wound, and maintain a moist environment. They are best used for wounds with significant exudate, especially useful for packing exudative wounds; do not physically inhibit wound contraction as does gauze and are highly absorbent. Alginates are used in the manufacture of textiles, paper, and cosmetics. The sodium salt of alginic acid, sodium alginate, is used in the food industry to increase viscosity and as an emulsifier. Alginates are found in food products such as ice cream and in slimming aids where they serve as appetite suppressants. In dentistry, alginates are used to make dental impressions.

Fig. 2.42 Chemical structures and components of the alginic acid

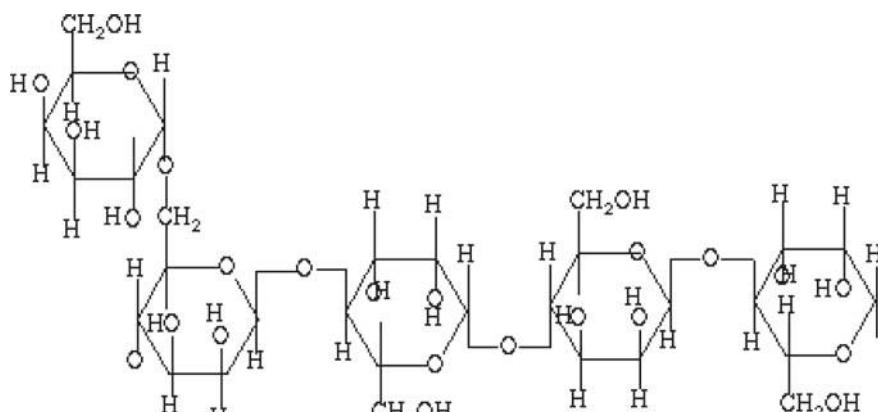


Galactomannan

Galactomannans are reserve polysaccharides consisting mainly of the monosaccharides mannose and galactose units (Fig. 2.43). The mannose elements form a linear chain a mannose backbone consisting of β -1 \rightarrow 4 linked-D-mannopyranosyl residues with α -1 \rightarrow 6 linked D-galactopyranosyl residues as side chain.

Galactomannans are present in several vegetable gums that are used to increase the viscosity of food products. Several galactomannans are known from natural sources and they may be arranged into four groups according to their molecular mannose to galactose ratio, e.g., fenugreek gum (\sim 1:1), guar gum (\sim 2:1), tara gum (\sim 3:1), locust bean gum (LBG) or carob gum (\sim 4:1) etc. These galactomannans are obtained from four main plant sources of Fabaceae, e.g., fenugreek from the seeds of *Trigonella foenum-graecum*, guar gum from the seeds of *Cyamopsis tetragonoloba*, Tara gum from seeds of *Cesalpinia spinosa* and LBG from the seeds of *Ceratonia siliqua* (Carob tree). Extraction of galactomannans involves de-hulling of seeds, crushing to remove the embryo, followed by milling of the endosperm to produce crude flour. The flour can be purified by dissolving in hot water followed by filtration and precipitation with isopropanol to remove impurities.

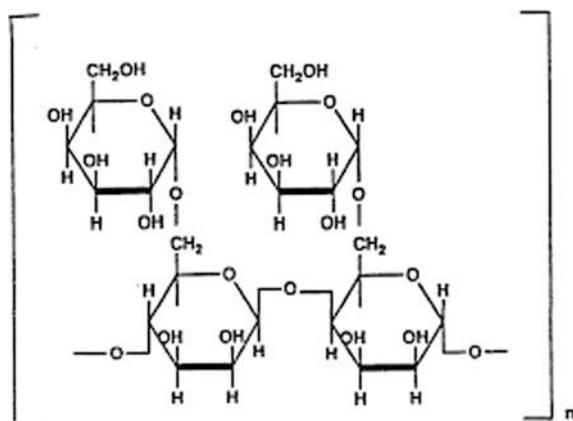
Galactomannans, common food fibers, are often used in food products to increase the viscosity of the water phase and also used in foods as stabilizers. Guar and LBG are commonly used in ice cream to improve texture and reduce ice cream meltdown. LBG is also used extensively in cream cheese, fruit preparations, and salad dressings. The use of tara gum as a food ingredient growing but is still to a much lesser extent than guar or LBG. Medical potential of mannans is as a drug nanocarrier system.



Galactomannan

Fig. 2.43 A generalized structure of locust bean gum galactomannan- β -1 \rightarrow 4-linked-D-mannopyranose backbone with a branch of α -1 \rightarrow 6-linked D-galactopyranose

Fig. 2.44 Fenugreek gum, mannose:galactose 1:1



The properties of galactomannans from different source species vary depending on the ratios of mannose to galactose, number and distance of the side chains on mannose backbone chain.

Fenugreek gum, mannose:galactose 1:1 (Fig. 2.44)

Fenugreek (seeds of *T. foenum-graecum* of Fabaceae) is a good source of soluble fiber, it prevents the post prandial glucose surge, lowers the Glycemic Index (GI) level of the food product, eases heartburn, minimizes the risk of heart disease, suppresses appetite, helps in weight and cholesterol management, prevent constipation, smoothes menstrual pain, and increases libido, etc. In addition, it is an ideal gum to be used as thickener or to increase viscosity in gravies, sauces, spread, and beverages.

Guar gum, mannose:galactose 2:1 (Fig. 2.45)

Guar gum, or guaran is a galactomannan. Guar is the main component in guar gum. It is primarily the ground endosperm of guar or cluster beans (*C. tetragonoloba* of Fabaceae). The guar seeds are dehusked, milled, and screened to obtain the guar gum. Guar gum is water soluble and exhibits a viscosifying effect in water, almost eight times the water-thickening potency of corn starch. Guar gum can be

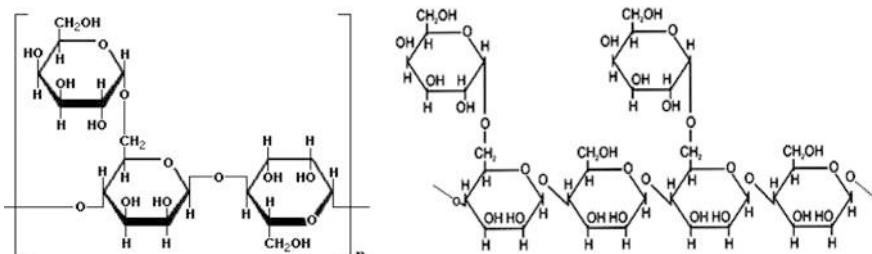


Fig. 2.45 Guar, the main component in guar gum; Guar gum, mannose: galactose 2:1

used as an emulsifier or as a stabilizer in various multiphase formulations. Guar gum retards ice crystal growth nonspecifically by slowing mass transfer across the solid/liquid interface. It shows good stability during freeze–thaw cycles. Guar has been used as an appetite depressant. Its thickening ability is utilized in various lotions and creams. Coarse Guar gum is often used as a binding and disintegrating ingredient in compressed tablets.

Tara gum, mannose:galactose 3:1 (Fig. 2.46)

Tara gum is produced by separating and grinding the seed endosperm of *C. spinosa* of Fabaceae. Tara gum consists of a linear main chain of β -1 \rightarrow 4-D-mannopyranose units attached by α -1 \rightarrow 6 linkages with D-galactopyranose units. The ratio of mannose to galactose in Tara gum is 3:1. Tara gum is safe for human consumption as a food additive in the form of thickening agent and stabilizer. Generally, Tara gum presents a high viscosity, an intermediate acid stability, and resists the depolymerization effect of organic acids down to a pH of 3.5, stable to high temperature heat treatment (up to 145 °C).

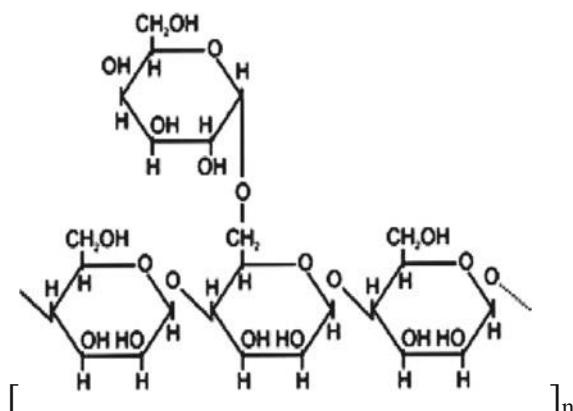
Medicinal uses include gargling infusions of the pods for inflamed tonsils or washing wounds in and also used to treat fevers, colds, and stomach aches. The tree can also be a source of lumber and firewood, and as a live fence. Water from boiled dried pods is used as insecticides. The seeds can be used to produce black dye while dark blue dye can be obtained from the roots.

Locust bean gum or carob gum, mannose:galactose 4:1 (Fig. 2.47)

LBG is extracted from the seeds of the carob tree (*C. siliqua* L.), mostly found in the Mediterranean region.

Guar is a legume that has been traditionally cultivated as livestock feed. Guar gum is also known by the name *C. tetragonoloba* which is the Latin taxonomy for the guar bean or cluster bean. Guar gum is the ground endosperm of the seeds. Approximately 85% of guar gum is guaran, a water-soluble polysaccharide consisting of linear chains of mannose with β -1 \rightarrow 4 linkages to which galactose units

Fig. 2.46 Tara gum, mannose:galactose 3:1



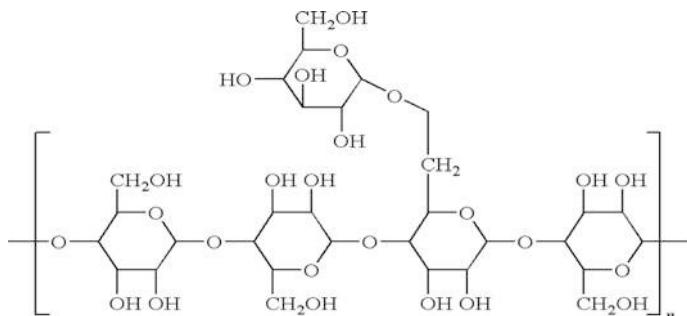


Fig. 2.47 Locust bean gum, mannose to galactose ratio = 4:1

are attached with α -1 \rightarrow 6 linkages. The ratio of mannose to galactose is 2:1. Guar gum has five to eight times the thickening power of starch and has many uses in the pharmaceutical industry, as a food stabilizer, and as a source of dietary fiber.

Pectin

Pectin is a polymer of 300–1000 α -D-galacturonic acid units with a variable number of methyl ester groups and joined by α -1 \rightarrow 4 glycoside linkages (Fig. 2.48). The degree of esterification (DE) affects the gelling properties of pectin. The structure shown below has three methyl ester forms ($-COOCH_3$) for every two carboxyl groups ($-COOH$), hence it is has a 60% DE, normally called a DE-60 pectin.

Pectin acts as a cementing material in the cell wall and middle lamella of all plant tissues. Pectin is present in all plants but the content and composition vary depending on the species, variety, maturity of the plant, plant part, tissue, and growing condition. Pectin is higher in legumes and citrus fruits than cereals. Other sources of pectin include banana, beets, cabbage, and carrots. Fruits that are high in pectin include apples, crab apples, blackberries, guava, gooseberries, cranberries, grapes, medlars, plums, and quince. Pectin content is low in fruits like apricots, blueberries, cherries, peaches, pears, raspberries, rhubarb, and strawberries. Any citrus fruit peel is also very high in pectin. The white portion of the rind of lemons and oranges contains approximately 30% pectin. Generally, unripe fruit will have more pectin than ripe fruit. Pectin is an important ingredient in industrial yogurt, cakes, ketchup, fruit jellies, jams, and fruit preserves.

Pectin is a soluble dietary fiber and it has several health benefits. It lowers serum cholesterol (LDL), blood glucose levels, heart disease and gallstones,

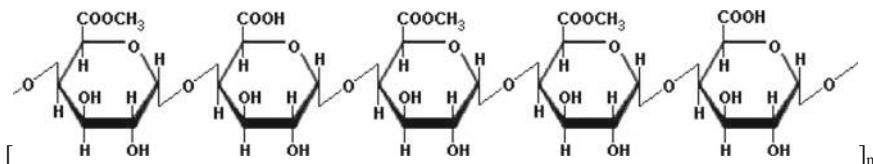


Fig. 2.48 A pectinmolecule with 3/5th degree (60%) of esterification

improves insulin resistance, constipation, and relieves diarrhea. Pectin acts as detoxicant, regulator, and protectant of the gastrointestinal tract, immune system stimulant, antiulcer, and antinephrotic agent. Several studies have reported significant decrease in serum LDL and increase or no change in HDL cholesterol in people by pectin rich dietary supplement like fruits and vegetables (e.g., apples, carrots etc.). Since pectin is fermented only in the colon and releases fatty acids that lowers the risks of colon cancer and also for that pectin is one of the strong candidates for coating colon-specific oral drug delivery systems.

Xanthan gum

Xanthan gum, secreted by the bacterium *Xanthomonas campestris*, is a polysaccharide with a β -D-glucose backbone like cellulose, but every second glucose unit is attached to a trisaccharide consisting of mannose, glucuronic acid, and mannose and each of the repeat units is composed of glucose, mannose, and glucuronic acid in the molar ratio 2:2:1 (Fig. 2.49). The mannose closest to the backbone has an acetic acid ester on carbon 6, and the mannose at the end of the trisaccharide is linked through carbons 6 and 4 to the second carbon of pyruvic acid.

Xanthan gum produced by the bacterium *X. campestris* as slimy substance on cruciferous vegetables such as broccoli, cabbage and cauliflower causing black rot. Unlike other gums, it is very stable under a wide range of temperatures and pH. The negatively charged carboxyl groups on the side chains because the molecules to form very viscous fluids when mixed with water. Xanthan gum is commonly used as a food additive, thickener (rheology modifier) for sauces, in salad dressing, to

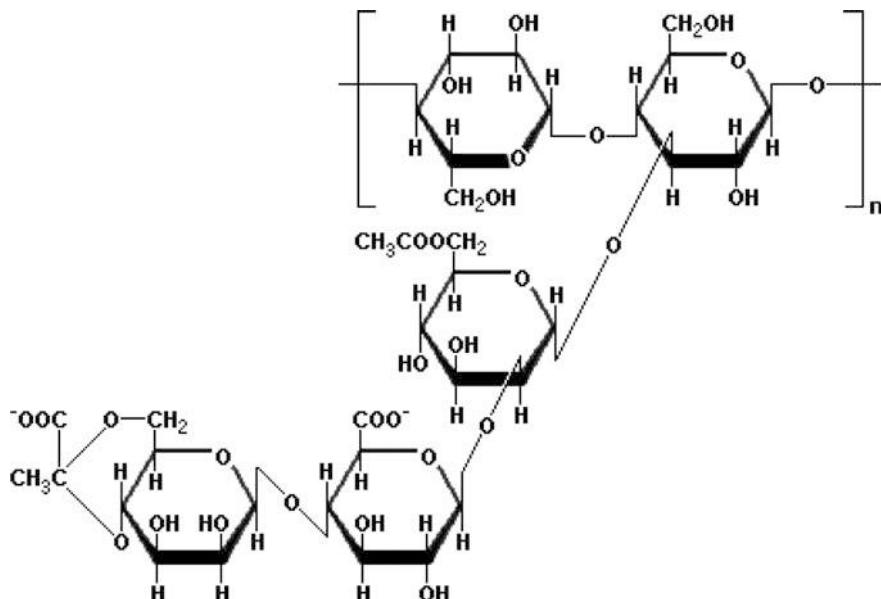


Fig. 2.49 The repeating unit of xanthan gum (2- β -D-glucose units backbone + 1 mannose + 1 glucuronic acid + 1 mannose

prevent ice crystal formation in ice cream, and as a low-calorie substitute for fat, stabilizer in cosmetic products and as a binder in toothpaste. Xanthan gum is frequently mixed with guar gum because the viscosity of the combination is greater than when either one is used alone. Xanthan gum is a highly efficient laxative but may act as common allergen to sensitive people.

Glucomannan

Glucomannan is mainly a straight chain polysaccharide with a small amount of branching consists of D-glucose (G) and D-mannose (M) in a proportion of 5:8 joined by β -1 \rightarrow 4 glycoside linkages (Fig. 2.50). The degree of branching is about 8% through β -1 \rightarrow 6-glucosyl linkages. The basic polymeric repeating unit has the GGMMGMMMMGGM pattern. Short side chains of 11–16 monosaccharides occur at intervals of 50–60 units of the main chain attached by β -1 \rightarrow 3 linkages as well as acetate groups on carbon 6 occur at every 9–19 units of the main chain. Hydrolysis of the acetate groups favors the formation of intermolecular hydrogen bonds that are responsible for the gelling action.

Glucomannan is a dietary fiber obtained from tubers or corm (40%) of *Amorphophallus konjac* of Araceae cultivated in Asia. Another culinary source is salep, ground from the roots of certain orchids and used in Turkish cuisine. Glucomannan (a hemicellulose) is present in large amounts in the wood of conifers and in smaller amounts in the wood of dicotyledons. Glucomannan is also a constituent of bacterial, plant, and yeast membrane with differences in the branches or glycosidic linkages in the linear structure. Glucomannan, a food additive, is used as an emulsifier and thickener. It has some clinically supported health benefits and different products containing glucomannan are sold as nutritional supplements for constipation, obesity, high cholesterol, acne vulgaris, and type 2 diabetes. The U.S. FDA did not but the Canadian authority approved some of the products containing glucomannan for the purposes of appetite reduction, weight management, and treatment of constipation and management of high cholesterol levels. Flour from the konjac tubers is used to make Japanese shirataki noodles, also called konnyaku noodles, which are very low in calories. Glucomannan is used as a hunger suppressant because it produces a feeling of fullness by creating very viscous solutions that retard absorption of the nutrients in food. One gram of this soluble polysaccharide can absorb up to 200 ml of water, so it is also used for absorbent articles such as disposable diapers and sanitary napkins.

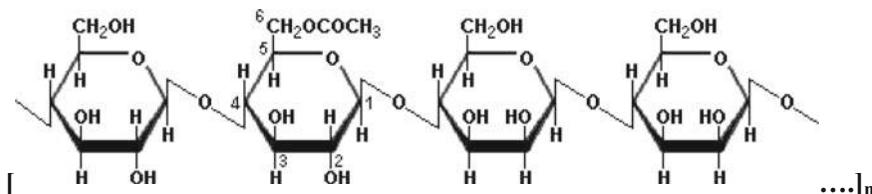


Fig. 2.50 A portion (GGMM) of the glucomannan repeating unit, an acetate group on C6 of 2nd glucose

2.3.1.3 Lipids (C, H, O, N, S & P)

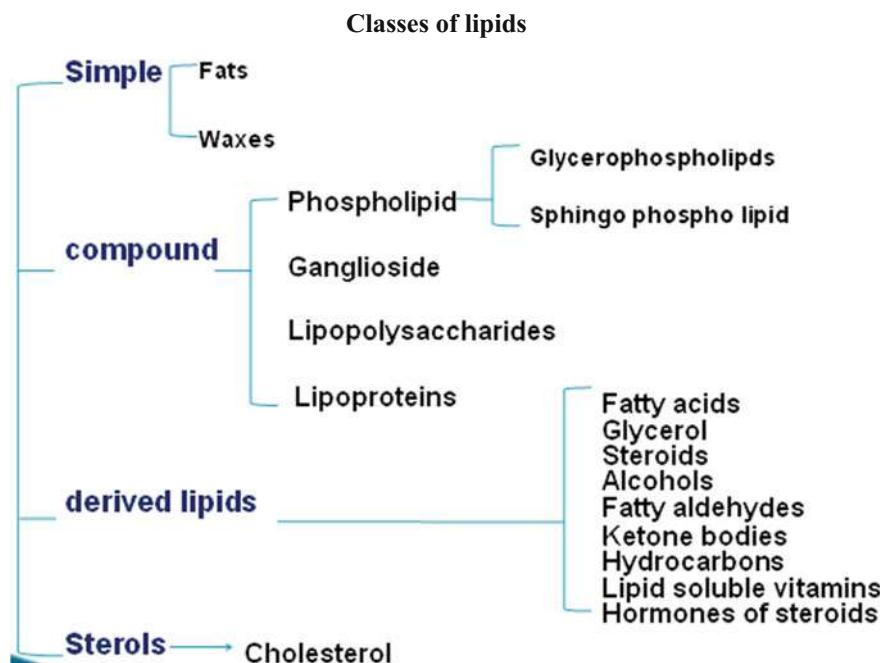
Lipids are a heterogeneous group of compounds including esters of fatty acids and glycerol or alcohols and their derivatives, and the fat-like substances that are soluble in nonpolar organic solvents. These heterogeneous groups of substances are of plant and animal origin and share a common character of solubility in organic solvents like alcohol, ether, chloroform, benzene etc., but not in water. Lipids are one of the four major classes of biologically essential organic molecules (e.g., carbohydrates, proteins and nucleic acids) found in living organisms; their amounts and quality in diet are able to influence cell, tissue and body physiology. Lipids are macromolecules, but not polymers, with a molecular weight 100–5000 D, vary considerably in polarity, including hydrophobic (triglycerides, sterol esters) and hydrophilic molecules (phospholipids). The little or absent water solubility of many of them means that they are subject to special treatments at all stages of their utilization during digestion, absorption, transport, storage, and use. Lipids are the most energy-rich and important sources of metabolic energy (9.50 kcal/g) compared to protein (5.60 kcal/g) and carbohydrate (4.10 kcal/g).

Classification

Generally, lipids may be classified as (a) True lipids—esters of fatty acids and glycerol, (b) Lipoids or lipid-like substances and (c) Pseudolipids—substances soluble in lipid solvents. The esters of fatty acids and glycerol (triglycerides) and fatty acids and long chain monobasic alcohols (waxes) are simple or true lipids; true lipids molecules in association with phosphoric acid, carbohydrate, protein, etc. constitute complex lipids (e.g., phospholipid, glycolipid, lipoprotein, etc.). Lipid degradation products (e.g., fatty acids, glycerol, alcohol, fatty aldehydes and ketone bodies, etc.), organic solvent soluble substance (pigments), lipid soluble vitamins (A, D, E and K), and steroids (e.g., hormones, cholesterol, etc.) constitute derived lipids. Aliphatic hydrocarbon and terpenes and similar other fat-soluble substances are grouped as miscellaneous or pseudolipids. Lipids are also classified as glycerol-based and non-glycerol-based lipids. Glycerol-based lipids include simple (e.g., fats, oils, etc.) and complex lipids (e.g., phospholipids, sulfolipids, glycolipids, lipoprotein, etc.) while non-glycerol-based lipids include waxes, cerebrosides, steroids, terpenes, sphingomyeline, etc. (Fig. 2.51).

On the basis of structural features, lipids may be grouped as single component lipids or lipid monomers including higher hydrocarbon, fatty acids, aliphatic alcohols, amino alcohols, aldehydes, ketones, isoprene compounds, polyols etc. and multicomponent lipids including simple or true lipids (e.g., oils, fats, waxes, etc.) and heterolipids (e.g., phospholipids, glycolipids, lipoprotein, sphingophospholipids, etc.) (Table 2.4).

Hydrocarbons form the simplest form of lipids and include alkanes, alkenes, cyclic hydrocarbons, and others. Several hydrocarbons may be substituted with oxygen-containing groups. These molecules are found mainly in petroleum but living organisms, eukaryotic or prokaryotic, and contain frequently hydrocarbons which are directly derived from fatty acids. They are distinct from the terpenoid

**Fig. 2.51** Broad classes of lipids**Table 2.4** Classification of lipids with properties and examples

Classes of lipids	Properties and examples
Class	
I. One component lipids or lipid monomers	1.1 Aliphatic hydrocarbons, the simplest form of lipids, e.g., alkanes, alkenes, cyclic hydrocarbons, etc. 1.2 Aliphatic alcohols, aldehydes and ketones, e.g., long chain monohydric/dihydric alcohols of beeswax, insect sex hormone pheromones 1.3 Isoprenoids and their derivatives (terpenes—pigments, sterols—cholesterol, steroid hormones—vitamins—glycosides—alkaloids) 1.4 Fat-soluble vitamins (A, D, E and K) 1.5 Amino alcohols (sphingosines) 1.6 Polyols 1.7 Fatty acids (saturated and unsaturated fatty acids), aldehydes and ketone bodies

(continued)

Table 2.4 (continued)

Classes of lipids	
Class	Properties and examples
II. Multicomponent lipids	<p>1. Simple lipids (esters composed of lipid monomers—fatty acids with glycerol), fats and oils are true lipids, fats contain long chain saturated fatty acids, solid at room temperature (lard, tallow) while oils contain relatively short chain unsaturated fatty acids, liquid at room temperature (olive oil, soybean oil)</p> <p>1.1 Waxes (esters of fatty acids with long chain mono or dihydric alcohols)</p> <p>1.2 Simple diol lipids or acyl diols (esters of dibasic alcohols)</p> <p>1.3 Glycerides or acyl glycerides (esters of fatty acids with tribasic alcohols glycerol)</p> <p>1.4 Sterids, esters of sterols with fatty acids, cholesterol esters; stigmasterol, ergosterol, and β-sitosterol are plant sterols</p> <p>2. Complex lipids or heterolipids</p> <p>2.1 Phospholipids (triesters of glycerol that contain charged phosphate diester groups)</p> <p>2.1.1 Phosphoglycerides (phosphoesters of glycerides)</p> <p>2.1.2 Diol phosphatides (phosphoesters of diol lipids)</p> <p>2.1.3 Sphingophosphatides or sphingophospholipids (phospholipids of <i>N</i>-acyl-sphingosine)</p> <p>2.2 Glycolipids (esters of fatty acids, glycerol and carbohydrates, a macromolecular complex)</p> <p>Lipoprotein (esters of fatty acids, glycerol and proteins; a macromolecular complex)</p>

hydrocarbons. They have usually a straight chain of up to about 36 carbon atoms but may also be branched, with one or more methyl groups attached at almost any point of the chain. Hydrocarbons are found at the outer surface in higher plant leaves. Resveratrol (3,4,5-trihydroxystilbene), a bicyclic hydrocarbon, is the most studied because of its presence in grapes and wine and some berries (blueberries, *Vaccinium*) and its numerous pharmacological properties (anticancer, antiviral, neuroprotective, antiaging, and anti-inflammatory).

Higher aliphatic alcohols, aldehydes, and ketones compounds occur in free state and also as structural constituents of multicomponent lipids (Fig. 2.52). They are oxygen derivatives of hydrocarbons. Higher aliphatic alcohols are compositional constituents of beeswax. Higher ketones occur in free form in bacteria while branched unsaturated ketones are structural components of insect sex hormone pheromones.

Isoprenoids and their derivatives constitute a vast group of biologically important lipids consisting of terpenes and steroids (Fig. 2.53). Cholesterol is synthesized from the triterpene squalene and lanosterol precursors. Carotenoids, structurally similar to tetraterpenes, are important and wide spread plant pigments, include

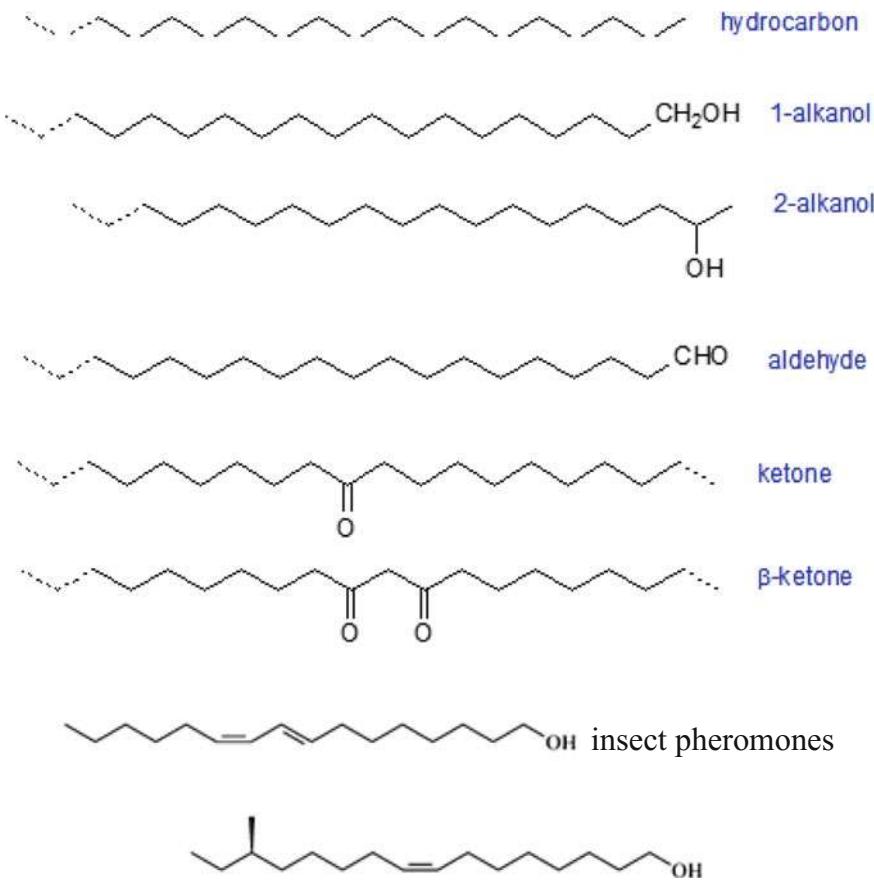


Fig. 2.52 Structures of higher aliphatic alcohols, aldehydes and ketones compounds, and insect pheromones

phytoene, lycopene, and α -, β -, γ -carotenes, of which β -carotene is the provitamin A. Isoprenoid alcohols include farnesol (sesquiterpene), geraniol, nerol, linalool (monoterpenes), etc. are used in the fabrication of perfumes. Menthol (monoterpene) is widely used in pharmaceutical and confectionary industries. Phytol (in chlorophyll) and retinol (in phylloquinone—vitamin K₁) are two diterpene alcohols. Camphor (monoterpene ketone) is used in drug; abscisic acid (monocyclic sesquiterpene derivative) is a phytohormone. Steroids (triterpene cyclic derivative) whose skeletal framework is that of gonane and steroids with an alcoholic group is called sterols, e.g., cholesterol, which is an important constituent of plasma membrane. Bile alcohols, bile acids (cholic acid, chenodeoxycholic acid), hormones (pregnenolone, progesterone), vitamins (vitamin D-calciferol), steroid glycosides (cardiac glycoside), steroid alkaloids (nitrogen-containing alkaloids of

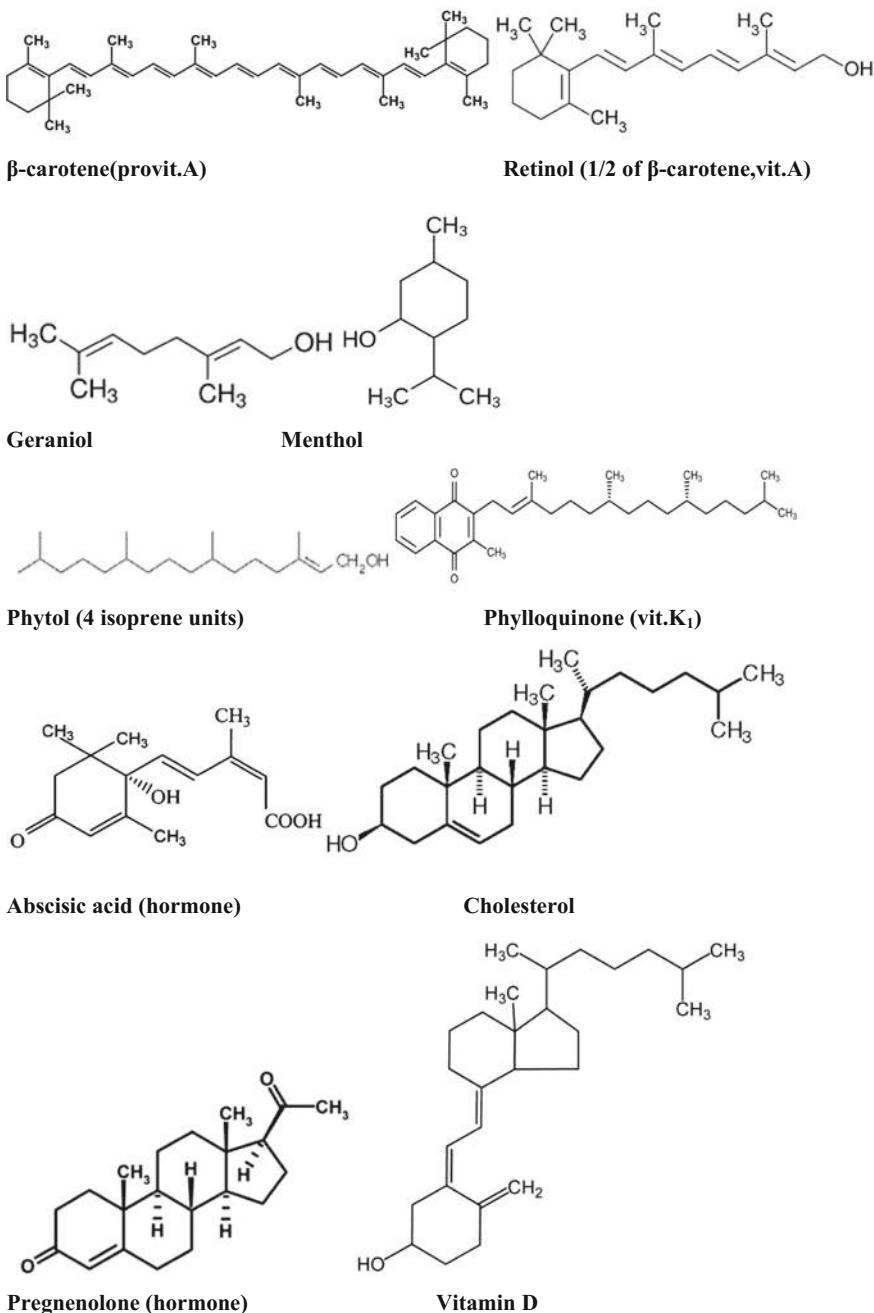
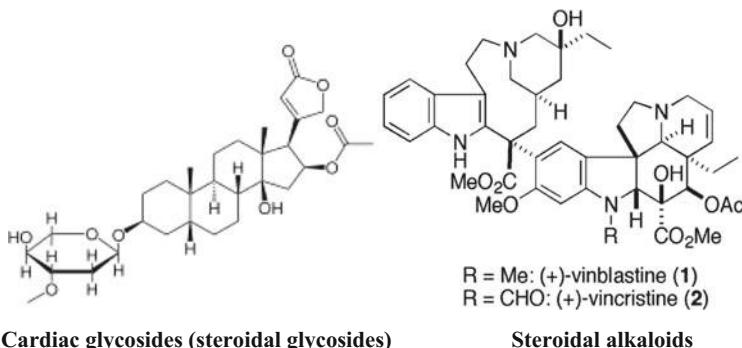


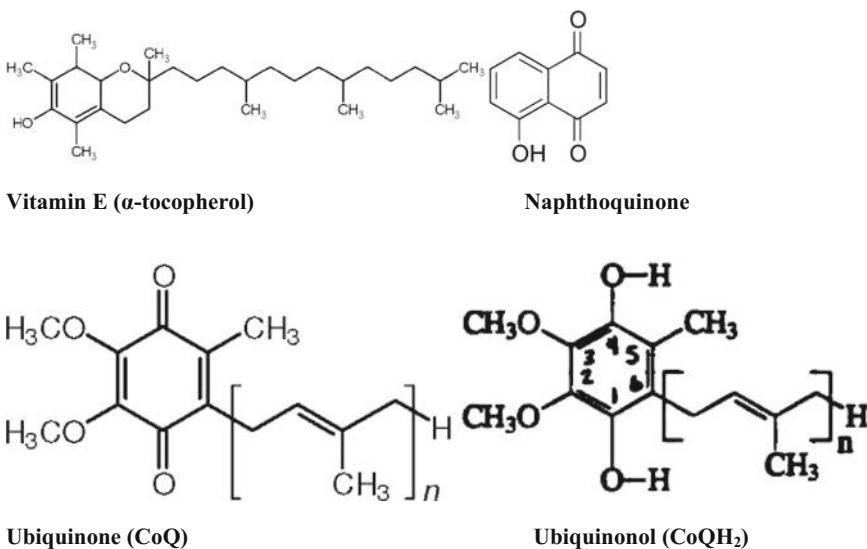
Fig. 2.53 Biologically important lipids consisting of terpenes and steroids

**Fig. 2.53** (continued)

nightshades, lilies, periwinkles, Phyllobates frog), etc., are important derivatives of cholesterol.

Vitamins that are soluble in fat are considered as lipids. This group of lipids include vitamin A (retinol), D (calciferols), E (α, β, γ -tocopherols), K (naphthoquinones) and vitaminoids ubiquinone (CoQ, CoQ₂H₂) and F (oleic, linoleic, linolenic acids) (Fig. 2.54). Toxic effects of vitamin A include drowsiness, headache, vomiting, peeling of the skin, sun sensitivity, that of vitamin D include hypercalcemia, E-supra oxidant effect, etc.

All fat-soluble vitamins are isoprenoid compounds and they are found in vegetable oils, colored vegetables, fruits, grains, nuts, seeds, meat, liver and fish

**Fig. 2.54** Lipids—fat-soluble vitamins

products, seafood, dairy products, etc. Fat-soluble vitamins can be stored in liver and in adipose tissue. They are not readily excreted in urine and excess consumption may cause excess accumulation leading to hypervitaminosis or vitamin intoxication. Vitamin A is important for vision, especially night vision, bone growth, and mucous membranes. As an antioxidant, it may reduce the risk of some forms of cancer. Vitamin D aids in the absorption of calcium and phosphorous. Teeth, bones, and cartilage require it. Vitamin E is also an antioxidant and helps to generate red blood cells and prevents blood from clotting. Vitamin K also works with the blood, aiding in the normal clotting process and bone maintenance.

Sphingosine and dihydrosphingosine, major membrane components, are examples of two unsaturated and saturated higher (18C) amino alcohols, respectively. They form part of multicomponent sphingolipids, a class of cell membrane lipids (Fig. 2.55). Sphingosine on phosphorylation leads to the formation of sphingosine-1-phosphate, a potent signaling molecule involved in diverse cellular processes.

A polyol is an alcohol containing multiple hydroxyl groups. Polyols are neither sugars nor alcohols, they are low-calorie carbohydrates (Fig. 2.56). Polyols like erythritol, HSH, isomalt, lactitol, maltitol, mannositol, sorbitol, and xylitol replace sugar in sugar-free foods and medicines. The sugar alcohols differ in chain length, most have five- or six-carbon chains as they are derived from pentoses and hexoses (six-carbon sugars), respectively. Some others have higher number of carbon atoms, e.g., volemitol (7C), isomalt (12C), maltitol (12C), lactitol (12C), maltotriitol (18C), maltotetraitol (24C), and polyglycitol (*n*C). They have one OH group attached to each carbon. Higher numbers of molecules create more viscous solutes, depending on the type of polyol. Higher polyols constitute a relatively small group of lipid monomers. They occur in microorganisms, plants and in animal tissues in the form of diol lipids.

Fatty acids are derivatives of aliphatic hydrocarbons with a carboxyl group. Over 200 fatty acids are known and they are the chief hydrophobic components of simple and complex lipids. They may be saturated or unsaturated and they also differ among themselves in chain length, number and position of double bonds, and also in the nature of substituents, e.g., oxy-, keto-, epoxy groups, cyclic structures, etc.

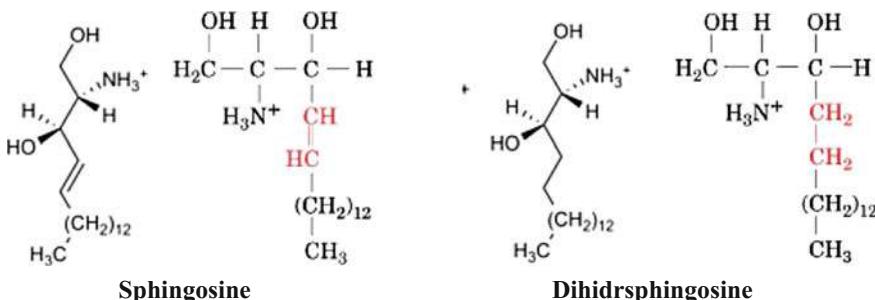


Fig. 2.55 Structure of a class of cell membrane lipids

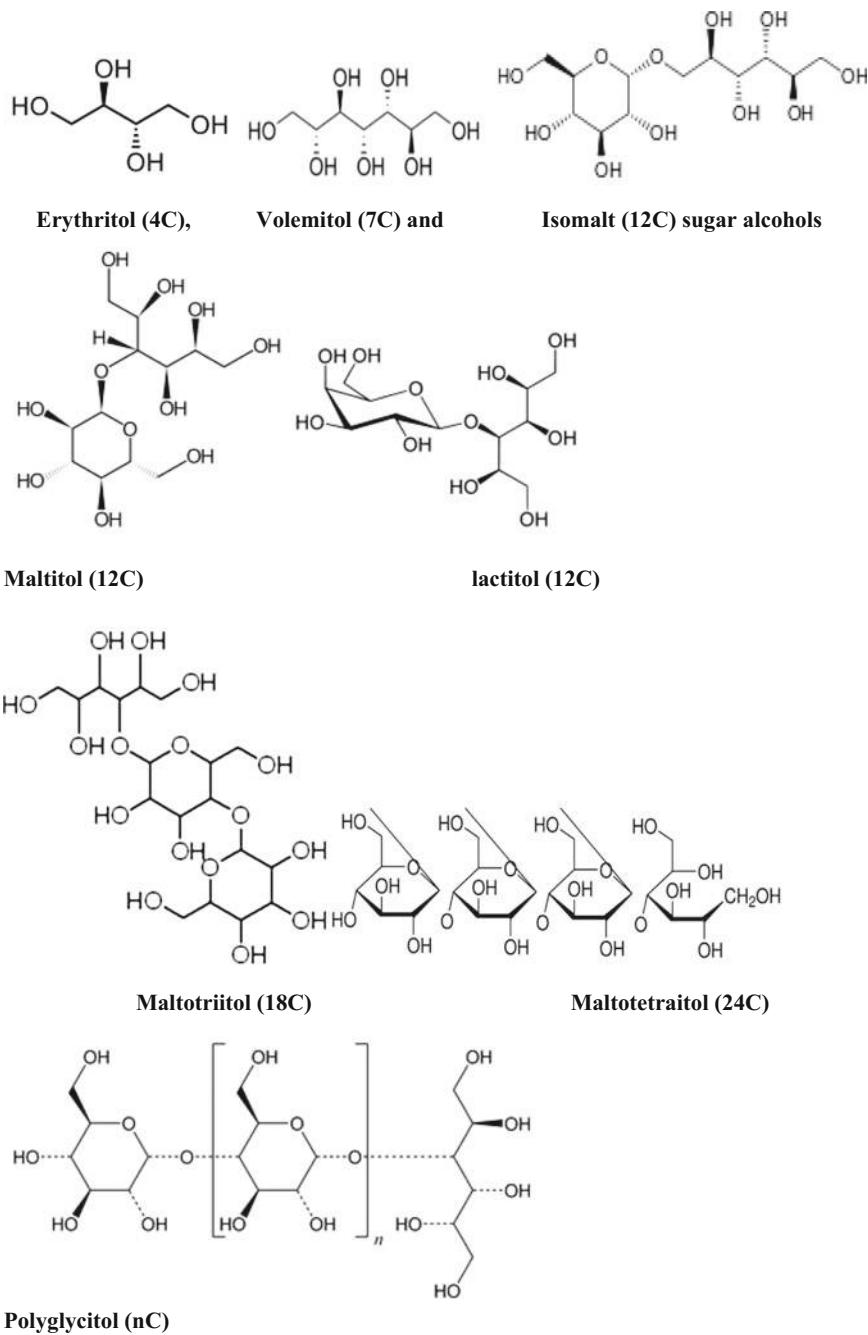
**Fig. 2.56** Structure of different polyols

Table 2.5 Some examples of saturated and unsaturated fatty acids

Type	Name of the acid	Source	Carbon atoms	Double bonds	Molecular formula	Melting point (°C)
Saturated	Lauric	Coconut oil	12	0	$\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$	44
	Myristic	Butter fats	14	0	$\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$	58
	Palmitic	Most fats and oils	16	0	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$	63
	Stearic	Most fats and oils	18	0	$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$	70
Unsaturated	Oleic	Olive oil	18	1	$\text{CH}_3(\text{CH}_2)_{7}\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}(cis)$	4
	Linoleic	Vegetable fats	18	2	$\text{CH}_3(\text{CH}_2)_{4}\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}(cis)$	-5
	Linolenic	Soybean and canola oils	18	3	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH CH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH(all cis)}$	-11
	Arachidonic	Lard	20	4	$\text{CH}_3(\text{CH}_2)_{4}(\text{CH}=\text{CHCH}_2)_4 \text{CH}_2\text{CH}_2\text{COOH(all cis)}$	-50

Fatty acids with odd number carbon are less frequent than that of even number, and palmitic (16C) and stearic (18C) acids are more frequent than others. Short chain fatty acids like butyric and capronic acids do not belong to lipids because they are water soluble. Some examples of fatty acids are shown in Table 2.5.

Multicomponent lipids contain more than one component or lipid monomer in the molecule. Molecules of simple and complex or hetero lipids are multicomponent lipids. They are eaters of fatty acids alcohols of different chain length, number of -OH group, etc. Fatty acid chains differ by length, e.g., SCFA with <6 carbons (i.e., butyric acid), medium chain fatty acids (MCFA) with 6–12 carbons, long chain fatty acids with 13–21 carbons (tallow or lard whose chains are 17 carbons long) and very long chain fatty acids with >22 carbons (hexacosanoic acid, a 26-carbon long chain saturated fatty acid).

Fatty acids

A fatty acid is a carboxylic acid with a saturated (without carbon–carbon double bonds) or unsaturated (having carbon–carbon double bonds) aliphatic tail, mostly of even number of carbon atoms ranging from 4 to 28 (Fig. 2.57). Fatty acids are usually derived from triglycerides or phospholipids. When they are not attached to other molecules, they are known as “free” fatty acids.

Fatty acids are important sources of cellular energy because they yield large quantities of ATP during their catabolism. Many cell types can use either glucose or fatty acids as for energy source (heart and skeletal muscle prefer fatty acids; brain cells also use fatty acids in addition to glucose and ketone bodies). Fatty acids with aliphatic tails of <6 carbon atoms are SCFA (e.g., butyric acid), with aliphatic tails of 6–12 carbon atoms are MCFA, with aliphatic tails of 13 to 21 carbon atoms are long chain fatty acids (LCFA), and with aliphatic tails > 22 carbon atoms are very long chain fatty acids (VLCFA).

Examples of some saturated and unsaturated fatty acids are shown in Table 2.6.

Fatty acids that are required by the human body but cannot be made in sufficient quantity from other substrates, and therefore must be obtained from food, are called essential fatty acids. There are two series of essential fatty acids: one has double-bond three-carbon atoms removed from the methyl end; the other has double-bond six-carbon atoms removed from the methyl end. Humans lack the ability to introduce double bonds in fatty acids beyond carbons 9 and 10, as counted from the carboxylic acid side. Two essential fatty acids are linoleic acid (LA) and alpha-linolenic acid (ALA). They are widely distributed in plant oils. The human body has a limited ability to convert ALA into the longer chain *n*-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which can also be

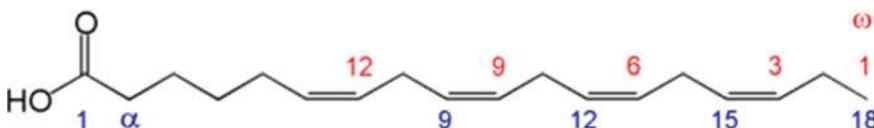


Fig. 2.57 The way of numbering of carbon atoms starting from carboxyl carbon onward and the number of double bond from other end

Table 2.6 Examples of saturated and unsaturated fatty acids

Saturated fatty acids	Chemical structure	C:D	Source
Butyric acid	CH ₃ (CH ₂) ₂ COOH	4:0	Butterfat
Caproic acid	CH ₃ (CH ₂) ₄ COOH	6:0	Butterfat
Caprylic acid, 8	CH ₃ (CH ₂) ₆ COOH	8:0	Coconut oil
Capric acid, 10	CH ₃ (CH ₂) ₈ COOH	10:0	Coconut oil
Lauric acid, 12	CH ₃ (CH ₂) ₁₀ COOH	12:0	Coconut oil
Myristic acid, 14	CH ₃ (CH ₂) ₁₂ COOH	14:0	Palm kernel oil
Palmitic acid, 16	CH ₃ (CH ₂) ₁₄ COOH	16:0	Palm oil
Stearic acid, 18	CH ₃ (CH ₂) ₁₆ COOH	18:0	Animal fats
Arachidic acid, 20	CH ₃ (CH ₂) ₁₈ COOH	20:0	Peanut oil, fish oil
Behenic acid, 22	CH ₃ (CH ₂) ₂₀ COOH	22:0	Rapeseed oil
Lignoceric acid, 24	CH ₃ (CH ₂) ₂₂ COOH	24:0	Small amounts in most fats
Cerotic acid	CH ₃ (CH ₂) ₂₄ COOH	26:0	beeswax
Unsaturated fatty acids	Chemical structure	C:D	Source
Myristoleic acid	CH ₃ (CH ₂) ₃ CH=CH(CH ₂) ₇ COOH	14:1	Nutmeg butter, palm oil, coconut oil, butter fat
Palmitoleic acid	CH ₃ (CH ₂) ₅ CH=CH(CH ₂) ₇ COOH	16:1	In animal oils, vegetable oils, and marine oils
Sapienic acid	CH ₃ (CH ₂) ₈ CH=CH(CH ₂) ₄ COOH	16:1	sebacous origin
Oleic acid	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₇ COOH	18:1	olive oil
Elaidic acid	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₇ COOH	18:1	in hydrogenated vegetable oils
Vaccenic acid	CH ₃ (CH ₂) ₅ CH=CH(CH ₂) ₉ COOH	18:1	butterfat
Linoleic acid	CH ₃ (CH ₂) ₄ CH=CHCH ₂ CH=CH(CH ₂) ₇ COOH	18:2	grape seed oil
Linoleaidic acid	CH ₃ (CH ₂) ₄ CH=CHCH ₂ CH=CH(CH ₂) ₇ COO	18:2	In partially hydrogenated vegetable oils

(continued)

Table 2.6 (continued)

Unsaturated fatty acids	Chemical structure	C:D	Source
α -Linolenic acid	$\text{CH}_3\text{CH}_2\text{CH}=\text{CH}\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	18:3	linseed oil
Arachidonic	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2$ $\text{CH}=\text{CHCH}_2$ $\text{CH}=\text{CH}(\text{CH}_2)_3\text{COOH}$	20:4	liver fats
Eicosapentenoic	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2$ $\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_3\text{COOH}$	20:5	fish oil
Erucic acid	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_11\text{COOH}$	22:1	rapeseed oil
Docosahexenoic	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHC H}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}$ $(\text{CH}_2)_2\text{COOH}$	22:6	fish oil

Table 2.7 Fatty acid composition of some common fats and oils (approximate)

Source	Type of fatty acids in fats and oils, %				
	Saturated	MyristicC14	PalmiticC16	StearicC18	Unsaturated
Animal fat	LauricC12	—	1	25	15
	Butter	2	10	25	10
	Human fat	1	3	25	8
	Whale blubber	—	8	12	3
Vegetable oil	Corn	—	1	8	4
	Olive	—	1	5	5
	Peanut	—	—	7	5
	Soybean	—	—	7	4
					34
					53

obtained from fish. Fatty acid composition (approximate) of some common animal fats and vegetable oils is shown in Table 2.7.

Simple diol lipids are esters of fatty acids with dibasic alcohols (ethyleneglycol). Small amount of diol lipids is present in plant and animal tissues and they are functionally active in cell generation and maturation of plant seeds. Glycerides or acylglycerides, examples of simple neutral lipids, are esters of fatty acids with tribasic alcohol glycerol (Fig. 2.58). Glycerides may be mono-, di-, and tri-acyl glycerides depending on the number of acyl groups ($\text{RCO}-$) in the molecule. Triglycerides are edible fats derived from oil seeds. Fats made up of shorter chain of unsaturated fatty acids are usually liquid at room temperature, whereas the fats with longer chain saturated fatty acids will be solid. Oil refers to fats that are liquids at normal room temperature, while fats are solids at normal room temperature, e.g., animal fats tallow and lard are high in saturated fatty acid content and are solids while olive and linseed oils are unsaturated and liquid. Fat is important foodstuff and serving structural, energy resource, and metabolic functions. Many cell types can use either glucose or fatty acids for this energy, heart, skeletal muscle, and brain cells prefer fatty acids as a source of fuel. Some fatty acids are essential because they cannot be synthesized in the body from simpler constituents. Two essential fatty acids (unsaturated) in human nutrition include α -linolenic acid (an omega-3 fatty acid) and LA (an omega-6 fatty acid). Unsaturated fatty acids of cis form are more common in nature than trans form. Consumption of *cis* fats is hygienic while *trans* fats increase the risk of coronary heart disease. Unsaturated fats can be altered by hydrogenation to fully saturated solid fat in order to increase the desirable physical properties, e.g., a desirable melting temperature (30–40 °C), shelf life, etc. However, trans fats are generated during hydrogenation as contaminants. Waxes are esters of higher monobasic alcohol and higher fatty acids. Waxes are hydrophobic and form water-repellent protective layer on plant leaves and fruits, skin and hair of animals, feather of birds, and external skeleton of insects.

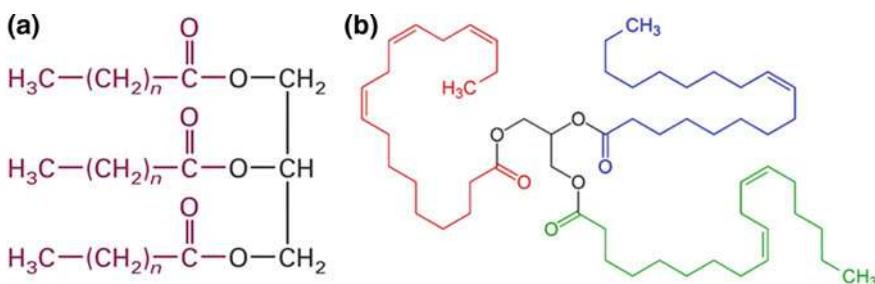


Fig. 2.58 **a** Triacylglycerol showing 3 ester bonds between $-\text{COOH}$ of fatty acids (left) and $-\text{OH}$ of glycerol (right). **b** Representative triglyceride found in a linseed oil, a triester (triglyceride) derived of linoleic acid (green, below, 18C), alpha-linolenic acid (red, left above, 18C), and oleic acid (blue, right above, 18C). Linseed oil is liquid at room temperature because all these fatty acids have some degrees of unsaturation

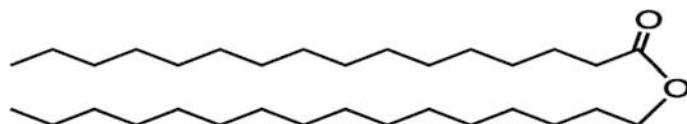


Fig. 2.59 Structure of wax esters consisting of long chain fatty acid (C₁₅ upper) and alcohol (C₁₅ lower)

Pharmaceutically, fixed oils and fats are used as emollients and vehicles for other medicaments. Some of them, such as castor oil and cod liver oil, possess therapeutic properties. Natural sources of fixed oils and fats include castor beans, olive fruits, peanuts, coconut seed kernel, cod liver, cacao seeds, and sheep wool. Industrially they are used as lubricants and in the manufacture of soaps, paints, and varnishes.

A wax, an ester of a long chain alcohol (C₁₆–C₃₂) and a fatty acid (C₁₆–C₂₆), is a simple lipid (Fig. 2.59). Waxes are found in nature as coatings on leaves and tender stems of plant, on skin, hair of mammals, feather of birds, and exoskeleton of insects also in fish and marine organisms (invertebrate-whale). The nature of the lipid constituents (also the chain length and degree of unsaturation and branching of the aliphatic constituents) varies greatly with the source of the waxy material and includes hydrocarbons, sterol esters, aliphatic aldehydes, primary and secondary alcohols, diols, ketones, β -diketones, triacylglycerols, etc. (Fig. 2.60).

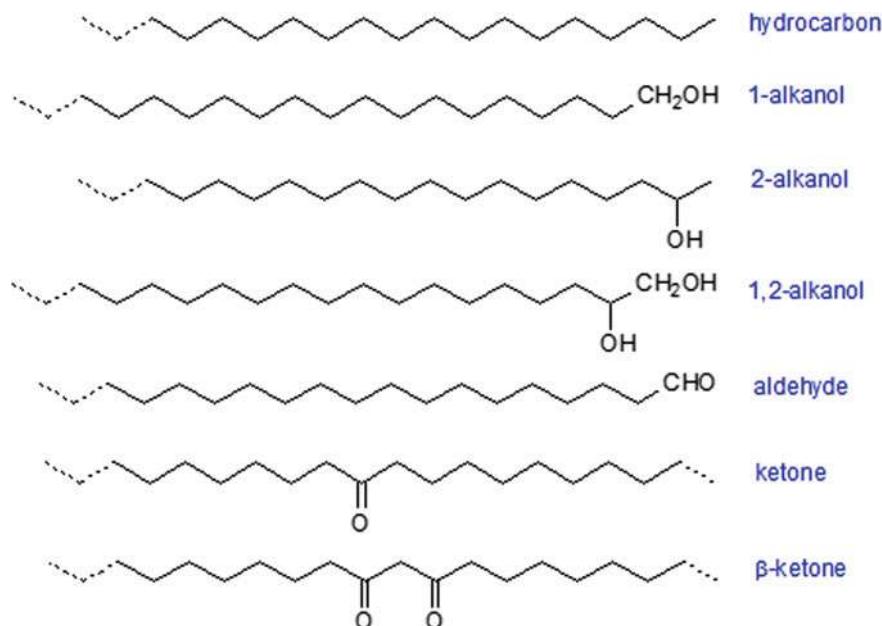


Fig. 2.60 The nature of the lipid constituents of the waxy material

Waxes on surface tissues of plants limit the diffusion of water and solutes, permit the controlled release of volatiles to deter pests or attract pollinating insects, provide protection from disease and insects, and help the plants resist drought. Waxes appear to have a variety of functions in fish, from serving as an energy source to insulation, buoyancy, and even echo location. Spermaceti or sperm whale oil (wax esters, 76%; triacylglycerols, 23%) was once in great demand as a lubricant but now is proscribed. The main purpose of the waxes is presumed to be to give a waterproof layer to the feathers. Waxes (microbial waxes) are not common in prokaryotes, but the pathogenic mycobacteria (e.g., *Mycobacterium tuberculosis* and *M. leprae*) produce waxes—mycoserulates (virulence factors consisting of branched chain alcohols C₃₄–C₃₆ chain length esterified with long chain fatty acids of C₁₈–C₂₆ chain length having 2–4 methyl branches). Sebaceous glands appear to be the only source of wax esters in mammalian tissues and these waxes keep skin surface moist and possess powerful antibacterial properties coating and prevent water loss. Carnauba wax is found on the leaves of Brazilian palm trees (*Copernicia cerifera*) as thick coating, harvested from the dried leaves and is used in floor and automobile waxes. It contains mainly wax esters (85%), accompanied by small amounts of free acids and alcohols, hydrocarbons and resins. The wax esters constitute C₁₆ to C₂₀ fatty acids linked to C₃₀ to C₃₄ alcohols, giving C₄₆ to C₅₄ molecular species. Jojoba is seed wax ester of jojoba plant (*Simmondsia chinensis*)—a significant crop of the semiarid regions of Mexico and the U.S.A. It consists mainly of C₁₈–C₂₂ fatty acids linked to C₂₀–C₂₄ fatty alcohols. The sunflower seed waxes (10–12%) from oil refineries are long chain fatty esters (C₃₈–C₅₄) comprised of fatty alcohol (C₁₈–C₃₀) and fatty acid (C₁₆–C₃₀). Lanolin or wool wax is obtained from the wool of sheep during the cleaning or refining process and is rich in wax esters (of 1- and 2-alkanols, and 1, 2-diols), sterol esters, triterpene alcohols, and free acids and sterols (contains up to 50% wax esters and 33% sterol esters). A high proportion of the sterol component is lanosterol. The fatty acid components are mainly saturated and *iso*- and *anteiso*-methyl-branched chain. Bees secret beeswax to make cells for honey and eggs. The wax is recovered as a by-product when the honey is harvested and refined. It contains a high proportion of wax esters (35–80%). The wax esters consist of C₄₀ to C₄₆ molecular species, some diesters with up to 64 carbons may be present, together with triesters, hydroxy-polyesters and free acids. Spermaceti wax is found in the head cavities and blubber of the sperm whale. Many of the waxes are used in ointments, hand creams, and cosmetics. Paraffin wax, used in some candles, is not based upon the ester functional group, but is a mixture of high-molecular-weight alkanes. Ear wax is a mixture of phospholipids and esters of cholesterol. Bees wax and Carnauba wax are examples of waxes. Waxes are used as hardeners of ointment and cosmetic creams. Structure of some waxes from different sources is given in Fig. 2.61.

Paraffin wax is a white or colorless soft solid derivable from petroleum, coal or oil shale that consists of a mixture of hydrocarbon molecules containing between 20 and 40 carbon atoms (Fig. 2.62). In chemistry, paraffin is used synonymously

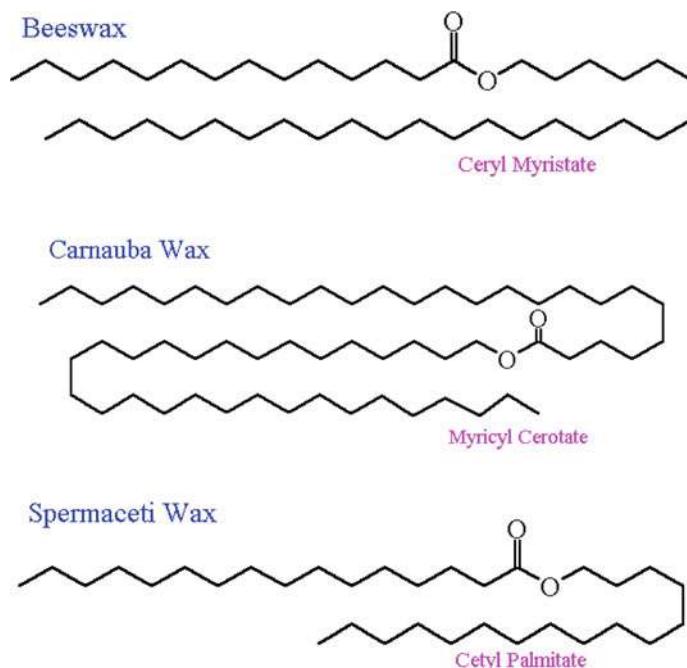
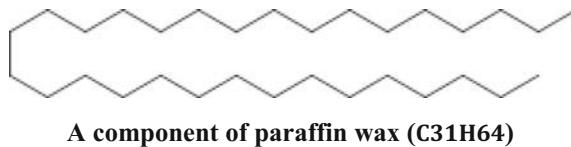


Fig. 2.61 Structure of different waxes. Beeswax (ceryl myristate), carnauba wax (myricyl cerotate) and spermaceti wax (cetyl palmitate)



A component of paraffin wax (C₃₁H₆₄)

Fig. 2.62 The hydrocarbon C₃₁H₆₄ (with the general formula C_nH_{2n+2}) is a typical component of paraffin wax

Table 2.8 Molecular composition of different waxes

Waxes		
Type	Alcohol	Fatty acid
Beeswax (ceryl myristate)	CH ₃ (CH ₂) ₂₈ CH ₂ -OH	CH ₃ (CH ₂) ₁₄ COOH
Carnauba (myricyl cerotate)	CH ₃ (CH ₂) ₂₈ CH ₂ -OH	CH ₃ (CH ₂) ₂₄ COOH
Spermacetic (cetyl palmitate)	CH ₃ (CH ₂) ₁₄ CH ₂ -OH	CH ₃ (CH ₂) ₁₄ COOH

with alkane, indicating hydrocarbons with the general formula C_nH_{2n+2}. Paraffins are unreactive in nature. Molecular composition of waxes from different sources is shown in Table 2.8.

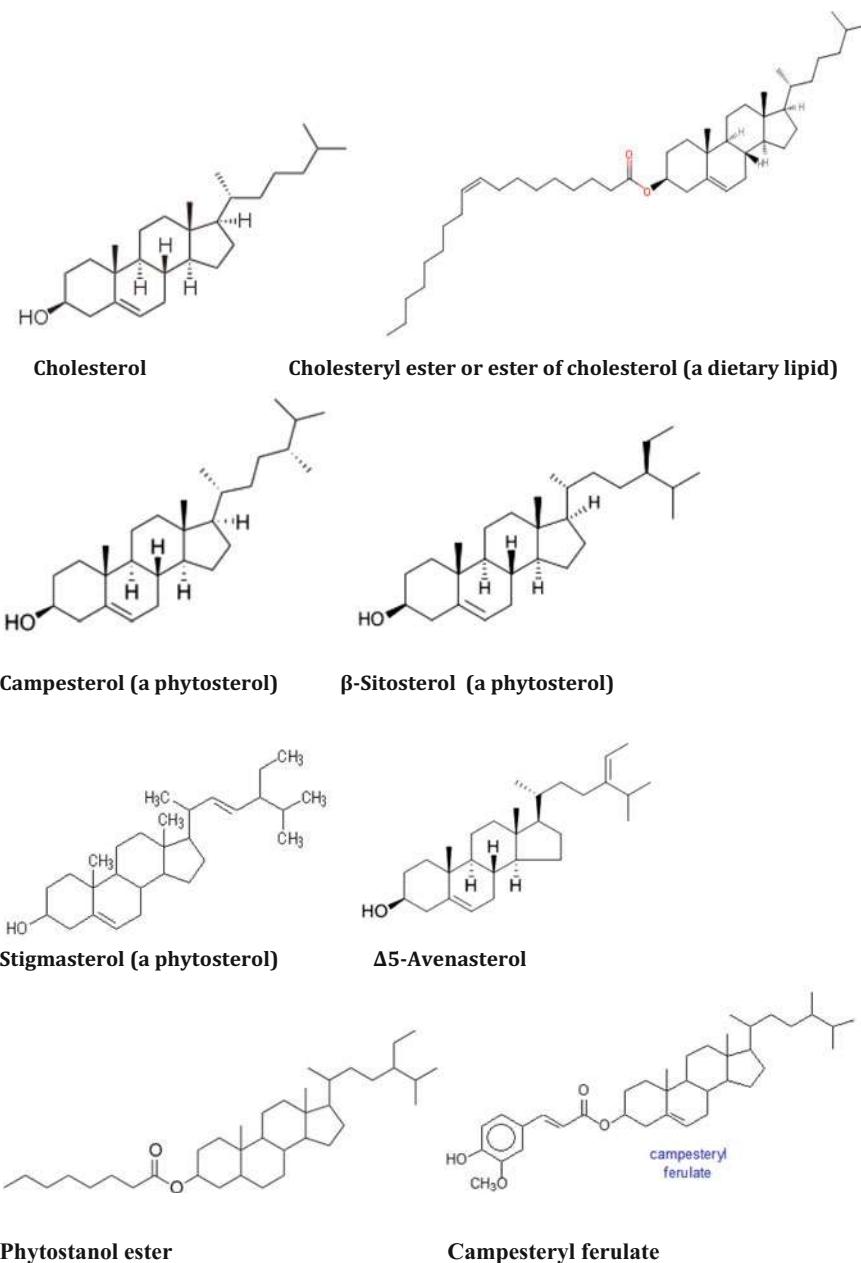


Fig. 2.63 Structure of sterols and sterids—cholesterol, cholesterol ester, campesterol, β -sitosterol, stigmasterol, Δ^5 -avenasterol, phytostanol ester and campesterol ferulate

Sterids are esters of sterols with fatty acids. Sterol esters constitute a heterogeneous group of chemical compounds (Fig. 2.63). The phytosterols (as opposed to zoosterols) include campesterol, β -sitosterol, stigmasterol, and $\Delta 5$ -avenasterol.

Certain distinctive phytosterol esters occur in the aleurone cells of cereal grains, including *trans*-hydroxycinnamate, ferulate (4-hydroxy-3-methoxy cinnamate) and *p*-coumarate esters. Similarly, rice bran oil is a rich source of esters of ferulic acid and a mixture of sterols and triterpenols, termed “ γ -oryzanol”. Leaf and other tissues in plants contain a range of sterol glycosides and acyl sterol glycosides, in which the hydroxyl group at C3 on the sterol is linked to the sugar by a glycosidic bond. Typical examples (glucosides of β -sitosterol and acyl β -sitosterol) are illustrated below (Fig. 2.64).

Phytosterols are bioactive compounds naturally occurring in vegetable oils and in cereal grains like corn and rice. In addition to the free form, plant sterols occur as different types of conjugates in which the 3β -OH group is (1) esterified to fatty acids, (2) hydroxycinnamic acids or (3) glycosylated with a hexose (Fig. 2.65). Phytosterols/stanols and their esters have received much attention because of their capacity to reduce cardiovascular risk of coronary heart disease. Several functional foods (e.g., margarine) enriched with plant steryl fatty acid esters are commercially available. Ferulic acid esters, e.g., γ -oryzanol in rice, exhibit cholesterol-lowering and antioxidative properties. Therefore, they are used in Asia for therapeutic reasons predominantly.

Plant sterols have a role in plants like that of cholesterol in mammals, e.g., forming cell membrane structures. Plant sterols fall into one of three categories: 4-desmethylsterols (no methyl groups); 4-monomethylsterols (one methyl group) and 4,4-dimethylsterols (two methyl groups). The most common plant sterols are β -sitosterol, campesterol and stigmasterol and structurally these are very like cholesterol, belonging to the class of 4-desmethylsterols (Figs. 2.63 and 2.66). They include mainly campesterol, sitosterol, stigmasterol, and their respective stanols, which chemically resemble cholesterol. They are present in a normal

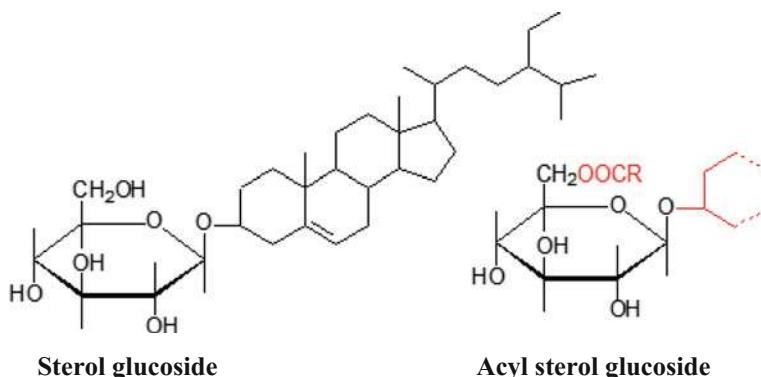


Fig. 2.64 Glucosides of β -sitosterol and acyl β -sitosterol

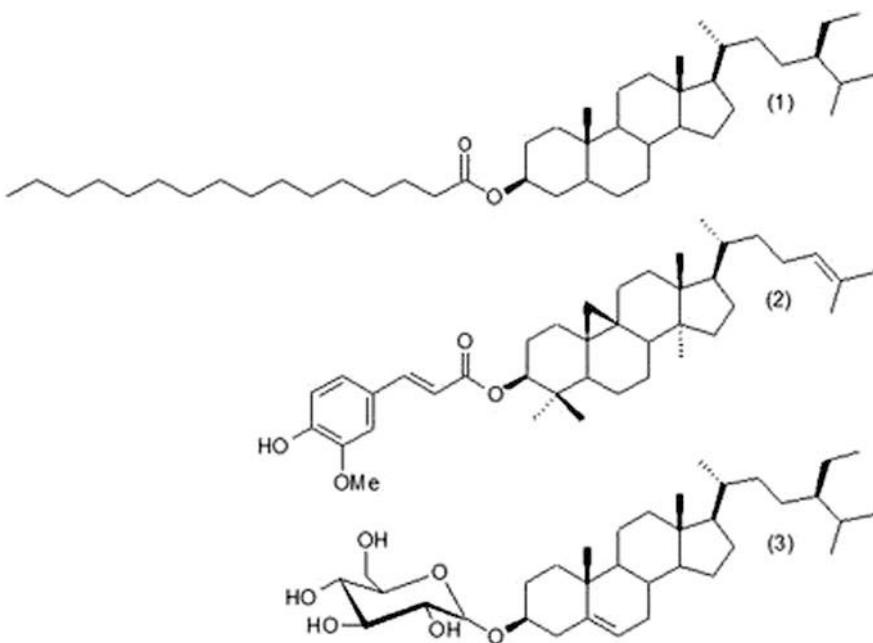


Fig. 2.65 Plant sterols as different types of conjugates in which the 3β -OH group is (1) esterified to fatty acids, (2) hydroxycinnamic acids or (3) glycosylated with a hexose

diet but less than 0.1% of serum sterols are plant sterols. A phytochemical-rich, plant-based diet is of importance in reducing risks of hormone-related neoplasms.

Stanol esters are a heterogeneous group of phytosterol esters with a saturated sterol ring structure known to reduce the level of low-density lipoprotein (LDL) cholesterol in blood when ingested. They can be found in trace amounts in every cell type but are highly enriched in foam cells (fat-laden immune cells of the type macrophage) and are common components of human skin oil. Cholesterol esters are of more frequent occurrence, e.g., butter and yolk contain cholesterol esters. In blood, cholesterol esters constitute the transport lipoprotein. Plant sterols are cholesterol-like molecules found in all plant foods, with the highest concentrations occurring in vegetable oils, are plant equivalents of cholesterol, and have a very similar molecular structure. According to their structure, plant sterols can be divided into sterols and stanols (a saturated subgroup of sterols). Plant sterols and stanols are substances that occur naturally in small amounts in many grains (rice, wheat, oat), vegetables (broccoli, cauliflower, tomato, brussels sprout), fruits (avocado, apples, blueberries, dill), legumes (pea, bean, lentil), nuts, and seeds (almond, peanuts, pecans, walnuts, sunflower seeds, pumpkin seeds, sesame seeds), oils (vegetable oils, rice bran oil, wheat germ oil) as well as some fortified foods (orange juice, margarine, cookies, energy bars, yogurt drinks). It is important to eat high sterol foods because these foods contain important vitamins and minerals, as

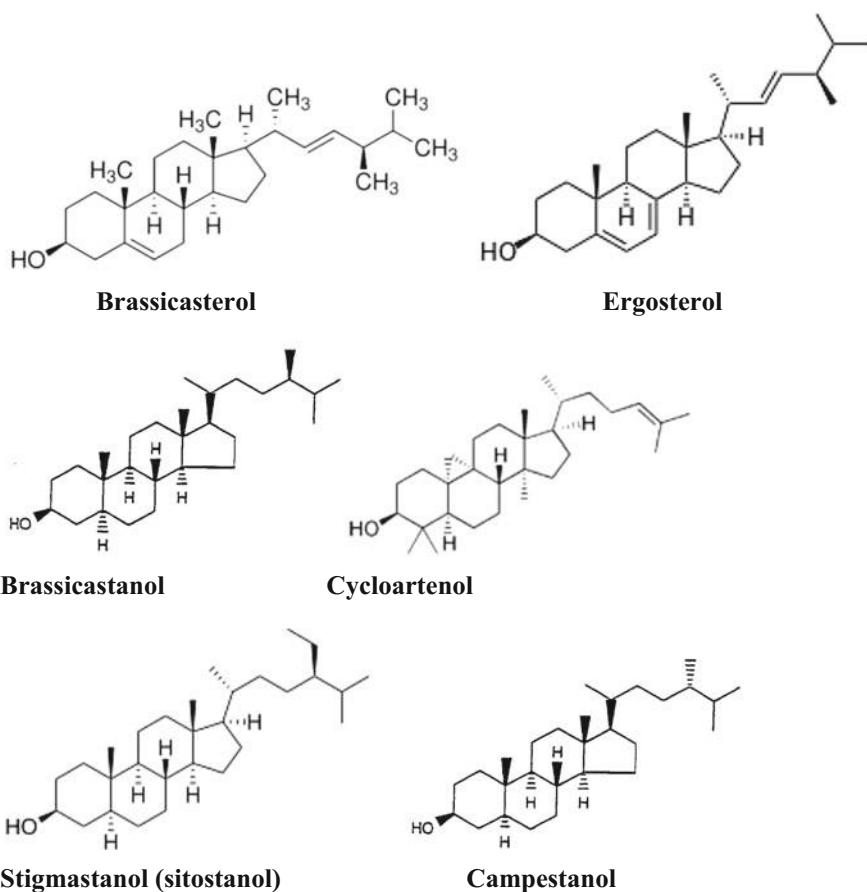


Fig. 2.66 Some common plant sterols and stanols—brassicasterol, ergosterol, brassicastanol, cycloartenol, stigmastanol (sitostanol) and campestanol

well as fiber. Plant sterol and stanol esters have been shown to reduce the level of low-density lipoprotein (LDL) cholesterol in blood, which helps to reduce the risk of heart disease. Plant sterol esters are used as dietary supplements and are added to certain oil-containing products like margarine, milk, or yogurt to make functional foods for controlling cholesterol levels. In the intestines, plant sterols interfere with cholesterol absorption and absorption rate of plant sterol is very low and more than 90% of sitosterol is passed through the fecal material. Cholesterol is esterified to long chain fatty acids and produces cholesterol esters. Cholesterol esters are much less polar than free cholesterol and appear to be the preferred form for transport in plasma and as a biologically inert storage (detoxification) form. They do not contribute to membranes but are packed into intracellular lipid particles. Cholesterol esters are major constituents of the adrenal glands, and they accumulate in the fatty lesions of atherosclerotic plaques. Stigmastanol is one of a

group of plant sterols that include β sitosterol, campesterol, ergosterol (provitamin D₂), brassicasterol, delta-7-stigmasterol, and delta-7-avenasterol that are chemically similar to animal cholesterol. Phytosterols are insoluble in water but soluble in most organic solvents and contain one alcohol functional group. Stigmasterol is an unsaturated plant sterol occurring in the plant fats or oils of soybean, calabar bean, and rapeseed, vegetables, legumes, nuts, seeds, and unpasteurized milk (pasteurization will inactivate stigmasterol) and in a number of medicinal herbs, including *Ophiopogon japonicus*, *Mirabilis jalapa*, and American Ginseng. Stigmasterol may be useful in the prevention of certain cancers, including ovarian, prostate, breast, and colon cancers, a diet high in phytosterols may inhibit the absorption of cholesterol, possesses potent antioxidant, hypoglycemic and thyroid inhibiting properties.

Sterols such as fatty acid esters of stigmasterol, ergosterol, and β -sitosterol are the important sterols. Complex or heterolipids contain nonlipid components like phosphate, carbohydrate, protein, etc., in the molecule and constitute phospholipid, glycolipid, and lipoprotein molecules, respectively. Phospholipids are phosphate substituted esters of diverse organic alcohols (glycerol, sphingosine, diols etc.). These polar lipids are predominantly present in the cell membrane. In phosphoglycerides, one of the -OH groups forms an ester bond with phosphate instead of a fatty acid. Phosphatidic acid is the naturally occurring simplest phosphoglyceride. Phosphatidylethanolamine, phosphatidylcholine, phosphatidyl linositides, cardiolipin, plasmogens, phosphatidylserines, etc. are some other examples of phosphoglyceride (Fig. 2.67).

Simple glycerides contain same fatty acids and mixed glycerides contain different fatty acids. Glycerophospholipids or phospholipids are similar to true lipid except that one hydroxyl group of glycerol is replaced by the ester of phosphoric acid and an amino alcohol (i.e., triesters of glycerol that contain charged phosphate diester groups depending on the amino alcohol, these can be lecithins (containing choline) or cephalines (containing ethanolamine or serine), abundant in cell membranes.); together with other lipids, they help to control the flow of molecules into and out of cells; sphingolipids or sphingomyelins are phospholipids of an 18 carbon amino alcohol (sphingosine) instead of glycerol and also contain charged phosphate diester groups, they are essential to the structure of cell membranes and are abundant in brain and nerve cell membranes (Fig. 2.68); steroids contain steroid nucleus, consisting of three cyclohexane rings and one cyclopentane ring fused together, e.g., cholesterol; Vitamins A, D₂, E, and K₁ are fat soluble, therefore, considered lipids. They have important roles in vision, bone growth, and blood clotting.

Sphingomyelins are contained in the nerve tissue in large amounts and also in other organs like lung, liver, kidney, spleen, blood, etc.

Glycolipid, a lipid that contains carbohydrate groups, usually galactose but also glucose, inositol, or others; while it can describe those lipids derived from glycerol or sphingosine, with or without phosphates, the term is usually used to denote the sphingosine derivatives lacking phosphate groups (glycosphingolipids). Glycolipids are glycoconjugates of lipids that are generally found on the extracellular face of

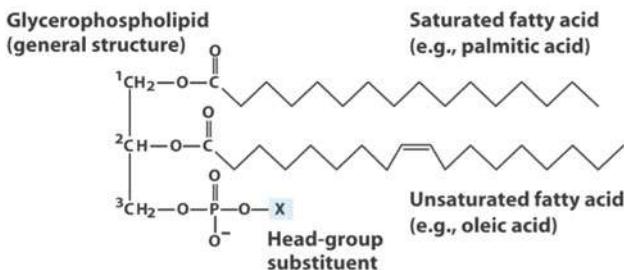
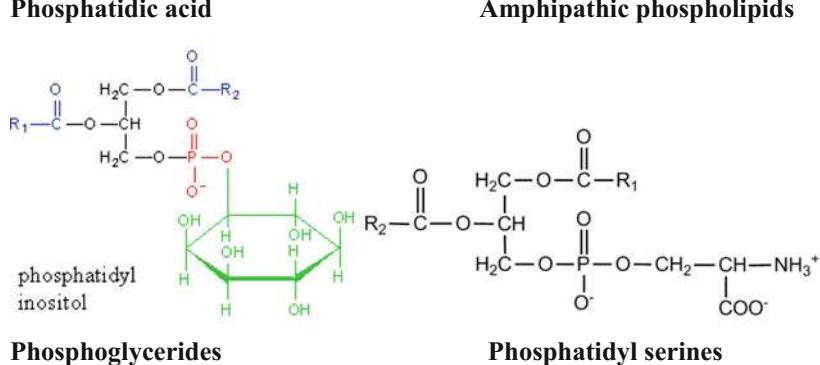
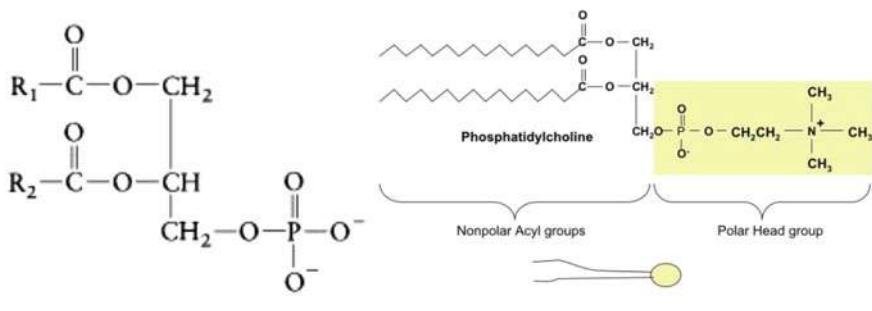
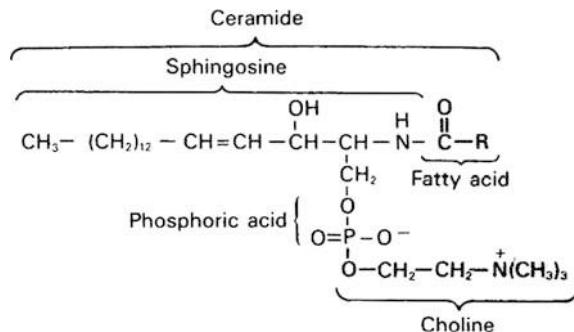


Fig. 2.67 Structure of phosphaglyceride—phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositides, cardiolipin, plasmogens, phosphatidyl serines, etc.

eukaryotic cellular membranes, and function to maintain stability of the membrane and to facilitate cell–cell interactions. Glycolipids can also act as receptors for viruses and other pathogens to enter cells. Gangliosides and cerebrosides that form glycosphingolipids (carbohydrate + sphingolipid) are two classes of glycolipids. Their role is to provide energy and also serve as markers for cellular recognition. The carbohydrates are found on the outer surface of all eukaryotic cell membranes. They extend from the phospholipid bilayer into the aqueous environment outside the cell where it acts as a recognition site for specific chemicals as well as helping to maintain the stability of the membrane and attaching cells to one another to form tissues. Glycolipids are membrane

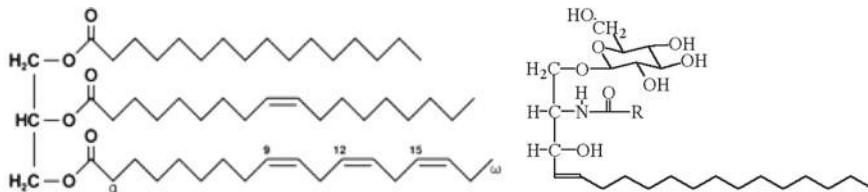
Fig. 2.68 Structure of sphingomyelins



components composed of lipids that are covalently bonded to monosaccharides or polysaccharides. Glycolipids include glyceroglycolipids (galactolipids and sulfolipids), glycosphingolipids (cerebrosides and galactocerebrosides) and others (Fig. 2.69).

Glycolipids, different amides derived from sphingosine, contain polar carbohydrate groups; on cell surfaces, the carbohydrate portion is recognized by and connects to intracellular messenger.

A lipoprotein is not a molecule but a biochemical assembly of particulate nature comprised of several thousand molecules of both proteins and lipids (Fig. 2.70). These particles solve the problem of lipid–water incompatibility via the amphiphatic nature of phospholipids. The monolayer lipids or their derivatives (e.g., cholesterol) may be covalently or non-covalently bound to the intrinsic proteins,



Unsaturated triesters of glycerol (palmitic, oleic & α -linolenic acids) Glycolipids

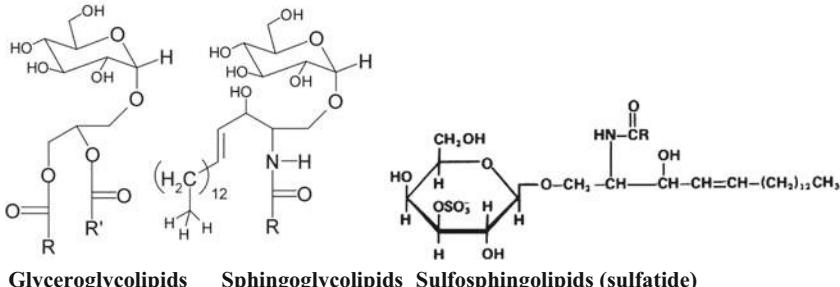


Fig. 2.69 Glycolipids, unsaturated triesters of glycerol, glyceroglycolipids, glycosphingolipids and sulfosphingolipids (sulfatide)

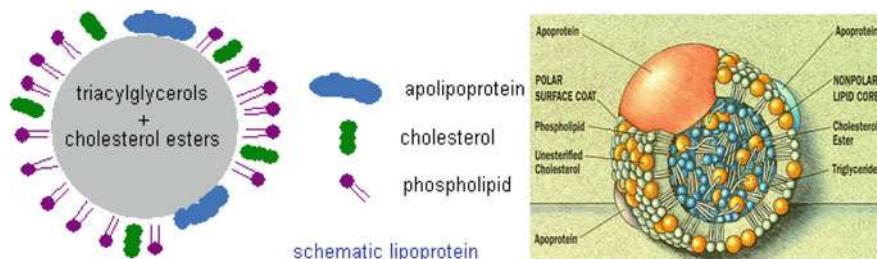


Fig. 2.70 General structure (schematic) of lipoprotein

which allow fats to move through the water inside and outside cells. The proteins serve to emulsify the lipid molecules. Examples include the plasma lipoprotein particles classified under high-density (HDL) and low-density (LDL) lipoproteins, which enable fats to be carried in the blood stream, many enzymes (lipoprotein lipase), transporters (chylomicrons, very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), LDL, HDL), structural proteins(α - and

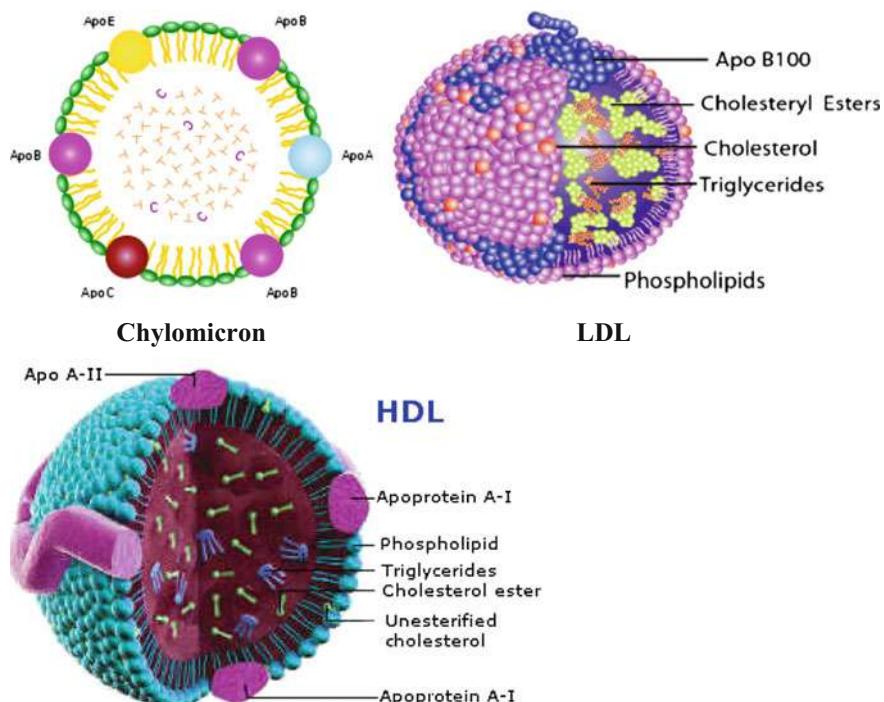


Fig. 2.71 Structure of lipoprotein chylomicron, LDL and HDL. ApoA, ApoB, ApoC, ApoE (apolipoproteins); T (triacylglycerol); C(cholesterol); outer phospholipid boundary (green in chylomicron or other colors in LDL, HDL)

Table 2.9 Size and molecular components of different transporter lipoproteins

Characteristic and molecular components lipoproteins						
Class	Density (g/ml)	Diameter (nm)	Protein (%)	Cholesterol (%)	Phospholipid (%)	Triglyceride (%)
HDL	>1.063	5–15	33	30	29	4
LDL	1.019–1.063	18–28	25	50	21	8
IDL	1.006–1.019	25–50	18	29	22	31
VLDL	0.95–1.006	30–80	10	22	18	50
Chylomicrons	<0.95	100–1000	<2	8	7	84

β -lipoproteins), antigens, adhesins and toxins, the transmembrane proteins of the mitochondrion and the chloroplast, and bacterial lipoproteins (Fig. 2.71).

The chief purpose of lipoproteins is to transport fats—mainly cholesterol and triglycerides—from place to place through the bloodstream. Fats are insoluble (they do not dissolve in water) so they have to be “packaged” in such a way that they can flow through the bloodstream. Lipoproteins form a container, made of specialized proteins called apolipoproteins, which enclose the fats, allowing them to be transported to their appropriate destinations. Chylomicrons are lipoproteins that deliver triglycerides from the intestines to the liver, muscle, and adipose (fat) tissue. The main apolipoprotein of chylomicrons is APO B-48. LDLs (low-density lipoproteins) carry cholesterol from the liver to tissues in the body. The main apolipoprotein of LDL is APO B-100. HDL (high-density lipoproteins) carries excess cholesterol from the body’s tissues back to the liver. The main apolipoprotein of HDL is APO-A.

The molecular components including their molecular diameter of different transporter lipoproteins are different (Table 2.9).

Lipoproteins are larger and less dense, if they consist of more fat than of protein. These lipid transporter lipoproteins characteristically carry different types of lipids to specific organs. Chylomicrons carry triglycerides (fat) from the intestines to the liver, skeletal muscle, and to adipose tissue. VLDL carry (newly synthesized) triacylglycerol from the liver to adipose tissue. Intermediate-density lipoproteins (IDL) are intermediate between VLDL and LDL. They are not usually detectable in the blood. Low-density lipoproteins (LDL) carry cholesterol from the liver to cells of the body. LDLs are sometimes referred to as the “bad cholesterol” lipoprotein. High-density lipoproteins (HDL) collect cholesterol from the body’s tissues, and bring it back to the liver. HDLs are sometimes referred to as the “good cholesterol” lipoprotein.

2.3.1.4 Plant Organic Acids

Plants contain an innumerable number of organic acids in the form of carboxylic acids, phenols etc.; they may be saturated or unsaturated and some of them are

volatile and others are nonvolatile. These acids are produced as metabolic intermediates and as amphibolites they participate in the synthetic or degradative pathways leading to the formation of more complex molecules or disintegrated into simple molecules in association with the liberation of energy. For example, Krebs cycle performs amphibolic (dual) functions, catabolic since the cycle degrades the acetyl residues into more simple substances (CO_2 and H_2O) with the liberation of energy and anabolic function since the cycle substrates are used in the synthesis of more complex materials (aspartic acid and glucose from oxaloacetate, glutamic acid from 2-oxoglutarate, heme from succinate). In plants, acids are produced in respiratory glycolytic pathway (pyruvic acid), Krebs cycle (at least 8 di-and tricarboxylic acids are produced, e.g., citric-, isocitric-, cisacconitic-, α -ketoglutaric-, succinic-, fumaric-, malic-, oxaloacetic-acids), pentose phosphate shunt; in photosynthetic Calvin-Basham, Hatch-Slack, CAM pathways; in lipid metabolic pathways, in secondary metabolic pathways like shikimic acid, mevalonic acid, etc. pathways.

The acids are found in the fruits, leaves, stem, and root stocks. The acid may occur in the free form, but is often combined as a salt or an ester. Oxalic acid occurs very frequently and widely distributed in plants, usually as the calcium salt, and apparently less frequently as the sodium and potassium salts. Succinic acid is found in many plants, and glutaric and adipic have been isolated from the sugar beet. Malic acid is found as the free acid and as the salts of malic acid in many plants, and particularly in apples and pears. Citric acid is found in tomatoes and it is found in smaller quantities in other foods, e.g., in cabbage, asparagus, and string beans. Tomatoes, which have the highest amount of acid of our common vegetables, contain about 0.42% citric acid. The most common acids in fruits, citric, and malic, may occur in different proportions or one alone may be present. The total acidity of most fruits varies with the variety and the degree of ripeness. The relative proportions of the various acids may also vary with the degree of ripeness, variety, and climatic and soil conditions. Rhubarb contains some oxalic acid; cranberries and plums some benzoic. Other acids sometimes found in small quantities are succinic, lactic, isocitric, and acetic. Gooseberry contains both citric and malic acids.

The citric acid of lemons, the tartaric acid of grapes, benzoic, cinnamic, salicylic, tannic acid, and some of their salts are of interest pharmacologically. Glycyrrhizin, the sweet principle of glycyrrhiza (licorice, *Glycyrrhiza glabra* of Fabaceae), is really glycyrrhizic acid, and is sweet to taste only in the form of alkaline salts. It is precipitated and rendered tasteless by acids.

Classification The plant acids may belong to different groups, e.g., the volatile and nonvolatile ones. Volatile acids volatilize and pass from the liquid to vapor state on exposure. The following acids of the $\text{C}_n\text{H}_{2n}\text{O}_2$ series are volatile, and are arranged according to their degree of volatileness from high to low; each acid contains only one carboxyl group per molecule and so they are also called monocarboxylic acids (Table 2.10).

Formic acid (methanoic acid) is the simplest carboxylic acid. It is an important intermediate in chemical synthesis and occurs naturally in ant and bee venom and

Table 2.10 Straight chain saturated monocarboxylic acids and fatty acids

Straight chain saturated monocarboxylic acids and fatty acids				
C-atom	Common name	IUPAC/other name	Molecular formula	Source and use
1	Formic acid	Methanoic acid	HCOOH	Ant, bee venom, hair of <i>Urtica dioica</i>
2	Acetic acid	Ethanoic acid	CH ₃ COOH	Vinegar, plant juice
2	Glycolic acid	2-Hydroxy ethanoic acid	HOCH ₂ COOH	Sugarcane
3	Propionic acid	Propanoic acid	CH ₃ CH ₂ COOH	Propionyl-CoA, fermentation by <i>Propionibacterium</i> , preservatives
4	Butyric acid	Butanoic acid	CH ₃ (CH ₂) ₂ COOH	Milk, rancid butter, cheese, human vomit, exhibit butyrate paradox
5	Valeric acid	Pentanoic acid	CH ₃ (CH ₂) ₃ COOH	<i>Valeriana officinalis</i>
6	Caproic acid	Hexanoic acid	CH ₃ (CH ₂) ₄ COOH	Goat or other barnyard animals fat
7	Ethanthetic acid	Heptanoic acid	CH ₃ (CH ₂) ₅ COOH	–
8	Caprylic acid	Octanoic acid	CH ₃ (CH ₂) ₆ COOH	Coconuts and breast milk
9	Pelargonic acid	Nonanoic acid	CH ₃ (CH ₂) ₇ COOH	<i>Peltargonium</i>
10	Capric acid	Decanoic acid	CH ₃ (CH ₂) ₈ COOH	Coconut and palm kernel oil
11	Undecylenic acid	Undecanoic acid	CH ₃ (CH ₂) ₉ COOH	–
12	Lauric acid	Dodecanoic acid	CH ₃ (CH ₂) ₁₀ COOH	Coconut oil and hand wash soaps
13	Tridecyclic acid	Tridecanoic acid	CH ₃ (CH ₂) ₁₁ COOH	–
14	Myristic acid	Tetradecanoic acid	CH ₃ (CH ₂) ₁₂ COOH	Nutmeg
15	–	Pentadecanoic acid	CH ₃ (CH ₂) ₁₃ COOH	–
16	Palmitic acid	Hexadecanoic acid	CH ₃ (CH ₂) ₁₄ COOH	Palm oil
17	Margaric acid	Heptadecanoic acid	CH ₃ (CH ₂) ₁₅ COOH	–
18	Stearic acid	Octadecanoic acid	CH ₃ (CH ₂) ₁₆ COOH	Chocolate, waxes, soaps and oils
20	Arachidic acid	Icosanoic acid	CH ₃ (CH ₂) ₁₈ COOH	Peanut oil
Straight chain unsaturated monocarboxylic fatty acids				
–	Acrilic acid	2 propenoic acid	–CH ₂ =CHCOOH	Used in polymer synthesis
6	Oleic acid		CH ₃ CH ₂ CH=CHCH ₂ COOH	

(continued)

Table 2.10 (continued)

Straight chain unsaturated monocarboxylic fatty acids			
18	Linolic acid	C ₁₈ H ₃₀ O ₂	
18	Linolenic acid	C ₁₈ H ₃₀ O ₂	
Other acids			
Straight chain mono- and dicarboxylic amino acids			
2	Glycine	Aminoethanoic acid	NH ₂ CH ₂ COOH
3	Alanine	2-amino propanoic acid	CH ₃ CH(NH ₂)COOH
5	Valine	2-amino-3-methylbutanoic acid	HOOCCH(NH ₂)CH(CH ₃) ₂
6	Leucine	2-amino-4-methylpentanoic acid	HOOCCH(NH ₂)CH ₂ CH(CH ₃) ₂
3	Serine	2-amino-3-hydroxypropanoic acid	HOOCCH(NH ₂)CH ₂ OH
4	Threonine	2-amino-3-hydroxybutanoic acid	HOOCCH(NH ₂)CH(OH)CH ₃
3	Cysteine,	2-amino-3-sulphydrylpropanoic acid	HOOCCH(NH ₂)CH ₂ SH
6	Cystine,	2-amino-3-(2-amino-2-carboxy-ethyl) disulfanyl-propanoic acid	{SCH ₂ CH(NH ₂)COOH} ₂

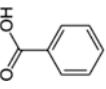
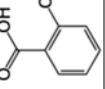
(continued)

Table 2.10 (continued)

Straight chain unsaturated monocarboxylic fatty acids			
5	Methionine	2-amino-4-(methylthio)butanoic acid	HOOCCH(NH ₂)CH ₂ CH ₂ SCH ₃
Cyclic aromatic, heterocyclic, hydroxy monocarboxylic amino acids			
9	Phenylalanine	2-amino-3-phenylpropanoic acid	
9	Tyrosine	L-2-amino-3-(4-hydroxyphenyl)propanoic acid	
11	Tryptophane	2-amino-3-(1H-indol-3-yl)propanoic acid	
	Histidine		
	Proline		
Mono- and dicarboxylic keto acids			
3	Pyruvic acid,	CH ₃ COCOOH,	Metabolites of glycolysis, acetoacetyl CoA, Krebs
4	Diacetic or	CH ₃ COCH ₂ COOH	cycle
4	Acetoacetic	COOHCOCH ₂ COOH, COOH	
5	acid,	(CH ₂) ₂ COCOOH	
	Oxalo acetic acid,		
	2-Oxoglutamate		

(continued)

Table 2.10 (continued)

Straight chain unsaturated monocarboxylic fatty acids			
Aromatic carboxylic acids			
7	Benzonic acid, Benzene carboxylic acid		
7	Salicylic acid	2-Hydroxy benzoic acid	Found in most berries, apples; used as an expectorant, analgesic, antiseptic and food preservative Found in white willow (<i>Salix alba</i>), active metabolite of aspirin (acetyl salicylic acid)
Straight chain saturated dicarboxylic acids			
2	Oxalic acid	COOH COOH	Leaves of Oxalis spp.
3	Malic acid	COOCH ₂ COOH	
4	Succinic acid	COOH(CH ₂) ₂ COOH	
4	Malic acid	COOCHOHCH ₂ COOH	Fruits of apple, pea, tomato, etc.
5	Glutaric acid	COOH(CH ₂) ₃ COOH	
4	Tartaric acid	COOH(CHOH) ₂ COOH	Fruits of tamarind, grape, tomato, etc.
6	Adipic acid	COOH (CH ₂) ₄ COOH	
Straight chain unsaturated dicarboxylic acids			
4	Fumaric acid	COOCH=CHCOOH	
Straight chain saturated tricarboxylic acids			
6	Citric acid	2-hydroxy propane-1,2,3-tricarboxylic acid	COOCH ₂ COHCOOCH ₂ COOH Fruits of <i>Citrus</i> spp.
6	Isocitric acid	1-hydroxypropane-1,2,3-tricarboxylic acid	COOCH ₂ CHCOOHCHOHCOOH
6	Aconitic acid	Prop-1-ene-1,2,3-tricarboxylic acid	COOCH=CCOOHCH ₂ COOH

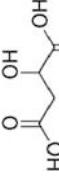
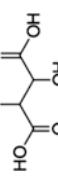
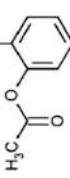
(continued)

Table 2.10 (continued)

Straight chain saturated tricarboxylic acids			
6	Propane-1,2,3-tricarboxylic acid (tricarballylic acid)	Propane-1,2,3-tricarboxylic acid	COOCH ₂ CHCOOHCH ₂ COOH
9	Trimesic acid	Benzene-1,3,5-tricarboxylic acid	
6	Ascorbic acid	(5R)-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one	
Alpha hydroxy acids (AHAs)			
3	Glyceric acid	2,3-dihydroxy propanoic acid	
2	Glycolic acid	2-hydroxyethanoic acid	
2	Lactic acid	2-hydroxy propanoic acid	

(continued)

Table 2.10 (continued)

Alpha hydroxy acids (AHAs)	
4	Malic acid, 
4	Tartaric acid 
9	Aspirin (acetylsalicylic acid) 

also in the stings of *Urtica dioica*. Formic and acetic acids have been obtained from plants during distillation. Esters, salts, and the anions derived from formic acid are referred to as formates. Formic acid is a colorless fuming liquid with a pungent penetrating odor; it irritates the mucous membranes and blisters the skin. It is miscible with water and most polar organic solvents, and soluble in hydrocarbons. In the vapor phase, it consists of hydrogen-bonded dimers but the gaseous formic acid does not obey the ideal gas law due to its hydrogen bond. Solid formic acid consists of an effectively endless network of hydrogen-bonded formic acid molecules. Formic acid is used as a preservative, as an acid reducing agent, as a coagulant in the production of rubber, in processing textiles and leather; applied on silage to promote the fermentation of lactic acid and to suppress the formation of butyric acid and as antibacterial agent in livestock feed because it arrests certain decay processes and causes the feed to retain its nutritive value longer. Formic acid has been shown to be an astringent, effective treatment against warts but may cause severe metabolic acidosis and ocular injury.

Acetic acid (ethanoic acid) is the second simplest carboxylic acid. Acetic acid has a distinctive sour taste and pungent smell. A dilute (4–8%) solution of acetic acid is called vinegar; a salt, ester, or acylal of acetic acid is called acetate. Pure acetic acid (glacial acetic acid) is a corrosive, colorless liquid, and completely miscible with water. Biologically, acetic acid is an important metabolic intermediate, and it occurs naturally in body fluids and in plant juices. Acetic acid is produced and excreted by acetic acid bacteria (*Acetobacter* sp., *Clostridium acetobutylicum*). It is an important chemical reagent and industrial chemical used in the production of cellulose acetate for photographic film and polyvinyl acetate for wood glue, as well as synthetic fibers and fabrics. In the food industry, acetic acid is used as food preservative, additive, acidity regulator, and condiment. Dilute acetic acid is often used in descaling agents. Acetic acid is produced industrially both synthetically (75% by the carbonylation of methanol), and by bacterial fermentation (10%). The biological route remains important for the production of vinegar because food purity laws claim vinegar of biological origin for food industry. Concentrated acetic acid is corrosive and attacks the skin. Diluted acetic acid is used in physical therapy using iontophoresis.

Propanoic acid (propionic acid) is a naturally occurring carboxylic acid. It is a clear liquid with a pungent odor. The anion, salts, and esters of propanoic acid are known as propanoates or propionates. In industry, propanoic acid is mainly produced by the hydrocarboxylation of ethylene using nickel carbonyl as the catalyst. Large amounts of propanoic acid were once produced as a by-product of acetic acid manufacture. Propanoic acid is produced biologically as its coenzyme A ester, propionyl-CoA, forms from the metabolic breakdown of fatty acids containing odd numbers of carbon atoms, and also from the breakdown of some amino acids. Bacteria of the genus *Propionibacterium* produce propanoic acid as

the end product of their anaerobic metabolism. This class of bacteria is commonly found in the stomachs of ruminants and the sweat glands of humans, and their activity is partially responsible for the odor of both Swiss cheese and sweat.

It is also biosynthesized in the large intestine of humans by bacterial fermentation of dietary fiber. Propanoic acid inhibits the growth of mold and some bacteria at the levels between 0.1 and 1% by weight. As a result, most propanoic acid produced is consumed as a preservative for both animal feed and food for human consumption.

Butyric acid (butanoic acid) is a fatty acid occurring in the form of esters in animal fats and plant oils. As a glyceride, it makes up 3–4% of butter; the disagreeable odor of rancid butter is that of hydrolysis of the butyric acid glyceride. Salts and esters of butyric acid are known as butyrates or butanoates. Butyric acid is found in milk, especially goat, sheep and buffalo milk, butter, Parmesan cheese, and as a product of anaerobic fermentation (including in the colon and as body odor). The acid is of considerable commercial importance as a raw material in the manufacture of esters of lower alcohols for use as flavoring agents; its anhydride is used to make cellulose butyrate, a useful plastic. Butyric acid is manufactured by catalyzed air oxidation of butanal (butyraldehyde). Butyric acid is present in, and is the main distinctive smell of, human vomit. Butyrate is produced as end product of a fermentation process solely performed by obligate anaerobic bacteria (e.g., *Clostridium*). The role of butyrate differs between normal and cancerous cells, called the “butyrate paradox” in which butyrate inhibits colonic tumor cells, and promotes healthy colonic epithelial cells; but the signaling mechanism is not well understood.

Valeric acid (pentanoic acid) is a straight chain alkyl carboxylic acid. Like other low-molecular-weight carboxylic acids, it has a very unpleasant odor. It is found naturally in the perennial flowering plant valerian (*Valeriana officinalis*), from which it gets its name. Its primary use is in the synthesis of its esters. Volatile esters of valeric acid tend to have pleasant odors and are used in perfumes and cosmetics. Ethyl valerate and pentyl valerate are used as food additives because of their fruity flavors.

Caproic acid (hexanoic acid) is the carboxylic acid derived from hexane with the general formula. It is a colorless oily liquid with an odor that is fatty, cheesy, waxy, and like that of goats or other barnyard animals. It is a fatty acid found naturally in various animal fats and oils, and is one of the chemicals that give the decomposing fleshy seed coat of the ginkgo its characteristic unpleasant odor. It is also one of the components of vanilla. The primary use of hexanoic acid is in the manufacture of its esters for artificial flavors, and in the manufacture of hexyl derivatives, such as hexylphenols. The salts and esters of this acid are known as hexanoates or caproates. Two other acids named after goats are caprylic (C8) and capric (C10) acids.

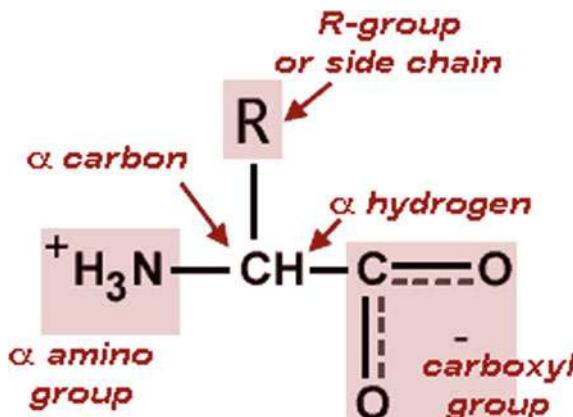
2.3.1.5 Amino Acids and Proteins (C, H, O, N, S & P)

Amino acids

Amino acids are organic acids consisting of an α -carbon (C_α) attached to a carboxyl functional ($\alpha\text{-COOH}$) group, an amino imino or amide functional group ($\alpha\text{-NH}_2$, $=\text{NH}$, $=\text{N}-$) group, a hydrogen atom, and a unique side chain (C_β), (R -group) (Fig. 2.72).

About 300–500 amino acids are known and can be classified according to the core structural functional groups' locations [alpha- (α)-, beta- (β)-, gamma- (γ)- or delta- (δ)-amino acids], on the basis of their polarity, acid/basic character, and side chain group type (aliphatic, acyclic, aromatic, containing hydroxyl or sulfur, etc.). Proteinogenic amino acids—PAAs, (amino acids that are precursors to proteins or building blocks of protein) are incorporated into proteins cotranslationally. There are 23 proteinogenic amino acids, but the genetic code (nuclear genes) of eukaryotes directly encodes only 20 standard amino acids ($L\text{-}\alpha$ -amino acids) for incorporation into proteins during translation. Selenocysteine and pyrrolysine are incorporated into proteins by distinct posttranslational biosynthetic mechanisms, and they are added in place of a stop codon when a specific sequence is present, UGA (uracyl, adenine, adenine) codon (one of 3 stop codons—UAA, UAG, and UGA) and selenocysteine insertion sequence (SECIS) element for selenocysteine, and UAG PYLIS (pyrrolysine insertion sequence) downstream sequence for pyrrolysine (Böck et al. 1991; Théobald-Dietrich et al. 2005). N -formylmethionine is often the initial amino acid of proteins in bacteria, mitochondria, and chloroplasts (often removed posttranslationally). Standard or proteinogenic 20 amino acids (in alphabetic order) are alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. Amino acids may also be classified as essential (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine), nonessential (alanine, asparagine,

Fig. 2.72 The general structure of an amino acid



aspartic acid, and glutamic acid), and conditional (arginine, cysteine, glutamine, tyrosine, glycine, ornithine, proline, and serine) amino acids. Essential amino acids cannot be synthesized within the body and as a result, they must be supplied through diet; body can synthesize nonessential amino acids while conditional amino acids are usually not essential, except in times of illness and stress.

Selenocysteine contains a selenol group on its β -carbon; Pyrrolysine is formed by joining to the ϵ -amino group of lysine a carboxylated pyrrolidine ring (Fig. 2.73).

There are various groups of amino acids such as (i) 20 standard pyrrolysine, (ii) 22 proteinogenic amino acids (20 standard amino acids plus 2 nonstandard selenocysteine and pyrrolysine amino acids), (iii) over 80 amino acids created abiotically in high concentrations, (iv) about 900 are produced by natural pathways, and (v) over 118 engineered amino acids have been placed into protein (Lu and Freeland 2006). Pyrrolysine, an α -amino acid, is used in the biosynthesis of proteins in some methanogenic archaea and bacteria (Rother and Krzycki 2010), and contains an α -amino group and a carboxylic acid group in the protonated ($-\text{NH}_3^+$) and deprotonated ($-\text{COO}^-$) forms, respectively under biological conditions.

In nature, there exist mostly α -amino acids with L conformation, but there is some nonalpha amino acids (amino group displaced further from the carboxylic acid end of the amino acid molecule and bonded to either α , β , or γ carbon) with non-L(D) conformation (Fig. 2.74).

These amino acids may perform various functions such as a precursor to coenzyme A (β -alanine), neurotransmitter in animals (γ -Aminobutyric acid (GABA), is found as an intermediate in tetrapyrrole biosynthesis in haem, chlorophyll, cobalamin, etc. (δ -Aminolevulinic acid) and as an intermediate in folate biosynthesis (p-Aminobenzoic acid (PABA). In some fungi, α -amino isobutyric acid is produced as a precursor to peptides, and some exhibit antibiotic properties (Gao et al. 2011). It is similar to alanine, but possesses an additional

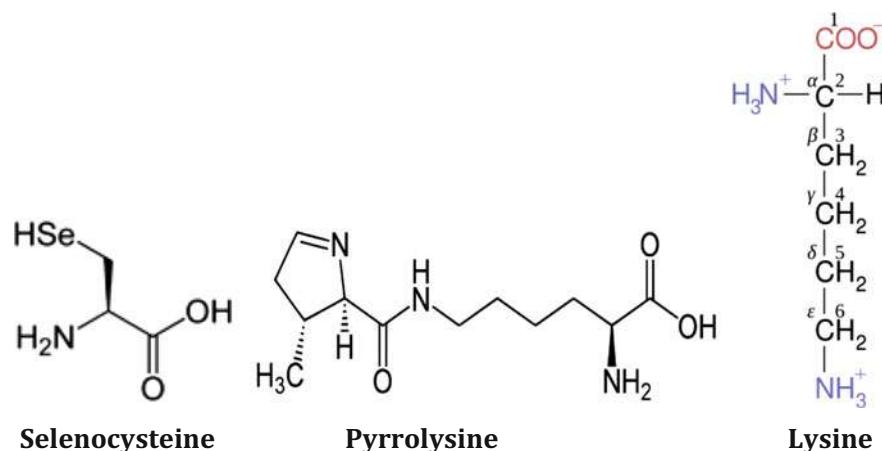


Fig. 2.73 Structure of selenocysteine, pyrrolysine and lysine

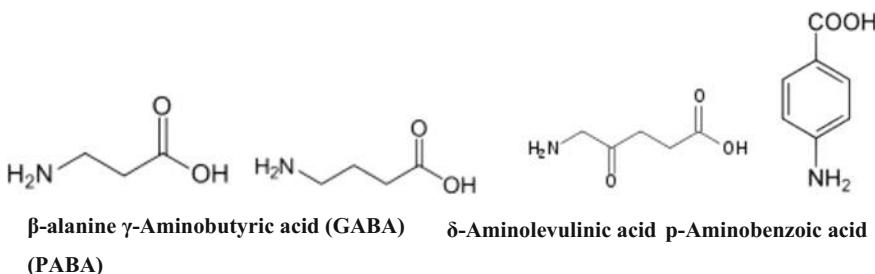
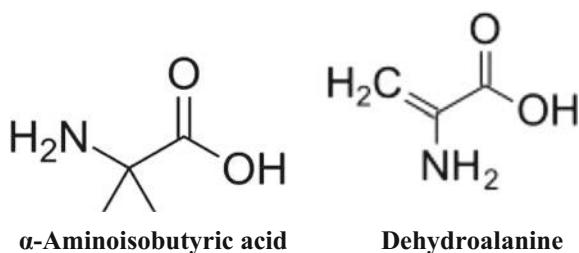


Fig. 2.74 Structure of nonalpha amino acid molecule bonded to either α , β , or γ carbon with non-L(D) conformation

Fig. 2.75 Structure of α -aminoisobutyric acid dehydroalanine



methyl group on the α -carbon instead of hydrogen. Dehydroalanine is similar to alanine without an α -hydrogen but a methylene side chain. It is one of several naturally occurring dehydroamino acids (Fig. 2.75). D-alanine and D-glutamate, D-lysine, D-serine, D-tyrosine, D-proline and D-phenylalanine, D-tryptophan, D-methionine, D-leucineetc are some common D-amino acids. D-amino acids singly or in combination exhibit antibacterial action, effective in preventing biofilm, etc (Kolodkin-Gal et al. 2010; Hochbaum et al. 2011; Moran-Palacio et al. 2014). In animals, some D-amino acids are neurotransmitters.

Taurine is an amino sulfonic acid but not an amino acid. However, it is often considered as amino acid because of its requirement is closer to those of essential amino acids to suppress amino acid auxotrophy in cats. The osmolytes, sarcosine, and glycine betaine are derived from amino acids, but have a secondary and quaternary amine respectively. Examples of posttranslationally incorporation or modification of amino acids into protein are carboxylation of glutamate (for better binding of calcium cations), hydroxylation of proline (critical for maintaining connective tissues), modification of a lysine residue (formation of hypusine) (Vermeer 1990; Bhattacharjee and Bansal 2005; Park 2006).

Classification:

The 20 proteinogenic amino acids are divided into following 5 groups on the basis of their side chains.

I. Nonpolar aliphatic side groups amino acids

Glycine (gly, G)—has a single hydrogen atom as a side chain; alanine (ala, A)—has a methyl group (CH_3) as a side chain; valine (val, V), leucine (leu, L), and isoleucine (ile, I)—have a branched aliphatic side chain; methionine (met, M)—has a sulfur-containing linear aliphatic side chain. Six amino acids with nonpolar aliphatic side groups are shown in Fig. 2.76.

II. Polar-uncharged side groups amino acids

Serine (ser, S) and threonine (thr, T)—have a hydroxyl group in their side chain; cysteine (cys, C)—has a thiol (sulfhydryl) group in its side chain; proline (pro, P)—has an aliphatic side chain which is covalently attached to the α -amino group; glutamine (gln, Q) and asparagine (asn, N)—have an amide group in their side chains. Six amino acids with polar-uncharged side groups are shown in Fig. 2.77.

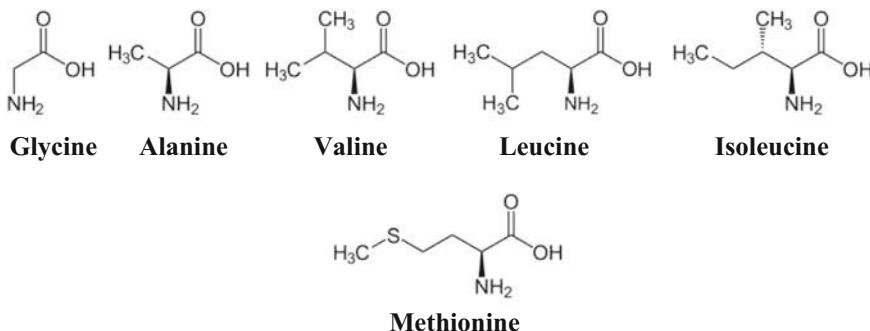


Fig. 2.76 Structure of six amino acids with nonpolar aliphatic side groups

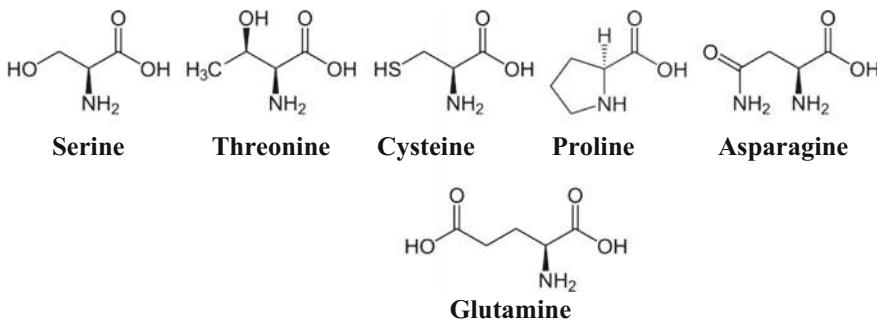


Fig. 2.77 Structure of six polar-uncharged side groups amino acids

III. Polar positively charged side groups amino acids

Lysine (lys, K) and asparagine (asn, N)—have nitrogen-containing groups in their side chains (amino group in lysine and guanidine group in arginine). These groups have high pKa and therefore tend to become protonated and positively charged at physiological pH. For this reason, lysine and arginine are referred to as basic amino acids. Histidine (his, H)—has an imidazole group in its side chain. This group has a pKa of ~6, and therefore has a 50% chance of being protonated (positively charged) or deprotonated (neutral) at physiological pH. This allows histidine to function in hydrogen transfer enzymatic catalysis, where it may function as the hydrogen donor, acceptor, or both. Three amino acids with positively charged side groups are shown in Fig. 2.78.

IV. Polar negatively charged side groups amino acids

Aspartate (asp, D) and glutamate (glu, E)—have a carboxyl group in their side chains. This group has a low pKa and tends to become deprotonated and negatively charged at physiological pH and therefore, aspartate and glutamate are referred to as acidic amino acids. Two amino acids with negatively charged side groups are shown in Fig. 2.79.

V. Aromatic side groups amino acids

Phenylalanine (phe, F)—has a phenyl group in its side chain; tyrosine (tyr, Y)—has a phenol group in its side chain; tryptophan (trp, W)—has an indole group in its side chain. Three amino acids with aromatic side groups are shown in Fig. 2.80.

Amino acids may also be classified on the basis of their chemical nature as acidic (aspartic acid, glutamic acid); basic (arginine, lysine, histidine); amides (asparagine, glutamine); aliphatic (alanine, glycine, isoleucine, leucine, valine); aromatic (phenylalanine, tryptophan, tyrosine); cyclic (proline); hydroxyl containing (serine, threonine, tyrosine); sulfur-containing (cysteine, methionine) amino acids; as well

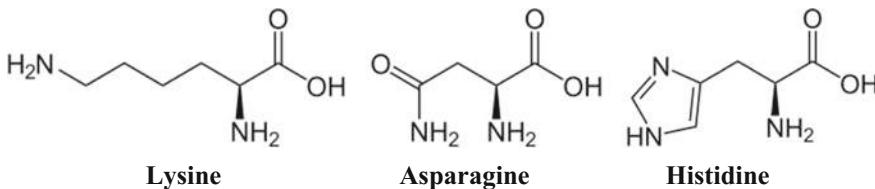


Fig. 2.78 Structure of polar positively charged side groups amino acids

Fig. 2.79 Structure of polar negatively charged side groups amino acids

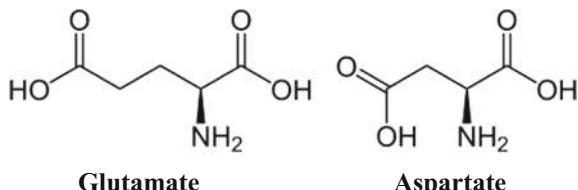




Fig. 2.80 Structure of aromatic side groups amino acids

as on the basis of their hydrophobic or nonpolar (with alkyl group side chain—alanine, glycine, isoleucine, leucine, valine, methionine, proline; with alkyl group side chain—phenylalanine, tryptophan) and hydrophilic or polar (neutral with polar side chains such as $-\text{OH}$, $-\text{SH}$ groups— serine, threonine, tyrosine cysteine, asparagine, glutamine; acidic-aspartic acid, glutamic acid; basic-arginine, lysine, histidine) properties.

Humans can synthesize 11 and the rest 9 must be supplied from outside through diet and so they are called essential amino acids. The 10 nonessential amino acids (body can synthesize them) are alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine and tyrosine. The nine essential amino acids include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Young 1994; Reeds 2000; Fürst and Stehle 2004). Amino acids arginine is essential for the young but adult can synthesize it. The human body cannot store excess amino acids for future use like starch and fat and therefore the essential amino acids must be supplied through food every day for necessary protein synthesis. Both L and D amino acids, the mirror image or enantiomers, exist in nature, but only L-amino acids are constituents of proteins. At isoelectric point (pI) ionizable amino acids form zwitterions.

Sources of amino acids

Amino acids are the building blocks of proteins and dietary proteins of plant and animal origin are the best sources of amino acids. Meat (beef, pork, lamb, goat, deer, etc.), poultry (chicken, turkey and others), fish and seafood (tilapia, halibut, tuna, salmon cod, sole, flounder, perch, etc.), eggs and dairy products (milk, yogurt, cheese, etc.) are some of the common sources of animal proteins and consequently, the most amino acids. Plant sources containing the highest quantity of these amino acids are almonds, avocados, figs, raisins, quinoa, *Spirulina*, watercress, green leafy vegetables, seeds of hemp, chia, soybeans, sesame, sunflower, pumpkin, wheat as well as sunflower butter. Outside protein synthesis, amino acids perform critical roles in processes such as neurotransmitter transport and biosynthesis. Biosynthetically PAAs are derived from keto acids like pyruvate (leucine, isoleucine, and valine), oxaloacetate (aspartic acid, asparagine, lysine, threonine, and methionine), α -ketoglutarate (glutamate, ornithine, arginine, proline, and hydroxyproline), and shikimate (phenylalanine, tyrosine, and tryptophan) or directly from the intermediate of the Calvin cycle (glycine, serine, cysteine). Biosynthesis of the heterocyclic amino acid histidine follows separate pathway (Goodwin and Mercer 1983) (Table 2.11).

Table 2.11 Main plant and animal food sources of 9 essential amino acids

Amino acids	Plant and animal food sources
1. Histidine	Soy protein, rice, wheat, rye, corn, seaweeds, beans, peanuts, cantaloupe, seeds of hemp and chia buckwheat, potatoes, cauliflower and sesame; eggs, parmesan
2. Isoleucine	Soy protein and tofu, rye, cashews, almonds, oats, lentils, beans, brown rice, cabbage, seeds of pumpkin, sunflower, sesame, hemp and chia, spinach, cranberries, quinoa, blueberries, apples, and kiwis; eggs, whitefish, pork, parmesan
3. Leucine	Seaweed, pumpkin, pea, rice, watercress, turnip greens, seeds of sunflower, sesame and kidney beans, figs, avocados, raisins, dates, apples, blueberries, olives and bananas; Eggs, whitefish, parmesan (cheese), smelts (fish)
4. Lysine	Beans, lentils, chickpeas, watercress, seeds of hemp and chia, spirulina, parsley, avocados, soybean, almonds, cashews; eggs, whitefish, parmesan (cheese), smelts (fish)
5. Methionine	Sunflower seeds and seed butter, seeds of hemp, sesame and chia, beans, soy protein, oats, wheat, rice, onions, figs, cacao, Brazil nuts, seaweed, and raisins; eggs, whitefish, smelts
6. Phenylalanine	Spirulina and seaweeds, pumpkin, beans, soy protein, peanuts, sesame, rice, avocado, almonds, peanuts, quinoa, figs, raisins, leafy greens, berries and olives; eggs, whitefish
7. Threonine	Spirulina, watercress, pumpkin, leafy greens, seeds of hemp, chia, soybeans, sesame, sunflower, soy protein and sunflower butter, almonds, avocados, figs, raisins, quinoa, and wheat; sprouted grains; eggs, whitefish, smelts
8. Tryptophan	Soy protein, sesame, beans, chickpeas, chia seeds, oats, seaweed, seeds of hemp and chia, pumpkin, sweet potatoes, beets, spinach, watercress, parsley, asparagus, mushrooms, lettuces, leafy greens, avocado, figs, winter squash, celery, peppers, carrots, onions, apples, oranges, bananas, quinoa, lentils, and peas; eggs
9. Valine	Soy protein, peanuts, seeds of sesame, hemp, and chia, beans, spinach, broccoli, whole grains, sprouted grains and seeds, figs, avocado, apples, blueberries, cranberries, oranges and apricots; eggs, parmesan, beef

On worldwide basis, α -amino acids, mainly protein amino acids (PAAs), are produced in large scale (~ 0.5 million tons per year) due to their wide use in medicine, agriculture (growth-stimulating food additives), and food industry (flavoring substances and preservatives), e.g., tryptophan (0.2–0.3 thousand tons), glycine (7–10 thousand tons), lysine (50 thousand tons), methionine (150–200 thousand tons), glutamic acid (>200 thousand tons) per year (Saghyan and Langer 2016). Methionine is used in medicine for the treatment and prevention of hepatotoxicity and diabetes, while a mixture of methionine and cysteine is used for the treatment of different kinds of poisoning. A mixture of glycine and glutamic acid is used to control gastric acidity. Pure glutamic acid is used for the treatment of CNS disorders (epilepsy, psychosis in children with polio, and mental retardation), and its sodium salt as flavoring and preservative in food.

Animal feed and human food industries are major consumers of amino acids. Many bulk components of animal feeds (e.g., soybeans) lack or contain low levels

of some essential amino acids like lysine, methionine, threonine, tryptophan, etc. and these are added in bulk quantities to feeds. Many amino acids are also used to chelate metal cations in order to improve the absorption of minerals in animals from supplements required for health improvement or production (Ashmead 1993; Leuchtenberger et al. 2005). The chelating ability of amino acids has been used in the human nutrition industry and in fertilizers to alleviate symptoms of mineral deficiencies in human (anemia) and plants (chlorosis) by facilitating the delivery of minerals such as iron. Glutamic acid and aspartame (aspartyl-phenylalanine-1-methyl ester) are used in food industry as flavor enhancer and low-calorie artificial sweetener, respectively (Stegink 1987; Garattini 2000). Amino acids produced industrially are also used in the synthesis of drugs and cosmetics (Leuchtenberger et al. 2005). Amino acid derivatives used in pharmaceutical industry include 5-HTP (5-hydroxytryptophan) for experimental treatment of depression (Turner et al. 2006), L-DOPA (L-dihydroxyphenylalanine) for treatment of Parkinson's disease (Kostrzewska et al. 2005) and eflornithine drug (inhibits ornithine decarboxylase) the treatment of sleeping sickness (Heby et al. 2007). Amino acids have been investigated as precursor chiral catalysts (Blaser 1992). Amino acids may be used as components of a range of water-soluble and biodegradable polymers (e.g., polypeptides, polyamides, polyesters, polysulfides, polyaspartate, polyurethanes, polycarbonates, etc.) for environmentally friendly packaging materials, drug delivery vehicle, disposable diapers, prosthetic implants, polycarbonates, etc (Sanda and Endo 1999; Gross and Kalra 2002; Bourke and Kohn 2003; Thombre and Sarwade 2005).

The 20 protein amino acids convey a vast array of chemical and functional diversity, e.g., they are either involved in synthesize proteins and other biomolecules or are oxidized to urea and carbon dioxide as a source of energy (Sakami and Harrington 1963). The exact and specific amino acid content, the sequence of amino acids in the protein molecule, etc., is determined by the sequence of the bases in the gene that encodes that protein through mRNA transcript. The chemical properties of the amino acids of proteins determine the biological and other cellular activities of the protein. The amino acid sequences in the protein contain the necessary information that determine the mechanism of protein fold into a three-dimensional structure as well as the stability of the resulting structure. The removal of amino group by a transaminase initiates the oxidation pathway; the amino group then enters the urea cycle and the keto acid (the other product of transamidation) enters the citric acid cycle (Brosnan 2000). Gluconeogenesis converts glucogenic amino acids into glucose (Young and Ajami 2001).

Amino acids carry out many important bodily functions in addition to their function in proteins synthesis as their building blocks. (i) Alanine is involved in sugar and acid metabolism, boosts up the immune system by producing antibodies, and provides energy for muscles tissues, brain, and the central nervous system. Alanine is used in pharmaceutical preparations for injection or infusion, in dietary supplement, and flavor compounds in Maillard reaction products and it is a stimulant of glucagon secretion. (ii) Arginine assists in wound healing and helps in burn treatment, enhances the production of T-cells necessary in normal immune system

activity, helps in vasodilation, chest pain, atherosclerosis (clogged arteries), heart disease or failure, erectile dysfunction, intermittent claudication/peripheral vascular disease, and vascular headaches (headache-inducing blood vessel swelling), enhancing sperm production, and preventing tissue wasting in people with critical illnesses. Arginine hydrochloride has high chloride content and has been used to treat metabolic alkalosis. (iii) Asparagine, along with glutamate, is an important neurotransmitter. Asparagine is required by the nervous system to maintain equilibrium and is also required for amino acid transformation from one form to the other. (iv) Aspartic acids are involved in transamination (aspartate \leftrightarrow oxaloacetate), also involved in immune system activity by promoting immunoglobulin production and antibody production, protects the liver and helps in detoxification of ammonia. Aspartate (conjugate base of aspartic acid) functions as a neurotransmitter. Along with few other amino acids, its primary role is to activate NMDA receptors (*N*-methyl-d-aspartate receptors) in brain (but not significant as glutamate's), used in coding of DNA. Aspartate plays important roles as acids in enzyme active centers, as well as in maintaining the solubility and ionic character of proteins. Aspartate, glycine, and glutamine are precursors of nucleotides (Stryer et al. 2007). (v) Cysteine can inactivate insulin in bloodstream (because of nucleophilic thiol groups), by reducing one of three disulfide bonds in insulin structure, and can be utilized in medicine and pharmaceutic in a patient experiencing hypoglycemia attack due to the high level of insulin. Cysteine promotes iron production in iron deficiency anemia, and assists in lung diseases by increasing production of red blood cells. It is a key active site residue in many important proteins (e.g., glyceraldehyde-3-phosphate dehydrogenase, glutathione reductases). (vi) Glutamine is the most abundant amino acid in the body, circulates in the blood and is able to cross the blood–brain barrier directly. Glutamine performs various functions such as protein synthesis, helps to maintain neutral pH in the liver by balancing the acid and base levels, is capable of fueling cell like glucose, donates nitrogen to cells via anabolic reactions and provides carbons in the citric acid cycle, provides energy to the small intestine as a primary energy source; provides energy to kidney, activated immune cells, and cancer cells (but not as a primary energy source). Within a cell, glutamine is essential for cell growth and protein translation. (vii) Glutamic acid is highly involved in metabolism in citric acid cycle, transamination (alpha-ketoglutarate with alanine or aspartate producing glutamate and pyruvate or oxalate respectively) and plays role in DNA synthesis. Glutamic acid assists in wound and ulcer healing, in the excitatory neurotransmitter and the metabolism of sugars and fats; aids potassium move through the blood–brain barrier and functions a source of fuel for the brain, can be used in correcting personality disorders and treating childhood behavioral disorders, in treating epilepsy, mental retardation, muscular dystrophy, ulcers, and hypoglycemic coma. Other minor uses include flavor enhancer, GABA precursor (gamma-aminobutyric acid precursor), nutrients, and fertilizers for plants. (viii) Glycine serves an important role in maintaining central nervous and digestive systems, prevents the breakdown of muscle by increase creatine, and keeps the skin firm and flexible. Glycine regulates blood sugar levels and helps to provide glucose for the body. Glycine serves as an inhibitory neurotransmitter in the central nervous system,

especially in the spinal cord. Glycine is a precursor of porphyrins such as heme (Shemin and Rittenberg 1946). (ix) Histidine is found in high concentrations in hemoglobin, it aids in the treatment of anemia and maintaining optimal blood pH; acts as a precursor of histamine and thus, involved in local immune responses. Histidine plays important roles in stimulating the inflammatory response of skin and mucous membranes; stimulates the secretion of the digestive enzymes gastrin and acts as the source and control for histamine levels. Histidine is required for growth and for the repair of tissues, as well as the maintenance of the myelin sheaths that act as protector for nerve cells. Histidine is also required to manufacture both red and white blood cells, it helps protect the body from damage caused by radiation and in removing heavy metals from the body, helpful in producing gastric juices, and people with a shortage of gastric juices or suffering from indigestion, arthritis and nerve deafness may benefit from this nutrient. (x) Isoleucine is needed for the formation of hemoglobin and to regulate blood sugar and energy levels; plays important roles in muscle strength and endurance, promotes muscle recovery after an intense workout and is a source of energy for muscle tissues. It is also involved in the formation of blood clots. Isoleucine deficiency may result in headaches, dizziness, fatigue, depression, confusion as well as irritability and may mimic the symptoms of hypoglycemia. (xi) Leucine is necessary in promoting growth in infant and regulating nitrogen concentration in adults. In many cases, functions of leucine are similar to that of isoleucine because of their similarity in branched hydrocarbon side chain. In addition, leucine facilitates skin healing and bone healing by modulating the release of natural pain reducers, enkephalins (a precursor of cholesterol) and increases the synthesis of muscle tissues by slowing down their degradation process. It is generally used as a flavor enhancer. (xii) Lysine inhibits viral growth and can be used in the treatment of Herpes Simplex and virus-associated Chronic Fatigue Syndrome, facilitates the formation of collagen (main component of fascia, bone, ligament, tendons, cartilage and skin), helps in absorption of calcium and thus facilitates the bone growth of infants, plays an essential role in the production of carnitine, converts fatty acids into energy, and helps to lower cholesterol. (xiii) Methionine is an antioxidant that neutralizes free radicals and removes waste in the liver, helps the breakdown of fat, reduces blood cholesterol levels, DNA and RNA synthesis. It is a precursor of several critical amino acids, hormones, and neurotransmitters in human body. Its AUG codon also serves as a “start” signal for ribosomal translation of mRNA. (xiv) Phenylalanine is a precursor of the amino acid tyrosine that gives rise to neurotransmitters (dopamine, norepinephrine and epinephrine), a powerful antidepressant and can enhance memory, thought, and mood, decreases blood pressure, promotes growth in infants and regulates nitrogen concentration in adults. Phenylalanine is a precursor of phenethylamine in humans and in plants, it is a precursor of various phenylpropanoids, which are important in plant metabolism. (xv) Proline plays role in protein's higher structure and function, it is important in healing, cartilage building, and in flexible joints and muscle support, helps reduce the sagging, wrinkling, and aging of skin. (xvi) Serine is a precursor of glycine and cysteine, found in phospholipids, active sites of trypsin and chymotrypsin; can synthesize pyrimidines and proteins, cysteine and tryptophan; involved in fat and

fatty acid formation, muscle synthesis. (xvii) Threonine is a precursor of isoleucine, aids the formation of elastin and collagen, aids in the formation of antibodies in the immune system, threonine, promotes growth and function thymus glands and absorption of nutrients. (xviii) Tryptophan is present in peptides, enzymes, and structural proteins. Tryptophan is a precursor of the neurotransmitter serotonin (Savelieva et al. 2008). (xix) Tyrosine helps in minimizing effects of the stress syndrome (depression treatment) as an adaptanogen, in drug detoxification, assists in treating vitiligo, pigmentation of skin, etc. Tyrosine is also an important precursor of epinephrine, norepinephrine, serotonin, dopamine, melanin, and enkephalins; affects the function of hormones by regulating thyroid, pituitary and adrenal glands. (xx) Valine is essential in muscle growth and development, muscle metabolism, and maintenance of nitrogen balance in the human body; can be used in the treatment of brain damage due to alcohol; can be used as an energy source in place of glucose.

Nonprotein amino acids (NPAAs)

The amino acids that are encoded directly by the codons of the universal genetic code are called standard or canonical amino acids while the nonstandard amino acids are nonproteinogenic except two such as selenocysteine (present in many non-eukaryotes and most eukaryotes) and pyrrolysine (present in some archaea and one bacterium). *N*-formylmethionine, a modified form of methionine, is often incorporated in place of methionine as the initial amino acid of proteins in bacteria, mitochondria, and chloroplasts. Nonprotein or nonproteinogenic or noncoded amino acids are not naturally encoded for protein synthesis or found in the genetic code of any organism. Despite the use of only 20 amino acids by the translational machinery to assemble proteins (the proteinogenic amino acids), over 140–200 amino acids are known to occur naturally in proteins and thousands more may occur in nature or be synthesized in the laboratory (Goodwin and Mercer 1983; Ambrogelly et al. 2007). Dietary exposure to the nonstandard amino acid beta-amino-L-alanine (BMAA) has been linked to human neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) (Holtcamp 2012; Cox et al. 2016), Parkinson's disease (PD), etc. However, nonprotein amino acids may have important roles as metabolic intermediates in humans such as in the biosynthesis of the neurotransmitter gamma-amino-butyric acid (GABA).

Many NPAAs are plant secondary metabolites and because of their similarity or resemblance in chemical structure, size, shape, and charge to protein amino acids (PAAs) and can be mistakenly used in protein synthesis, interfere in biochemical pathways, overstimulate receptors, or chelate metal ions (Rodgers et al. 2015). Because of structural similarity, nonprotein amino acid can be included in the composition of the protein, e.g., introduction of azetidine-2-carboxylic acid by tissues of *Phaseolus aureus* as a replacement of proline moieties in protein (Fowder and Lea 1979). NPAAs may be formed due to posttranslational modification of PAAs (5-hydroxytryptophan from tryptophan in legumes), modification of the synthetic pathways of PAAs (synthesis of β -parazylol-1-yl-alanine in cucurbits), or

independent biosynthetic pathway (formation of lathyrine in *Lathyrus* spp.) (Goodwin and Mercer 1983). Many nonprotein amino acids are incorporated in non-ribosomal peptides.

Some examples of NPAAs are citrulline, ornithine, arginosuccinic acid, theanine, thyroxine, triiodothyronine, trimethylglycine, taurine, homocysteine, DOPA, creatinine, β -alanine, γ -aminobutyric acid, etc. Many NPAAs are important because they are intermediates in biosynthesis, posttranslationally formed in proteins, possess a physiological role (e.g., components of bacterial cell walls, neurotransmitters, and toxins), natural or man-made pharmacological compounds, and are present in meteorites and in prebiotic experiments. About 700 amino acids are known from natural sources and at least 300 from plants. They are found mostly in a small number of families such as Fabaceae, seeds of Cucurbitaceae, Sapindaceae, Aceraceae, Hippocastanaceae microbes, seaweeds, from fungi, etc.

The nonprotein amino acids may play role in protecting plants against predators including insects, pathogens, and competing plant species (allelopathy). Nonprotein amino acids play an important role against the insect pests and high levels of nonprotein amino acids have been identified in certain plant groups (e.g., legumes and grasses) where they have been associated with resistance to insect herbivory, nitrogen storage (Huang et al. 2011). Generally, many of them found in food and fodder plants are found toxic to man and domestic animals (e.g., *Lathyrus*). They inhibit protein synthesis, disrupt urea synthesis and neurotransmission and are also incorporated into proteins with toxic effects. Specialized insect herbivores often possess specific mechanisms to avoid, detoxify nonprotein amino acids from their host plants or have evolved advanced tRNA synthetases that are able to discriminate between protein and nonprotein amino acids (Dunlop et al. 2015). In spite of these, some of them may have the potential of either as drugs or as leads to drugs in human and veterinary medicine (Bell 2003) (Fig. 2.81).

Nonprotein amino acids are common in plants and are present in widely consumed animal feeds and human foods, e.g., alfalfa (*Medicago sativa*) contains canavanine, lentil (*Lens culinaris*) contains homoarginine and *Lathyrus* species contain neurotoxic oxaryl-amino acid. Some occur in wild species that are inadvertently harvested with crop species. They may be passed along a food chain via animal intermediates (Nunn et al. 2010). Citrulline (in water melon), ornithine (in coconut, oats, soybean, wheat germs, gelatin, meat), theanine (in tea leaf), trimethylglycine (in beet, spinach, broccoli, whole grains, shellfish), taurine (in brewer's yeast, egg, fish and animal protein) are some of the examples of therapeutically important NPAAs. Citrulline is recommended for therapeutics against erectile dysfunction, ornithine acts as a detoxification agent in liver, theanine is a relaxant and aids in stress reduction, trimethylglycine assists in the formation of S-adenosylmethionine (SAM), an amino acid required for brain as antidepressant and taurine lowers cortisol levels, prevents diabetes, and fights against inflammation.

Vitamin B3 (pantothenic acid), which contains a fragment of the nonprotein amino acid β -alanine (3-aminopropionic acid) is used in polyneuritis, dermatoses, bronchitis, and venous ulcers. Nonprotein γ -aminobutyric acid acts as a mediator in

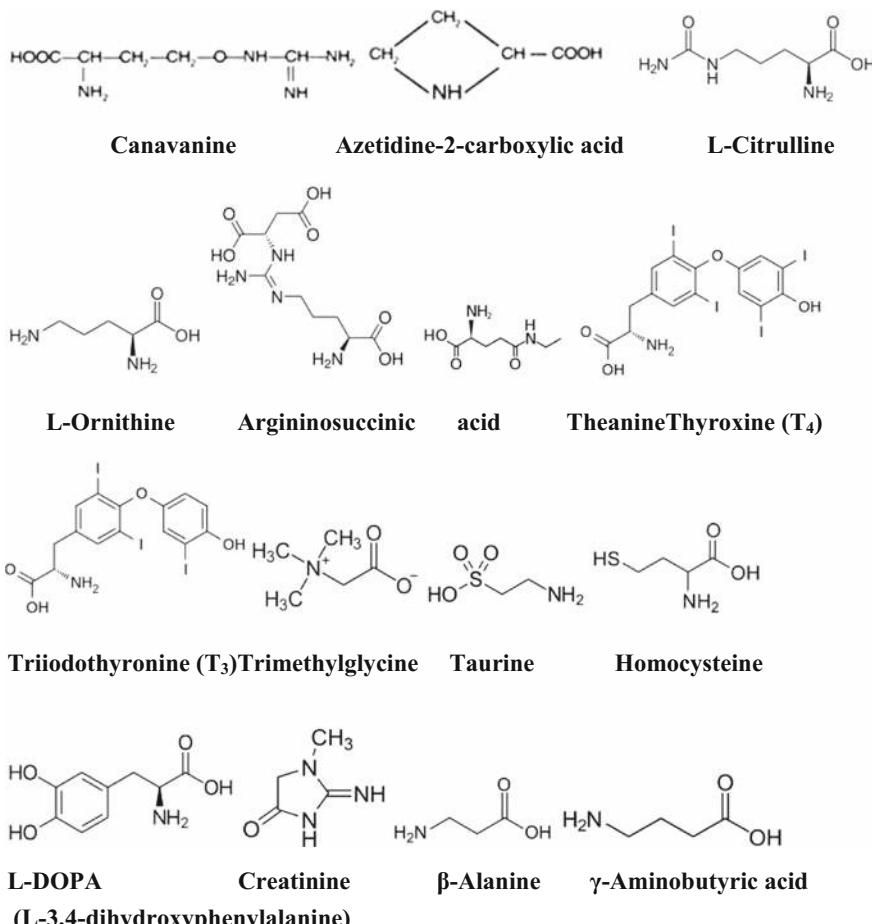


Fig. 2.81 Structure of nonprotein amino acids (NPAs)

the transmission of nerve impulses. γ -Aminobutyric acid (GABA) (aminolon, gammalon) is used to treat nervous system disorders, speech disorders, memory loss, cerebral vascular atherosclerosis, and mental retardation in children. 6-Aminohexanoic acid (ϵ -aminocaproic acid) is used in medicine to stop severe bleeding, as it helps in effective blood clotting.

Several oligomers, made up of α -amino acids, play an important role in body functions, and some of them are used in medical practice, e.g., methyl ether of L-asparagyl-L-phenylalanine dipeptide (aspartate, aspartame) is used for diabetes as low-calorie sugar substitute (150 times sweeter than glucose); a natural antibiotic *Gramicidin*, S-cyclic decapeptide—[Val-Orn-Leu-(D)-Phe-Pro]₂, produced by *Bacillus brevis*, has bacteriostatic and bactericidal action and is used to treat wounds, burns, and inflammatory diseases. *Gramicidin* is a polypeptide made up

from mixture of D- and L-amino acids (Ketcham et al. 1993). This antimicrobial peptide includes a D-form of phenylalanine and small peptides from several natural sources (e.g., leather tree frogs, snail's ganglion, poison spiders, etc.) were found to contain one or two D-amino acids. D-isomers are uncommon in live organisms but D-form of the amino acid moiety in such peptides greatly increases their resistance to hydrolytic action of exo- and endoproteases and this prolongates the action of oligopeptide drug substances (Soldatenkov et al. 2001). Tyrocidine and valinomycin also contain D-amino acids and these compounds disrupt bacterial cell walls, particularly in Gram-positive bacteria. Only 837 D-amino acids were found in Swiss-Prot database (187 million amino acids analyzed) (Khoury et al. 2011).

Proteins

Proteins are large polymeric molecules of 20 L- α -amino acids joined with each other by peptide bonds. Proteins are optically active, colloidal in nature, soluble in water or salt solution, show amphoteric property, and undergo denaturation on stress. Proteins in cell exist as enzymes, structural proteins and storage proteins. In cereal seeds, ~70% of the protein is gluten (consisting of equal amounts of prolammin and glutelin) and the rest consists of albumin and globulin almost in equal proportion. On the other hand, globulin dominates in oats. Albumin-globulin fraction dominates (>80%) in legume seeds. The proportion of amino acids in different protein fractions is also different, e.g., in barley seeds, albumin contains high proportion of glutamic acid, aspartic acid leucine and low amide-N, globulin contains high proportion of glutamic acid, glycine, arginine and low amide-N, porlamine contains high proportion of amide-N, proline and low lysine and glutelin contains high proportion of amide-N and proline. Major part of the leaf protein is located in chloroplast. Chloroplast protein in association with pigments forms chromoprotein and other proteins of the chloroplasts are enzymes. Enzyme proteins are also present in other cell organelles and cytoplasm of the cell (Goodwin and Mercer 1983). Proteins perform a vast array of functions in living organisms, e.g., provide cellular structure, cytoskeleton, and function as enzyme in metabolic reactions, DNA replication, signal transduction, antibody, storage, messenger or growth hormone and membrane transport.

Some plant protein sources do not always contain all the essential amino acids in required proportions, e.g., low or lacks one or more of the essential amino acids such as lysine, methionine, threonine, etc., rendering those incomplete proteins (Young and Pellett 1994). On the other hand, soybean, chia, seeds, etc. do contain all the essential amino acids while legumes (e.g., beans, lentils, peanuts, etc.) combined with cereal grains (e.g., wheat, rice, corn, etc.) yield a complete protein. An animal protein gelatin is incomplete because it lacks amino acid tryptophan. Foods of animal origin (e.g., meat, poultry, fish, whey, eggs, milk, cheese, yogurt, etc.) are considered complete protein sources. An incomplete protein source is one that is low in one or more of the essential amino acids, such as legumes, which lacks methionine. Many plant proteins are incomplete protein sources. Vegetarian meals may supply complete protein by the practice of protein combining which raises the amino acid profile through plant variety.

Classification of proteins based on composition and structure

(a) Based on composition, proteins classified as (i) Simple proteins, (ii) Conjugated proteins and (iii) Derived proteins.

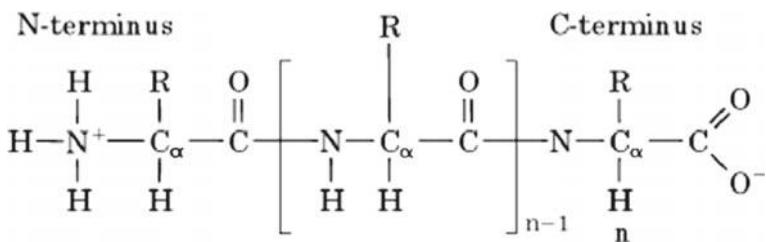
Simple Proteins are again classified according to solubility into Albumins, Globulins, Glutelins, Histories, Protamine, prolamines, and Scleroproteins. Conjugated proteins (polypeptide chain of amino acid + prosthetic group) are further divided into Glycoproteins, Chromoproteins, Lipoproteins, Nucleoproteins, and Phosphoprotein based on the nature of their prosthetic group. Derived proteins are the derivatives of proteins due to action of heat, enzymes, or chemical reagents and are grouped as primary derived and secondary derived proteins.

Structurally proteins are grouped as fibrous and globular while on the basis of function proteins are classified as storage, transport, structural material, metabolic growth regulator, control of physiological functions, catalytic activity, hormonal, and toxicity producing foreign proteins.

Proteins are divided into three main classes, e.g., globular proteins, fibrous proteins, and membrane proteins. Almost all globular proteins are soluble and many are enzymes. Fibrous proteins are often structural (collagen, elastin), the major component of connective tissue (cartilage), keratin (protein component of hair and nails). Membrane proteins often serve as receptors, carrier or provide channels for polar or charged molecules to pass through the cell membrane.

Animal sources of protein tend to deliver all the amino acids that body needs. Plant protein sources such as fruits, vegetables, grains, nuts and seeds, lack one or more essential amino acids. Nine out of the 20 amino acids are essential (e.g., phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine, and histidine, i.e., F V T W M L I K H; histidine is essential only for infants). Of the remaining 11 amino acids, 6 are conditional amino acids (e.g., arginine, cysteine, glycine, glutamine, proline and tyrosine (i.e., R C G Q P Y) because sick body or body under significant pathogenic and stresses may not be able to produce enough of these amino acids to meet needs. The remaining 5 (e.g., alanine, aspartic acid, asparagine, glutamic acid, and serine, i.e., A D N E S) are nonessential. An essential amino acid (or indispensable amino acid) cannot be synthesized de novo by the organism, and thus must be supplied in its diet (Young 1994). Conditionally essential amino acid synthesis can be limited under special pathophysiological conditions, such as prematurity in the infant or individuals in severe catabolic distress. Nonessential or dispensable amino acids can be synthesized in the body. Two or more amino acids may join with each other by peptide bonds ($-CO-NH-$) and thus develop di- or polypeptide chain with amino end (N-terminus) and carboxyl end (C-terminus) (Fig. 2.82).

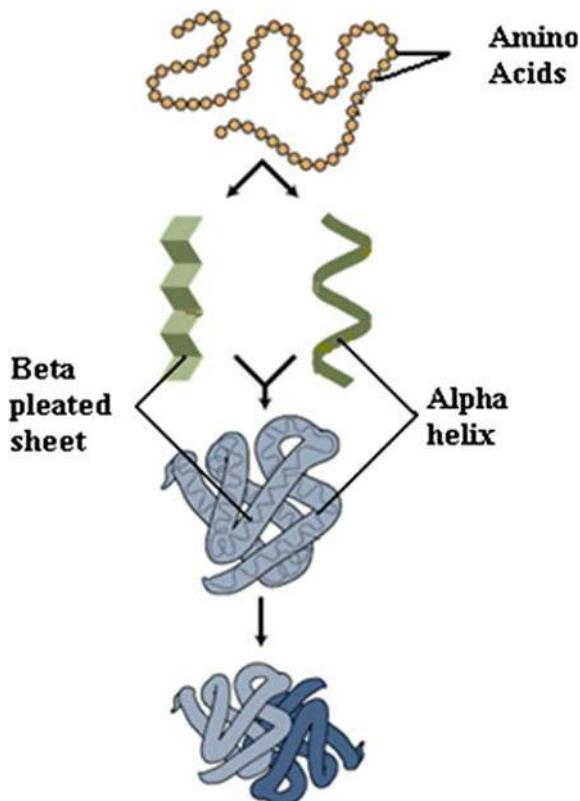
Biologically active proteins fold into one or more specific structural (spatial) conformations driven by a number of non-covalent interactions such as hydrogen bonding, ionic interactions, van der Waals forces, and hydrophobic packing. There are four structural levels of proteins such as primary, secondary, tertiary, and quaternary and a fully functional protein is assembled through these four levels of



Peptide chain with amino end (N-terminus) and carboxyl end (C-terminus)

Fig. 2.82 Structure of peptide chain with amino end (N-terminus) and carboxyl end (C-terminus)

Fig. 2.83 Structure of different structure levels of proteins



hierarchy (Fig. 2.83). Primary structure is the linear chain of amino acid sequence, a polypeptide or a polyamide chain. The sequence of amino acids determines the basic structure of the protein.

Primary (linear chain of amino acid sequence), secondary (alpha helix and beta pleated sheets—folded version of the linear polypeptide stabilized by hydrogen bonding), tertiary (several secondary structures are assembled together to develop tertiary structures in which, in addition to hydrogen bond, participate hydrophobic interactions, ionic interactions, salt bridges and disulfide bonds), and quaternary (several tertiary structures come together to develop a quaternary protein structure. The same forces of interactions operate in a quaternary structure as operate in a tertiary structure. The same forces of interactions operate in a quaternary structure as operate in a tertiary structure) structures of protein.

Unlike the rigid peptide bond, the other two bonds (bonds linking the amino group to the alpha carbon and the bond linking the alpha carbon to the carbonyl carbon) are free to rotate about the amide (peptide) bonds and allow the amino acids in the polypeptide chain to take on a variety of orientations. The enhanced freedom of rotation with regards to these two bonds allows proteins to fold into a variety of shapes to develop secondary protein structures (the folded version of the linear polypeptide stabilized by hydrogen bonding), which are essentially of two types—alpha helix and beta pleated sheets. These folded secondary structures are stabilized by the formation of hydrogen bonds between the amino acids. In an α helix, the amino acids get oriented in such a manner that the carbonyl, C=O, group of the n th amino acid can form a hydrogen bond with the amido, N—H, group of the ($n + 4$)th amino acid. This results in a strong hydrogen bond that has an optimum hydrogen to oxygen, H.... O, distance of 2.8 Å. The hydrogen bonds between the amino acids stabilize the α -helix structure. Unlike an α helix (where bonding occurs within the same polypeptide), in β sheets, hydrogen bonding occurs between neighboring polypeptide chains antiparallel (hydrogen-bonded chains extend in the opposite direction) and parallel (hydrogen-bonded chains extend in the same direction) manner to develop the antiparallel β sheet and the parallel β sheet, respectively.

Several secondary structures are assembled together to develop tertiary structures. In addition to hydrogen bond, amino acid side chains of the various secondary structures start interactions with each other including hydrophobic interactions, ionic interactions, and disulfide bonds. Nonlocal interactions generally stabilize tertiary structure, most commonly the formation of a hydrophobic core, but also through salt bridges, hydrogen bonds, disulfide bonds, and even posttranslational modifications. The tertiary structure is what controls the basic function of the protein.

Several tertiary structures come together to develop a quaternary protein structure, e.g., hemoglobin is a functional quaternary protein formed by the coming together of four tertiary structures, called globin proteins. The same forces of interactions operate in a quaternary structure as operate in a tertiary structure. Developmental assemblage of different structural levels of proteins from amino acids to hemoglobin, for example, may be comparable with that of a paragraph writing of a book (primary structure-alphabet, secondary structure-word, tertiary structure-sentence and quaternary structure-paragraph). Covalent bond (amide/peptide bond) is the lone bond of primary structure; in addition to covalent bond, hydrogen bonds are present in secondary structure; in addition to these two bonds,

ionic bonds, disulfide bonds, and hydrophobic interactions (van der Waals force) are operative in tertiary and quaternary structures of proteins.

Protein performs a wide array of important functions in human bodies, e.g., store amino acids, function as enzymes, antibodies, hormones (insulin), structural component (keratin and collagen connective tissue), and transporter (hemoglobin and myoglobin transport oxygen), etc. The human insulin is composed of 51 amino acids (5808 da), a dimer of an A-chain and a B-chain linked together by disulfide bonds (Fig. 2.84). Proteins are important for nutritional as well as for therapeutic purposes. The idea of protein therapy is similar to gene therapy, but unlike that, protein therapy delivers healing protein to a person in illness in specific amounts that would ordinarily be present absent in amount, to help repair illnesses, treat pain, or remake structures. It has wide-reaching healing possibilities in many fields such as diabetes, brain disease, and cancer (Frankel et al. 2002).

Until recently, pharmaceutical drugs were largely used based on relatively small organic molecules such as antibiotics, analgesics, hormones, and other pharmaceuticals synthesized by microbes, plants, animals, or by organic chemistry. But now protein-based large molecule drugs are the fastest growing class of drugs. These are used for the treatment of various diseases including infectious, diabetic,

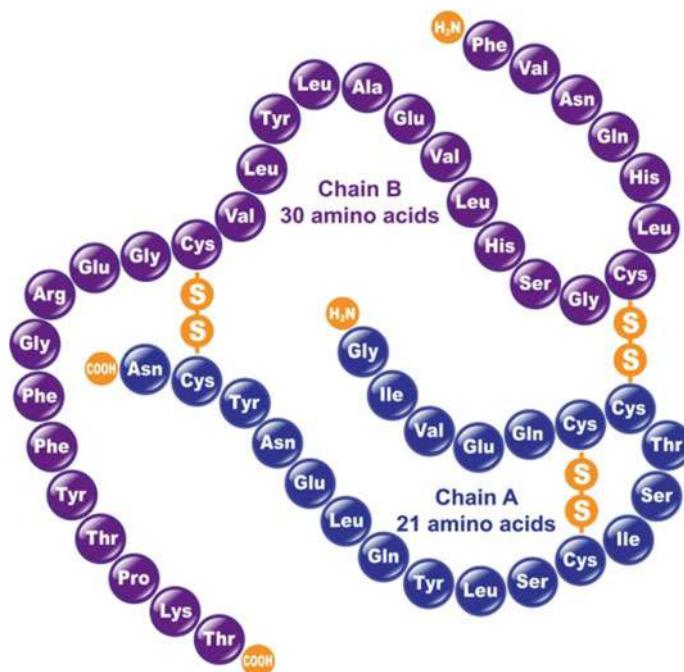


Fig. 2.84 Structure of human insulin, consisted of peptide chains A and B containing 21 and 30 amino acids, respectively. Chains A and B are linked together by two disulfide bonds and another disulfide bond is present within the A-chain (*source* Shutterstock)

inflammatory, cardiovascular, etc. diseases in humans. Traditionally used protein-based pharmaceuticals generally were manufactured through the expression of protein in bacterial, fungal, and mammalian cell cultures. More than 95 therapeutic proteins or peptides (biologics) have been licensed for production using bacterial, fungal, and mammalian cells grown in sterile cultures since 1982 and hundreds of additional therapeutic proteins are currently being developed and tested but it is anticipated that the capacity of cell culture facilities will fall far short of demand in the near future. Now, the production of more diverse pharmaceutical proteins, biologics or plant-made pharmaceuticals (PMPs), can be produced in transgenic plants developed through genetic engineering, i.e., plants engineered to produce specific proteins (Suslow et al. 2002; Thomas et al. 2002; Shama and Peterson 2004).

The use of transgenic plants will lower cost of production and easier expansion (simply by growing and harvesting additional plants) for large-volume production than cell culture systems (which require a large capital investment). About 50% of the total cost of production is in extraction and purification of the proteins common in both the systems. Plant expression systems can potentially produce hundreds of kilograms per year of a purified protein with tolerable investment while the cost of a similar production capacity using mammalian cell cultures may be simply prohibitive. In addition, unlike mammalian cells and bacterial cells, plant cells offer several advantages such as posttranslational modification, glycosylation, etc. in the production of diverse bioactive proteins for pharmaceutical purposes and to date, a large number of plants including tobacco, potato, tomato, corn, soybeans, alfalfa, rice, wheat are now available biofactories (Fischer et al. 2003; Ma et al. 2003; Streatfield et al. 2003; Goldstein and Thomas 2004). Plant-made pharmaceuticals like vaccines have several advantages over the conventional such as they are free from the risk of viral contamination, heat stable, edible, storable in seeds (Walmsley and Arntzen 2000; Daniell et al. 2001; Sala et al. 2003).

Recombinant protein therapies have many advantages compared to chemically synthesized drugs, e.g., high specificity, correct function, non-interpretation with other biological reactions, and non-induction of immunological responses (Leader et al. 2008). Plasma protein therapies are unique, biologic medicines that are either infused or injected to treat a variety of rare, life-threatening, chronic and genetic diseases including bleeding disorders, immune deficiencies, pulmonary disorders, neurological disorders, shock and trauma, liver cirrhosis and infectious diseases such as tetanus, hepatitis, and rabies. Protein-based therapeutics are highly successful in clinic and more than 100 genuine and similar number of modified therapeutic proteins are approved for clinical use in the European Union and the USA with 2010 sales of US\$108 bln. (Dimitrov 2012).

The protein therapeutics can be enzymes (digestion enzymes or other enzymes like urokinase), clotting factors (recombinant proteins produced in bacteria or in cell cultures), therapeutic antibodies (trastuzumab, or herceptin-antibody-dependent cell-mediated cytotoxicity, ADCC, therapeutic proteins are targeted against the tumor cells), antibody mimetics (Ecballantide, a 60-amino acid polypeptide),

vaccines (vaccines against human papilloma virus (HPV) and hepatitis B), and small peptide-based drugs (peptide hormones like insulin, glucagon).

Based on their pharmacological activity, they can be divided into five groups: (a) replacing a protein that is deficient or abnormal; (b) augmenting an existing pathway; (c) providing a novel function or activity; (d) interfering with a molecule or organism; and (e) delivering other compounds or proteins, such as a radionuclide, cytotoxic drug, or effector proteins (Dimitrov 2012).

2.3.1.6 Nucleic Acids (C, H, O, N & P)

Nucleic acids, polymeric molecules of nucleotides, are of two types, e.g., deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is usually double stranded, while RNA is usually single stranded. DNA consists of four types of nucleotides, e.g., adenine (A), guanine (G), thymine (T), and cytosine (C) while RNA, instead of thymine, contains uracil (U). Besides this, RNA contains ribose structure but 2-deoxy ribose is present in DNA molecule. DNA is found in the nucleus and mitochondria and chloroplasts and stores genetic information used for the synthesis of proteins (including enzymes). RNA is found in the nucleus, cytosol and mitochondria and they are of several types and performs different functions, e.g., messenger RNA (mRNA) carries genetic information obtained from DNA to sites that translate the information into a protein, transfer RNA (tRNA) carries activated amino acids to sites where the amino acids are linked together to form polypeptides, ribosomal RNA (rRNA) is a structural component of ribosomes (sites for protein synthesis), small nuclear RNA (snRNA) is a component of small nuclear ribonucleoprotein particles, which process heterogeneous RNA (hnRNA, the immature form of mRNA) into mature mRNA. MicroRNAs (miRNAs) are small RNAs that help to regulate gene expression. In some viruses, HIV, influenza, polio, RNA functions as the storage house of genetic information. The flow of genetic information involves a unidirectional flow from DNA to RNA to Protein (Fig. 2.85).

They are composed of nucleotides monomers, a nucleotide is made of three components: a 5-carbon sugar (ribose/2-deoxyribose sugar), a phosphate group, and a nitrogenous base (1 nitrogen base/nucleotide out of 5 bases). Nucleic acids are found in abundance in all life forms including eukaryotic and prokaryotic cells, cell organelles like mitochondria, chloroplasts and acellular viruses, and viroids, where they function in encoding, transmitting, and expressing genetic information. The encoded information is contained and conveyed via the nucleic acid sequence, which provides the “ladder-step” ordering of nucleotides within the molecules of RNA and DNA. Strings of nucleotides strung together in a specific sequence are the

DNA ----->mRNA----->Protein

Fig. 2.85 The unidirectional flow of genetic information from DNA to mRNA to protein

mechanism for storing and transmitting hereditary or genetic information and they (nucleotides) are bonded to form helical backbones (one for RNA and two for DNA) and assembled into chains of base pairs selected from the five primary, or canonical, nucleobases, (e.g., adenine, cytosine, guanine, thymine, and uracil; thymine occurs only in DNA and uracil only in RNA) (Fig. 2.86). Using amino acids and the process known as protein synthesis, the specific sequencing in DNA of these nucleobase pairs enables storing and transmitting coded instructions as genes. In RNA, base pair sequencing provides for manufacturing new proteins that determine the frames and parts and most chemical processes of all life forms.

Nucleic acids are macromolecules and DNA molecules are perhaps the largest individual molecules ever known. Nucleic acid molecules may range in size from 21 nucleotides (e.g., interfering RNA) to as large as 247 million base pairs constituting a single molecule of DNA (e.g., in human chromosome 1) (Gregory et al. 2006).

Naturally occurring DNA molecules are double-stranded (dsDNA) but may also be single-stranded DNA (ssDNA) while the RNA molecules are single-stranded (ssRNA). However, dsRNA viruses are known, e.g., rotaviruses in human, blue-tongue virus in cattle and sheep. Only a few human and animal pathogenic viruses are known that have a single-stranded DNA (ssDNA) genome (e.g., in Anelloviridae, Circoviridae). A circular ssDNA virus (e.g., torque teno virus) of the family Anelloviridae was first isolated from human in 1997.

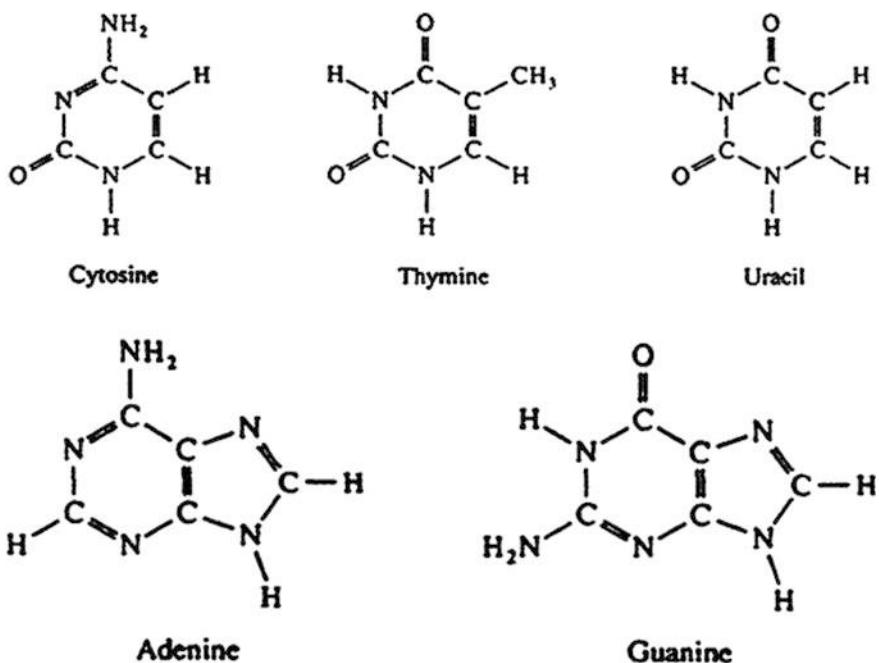


Fig. 2.86 Structure of different nitrogen bases

Nucleic acids are linear polymers (chains) of nucleotides. Each nucleotide consists of three components: a purine or pyrimidine nucleobase (sometimes termed *nitrogenous base* or simply *base*), a pentose sugar, and a phosphate group. The substructure consisting of a nucleobase plus sugar is termed a nucleoside. Nucleic acid types differ in the structure of the sugar in their nucleotides—DNA contains 2'-deoxyribose while RNA contains ribose (where the only difference is the presence of a hydroxyl group). Also, the nucleobases found in the two nucleic acid types are different: adenine, cytosine, and guanine are found in both RNA and DNA, while thymine occurs in DNA and uracil occurs in RNA.

The sugars and phosphates in nucleic acids are connected to each other in an alternating chain (sugar-phosphate backbone) through phosphodiester linkages (Stryer et al. 2007). In conventional nomenclature, the carbons to which the phosphate groups attach are the 3'-end and the 5'-end carbons of the sugar. This gives nucleic acids directionality, and the ends of nucleic acid molecules are referred to as 5'-end and 3'-end. The nucleobases are joined to the sugars via an N-glycosidic linkage involving nucleobase ring nitrogen (N-1 for pyrimidines and N-9 for purines) and the 1' carbon of the pentose sugar ring.

Nonstandard nucleosides are also found in both RNA and DNA and usually arise from modification of the standard nucleosides within the DNA molecule or the primary (initial) RNA transcript. Transfer RNA (tRNA) molecules contain a particularly large number of modified nucleosides (Rich and Raj Bhandary 1976).

Adenine and guanine are double-ring purine bases; thymine, cytosine, and uracil are single ring pyrimidine bases.

In nucleic acids chain, ribose (or 2-deoxy ribose) sugar and phosphoric acid form the backbone and phosphoric acids connect sugars by joining at its 3C or 5C position (3→5 or 5→3 direction) and each ribose sugar of the chain holds a nitrogen base at its 1C position. Sugar and nitrogen constitute nucleoside while phosphoric acid, sugar and nitrogen constitute nucleotide of the nucleic acid (Fig. 2.87, Table 2.12).

Nucleotides structure

Primary structure of nucleic acids

The sequence or order of the nucleotides defines the primary structure of DNA and RNA. When nucleotides polymerize to form nucleic acids, they follow a definite sequence such as the hydroxyl group attached to the 3' carbon of a sugar of one nucleotide forms an ester bond to the phosphate of another nucleotide with the elimination of a molecule of water (Fig. 2.88).

This condensation reaction may be comparable with peptide bond of protein formed between two amino acids. A single nucleic acid strand is a phosphate-pentose polymer (a polyester) with purine and pyrimidine bases as side groups. The ester bonds between the nucleotides are called phosphodiester bonds. A nucleic acid strand (similar to peptide chain) has an end-to-end chemical orientation: the 5' end has a free hydroxyl or phosphate group on the 5' carbon of its terminal sugar; the 3' end has a free hydroxyl group on the 3' carbon of its terminal

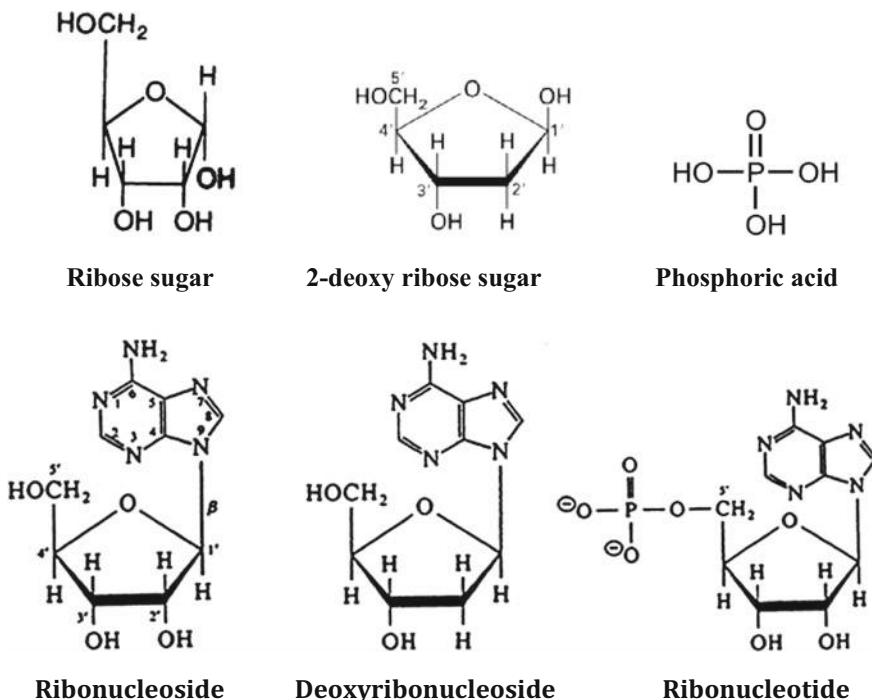


Fig. 2.87 Structure of different nucleotides

Table 2.12 Different nucleosides and nucleotides based on nitrogen base composition

Base	Ribonucleoside	Ribonucleotide
<i>N</i> -base	(Base + Ribose)	(Base + Ribose + Phos.)
Adenine (A)	Adenosine	Adenosine 5'-monophosphate (AMP)
Guanine (G)	Guanosine	Guanosine 5'-monophosphate (GMP)
Cytosine (C)	Cytidine	Cytidine 5'-monophosphate (CMP)
Thiamine	Thymidine	Thymidine 5'-monophosphate (TMP)
Uracil (U)	Uridine	Uridine 5'-monophosphate (UMP)

sugar (Fig. 2.89). This is why polynucleotide sequences are written and read in the 5'→3' direction (from left to right), e.g., the sequence AUG is assumed to be (5') AUG (3'). The 5'→3' directionality of a nucleic acid strand is an extremely important property of the molecule. The oxygen and nitrogen atoms in the backbone give DNA and RNA polarity.

Secondary structure of nucleic acids

Two nucleotides on opposite complementary DNA or RNA strands that are connected via hydrogen bonds are called a base pair (bp) and following the Watson-Crick base pairing model, a purine base always pairs with a pyrimidine

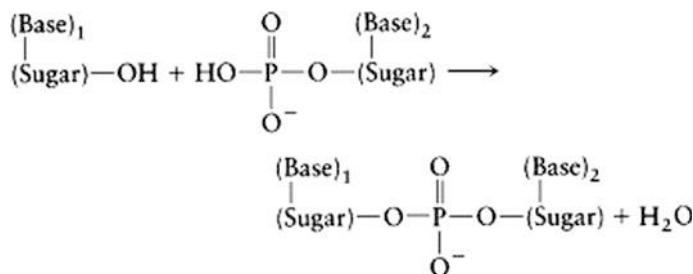


Fig. 2.88 End-to-end chemical orientation of nucleic acid strand: the *5' end* has a free hydroxyl or phosphate group on the *5' carbon* of its terminal sugar; the *3' end* has a free hydroxyl group on the *3' carbon* of its terminal sugar

base such as adenine (A) forms a base pair with thymine (T) and guanine (G) forms one with cytosine (C) in DNA and in RNA, thymine is replaced by uracil (U) (Fig. 2.90).

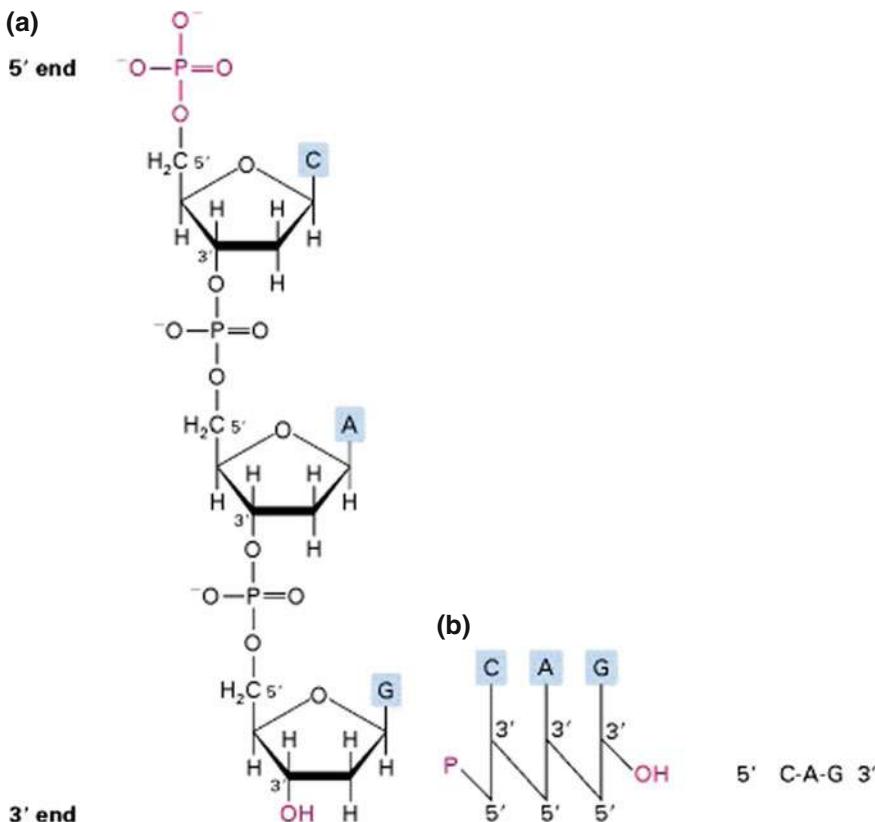
A purine base always pairs with a pyrimidine base or more specifically Guanosine (G) with cytosine (C) and adenine (A) with thymine (T) or uracil (U).

The G-C pair has three hydrogen bonds while the A-T pair has two hydrogen bonds. Alternate hydrogen bonding patterns (wobble base pair and Hoogsteen base pair) may also occur in RNA giving rise to complex and functional tertiary structures.

The secondary structure of DNA consists of two polynucleotide chains wrapped around one another to form a double helix. The orientation of the helix is usually right handed with the two chains running antiparallel to one another (Fig. 2.91). Structure of tRNA represents the secondary structure of RNA (Fig. 2.92).

The completion of human genome sequencing and elucidation of many molecular pathways of gene function related to diseases have provided unprecedented opportunities for the development of nucleic acid therapeutics, which include DNA therapeutics, oligonucleotide therapeutics, etc. The nucleic acid therapeutics has created an opportunity to handle many diseases that were generally considered undruggable by small molecule or protein therapeutics. The nucleic acid-based drugs have emerged in recent years and they are in early stages of clinical trials but they appeared to be extremely promising candidates for drug therapy to a wide range of diseases, including cancer, infectious diseases, diabetes, cardiovascular, inflammatory, and neurodegenerative diseases, cystic fibrosis, hemophilia, and other genetic disorders (Alvarez-Salas 2008; Pushpendra et al. 2012).

Therapeutic nucleic acids (TNAs) and its precursors are applied in medical treatment. TNA-based therapy can be classified into three main groups: (i) Therapeutic nucleotides and nucleosides; (ii) Therapeutic oligonucleotides; and (iii) Therapeutic polynucleotides. Therapeutic nucleotides and nucleosides that interfere with nucleic acid metabolism and DNA polymerization have been successfully used as anticancer and antiviral drugs, but they often produce toxic secondary effects related to dosage and continuous use. The use of oligonucleotides



A single strand of DNA with 5' end and 3' end

Fig. 2.89 Structure of a single strand of DNA **a** a trinucleotide containing only three bases: cytosine (C), adenine (A), and guanine (G) with free hydroxyl group at the 3' end and free phosphate group at the 5' end; **b** two common simplified methods of representing polynucleotides. Left—in the “stick” diagram, the sugars are indicated as vertical lines and the phosphodiester bonds as slanting lines; the bases are denoted by their single-letter abbreviations. Right in the simplest representation, the bases are indicated by single letters. By convention, a polynucleotide sequence is always written in the 5'→3' direction (left to right)

such as ribozyme and antisense oligodeoxynucleotides (AS-ODNs) promised as therapeutic moieties but faced several issues such as nuclease sensitivity, off-target effects and efficient delivery. Immuno stimulatory oligodeoxynucleotides and AS-ODNs represent the most successful group of therapeutic oligonucleotides in the clinic. A newer group of therapeutic oligonucleotides, the aptamers, is rapidly advancing towards early detection and treatment alternatives have reached the commercial interest.

DNA-based therapeutics include plasmids, oligonucleotides for antisense and antigenic applications, deoxyribonucleic acid aptamers, and deoxyribonucleic

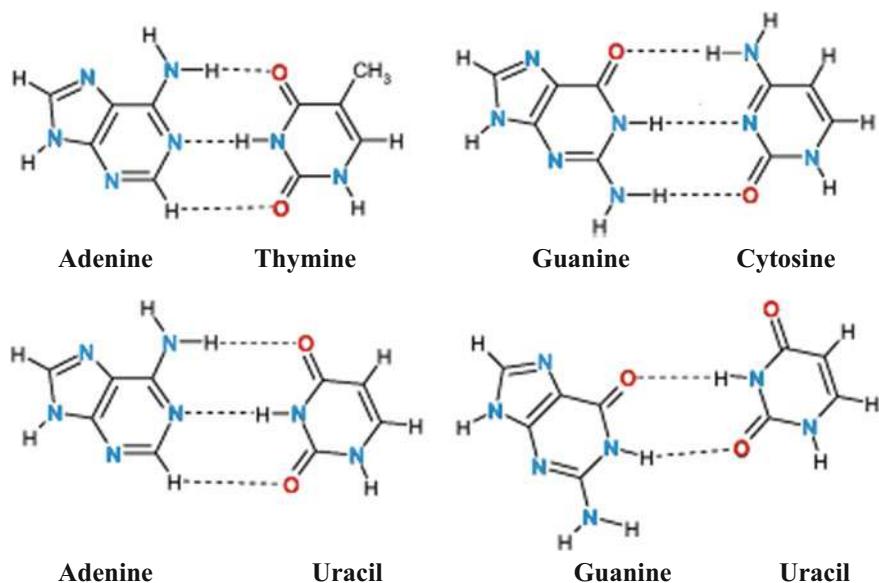


Fig. 2.90 Structure of complementary base pair of DNA or RNA strands

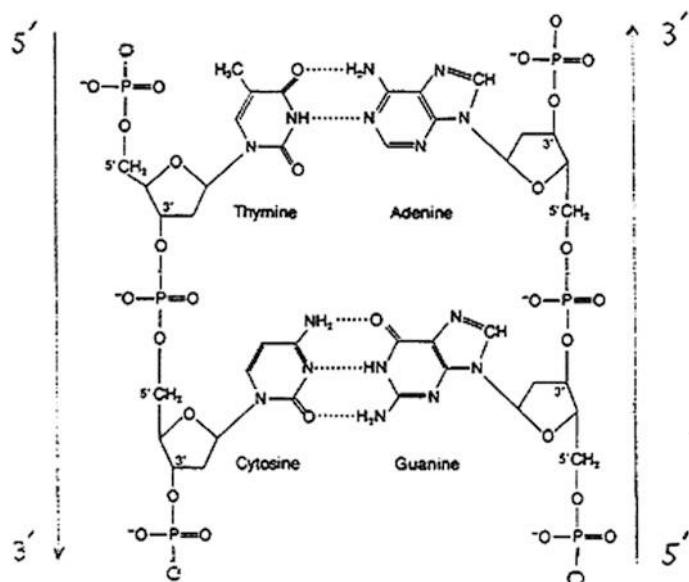


Fig. 2.91 The secondary structure of DNA

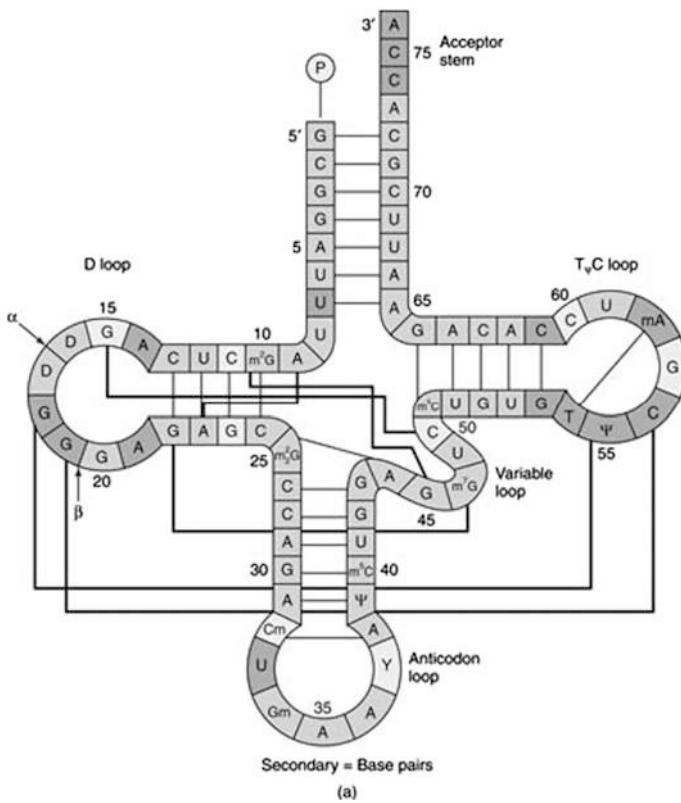


Fig. 2.92 Secondary structure of RNA—tRNA represents the secondary structure of RNA

acidzymes, while RNA based therapeutics includes ribonucleic acid aptamers, ribonucleic acid decoys, antisense ribonucleic acid, ribozymes, small interfering ribonucleic acid, and micro-ribonucleic acid. Constructed double-stranded DNA of plasmids containing transgenes may be used to biosynthesize therapeutic protein (Uherek and Wels 2000) and plasmids can be used as DNA vaccines for genetic immunization (Johnston et al. 2002). Plasmid-based gene therapy protocol in human was initiated in 1990 for the treatment of adenosine deaminase deficiency (Anderson 1998) and since then, >500 gene therapy protocols have been approved or implemented including severe combined immunodeficiency (SCID), head and neck squamous carcinoma (Vorburger and Hunt 2002; Otsu and Candotti 2002). Diseases with complex etiologies such as cancer, Alzheimer's and Parkinson's diseases (i.e., neurodegenerative diseases) are also considered for DNA-based therapeutics (Baekelandt et al. 2000; Galanis and Russell 2001; Mulherkar 2001). DNA vaccines for malaria, AIDS, allergic response, etc. are in development (Bunnel and Morgan 1996; Horner et al. 2001). DNA aptamers are double-stranded nucleic acid segments that can directly interact with proteins (Stull and Szoka 1995), interfere with the

molecular functions of disease-implicated proteins or those that participate in the transcription or translation processes and are preferred over antibodies in protein inhibition owing to their specificity, nonimmunogenicity, and stability of pharmaceutical formulation (Jayasena 1999). DNA aptamers have demonstrated promise in intervention of pathogenic protein biosynthesis against HIV-1 integrase enzyme (de Soultrait et al. 2002).

Oligonucleotides, short single-stranded segments of DNA, are used for antisense and antigenic applications as they interact and form a duplex with the mRNA or the pre-mRNA and inhibit its translation and protein synthesis finally. Antisense oligonucleotides are single-stranded nucleic acid analogs (14–20 nucleotides in length) that also silence genes by targeting individual mRNAs. An effective antisense RNA must bind to a specific mRNA and prevent translation of the protein. Phosphorothioate antisense oligonucleotides (*S*-oligos) are the first generation agents, alkyl modifications at the 2' position of the ribose (2'-*O*-methyl ribose) are second generation agents while the third generation antisense oligonucleotides contain a variety of modifications within ribose and phosphate backbone. Oligonucleotides, in therapy, can be used to selectively block the expression of proteins that are implicated in diseases (Akhtar et al. 2000), e.g., the first oligonucleotides antisense drug, fomivirsen sodium, was approved for the treatment of cytomegalovirus retinitis in AIDS patients in 1998 (Crooke 1998); MG98 and ISIS 5132 are in human clinical trials for cancer (Mardan et al. 2002).

Ribozymes are naturally occurring catalytic RNA molecules (~40 to 50 nucleotides in length) and maintain separate catalytic and substrate-binding domains and can be engineered to specifically cleave any mRNA sequence. Ribozymes can inhibit the expression of a variety of viral genes and the proliferation of organisms. DNAzymes are analogs of ribozymes with greater biological stability (Akhtar et al. 2000). The RNA backbone chemistry is replaced by the DNA motifs that confer improved biological stability. DNAzyme directed against vascular endothelial growth factor receptor 2 was confirmed to be capable of tumor suppression by blocking angiogenesis upon intratumoral injections in mice (Zhang et al. 2002).

Aptamers are oligonucleotide that binds well to proteins, amino acids, etc. and RNA aptamers (single-stranded nucleic acid segments) can directly interact with proteins (Stull and Szoka 1995) and recognize their targets on the basis of shape complementarity (Kaur and Roy 2008) and have demonstrated promise in the intervention of pathogenic protein biosynthesis against HIV-1 transcriptase (Chaloin et al. 2002). The RNA decoys can prevent translation or induce instability and, ultimately, destruction of the mRNA. Antisense RNA and ribozymes can selectively bind to target mRNAs and form a duplex having highly distorted confirmation that is easily hydrolyzed, and this hydrolysis of mRNA may be used for targeted suppression of specific gene (Mardan et al. 2002). Ribozymes can be used for knockout gene therapy by targeting overexpressed oncogenes such as the human epidermal growth factor receptor type-2 gene implicated in breast cancer (Aigner et al. 2001) and human papilloma virus infection. RNA interference (RNAi) is a method to achieve gene silencing by double-stranded RNA (dsRNA) segment. It is

a posttranscriptional mechanism of gene silencing through chromatin remodeling, inhibition of protein translation, or direct mRNA degradation (Caplen 2004; Dorsett and Tuschl 2004; Shankar et al. 2005). Small interfering RNAs (siRNAs) are double-stranded RNA analogs (two 22–27 nucleotide strands) and can be used for downregulation of disease-causing genes through RNA interference. Short hairpin RNA (shRNA) expressed by plasmid shows RNAi effect. MicroRNAs (miRNAs) are a class of naturally occurring, single-stranded small noncoding RNA molecules 21–25 nucleotides in length. These molecules are partially complementary to messenger RNA (mRNA) molecules, and their main function is downregulation of gene expression via translational repression, mRNA cleavage, and deadenylation.

Gene therapy is a technique for correcting defective genes responsible for disease development and theoretically, introduction of a normal gene following gene therapy into a cell with a defective gene may correct the disorder. Nucleic acid-based molecules (DNA, cDNA, complete genes, RNA, and oligonucleotides) are utilized as research tools within the broad borders of gene therapy and the emerging field of molecular medicine. Gene therapy may be classified into two types: somatic and germ line gene therapy. It is an extremely promising field of therapy to a wide range of diseases, including cancer, infectious diseases, diabetes, cardiovascular, inflammatory, and neurodegenerative diseases, cystic fibrosis, hemophilia, and other genetic disorders. The first human gene therapy trial was initiated in two adenosine deaminase (ADA)-deficient patient (a genetic disease of a 4-year-old girl made her defenseless against infections) on 14 September 1990 at the NIH Clinical Center. The patient received large dose of her own cells that had been engineered to carry a functional ADA gene. Ornithine transcarbamylase (OTC) deficient 18-year-old boy, for gene therapy at the University of Pennsylvania in 1999, was given a large dose of an adenoviral vector carrying the OTC gene but the case was fatal after 4 days due to a massive immune response. Now, various nonviral gene transfer systems as well as pure DNA constructs have been devised. Current gene therapy is experimental and has not proven very successful in clinical trials.

2.4 Sources, Chemistry, and Health Effects of the Bioactive Compounds of Primary Metabolic Origin

Higher plants produce a large number of diverse group of chemical compounds (>200,000 different structures). These compounds can be classified as belonging to primary or secondary metabolites, also called natural products. Primary metabolites are ubiquitous in all plants and fulfill essential metabolic roles. Primary metabolism governs all basic physiological processes that allow a plant to grow and set seeds, by translating the genetic code into proteins, carbohydrates, and amino acids, while secondary metabolism is connected to primary metabolism by using building blocks

and biosynthetic enzymes derived from primary metabolism. Primary plant metabolites are simple molecules or polymers of simple molecules (viz., chlorophyll, sugar, starch, total protein, ascorbic acid, organic acids, etc.), generally do not possess therapeutic property as such but they are essential for life activity of plants and they contain high-energy bonds. Primary metabolites determine the nutritional potential of plants and also serve as precursors (used up) for the synthesis of secondary metabolites (Tatsuta and Hosokawa 2006; Vijayvergia and Kumar 2007; Harada and Fukusaki 2009). Plants are the sources of many bioactive compounds containing many primary metabolites like, carbohydrates—starch and sugar, energy-providing carbohydrates, proteins, lipids, essential amino acids, fatty acids, ascorbic acid, chlorophyll, etc., are useful ingredients in medicine, nutraceuticals, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs. Therapeutic application of folate is important because its deficiency induces neural tube defects during early pregnancy; some fatty acids are critical in metabolism, cardiovascular health, inflammatory responses, and blood pressure regulation. The health benefits of green tea and its pleasant taste are due to the presence of bioactive compounds predominantly derived from secondary metabolic pathway, however, the composition of primary metabolite and secondary metabolites determines the ultimate quality of green tea. Among the tea quality parameters, tea polyphenol, free amino acid, and theanine concentrations increased, while the caffeine concentration decreased after CO₂ enrichment; CO₂ enrichment on photosynthesis and respiration in tea plants eventually modulated the biosynthesis of key secondary metabolites towards the production of a quality green tea.

In microorganisms, primary metabolites are typically formed during the growth phase and include glucides, lipids, alcohol (ethanol), organic acids (acetic acid, lactic acid, citric acid), nucleotides (5'guanylic acid), antioxidants (isoascorbic acid), amino acids (aspartic acid, L-glutamate and L-lysine) and amino acid derivatives, vitamins (B₂), and polyols (glycerol). Many of these metabolites can be used in industrial microbiology to obtain amino acids, develop vaccines and antibiotics, and isolate chemicals necessary for organic synthesis.

Microalgae, autotrophic prokaryotic, and eukaryotic microorganisms (~3–10 µm in length/dia), in freshwater or marine with different morphological, physiological, and genetic traits, are able to produce different biologically active metabolites. Microalgae including *Arthrospira* (*Spirulina*), *B. braunii*, *Chlorella vulgaris*, *Dunaliella salina*, *Haematococcus pluvialis*, *Nostoc*, etc., have been investigated for bioactive compounds (Mendes et al. 2006; Nobre et al. 2006; Palavra et al. 2011). Bioactive compounds of microalgal origin can be sourced directly from primary metabolism, such as proteins, fatty acids, vitamins, and pigments, or can be synthesized from secondary metabolism and such compounds can present antifungal, antiviral, antialgal, antienzymatic, or antibiotic actions (Volk 2008). Many compounds including cyanovirin, oleic acid, linolenic acid, palmitoleic acid, vitamin E, B12, β-carotene, phycocyanin, phycobilins, polysaccharides, sterols, lutein, zeaxanthin, etc., have antimicrobial antioxidant, and anti-inflammatory capacities, with the potential for the reduction and prevention of diseases (Smee et al. 2008; Ibañez and Cifuentes 2013; Markou and Nerantzis

2013). Bioactive metabolites of microalgal origin are of special interest in the development of new products for medical, pharmaceutical, cosmetic, and food industries. Microalgae are photosynthetic organisms that play a key role in aquatic ecosystems and ~40% of global photosynthesis is due to these microorganisms (Moreno-Garrido 2008). As microalgal metabolism reacts to changes in the external environment with changes in its intracellular environment, the manipulation of the culture conditions, or the presence or absence of certain nutrients, stimulates the biosynthesis of specific compounds.

References

- Aggarwal BB, Sundaram C, Malani N, Ichikawa H (2007) Curcumin: the Indian solid gold. *Adv Exp Med Biol* 595:1–75
- Aigner A, Juhl H, Malerczyk C, Tkybusch A, Benz CC, Czubayko F (2001) Expression of a truncated 100 kDa HER2 splice variant acts as an endogenous inhibitor of tumor cell proliferation. *Oncogene* 20:2101–2111
- Akhtar S, Hughes MD, Khan A (2000) The delivery of antisense therapeutics. *Adv Drug Deliv Rev* 44:3–21
- Albert A, Sareedenchai V, Heller W, Seidlitz HK, Zidorn C (2009) Temperature is the key to altitudinal variation of phenolics in *Arnica montana* L. c.v ARBO. *Oecologia* 160:1–8
- Alonso-Amelot ME, Oliveros-Bastidas A, Calcagno-Pisarelli MP (2007) Phenolics and condensed tannins of high altitude *Pteridium arachnoideum* in relation to sunlight exposure, elevation and rain regime. *Biochem Syst Ecol* 35:1–10
- Alvarez-Salas LM (2008) Nucleic acids as therapeutic agents. *Curr Top Med Chem* 8:1379–1404
- Ambrogelly A, Palioura S, Söll D (2007) Natural expansion of the genetic code. *Nat Chem Biol* 3(1):29–35
- Anan T, Nakagawa N (1974) Effect of light on chemical constituents in the tea leaves. *Nippon Nogeikagaku Kaishi* 48:91–98
- Anderson WF (1998) Human gene therapy. *Nature (Lond)* 392:25–30
- Ashihara H, Sano H, Crozier A (2008) Caffeine and related purine alkaloids: biosynthesis, catabolism, function and genetic engineering. *Phytochemistry* 69:841–856
- Ashmead HD (ed) (1993) The role of amino acid chelates in animal nutrition. Noyes Publications, Westwood
- Azuma H, Toyota M, Asakawa Y, Kawano S (1996) Naphthalene—a constituent of Magnolia flowers. *Phytochemistry* 42:999–1004
- Baekelandt V, De Strooper B, Nuttin B, Debysen Z (2000) Gene therapeutic strategies for neurodegenerative diseases. *Curr Opin Mol* 2:540–554
- Bell EA (2003) Nonprotein amino acids of plants: significance in medicine, nutrition and agriculture. *J Agric Food Chem* 51(10):2854–2865
- Bernays EA, Chapman RF (2000) Plant secondary compounds and grasshoppers: beyond plant defences. *J Chem Ecol* 26:1774–1794
- Bhattacharjee A, Bansal M (2005) Collagen structure: the Madras triple helix and the current scenario. *IUBMB Life (Int Union Biochem Mol Biol Life)* 57(3):161–172
- Blaser HU (1992) The chiral pool as a source of enantioselective catalysts and auxiliaries. *Chem Rev* 92(5):935–952
- Böck A, Forchhammer K, Heider J, Baron C (1991) Selenoprotein synthesis: an expansion of the genetic code. *Trends Biochem Sci* 16(12):463–467
- Bourke SL, Kohn J (2003) Polymers derived from the amino acid L-tyrosine: polycarbonates, polyarylates and copolymers with poly (ethylene glycol). *Adv Drug Deliv Rev* 55(4):447–466

- Briskin DP (2000) Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. *Plant Physiol* 124:507–514
- Brosnan JT (2000) Glutamate, at the interface between amino acid and carbohydrate metabolism. *J Nutr* 130(4S Suppl):988S–990S
- Brown GD (2010) The biosynthesis of artemisinin (Qinghaosu) and the phytochemistry of *Artemisia annua* L. (Qinghao). *Molecules* 15:7603–7698
- Bunnel BA, Morgan RA (1996) Gene therapy for HIV infection. *Drugs Today* 32:209–224
- Burchard P, Bilger W, Weissenböck G (2000) Contribution of hydroxycinnamates and flavonoids to epidermal shielding of UV-A and UV-B radiation in developing rye primary leaves as assessed by UV induced chlorophyll fluorescence measurements. *Plant Cell Environ* 23: 1373–1380
- Caplen NJ (2004) Gene therapy progress and prospects. Downregulating gene expression: the impact of RNA interference. *Gene Ther* 11:1241–1248
- Chaloin L, Lehmann MJ, Szczakiel G, Restle T (2002) Endogenous expression of a high-affinity pseudoknot RNA aptamer suppresses replication of HIV-1. *Nucleic Acids Res* 30:4001–4008
- Christie RJ, Alfinito MR, Walbot V (1994) Impact of low temperature stress on general phenylpropanoid and anthocyanin pathways: enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. *Planta* 194:541–549
- Coley PD, Bryant JP, Chapin SF (1985) Resource availability and plant antiherbivore defence. *Science* 230:895–899
- Cox PA, Davis DA, Mash DC, Metcalf JS, Banack SA (2016) Dietary exposure to an environmental toxin triggers neurofibrillary tangles and amyloid deposits in the brain. *Proc Biol Sci* 283(1823):20152397
- Crooke ST (1998) Vitravene another piece in the mosaic. *Antisense Nucleic Acid Drug Dev* 8:vii–viii
- Daniell H, Streatfield SJ, Wycoff K (2001) Medical molecular farming: production antibodies, biopharmaceuticals and edible vaccines in plants. *Trends Plant Sci* 6:219–226
- de Soultrait VR, Lozach PY, Altmyer R, Tarrago-Litvak L, Litvak S, Andreola ML (2002) DNA aptamers derived from HIV-1 RNase H inhibitors are strong anti-integrase agents. *J Mol Biol* 324:195–203
- Dimitrov DS (2012) Therapeutic proteins. *Methods Mol Biol* 899:1–26
- Dixon RA, Paiva NL (1995) Stress-induced phenylpropanoid metabolism. *Plant Cell* 7:1085–1097
- Dorsett Y, Tuschl T (2004) siRNAs: applications in functional genomics and potential as therapeutics. *Nat Rev Drug Discov* 3:318–329
- Dunlop RA, Main BJ, Rodgers KJ (2015) The deleterious effects of non-protein amino acids from desert plants on human and animal health. *J Arid Environ* 112(Part B):152–158
- Eckey-Kaltenbach H, Ernst D, Heller W, Sandermann H (1994) Biochemical plants responses to ozone. IV. cross-induction of defensive pathways in parsley (*Petroselinum crispum* L.) plants. *Plant Physiol* 104:67–74
- Eisner T, Meinwald J (eds) (1995) Chemical ecology: the chemistry of biotic interaction. National Academy Press, Washington, DC. <https://doi.org/10.17226/4979>
- EC, European Commission (2002) Opinion of the scientific committee on food on the risks to human health of polycyclic aromatic hydrocarbons in food. SCF/CS/CNTM/PAH/29
- Fischer R, Twyman RM, Schillberg S (2003) Production of antibodies in plants and their use for global health. *Vaccine* 21:820–825
- Fournier AR, Proctor JTA, Gauthier L, Khanizadeh S, Belanger A, Gosselin A et al (2003) Understory light and root ginsenosides in forest-grown *Panax quinquefolius*. *Phytochemistry* 63:777–782
- Fowler L, Lea PJ (1979) The nonprotein amino acids of plants. *Adv Enzymol* 50:117
- Frankel AE, Powell BL, Duesbery NS, Vande Woude GF, Leppla SH (2002) Anthrax fusion protein therapy of cancer. *Curr Protein Pept Sci* 3(4):399–407
- Fürst P, Stehle P (2004) What are the essential elements needed for the determination of amino acid requirements in humans? *J Nutr* 134(6 Suppl):1558S–1565S

- Galanis E, Russell S (2001) Cancer gene therapy clinical trials: lessons for the future. *Br J Cancer* 85:1432–1436
- Ganzena M, Guggenberger M, Stuppner H, Zidorn C (2008) Altitudinal variation of secondary metabolite profiles in flowering heads of *Matricaria chamomilla* cv BONA. *Planta Med* 74:453–457
- Gao X, Chooi YH, Ames BD, Wang P, Walsh CT, Tang Y (2011) Fungal indole alkaloid biosynthesis: genetic and biochemical investigation of the tryptoquinalanine pathway in *Penicillium aethiopicum*. *J Am Chem Soc* 133(8):2729–2741
- Garattini S (2000) Glutamic acid, twenty years later. *J Nutr* 130(4S Suppl):901S–909S
- Glynn C, Ronnberg-Wastljung AC, Julkunen-Tiitto R, Weih M (2004) Willow genotype, but not drought treatment, affects foliar phenolic concentrations and leaf-beetle resistance. *Entomol Exp Appl* 113:1–14
- Goldstein DA, Thomas JA (2004) Biopharmaceuticals derived from genetically modified plants. *QJM Int J Med* 97:705–716
- Goodwin TW, Mercer EI (1983) Introduction to plant biochemistry, 2nd edn. Pergamon Press, Oxford, pp 328–399
- Grass S, Zidorn C, Blattner FR, Stuppner H (2006) Comparative molecular and phytochemical investigation of *Leontodon autumnalis* (Asteraceae, Lactuceae) populations from central Europe. *Phytochemistry* 67:122–131
- Grayer RJ, Harborne JB (1994) A survey of antifungal compounds from higher plants 1982–1993. *Phytochemistry* 37:19–42
- Gregory SG, Barlow KF, McLay KE, Kaul R, Swarbreck D, Dunham A, Scott CE et al (2006) The DNA sequence and biological annotation of human chromosome 1. *Nature* 441 (7091):315–321
- Gross RA, Kalra B (2002) Biodegradable polymers for the environment. *Science* 297(5582): 803–807
- Hagerman AE, Butler LG (1991) Tannins and lignins. In: Rosenthal GA, Berenbaum MR (eds) *Herbivores: their interactions with secondary plant metabolites*, vol 1, 2nd edn. The chemical participants. Academic Press, New York, pp 355–388
- Harada K, Fukusaki E (2009) Profiling of primary metabolite by means of capillary electrophoresis-mass spectrometry and its application for plant science. *Plant Biotech* 26:47–52
- Harvell CD, Tollrian R (1999) Why inducible defenses? In: Tollrian R, Harvell CD (eds) *The ecology and evolution of inducible defenses*. Princeton University Press, Princeton, pp 3–9
- Harvey RG (1997) Polycyclic aromatic hydrocarbons. Wiley-VCH, New York, xiii-667pp
- He X, Huang W, Chen W, Dong T, Liu C, Chen Z et al (2009) Changes of main secondary metabolites in leaves of *Ginkgo biloba* in response to ozone fumigation. *J Environ Sci* 21: 199–203
- Heby O, Persson L, Rentala M (2007) Targeting the polyamine biosynthetic enzymes: a promising approach to therapy of African sleeping sickness, Chagas' disease, and leishmaniasis. *Amino Acids* 33(2):359–366
- Heftmann E (1975) Function of steroids in plants. *Phytochemistry* 14:891–901
- Hochbaum AI, Kolodkin-Gal I, Foulston L, Kolter R, Aizenberg J, Losick R (2011) Inhibitory effects of d-amino acids on *Staphylococcus aureus* biofilm development. *J Bacteriol* 193 (20):5616–5622
- Holtcamp W (2012) The emerging science of BMAA: do cyanobacteria contribute to neurodegenerative disease? *Environ Health Perspect* 120(3):A110–A116
- Horner AA, van Uden JH, Jubeldia JM et al (2001) DNA-based immunotherapeutics for the treatment of allergic disease. *Immunol Rev* 179:102–118
- Howsam M, Jones K (1998) Sources of PAHs in the environment. In: Neilson AH (ed) PAHs and related compounds. Springer, Berlin, pp 137–174
- Huang T, Jander G, de Vos M (2011) Non-protein amino acids in plant defense against insect herbivores: representative cases and opportunities for further functional analysis. *Phytochemistry* 72(13):1531–1537

- Huang ZA, Zhao T, Fan HJ, Wang N, Zheng SS, Ling HQ (2012) The up-regulation of ntan 2 expression at low temperature is required for anthocyanin accumulation in juvenile leaves of lc-transgenic tobacco (*Nicotiana tabacum* L.). *J Genet Genomics* 20:149–156
- Hughes DA (1999) Effects of carotenoids on human immune function. *Proc Nutr Soc* 58(3): 713–718
- Ibañez E, Cifuentes A (2013) Benefits of using algae as natural sources of functional ingredients. *J Sci Food Agric* 93(4):703–709
- Jaakola L, Maatta-Riihinne K, Karenlampi S, Hohtola A (2004) Activation of flavonoid biosynthesis by solar radiation in bilberry (*Vaccinium myrtillus* L.) leaves. *Planta* 218:721–728
- Jayasena SD (1999) Aptamers: an emerging class of molecules that rival antibodies in diagnostics. *Clin Chem* 45:1628–1650
- Jenkins GI (2009) Signal transduction in responses to UV-B radiation. *Annu Rev Plant Biol* 60:407–413
- Johnston SA, Talaat AM, McGuire MJ (2002) Genetic immunization: what's in a name? Review article. *Arch Med Res* 33:325–329
- Jordan DN, Green TH, Chappelka AH, Lockaby BG, Meldahl RS, Gjerstad DH (1991) Response of total tannins and phenolics in loblolly pine foliage exposed to ozone and acid rain. *J Chem Ecol* 17(3):505–513
- Karban R, Baldwin IT (1997) Induced responses to herbivory. University of Chicago Press, Chicago
- Kataoka H, Ishizaki A, Saito K (2010) On-line automated analysis of polycyclic aromatic hydrocarbons—applications to herbal medicines. *Chimica Oggi—Chem Today* 28:21–24
- Kaur G, Roy I (2008) Therapeutic applications of aptamers. *Expert Opin Invest Drugs* 17(1):43–60
- Kavanaugh CJ, Trumbo PR, Ellwood KC (2007) The U.S. food and drug administration's evidence-based review for qualified health claims: tomatoes, lycopene, and cancer. *J Natl Cancer Inst* 99(14):1074–1085
- Kazan K, Manners JM (2011) The interplay between light and jasmonate signalling during defence and development. *J Exp Bot* 62:4087–4100
- Ketcham RR, Hu W, Cross TA (1993) High-resolution conformation of gramicidin A in a lipid bilayer by solid-state NMR. *Science* 261(5127):1457–1460
- Khoury GA, Balibar RC, Floudas CA (2011) Proteome-wide post-translational modification statistics: frequency analysis and curation of the swiss-prot database. *Sci Rep* 1, Article number: 90
- Klein RM (1987) The green world: an introduction to plants and people. Harper and Row, New York
- Kliebenstein DJ (2004) Secondary metabolites and plant/environment interactions: a view through *Arabidopsis thaliana* tinged glasses. *Plant Cell Environ* 27:675–684
- Kolodkin-Gal I, Romero D, Cao S, Clardy J, Kolter R, Losick R (2010) D-amino acids trigger biofilm disassembly. *Science* 328(5978):627–629
- Kostrzewska RM, Nowak P, Kostrzewska JP, Kostrzewska RA, Brus R (2005) Peculiarities of L:DOPA treatment of Parkinson's disease. *Amino Acids* 28(2):157–164
- Kouki M, Manetas Y (2002) Resource availability affects differentially the levels of gallotannins and condensed tannins in *Ceratonia siliqua*. *Biochem Syst Ecol* 30:631–639
- Kovács A, Vasas A, Hohmann J (2008) Natural phenanthrenes and their biological activity. *Phytochemistry* 69(5):1084–1110
- Krajian H, Odeh A (2013) Polycyclic aromatic hydrocarbons in medicinal plants from Syria. *Toxicol Environ Chem* 95:942–953
- Lavoie AV, Staudt M, Schnitzler JP, Landais D, Massol F, Rocheteau A et al (2009) Drought reduced monoterpane emissions from *Quercus ilex* trees: results from a throughfall displacement experiment within a forest ecosystem. *Biogeosciences* 6:863–893
- Leader B, Baca QJ, Golan DE (2008) Protein therapeutics: a summary and pharmacological classification. *Nat Rev Drug Discov* 7(1):21–39

- Lee WH, Lin RJ, Lin SY, Chen YC, Lin HM, Liang YC (2011) Osthole enhances glucose uptake through activation of AMP-activated protein kinase in skeletal muscle cells. *J Agric Food Chem* 59(24):12874
- Leuchtenberger W, Huthmacher K, Drauz K (2005) Biotechnological production of amino acids and derivatives: current status and prospects. *Appl Microbiol Biotechnol* 69(1):1–8
- Lu Y, Freeland S (2006) On the evolution of the standard amino-acid alphabet. *Genome Biol* 7(1):1167
- Ma JK-C, Drake PMW, Christou P (2003) The production of recombinant pharmaceutical proteins in plants. *Nat Rev Genet* 4:794–805
- Mardan T, Kopecek J, Kissel T (2002) Prospects for cationic polymers in gene and oligonucleotide therapy against cancer. *Adv Drug Deliv Rev* 54:715–758
- Markou G, Nerantzis E (2013) Microalgae for high-value compounds and biofuels production: a review with focus on cultivation under stress conditions. *Biotechnol Adv* 31(8):1532–1542
- Mauricio R (1998) Costs of resistance to natural enemies in field populations of the annual plant *Arabidopsis thaliana*. *Am Nat* 151(1):20–28
- Mendes RL, Reis AD, Palavra AF (2006) Supercritical CO₂ extraction of γ-linolenic acid and other lipids from *Arthrospira* (*Spirulina*) maxima: comparison with organic solvent extraction. *Food Chem* 99(1):57–63
- Miean KH, Mohamed S (2001) Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) of edible tropical plants. *J Agric Food Chem* 49(6):3106–3112
- Moran-Palacio EF, Tortoledo O, Yanez-Farias GA, Alfredo Rosas-Rodríguez JA, Zamora-Álvarez LA, Stephens-Camacho NA et al (2014) Determination of amino acids in medicinal plants from Southern Sonora, Mexico. *Trop J Pharm Res* 13(4):601–606
- Moreno-Garrido I (2008) Microalgae immobilization: current techniques and uses. *Bioresour Technol* 99(10):3949–3964
- Mulherkar R (2001) Gene therapy for cancer. *Curr Sci* 81:555–560
- Müller DG, Jaenicke L, Donike M, Akintobi T (1971) Sex attractant in brown algae: chemical structure. *Science* 171(3973):815–817
- Ncube B, Finnie JF, Van Staden J (2011) Seasonal variation in antimicrobial and phytochemical properties of frequently used medicinal bulbous plants from South Africa. *S Afr J Bot* 77: 387–396
- Niinemets Ü (2010) Mild versus severe stress and BVOCs: thresholds, priming and consequences. *Trends Plant Sci* 15:145–153
- Nobre B, Marcelo F, Passos R et al (2006) Supercritical carbon dioxide extraction of astaxanthin and other carotenoids from the microalga *Haematococcus pluvialis*. *Eur Food Res Technol* 223 (6):787–790
- Nunn PB, Bell EA, Watson AA, Nash RJ (2010) Toxicity of non-protein amino acids to humans and domestic animals. *Nat Prod Commun* 5(3):485–504
- Otsu M, Candotti F (2002) Gene therapy in infants with severe combined immunodeficiency. *BioDrugs* 16:229–239
- Palavra AMF, Coelho JP, Barroso JG et al (2011) Supercritical carbon dioxide extraction of bioactive compounds from microalgae and volatile oils from aromatic plants. *J Supercrit Fluids* 60:21–27
- Park MH (2006) The post-translational synthesis of a polyamine-derived amino acid, hypusine, in the eukaryotic translation initiation factor 5A (eIF5A). *J Biochem* 139(2):161–169
- Parker J (1977) Phenolics in black oak bark and leaves. *J Chem Ecol* 3:489–496
- Pavarini DP, Pavarini SP, Niehues M, Lopes NP (2012) Exogenous influences on plant secondary metabolite levels. *Anim Feed Sci Technol* 176:5–16
- Pennycooke JC, Cox S, Stushnoff C (2005) Relationship of cold acclimation, total phenolic content and antioxidant capacity with chilling tolerance in petunia (*Petunia* × *hybrida*). *Environ Exp Bot* 53:225–232
- Pesci EC, Milbank JB, Pearson JP, McKnight S, Kende AS, Greenberg EP et al (1999) Quinoline signaling in the cell to cell communication system of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* 96(20):11229–11234

- Prasad TK (1996) Mechanisms of chilling-induced oxidative stress injury and tolerance in developing maize seedlings: changes in antioxidant system, oxidation of proteins and lipids and protease activities. *Plant J* 10:1017–1026
- Pushpendra S, Arvind P, Anil B (2012) Nucleic acids as therapeutics. In: Erdmann VA, Barciszewski J (eds) From nucleic acids sequences to molecular medicine. RNA Technologies, Springer, Berlin, pp 19–45
- Ramani S, Chelliah J (2007) UV-B-induced signaling events leading to enhanced-production of catharanthine in *Catharanthus roseus* cell suspension cultures. *BMC Plant Biol* 7:61
- Reeds PJ (2000) Dispensable and indispensable amino acids for humans. *J Nutr* 130(7):1835S–1840S
- Reif C, Arrigoni E, Schärer H, Nyström L, Hurrell RF (2013) Carotenoid database of commonly eaten Swiss vegetables and their estimated contribution to carotenoid intake. *J Food Compos Anal* 29:64–72
- Rich A, RajBhandary UL (1976) Transfer RNA: molecular structure, sequence, and properties. *Annu Rev Biochem* 45:805–860
- Rodgers KJ, Samardzic K, Main BJ (2015) Toxic nonprotein amino acids. *Plant toxins*. Springer Science, pp 1–20
- Rosenthal GA (1991) The biochemical basis for the deleterious effects of L-canavanine. *Phytochemistry* 30:1055–1058
- Rother M, Krzycki JA (2010) Selenocysteine, pyrrolysine, and the unique energy metabolism of methanogenic archaea. *Archaea* 1–14
- Rozema J, Van de Staaij J, Björn LO, Calwell M (1997) UV-B as an environmental factor in plant life: stress and regulation. *Trends Ecol Evol* 12:22–28
- Saghyan AS, Langer P (2016) Asymmetric synthesis of non-proteinogenic amino acids. Wiley, New York
- Sakami W, Harrington H (1963) Amino acid metabolism. *Annu Rev Biochem* 32(1):355–398
- Sala F, Rigano MM, Barbante A, Basso B, Walmsley AM, Castiglione S (2003) Vaccine antigen production in transgenic plants: strategies, gene constructs and perspectives. *Vaccine* 21: 803–808
- Sanda F, Endo T (1999) Syntheses and functions of polymers based on amino acids. *Macromol Chem Phys* 200(12):2651–2661
- Santos RM, Fortes GAC, Ferri PH, Santos SC (2011) Influence of foliar nutrients on phenol levels in leaves of *Eugenia uniflora*. *Rev Bras Farmacogn* 21:581–586
- Savelieva KV, Zhao S, Pogorelov VM, Rajan I, Yang Q, Cullinan E et al (2008) Genetic disruption of both tryptophan hydroxylase genes dramatically reduces serotonin and affects behavior in models sensitive to antidepressants. *PloS One* 3(10):e3301. <https://doi.org/10.1371/journal.pone.0003301>
- Schafer H, Wink M (2009) Medicinally important secondary metabolites in recombinant microorganisms or plants: progress in alkaloid biosynthesis. *Biotechnol J* 4(12):1684–1703
- Shama LM, Peterson RKD (2004) The benefits and risks of producing pharmaceutical proteins in plants. *Risk Manag Matters* 2(4):28–33
- Shankar P, Manjunath N, Lieberman J (2005) The prospect of silencing disease using RNA interference. *J Am Med Assoc* 293:1367–1373
- Sharkey TD, Loreto F (1993) Water-stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of Kudzu leaves. *Oecologia* 95:328–333
- Sharkey TD, Yeh SS (2001) Isoprene emission from plants. *Annu Rev Plant Physiol Plant Mol Biol* 52:407–436
- Shemin D, Rittenberg D (1946) The biological utilization of glycine for the synthesis of the protoporphyrin of hemoglobin. *J Biol Chem* 166(2):621–625
- Shiga T, Shoji K, Shimada H, Hashida SN, Goto F, Yoshihara T (2009) Effect of light quality on rosmarinic acid content and antioxidant activity of sweet basil *Ocimum basilicum* L. *Plant Biotechnol* 26:255–259
- Siemens DH, Garner SH, Mitchell-Olds T, Callaway RM (2002) Cost of defense in the context of plant competition: *Brassica rapa* may grow and defend. *Ecology* 83(2):505–517

- Singaa EL, Sharkey TD (2000) The effects of high temperature on isoprene synthesis in oak leaves. *Plant Cell Environ* 23:751–757
- Slama K (1980) Animal hormone and antihormones in plants. *Biochem Physiol Pflanzen* 175:177–193
- Smee DF, Bailey KW, Wong MH, O'Keefe BR, Gustafson KR, Mishin VP et al (2008) Treatment of influenza A (H1N1) virus infections in mice and ferrets with cyanovirin-N. *Antiviral Res* 80 (3):266–271
- Soldatenkov AT, Kolyadina NM, Shendrik IV (2001) Fundamentals of organic chemistry of drugs. Khimiya, Moscow, p 36
- Stegink LD (1987) The aspartame story: a model for the clinical testing of a food additive. *Am J Clin Nutr* 46(1 Suppl):204–215
- Stiling P, Cornelissen T (2007) How does elevated carbon dioxide (CO_2) affect plant-herbivore interactions? A field experiment and meta-analysis of CO_2 -mediated changes on plant chemistry and herbivore performance. *Glob Change Biol* 13:1823–1842
- Streatfield SJ, Lane JR, Brooks CA, Barker DK, Poage ML, Mayor JM et al (2003) Corn as a production system for human and animal vaccines. *Vaccine* 21:812–815
- Stryer L, Berg JM, Tymoczko JL (2007) Biochemistry, 6th edn. W.H. Freeman, San Francisco, pp 679–706
- Stull RA, Szoka FC Jr (1995) Antigene, ribozyme and aptamer nucleic acid drugs: progress and prospects. *Pharm Res* 12:463–465
- Suslow TV, Thomas BR, Bradford KJ (2002) Biotechnology provides new tools for planting. University of California Division of Agriculture and Natural Resources, Publication 8043. <http://anrcatalog.ucdavis.edu>
- Szakia A, Pączkowski C, Henry M (2011) Influence of environmental biotic factors on the content of saponins in plants. *Phytochem Rev* 10(4):493–502
- Taiz L, Zeiger E (2006) Plant physiology, 5th edn. Sinauer Associates Inc, Sunderland, MA, USA, p 700
- Tatsuta K, Hosokawa S (2006) Total syntheses of bioactive natural products from carbohydrates. *A Rev Sci Technol Adv Mat* 7:397–410
- Théobald-Dietrich A, Giegé R, Rudinger-Thirion JL (2005) Evidence for the existence in mRNAs of a hairpin element responsible for ribosome dependent pyrolysine insertion into proteins. *Biochimie* 87(9–10):813–817
- Thomas BR, Van Deynze A, Bradford KJ (2002) Production of Therapeutic proteins in plants. Agricultural biotechnology in California Series, Publication 8078. Division of Agriculture and Natural Resources, University of California-Davis, pp 1–12. <http://anrcatalog.ucdavis.edu>
- Thombre SM, Sarwade BD (2005) Synthesis and biodegradability of polyaspartic acid: a critical review. *J Macromol Sci Part A* 42(9):1299–1315
- Turner EH, Loftis JM, Blackwell AD (2006) Serotonin a la carte: supplementation with the serotonin precursor 5-hydroxytryptophan. *Pharmacol Ther* 109(3):325–338
- Uherek C, Wels W (2000) DNA-carrier proteins for targeted gene delivery. *Adv Drug Deliv Rev* 44:153–166
- Van Etten HD, Mansfield JW, Bailey JA, Farmer EE (1994) Two classes of plant antibiotics: phytoalexins versus phytoanticipins. *Plant Cell* 6:1191–1192
- Vermeer C (1990) Gamma-carboxyglutamate-containing proteins and the vitamin K-dependent carboxylase. *Biochem J* 266(3):625–636
- Vijayvergia R, Kumar J (2007) Quantification of primary metabolites of *Nerium indicum* Mill. *Asian J Exp Sci* 21:123–128
- Volk RB (2008) A newly developed assay for the quantitative determination of antimicrobial (anticyanobacterial) activity of both hydrophilic and lipophilic test compounds without any restriction. *Microbiol Res* 163(2):161–167
- Vorburger SA, Hunt KK (2002) Adenoviral gene therapy. *Oncologist* 7:46–59
- Wallaart TE, Pras N, Beekman AC, Quax WJ (2000) Seasonal variation of artemisinin and its biosynthetic precursors in plants of *Artemisia annua* of different geographical origin: proof for the existence of chemotypes. *Planta Med* 66:57–62

- Walmsley AM, Arntzen CJ (2000) Plants for delivery of edible vaccines. *Curr Opin Biotechnol* 11:126–129
- Wink M (ed) (1999) Functions of plant secondary metabolites and their exploitation in biotechnology. In: Annual plant reviews, vol 3. CRC Press, Sheffield Academic Press, New York, pp 362
- Young VR (1994) Adult amino acid requirements: the case for a major revision in current recommendations. *J Nutr* 124(8 Suppl):1517S–1523S
- Young VR, Ajami AM (2001) Glutamine: the emperor or his clothes? *J Nutr* 131(9 Suppl):2449S–2459S
- Young VR, Pellett PL (1994) Plant proteins in relation to human protein and amino acid nutrition. *Am J Clin Nutr* 59(5 Suppl):1203S–1212S
- Zhang L, Gasper WA, Stass SA, Ioffe OB, Davis MA, Mixson AJ (2002) Angiogenic inhibition mediated by a DNAzyme that target vascular endothelial growth factor receptor 2. *Cancer Res* 62:5463–5469
- Zhang XX, Li CJ, Nan ZB (2011) Effects of salt and drought stress on alkaloid production in endophyte-infected drunken horse grass (*Achnatherum inebrrians*). *Biochem Syst Ecol* 39:471–476
- Zidorn C, Stuppner H (2001) Evaluation of chemosystematic characters in the genus Leontodon. *Taxon* 50:115–133
- Zobayed SMA, Afreen F, Koza T (2005) Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. John's Wort. *Plant Physiol Biochem* 43:977–984
- Zongyan C, Na G, Yanzhong C, Jinjie Z, Yongming L, Le Z (2014) Investigation and assessment of polycyclic aromatic hydrocarbons contamination in Chinese herbal medicines. *Environ Chem* 33(5):844–849

Chapter 3

Secondary Metabolites: Secondary Metabolic Products Consisting of C and H; C, H, and O; N, S, and P Elements; and O/N Heterocycles



Abstract Terpenes and terpenoids, steroids and sterols, volatile oils, miscellaneous isoprenoids, phenols and phenyl propanoids, alkaloids, glycosides, bitter principles, resins, saponins, cardioactive compounds, etc., are important groups of secondary metabolites of plant origin. Terpenes and terpenoids are naturally occurring hydrocarbons, and ~2000 plant species of 60 families produce more than 55,000 terpenes and their derivatives. Terpenes are built from isoprene monomer (C_5H_8), and $(C_5H_8)_n$ is the basic molecular formula. The oxygen-containing terpenes are called terpenoids or isoprenoids while steroids are cyclic terpenoids, and sterols are steroid alcohols. Terpenoids have significant importance in food, pharmaceutical, and cosmetic industry. Terpenoids contribute to plant essential oils (eucalyptus, lavender, thyme, and mint), flavors (cinnamon, cloves, and ginger), color (yellow—sunflowers, red—tomatoes), etc. They protect plant against predators and pests (e.g., from herbivores, insects, fungi, microorganisms, etc.), aid to pollination and dispersal of spores, and in living organisms function range from pigments and fragrances to vitamins and precursors of sex hormones. Plant sterols, including campesterol, inhibit the absorption of cholesterol in the intestines and thereby reduce LDLs or cholesterol level. Phenols or phenolics are a class of chemical compounds with a benzene nucleus supporting a hydroxyl group and range from simple substances like phenolic acids or phenols, cumarines, flavonoids, and quinines to very complex ones such as lignins and tannins. Phenol and its chemical derivatives are used in the production of detergents, phenoxy herbicides, numerous pharmaceutical drugs, and many industrial synthetic goods. Alkaloids are cyclic bitter organic compounds containing nitrogen in a negative state of oxidation having a marked physiological action on man and other animals. A large variety of organisms produce alkaloids, including bacteria, fungi, plants, and animals. Alkaloids like caffeine, ephedrine, codeine, colchicine, nicotine, pilocarpine, opium, quinine, reserpine, cocaine, psilocin morphine, atropine, berberine, vincristine, yohimbine, etc., are some common examples of drugs principles of pharmaceutical importance and often are used as recreational drugs, or in entheogenic rituals. A glycoside is a heteromolecule consisting of a non-sugar (aglycone) and a sugar part (glycone) components. The glycone may be monosaccharide or oligosaccharide, and the aglycone may be an alcohol, anthraquinone derivative,

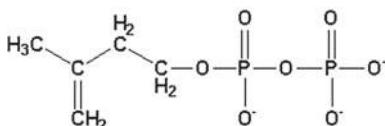
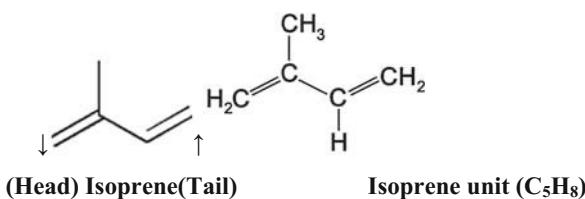
phenol, aldehyde, acid, ester, or another compound. Glycosides play numerous important roles in living organisms, and many such plant glycosides are used as medications, e.g., the active principles of digitalis, strophanthus, cascara, willow, and poplar barks are being among the most valued remedies. The bitter principles are mostly terpenoid, especially the sesquiterpene lactones, monoterpene iridoids, and the secoiridoids. Diterpene bitters are found in columbo root and white horehound, and triterpenoids are the cause of bitterness in Cucurbitaceous plants, which is due to cucurbitacins. Plant lignans are diphenolic compounds (phenylpropanoids dimers) whose structure is the union of two units of phenylpropane. Tannins are non-nitrogenous bitter plant polyphenolic compounds having a molecular weight between 500 and 3000 (gallic acid esters) and up to 20,000 (proanthocyanidins). They are non-crystallisable colloidal compounds and may be (i) hydrolyzable tannins, which consist of gallic acid or related polyhydric compounds esterified with glucose, and they are readily hydrolysed to yield the phenolic acids and the sugar; and (ii) non-hydrolyzable or condensed tannins contain only phenolic nuclei and most of such tannins are formed by the condensation of two or more flavanols, such as catechin. Pharmaceutically, tannins have antibacterial, antiviral, antiparasitic, astringent, and antiseptic properties, and may be used in the treatment of hemorrhages (constrict of blood vessels), burns (cicatrizing), diarrhea, and as an antidote for alkaloid poisoning because of their ability to precipitate alkaloids; effective against 6-hydroxydopamine-induced toxicity and also have anti-inflammatory and antiulcer activity. Quinones are cyclic organic compounds (aromatic diketones) and are found in bacteria, in certain fungi, in various higher plant forms, and in a few animals (e.g., sea urchins, aphids, lac insects, and certain scale insects). It is highly active anti-microbacterial, antifungal agent and highly toxic and fatal if swallowed, inhaled, or absorbed through the skin and widely used in medicine, herbicides, chemical reagents, dyes, and tanning agents. Saponins are amphiphilic glucoside molecules composed of hydrophilic glycoside glycone and lipophilic triterpene or steroid aglycone. Saponins have been used in medicine, foaming agents, in fire extinguishers, and fish poisons. Drugs that influence heart or drugs having an influence on the heart are cardioactive drugs. (i) Beta-adrenoceptor antagonists, (ii) calcium channel blocking drugs, and (iii) cardiac glycosides are three major classes of cardioactive drugs. Cardiac glycosides are also important in the pathogenesis and therapy of different human diseases (e.g., stroke, diabetes, neurological diseases, cancer, etc.). Cardioactive steroids are a class of animal and plant-derived compounds with a steroid nucleus and a specific inotropic, chronotropic, and dromotropic effect. Cardioactive steroids (CAS) became the mainstay of treatment for congestive heart failure and to control the ventricular response rate in atrial tachydysrhythmias. Antibiotics are produced by different groups of microorganisms like bacteria, fungi, and actinomycetes and in many cases by higher plants. Antibiotics in low concentration are capable of inhibiting the growth of microorganisms through an antimetabolic mechanism. They differ from antiseptics and disinfectants in their mode of action, chemical, and physical properties. The development of resistance among the microorganisms on prolonged

contact with the drug is the present-day problems in the field of antibiotics. The microbial and plant sources from the terrestrial and marine environments are now providing natural products with antitumor activity.

3.1 Secondary Products Consisting of C, H, and O Elements

3.1.1 Terpenes and Terpenoids

Terpenes and terpenoids are naturally occurring hydrocarbons and their derivatives (e.g., alcohols, glycosides, ethers, aldehydes, ketones, carboxylic acids, esters, etc.) all and about 2000 plant species of 60 families including Lamiaceae, Asteraceae, Rutaceae, Myrtaceae, Apiaceae, Pinaceae, etc., produce more than 55,000 terpenes and their derivatives. Buckingham (2004) in a conservative estimate suggests that at least 40,000 different terpenoids produced by a variety of plants, particularly conifers, though also by some insects (termites or swallowtail butterflies), which emit terpenes from their osmeteria. They are often strong smelling, many of which are of plant origin. Terpenes usually contain one or more C=C double bonds and are built from isoprene monomer, a hydrocarbon made up of five carbon atoms (C_5) attached to eight hydrogen atoms ($CH_2=C(CH_3)-CH=CH_2=C_5H_8$) and they are linked with each other to form linear chains or they may be arranged to form rings. According to isoprene rule or the C_5 rule, the basic molecular formula of terpenes is multiple of isoprene or $(C_5H_8)_n$, nature's common building blocks, and the natural-occurring terpenes and terpenoids obey the isoprene rule (Fig. 3.1). The oxygen-containing terpenes are called terpenoids or isoprenoids. Terpenoids form a large and diverse class of naturally occurring lipid-like chemicals and form the



Terpenoid-isopentenyl pyrophosphate(2-methyl-1,3-butadiene)

Fig. 3.1 Isoprene (C_5H_8)—building block of terpenes and terpenoids

largest class of natural products. The isopropyl part of isoprene (2-methylbutane) is defined as the head, and the ethyl residues the tail. In mono-, sesqui-, di-, and sesterpenes, the isoprene units are linked to each other from head to tail; tri- and tetraterpenes contain one tail-to-tail connection in the center.

Terpenoids have significant importance in food, pharmaceutical, and cosmetic industry. Terpenoids contribute to plant essential oils (eucalyptus, lavender, thyme, mint), flavors (cinnamon, cloves, and ginger), color (yellow—sunflowers, red—tomatoes), etc. They include essential oils, iridoids, lactones, sesquiterpenics, saponins, and cardiotonic heterosides. Citral, menthol, camphor, turpentine, salvianorin A (in *Salvia divinorum*), cannabinoids (in *Cannabis* spp.), ginkgolide and bilobalide (in *Ginkgo biloba*), and the curcuminooids (in *Curcuma longa* and also in *Brassica* spp.) are some of the examples of much known terpenoids of pharmaceutical importance. They protect plant against predators and pests (e.g., from herbivores, insects, fungi, microorganisms, etc.), aid to pollination and dispersal of spores, and in living organisms function range from pigments and fragrances to vitamins and precursors of sex hormones.

Resin and turpentine contain terpenes. Many terpenes are used as major biosynthetic building blocks steroids (derivatives of triterpene squalene), vitamin A (derivatives of tetraterpenoid carotene). Biologically active terpenoids span various orders of magnitude including natural flavor additives for food or fragrances in perfumery and in traditional and alternative medicines as aromatherapy. Application of taxol derivatives, paclitaxel, and docetaxel in cancer chemotherapy is the most comprehensively studied case. In addition to cancer therapies, there are so many important aspects of the pharmacological usage of natural terpenoids including antimicrobial, antifungal, antiviral, antiparasitic, anti-allergenic, antihyperglycemic, anti-inflammatory, antioxidants, antiseptics, expectorants, gastrointestinal disorder, pain relievers, immunomodulatory, and skin permeation enhancer, cholesterolemia, tracheal and bronchial disorders, arthritis, rheumatism, and also to have properties (Wagner and Elmadfa 2003). Epidemiological and experimental studies suggest that monoterpenes may be helpful in the prevention and therapy of several cancers, including mammary, skin, lung, forestomach, colon, pancreatic, and prostate carcinomas (Gould 1997; Crowell 1999). Numerous preclinical efficacy studies have provided extensive evidence that both naturally occurring and synthetic derivatives of triterpenoids possess chemopreventive and therapeutic effects against colon, breast, prostate, and skin cancer (Liby et al. 2007; Rabi and Gupta 2008; Bishayee et al. 2011). A large number of triterpenoids have been shown to suppress the growth of a variety of cancer cells without exerting any toxicity in normal cells (Setzer and Setzer 2003; Petronelli et al. 2009).

Terpenes and terpenoids appear in the leaf, bark, wood, root, rhizome, flower, fruit, and seed of the medicinal and aromatic plants such as bay leaf, cinnamon bark, ginger, sandalwood, nutmeg, thyme, clover, eucalyptus, *Cannabis sativa*, etc. The livers of fishes and other animals are particularly rich in oils that are largely acyclic triterpenoid hydrocarbons, especially squalene. Many of the terpenoid molecules, however, are only found in very low levels in nature. Synthetic biology

and metabolic engineering may provide innovative approaches to increase the production of terpenoids in natural sources. Iridoids are monoterpenic compounds, from a type of Australian ant (*Iridomyrmex* genus), in plants as glycosides, prevalent in the plant root of subclass Asteridae (Ericaceae, Loganiaceae, Gentianaceae, Rubiaceae, Verbenaceae, Lamiaceae, Oleaceae, Plantaginaceae, Scrophulariaceae, Valerianaceae, and Menyanthaceae), harpagosides from the tuberous roots of the harpagophytum, also known as grapple plant, wood spider or devil's claw (*Harpagophytum procumbens* of Pedaliaceae), oleuropeoside in olive leaf (*Olea europaea* of Oleaceae), and genciopicroside in the roots of the genciana (*Gentiana lutea* of Gencianaciae).

Classification

Terpenes may be classified on the basis of the number of isoprene (5C) units in the molecule as (Table 3.1).

Table 3.1 Different classes of terpenes with information about isoprene units, number of carbon atoms, class name, and examples

Classes of terpenes			
Isoprene units	Number of carbon atoms	Classes of terpenes	Examples
1	5	Hemiterpene (2-methylbutane)	Isoprene; prenol, and isovaleric acid are hemiterpenoids
2	10	Monoterpene (2,6-dimethyloctane)	Geraniol, limonene, terpineol
3 (1.5)	15	Sesquiterpene (2,6,10-trimethyldodecane)	Humulene farnesane, farnesol
4	20	Diterpene (2,6,10,14-tetramethylhexadecane)	Cafestol, phytene, kahweol, cembrene, and taxadiene
5 (2.5)	25	Sesterterpene (2,6,10,14,18-pentamethyl icosane)	Manoalide
6	30	Triterpene (2,6,10,15,19,23-hexamethyl tetracosane)	Squalene
7	35	Sesquarterpenes ($C_{35}H_{56}$)	Ferrugicadiol, tetraprenylcurcumene
8	40	Tetraterpene ($C_{40}H_{64}$)	α, β -bicyclic & γ -monocyclic carotenes, acycliclycopene
>8	>40	Polyisoprene(C_5H_8) _n n > 8	Rubber with cis and gutta-percha with trans double bonds

3.1.1.1 Hemiterpenes

Hemiterpenes consist of a single isoprene unit (C_5H_8). Isoprene itself is considered the only hemiterpene, but oxygen-containing derivatives such as prenol and isovaleric acids are hemiterpenoids (Fig. 3.2).

Prenol (3-methyl-2-but-en-1-ol) is a natural alcohol and a representative of the simplest terpenoids. It is clear colorless oil that is reasonably soluble in water and miscible with most common organic solvents. It has a fruity odor and is used occasionally in perfumery. Prenol occurs naturally in citrus fruits, cranberry, bilberry, currants, grapes, raspberry, blackberry, tomato, white bread, hop oil, coffee, arctic bramble, cloudberry, and passion fruit. Isovaleric acid [$(CH_3)_2CHCH_2COOH$] is a colorless liquid that is sparingly soluble in water, but highly soluble in most common organic solvents. It has a strong pungent cheesy or sweaty smell, but its volatile esters have pleasing scents and are used widely in perfumery. Isovaleric acid is seen as the primary cause of the flavors added to wine caused by Brettanomyces yeasts.

3.1.1.2 Monoterpene

Monoterpene consists of two isoprene units ($C_{10}H_{16}$). Monoterpene may be linear or acyclic and contain rings or cyclic (Fig. 3.3). Besides, biochemical modifications such as oxidation or rearrangement produce the related monoterpeneoids acyclic monoterpene, and monoterpeneoids include geraniol, ocimene, myrcenes and their oxidative products citral, citronellal, citronellol, linalool, and many others. Halomon is a halogenated monoterpene found in marine organisms. Classic examples of cyclic monoterpene are limonene, phellandrenes, terpinolene, carvone, etc. Menthol, thymol, carvacrol, and many others are terpenoids derived from monocyclic terpenes. Bicyclic monoterpene include pinene, carene, sabinene, camphene, iridoids, and thujene. Camphor, borneol, and eucalyptol are examples of bicyclic monoterpeneoids containing ketone, alcohol, and ether functional groups, respectively.

Geraniol is a monoterpeneoid alcohol. It is the primary part of rose oil, palmarosa oil, and citronella oil (Java type). It also occurs in small quantities in geranium, lemon, and many other essential oils. Geraniol appears to be an effective

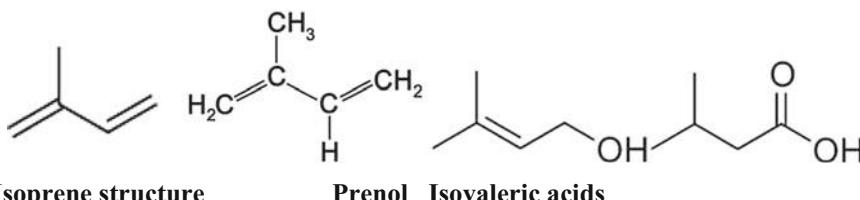


Fig. 3.2 Structure of hemiterpene and hemiterpenoids

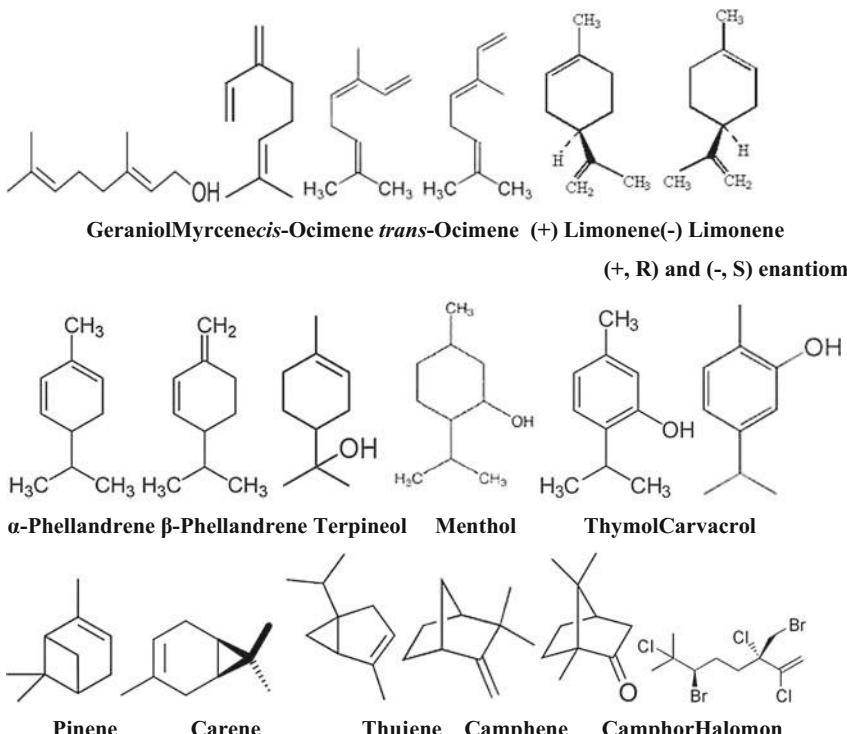


Fig. 3.3 Structure of different monoterpenes and monoterpenoids of plant origin

plant-based mosquito repellent. Limonene is an odor contributing colorless liquid cyclic terpene of the citrus fruits. Limonene is a chiral molecule, and biological sources produce one enantiomer, orange contains D-limonene ((+)-limonene), which is the (R)-enantiomer while lemon contains L-limonene ((-)-limonene), which is the (S)-enantiomer. The symbol R comes from the Latin rectus for right, and S from the Latin sinister for left. The more common D-isomer possesses a strong smell of oranges. Limonene is common in cosmetic products. D-limonene is used in food manufacturing and some medicines, e.g., as a flavoring to mask the bitter taste of alkaloids, and as a fragrant in perfumery, after shave lotions, bath products and other such products that include fragrance to their products; it is also used as botanical insecticide, particularly the (R)-(+)-enantiomer is most active as an insecticide. It is added to cleaning products such as hand cleansers to give a lemon-orange fragrance (see orange oil) and because of its ability to dissolve oils. L-limonene has a piney, turpentine-like odor. In alternative medicine, D-limonene is marketed to relieve gastroesophageal reflux disease and heartburn. Terpineol is a naturally occurring monoterpene alcohol that has been isolated from a variety of sources such as cajunut oil, pine oil, and petitgrain oil. There are four isomers, α -, β -, γ -terpineol, and terpinen-4-ol. Terpineol is usually a mixture of these

isomers with α -terpineol as the major constituent. Terpineol has a pleasant odor similar to lilac and is a common ingredient in perfumes, cosmetics, and flavors. α -terpineol is one of the two most abundant aroma constituents of lapsang sou-chong tea; the α -terpineol originates in the pine smoke used to dry the tea. Myrcene, or β -myrcene, is an olefinic monoterpene from *Myrcia* genus of Myrtaceae. It is a component of the essential oil of several plants including bay, cannabis, ylang-ylang, wild thyme, parsley, and hops. Myrcene has an analgesic effect and is likely to be responsible for the medicinal properties of lemongrass tea. It has anti-inflammatory properties through Prostaglandin E2.

Pinene ($C_{10}H_{16}$) is a bicyclic monoterpene chemical compound. There are two structural isomers of pinene found in nature: α -pinene and β -pinene. As the name suggests, both forms are important constituents of pine resin; they are also found in the resins of many other conifers, as well as in non-coniferous plants such as big sagebrush (*Artemisia tridentata*). Carene is a bicyclic monoterpene which occurs naturally as a constituent of turpentine, with a content as high as 42% depending on the source. Carene has a sweet and pungent odor, not soluble in water, but miscible with fats and oils. Natural sources of carene include turpentine, rosemary, and cedar. In higher concentrations, carene can be a skin irritant or central nervous system depressant. Thujene is a natural organic compound classified as a monoterpene. It is found in the essential oils of a variety of plants and contributes pungency to the flavor of some herbs such as Summer savory. Camphene is a bicyclic monoterpene. It is nearly insoluble in water, but very soluble in common organic solvents. It volatilizes readily at room temperature and has a pungent smell. It is a minor constituent of many essential oils such as turpentine, cypress oil, camphor oil, citronella oil, neroli, ginger oil, and valerian. It is produced industrially by catalytic isomerization of the more common alpha-pinene. Camphene is used in the preparation of fragrances and as a food additive for flavoring. Camphor is a terpenoid with the chemical formula $C_{10}H_{16}O$. It is a waxy, flammable, white or transparent solid with a strong aromatic odor found in the wood of the camphor laurel—*Cinnamomum camphora*, *Ocotea usambarensis*, *Rosmarinus officinalis*, etc. It can also be synthetically produced from oil of turpentine. It is used for its scent, as an ingredient in cooking (mainly in India), as an embalming fluid, for medicinal purposes, and in religious ceremonies. Camphor is readily absorbed through the skin and produces a feeling of cooling similar to that of menthol, and acts as slight local anesthetic and antimicrobial substance. There are anti-itch gels and cooling gels with camphor as the active ingredient. Camphor is an active ingredient (along with menthol) in vapor-steam products, such as Vicks VapoRub. Camphor may also be administered orally in small quantities (50 mg) for minor heart symptoms and fatigue. Halomon is a polyhalogenated monoterpene first isolated from the marine red algae *Portieria hornemannii*. Halomon has attracted research interest because of its promising profile of selective cytotoxicity that suggests its potential use as an antitumor agent.

3.1.1.3 Sesquiterpenes

Sesquiterpenes consist of three isoprene units ($C_{15}H_{24}$). Sesquiterpenes and sesquiterpenoids include farnesol and farnesenes (acyclic); zingiberene, humulene, and bisabolol, (monocyclic), β -caryophyllene (bicyclic); artemisinin, longifolene, copaene, and alcohol patchoulol (tricyclic) (Fig. 3.4). Zingiberene, a monocyclic sesquiterpene, is the predominant constituent of the oil of ginger, and caryophyllene, a natural bicyclic sesquiterpene, is a constituent of many essential oils, especially clove oil, the oil from the stems and flowers of *Syzygium aromaticum*, the essential oil of *Cannabis sativa*, rosemary, hops, black pepper, etc. Bisabolol has a weak sweet floral aroma and is used in various fragrances. It has also been used for hundreds of years in cosmetics because of its perceived skin healing properties. Bisabolol, a natural monocyclic sesquiterpene alcohol from *Matricaria recutita* and *Myoporum crassifolium*, is known to have anti-irritant, anti-inflammatory, and antimicrobial properties. Artemisinin, an antimalarial principle from *Artemisia annua L.*, is a cyclic sesquiterpene lactone-containing an unusual peroxide bridge, which is believed to be responsible for the drug's mechanism of action.

Humulene, also known as α -humulene or α -caryophyllene, is a naturally occurring monocyclic sesquiterpene ($C_{15}H_{24}$), which is an 11-membered ring, consisting of three isoprene units containing three nonconjugated C=C double bonds: two of them being triply substituted and one being doubly substituted terpenoid. Humulene is one of the essential oils made in the flowering cone of the hops plant (*Humulus lupulus*) and was first found in the essential oils of hops plant from which it derives its name. Humulene is an isomer of β -caryophyllene, and the two are often found together as a mixture in many aromatic plants. β -caryophyllene is a constituent of many essential oils, especially clove oil (*Syzygium aromaticum*), the essential oil of hemp (*Cannabis sativa*), rosemary (*Rosmarinus officinalis*), and hops. Proven α -humulene emitters into the atmosphere are pine trees, orange orchards, marsh elders, tobacco, and sunflower fields. α -Humulene is contained in

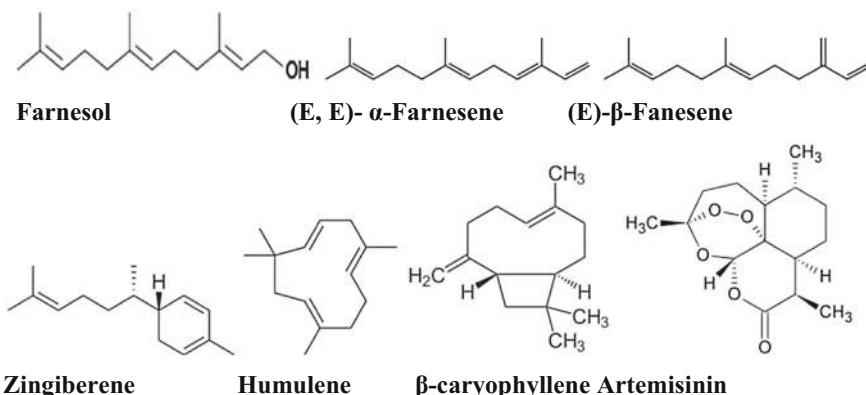


Fig. 3.4 Structure of different sesquiterpenes and sesquiterpenoids

the essential oils of aromatic plants such as common sage, culinary sage (*Salvia officinalis*), Uyaku or Japanese spicebush (*Lindera strychnifolia*), ginseng species (*Panax* spp.), *Mentha spicata*, members of ginger family (Zingiberaceae), Chinese laurel tree (*Litsea mushaensis*), and Brazilian coastal bush erva baleeira (*Cordia verbenacea*). Humulene has been found to produce anti-inflammatory effects in mammals and has potential to be a tool in the management of inflammatory diseases. It produces similar effects to dexamethasone and is found to decrease the edema formation caused by histamine injections. Humulene produced inhibitory effects on tumor necrosis factor- α (TNF α) and interleukin-1 β (IL1 β) generation in carrageenan-injected rats.

The term farnesene includes a set of six closely related sesquiterpenes. α -Farnesene and β -farnesene are isomers, differing by the location of one double bond. α -Farnesene is 3,7,11-trimethyl-1,3,6,10-dodecatetraene, and β -farnesene is 7,11-dimethyl-3-methylene-1,6,10-dodecatriene. The alpha form can exist as four while the beta isomer exists as two stereoisomers about the geometry of its central double bond. (E, E)- α -Farnesene is the most common isomer. It is found in the coating of apples, and other fruits, and it is responsible for the characteristic green apple odor. Its oxidation by air gives compounds that are damaging to the fruit. The oxidation products injure cell membranes which eventually causes cell death in the outermost cell layers of the fruit, resulting in a storage disorder known as scald. (E) β -Farnesene has one naturally occurring isomer. The E isomer is a constituent of various essential oils. It is also released by aphids as an alarm pheromone upon death to warn away other aphids. Several plants, including potato species, have been shown to synthesize this pheromone as a natural insect repellent. Farnesol is present in many essential oils such as citronella, neroli, cyclamen, lemongrass, tuberose, rose, musk, balsam, and tolu. It is used in perfumery to emphasize the odors of sweet floral perfumes. Its method of action for enhancing perfume scent is as a co-solvent that regulates the volatility of the odorants. It is especially used in lilac perfumes. Farnesol is a natural pesticide for mites and is a pheromone for several other insects.

Sesquiterpene lactones are found in abundance in the species of Asteraceae, Lauraceae, and Magnoliaceae families, and are responsible for the bitter taste of many drugs; the holy thistle (*Cnicus benedictus*), absinthe (*Artemisia absinthium*), or dandelions (*Taraxacum officinale*). They are antibacterial and antifungal. Some produce dermatitis as they cause the formation of allergens. Artemisinin, an anti-malarial sesquiterpenoid pharmaceutical from annual wormwood (*Artemisia annua*) that is being explored for production in metabolically engineered microbial fermentation systems and transgenic plants; taxol, a high-value diterpenoid-derived anticancer drug of limited supply from its initial natural source, the bark of the Pacific yew tree (*Taxus brevifolia*—a conifer of Taxaceae).

3.1.1.4 Diterpenes

Diterpenes composed of four isoprene units ($C_{20}H_{32}$). They derive from geranyl-geranyl pyrophosphate. Diterpenes and diterpenoids include cafestol, ginkgolides, kahweol, cembrene, forskolin, aphidicolin, salvianorin A, taxol, phytol and taxadiene (precursor of taxol), and also diterpenes form the basis for biologically important compounds such as retinol, retinal, and phytol (Fig. 3.5).

Retinol is a diterpenoid alcohol. It is one of the animal forms of vitamin A. Retinol is convertible to other forms of vitamin A. Retinyl ester derivative serves as the storage form of the vitamin in animals. Retinaldehyde form of vitamin A is essential for vision, and retinoic acid is essential for skin health, teeth remineralization, and bone growth. These chemical compounds are collectively known as retinoids. Retinoids may be grouped as first-generation retinoids (retinol, retinal, retinoic acid, Retin-A, isotretinoin, alitretinoin); second-generation retinoids (etretinate, acitretin, etc.); and third-generation retinoids (tazarotene, bexarotene, adapalene, etc.).

Structurally, all retinoids also possess a β -ionone ring and a polyunsaturated side chain, with either an alcohol (retinol), aldehyde (retinal), a carboxylic acid group (retinoic acid) or an ester group (retinyl ester). The side chain is composed of four isoprenoid units, with a series of conjugated double bonds which may exist in trans- or cis-configuration (geometric isomerism describing the relative orientation of functional groups within a molecule, cis=on this side and trans="on the other side or" across). Retinol is produced in the body from the hydrolysis of retinyl esters, and from the reduction of retinal. Retinol in turn is ingested in a precursor form; animal sources (liver and eggs) contain retinyl esters, whereas plants (carrots, spinach) contain provitamin A carotenoids. Hydrolysis of retinyl esters results in retinol, while provitamin A carotenoids can be cleaved to produce retinal by carotene dioxygenase in the intestinal mucosa. Retinal (retinaldehyde or vitamin A aldehyde) is one of the many forms of vitamin A (the number of which varies from species to species). Retinal is a polyene chromophore, and bound to proteins called opsins is the chemical basis of animal vision. Bound to proteins called type 1 rhodopsins, retinal allows certain microorganisms to convert light into metabolic energy. Vertebrate animals ingest retinal directly from meat, or produce retinal from one of two carotenes (α -carotene, β -carotene), and also from another type of carotenoid known as β -cryptoxanthin (a type of xanthophyll), these must be obtained from plants or other photosynthetic organisms. No other carotenoids can be converted by animals to retinal, and some carnivores cannot convert any carotenoids at all. The other main forms of vitamin A, retinol, and retinoic acid may be produced from retinal. Retinoids regulate epithelial cell growth and diverse functions throughout the body including vision, cell proliferation and differentiation, growth of bone tissue, immune function, and activation of tumor suppressor genes. Retinoids are used in the treatment of many diverse diseases and are effective in the

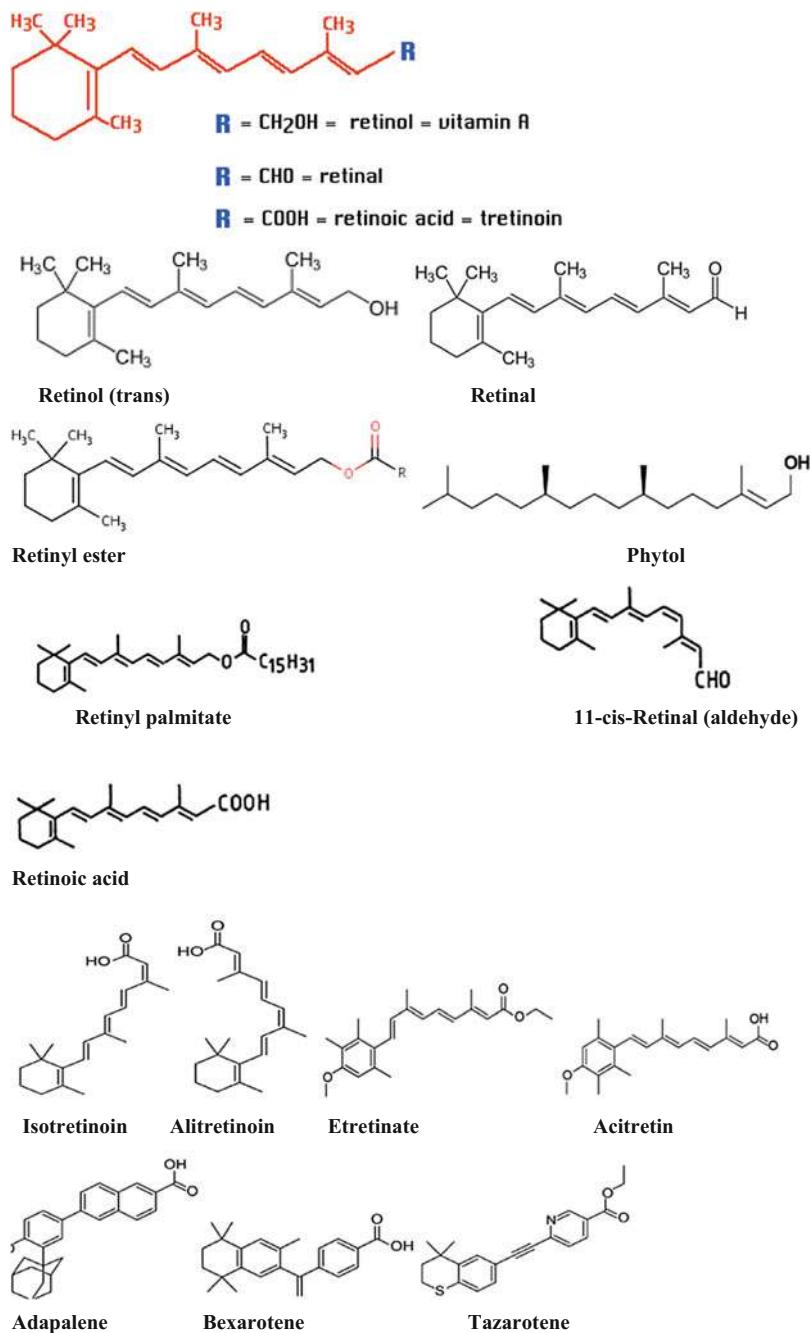


Fig. 3.5 Structure of different diterpenes

treatment of many dermatological conditions such as inflammatory skin disorders, skin cancers, disorders of increased cell turnover (e.g., psoriasis), and photoaging.

Phytol is an acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. In ruminants, the gut fermentation of ingested plant materials liberates phytol, a constituent of chlorophyll, which is then converted to phytanic acid and stored in fats. Phytol is used in the fragrance industry and used in cosmetics, shampoos, toilet soaps, household cleaners, and detergents. Its worldwide use has been estimated to be approximately 0.1–1.0 metric tons per year. The 11 conjugated double bonds form the chromophore of the β -carotene molecule. On enzymatic hydrolysis and reduction, it produces retinol in the following way (Fig. 3.6).

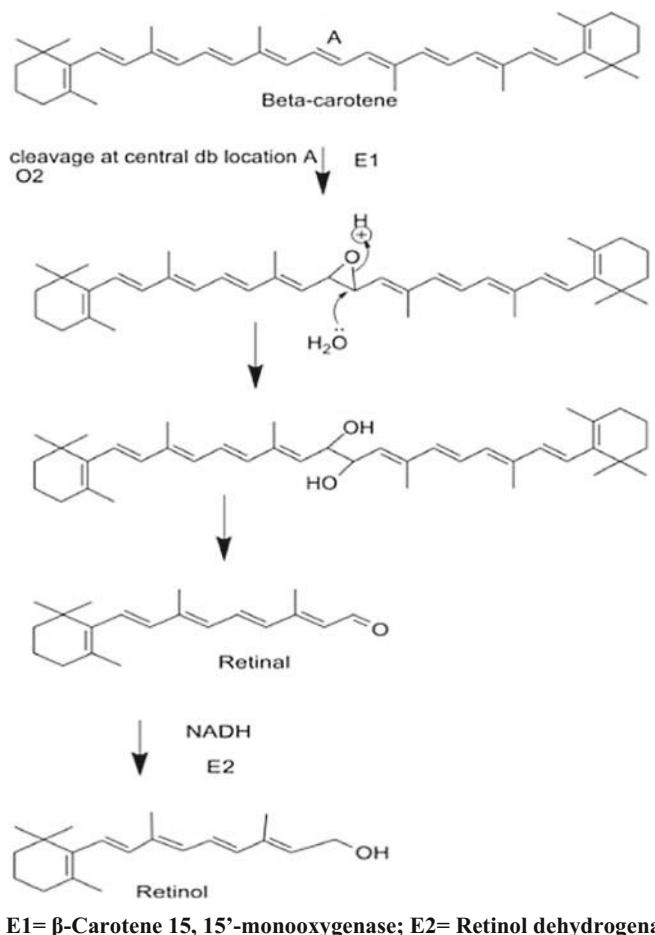


Fig. 3.6 Enzymatic formation of retinol from β -Carotene

3.1.1.5 Sesterterpenes

Sesterterpenes ($C_{25}H_{40}$) are derived from geranyl farnesol pyrophosphate and have five isoprene units or 25 carbon atoms (prefix sester means half to three, i.e., 2.5) and they are rare relative to the other terpenes. Manoalide (1), secomanoalide (2), (*E*)-neomanoalide (3), and (*Z*)-neomanoalide (4) are representatives of bioactive sesterterpenes derived from different marine sponges and could lead to potential new drug candidates (Ebada et al. 2010). An example of a sesterterpenoid is geranyl farnesol (Fig. 3.7).

3.1.1.6 Triterpenes

Triterpenes consist of six isoprene units ($C_{30}H_{48}$). Triterpenes are a class of natural products present in all organisms, especially in plants. They include squalene, lanosterol or cycloartenol, sterols, steroids, and lupeol (Fig. 3.8). The triterpene acids (e.g., betulinic, ursolic, oleanolic acids, etc.) exhibit unique and important biological and pharmacological activities like anti-inflammatory, antimicrobial, antiviral, cytotoxic, and cardiovascular effects. The linear triterpene squalene, the major constituent of shark liver oil, is derived from the reductive coupling of two molecules of farnesyl pyrophosphate. From squalene, one may get lanosterol or cycloartenol, the structural precursors to all the steroids, through biosynthetic processes. The pentacyclic triterpenes can be classified into lupane, oleanane or ursane groups. A notable pentacyclic triterpene is boswellic acid. Animals, plants, and fungi, create triterpenes, like, squalene, ambrein, and ganoderic acid. Triterpenoids are thought of as modified triterpenes, such as lanosterol.

Squalene is obtained for commercial purposes primarily from shark liver oil (hence its name). Plant sources (primarily vegetable oils) including amaranth seed, rice bran, wheat germ, and olives are now used also. It is also found in high concentrations in the stomach oil of birds in the order Procellariiformes. Squalene is produced as a biochemical intermediate in all plants and animals including humans, and it is a vital part of the synthesis of all plant and animal sterols, including

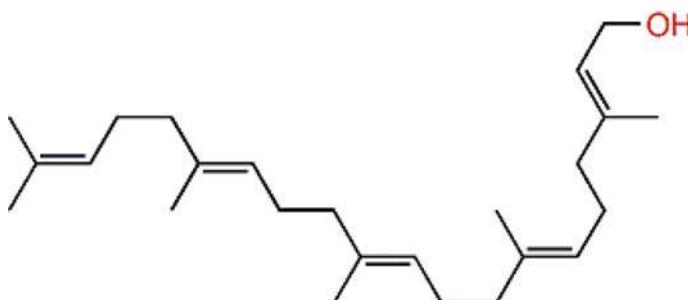


Fig. 3.7 Geranyl farnesol, an acyclic C_{25} isoprenoid alcohol found in insect wax

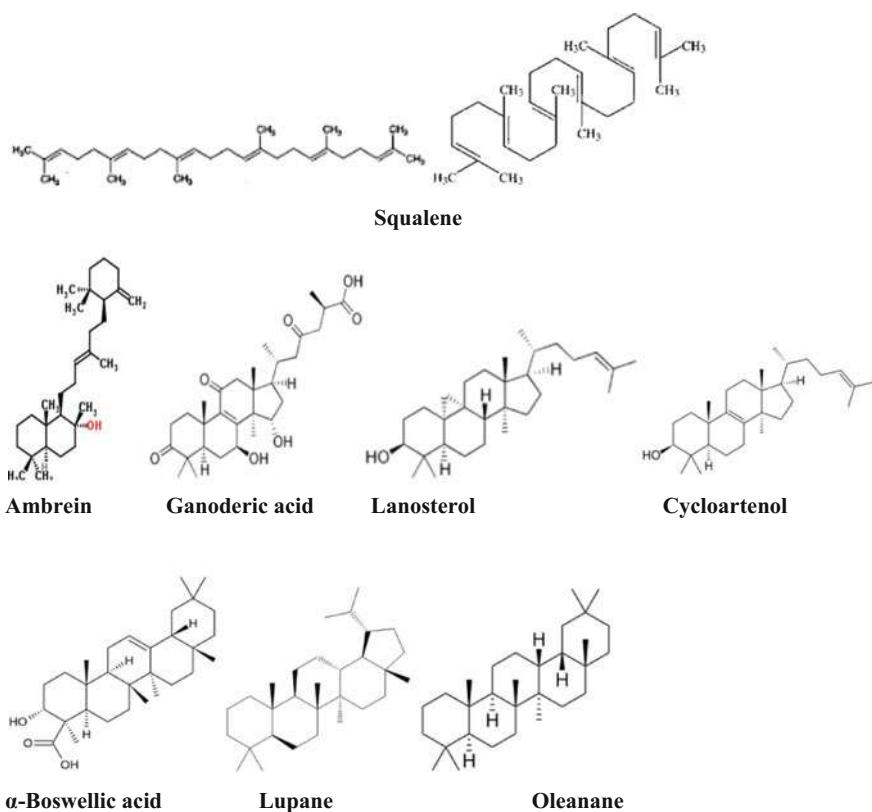


Fig. 3.8 Structure of different triterpenes including squalene, lanosterol or cycloartenol, sterols, steroids, and lupeol

cholesterol, steroid hormones, and vitamin D in the human body. Squalene is used in cosmetics, recently as an immunologic adjuvant in vaccines and as a chemopreventive substance against cancer. It has been suspected that sharks are resistant to cancer due to high tissue levels of squalene. Ambrein is a triterpene alcohol that is the chief constituent of ambergris, a secretion from the digestive system of the sperm whale, and has been suggested as the possible active component producing the supposed aphrodisiac effects of ambergris. It serves as a biological precursor for a number of aromatic derivatives (ambroxan) and is thought to possess fixative properties for other odorants. It has been shown to act as an analgesic. Ganoderic acids are a class of closely related triterpenoids (derivatives from lanosterol) found in *Ganoderma* mushrooms. There are dozens of ganoderic acids (ganoderic acid A, B, etc.). Some ganoderic acids have been found to possess biological activities including hepatoprotection, antitumor effects, and 5-alpha reductase inhibition.

Boswellic acids are a series of pentacyclic triterpene molecules that are produced by plants in the genus *Boswellia*. Boswellic acids appear in the resin of the plant

that exudes them and it makes up 30% of the resin of *Boswellia serrata*. A boswellic acid consists of a pentacyclic triterpene, a carboxyl group, and at least one other functional group. α -Boswellic acid and β -boswellic acid both have an additional hydroxyl group; they differ only in their triterpene structure. Acetyl- α -boswellic acid and acetyl- β -boswellic acid, replace the hydroxyl group with an acetyl group. β -Boswellic acid, keto- β -boswellic acid, and acetyl-keto- β -boswellic acid have been indicated in apoptosis of cancer cells, brain tumors, and cells affected by leukemia or colon cancer. Boswellic acids are also thought to decrease the symptoms of asthma.

Oleanane is a natural triterpene. It forms the central core for a wide variety of chemical compounds found in flowering plants which are referred to collectively as oleanane triterpenes. Some oleanane triterpenes have a suppressing effect on insect pests. They are considered a key marker differentiating flowering plants from other life and have been used in the effort to study their evolution, which is as yet poorly documented in the fossil record. Cycloartenol is an important type of stanol found in plants. It is also found in dandelion coffee. The biosynthesis of cycloartenol starts from the triterpenoid squalene. It is the first precursor in the biosynthesis of other stanols and sterols, referred to as phytostanols and phytosterols in photosynthetic organisms and plants.

3.1.1.7 Sesquiterpenes

Sesquiterpenes composed of seven isoprene units ($C_{35}H_{56}$). Many sesquiterpenes are typically microbial in their origin. Examples of sesquiterpenoids are ferrugicadiol and tetraprenylcurcumene.

3.1.1.8 Tetraterpenes

Tetraterpenes contain eight isoprene units ($C_{40}H_{64}$) and include carotenoids. Carotenoids containing oxygen such as lutein and zeaxanthin are known as xanthophylls while the oxygen-free carotenoids such as α -carotene, β -carotene, γ -carotene, and lycopene are known as carotenes (Fig. 3.9). The color of this group of pigments, ranging from pale yellow through bright orange to deep red, is directly linked to their structure. Xanthophylls are often yellow, hence their class name. Some of the best sources of xanthophylls are spinach, kale, dandelion, chard, chicory, collards, watercress and parsley, orange-red bell peppers, peas, pumpkin, corn, squash, broccoli, brussels sprouts, chlorella, and spirulina. Vegetables and fruits with yellow and orange pigments and more dark green leafy vegetables are good sources of xanthophylls. The lutein and zeaxanthin in egg yolks is very bioavailable.

The xanthophylls include astaxanthin, canthaxanthin, cryptoxanthin, lutein, zeaxanthin, etc. They are phytonutrient pigments with many health benefits, e.g., function as powerful antioxidants, and protect body cells from free radicals.

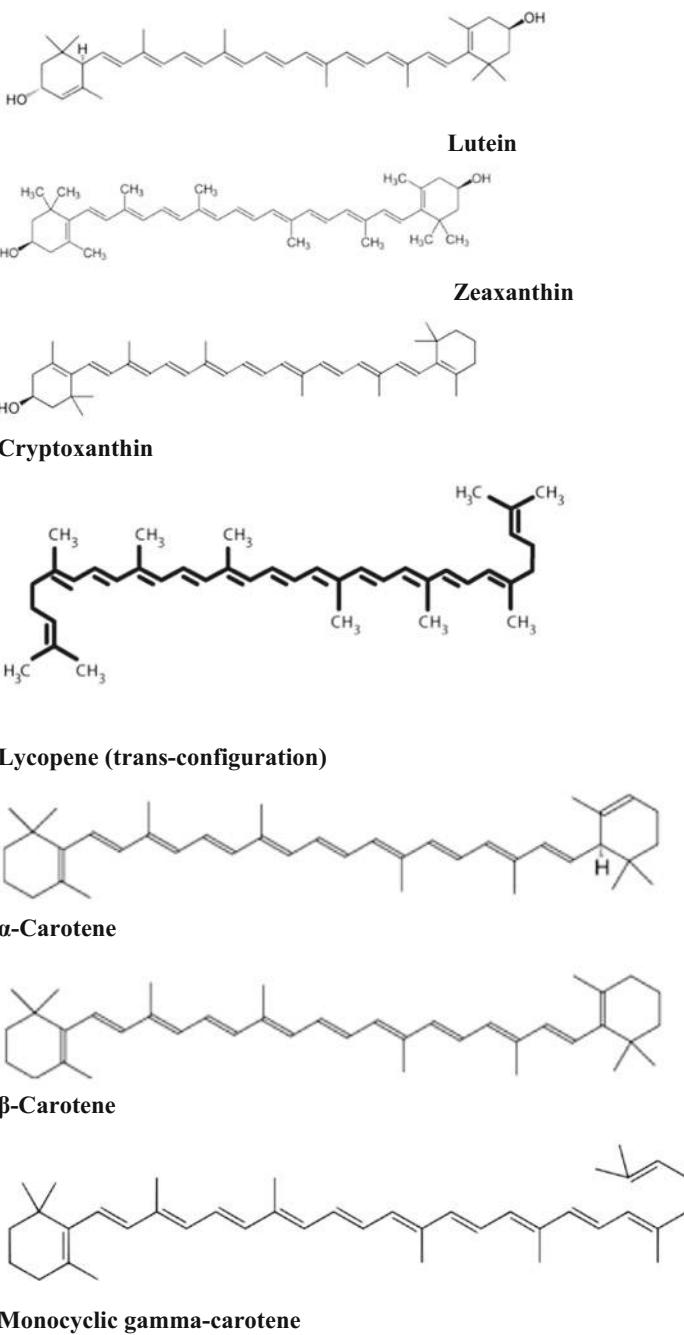


Fig. 3.9 Structure of different tetraterpenes—carotenoids

Cryptoxanthin, a provitamin A, helps to maintain healthy vision and xanthophylls boost up immune system and protect body against a number of disorders. Lutein, zeaxanthin, and cryptoxanthin are major xanthophyll carotenoids in human plasma and reduce the risk of cancers, cardiovascular disease, age-related macular degeneration, and cataract formation. Anyone who spends a lot of time looking at the computer screen could likely benefit from a good intake of lutein and zeaxanthin. Canthaxanthin and astaxanthin also have considerable importance in aquaculture for salmonid and crustacean pigmentation, and are of commercial interest for the pharmaceutical and food industries.

Carotenes and several related unsaturated hydrocarbon substances ($C_{40}H_x$) are synthesized by plants but cannot be made by animals. It is an orange photosynthetic pigment and vegetables and fruits like sweet potatoes, chanterelle, and orange cantaloupe melon contain carotene pigments. Carotenes contribute to photosynthesis by transmitting the light energy they absorb to chlorophyll. They also protect plant tissues by helping to absorb the energy from singlet oxygen, an excited form of the oxygen molecule O_2 which is formed during photosynthesis.

β -Carotene is composed of two retinyl groups and is broken down in the mucosa of the human small intestine by β -carotene 15,15'-monooxygenase to retinal, a form of vitamin A. β -Carotene can be stored in the liver and body fat and converted to retinal as needed, thus making it a form of vitamin A for humans and some other mammals. The carotenes α -carotene and γ -carotene, due to their single retinyl group (β -ionone ring), also have some vitamin A activity (though less than β -carotene), as does the xanthophyll carotenoid β -cryptoxanthin. All other carotenoids, including lycopene, have no beta-ring and thus no vitamin A activity (although they may have antioxidant activity and thus biological activity in other ways)

Lycopene is an acyclic isomer of β -carotene. It is an open chain polyisoprenoid with 11 conjugated double bonds. The structural formula of lycopene is represented in the diagram above. Lycopene is a bright red carotenoid pigment found in tomatoes and other red fruits and vegetables, such as red carrots, watermelons, Vietnamese gac fruit, papayas, pink grapefruits, apricots, pink guavas (in strawberries, red bell peppers, or cherries) as well as in brown beans, parsley, etc., although they are not red. In plants, algae, and other photosynthetic organisms, lycopene is an important intermediate in the biosynthesis of many carotenoids, including beta-carotene, which is responsible for yellow, orange, or red pigmentation, photosynthesis, and photo protection. Lycopene's 11 conjugated double bonds give its deep red color and its antioxidant activity but it has no vitamin A activity. Owing to the strong color and non-toxicity, lycopene is a useful food coloring. Preliminary research has shown that people who consume tomatoes may reduce the risk of prostate, breast, lung, bladder, ovaries, colon, and pancreas cancer, possibly due to lycopene. Consumption of tomato paste may decrease heart disease, atherosclerosis, age-related eye disorders, and sun damage by UV radiation through the action of lycopene. Lycopene is also used for treating human papillomavirus (HPV) infection, which is a major cause of uterine cancer. Some people also use lycopene for cataracts and asthma. All

health-related activities of lycopene are due to its powerful antioxidant activities that may help protect cells from damage.

γ -Carotene is a carotenoid and is a biosynthetic intermediate for cyclized carotenoid synthesis in plants. It is formed from cyclization of lycopene by lycopene cyclase epsilon. Along with several other carotenoids, γ -Carotene is a vitamer of vitamin A in herbivores and omnivores.

α -Carotene is a form of carotene with a β -ionone ring at one end (left end ring) and an α -ionone ring at the opposite end. It is the second most common form of carotene. Vegetables rich in α -carotene include yellow-orange vegetables like carrots sweet potatoes, pumpkin, winter squash, etc., as well as dark green vegetables like broccoli, green beans, green peas, spinach, turnip greens, collards, lettuce, avocado, etc. α -Carotene is a strong antioxidant agent and research findings support the view that blood levels of α -carotene lowers the risk of death from cancer, cardiovascular, and some other diseases. For example, people with 9 $\mu\text{g}/\text{dL}$ or more blood levels of α -carotene had a 39% lower risk of premature death than people with 0–1 $\mu\text{g}/\text{dL}$ blood levels of α -carotene.

β -Carotene is a strongly colored red-orange pigment abundant in vegetables and fruits like carrots, apricot, pumpkins, sweet potatoes, and also crude palm oil. It is chemically classified as a hydrocarbon, specifically as a terpenoid (isoprenoid), reflecting its derivation from isoprene units. β -carotene is distinguished from others by having beta-rings at both ends of the molecule. Absorption of β -carotene is enhanced if eaten with fats, as carotenes are fat soluble. In nature, β -carotene is a precursor (inactive form) to vitamin A via the action of beta-carotene 15,15'-monooxygenase. β -Carotene is effective in erythropoietic protoporphyria treatment and assumed, but not proved, that it reduces the risk of breast cancer before menopause, age-related cataract and the risk of age-related macular degeneration (AMD). The common side effect of excessive β -carotene consumption is carotenodermia, a physically harmless condition that presents as a conspicuous orange skin tint arising from deposition of the carotenoid in the outermost layer of the epidermis.

3.1.1.9 Polyterpenes

Polyterpenes consist of long chains of many isoprene units. Natural rubber consists of polyisoprene in which the double bonds are cis and some plants produce a polyisoprene with trans double bonds, known as gutta-percha (Fig. 3.10).

Chemically, gutta-percha is a polyterpene, a polymer of isoprene, or polyisoprene, specifically (trans-1, 4-polyisoprene). The cis structure of polyisoprene is the common latex elastomer or elastic polymers. While latex rubbers are amorphous in molecular structure, gutta-percha (the trans structure) crystallizes, leading to a more rigid material. Gutta-percha latex is biologically inert, resilient, and is a good electrical insulator with a high dielectric strength, an exudate isolated from several species of the tropical tree of the genus *Palaquium* (Sapotaceae), particularly from *Palaquium gutta*. Natural rubber is elastomer of polyisoprene, derived from the

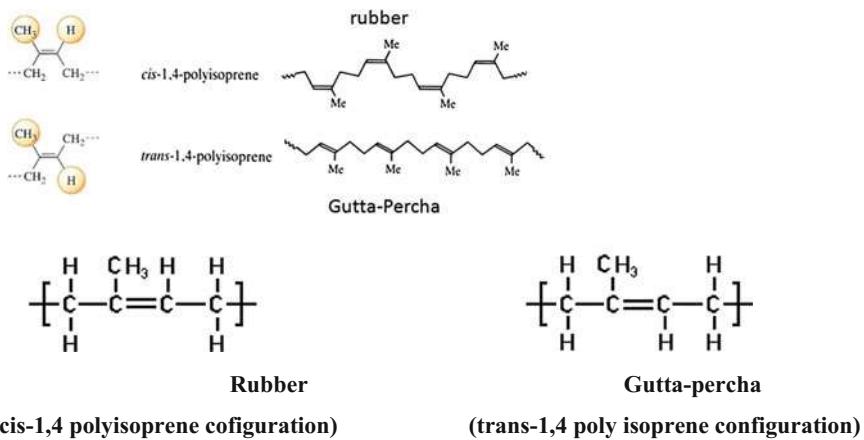


Fig. 3.10 Structure of polyterpenes—rubber and gutta-percha

latex of *Hevea brasiliensis* (Euphorbiaceae). Gutta-percha has been used in Endodontics for over 100 years and is currently the most frequently used core material for permanent obturation of root canals.

3.1.1.10 Norisoprenoids

Norisoprenoids are carotenoid breakdown products, e.g., C₁₃-norisoprenoids 3-oxo- α -ionol, present in Muscat of Alexandria leaves, and 7,8-dihydroionone derivatives, such as megastigmane-3,9-diol and 3-oxo-7,8-dihydro- α -ionol found in leaves of *Vitis vinifera*, can be produced by fungal peroxidases or glycosidases. Norisoprenoids originate in large carotenoid molecules found in grapes, such as β -carotene and lutein. These compounds accumulate during ripening, but break down into smaller compounds as the grapes reach maturity.

Norisoprenoids contribute to the varietal character of many aromatic varieties of wines, e.g., β -damascenone, 1,1,6,-trimethyl-1,2-dihydronaphthalene (TDN), and vitispirane. β -ionone, actinidiol, 3-oxo- α -ionol, and 2,2,6-trimethylcyclohexanone are other members of this class that are found in wine (Fig. 3.11).

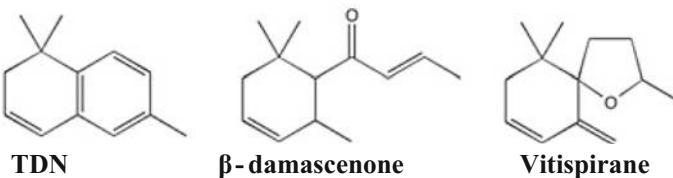


Fig. 3.11 Structures of three major C₁₃—norisoprenoids TDN, β -damascenone, and vitispirane

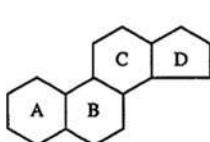
In the vineyard, increased sunlight exposure to the grapes seems to encourage the development of carotenoids, and subsequently increase the levels of norisoprenoids in finished wine. This effect probably occurs because the carotenoids help protect the grape tissue from ultraviolet light. In the laboratory, norisoprenoids are most often measured by gas chromatography–mass spectroscopy.

3.1.2 Steroids and Sterols

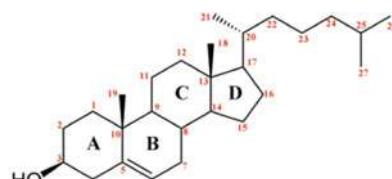
Steroids are cyclic terpenoids, and the term simply refers to a chemical molecule of 17 carbon atoms arranged in a fused four-ring structure consisting of three 6-carbon rings (A, B, C) and one 5-carbon ring (D) while sterols are steroid alcohols, with a hydroxyl group at the 3-position of the A-ring. When an eight-carbon side chain is put on carbon 17, the compound becomes typical animal sterol, the cholesterol (Fig. 3.12).

Sterols are amphipathic lipids, the hydroxyl group on the A-ring is polar and the rest aliphatic chain is nonpolar. Sterol is nearly ubiquitous among eukaryotes and almost completely absent in prokaryotes. So far, over 200 phytosterols have been reported, and among them, campesterol, β -sitosterol, stanols, and stigmasterol are most prominent; sterols of animal include cholesterol and steroid hormones like ecdysone, ecdysterone or 20E, cortisone, cortisol, and estradiol (E2) while ergosterol is fungal origin. Considerable variability in the concentration of free sterols was observed among different oils. While concentrations lower than 100 mg/100 g are found in oils from coconut, palm, olive, and avocado whereas concentrations between 100 and 200 mg/100 g are found in oils from peanut, safflower, soybean, borage, cottonseed, and sunflower, and concentrations between 200 and 400 mg/100 g are found in oils from sesame, canola, rapeseed, corn, and evening primrose (Fig. 3.13).

Campesterol is a phytosterol whose chemical structure is similar to that of cholesterol. It is so named because it was first isolated from the rapeseed (*Brassica campestris*). Many vegetables, fruits, nuts, and seeds contain campesterol, but in low concentrations. Banana, pomegranate, pepper, coffee, grapefruit, cucumber, onion, oat, potato, and lemongrass (citronella) are common sources containing

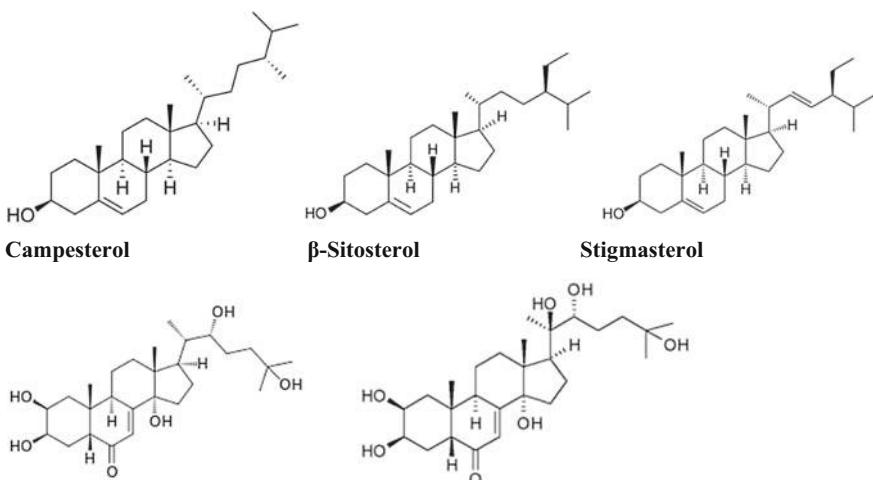


Four ring structure of steroid



Structure of cholesterol

Fig. 3.12 Four ring structure of steroid and cholesterol



Ecdysone (a steroidal prohormone) Ecdysterone or 20E

Fig. 3.13 Structure of different sterols—campesterol, β -sitosterol, and stigmasterol; steroid hormones—ecdysone and ecdysterone or 20E

campesterol at \sim 1–7 mg/100 g of the edible portion. Canola and corn oil contain as much as 16–100 mg/100 g. It is also found in dandelion coffee. It is thought to have anti-inflammatory effects, protects osteoarthritis-induced cartilage degradation. Being a steroid, campesterol is a precursor of anabolic steroid boldenone. Boldenone undecylenate is commonly used in veterinary medicine to induce growth in cattle but it is also one of the most commonly abused anabolic steroids in sports. Plant sterols, including campesterol inhibit the absorption of cholesterol in the intestines and thereby reduce LDLs or cholesterol level. The presence of phytosterols in the blood appears to be beneficial and is thought to reduce the chance of developing cardiovascular disease.

β -Sitosterol is structurally similar to that of cholesterol. Sitosterols are white, waxy powders with a characteristic odor. It is found in pecans, avocados, *Cucurbita pepo* seeds, cashew fruit, rice bran, wheat germ, corn oils, soybeans and dandelion coffee. β -Sitosterol is used for boosting the immune system and for preventing colon cancer, gallstones, common cold and flu (influenza), asthma, bronchitis, HIV and AIDS, rheumatoid arthritis, tuberculosis, psoriasis, allergies, cervical cancer, fibromyalgia, systemic lupus erythematosus (SLE), hair loss, migraine headache and chronic fatigue syndrome and benign prostatic hyperplasia. However, β -sitosterol enriched food should be avoided during pregnancy, and breastfeeding is also not recommended for individuals with sitosterolemia (fat storage disease) as well as β -sitosterol enriched food is not recommended for people suffered from heart attacks.

Stigmasterol (anti-stiffness factor) is chemically like animal cholesterol. Phytosterols are insoluble in water but soluble in most organic solvents and contain one alcohol functional group. Stigmasterol is found various vegetables, legumes,

nuts, seeds, and unpasteurized milk, in the plant fats or oils of soybean, calabar bean, and rape seed, and in a number of medicinal herbs, including the Chinese herbs *Ophiopogon japonicus* and American Ginseng. Stigmasterol is used as a precursor in the manufacture of semisynthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. It is also used as the precursor of vitamin D₃. Stigmasterol like other plant sterols inhibits hepatic synthesis and competes with cholesterol for intestinal absorption to limit absorption and lower plasma concentrations of cholesterol. Stanols are a saturated subgroup of sterols. Stanol esters are a heterogeneous group of phytosterol esters with a saturated sterol ring structure known to reduce the level of low-density lipoprotein (LDL) cholesterol in blood when ingested. However, no evidence of any beneficial effect on cardiovascular disease exists. Their main sources are whole-grain foods, mostly wheat and rye. The LDL lowering efficacy of plant stanol ester is dose dependent, but the same effect was not found with plant sterols (Fig. 3.14).

Cholesterol is the principal sterol synthesized by animals. Cholesterol is an essential structural component of animal cell membranes that is required to maintain membrane structural integrity, fluidity and viability. Sterols and related compounds play essential roles in the physiology of organisms as cell membrane component, signaling compounds and as a precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D. Sterols may be found either as free sterols (cholestane, cholesterol), acylated (sterol esters), alkylated (steryl alkyl ethers), sulfated (sterol sulfate), or linked to a glycoside moiety (steryl glycosides) which can be itself acylated (acylated sterol glycosides). Major dietary sources of cholesterol include cheese, egg yolks, beef, pork, poultry, fish, and shrimp. Human

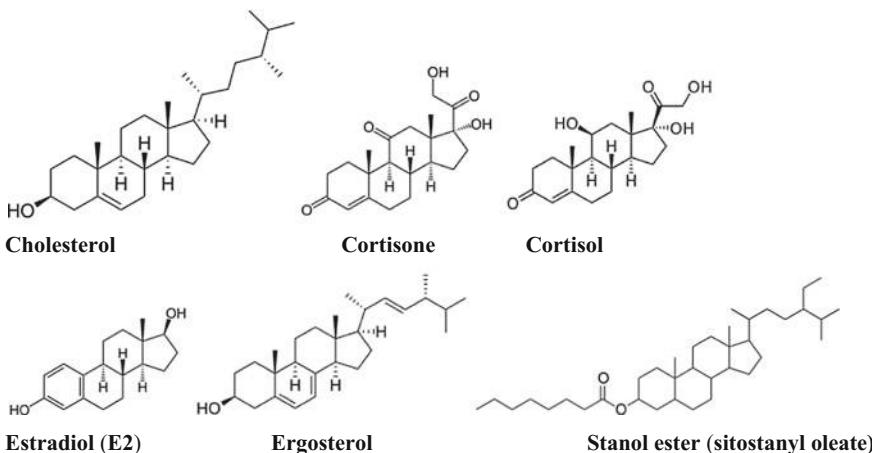


Fig. 3.14 Structure of cholesterol, cortisone, cortisol, estradiol (E2), ergosterol, and stanol ester (sitostanyl oleate)

breast milk also contains significant quantities of cholesterol. From a dietary perspective, cholesterol is not found in significant amounts in plant sources. In addition, plant products such as flax seeds and peanuts contain cholesterol-like compounds called phytosterols, which are believed to compete with cholesterol for absorption in the intestines. Cholesterol also serves All kinds of cells in animals can produce it. In vertebrates, the hepatic cells typically produce greater amounts than other cells. It is almost completely absenting among prokaryotes (bacteria and archaea), although there are some exceptions such as Mycoplasma, which require cholesterol for growth.

Cortisone is a 21-carbon steroid hormone. It is one of the main hormones released by the adrenal gland in response to stress. In chemical structure, it is a corticosteroid closely related to cortisol. It is used to treat a variety of ailments and can be administered intravenously, orally, intra-articularly (into a joint), or transcutaneously. Cortisone suppresses the immune system, thus reducing inflammation and attendant pain, and swelling at the site of the injury. Risks exist, in particular in the long-term use of cortisone. Cortisol is a steroid hormone, more specifically a glucocorticoid, which is produced by the zona fasciculata of the adrenal cortex. It is released in response to stress and a low level of blood glucose. Its functions are to increase blood sugar through gluconeogenesis, to suppress the immune system, and to aid the metabolism of fat, protein, and carbohydrate. It also decreases bone formation. Hydrocortisone is a name for cortisol when it is used as a medication. Hydrocortisone is used to treat people who lack adequate naturally generated cortisol.

Estradiol, or more precisely, 17β -estradiol, is a human sex hormone and steroid, and the primary female sex hormone. It is named for and is important in the regulation of the estrous and menstrual female reproductive cycles. Estradiol is essential for the development and maintenance of female reproductive tissues. Estradiol is found in most vertebrates as well as many crustaceans, insects, fish, and other animal species.

3.2 Volatile Oils

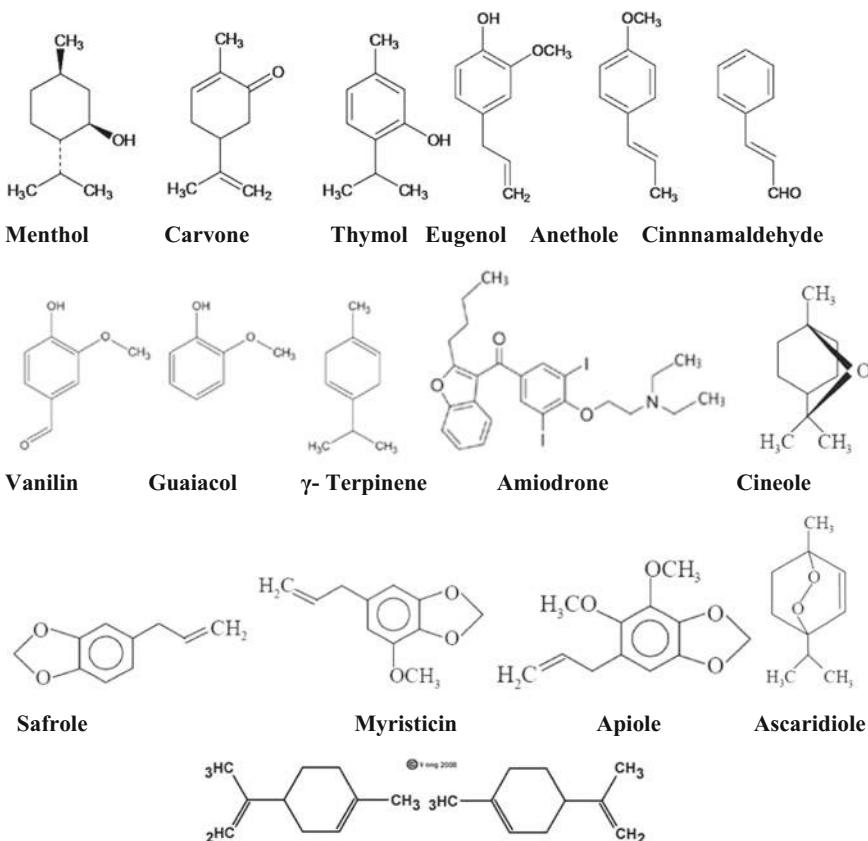
Volatile oils are the odoriferous principles present in various plant organs, e.g., flowers (rose), leaves (mint), fruits (lemon), bark (cinnamon), wood (cedar), root (ginger) or seeds (cardamom), and many resinous exudations as well. They are present in over 60 families, especially in the members of Lauraceae, Myrtaceae, Apiaceae, Lamiaceae, and Asteraceae. They are very complex in chemical nature (>200 components); hydrocarbon terpenes and their oxygenated and sulphured products are two main groups of chemicals. The quantity of oil varies from a very small amount to as much as 1–2%. Volatile oils evaporate when exposed to air at normal temperature and pressure and since they represent the essences or odors of the plant or plant part, they are also called essential oils. Essential oils are also known simply as the oil of the plant from which they were extracted (oil of clove).

Essential oils occur in glandular hairs, modified parenchymatous cells, oil tubes (vittae) and in some special oil ducts and are extracted by water (rose water) or steam distillation (eucalyptus oil), expression (grapefruit oil), solvent extraction (rose absolute and other lipophilic material) and florasol extraction (temperature sensitive material). Volatile oils are mixtures of hydrocarbons and their oxygenated derivatives. The odor and taste of volatile oils are mainly determined by their water soluble oxygenated constituents. This very fact makes it possible to prepare aromatic waters (rose water, orange water) by shaking volatile oils with water.

Volatile oils differ from the fatty or fixed oils both in their chemical composition and physical properties. The principal points of difference between volatile and fixed oils are as follows: (i) volatile oils are hydrocarbon terpenes and their oxygenated derivatives with a pleasant taste and strong aromatic odor and they are mixture of monosesquiterpenes (stereoptenes and oleoptenes) products and not glyceryl esters of fatty acids, (ii) they evaporate or volatilize on contact with the air and do not leave any permanent grease spot on paper, (iii) cannot be saponified with alkalis, (iv) volatile oils are usually extracted from their natural sources by water or steam distillation whereas fixed oils are extracted by hot or cold expression or with organic solvents, (v) volatile oils have high refractive index and optically active while fixed oils have low refractive index and optically inactive, and (vi) volatile oils do not become rancid as do the fixed oils, but instead, on exposure to light and air, they oxidize and resinify.

There are about 100 commercially valuable volatile oils. They are grouped on the basis of their synthetic pathways as (i) terpene derivatives (synthesized via the acetate–mevalonic acid pathway), e.g., peppermint oil (menthol), caraway oil (carvone), thyme oil (thymol); (ii) aromatic compounds (synthesized via the shikimic acid–phenylpropanoid pathways), e.g., clove oil (eugenol), anise oil (anethole), cinnamon oil (cinnamaldehyde) and (iii) others (follow some miscellaneous route). Figure 3.15 shows structures of plant essential oil constituents with closely related precursors—menthol, carvone, thymol, eugenol, anethole, cinnamaldehyde, vanillin, guaiacol, γ -terpinene, amiodrone, cineole, safrole, myristicin, apiole, and ascaridiole; stereoisomers—L-limonene and D-limonene.

Safrole is a constituent of many volatile oils, the dried bark of the roots of *Sassafras albidum* of Lauraceae and in a variety of other plant sources, namely: *Acorus calamus*, Araceae; *Angelica polymorpha*, Apiaceae; *Cananga odorata*, Annonaceae; *Cinnamomum comphora*, Lauraceae; *Illicium verum*, Magnoliaceae; *Myristica fragrans*, Myristicaceae; *Ocimum basilicum*, Lamiaceae; *Piper nigrum*, Piperaceae; *Theobromacacao*, Sterculiaceae. Botanical sources of the aromatic ether myristicin include black pepper (*Piper nigrum*, Piperaceae); mace, nutmeg (*Myristica fragrans*, Myristicaceae), French parsley (*Petroselinum crispum*, Apiaceae); carrots (*Daucus carota*) and dill seed (*Anethum graveolens*, Apiaceae); sassafras (*Sassafras albidum*, Lauraceae). Apiole (a phenylpropene) occurs abundantly in dill oil (*Anethum graveolens*, Apiaceae); parsley seed oil (*Petroselinum crispum*, Apiaceae). Cineole (an essential oil) is the chief constituent of oil of eucalyptus obtained from the leaves of *Eucalyptus globulus* (Myrtaceae) and other species of Eucalyptus. Ascaridiole (a bicyclic monoterpene) is the major constituent (65–70%) in the chenopodium oil, i.e., a volatile oil of *Chenopodium ambrosioides* of Chenopodiaceae.



Stereoisomers: L-limonene and D-limonene mirror images

Fig. 3.15 Structures of plant essential oil constituents with closely related precursors—menthol, carvone, thymol eugenol, anethole, cinnamaldehyde, vanillin, guaiacol, γ -terpinene, amiodrone, cineole, safrole, myristicin, apiole, and ascaridiole. Stereoisomers—L-limonene and D-limonene

Volatile oils have no physiological significance to plants; they represent byproducts rather than foods. However, the characteristic aromas have some advantage in attracting insects and other animals for pollination and dispersal of fruits and seeds and in some cases for repelling the herbivore enemies. There is some evidence that they play an even more vital role as hydrogen donors in oxidoreduction reactions, as potential sources of energy, or in affecting transpiration and other physiological processes. The oils may also have some antiseptic and bactericidal value.

Volatile oils are mainly used as flavoring agents in cosmetics (perfumes, soaps and other products), in food products (food and drink), in household cleaning products, and in pharmaceuticals. They possess a carminative action and some of them also have other therapeutic properties like antiseptic and anesthetic properties.

They are commonly used in both modern allopathic and traditional systems of medicine. The important volatile oil-containing drugs of natural origin include Peppermint (*Mentha piperita*), Cinnamon (*Cinnamomum zeylanicum*), Lemon peel (*Citrus limon*), Camphor (*Cinnamomum camphora*), Clove (*Eugenia caryophyllus*), Anise (*Pimpinella anisum*), and Eucalyptus (*Eucalyptus globulus*). Plants grown for their unique essential oils include *Rosa damascena* (Rosaceae), *Jasminum grandiflorum* (Oleaceae), *Pelargonium* spp. (Geraniaceae), *Gardenia* spp. (Rubiaceae), and *Viola odorata* (Violaceae). Lavender comes from the species of *Lavandula* (Lamiaceae). Orange blossom perfume comes from *Citrus aurantium* and bergamot is from the fruit rinds of *C. bergamia* (Rutaceae). In addition to that, they are also utilized for many other industrial and commercial purposes. Volatile oils are used in aromatherapy, a form of alternative medicine that uses essential oils and other aromatic compounds for the purpose of altering one mood, cognitive, psychological or physical wellbeing, through topical application, (general massage, baths, compresses, therapeutic skin care massage), aerial diffusion (environmental fragrancing or aerial disinfection), direct inhalation (respiratory disinfection, decongestion, expectoration as well as psychological effects) or water immersion to stimulate a desired response.

3.3 Miscellaneous Isoprenoids

3.3.1 Resins

Resins are complex viscous exudates (containing essential oils, oxygenated products of terpenes and carboxylic acids) of many plants, particularly in the schizogenous ducts of coniferous trees, resin cells of ginger and glandular hairs of Cannabis. Distribution of resins in Cryptogam is absent (including sea weeds and fungi) and present in a few Phanerogam families like Pinaceae, Araceae, Dracaenaceae, Berberidaceae, Plumbaginaceae, Moraceae, Fabaceae, Dipterocarpaceae, Burseraceae, Apiaceae, Anacardiaceae, Piperaceae, etc. In plants, resins occur in different secretory zones or structures, e.g., in resin cells of ginger, in schizogenous ducts or cavities of pine wood and in glandular hairs of cannabis. Chemically, resins are a complex mixture of many components and the major chemical constituents of resins may be grouped under three heads: of (i) resin acids, (ii) resin esters, i.e., esters of resin alcohols (resinols) and resin phenols (resinotannols), and (iii) chemically inert compounds resenes. Acid resins include colophony (abietic acid), copaiba (copaivic and oxycopaivic acid), sandarac (sandracolic acid), shellac (aleuritic acid), and myrrh (commiphoric acid); ester resins include benzoin (benzyl benzoate), storax (cinnamyl cinnamate), balsam of Peru (peruresinotannol), guaiacum resin (guaic resinol), and gurjun balsam (gurjuresinol); and resene resins include dragon's blood (dracoresene), gutta-percha (fuavil) dammar, mastic, myrrh, and olibanum. Resins may also be grouped as

Coniferous (colophony, sandarac), Berberidaceae (*Podophyllum*), and Zygophyllaceae (*Guaiacum*) resins. Aroma pertains to different volatile fluid terpenes while the dissolved nonvolatile solids make resins thick and sticky. Some resins also contain a high proportion of resin acids. The volatile components of resins from Jeffrey Pine and Gray Pine are largely pure *n*-heptane with little or no terpenes. Resins often occur in mixtures with volatile oils (oleoresins) or with gums (gum resins) or with both gum and volatile oils (oleo-gum-resins). Acid resins may be abietane (abietic acid), pimarane (pimamic acid), labdane (communic acid, ozoic acid), kaurane (trachylobanic acid), and clerodane (hardwickiic acid) types having the characteristic acids noted inside the parenthesis. Resins are also grouped as hard resins (copal, damar, mastic, dragon's blood, etc.) and soft resins and balsams (benzoin, styrax, balsams of tolu and Peru, copaiba, elemi, asafetida and galbanum, etc.). Resins are usually hard and transparent or translucent substances, which are insoluble in water but soluble in alcohol and other organic solvents. True balsams are oleoresins, but the resinous mixtures that contain cinnamic or benzoic acid or both or esters of these acids are also known as balsams. Hence, all balsams are oleoresins but not all resins are balsams. Canada balsam (from fir tree, *Abies balsamea*, a widely used Christmas tree), larch balsam (often called larch turpentine is exudate of the *Larix europaea*), and copaiba balsam (from the trunk exudate of South American leguminous trees genus *Copaifera*) are terpenes, containing the characteristic resin in solution, and are not regarded as true balsams. Resins and balsams are used in pharmacy as stiffening agents, purgatives, and cathartics. Important resin-containing drugs of natural origin include Rosin (from *Pinus palustris*), *Podophyllum*, Jalap (roots of *Exogonium purga*), Cannabis (*Cannabis sativa*) and Ginger (*Zingiber officinale*). Copaiba balsam is medicinally important due to its anti-inflammatory, antitumor, anti-tetanus, antiseptic and antihemorrhagic properties. Galbanum, used in medicine, is a gum resin from the perennial herb *Ferula galbaniflua* of western Asia. Creosote bush resin is obtained from the leaves and small twigs of the greasewood bush, *Larrea tridentata*, or creosote bush, *L. divaricata*, of the desert regions of Mexico and the southwestern United States. It is used in adhesives, insecticides, core binders, insulating compounds, and pharmaceuticals. Okra gum is from the fruits of *Hibiscus esculentus* is edible and is used as a thickening agent in foodstuffs and pharmaceuticals. It has antioxidant properties and acts as a stabilizer and a gelling agent. Okra gum is also used in plating baths as a brightener. Ammoniac is a gum resin from the stems of *Dorema ammoniacum*, a desert perennial plant of Persia and India. It is used in adhesives, in perfumery, and as a stimulant in medicine. Amber and copal are fossil resins from ancient trees, which have been chemically altered by long exposure.

Resin alcohols may occur in free state or esters, e.g., balsam of Peru with peruresinotannol and guaiacum resin with guaic resinol. Resins (colophony, cannabis); oleoresins (copaiba, ginger); oleo-gum-resins (asafetida, myrrh); balsams (balsam of Peru, balsam of tolu); glycoresins (jalap); and resenes (asafetida, colophony) are known from different plant sources. The oleo-gum-resin yields about 30% alcohol soluble extract and contains phenolic compounds such as pyrocatechin and protocatechuic acid, and gum is alcohol insoluble and comprised of protein

(18%) and carbohydrate (64%) made up of arabinose, galactose, and glucuronic acid and associated with an oxidase enzyme. Myrrh contains volatile oil (7–17%), resin (20–25%), gum (57–61%). The volatile oil consists of eugenol, m-cresol, and cuminaldehyde. The resin is found to consist of a mixture of α -, β -, and γ -commiphoric acids (resin acids) which are ether soluble, and also two ether insoluble phenolic resins α - and β -herrabomyrhololic acids.

Resin acid refers to mixtures of several related carboxylic acids, and basic skeleton is made up of three fused rings (diterpenes composed of four isoprene units) with the empirical formula $C_{19}H_{29}COOH$. Acid resins contain abietic acids, sandracolic acid, commiphoric acid, copaivic acid, etc. A few typical examples of resin acids are shown below (Fig. 3.16).

The α - and β -amyrins are commonly found in wood resins and the bark of many trees (Fig. 3.17).

Rosin (also called colophony or resin from the pine trees of Colophon), a semitransparent, yellow to black solid form of resin, is obtained mostly from conifers. It chiefly consists of different resin acids, especially abietic acid. Rosin is an ingredient in printing inks, photocopying and laser printing paper, varnishes, adhesives (glues), soap, paper sizing, soda, soldering fluxes, and sealing wax. Rosin can be used as a glazing agent in medicines and chewing gum, can be used as an emulsifier in soft drinks. In pharmaceuticals, rosin forms an ingredient in several plasters and ointments, also used for tablet film and enteric coating purpose. Rosins have also been used to formulate microcapsules and nanoparticles.

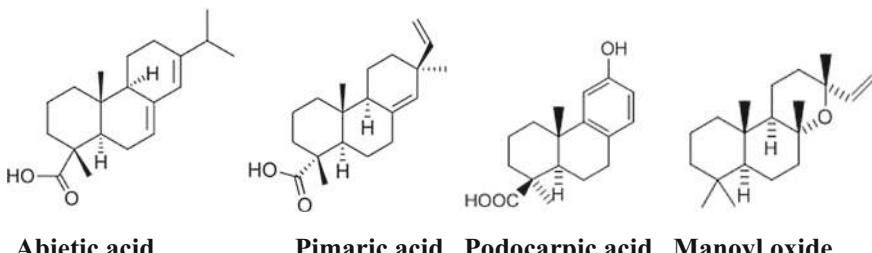
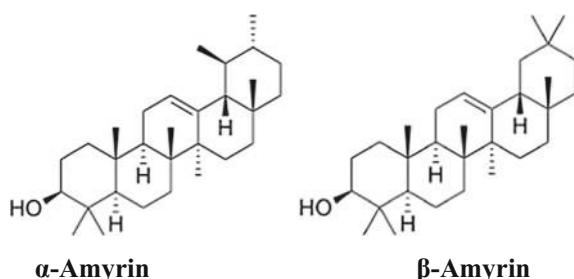


Fig. 3.16 Abietic acid, pimamic acid, podocarpic acid, and manoyl oxide

Fig. 3.17 α - and β -amyrins are commonly found in wood resins



3.4 Phenols and Phenylpropanoids

3.4.1 *Phenol, Polyphenol, Phenolic Acids and Phenylpropanoids*

Phenols or phenolics, are a class of chemical compounds consisting of a hydroxyl group ($-OH$) bonded directly to an aromatic hydrocarbon group. The simplest of the class is phenol, which is also called carbolic acid C_6H_5OH . Unlike alcohols, phenols can contribute proton in solution since the hydroxyl group is bonded to an unsaturated carbon atom having tight coupling with the oxygen and a relatively loose bond between the oxygen and hydrogen. The acidity of the hydroxyl group in phenols is commonly intermediate between that of aliphatic alcohols and carboxylic acids, and their pK_a is usually between 10 and 12. Loss of H^+ from the hydroxyl group of a phenol forms a corresponding negative phenolate ion or phenoxide ion, and the corresponding salts are called phenolates or phenoxides. Phenols are aromatic compounds and are classified as simple phenols or polyphenols based on the number of phenol units in the molecule; occur naturally in many fruits, vegetables and also in some essential oils of plants and show powerful antiseptic and antibacterial properties (Table 3.2). Polyphenols are a structural class of mainly natural organic compounds characterized by the presence of large multiples of phenol structural units while phenols are substances with a benzene nucleus supporting a hydroxile group. They range from very simple substances to very complex ones such as lignins and tannins. The main groups in this category are phenolic acids or phenols, cumarines, flavonoids, lignanes, tannins, and quinines. The historically important chemical class of tannins is a subset of the polyphenols. Phenols are aromatic compounds and these fragrant compounds can act to stimulant nerves and immune system, and can sometimes cause liver damage and skin irritation.

Phenolic acids are aril-carboxilic and contain one or more OH groups in the aril. They have various pharmacological properties and uses: antioxidant, analgesics, choleric, etc. Eugenol, for example, is an antiseptic and also a local anesthetic used in dentistry.

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. Phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecule. Phenol (carbolic acid) is an aromatic organic compound with the molecular formula C_6H_5OH . It is a white crystalline solid but volatile. It is mildly acidic and may cause chemical burns. The molecule consists of a phenyl group ($-C_6H_5$) bonded to a hydroxyl group ($-OH$). Similar to alcohols but unlike alcohols the hydroxyl group in phenol is attached to an unsaturated aromatic hydrocarbon ring and phenols can donate H^+ insolution to produce conjugate base. Loss of H^+ from the hydroxyl group of a phenol forms a corresponding phenoxide ion, and the corresponding salts are called phenolates or phenoxides. Phenols with two or more hydroxyl groups bonded to one or several aromatic ring or rings of the same molecule are called polyphenols (quercetin). The simplest examples are the three benzenediols, each having two

Table 3.2 Classification of phenol, polyphenol, and phenolic acids with their structure, source, and function

Classes	Name	Molecular formula	Structure	Source	Function
1. Simple- and poly-phenols	Carbolic acid	C ₆ H ₆ O		Berries, tea, cocoa, coffee, fruits, spices, and vegetables	Antioxidant, precursors of aspirin, herbicides and pharmaceutical drugs, and antiseptic
	Catechol	C ₆ H ₄ (OH) ₂		<i>Mimosa catechu</i>	Precursor to pesticides, flavors, and fragrances
	Hydroquinone	C ₆ H ₄ (OH) ₂		Active toxin in <i>Agaricus hondensis</i> mushrooms	Reducing agent, a potential carcinogen suspect
	2,6-dimethoxybenzoquinone	C ₈ H ₈ O ₄		<i>Rauvolfia vomitoria</i> , <i>Tibouchina pulchra</i>	Mutagenic, cytotoxic, genotoxic, and hepatotoxic
	Quercetin, a flavonol.				

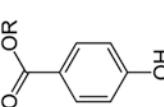
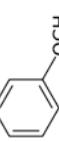
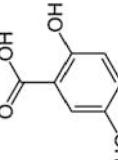
(continued)

Table 3.2 (continued)

Phenol, polyphenol, and phenolic acids					
Classes	Name	Molecular formula	Structure	Source	Function
	Cyanidin, an anthocyanidin				Ease aches, pains, and reduce fevers
2. Derivatives of benzoic acid: a. Mono hydroxy benzoic acids	Salicylic acid	$C_7H_6O_3$		<i>Salix alba</i>	Shows antifungal, antimutagenic, antisticking, and estrogenic antimicrobial activities
	3-hydroxybenzoic acid	$C_7H_6O_3$		<i>Citrus paradise</i> , <i>Olea europaea</i> , <i>Mespilus germanica</i> , and <i>Castor canadensis</i>	
	4-hydroxybenzoic acid	$C_7H_6O_3$		<i>Vitex agnus-castus</i> , <i>V. negundo</i> , and <i>Hypericum perforatum</i>	Used as intermediate for pesticide, antispasmodics, and pharmaceuticals

(continued)

Table 3.2 (continued)

Phenol, polyphenol, and phenolic acids					
Classes	Name	Molecular formula	Structure	Source	Function
	Paraben (methyl propyl parabens)	Ester of 4/ para-hydroxybenzoate, R=an alkyl group— methyl, propyl		Commercial parabens are synthetic; methylparaben is found in blueberries	Antimicrobial agent; used as food additives, as preservatives by cosmetic and pharmaceutical industries, breast cancer suspect
b. Dihydroxy benzoic acids	Vanillin, phenolic aldehyde	C ₈ H ₈ O ₃		Vanilla planifolia orchid bean seed	Flavoring foods, icecream, baked goods, and medicines
	Vanillic acid, an oxidized form of vanillin	C ₈ H ₈ O ₄		Angelica sinensis, a Chinese herb	Used as a flavoring agent
	Gentisic acid, a dihydroxybenzoic acid	C ₇ H ₆ O ₄		African tree Alchornea cordifolia	Used as an antioxidant excipient in pharmaceutical preparations

(continued)

Table 3.2 (continued)

Phenol, polyphenol, and phenolic acids					
Classes	Name	Molecular formula	Structure	Source	Function
	Protocatechuic acid, a dihydroxybenzoic acid	C ₇ H ₆ O ₄		<i>Hibiscus sabdariffa</i> , green tea	Antioxidant and anti-inflammatory agent
c. Trihydroxybenzoic acids	Gallic acid	C ₇ H ₆ O ₅		In gallnuts, sumac, witchhazel, tea leaves, oak bark	Antifungal, antiviral, antioxidant, and anticarcinogenic
	Ellagic acid	C ₁₄ H ₁₀ O ₈		Blackberries, cranberries, pecans, pomegranates, raspberries, strawberries, walnuts, wolfberries, grapes, <i>Quercus alba</i>	Antiproliferative and antioxidant
	Syringic acid	C ₉ H ₁₀ O ₅		<i>Aralia elliptica</i> , <i>Eurycoma longifolia</i>	

(continued)

Table 3.2 (continued)

Phenol, polyphenol, and phenolic acids					
Classes	Name	Molecular formula	Structure	Source	Function
	Eudesmic acid	C ₁₀ H ₁₂ O ₅		<i>Eucalyptus</i> spp.	
	Phloroglucinol carboxylic acid	C ₇ H ₆ O ₅		Produced by <i>Pseudomonasfluorescen-</i> , <i>Acinetobactercalcoaceticus</i>	
3. Derivatives of cinnamic acid	Cinnamic acid				
	Caffeic acid				

(continued)

Table 3.2 (continued)

Phenol, polyphenol, and phenolic acids					
Classes	Name	Molecular formula	Structure	Source	Function
	Coumaric acid				
	Ferulic acid				

hydroxyl groups on a benzene ring. Phenol and its chemical derivatives are used in the production of detergents, phenoxy herbicides, numerous pharmaceutical drugs and many industrial synthetic goods.

Phenolic acids are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants (>8000 phenolic structures currently known) ranging from simple molecules (phenolic acids) to highly polymerized substances (tannins). Phenolic acids or phenolcarboxylic acids are types of aromatic acid compound containing a phenolic ring and an organic carboxylic acid function (C_6-C_1 skeleton). Phenolic acids can be divided into two classes, e.g., derivatives of benzoic acid (gallic acid), and derivatives of cinnamic acid (coumaric, caffeic and ferulic acid). The benzoic acid derivatives of phenolic acids include mono-hydroxybenzoic acids (salicylic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid and also esters of this group like paraben, methyl paraben, propyl paraben, etc.); dihydroxybenzoic acids (vanillin, vanillic acid, gentisic acid, protocatechuic acid, etc.); and trihydroxybenzoic acids (gallic acid, ellagic acid, syringic acid, eudesmic acid, phloroglucinol carboxylic acid). Phenolic acids can be found in many plant species. Natural phenols in horse grams (*Macrotyloma uniflorum*) are mostly phenolic acids (3,4-dihydroxybenzoic, *p*-hydroxybenzoic, vanillic, caffeic, *p* coumaric, ferulic, syringic, and sinapinic acids). Phenolic acids can be found in mushroom, in humic substances of soil humus and also in human urine. The diverse classes of phenolic compounds made by plants are known to play multifunctional roles in rhizospheric plant–microbe interactions. Phenolic acids are the main polyphenols made by plants. Phenolic compounds act as signaling molecules in the initiation of legume rhizobia symbioses, establishment of arbuscular mycorrhizal symbioses and can act as agents in plant defense. Caffeic acid is the most abundant phenolic acid in many fruits and vegetables, most often esterified with quinic acid as in chlorogenic acid, which is the major phenolic compound in coffee. Another common phenolic acid is ferulic acid, which is present in cereals and is esterified to hemicelluloses in the cell wall.

The phenylpropanoids, the name indicates a phenyl ring and a propene tail (C_6-C_3), include a diverse group of organic compounds they are synthesized in plants from the phenylalanine and tyrosine (Fig. 3.18). Phenylpropanoids are found throughout the plant kingdom and serve as essential components of a number of structural polymers, provide protection from ultraviolet light, defend against herbivore and pathogen predators, and attract pollinators as floral pigments and scent compounds. Concentrations of phenylpropanoids within plants are also altered by changes in resource availability (Davey et al. 2004). Plant-derived phenylpropanoids (PPPs) are parent molecules for biosynthesis of numerous structurally and functionally diverse plant polyphenols, E.G, phenolic acids and esters, glycosylated derivatives of primary PPPs, flavonoids, isoflavonoids, stilbenes, coumarins, curcuminoids, lignans, etc., and play multiple essential roles in plant physiology.

Phenylpropanoids have been identified as potential radiotherapeutic agents due to their anticancer activity and relatively safe levels of cytotoxicity and based on experimental findings, it is expected that these compounds could not only sensitize

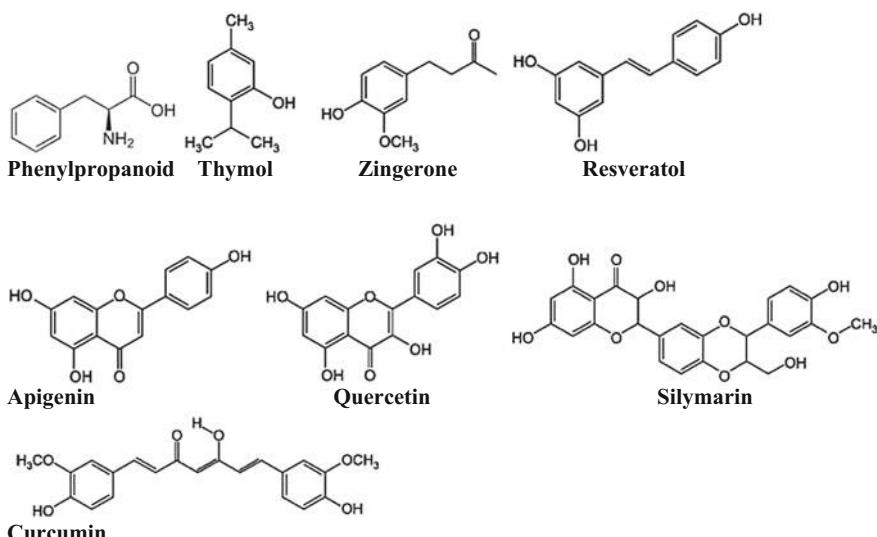


Fig. 3.18 Structure phenylpropanoid and examples of several other phenylpropanoids with radioregulation properties

cancer cells to radiation resulting in inhibition of growth and cell death but also protect normal cells against radiation-induced damage. Radiosensitizing properties of phenylpropanoids include inhibition of inflammatory response, antiapoptotic activity, ROS regulation, inhibition of NF- κ B activation, ERK activation, inhibition of AKT activation, cell cycle arrest, etc. (Kim et al. 2011).

3.5 Alkaloids

Alkaloids include a vast and diverse group of chemical arsenals of secondary metabolic origin with alkali-like reaction. A clear-cut boundary between alkaloids and naturally occurring complex amines is absent and also a precise definition of alkaloids. However, alkaloids (alkali-like) may be defined as basic nitrogenous heterocyclic compounds with bitter taste, plant origin and possess a marked physiological action on man and other animals. Exceptions to this definition are known, e.g., (i) several alkaloids like colchicine, piperine, and quaternary alkaloids (tubercurarine) are not basic and in some others basicity may be weak base (caffeine), strong base (atropine), amphoteric (morphine, narceine, theobromine and theophylline) or neutral (colchicine); (ii) nitrogen in ephedrine, colchicine, hordenine and mescaline is not in a heterocyclic ring; and (iii) alkaloids are not confined to plant kingdom only, some alkaloids are also found in bacteria, fungi, frogs, insects and other animals. Considering all these exceptions, now alkaloids are defined as

cyclic organic compounds containing nitrogen in a negative state of oxidation with limited distribution among living organisms.

Alkaloids may be solid crystalline (majority), amorphous (emetine), volatile liquid (nicotine, coniine) or nonvolatile (pilocarpine, hyoscine). Alkaloids may contain other elements (oxygen, sulfur and more rarely chlorine, bromine, and phosphorus) in the molecule in addition to CHN and oxygen-containing (majority) alkaloids are usually colorless crystals at ambient conditions while oxygen-free alkaloids (e.g., nicotine, coniine, spartine, etc.) are typically volatile, colorless, oily liquids. Some alkaloids may be yellow (berberine, colchicine), brown (nicotine), orange (betanidine, sanguinarine) or copper red (salts of sanguinarine), etc.

Alkaloids may occur as free bases, salts with organic acids (oxalic, acetic acids), inorganic acids (HCl , H_2SO_4), salts with special acids (maconic acid in opium, quinic acid in Cinchona), glycosides (solanine in Solanum), etc. Alkaloid bases are generally soluble in most organic solvents (alcohol, diethyl ether, chloroform or 1,2-dichloroethane) and sparingly soluble or insoluble in water. However, bases like caffeine, ephedrine, codeine, colchicine, nicotine, pilocarpine, etc., are moderately water soluble, whereas morphine and yohimbine are highly water soluble. Some bases are insoluble or sparingly soluble in organic solvents like ether (morphine) and benzene (theobromine, theophylline). Salts are usually soluble in water including quinine sulfate and lobeline salts are soluble in organic solvent like chloroform. Alkaloids give a precipitate with metal or heavy metal iodides (iodine in potassium iodide of Wagner's, potassium mercuric iodide of Mayer's, potassium bismuth iodide of Dragendorff's and potassium cadmium iodide of Marme's reagents as well as noniodide reagents like picric acid (Hager's reagent), phosphomolybdic acid (Sonnenschein's reagent), gold chloride, mercuric chloride, tannic acid except caffeine, which does not precipitate like most alkaloids, form salts with acids, may exist in the free state, salts or as N-oxides, occur in a limited number of plants.

The boundary between alkaloids and other nitrogen-containing non-alkaloid natural compounds like amino acid peptides, proteins, nucleotides, nucleic acid, amines, and antibiotics is not clear-cut. Natural compounds containing nitrogen in the exocyclic position (mescaline, serotonin, dopamine, etc.) are usually attributed to amines rather than alkaloids. Some authors, however, consider alkaloids a special case of amines. A large variety of organisms produces alkaloids, including bacteria, fungi, plants, and animals, and are part of the group of many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local anesthetic and stimulant cocaine, the psychedelic psilocin, the stimulant caffeine, nicotine, the analgesic morphine, the antibacterial berberine, the anticancer compound vincristine, the antihypertension agent reserpine, the cholinomimetic galantamine, the anticholinergic agent atropine, the vasodilator vincamine, the antiarrhythmia compound quinidine, the antiasthma therapeutic ephedrine, and the antimalarial drug quinine. There is no unique method of naming alkaloids. In Trivial system, names are formed by adding the suffix "ine" to botanical genus (atropine from *Atropa belladonna*, strychnine from *Strychnos nux-vomica*), species

(cocaine from *Erythroxylon coca*), common name of the drug (ergotamine from ergot), name of the discoverer (pelletierine from the discoverer Pelletier), after the name of physical action (emetine that acts as emetic, morphine that acts as narcotic), prominent physical character (hygrine from hygroscopic character), etc. If several alkaloids are extracted from one plant then their names often contain suffixes idine, anine, aline, inine, etc. There are at least 86 alkaloids containing the roots of *Catharanthus roseus*. Suffixes "dine" designates isomerism (quinidine, cinchonidine), "ine" indicates a lower pharmacological activity (ergotaminine is less potent than ergotamine), etc. Prefix "Nor" designates *N*-demethylation or *N*-demethoxylation (norpseudoephedrine, nornicotine), "Apo" designates dehydration (apomorphine) while "iso-", "pseudo-", "neo-", "epi-" indicate different types of isomers. Alkaloids may be of primary-(R-NH₂, morphine), secondary-(R₂-NH, ephedrine), tertiary-(R₃-N, atropine) amines and quaternary ammonium salts (R₄-N, D-tubocurarine) and their basicity may be graded as R₂-NH>R-NH₂>R₃-N. Saturated heterocyclic amines are more basic than aromatic amines.

The role of alkaloids for living organisms that produce them is still not clear. Alkaloids may act as protective against pathogen, insects and herbivores due to their bitter principles and toxicity (e.g., liriodenine protects tulip tree from parasitic mushrooms, pyrrolizidine alkaloids render larvae and adult ornate moths unpalatable to their natural enemies like coccinellid beetles, green lacewings, insectivorous hemiptera, and insectivorous bats). Recent research has proved that they are not toxic to the organisms that produce them. Biotoxicity is directed only towards foreign organisms or cells and it is selective. Some animals are adapted to alkaloids and even use them in their own metabolism (serotonin, dopamine and histamine are important neurotransmitters). Alkaloids were the final products of detoxification (waste products) products of nitrogen metabolism in plants as urea in mammals, but this hypothesis is refuted because alkaloid concentrations in plants varies over time. They play a very important role in the immune systems of animals and plants, they are, in certain cases, source of nitrogen in case of nitrogen deficiency; sometimes, they act as growth regulators in certain metabolic systems, show allelopathic activity, and they may be utilized as a source of energy in case of deficiency in CO₂ assimilation. They are biologically significant as active stimulators, inhibitors and terminators of growth, a part of an endogenous security and regulation mechanism. Although the physiological role of alkaloids in the organisms that produce them is obscure but their therapeutic and pharmacological activities are highly significant. Some alkaloids have remarkable structural similarities with neurotransmitters (e.g., dopamine, serotonin, acetylcholine, etc.), some possess analgesic, hallucinogenic effects and some create serious addictions. Most of the natural drugs are obtained from the alkaloid-containing plants and alkaloids always play important role in herbal, allopathic and homeopathy systems of medicine as well as they play role as biopesticides.

Alkaloid-containing plants have been used by humans since antiquity for therapeutic and recreational purposes. Studies on alkaloids began in the early part of nineteenth century that led the discovery of xanthine (1817), strychnine (1818), atropine (1819), caffeine (1820), quinine (1820), coniine (1827), nicotine (1828),

colchicine (1833), sparteine (1851), and cocaine (1860) and up to now more than 12,000 alkaloids have been identified. Raffauf (1996) earlier, however, wrote about the discovery of >10,000 different alkaloids in plant species from over 300 plant families. Medical use of alkaloids has a long history and when the first alkaloids were isolated in the nineteenth century, they immediately found application in clinical practice. Many alkaloids such as ajmaline (antiarrhythmic), atropine, scopolamine, hyoscyamine (anticholinergic), caffeine(stimulant, adenosine receptor antagonist), codeine (cough medicine, analgesic), colchicine (remedy for gout), emetine (antiprotozoal agent), ergot alkaloids (sympathomimetic, vasodilator, antihypertensive), morphine (analgesic), nicotine (stimulant, nicotinic acetylcholine receptor agonist), physostigmine (inhibitor of acetylcholinesterase), quinidine (antiarrhythmic), quinine (antipyretics, antimalarial), reserpine (antihypertensive), tubocurarine (muscle relaxant), vinblastine, vincristine (antitumor), vincamine (vasodilating, antihypertensive), and yohimbine (stimulant, aphrodisiac) are still used in medicine, usually in the form of salts. Cocaine, caffeine, and cathinone are stimulants of the central nervous system. Mescaline and many of indole alkaloids (such as psilocybin, dimethyltryptamine and ibogaine) have hallucinogenic effect. Morphine and codeine are strong narcotic pain killers. All these are used as psychoactive drugs. Many synthetic and semisynthetic drugs are structural modifications of the alkaloids, designed to enhance or change the primary effect of the drug and reduce unwanted side effects (thebaine of opium modified to naloxone, an opioid receptor antagonist). Ephedrine and pseudoephedrine are used to produce methcathinone and methamphetamine and thebaine is used in the synthesis of oxycodone for enhancing their effects. Salts of nicotine and anabasine are insecticides in agriculture but their use is limited by their high toxicity to humans.

Distribution

Alkaloids, especially true alkaloids are of rare occurrence in lower plants, in fungi, the psilocybin in the genus *Psilocybe*, the lysergic acid derivatives and the sulfur-containing alkaloids (gliotoxins) are the best known and in animals—bufotenin in the skin of toad—*Bufo alvarius*. About 300 alkaloids under 24 classes are known to occur in the skins of amphibians including the potent neurotoxic alkaloids of frogs—*Phyllobates*, which are among some of the most poisonous substances known. Other reptilian alkaloids are strongly antimicrobial. Alkaloids derived from mammals include ones of indole and isoquinoline classes. Many marine organisms also contain alkaloids. Estimates for the distribution of alkaloids in vascular plants have been placed as high as 15–20%, although this figure appears somewhat high with respect to data derived from several extensive phytochemical screening programs, 9–10% seems to be the more logical estimate representing alkaloids yielding plant species.

Alkaloids appear to have a restricted and uneven distribution in the plant kingdom; approximately 5000 alkaloids are known to occur in 15% of all land plants under about 150 families of angiosperm. Among the pteridophytes and gymnosperms, the lycopodium, ephedra, and *Taxus* alkaloids have medicinal interest. Alkaloid distribution in the angiosperms is uneven, about 10–25% of

higher plants contain alkaloids and dicots are richer than monocots. Apocynaceae, Berberidaceae, Boraginaceae, Campanulaceae, Chenopodiaceae, Convolvulaceae, Lauraceae, Loganiaceae, Magnoliaceae, Menispermaceae, Ranunculaceae, Rubiaceae, Rutaceae, Solanaceae, Papilionaceae, Papaveraceae, Fumariaceae, etc., families of dicotyledons are rich in alkaloids. Amaryllidaceae and Liliaceae of monocotyledons are rich in alkaloids. The Lamiaceae and Rosaceae are almost free from alkaloids, and the monocotyledons (except Amaryllidaceae and Liliaceae) and gymnosperms (except Taxaceae) are poor in alkaloids. A specific alkaloid is usually confined to a specific plant family (e.g., hyoscyamine in Solanaceae, colchicine in Liliaceae) except caffeine, berberine and nicotine, which are found in a number of widely scattered plant families.

Alkaloids may be distributed in all plant parts (*Datura metel*), leaves (*Nicotiana tabacum*), underground stem (*sanguinaria*), roots (*Aconitum napellus*, *Rauwolfia serpentina*), rhizomes (ipecac, hydrastis), bark (*Cinchona officinalis*), fruits (*Piper nigrum*), seeds (*Strychnos nux-vomica*), and latex (*Papaver somniferum*). Furthermore, different tissues of the same plants may contain different alkaloids. Factors influencing the alkaloid distribution in plants include age, climate, habitat, season, time of harvest, chemical races of plants, etc. For example, broad leaf form of *Geijera salicifolia* (Rutaceae) gives better alkaloid tests than narrow leaf form even they grow side by side in the field.

Classification

Alkaloids are characterized by a great structural diversity and presence of nitrogen in the molecule is the only unifying character for various classes of alkaloids. There is no uniform classification of alkaloids and they are classified in various ways on the basis of their (a) biogenic precursors like nonamino acid (e.g., purine) or amino acids (e.g., phenylalanine, ornithine, lysine, tyrosine, tryptophan, histidine, anthranilic acid), etc.; (b) biosynthetic carbon skeleton (e.g., indole-, isoquinoline-, and pyridine-trigonelline); (c) the presence of the basic heterocyclic nucleus, the chemical entity (e.g., pyridine—trigonelline; pyrrolidine alkaloids—hygrine, nicotine, stachydrine; pyridine—arecoline, ricinine, trigonelline; piperidine alkaloids—connine, lobeline, pelletierine; pyrrolizidine alkaloids—senecionine; tropane alkaloids—atropine, cocaine, hyoscyamine; quinoline alkaloids—quinine, quinidine, cuspareine; isoquinoline alkaloids—papaverine, berberine, emetine, corydaline, tubocurarine, narcine, berberine; aporphine alkaloids—boldine; indole alkaloids—strychnine, reserpine, ergometrine); phenanthrene group—morphine, codeine; phenethylamine group—ephedrine, hordenine, capsaicin, mescaline, narceine; purine group—caffeine; steroid alkaloids—conicine, withanine. (d) pharmacological characteristics (e.g., morphine as narcotic analgesic; quinine as antimalarial; strychnine as reflex excitability; lobeline as respiratory stimulant; boldine as choleric and laxatives; aconitine as neuralgia); (e) taxonomic category (e.g., Cannabinaceous alkaloids—*Cannabis sativa* Linn.—hemp, marijuana; Rubiaceous alkaloids—*Cinchona* sp.,—quinine, *Mitragyna speciosa* Korth—katum, kratum, kutum, *Pausinystalia johimbe*—yohimbe; Solanaceous alkaloids—*Atropa belladonna* L.—belladonna), etc., and on many more number of modes and means.

However, they require compromises in borderline cases; for example, nicotine contains a pyridine fragment from nicotinamide and pyrrolidine part from ornithine and therefore can be assigned to both classes.

(a) Classification based on biogenic precursors

Based on biogenesis, alkaloids are classified into true alkaloids and pseudo alkaloids. The true alkaloids are derived from α -amino acid precursors; and true alkaloids without nitrogen heterocyclic ring in the molecule are called proto alkaloids. Pseudo alkaloids are derived from nonamino acid precursors such as terpenes, steroids, etc. The classification based on biogenic precursors is shown in the Fig. 3.19.

(i) Protoalkaloids originate from amino acids but do not possess heterocyclic ring in the molecule, they acquire their nitrogen atom through transamination and not from their originating amino acid, e.g., hordenine, ephedrine, colchicine, mescaline, adrenaline, cathinone, tyramine, pseudoephedrine, catecholamines, etc. (Fig. 3.20).

Hordenine (*N,N*-dimethyltyramine) occurs naturally in a variety of plants and its name came from the genus *Hordeum*. It is a stimulant of the central nervous system and can promote weight loss by enhancing metabolism but these are proved scientifically. Ephedrine is an alkaloid with a phenethylamine skeleton found in various plants in the genus *Ephedra*. The Chinese herb *Ephedra sinica* contains

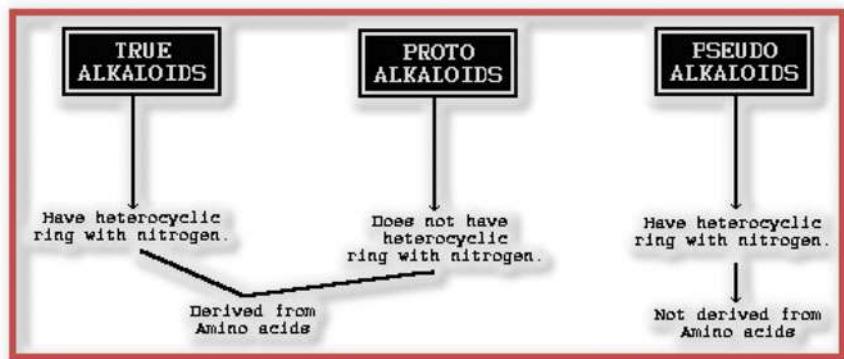


Fig. 3.19 Classification of alkaloids based on biogenic precursors

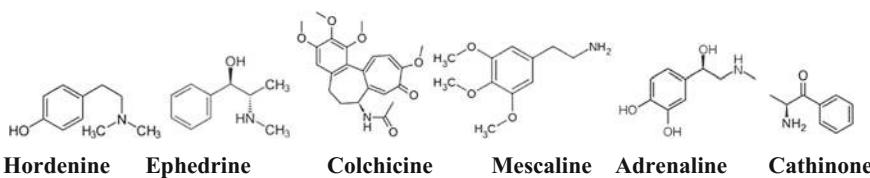


Fig. 3.20 Structure of different protoalkaloids—hordenine, ephedrine, colchicine, mescaline, adrenaline, and cathinone

ephedrine and pseudoephedrine as its principal active constituents. It is a sympathomimetic amine commonly used as a stimulant, concentration aid, decongestant, appetite suppressant, and to treat hypotension associated with anesthesia. Cathinone is a monoamine alkaloid found in the shrub *Catha edulis* and is chemically similar to ephedrine, cathine, methcathinone, and other amphetamines. Cathinone has stimulant and euphoriant effects and may be used to treat obesity and prevent hunger in areas with meager food supplies. Colchicine is a toxic natural product, originally extracted from plants of the genus *Colchicum*. It is used to treat gout, familial Mediterranean fever, pericarditis, and Behçet's disease. Adverse effects are primarily gastrointestinal upset at high doses. Mescaline (3,4,5-trimethoxyphenethylamine) is a naturally occurring psychedelic alkaloid and is known for its hallucinogenic effects similar to those of psilocybin. It occurs naturally in different members of the Cactaceae including *Lophophora williamsii*, *Echinopsis pachanoi*, *Echinopsis peruviana*, and also in small amounts *Acacia berlandieri*. Adrenaline (β , 3,4-trihydroxy-N-methylphenethylamine) is made in the adrenal gland of the kidney. Its biological name is epinephrine, from the Greek nephros for kidney. It works as hormone and neurotransmitter. Adrenaline is used to treat a number of conditions including cardiac arrest, anaphylaxis, and superficial bleeding.

(ii) True alkaloids are derived from the amino acids and have nitrogen in a heterocyclic ring, e.g., atropine, nicotine, morphine, ergotamine, coniine, coniceine, etc. (Fig. 3.21). Some members of this group may contain terpene (e.g., evonine) or peptide fragments (e.g., ergotamine) in addition to nitrogen heterocycle, and also includes coniine and coniceine alkaloids although they do not originate from amino acids, get N through transamination reaction. Alkaloids are derived from amino acid precursors include ornithine (pyrrolidine—cuscohygrine, hygrine, hygroline, stachydrine; tropine—atropine, scopolamine, hyoscyamine and also cocaine, ecgonine; pyrrolizidine alkaloids—retronecine, heliotridine, laburnine, indicine,

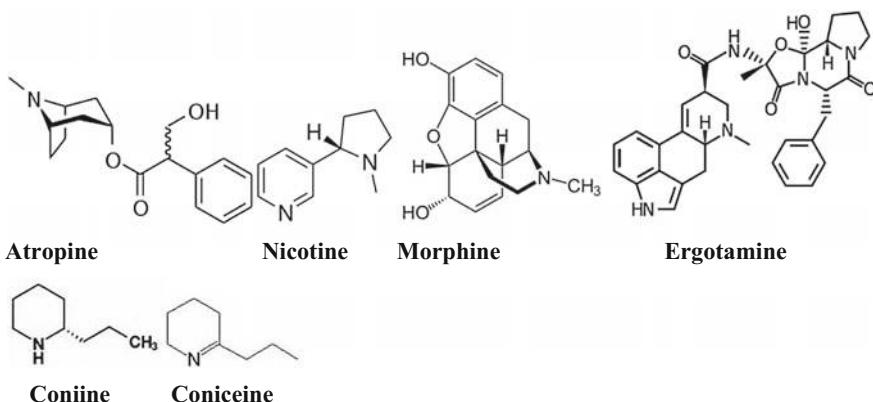


Fig. 3.21 Structure of different true alkaloids—atropine, nicotine, morphine, ergotamine, coniine, and coniceine

lindelophin, sarracine, platiphylline, trichodesmine, loline, *N*-formylloline, and *N*-acetylloline); lysine (piperidine—sedamine, lobeline, anaferine, piperine, coniine, coniceine; quinolizidine—lupinine, nupharidin, cytosine, sparteine, luponine, anahygrine, matrine, oxymatrine, allomatridine, ormosanine, piptantine; and indolizidine alkaloids—swainsonine, castanospermine); nicotinic acid (pyridine alkaloids—trigonelline, ricinine, arecoline, nicotine, nornicotine, anabasine, anatabine, actinidine, gentianine, pediculinine, evonine, hippocrateine, triptonine); tyrosine (phenylethylamines, isoquinoline—salsoline, lophocerine, papaverine, laudanosine, sendaverine, cularine, yagonine, berberine, canadine, ophiocarpine, mecambridine, corydaline, morphine, codeine, thebaine, sinomenine; tetrahydroisoquinoline alkaloids; Amaryllidaceae includes *Amaryllis*, *Narcissus*, and *Galanthus*, and the alkaloid content of bulbs from most members makes these toxic, alkaloids—lycorine, galanthamine, crinine, etc.); tryptophan (simple indole-serotonin, psilocybin, dimethyltryptamine, bufotenin; β -carboline-harmane, harmine, harmaline, eleagnine; terpenoid indole-ergotamine, ergobasine, ergosine, ajmalicine, sarapagine, vobasine, ajmaline, yohimbine, reserpine, mitragynine, strychnine and strychnine brucine, aquamicine, vomicine, ibogamine, ibogaine, voacangine, vincamine, vinca alkaloids, vincotine, aspidospermine; quinolone; pyrroloindole—physostigmine (eserine), etheramine, physovenine, eptastigmine; and ergot alkaloids); phenylalanine—L-phenylalanine is usually contributes only carbon atoms, e.g., C_6C_3 , C_6C_2 , or C_6C_1 units, without providing a nitrogen atom from its amino group, e.g., ephedrine, norpseudoephedrine (cathine), capsaicin, colchicine, lobeline, etc.; anthranilic acid is a key intermediate in the biosynthesis of L-tryptophan and so contributes to the elaboration of indole alkaloids (quinazoline, quinolone and acridine alkaloids, while histidine gives imidazole derivatives (histamine, pilocarpine, isopilocarpine, pilosene, stevensine, etc.).

Atropine, a naturally occurring tropane alkaloid, may be obtained from *Atropa belladonna*, *Datura stramonium*, *Mandragora officinarum*, and other members of Solanaceae. It serves as a drug with a wide variety of effects. Atropine dilates the pupils, increases heart rate, and reduces salivation and other secretions. Nicotine is named after the tobacco plant *Nicotiana tabacum*. It is made in the roots of and accumulates in the leaves. It constitutes approximately 0.6–3.0% of the dry weight of tobacco and is present in the range of 2–7 $\mu\text{g}/\text{kg}$ of various edible plants. It functions as an antiherbivore chemical; consequently, nicotine was widely used as an insecticide in the past. In lesser doses (an average cigarette yields about 1 mg of absorbed nicotine), the substance acts as a stimulant in mammals, while high amounts (50–100 mg) can be harmful. Morphine is an analgesic and psychoactive drug found in opium *Papaver somniferum*. In clinical medicine, morphine is regarded as the gold standard of analgesics used to relieve intense pain, morphine acts directly on the central nervous system (CNS) to relieve pain. Ergotamine is an ergot fungus alkaloid. It has been used to prevent postpartum hemorrhage (bleeding after childbirth). Coniine is a poisonous alkaloid found in hemlock (*Conium maculatum*) and yellow pitcher plant (*Sarracenia flava*) as a mixture of the R-(−)- and S-(+)-enantiomers with the predominance of S-enantiomer. It is a neurotoxin, disrupts the peripheral nervous system, and causes death by respiratory paralysis.

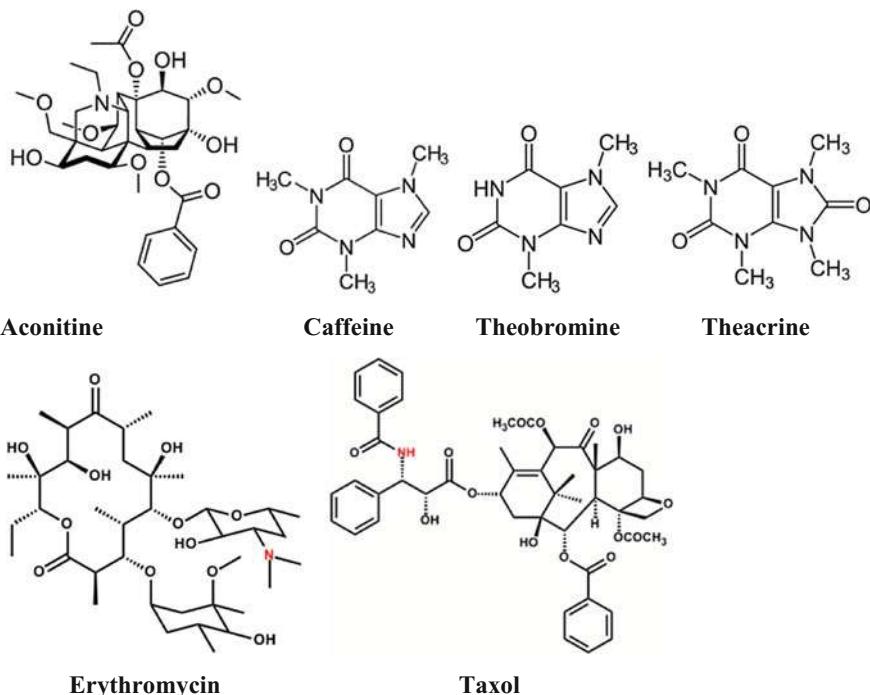


Fig. 3.22 Structure of different pseudo alkaloids—aconitine, caffeine, theobromine, theacrine, erythromycin, and taxol

Socrates was put to death by means of this poison in 399 BC. Coniceine is also found in hemlock.

(iii) Pseudo alkaloids are not derived from amino acids but have nitrogen in a heterocyclic ring, e.g., caffeine, colchicines, aconitine, etc. This group includes terpene alkaloids—aconitine, delphinine; steroid alkaloids—solasodine, solanidine, veralkamine, batrachotoxin as well as purine-like alkaloids such as caffeine, theobromine, theacrine and theophylline (Fig. 3.22). Some authors include ephedrine and cathinone in pseudalkaloids although they originate from the amino acid phenylalanine but acquire their nitrogen not from the amino acid but through transamination.

Aconitine is a toxic alkaloid produced by the *Aconitum* plant. It has antipyretic and analgesic effects and has some limited application in herbal medicine In China, aconitine is used as a herbal medicine against pain. Caffeine is a bitter, white crystalline trimethyl xanthine (purine base) alkaloid found in various seeds, leaves, nuts, and berries. Common sources of caffeine are coffee, tea, soft drinks and energy drinks, caffeine supplements, and chocolate derived from cocoa beans. Also include the yerba mate, guarana and ilex guayusa plants. Caffeine is a CNS and metabolic stimulant and widely consumed legal psychoactive drug. It produces increased wakefulness, faster and clearer flow of thought, increased focus, and

better general body coordination. Theobromine is a crystalline bitter dimethyl xanthine (purine base) alkaloid. It is similar to caffeine but differs in degree of methylation. It is found in chocolate, tea, and the cola nut. It has a similar effect to caffeine in the effect on human nervous system but in lesser extent making it a lesser homologue. Theobromine is an isomer of theophylline and paraxanthine. Theacrine is a purine alkaloid found in *Theobroma grandiflorum* and in *Camellia assamica* var. kucha). It shows anti-inflammatory and analgesic activities. Erythromycin is an antibiotic from *Streptomyces erythreus*, jurubin, a steroid with 3-amino group from *Solanum paniculatum*, and taxol, a modified diterpene pseudo alkaloid from *Taxus brevifolia* (Taxaceae) is used in the treatment of ovarian cancer, breast cancer and non-small cell lung cancer, pachysandrine A, a steroid with N-containing C-17 side chain from *Pachysandra terminalis* (Buxaceae).

Terpenoid alkaloids

Terpenoid alkaloids based on mono-, sesqui-, di-, and tri-terpenoid skeletons are known. It has been observed that the monoterpene alkaloids are derived from the structurally related iridoid materials, wherein the O-atom in the heterocyclic ring is replaced by an N-containing ring. Typical examples of the terpenoid alkaloids are aconine and actinitine (Fig. 3.23).

Aconine is used in the treatment of neuralgia, sciatica, rheumatism, and inflammation. It is employed occasionally as analgesic and cardiac depressant. Aconitine is exclusively used in producing heart arrhythmia in experimental animals. It has also been used topically in neuralgia.

Steroidal alkaloids

Steroidal alkaloids represent an important class of alkaloids that essentially afford a close structural relationship to sterols (they contain a perhydro-1,2-cyclopentanoperphenanthrene nucleus) and occur in the plant kingdom as glycosidal combination with carbohydrate moieties, e.g., solasonine is a glycoside of solasodine with carbohydrate moieties such as L-rhamnose, D-galactose, D-glucose; α -tomatine consists of two D-glucose units, a D-galactose unit, and a D-xylose unit (Fig. 3.24). The solanum and veratrum alkaloids are two major groups of steroidal alkaloids.

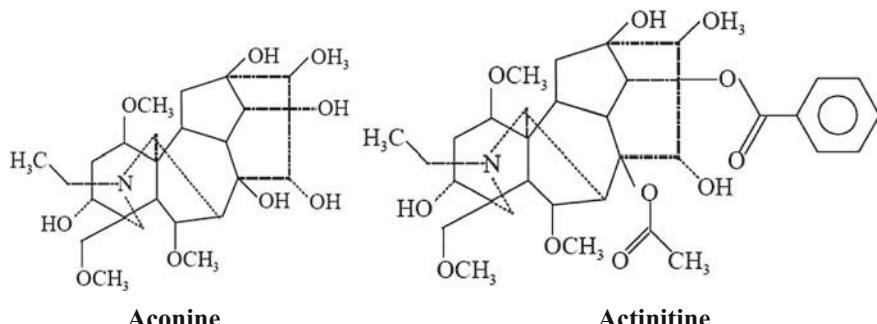


Fig. 3.23 Structure of terpenoid alkaloids—aconine and actinitine

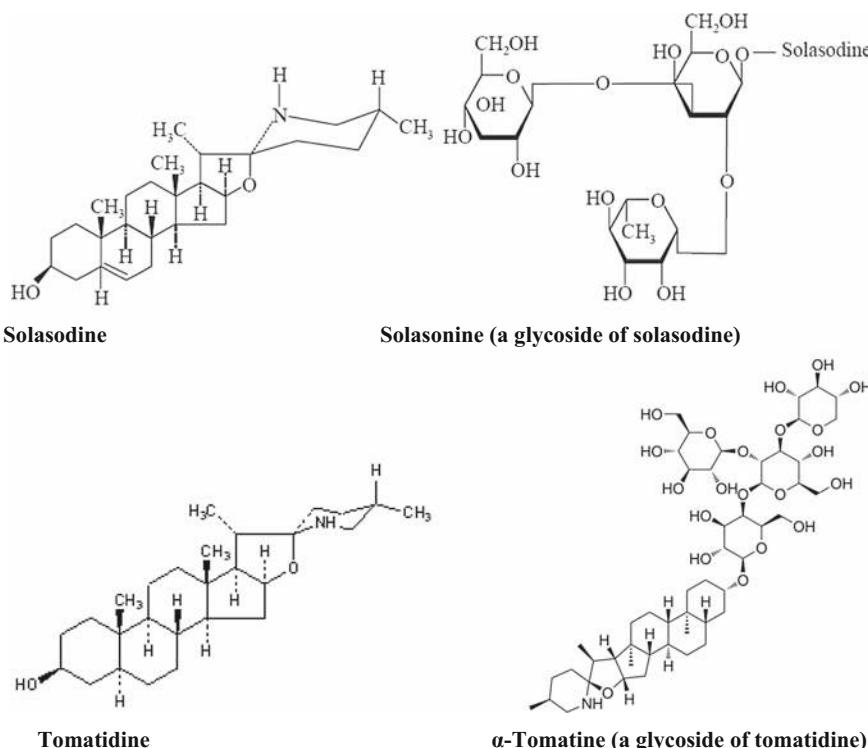


Fig. 3.24 Structure of steroidal alkaloids—solasodine and tomatidine and their glycosides

Several steroidal alkaloids are based on a C₂₇ cholestane skeleton, e.g., solasodine, tomatidine, solanidine and occur in a wide variety of the genus *Solanum* (*S. laciniatum*, *S. dulcamara*, *S. nigrum*, *S. torvum*, *S. tuberosum*, *S. aviculare*, *Lycopersicon esculentum*, etc.). Solasodine is invariably used as a starting material for steroidal drugs.

The veratrum alkaloids represent the most important and medicinally significant class of steroidal alkaloids obtained from the rhizomes of *Veratrum viride*, *V. grandiflorum* and *V. eschscholtzii* (Liliaceae). The basic ring systems present in the veratrum alkaloids, however, are not quite the same as seen in the usual steroidal nucleus as present either in the cholesterol or in the aglycone residues of the cardiac glycosides. In veratrum alkaloids, the ring ‘C’ is a five-membered ring while ring ‘D’ is a six-membered, ring (B) is just the reverse of the pattern in the regular steroidal nucleus. Cavaratum alkaloids (protoveratrines, veratridine, cevadine, germine, etc.) and jeveratum alkaloids (veratramine, jervine and pseudojervine, etc.) are two major categories of veratrum alkaloids (Fig. 3.25).

Protoveratrines is used as an antihypertensive agent which exerts its action through reflex inhibition of pressor receptors in the heart and carotid sinus. It also possesses emetic action. It is used in the treatment of toxemia of pregnancy.

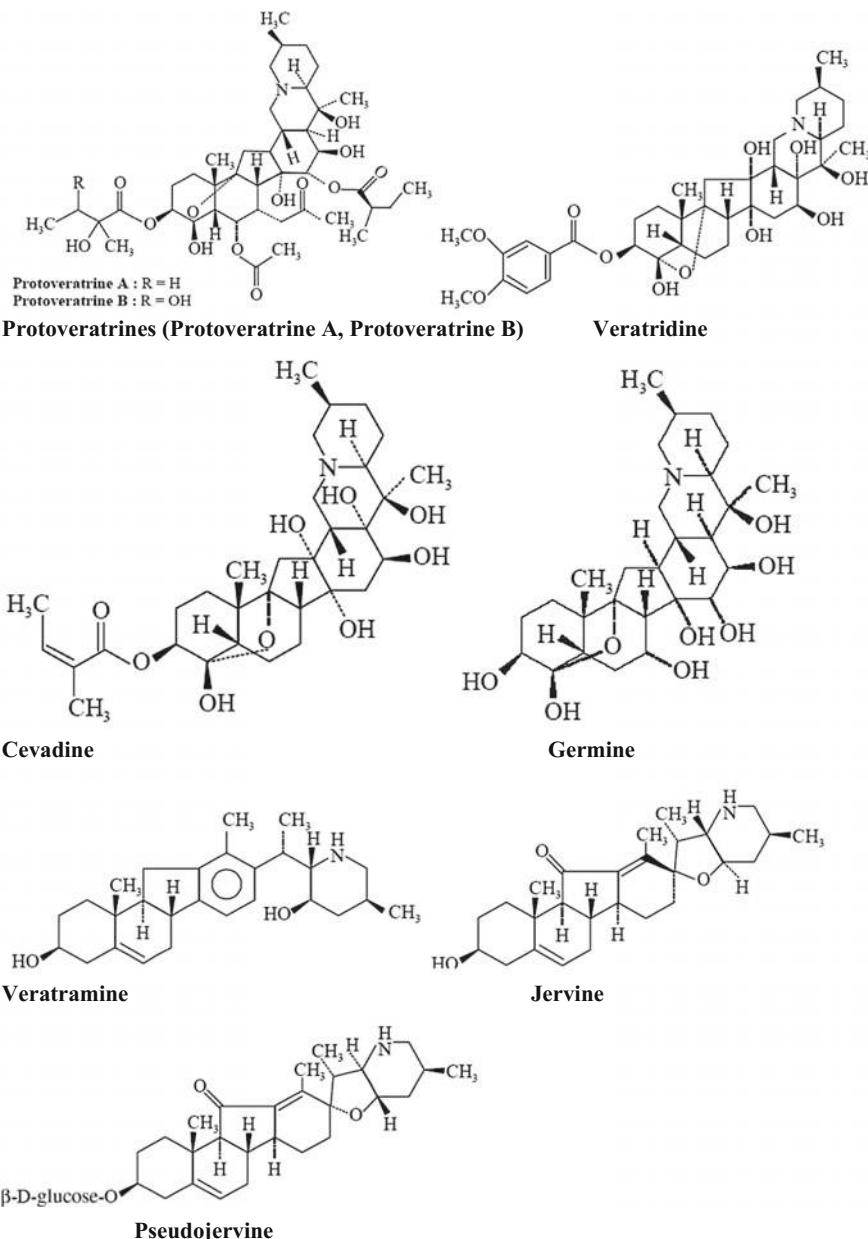


Fig. 3.25 Structure of steroidal veratrum alkaloids—cevaratum alkaloids, e.g., protoveratrines, veratridine, cevadine, germine, etc., and Jeveratum alkaloids, e.g., veratramine, jervine and pseudojervine, etc.

(vi) False alkaloids are a variety of compounds that give false-positive alkaloid reactions with Dragendorff's reagent. The most frequent false-positive reactions have been attributed to the presence of proteins which precipitate on the addition of heavy metal containing reagents. Included in this category are ptomaine's, certain glycosides, carbohydrate, betaine, choline, purines, methylated amines, tannins, ammonium salts, etc. The false-positive alkaloid tests in *Piper methysticum* Forst. (Piperaceae) has been reported to be due to certain non-nitrogenous α -pyrone compounds.

(b) Classification based on biosynthetic carbon skeleton

In this instance, the significance solely lies to the precursor from which the alkaloids in question are produced in the plant biosynthetically. Therefore, it is quite convenient and also logical to group together all alkaloids having been derived from the same precursor but possessing different taxonomic distribution and pharmacological activities. Examples include:

(i) **Alkaloids derived from ornithine** include pyrrolidine (hygrine, cuscohygrine, hygroleine, stachydrine); tropane (atropine, scopolamine, hyoscyamine and also cocaine, ecgonine) and pyrrolizidine (retronecine, heliotridine, laburnine, indicine, lindelophin, sarracine, platy, phylline, trichodesmine, loline, *N*-formylloline, *N*-acetylloline) alkaloids.

About 80 pyrrolidine alkaloids are known. The three glaring examples of pyrrolidine alkaloids are hygrine, cuscohygrine and stachydrine (Fig. 3.26).

Pyrrolidine is a saturated heterocycle. Hygrine is a pyrrolidine alkaloid, found mainly in *Erythroxylon coca* leaves (0.2%) accompanying and in *Withania somnifera* roots. Hygrine is extracted as thick yellow oil, having a pungent taste and odor. It basically stimulates the salivary gland. The drug is broadly used as a sedative, hypnotic laxative and diuretic. Cuscohygrine is a pyrrolidine alkaloid found in coca, *Atropa belladonna*, *Datura inoxia* and *D. stramonium*. Cuscohygrine usually comes with other, more potent alkaloids like atropine or cocaine. Stachydrine is a Betony alkaloid. Stachydrine is also present also in Yarrow, Motherwort, Alfalfa, Chrysanthemum and Citrus plants. It is an osmoprotectant capable of helping organisms to survive extreme osmotic stress. Motherwort contains lionurine and stachydrine alkaloids that help lower blood

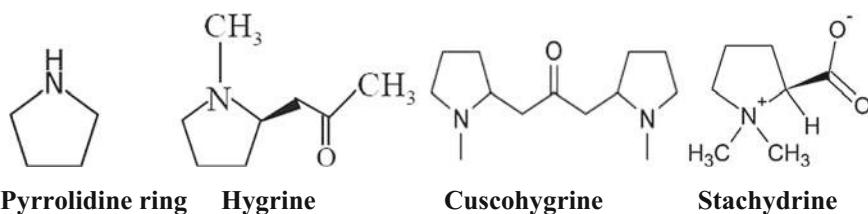


Fig. 3.26 Structure of alkaloids derived from pyrrolidine—hygrine, cuscohygrine, and stachydrine

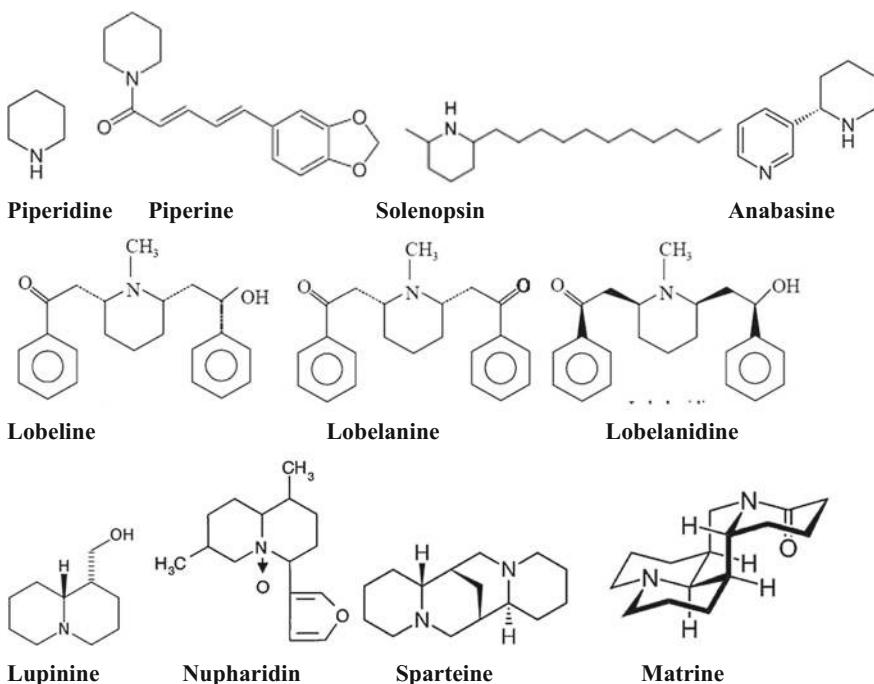


Fig. 3.27 Structure of alkaloids derived from lysine—piperine, solenopsin, anabasine, lobeline, lobelanine, lobelanidine, lupinine, nupharidin, sparteine, and matrine

pressure and have a sedating effect on the central nervous system, which supports motherwort's traditional use as a treatment for depression anxiety.

Tropane alkaloids include atropine, scopolamine, hyoscyamine and also cocaine, ecgonine, etc. Pyrrolizidine alkaloids are retronecine, heliotridine, laburnine, indicine, lindelophin, sarracine, platy, phylline, trichodesmine, loline, *N*-formylloline and *N*-acetylloolloline.

(ii) **Alkaloids derived from lysine** are piperidine (sedamine, lobeline, anaferine, piperine, coniine, coniceine); quinolizidine (lupinine, nupharidin, cytosine, sparteine, lupanine, anahygrine, matrine, oxymatrine, allomatridine, ormosanine, pippantidine); and indolizidine alkaloids (swainsonine, castanospermine) (Fig. 3.27). Piperidine alkaloids are identified by their saturated heterocyclic ring, i.e., piperidine nucleus. This heterocyclic amine consists of a six-membered ring containing five methylene bridges ($-\text{CH}_2-$) and one amine bridge ($-\text{NH}-$). It is a colorless fuming liquid with an odor described as ammoniacal, pepper-like, or semen-like; the name comes from the genus name *Piper*, which is the Latin word for pepper.

Piperidine itself has been obtained from *Piper nigrum*, from *Psilocaulon absimile* and in *Petrosimonia monandra*. The piperidine structural motif is present in numerous natural alkaloids. These include piperine, which gives black pepper its spicy taste. Other examples are the fire ant toxin solenopsin, the nicotine analog

anabasine of the *Nicotiana glauca*, lobeline, lobelanine of *N. tabacum*, and the toxic alkaloid coniine from poison hemlock. Piperine has been used in some forms of traditional medicine and as an insecticide. It has shown ‘antidepressant like activity’, and cognitive enhancing effects in rats. Piperine has shown anti-inflammatory and anti-arthritis effects in human interleukin-1beta-stimulated fibroblast-like synoviocytes and in rat arthritis models. Piperine also possesses antiangiogenic activities. Solenopsin inhibits angiogenesis via the phosphoinositol-3 kinase (PI3-K) signaling pathway, contributes to the toxic effect of fire ant venom and has cytotoxic, hemolytic, necrotic, insecticidal, antibacterial, antifungal, and anti-HIV properties. Principal industrial use of anabasine is as an insecticide.

Quinolizidine alkaloids are lupinine, nupharidin, sparteine, luponine, anahygrine, matrine, oxymatrine, allomatridine, ormosanine and piptantine. Lupinine is a bitter tasting quinolizidine alkaloid present in *Lupinus* species (lupins), plants of the family Fabaceae. Sparteine is the predominant alkaloid in *Lupinus mutabilis*. It is an antiarrhythmic agent and a sodium channel blocker but FDA did not approve it for human use as an antiarrhythmic agent. Matrine is found in plants from the *Sophora* genus. It has anticancer effects, and action as a kappa opioid receptor and μ -receptor agonist. Matrine possesses strong antitumor activities in vitro and in vivo. Inhibition of cell proliferation and induction of apoptosis are the likely mechanisms responsible for matrine’s antitumor activities. Matrine is a component of the traditional Chinese medical herb *Sophora flavescens*.

Phenylethylamines, isoquinoline alkaloids are salsoline, lophocerine, papaverine, laudanosine, sendaverine, cularine, yagonine, berberine, canadine, ophiocarpine, mecambridine, corydaline, morphine, codeine, thebaine and sinomenine. Indolizidine alkaloids are swainsonine and castanospermine.

(iii) **Alkaloids derived from tyrosine** include phenylethylamines (ephedrine, hordenine, mescaline and narceine), isoquinoline (salsoline, lophocerine, papaverine, laudanosine, sendaverine, cularine, yagonine, berberine, canadine, ophiocarpine, mecambridine, corydaline, morphine, codeine, thebaine, sinomenine) and tetrahydroisoquinoline alkaloids (Fig. 3.28). The important alkaloids belonging to phenylethylamine group are ephedrine, hordenine, mescaline and narceine.

Phenylethylamine, phenethylamine or β -phenethylamine is a natural monoamine alkaloid. It has psychoactive and stimulant effects. Phenylethylamine functions as a neuromodulator or neurotransmitter in the mammalian central nervous system. It is sold as a dietary supplement for purported mood and weight loss-related therapeutic benefits. Phenethylamine is widely distributed throughout the plant kingdom and also present in animals, such as humans. Narceine is obtained from the dried latex opium (*Papaver somniferum*) to the extent of 0.1–0.5%. Narcyl is used as a narcotic analgesic and also employed as an antitussive agent.

Isoquinoline alkaloids are salsoline, lophocerine, papaverine, laudanosine, sendaverine, cularine, yagonine, berberine, canadine, ophiocarpine, mecambridine, corydaline, morphine, codeine, thebaine and sinomenine.

Papaverine is an opium poppy alkaloid antispasmodic drug, used primarily in the treatment of visceral spasm, vasospasm, and occasionally in the treatment of erectile dysfunction. Papaverine differs in both structure and pharmacological action from the analgesic opium alkaloids (morphine). Morphine is an opioid analgesic drug and acts directly on the central nervous system (CNS) to relieve pain. A minor constituent of opium, thebaine is chemically similar to both morphine and codeine, but has stimulatory rather than depressant effects.

Tetrahydroisoquinoline alkaloid is a secondary amine. The tetrahydroisoquinoline skeleton is commonly encountered in pharmaceutical drugs, notably quaternary ammonium muscle relaxants such as tubocurarine. Tubocurarine (*p*-tubocurarine or DTC) is a toxic alkaloid and skeletal muscle relaxant in the category of non-depolarizing neuromuscular-blocking drugs, used adjunctively in anesthesia to provide skeletal muscle relaxation during surgery or mechanical ventilation.

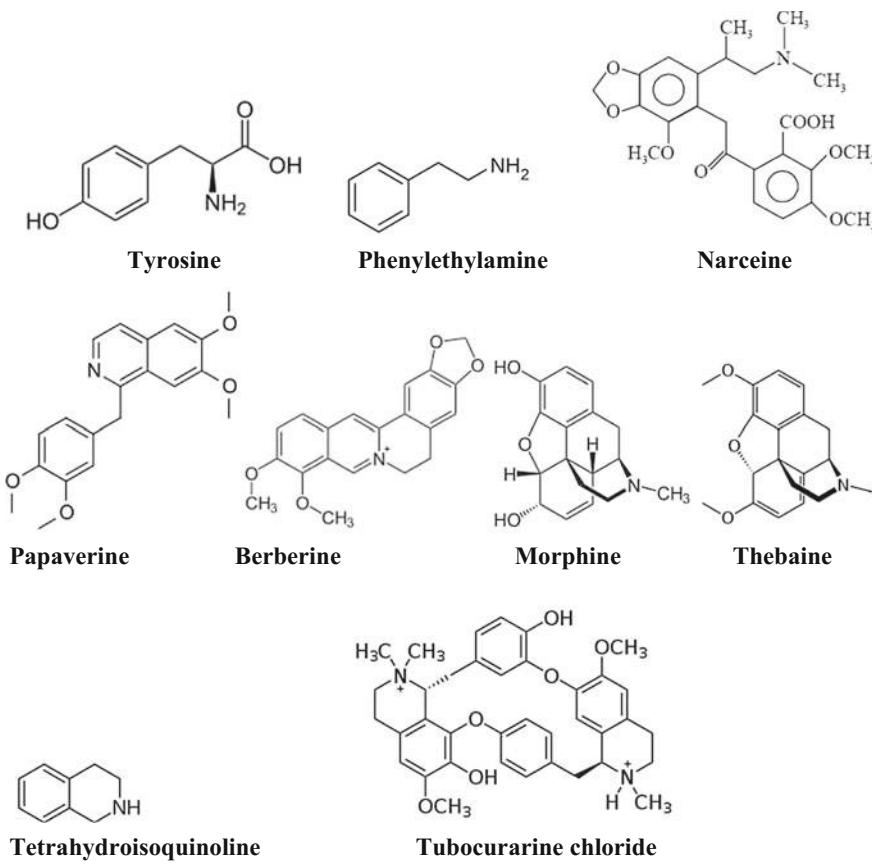


Fig. 3.28 Structure of alkaloids derived from tyrosine

(iv) **Alkaloids derived from tryptophan** are indole alkaloids. With respect to their structural features, tryptophan derived indole alkaloids can be divided into non-isoprenoid and isoprenoid indole alkaloids (derived from tryptamine and secologanin). The first group includes three types, e.g., (i) simple indole, (ii) β -carboline and (iii) ergot alkaloids and the second group include eight types, e.g., (i) corynanthean or C-type (sarpagine, yohimbine, ajmalicine), (ii) vincosan or d-type (vincoside), (iii) vallesiachotamon or V-type (vallesiachotamine), (iv) strychnan or S-type (vomicine), (v) aspidospermatan or A-type (condylcarpine), (vi) eburnan or E-type (vincamine), (vii) plumeran or P-type (kopsine) and (viii) ibogan or J-type (voaluteine).

Simple indole alkaloids include serotonin, psilocybin, dimethyltryptamine, bufotenin, etc. (Fig. 3.29).

The indole structure consists of a pyrrole ring and a benzene ring fused together to form two isomeric benzopyrroles. Serotonin is found in mushrooms, fruits—in nuts of the walnut and hickory (25–400 mg/kg) in plantains, pineapples, banana, kiwifruit, plums, and tomatoes (3–30 mg/kg) have been found. and vegetables (0.1–3 mg/kg). Serotonin is one compound of the poison contained in stinging nettles (*Urtica dioica*), where it causes pain on injection in the same manner as its presence in insect venoms. Serotonin, 5-hydroxytryptamine (5-HT), is a mono-amine neurotransmitter, serotonin is primarily found in the gastrointestinal tract (GI tract), platelets, and the central nervous system (CNS) of animals, including humans. It is popularly thought to be a contributor to feelings of well-being and happiness. The ergot alkaloids are mycotoxins produced by several species of fungi in the genus *Claviceps*. There are four main groups of ergot alkaloids: the clavines, the lysergic acids, the lysergic acid amides, and the ergo peptides. Psilocybin mushrooms contains derivatives of tryptamine and *Claviceps* contains derivatives of lysergic acid. Psilocybin is a naturally occurring psychedelic compound produced by more than 200 species of mushrooms, collectively known as psilocybin mushrooms (*Psilocybe azurescens*, *P. semilanceata*, *P. cyanescens*, etc.). In general, the effects include euphoria, visual and mental hallucinations, changes in perception, a distorted sense of time, and spiritual experiences, and can include possible adverse reactions such as nausea and panic attacks. Bufotenin, a dimethyl serotonin, are found in the skin of some species of toads; in mushrooms, higher plants, and mammals. Bufotenine is an indole hallucinogen capable of blocking the action of serotonin (an indole amine neurotransmitter) and is a powerful constrictor of blood vessels, causing a rise in blood pressure.

β -carboline alkaloids consist of pyridine ring that is fused to an indole skeleton producing a three-ringed structure. β -Carboline alkaloids are widespread in plants and animals and include harmane, harmine, harmaline, eleagnine, etc. β -carboline alkaloids are a large group of natural indole alkaloids with different degrees of aromaticity, distributed in nature, including various plants, foodstuffs, marine creatures, insects, mammalians as well as human tissues and body fluids. They show diverse biological activities, e.g., intercalate into DNA, inhibit CDK, topoisomerase, and monoamine oxidase, interact with benzodiazepine receptors and

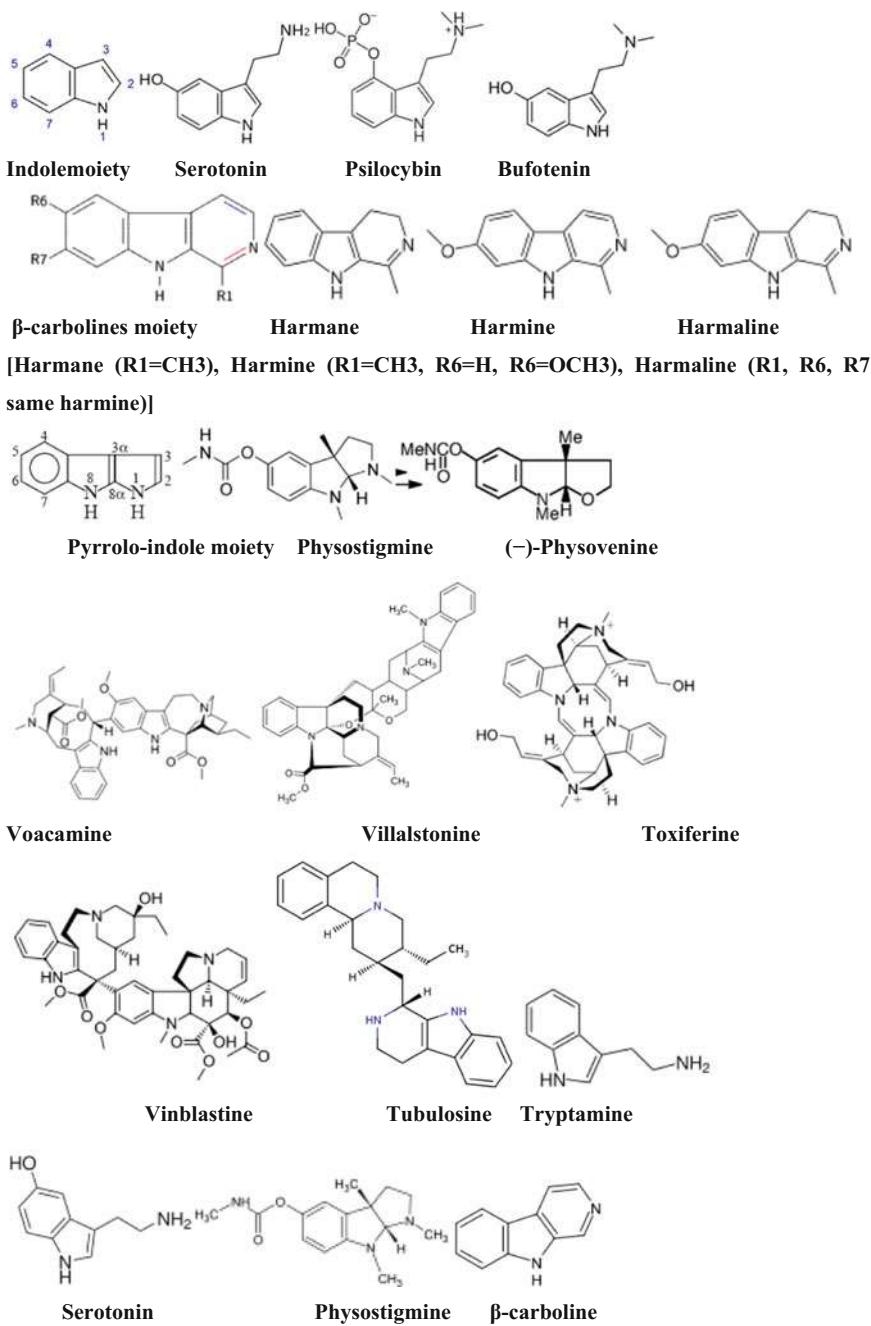


Fig. 3.29 Structure of alkaloids derived from tryptophan

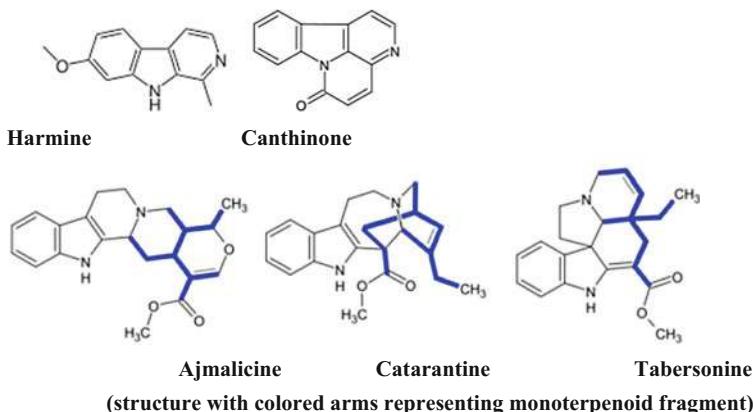


Fig. 3.29 (continued)

5-hydroxy serotonin receptors. They have a broad spectrum of pharmacological properties including sedative, anxiolytic, hypnotic, anticonvulsant, and antitumor, antiviral, antiparasitic as well as antimicrobial activities. The β -carbolines harmine, harmaline, and tetrahydroharmine play a pivotal role in the pharmacology of the indigenous psychedelic drug. β -carboline is a benzodiazepine receptor inverse agonist and can therefore have convulsive, anxiogenic and memory enhancing effects.

The plants rich in non-isoprenoid indole alkaloids include *Peganum harmala* (harmane, harmine, and harmaline), and *Physostigma venenosum* (physostigmine). Some members of the family Convolvulaceae, in particular *Ipomoea violacea* and *Turbina corymbosa*, contain ergolines and lysergamides.

Pyrroloindole includes physostigmine (eserine), etheramine, physovenine, eptastigmine; and ergot alkaloids). Physostigmine is a parasympathomimetic alkaloid, specifically, a reversible cholinesterase inhibitor. It occurs naturally in the Calabar bean.

Isoprenoid indole alkaloids contain residues of tryptophan and isoprenoid building blocks derived from the dimethylallyl pyrophosphate and isopentenyl pyrophosphate and include ergot and monoterpenoid alkaloids. Beside these, bisindole alkaloids are produced in living organisms through dimerization of monomeric indole bases.

Isoquinoline indole alkaloids are ergotamine, ergobasine, ergosine, ajmalicine, sarpagine, vobasine, ajmaline, yohimbine, reserpine, mitragynine, strychnine and strychnine brucine, aquamicine, vomicine, ibogamine, ibogaine, reserpine, strychnine, physostigmine, strychnine, brucine; quinolone alkaloid-cinchonine, quinine, quinidine, campotothecin; vinca alkaloids—voacangine, vincamine, vincristine, vinblastine, vincamine, aspidospermine; quinolone. Depending on their biosynthesis, they are grouped into hemiterpenoids (e.g., ergot alkaloids—ergine, ergotamine, ergosine, ergostine, ergoptine, ergonine, ergocristine, α -ergocryptine,

β -ergocryptine, and ergocornine) and three classes of monoterpenoids (e.g., corynanthe—ajmaline, aquamycin, strychnine, brucine, ajmalicine, yohimbine, reserpine, sarpagine, and mitragynine; iboga—ibogaine, ibogamin, and voacangine; aspidosperma—eburnamin, tabersonine, vindolin, and vincamine).

Despite the considerable structural diversity, most of monoterpenoid indole alkaloids is localized in three families of dicotyledon plants: Apocynaceae (Alstonia, Aspidosperma, Rauwolfia and Catharanthus), Rubiaceae (Corynanthe) and Loganiaceae (Strychnos).

(v) **Alkaloids derived from nicotinic acid** are pyridine alkaloids (trigonelline, ricinine, arecoline, nicotine, nornicotine, anabasine, anatabine, actinidine, gentianine, pediculinine, evonine, hippocrateine, triptonine) (Fig. 3.30).

Nicotine is a potent parasympathomimetic alkaloid found in the members of Solanaceae (Belladonna, Nictiana) and also in various edible plants. It is made in the roots of and accumulates in the leaves. Nicotine appears to have significant performance enhancing effects, particularly in fine motor skills, attention, and memory. These beneficial cognitive effects may play a role in the initiation and maintenance of tobacco dependence. It functions as an antiherbivore chemical; consequently, nicotine was widely used as an insecticide in the past and nicotine analogs such as imidacloprid are currently widely used. In lesser doses (about 1 mg), the substance acts as a stimulant in mammals, while high amounts (50–100 mg) can be harmful. Anabasine is found in the tree tobacco (*Nicotiana glauca*) plant, structurally and chemically similar to nicotine. Its principal industrial use is as an insecticide. Anabasine is present in trace amounts in tobacco smoke, and can be used as an indicator of a person's exposure to tobacco smoke. Anabasine is a nicotinic acetylcholine receptor antagonist. In high doses, it produces a depolarizing block of nerve transmission, which can cause symptoms similar to those of nicotine poisoning and, ultimately, death by asystole.

(vi) **Phenylalanine** or L-phenylalanine usually contributes only carbon atoms, e.g., C₆C₃, C₆C₂, or C₆C₁ units, without providing a nitrogen atom from its amino group, e.g., ephedrine, norpseudoephedrine (cathine), capsaicin, colchicine, lobeline, etc. (Fig. 3.31).

Capsaicin is an active component of chili peppers (*Capsicum* sp.). Capsaicin is used as an analgesic in topical ointments, nasal sprays, and dermal patches to relieve pain

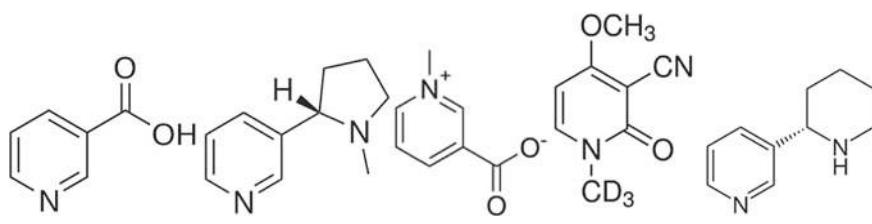


Fig. 3.30 Structure of alkaloids derived from nicotinic acid

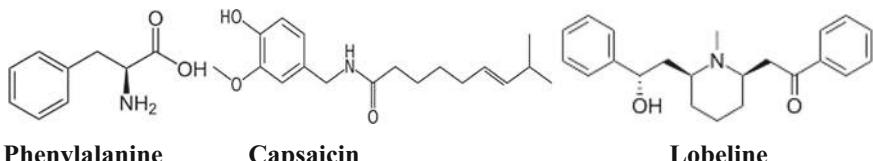


Fig. 3.31 Structure of alkaloids derived from phenylalanine

at low concentration (0.025–0.25%). It may be applied in cream form for the temporary relief of minor aches and pains of muscles and joints associated with arthritis, backache, strains other *Lobelia* spp. Lobeline has been used as a smoking cessation aid, and may have application in the treatment of other drug addictions such as addiction to amphetamines, cocaine, or alcohol.

(vii) **Alkaloids derived from histidine** are imidazole alkaloids. Imidazole is an aromatic heterocycle and alkaloids containing one or more imidazole moieties as part of its structure (pilocarpine, pilosine) are imidazole alkaloids (Fig. 3.32).

Pilocarpine is a drug used to treat dry mouth and glaucoma. It is a parasympathomimetic alkaloid obtained from the leaves of tropical American shrubs from the genus *Pilocarpus*. It is a nonselective muscarinic receptor agonist in the parasympathetic nervous system, which acts therapeutically at the muscarinic acetylcholine receptor M3 due to its topical application, e.g., in glaucoma and xerostomia. Pilocarpine stimulates the secretion of large amounts of saliva and sweat. It is used to treat dry mouth (xerostomia) particularly in Sjögren's syndrome, but also as a side effect of radiation therapy for head and neck cancer.

(viii) **Alkaloids derived from anthranilic acid** include quinazoline, quinoline, and acridine alkaloids. Anthranilic acid is a key intermediate in the biosynthesis of L-tryptophan and so contributes to the elaboration of indole alkaloids (quinazoline-vasicine, vasicinone; quinolone and acridine alkaloids—melicopicine, melicopidine, and melicopine, while histidine gives imidazole derivatives, histamine, pilocarpine, isopilocarpine, pilosine, stevensine, etc.) (Fig. 3.33).

Vasicine and also vasicinone are obtained from the leaves of *Adhatoda vasica* (L.) and the seeds of *Peganum harmala*. Vasicine is mostly used as an expectorant and bronchodilator, shows oxytocic properties, abortifacient action, etc. Vasicinone is used mainly as an expectorant which action is solely due to stimulation of the

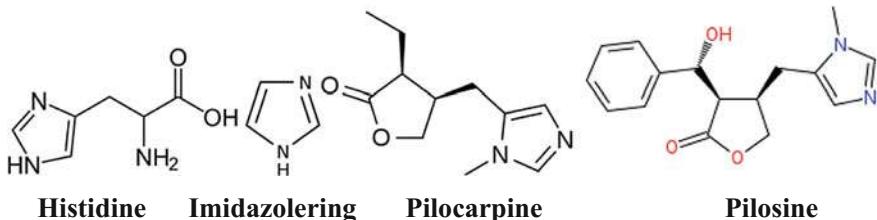


Fig. 3.32 Structure of alkaloids derived from histidine

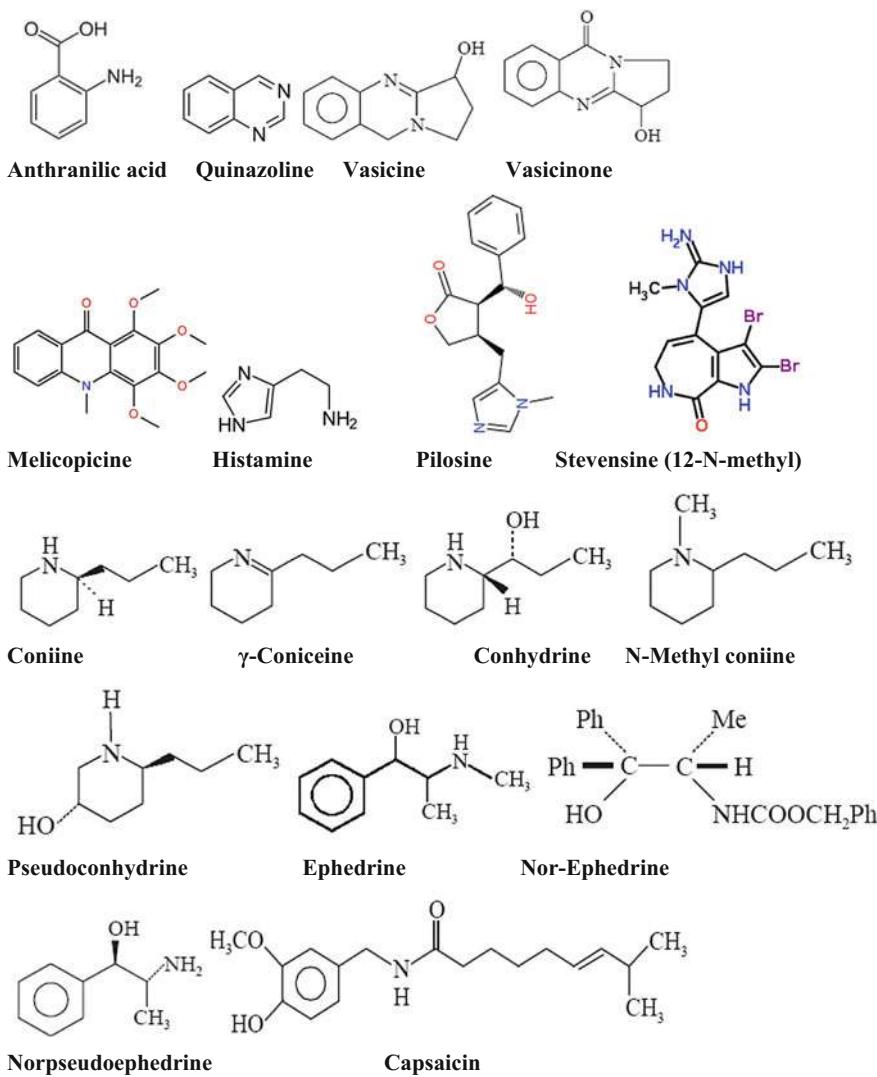


Fig. 3.33 Structure of alkaloids derived from anthranilic acid

bronchial glands. Histamine is involved in local immune responses to foreign pathogens, as well as regulating physiological function in the gut and acting as a neurotransmitter. Histamine is involved in the inflammatory response. Histamine increases the permeability of the capillaries to white blood cells and some proteins, to allow them to engage pathogens in the infected tissues. Stevensine is a bioactive bromopyrrole alkaloid isolated from marine sponge.

Alkaloids derived from amination reactions include acetate-derived alkaloids, phenylalanine-derived alkaloids, terpenoid alkaloids and steroidal alkaloids.

Amination reaction is the process by which an amine group is introduced into an organic molecule; enzymes which catalyze this reaction are termed aminases. A large number of alkaloids are derived from amino acids (an N-atom as well as an amino acid carbon skeleton or a major part of it). However, a good number of amino acids are synthesized from nonamino acid precursors (predominantly based on terpenoid and steroidal skeleton) and they derive their N-atom later through transamination reaction (late amination processes) using an appropriate aldehyde or ketone.

Acetate-derived alkaloids include coniine, γ -coniceine, conhydrine, *N*-methyl coniine and pseudoconhydrine derived from hemlock plant (*Conium maculatum*, Apiaceae). It also occurs in the plant *Aethusa cynapium* (Apiaceae) and *Cicuta maculata* (Apiaceae) (Water Hemlock). Socrates was made to drink the decoction of the hemlock plant and died soon after.

Externally, the coniine salts are used as ointments and infrequently employed for their local analgesic action in the symptomatic relief of pruritis, hemorrhoids and fissures.

Phenylalanine derived alkaloids where L-phenylalanine contributes carbon atoms only (C_6C_1 , C_6C_2 or C_6C_3 units) without contributing its N-atom from its amino function. The various typical examples of phenylalanine-derived alkaloids are: ephedrine, norpseudoephedrine (cathine) and capsaicin. Ephedrine exists singly in *Ephedra sinica* (1–3%) and *E. equisetina* (2%), pseudoephedrine) in *E. vulgaris*, norpseudoephedrine from *Catha edulis* Forsk., *Maytenus krukovi* A.C. Smith (Celastraceae) and capsaicin from *Capsicum annum* L. (Solanaceae).

The L-ephedrine is extensively used as a bronchodilator. The D-pseudoephedrine is employed widely as a decongestant. Norpseudoephedrine is widely employed as an anorexic. It is also used in the optical resolution of externally compensated acids. Capsaicin is used as a topical analgesic.

It is often employed as a tool in neurobiological research. It is used in creams to counter neuralgia caused by herpes infections and in other pain-relieving formulations.

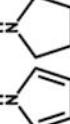
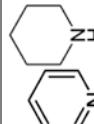
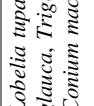
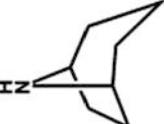
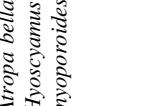
(c) Chemical classification on the basis of heterocyclic ring structure

It is probably the most widely accepted and common mode of classification of alkaloids (into 16 groups) for which the main criterion is the presence of the basic heterocyclic nucleus (i.e., the chemical entity) (Table 3.3).

(d) Pharmacological classification

Alkaloids exhibit a broad range of pharmacological characteristics such as analgesics, cardiovascular stimulants, CNS stimulants and depressants, dilation of pupil of eye, mydriatics, anticholinergics, sympathomimetics, antimalarials, antineoplastic, antidiarrheal, antihypertensive, antihyperglycemic, purgatives, etc., and they are classified on the basis of such pharmacological characteristics (Table 3.4). Perhaps this might also be used as a strong basis for the general classification of the wide-spectrum of alkaloids derived from the plant kingdom. However, such a classification is not quite common and broadly known.

Table 3.3 Classification of Alkaloids on the basis of heterocyclic ring structure

Alkaloids	Serial number	Type	Structure	Example	Plant source
1.	Pyrrole, pyrrolidine alkaloids				<i>Nicotiana tabacum, Erythroxylum coca</i>
2.	Pyridine and piperidine alkaloids			 Nicotine (Pyridine + Pyrrolidine) Nicotine Lobeline Piperine Conine Trigonelline arecoline, anabasine	<i>Lobelia tupa, Areca catechu, Nicotiana glauca, Trigonella foenum-graecum, and Conium maculatum</i>
	Tropane (piperidine/ <i>N</i> -methyl-pyrrolidine)				<i>Atropa belladonna, Datura stramonium, Hyoscyamus niger, Duboisia myoporoides, and Erythroxylum coca</i>

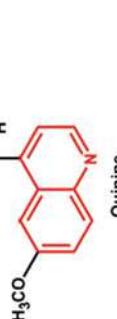
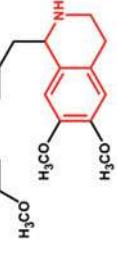
(continued)

Table 3.3 (continued)

Alkaloids		Serial number	Type	Structure	Example	Plant source
3.	Pyrrolizidinealkaloids					<i>Senecio vulgaris, Echium plantagineum, Senecio jacobaea, and Crotalaria juncea</i>
4.	Tropanealkaloids					<i>Atropa belladonna, Datura stramonium</i>

(continued)

Table 3.3 (continued)

Alkaloids		Serial number	Type	Structure	Example	Plant source
5.	Quinolinealkaloïds				 Quinine	<i>Cinchonapubescens, C. officinalis</i>
6.	Isoquinolinealkaloïds				 Emetine	<i>Papaver somniferum</i> Emetine, morphine, cephaline, narcotine, narceine, D-tubocurarine papaverine, narcotine, and codeine

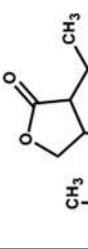
(continued)

Table 3.3 (continued)

Alkaloids	Serial number	Type	Structure	Example	Plant source
7.	Aporphine (reduced isoquinoline/naphthalene)			Boldine Boldine, bulbocapnine, glaucine, corytuberine	<i>Lindera aggregate</i> , <i>Corydalis</i> spp., <i>Glaucium</i> spp.
8.	Indole or benzopyrrole			Physostigmine Physostigmine ergometrine, erotamine, reserpine, vincristine, vinblastine, strychnine, brucine yohimbine, serpentine	<i>Claviceps paspali</i> (Ascomycetaceae), <i>Rauvolfia serpentina</i> , <i>Catharanthus roseus</i> , and <i>Strychnos nux-vomica</i>

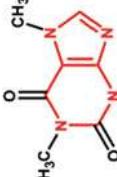
(continued)

Table 3.3 (continued)

Alkaloids		Serial number	Type	Structure	Example	Plant source
9.	Imidazole alkaloids Imidazole or glyoxaline				 Pilocarpine	<i>Pilocarpus</i> sp.
10.	Norlupanine=Quinolizidine				 Cytisine	<i>Laburnum</i> sp., <i>Cytisus</i> sp., <i>Lupinus mutabilis</i> , <i>Anagyris</i> sp., <i>Thermopsis</i> sp., <i>Genista</i> sp., <i>Retama</i> sp., <i>Sophora</i> sp., and <i>Nymphaea</i> lotus
11.	Diazocin alkaloids				 Lupanine	<i>Lupinus albus</i>

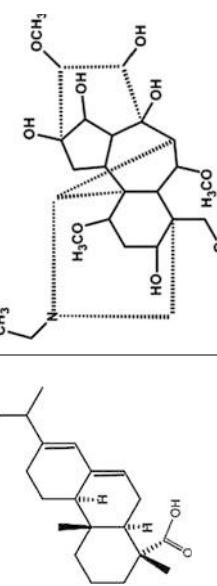
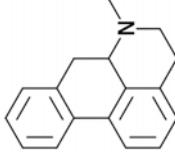
(continued)

Table 3.3 (continued)

Alkaloids		Serial number	Type	Structure	Example	Plant source
12.	Purine alkaloids (pyrimidine/imidazole)				Caffeine Caffeine, theobromine, theophylline, cocaine, nicotine	<i>Coffea</i> spp., <i>Camellia sinensis</i> , <i>Ilex paraguariensis</i> , <i>Erythroxylum coca</i> , and <i>Nicotiana tabacum</i>
13.	Steroidal alkaloids Steroidal (some combined as glycosides)*					<i>Solanum</i> spp., <i>Capsicum annuum</i> , <i>Lycopersicon esculentum</i> , <i>Veratrum</i> spp., and <i>Zigadenus</i> spp.

(continued)

Table 3.3 (continued)

Alkaloids	Serial number	Type	Structure	Example	Plant source
14.	Amino alkaloids			Ephedrine	<i>Ephedra sinica</i>
15.	Diterpene alkaloids Terpenoid			Aconitum heterophyllum, <i>Inula royleana</i> , and <i>Spiraea japonica</i>	
16.	Aporphine (reduced isoquinoline naphthalene)			Boldine	<i>Lindera aggregata</i>

* some combines as glycosides

Table 3.4 Pharmacological classification of alkaloids

Pharmacological characteristics	Example	Plant source
i. Narcotic, analgesic	Morphine	Capsule of <i>Papaver somniferum</i>
ii. Antimalarial	Quinine, artemisinins (WHO only recommends artemisinins)	Bark of <i>Cinchona</i> spp., glandular trichomes of the leaves, stems, and inflorescences of <i>Artemisia annua</i>
iii. Reflexexcitability	Strychnine	Seeds of <i>Strychnos nux-vomica</i>
iv. Respiratory stimulant	Lobeline	Leaf and flower of <i>Lobelia inflata</i>
v. Choleretics and laxatives	Boldine	Leaf and bark of <i>Lindera aggregata</i>
vi. Neuralgia	Aconitine	Whole herb of <i>Aconitum heterophyllum</i>
vii. Antiglaucoma and xerostomia agents	Pilocarpine	Leaf of <i>Pilocarpus microphyllus</i>
viii. Oxytocic	Ergonovine	Seeds of <i>Ipomea violacea</i>
ix. Bronchodilator	Ephedrine, vasicine	Whole shrub of <i>Ephedra sinica</i> , Leaf of <i>Justicia adhatoda</i>
x. Analgesic (narcotic), antitussive	Narceine, codeine (3-methylmorphine)	Capsule of <i>Papaver somniferum</i>
xi. CNS stimulant	Caffeine	Seeds of <i>Coffea</i> spp., leaf of <i>Camellia sinensis</i>
xii. Antihypertensive	Reserpine	Root of <i>Rauvolfia serpentine</i>
xiii. Anticancer	Vinblastine, vincristine	Leaf of <i>Catharanthus roseus</i>
xiv. Antiarrhythmic	Quinidine	Bark of <i>Cinchona</i> spp.
xv. Anticholinergic	Atropine	Foliage and berries of <i>Atropa belladonna</i>

(e) Taxonomic classification

The taxonomic classification of alkaloids essentially deals with the taxon—the taxonomic category. The most common taxa are the genus, subgenus, species, subspecies, and variety. Therefore, the taxonomic classification encompasses the plethora of alkaloids exclusively based on their respective distribution in a variety of Plant Families. A few typical examples of plant families and the various species associated with them are stated below.

(i) Amariillidaceae alkaloids

Amariillidaceae, a monocot family, include 75 genera and about 1100 species. Plants of this family are among the top 20 in the most widely considered medicinal plant families. Different pharmacologically active compounds such as phenols, alkaloids, lectins, peptides, etc., are present in the members of this family. About 500 structurally diverse Amariillidaceae alkaloids have been isolated to date. These structurally diverse Amariillidaceae alkaloids are classified into nine skeleton types, for which the representative alkaloids are: norbelladine, lycorine, homolycorine, crinine, haemanthamine, narciclasine, tazettine, montanine and galanthamine.

Amaryllidaceae alkaloids have important pharmacological properties such as acetylcholinesterase inhibitory activity, cytotoxicity and antitumoral activity (Bastida et al. 2006). Galanthamine, an isoquinoline alkaloid, is obtained from *Galanthus* sp., *Narcissus* spp. and *Leucojum aestivum*. Lycorine, a pyrrolophenanthridine alkaloid, displays a strong antiviral effect against poliovirus, measles and herpes simplex type 1 viruses, as well as high antiretroviral and strong antimitotic activities. Galanthamine is a long-acting, selective, reversible and competitive inhibitor of acetylcholinesterase, and is used for the treatment of Alzheimer's disease. Amaryllidaceae alkaloids have shown much promise as remarkably potent and selective anticancer agents. Due to interesting biological properties of Amaryllidaceae alkaloids and their abundance in different members, plants of the Amaryllidaceae have provided a diverse and accessible platform for phytochemical-based drug discovery.

(ii) Liliaceous alkaloids

The Liliaceae is one of the largest plant families with about 280 genera and 4000 species distributed throughout the world. They are mostly perennial herbs with starchy rhizomes, corms, tuber or bulbs. Plants belonging to the Liliaceae family have been the topic of research in many phytochemical and pharmacological laboratories because they contain structurally complex and biologically fascinating steroidal alkaloids. Medicinally important genera of the genera are *Asphodelus aestivus*, *Asparagus aphyllus*, *Draceana* spp., *Drimia maritima*, *Smilax aspera*, *Ruscus hypophyllum*, *R. aculeatus*, *Muscari comosum*, *Lilium candidum*, *Hyacinthus orientalis*, *Aloe vera*, *Allium sativum*, *Allium cepa*, *Colchicum cupani*, *C. autumnale*, etc. Liliaceae is a rich source of steroidal glycosides and alkaloids.

(iii) Cannabinaceous alkaloids

Cannabis is a psychoactive plant belonging to Cannabaceae family. The two principal varieties are *Cannabis sativa* or textile hemp and the *Cannabis sativa* indica or Indian hemp (Hemp, Marijuana).

(iv) Rubiaceous alkaloids

Rubiaceae family contains 611 genera and about 13,000 species. It is the fourth largest angiosperm family and contains economically important plants like *Coffea* spp. (caffein), *Cinchona officinalis* (quinine), *Rubia* spp., *Gardenia* spp., *Ixora* spp., *Pentas lanceolata*, *Mitragyna speciosa* (mitragynine, mitraphylline, 7-hydroxymitragynine, mitragynine pseudoindoxyl, raubasine, and some yohimbe alkaloids such as corynantheidine); *Pausinystalia johimbe* (yohimbine, corynanthine).

(v) Solanaceous alkaloids

The family Solanaceae consists of about 98 genera and some 2700 species with a great diversity of habitats, morphology, and ecology. Certain species are universally known for their medicinal uses and their psychotropic effects. Many species contain a variety of alkaloids including solanine, tropane, nicotine, capsaicin, scopolamine, atropine, hyoscyamine, nicotine, etc. Some of the medicinally important members of this family are *Nicotiana tabacum* (nicotine, anatabine, anabasine, etc.), *Petunia* spp., *Browallia* spp., and *Lycianthes* spp., the source of psychoactive alkaloids,

Datura stramonium (tropine alkaloids—atropine, hyoscyamine, scopolamine, etc.), *Mandragora officinarum* (atropine, hyoscyamine, scopolamine, scopine, cuscohygrine, etc.), *Atropa belladonna* (atropine, hyoscine scopolamine, hyoscyamine, etc.) and *Hyoscyamus albus* (piperidone alkaloid, tropine alkaloid, hyalbidone, littorine, etc.), *Brunfelsia uniflorus*, *Capsicum annum*, *Duboisia myoporoides*, *H. niger*, *Nicotiana glauca*, *Seopolia carniolica*, *Solanum dulcamara*, *Withania somniferum*, etc.

Invariably, alkaloids are grouped together according to the name of the genus wherein they belong to, such as coca, cinchona, and ephedra. Some phytochemists have even gone a step further and classified the alkaloids based on their chemotaxonomic classification. In the recent past, the alkaloids have been divided into two major categories based on the analogy that one containing a nonheterocyclic nucleus, while the other having the heterocyclic nucleus. These two classes of alkaloids, their major groups, synthetic precursors, examples and plant sources are shown in the Table 3.5.

The biological role

The role of alkaloids for living organisms that produce them is still unclear. It was initially assumed that the alkaloids are the final products of nitrogen metabolism in plants, as urea in mammals. It was later shown that alkaloid concentration varies over time, and this hypothesis was refuted. Most of the known functions of alkaloids are related to protection. For example, aporphine alkaloid liriodenine produced by the tulip tree protects it from parasitic mushrooms. In addition, presence of alkaloids in the plant prevents insects and chordate animals from eating it. However, some animals adapted to alkaloids and even use them in their own metabolism. Such alkaloid-related substances as serotonin, dopamine and histamine are important neurotransmitters in animals. Alkaloids are also known to regulate plant growth. Another example of an organism that uses alkaloids for protection is the *Uteheisa ornatrix*, more commonly known as the Ornate Moth. Pyrrolizidine alkaloids render these larvae and adult moths unpalatable to many of their natural enemies like coccinellid beetles, green lacewings, insectivorous hemiptera and insectivorous bats.

Applications of alkaloids

In medicine

Medical use of alkaloid-containing plants has a long history, and, thus, when the first alkaloids were isolated in the nineteenth century, they immediately found application in clinical practice. Many alkaloids are still used in medicine. Ajmaline—antiarrhythmic; atropine, scopolamine, hyoscyamine—anticholinergic; caffeine—stimulant, adenosine receptor antagonist; codeine—cough medicine, analgesic; colchicine—remedy for gout; emetine—antiprotozoal agent; ergot alkaloids—sympathomimetic, vasodilator, antihypertensive; morphine—analgesic; nicotine—

Table 3.5 Alkaloids with heterocycle and nonheterocycle rings nucleus

Class	Major groups	Synthetic precursors	Examples and plant sources
<i>Alkaloids with nitrogen heterocycles (true alkaloids)</i>			
Pyrrolidine derivatives		Ornithine or arginine	Cuscohygrine, hygrine, hygroline, and stachydine
Tropane derivatives	Atropine group Cocaine group	Ornithine or arginine	Atropine, scopolamine, and hyoscyamine Cocaine, ecgonine
Pyrrrolizidine derivatives	Non-esters Complex esters of monocarboxylic acids Macroyclic diesters L-amino pyrrolizidines (loline)	In plants: ornithine or arginine In fungi: L-proline + L-homoserine	Retronecine, heliotridine, laburnine Indicine, lindelophin, and saracine Platiphylline, trichodesmine Loline, N-formylloline, and N-acetyloline
Piperidine derivatives		Lysine Octanoic acid	Sedamine, lobeline, anaferine, and piperine Coniine, coniceine
Quinolizidine derivatives	Lupinine group Cytidine group Sparteine group Matrine group Ormosanine group	Lysine	Lupinine, nupharidin Cytidine Sparteine, lupanine, and anahygrine Matrine, oxymatrine, and allomatridine Ormosanine, pipantine

(continued)

Table 3.5 (continued)

Class	Major groups	Synthetic precursors	Examples and plant sources
Indolizidine derivatives		Lysine	Swainsonine, castanospermine
Pyridine derivatives	Simple derivatives of pyridine Polyyclic noncondensing pyridine derivatives Polyyclic condensed pyridine derivatives	Nicotinic acid	Trigonelline, ricinine, and arecoline Nicotine, nomicotine, anabasine, and anatabine Actindine, gentianine, and pediculinine
	Sesquiterpene pyridine derivatives	Nicotinic acid, isoleucine	Evonine, hippocrateine, and triptamine
Isoquinoline derivatives and related alkaloids	Simple derivatives of isoquinoline Derivatives of 1- and 3-isoquinolines Derivatives of 1- and 4-phenyltetra hydroisoquinolines Derivatives of 1- and 4-phenyltetra hydroisoquinolines	Tyrosine or phenylalanine	Salsoline, lophocerine N-methylcoridaldine, noroxyhydrastinine Cryptostilbin
	Derivatives of 5-nitroisoquinoline Derivatives of 1- and 2-benzyl-isoquinolines Cularine group Pavines and isopavines Benzopyrrolines		Ancistrocladine Papaverine, laudanosine, and sendaverine Cularine, yagonine Argemone, amurensine Cryptostilbin

(continued)

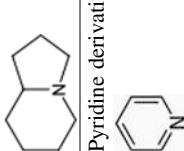
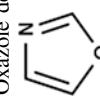


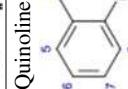
Table 3.5 (continued)

Class	Major groups	Synthetic precursors	Examples and plant sources
Protoberberines			Berberine, canadine, ophiocarpine, mecambridine, and corydaline
Phthalidisoquinolines			Hydrastine, narcotine (Noscapine)
Spirobenzylisoquinolines			Fumarcine
Ipecacuanha alkaloids		Emetine, protoemetine, and ipescide	
Benzophenanthridines		Sanguinarine, oxytutidine, and corynoloxine	
Aporphines		Glaucine, coridine, and liriodenine	
Proaporphines		Pronuciferine, glaziovine	
Homoaporphines		Kreysiginine, multifloramine	
Homoproaporphines		Bulbocodeine	
Morphines		Morphine, codeine, thebaine, and sinomenine	
Homomorphines		Kreysiginine, androcymbine	
Tropoloisoquinolines		Inerubrine	
Azofluoranthenes		Rufescine, imeluteine	
Amaryllis alkaloids		Lycorine, ambelline, tazettine, galantamine, and montamine	
Erythrina alkaloids		Erysodine, erythroidine	
Phenanthrene derivatives		Atherosperminine	
Protopins		Protopine, oxomurarine, and corycavidine	
Aristolactam		Doriflavin	
Oxazole derivatives	Tyrosine		Annuloline, halfordinol, texaline, and texamine



(continued)

Table 3.5 (continued)

Class	Major groups	Synthetic precursors	Examples and plant sources
Isoxazole derivatives 		Ibotenic acid	Ibotenic acid, muscimol
Thiazole derivatives 		1-deoxy-D-xylulose 5-phosphate (DOXP), tyrosine, cysteine	Nostocyclamide, thiostreptone
Quinazoline derivatives 	3,4-dihydro-4-quinazolone derivatives 1,4-dihydro-4-quinazolone derivatives Pyrrolidine and piperidine quinazoline derivatives	Anthranilic acid or phenylalanine or ornithine	Febrifugine Glycorine, arbamine, and glycosamine Vazine (peganine)
Acridinedervatives 		Anthranilic acid	Rutacridone, acronicine
Quinoline derivatives 	Simple derivatives of quinoline derivatives of 2-quinolones and 4-quinolone Tricyclic terpenoidss Furanquinoline derivatives Quinines	Anthranilic acid	Cusparine, echinopsine, and evocarpine Flindersine Dictamnine, fagarine, and skimmianine Quinine, quinidine, cinchonine, and cinchonidine

(continued)

Table 3.5 (continued)

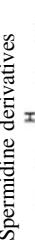
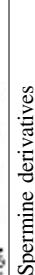
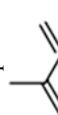
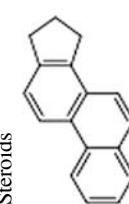
Class	Major groups	Synthetic precursors	Examples and plant sources
Indole derivatives			
Non-isoprene indole alkaloids	Tryptophan	Bufotenin	Serotonin, psilocybin, dimethyltryptamine (DMT), and bufotenin
Simple indole derivatives		Harmaline, harmine, harmaline, and eleagnine	
Simple derivatives of β-carboline			
Pyrroloindole alkaloids		Phystostigmine (eserine), ethera mine, physostigmine, and epatristigmine	
Semiterpenoid indole alkaloids			
Ergot alkaloids	Tryptophan		Ergotamine, ergobasine, and ergosine
Monoterpenoid indole alkaloids			
Corynanthe type alkaloids	Tryptophan	Ajmalicine, sarpagine, vobasine, ajmaline, yohimbine, reserpine, mitragynine, groupstrychnine and Strychnine brucine, aquamicine, and vomicine	
Iboga-type alkaloids		Ibogamine, ibogaine, and voacangine	
Aspidosperma-type alkaloids		Vincamine, vinca alkaloids, vincotine, and aspidospermine	
Imidazolederivatives			
		Directly from histidine	Histamine, pilocarpine, pilosine, stevensine
		Xanthosine (formed in purine biosynthesis)	Caffeine, theobromine, theophylline, and saxitoxin
Purinederivatives			
			(continued)

Table 3.5 (continued)

Class	Major groups	Synthetic precursors	Examples and plant sources
<i>Alkaloids with nitrogen in the side chain (protoalkaloids)</i>			
β -Phenylethylamine derivatives		Tyrosine or phenylalanine	Tyramine, ephedrine, pseudoephedrine, mescaline, cathinone, and catecholamines (adrenaline, noradrenaline, and dopamine)
Colchicine alkaloids		Tyrosine or phenylalanine	Colchicine, colchamine
Muscarine		Glutamic acid	Muscarine, allomuscarine, epimuscarine, and epiallomuscarine
Benzylamine		Phenylalanine with valine, leucine or isoleucine	Capsaicin, dihydrocapsaicin, nordihydrocapsaicin, and vanillylamine

(continued)

Table 3.5 (continued)

Class	Major groups	Synthetic precursors	Examples and plant sources
<i>Polyamines</i> alkaloids			
Putrescine derivatives		Ornithine	Paucine
			Lunarine, codonocarpine
Spermidine derivatives			
			
Spermine derivatives			Verbascenine, aphelandrine
			
<i>Peptide (cyclopeptide) alkaloids</i>			
Peptide alkaloids with a 13-membered cycle	Nummularine C type Ziziphine type	From different amino acids	Nummularine C, Nummularine S Ziziphine A, sativanine H
Peptide alkaloids with a 14-membered cycle	Frangulanine type Scutianine A type Integerrine type Amphibine F type Amfibine B type		Frangulanine, scutianine J Scutianine A Integerrine, discarine D Amphibine F, spinanine A Amphibine B, lotusine C
Peptide alkaloids with a 15-membered cycle	Muctonine A type	From different amino acids Amino acids	Mucronine A
<i>Pseudoalkaloids (terpenes and steroids)</i>			
Diterpenes	Lycocotonine type	Mevalonic acid	Aconitine, delphinine
			
Steroids		Cholesterol, arginine	Solasodine, solanidine, veralkamine, and batrachotoxin
			

stimulant, nicotinic acetylcholine receptor agonist; physostigmine— inhibitor of acetylcholinesterase; quinidine—antiarrhythmic; quinine—antipyretics, antimalarial; reserpine—antihypertensive; tubocurarine—muscle relaxant; vinblastine, vincristine—antitumor; vincamine—vasodilating, antihypertensive; and yohimbine—stimulant, aphrodisiac.

In agriculture

Prior to the development of a wide range of relatively low-toxic synthetic pesticides, some alkaloids, such as salts of nicotine and anabasine, were used as insecticides. Their use was limited by their high toxicity to humans.

Use as psychoactive drugs

Preparations of plants containing alkaloids and their extracts, and later pure alkaloids, have long been used as psychoactive substances. Cocaine, caffeine, and cathinone are stimulants of the central nervous system. Mescaline and many of indole alkaloids (such as psilocybin, dimethyltryptamine and ibogaine) have hallucinogenic effect. Morphine and codeine are strong narcotic pain killers. There are alkaloids that do not have strong psychoactive effect themselves, but are precursors for semisynthetic psychoactive drugs. For example, ephedrine and pseudoephedrine are used to produce methcathinone and methamphetamine. Thebaine is used in the synthesis of many painkillers such as oxycodone.

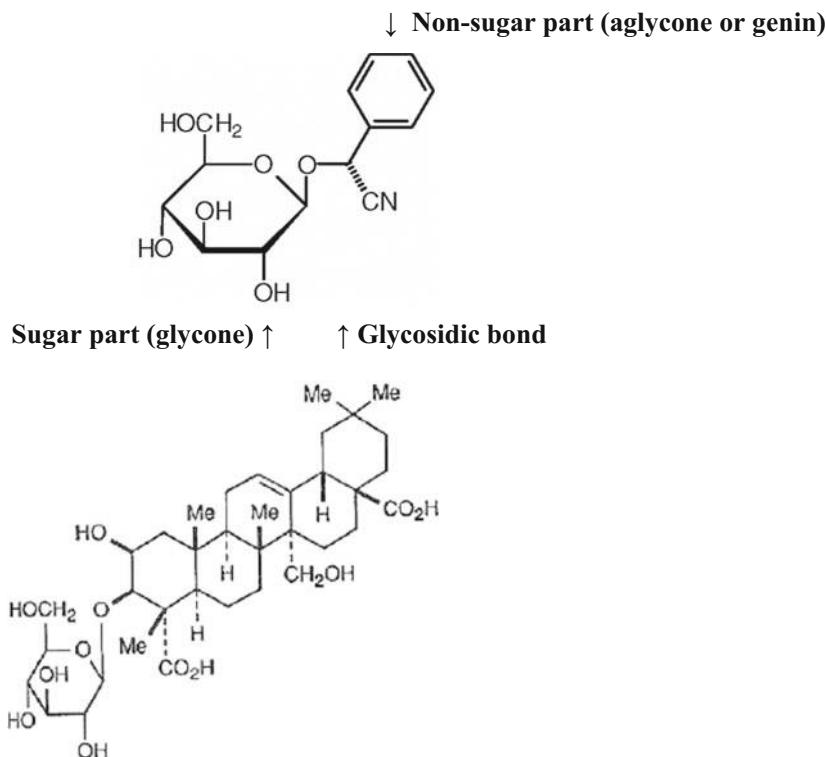
Artificial Alkaloids

A number of alkaloids can be prepared artificially, and theophylline, which occurs naturally in minute quantity in tea leaves, was the first to be produced synthetically on a commercial scale. Suprarenine, a synthetic with the actions of epinephrine, is also marketed. In addition, the pharmacopoeia recognizes four bodies which are manufactured from plant alkaloids, viz., apomorphine, prepared from morphine by dehydration; cotarnine, prepared by hydrolyzing narcotine; homatropine, which results from the action of mandelic acid upon tropine, the mother substance of atropine; and hydrastinine, obtained by the oxidation of hydrastine. Two other artificial substances of the Pharmacopoeia, hexamethylenamine, or urotropine, and antipyrine, have close chemic affiliations with the alkaloid group.

3.6 Glycosides

Glycosides and bitter principles

Besides acid and basic substances, plants furnish a large number of proximate principles which are chemically neutral. The most important are the glycosides (heterosides). A glycoside is a molecule in which a sugar (glycone) group by means of its anomeric carbon (C-1 carbon) bonded to a non-sugar (aglycone or genin) component. Glycosides on hydrolysis yield a sugar part (glycone) and a non-sugar part (aglycone or genin) as shown in the Fig. 3.34 with an example of Ginsenoside.



Ginsenoside-glycoside of Ginseng

Fig. 3.34 General structure of a glycoside showing sugar part (glycone) and non-sugar part (aglycone or genin); ginsenoside—glycoside of ginseng

Many plants store chemicals in the form of inactive glycosides. These can be activated by enzymehydrolysis or other decomposing agents such as heat, dilute acids, strong alkalies, bacteria, or fungi, which causes the sugar (glycone) part to be broken off, making the chemical (aglycon, the truly active constituent) available for use. Most of the glucosides yield glucose and a few of them yield other sugars. The glucosidal nature of glycosidic heterosides may be readily demonstrated by warming their mixture with Fehling's solution following hydrolysis by dilute hydrochloric acid. They are classified according to the nature of the non-sugar or aglycone. The names end in—oside, although some prefer to use the traditional names ending—ine (digoxin).

Chemically, glycosides are a loose group, and beyond their readiness of decomposition and their power to yield sugar, have no essential characters in common. They follow no rules as to solubility, taste or importance. Some of them are bitter, soluble in water or alcohol and inert pharmacologically while others are

not. Glycosides are classified based on their therapeutic effects, glycone–aglycon parts, bond between glycone–aglycon parts, name of the source plant, etc.

The glycone can consist of a single sugar group (monosaccharide) or several sugar groups (oligosaccharide). When the glycone group is glucose, fructose or glucuronic acid then the resulting glycoside molecule is a glucoside, fructoside or glucuronide, respectively. The aglycone may be an alcohol, anthraquinone derivative, phenol, aldehyde, acid, ester, or another compound. There are four types of linkages present between glycone and aglycone, e.g., O–, N–, S–, and C–glycosidic bond.

Glycosides play numerous important roles in living organisms. Many such plant glycosides are used as medications. The active principles of digitalis, strophanthus, cascara, willow and poplar barks are being among the most valued remedies. Salicin (named after the genus *Salix*) is an alcoholic glycoside found in willow and poplar barks. Salicin is converted in the body into salicylic acid, which is closely related to aspirin and has analgesic, antipyretic, and anti-inflammatory effects. Other glycosides are anthraquinones, cardiac glycoside, cyanogenics, coumarine, phenol, flavonoids, ranunculosides, saponins, and sulphurates. The most important groups are the anthraquinonics, cyanogenics, cardiotonics, and cumarinics. In the body, toxic substances are often bonded to glucuronic acid to increase their water solubility; the resulting glucuronides are then excreted.

Classification

Glycosides may be classified based on the characteristics of (a) glycone, (b) glycosidic bond and (c) aglycone, (d) correlation to the parent natural glycoside. A therapeutic classification, although excellent from a pharmaceutical viewpoint, omits many glycosides of pharmacognostic interest, e.g., cardiac glycosides.

(a) Based on the characteristics of glycone

The glycone part of a glycoside may be glucose, galactose, fructose, mannose, arabinose, rhamnose, glucuronic acid, etc., and the corresponding glycoside may be grouped as a glucoside, galacoside, fructoside, mannoside, arabinoside, rhamnoside, glucuronide, etc., respectively. Other monosaccharides include digitoxose, acetyldigitoxose, D-cymarose, L-oleandarose, etc. In the body, toxic substances are often bonded to glucuronic acid to increase their water solubility; the resulting glucuronides are then excreted. Sugar part of the molecule may be consisted of one monosaccharide (monosides, e.g., salicin), two monosaccharides (biosides, e.g., gentobioside), three monosaccharides (triosides, e.g., strophantothriose), four-, five monosaccharides (triosides, tetrosides, pentosides).

(b) Based on the characteristics of glycosidic bond

Glycosides are classified as α -glycosides or β -glycosides depending on the position of the glycosidic bond below or above the plane of the cyclic sugar molecule. Enzyme α -amylase hydrolyzes α -linkages while emulsin hydrolyzes β -linkages only. Most of the naturally occurring glycosides are of the β -type.

(c) Based on the chemical group of the aglycone involved in glycosidic bond

There are four types of chemical groups of the aglycone involved in glycosidic bond formation:

- (i) OH group (O-glycoside),
- (ii) SH group (S-glycoside),
- (iii) NH group (N-glycoside), and
- (iv) CH group (C-glycoside).

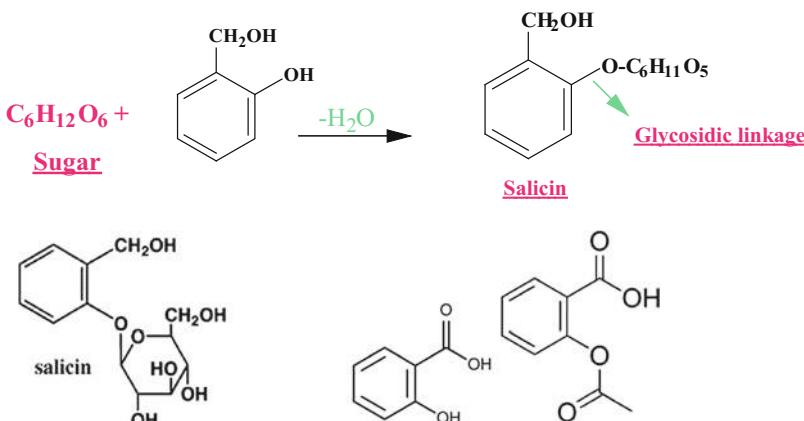
(d) Based on the characteristics of aglycone

Glycosides are classified on the basis of the chemical nature of the aglycone moiety. Classification by this method is very helpful for the purposes of biochemistry and pharmacology. When the chemical nature of the aglycone group is used as the basis of systematization, the classification is as follows:

- (i) Alcoholic glycosides (O-glycosides),
- (ii) Anthracene glycosides (O-glycosides or C-glycosides),
- (iii) Phenol glycosides (O-glycosides),
- (iv) Steroid glycosides,
- (v) Flavonoid glycosides,
- (vi) Coumarin and Furanocoumarin glycosides,
- (vii) Cyanogenetic glycosides,
- (viii) Sulfur-containing or Thioglycosides (S-glycosides),
- (ix) Saponin glycosides, and
- (x) Aldehyde glycosides.

(i) Alcoholic glycosides (O-glycosides)

Salicin found in the genus *Salix* (willows) is a good instance of an alcoholic β -glycoside (Fig. 3.35). Salicin is converted in the body into salicylic acid, which is



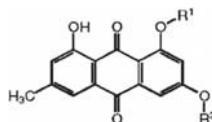
Salicin (salicylic acid + glucose), salicylic acid and aspirin (acetylsalicylic acid)

Fig. 3.35 Showing salicin as example of alcoholic β -glycoside (O-glycoside linkage)

closely related to aspirin and possesses analgesic (pain killing), antipyretic (alleviating fever) as well as anti-inflammatory properties.

(ii) Anthraquinone glycosides

These glycosides enclose an aglycone group that is a derivative of anthraquinones. Anthraquinones are yellow-brown pigments, most commonly occurring as O-glycosides or C-glycosides. The aglycone portions consist of two or more phenols linked by a quinone ring (anthracene derivative). They are found in many families of dicot (e.g., Ericaceae, Euphorbiaceae, Fabaceae, Lythraceae, Polygonaceae, Rhamnaceae, Rubiaceae, Saxifragaceae, Scrophulariaceae, Verbenaceae, etc.), one family of monocot (Liliaceae) plants as well as in certain fungi and lichen. Anthraquinone glycosides (anthraquinones without -COOH moiety) include chrysophanol, physcion, emodin, aloe-emodin, barbaloin, rhein, sennosides, etc., and (anthraquinones with -COOH moiety) include rhein, glucorhein, etc., are found in many herbal drugs, e.g., cascara sagrada (barks of *Rhamnus catharticus*, *R. frangula* and *Rhamnus purshiana* of *Rhamnaceae* contains cascarosides, emodin, etc.), rhubarb (rhizome of *Rheum palmatum* of *Polygonaceae* contains chrysophanol, emodin, rhein, etc.), senna (dried leaf lets of *Cassia senna*, *C. angustifolia* of *Caesalpiniaceae* contain sennoside), aloes (leaf of *Aloe barbadensis*, *A. vera* contains aloin, aloe-emodin, barbaloin, etc.). Chrysophanol is a dihydroxy methyl anthraquinone, emodin is a trihydroxy methyl derivative, aloe-emodin is a primary alcohol derived from chrysophanol and rhein is an acid derived from aloe-emodin. These glycosides are important laxative and cathartic drugs. Chrysophanic acid or chrysophanol, a fungal isolate and from the root of *Rheum wittrochii*, is a natural anthraquinone with anticancer activity, induces the necrosis of cancer cells via a reduction in ATP levels, attenuates the effects of lead exposure in mice by reducing hippocampal neuronal cytoplasmic edema, enhancing mitochondrial crista fusion, significantly increasing memory and learning abilities, reducing lead content in blood, heart, brain, spleen, kidney and liver, promoting superoxide dismutase and glutathione peroxidase activities and reducing malondialdehyde level in the brain, kidney and liver. Emodin (1,3,8-trihydroxy-6-methylantraquinone) is a naturally occurring anthraquinone present in the roots and barks of numerous plants, molds, and lichens, and an active ingredient of various Chinese herbs. It ameliorates diabetes and insulin resistance, emodin from rhubarb exhibits anticancer effects on several human cancers, including human pancreatic cancer and neuroprotective properties against glutamate toxicity. Aloe-emodin, a hydroxyanthraquinone, is present in aloe leaf exudate of *Aloe vera*, in the bark of *Rhamnus frangula* and *Rhamnus purshiana*, in the leaves of *Cassia angustifolia*, and in the rhizome of *Rheum rhabonticum*. It has a strong stimulant-laxative action. It has a marked antiviral effect in vitro against both herpes simplex virus (HSV) types 1 and 2 and has a specific in vitro and in vivo antineuroectodermal tumor activity. Figure 3.36 shows the structures of anthraquinone and different anthraquinone glycosides.



Structure of anthraquinone (R^1R^2 in frangula emodin = HH, in frangulin A = HRha, glucofrangulin A = GlcRha, frangulin B = HApi, in glucofrangulin B = GlcApi; Api = D-apio- β -D-furanosyl, Glc = β -D-glucopyranosyl, Rha = 6-deoxy- α -L-mannopyranosyl)

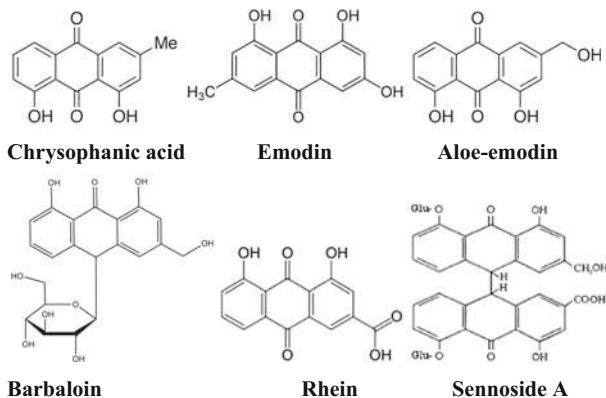


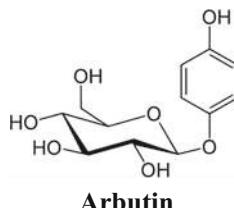
Fig. 3.36 Showing structures of anthraquinone and different anthraquinone glycosides

Barbaloinis or aloin, C-glucoside, is as a bitter, yellow-brown colored compound noted in the exudate of at least 68 *Aloe* spp. at levels from 0.1 to 6.6% of leaf dry weight (making between 3 and 35% of the total exudate). It is used as a stimulant laxative; remove constipation by inducing bowel movements. Rhein, castic acid, is a substance in the anthraquinone group obtained from rhubarb (*Rheum undulatum R. palmatum*) as well as in *Cassia reticulata*. Rhein is commonly found as a glycoside such as rhein-8-glucoside or glucorhein. Like all such substances, rhein is a cathartic. They are dimeric glycosides named after their abundant occurrence in plants of the genus Senna. Sennosides or Senna glycosides are many anthraquinone derivatives useful as a laxative.

(iii) Phenol glycosides (O-glycosides)

In phenolic glycosides (simple), the aglycone is a simple phenolic structure, e.g., salicin, arbutin, etc. (Fig. 3.37). Arbutin (a glycosylated hydroquinone) is found in

Fig. 3.37 Showing structures of phenol glycoside arbutin



the common bearberry (*Arctostaphylos uva-ursi*) and therefore bearberry is a traditional treatment for urinary tract infections. Among the medicinal plants whose activity is related to flavonoid content are the passion flower (*Passiflora incarnata*), chamomile (*Chamaemelum glabra*), aquilea (*Achillea millefolium*), liquorice (*Glycyrrhiza glabra*), gingko (*Ginkgo biloba*), thistle (*Silybum marianum*) and white thorn (*Crataegus monogyna*). Phenolic glycosides possess urinary antiseptic effect. It inhibits tyrosinase and thus prevents the formation of melanin. Arbutin is therefore used as a skin-lightening agent. Arbutin is found in wheat, and is concentrated in pear skins. It is also found in *Bergenia crassifolia*.

(iv) Steroidal glycosides or cardiac glycosides

The aglycone component here is a 17-carbon steroid nucleus consisted of four-ring (e.g., ABCD) structure (three 6-carbon rings-ABC and one 5-carbon ring-D) (Fig. 3.38). Steroidal glycosides or cardiac glycosides (CGs) are of two types, e.g., cardenolide type and bufadienolide type and may be obtained from both plants and animals. Cardiac glycosides (CGs) are present in the leaves of *Digitalis purpurea* and *D. lanata* (Scrophulariaceae). The important glycosides of this group include digoxin, digitoxigenin, digoxigenin, lantoside C, ouabain, strophanthin, ouabagenin and scillarenin A. Seeds of *Strophantus gratus*, *S. kombe* (Apocynaceae), *Adonis vernalis* (Ranunculaceae), bulb of *Scilla siberica* (Asparagaceae), dried scales of bulbs of *Urginea maritima* (Liliaceae) and *Nerium oleander* (Apocynaceae) are sources of cardioglycosides. These are chemically related to the sex hormones, vitamin D and venom of some toads. Cardiac glycosides are highly esteemed for their unique ability to increase the force of systolic contraction of the heart muscle. In small doses, CG can show a specific effect on the heart muscle. They are therefore highly valuable in the treatment of congestive heart failure and arrhythmia (trouble in the rhythm of the heartbeat), but now instead of CG other agents are preferred. Lanatoside C is a CG composed of four monosaccharides (glucose, 3-acetylglucosidose and two digitoxoses) and an aglycon named digoxigenin. Figure 3.38 given below shows the structures of a steroid skeleton and three glycosides.

Lanatoside C, a cardiac glycoside available in various species of *Digitalis*, can be used in the treatment of congestive heart failure and cardiac arrhythmia. Ouabain, also known as g-strophanthin, is a cardiac glycoside and in lower doses, can be used medically to treat hypotension and some arrhythmias. It acts by inhibiting the Na/K-ATPase, also known as the sodium-potassium ion pump. It is a plant-derived toxic substance that was traditionally used as an arrow poison in eastern Africa for both hunting and warfare. k-Strophanthin, an analog of ouabain, is a cardenolide in plants of the genus *Strophantus*.

Steviol glycosides

Steviol is a diterpene and occurs in the plant as steviol glycosides, sweet compounds that have found widespread use as sugar substitutes (Fig. 3.39). Steviol glycosides from *Stevia rebaudiana* of Asteraceae have been reported to be between

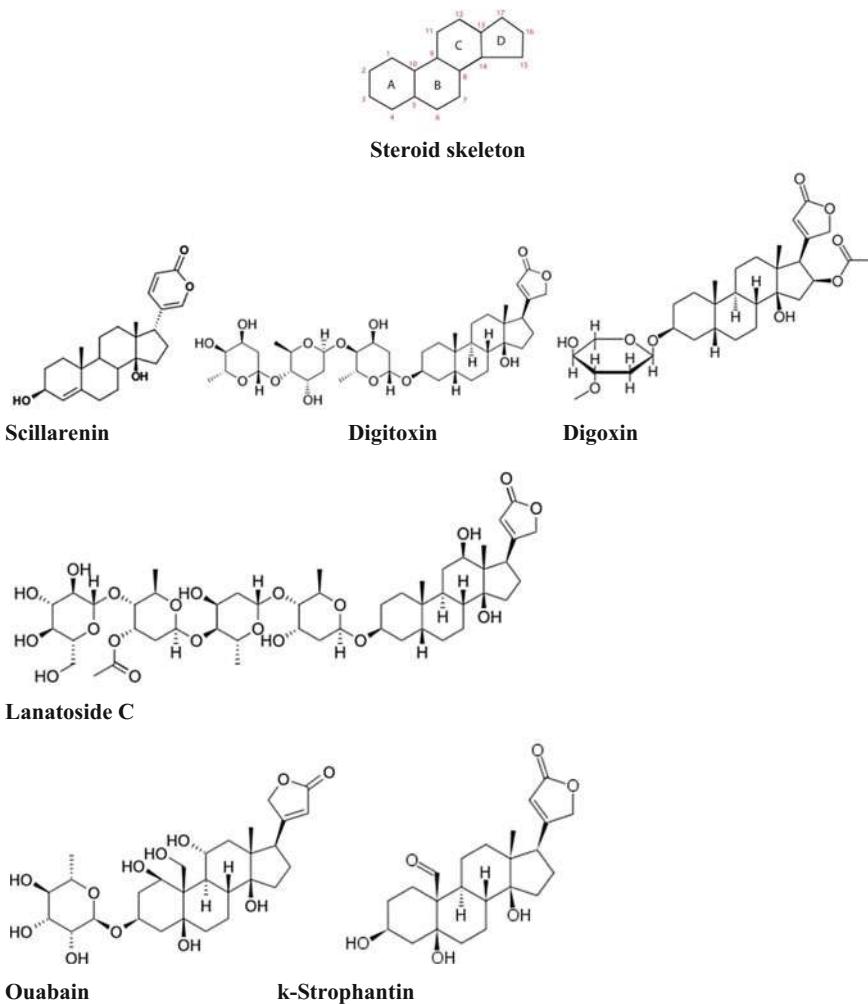


Fig. 3.38 Showing structures of a steroid skeleton and several glycosides—scillarenin, digitoxin, digoxin, lanatoside C, ouabain, and k-strophanthin. Each glycoside has three basic structural components: an unsaturated lactone ring, a steroid nucleus, and sugar moieties

30 and 320 times sweeter than sucrose. Steviol glycosides also occur in *Stevia phlebophylla* and in *Rubus chingii* (Rosaceae). These glycosides have steviol as the aglycone part. Glucose or rhamnose–glucose combinations are bound to the ends of the aglycone to form the different compounds. Stevioside and rebaudioside A are two natural sweeteners used in many countries. Rebaudioside A is a steviol glycoside that is 200 times sweeter than sugar. The steviol glycosides are stable to heat, pH changes and are also to fermentation. When these glycosides are consumed,

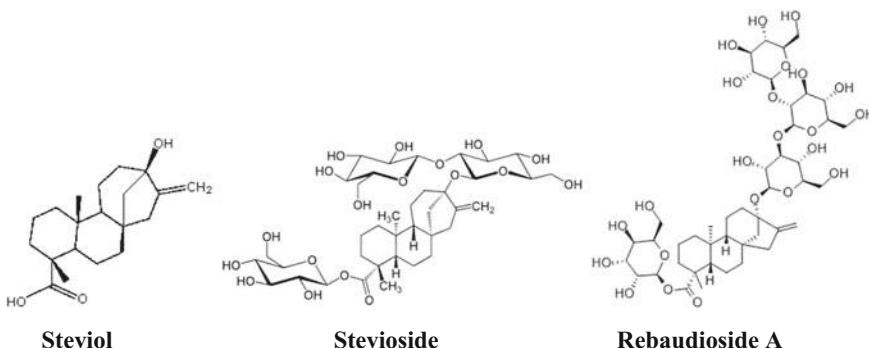


Fig. 3.39 Showing structures of steviol and steviol glycosides—stevioside and rebaudioside A

they also do not stimulate any glycemic response and as a result of this, steviol glycosides are preferred as natural sweeteners for diabetic patients as well as others who take low carbs diets.

(v) Flavonoid glycosides

In flavonoid glycosides, the aglycone components are flavonoids, yellow pigments derived from phenil-benzo γ pirona or phenil cromome. Flavonoids are phenolic compounds composed of three benzene rings with hydroxyl groups and include 6 main classes, e.g., chalcones, flavones, flavonols, anthocyanidins, and condensed tannins, as well as two others, xantones, and aurones; some of the examples of flavonoid glycosides include naringin (aglycone: naringenin, glycone: rutinose), hesperidin (aglycone: hesperetin, glycone: rutinose), quercitrin (aglycone: quercetin, glycone: rhamnose) and rutin(aglycone: quercetin, glycone: rutinose) (Fig. 3.40). Flavonoid glycosides occur in significant amounts usually in the form of heterosides, and they are frequently found in the plant kingdom such as in fruits —apple, berries, cherry, grape, grapefruit, plum; vegetables-cabbage, parsley, seeds, legumes, soy products, onions, black and green tea, red wine, etc. More than 4000 different flavonoids have been identified, some of which are believed to have beneficial effects on human health. Some of the health-related benefits associated with flavonoid glycosides include antioxidant properties (free radical scavenger), strengthening of the immune system, protection against cancer, dilation of blood vessels and reduction in capillary weakness or fragility.

(vi) Coumarin and Furanocoumarin glycosides

Coumarin glycosides

Coumarin is a fragrant organic chemical compound in the benzopyrone (benzo- α pyrones, phenolic active components) chemical class, which is a colorless crystalline substance in its standard state. It is a natural substance found in many

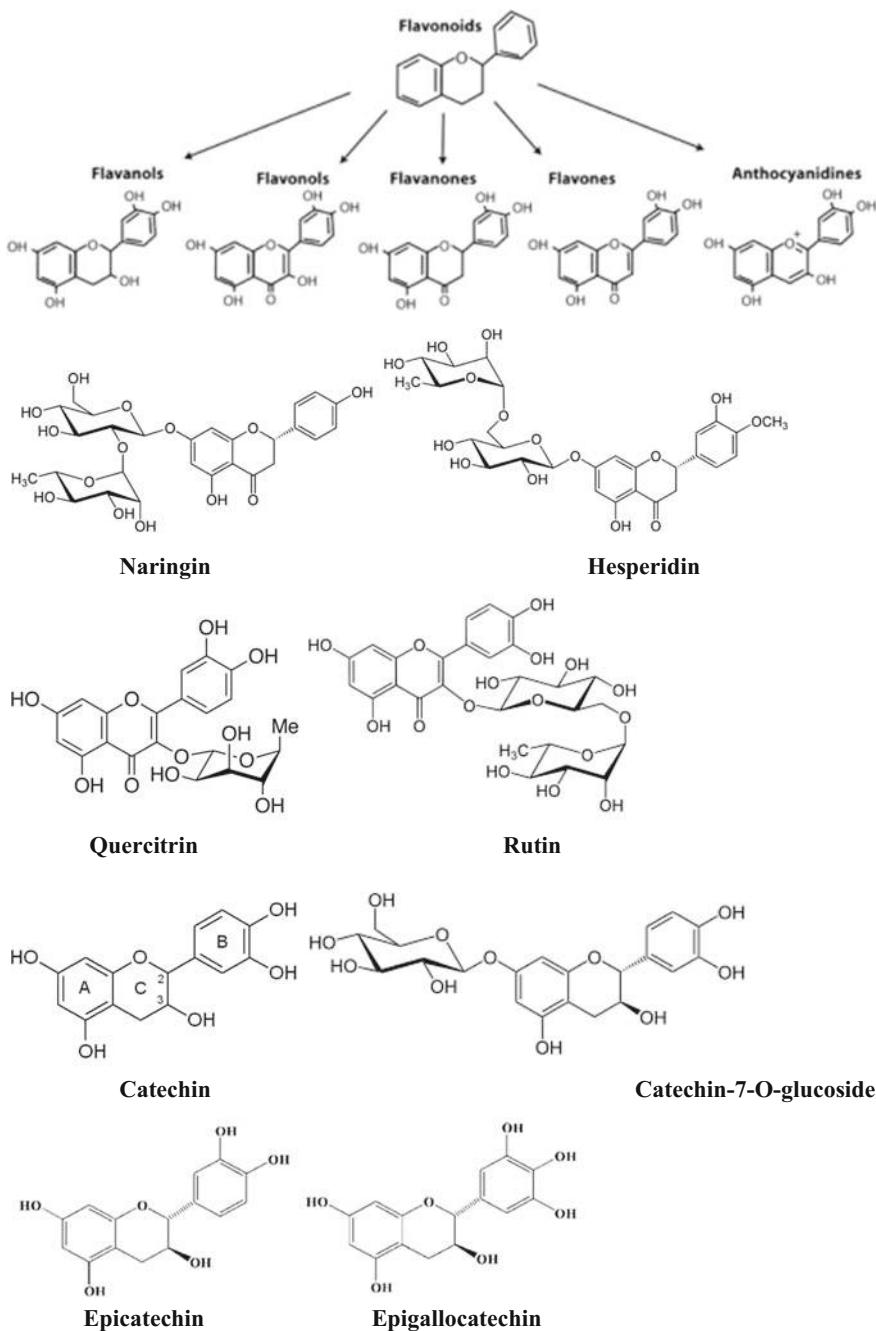


Fig. 3.40 Showing classes of flavonoids and structures of different flavonoid glycosides

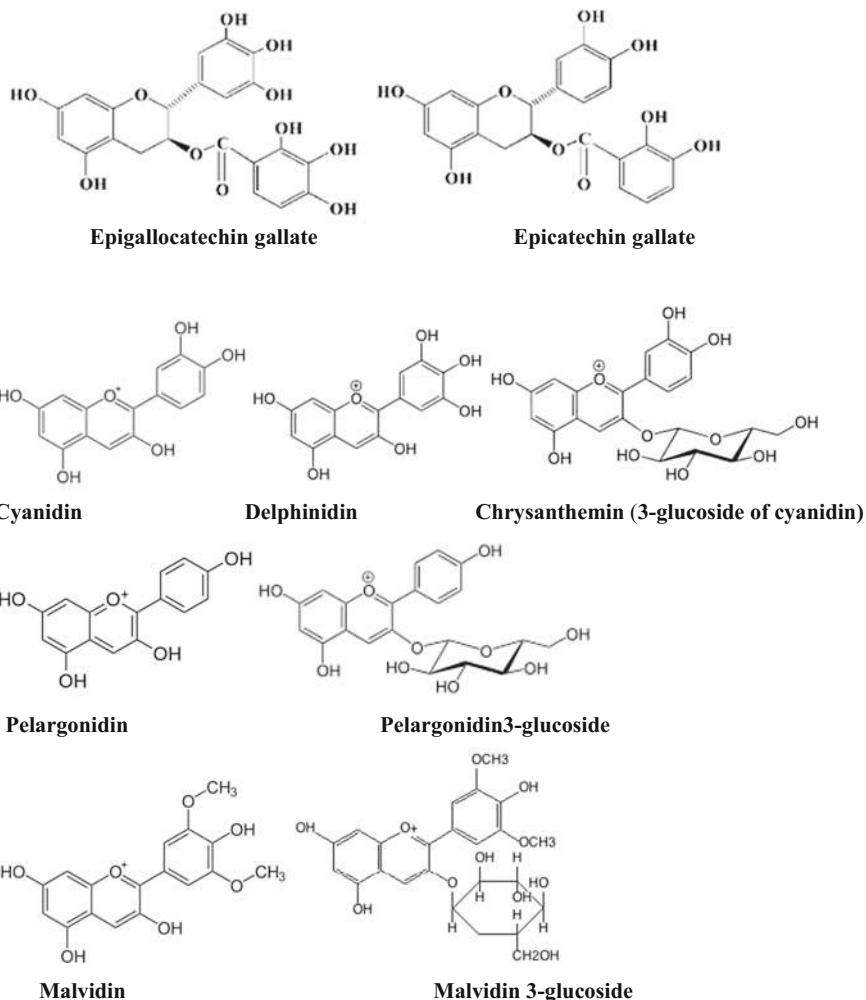


Fig. 3.40 (continued)

medicinal plants (*Dipteryx odorata*, *Anthoxanthum odoratum*, *Galium odoratum*, *Verbascum*spp., *Hierochloe odorata*, *Melilotus officinalis*, and *Dicranthelium clandestinum*, *Cinnamomum cassia* but true cinnamon or Ceylon cinnamon—*Cinnamomum zeylanicum* contains little coumarin). Coumarin is also found in extracts of *Justicia pectoralis*. Coumarins are effective on the vascular system (both on arteries and veins), against venolymphatic insufficiency and in the treatment of psoriasis due to its photosensitizing properties. Esculetin, found in Indian chestnuts (*Aesculus hippocastanum*), acts as both a tonic for the veins and a protector of the cell wall. Visnadinine is a dilator of the blood vessels found in the fruit of the visnaga (*Amni visnaga*). Dicumarol is an anticoagulant which forms in *Melilotus*

when conditions for conservation are bad. Furanocumarins are photosensitizing and are used to treat psoriasis. Sometimes they are used in sun creams as they enhance melanin production (photodynamic), for example, essence of bergamot (*Citrus bergamia*). Coumarin glycosides contain coumarin or a derivative as aglycone, e.g., apterin is a coumarin glycoside.

It is a furanocoumarin, the glucoside of vaginol. It has been isolated from the root of plants in the Apiaceae (*Angelica* spp. *Zizia aptera*, etc.). Apterin is said to expand the coronary arteries and also functions as a calcium channel blocker. Aesculin is a poisonous coumarin glycoside that naturally occurs in the horse chestnut (*Aesculus hippocastanum*), California buckeye (*Aesculus californica*), prickly box (*Bursaria spinosa*), in daphnin (*Daphne mezereum*) and in dandelion coffee. It is used as a laboratory to aid in the identification of bacterial species. Warfarin is an anticoagulant normally used in the prevention of thrombosis and thromboembolism. Warfarin is an antagonist of vitamin K and it prevents it from synthesizing clotting factors, thereby creating an anticoagulation effect. Figure 3.41 shows the structures of coumarin and different coumarin glycosides.

Coumarins along with its derivatives are abundantly found in plant families like Orchidaceae, Apiaceae, Asteraceae, Clusiaceae, Leguminaceae Rutaceae, Solanaceae and Thymalaceae. Apterin, a furanocoumarin glucoside, isolated from the root of plants in the genus *Angelica*, including Garden Angelica and in *Zizia aptera* of Apiaceae, dilates the coronary arteries as well as blocks calcium channels.

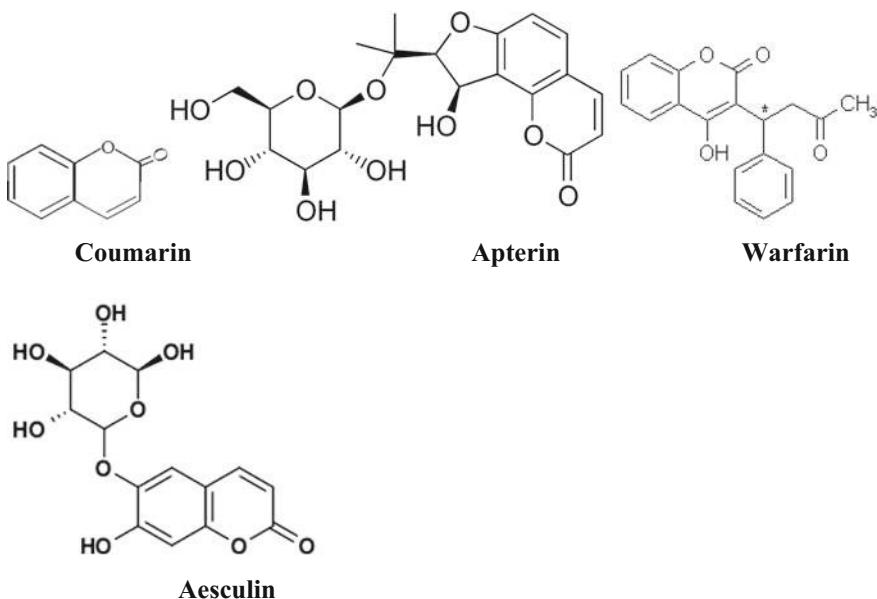


Fig. 3.41 Showing structures of coumarin and different coumarin glycosides

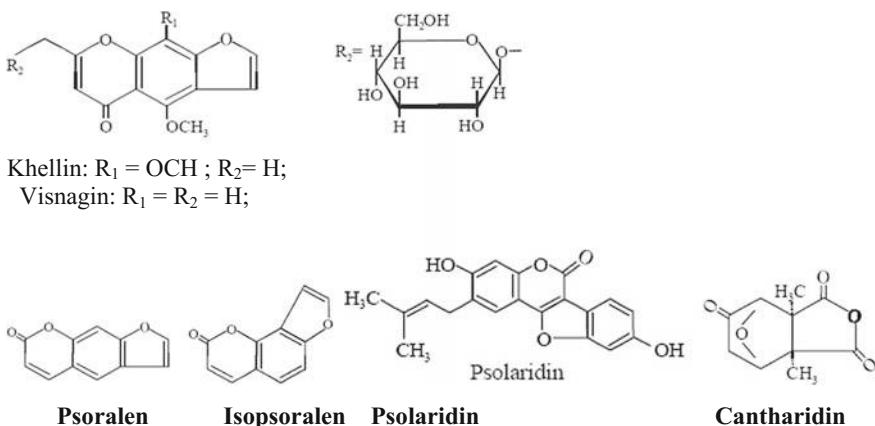


Fig. 3.42 Showing structures of furanocoumarin glycosides

In chromone glycosides (isolated from leaves of *Cassia multijuga* of Leguminosae, *Hypericum japonicum* of Clusiaceae), the aglycone is benzo-gamma-pyrone. Coumarin glycosides from *Daucus carota* have been used in traditional medicine to treat hypertension.

Furanocoumarin glycosides

The furanocoumarins are obtained by the fusion of the furan ring to the coumarin nucleus either at C-6 and C-7 positions or at C-7 and C-8 positions. A few typical examples of furanocoumarin glycosides are khellol glucoside; psoralea and cantharides (Fig. 3.42). Khellol glucoside is obtained from the seeds of *Eranthis hyemalis* of Ranunculaceae, *Ammi visnaga* Lamiaceae. Psoralen is found in dried ripe fruits of *Psoralea corylifolia* of Fabaceae and also found naturally in bergernot, limes, cloves, figs, etc. Cantharides comprises of the dead and dried insects of *Cantharis vesicatoria* Meloidae. Cantharides contains the furanocoumarin derivatives cantharidin ranging from 0.6 to 1%.

(vii) Cyanogenic glycosides

In cyanogenic glycosides, the aglycone contains a benzene ring having a cyanide group. Amygdalin obtained from almonds is a good example of cyanogenic glycosides. Dhurrin, linamarin, lotaustralin, and prunasin are also classified as cyanogenic glycosides. A number of fruits as well as floppy (wilting) leaves of apples, wild cherries, plums, peaches, almonds, apricots, crabapples and raspberries contain cyanogenic glycosides. Root of *Cassava*, a significant food plant found in South America, South East Asia and Africa, also contains cyanogenic glycosides and hence it is essential to carefully wash it under running water and ground before it can be ingested. Even sorghum (*Sorghum bicolor*) possesses cyanogenic glycosides in its roots and hence, it is resistant to rootworms (*Diabrotica* spp.). Plants store cyanogenic glycosides in the vacuole in inactive form and release them in the

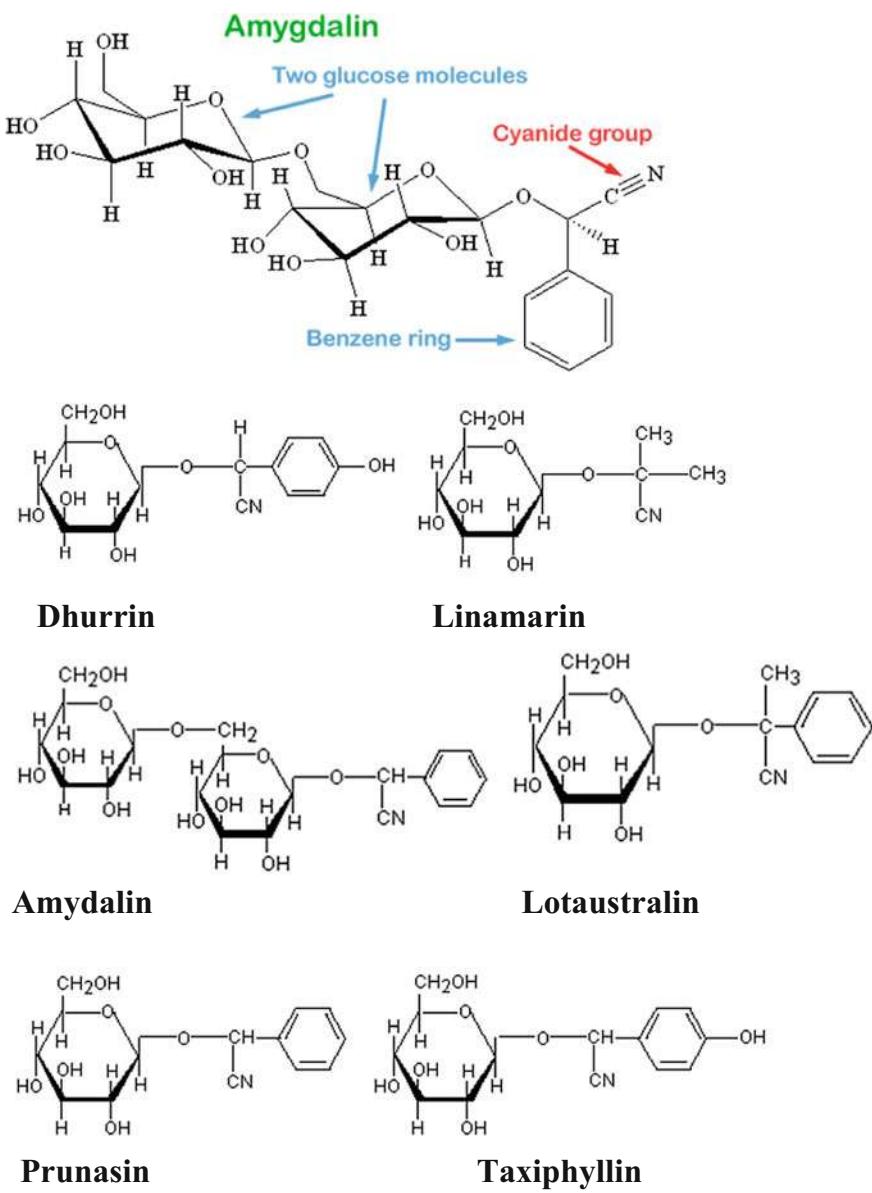


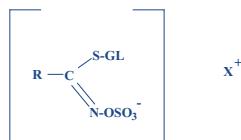
Fig. 3.43 Showing structures of different cyanogenetic glycosides

cytoplasm for defensive purposes when enzymes remove the sugar part set free the toxic hydrogen cyanide. Storing them in inactive forms in the vacuole prevents them from damaging the plant under normal conditions. Increase of CO_2 levels in the atmosphere resulted in much higher levels of cyanogenic glycoside production

in sorghum and cassava plants, making them highly toxic and inconsumable. Preparations from plants containing cyanogenic glycosides are widely used as flavoring agents. Some of them have been claimed to possess anticancer properties and others have been suggested as possible agents for control of sickle cell anemia. Figure 3.43 shows structures of different cyanogenetic glycosides.

(viii) Sulfur-containing or thioglycosides (S-glycosides)

These glycosides enclose sulfur. Sinigrin and sinalbin, the two glucosinolates (compounds that contain sulfur and nitrogen) present in Indian or brown and black (*Brassica juncea* or *Brassica nigra*) and white (*Sinapis alba*, *S. hirta*) mustard of the Brassicaceae family, respectively, are good examples of two thioglycosides. Sinalbin is found in the seeds of white mustard and in many wild plant species. In contrast to sinigrin of black mustard seeds, sinalbin from white mustard seeds has only a weakly pungent taste. The glucosinolates also occur in other plants such as *Phyllanthus emblica*, *Armoracia lapathifolia*, *Wasabia japonica*, etc. Glucosinolates protect plants from fungal, nematodes and other pathogens and herbivores threats. Sinigrin is a precursor of the anticancer compound allyl isothiocyanate and antimicrobial, anticancer and antilipidimic (lower plasma triglyceride level) activities. Both the seeds and leaves traditionally have been used for medicinal purposes, including historical use as a curative for the common cold and applications in mustard plasters, baths, and treatments for chilblains (Herbst 2001; Downey 2003). Sinigrin is composed of glucose, allylisothiocyanate (volatile oil of mustard) and potassium acid sulfate and sinalbin is consisted of a phenolic isothiocyanate (acrinal isothiocyanate), glucose and the acid sulfate of a quaternary alkaloid, sinapine⁺. Figure 3.44 shows general structure of thioglycosides and structures of different sulfur-containing glycosides or thioglycosides.



The general structure of thioglycosides. The anion is called the glucosinolate ion; R may be aliphatic or aromatic. The cation (X) may be a simple metal ion or a complex organic cation, e.g., sinapine ion of sinalbin.

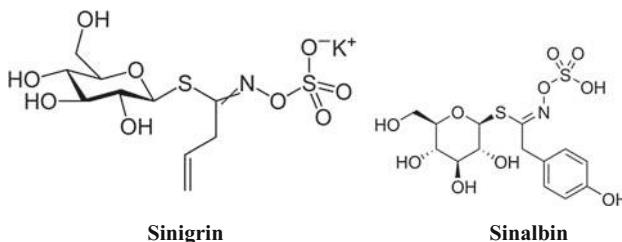
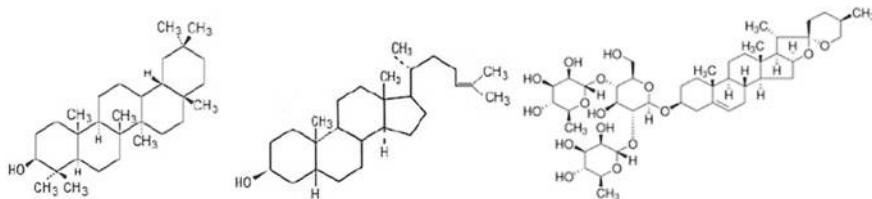


Fig. 3.44 Showing general structure of thioglycosides and structures of different sulfur-containing glycosides or thioglycosides

(ix) Saponin glycosides

Glycosides with foaming features are known as saponins. Saponins comprise polycyclic aglycones (terpenoids, steroids) bound to one or many sugar side chains (Fig. 3.45). Most of the saponins are neutral and soluble in water; contain a dextrose sugar glycone and an aglycone, called sapogenin. The sapogenins are insoluble in water, but soluble in weak alcohol. Saponins are found in soapwort (*Saponaria officinalis* of Caryophyllaceae), soapberry or soapnut (*Sapindus mukorossi* of Sapindaceae), sugar maples (*Acer saccharum* of Aceraceae), jiaogulan (*Gynostemma pentaphyllum* of Cucurbitaceae), soapbark tree (*Quillaja saponaria* of Quillajaceae) and ginseng or red ginseng (*Panax ginseng* of Araliaceae) in various parts of the plant, e.g., leaves, stems, roots, bulbs, blossom, and fruit. Triterpenic saponins (acid) are found in the seed of the Indian chestnut (*Aesculus indica* of Sapindaceae), in liquorice (*Glycyrrhiza glabra* of Fabaceae), the Asian centella (*Centella asiatica* of Apiaceae), and in ginseng (*Panax ginseng* of Araliaceae). Steroidal saponins (neutral) are in Ruscus (*Ruscus aculeatus* of Asparagaceae), agave (*Agave sisalana* of Asparagaceae), and in dioscorea (*Dioscorea* spp. of Dioscoreaceae). Saponins taste bitter and some of them are poisonous. The toxic saponins are called sapotoxins. The compounds also result in the hemolysis of the red blood cells (the breaking down of red blood cells with liberation of hemoglobin). Many saponins are used as fish poisons. Saponin glycosides have a prominent therapeutic benefit. Medicinal value of saponins is due to their expectorant, corticoid and anti-inflammatory effects. Steroid saponins from *Dioscorea* dioscin is an important starting material for production of semisynthetic glucocorticoids and other steroid hormones such as progesterone. Glycyrrhizin, a sweet compound that is 50 times higher than sugar, increases fluid and sodium retention and promotes potassium depletion in blood (Fig. 3.46). Saponins cause a reduction of blood cholesterol by preventing its reabsorption. Saponins have anti-tumor and antimutagenic activities and can lower the risk of human cancers by preventing cancer cells from growing.



Tri-terpenoidal skeleton Steroidal skeleton

Dioscin (steroid saponin)

Fig. 3.45 Pentacyclic tri-terpenoidal and tetracyclic steroidal skeletons of saponins. Dioscin—a saponin with steroidal nucleus

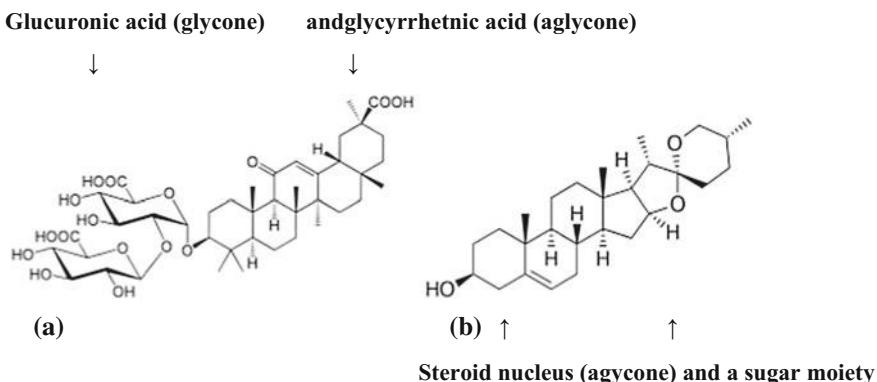


Fig. 3.46 **a** Glycyrrhizin or glycyrrhetic acid (glycoside), and **b** diosgenin

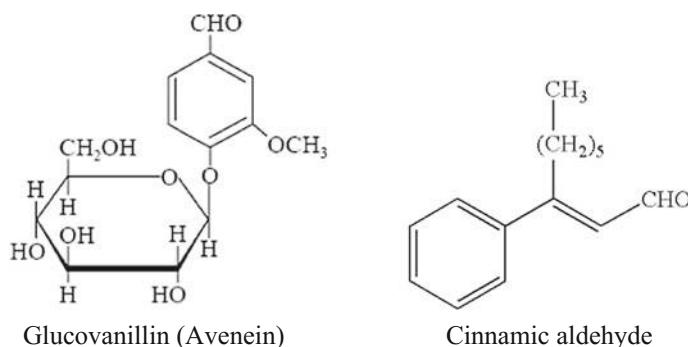


Fig. 3.47 Showing structures of different aldehyde glycosides

(x) Aldehyde glycosides

Vanilla pod is the most glaring example of a naturally occurring plant that contains an aldehyde glycoside, e.g., glucovanillin, and cinnamon bark is another important example which contains cinnamic aldehyde (Fig. 3.47).

3.7 Bitter Principles

Bitter principles, alkaloidal glycosides, and miscellaneous compounds

The chemical composition of bitter principles includes a complex pattern of molecular structures. Structurally they are mostly terpenoid, especially the sesquiterpene lactones, monoterpene iridoids and the secoiridoids (Fig. 3.48). Iridoids are responsible for the chief bitter constituents of *Cichorium intybus*

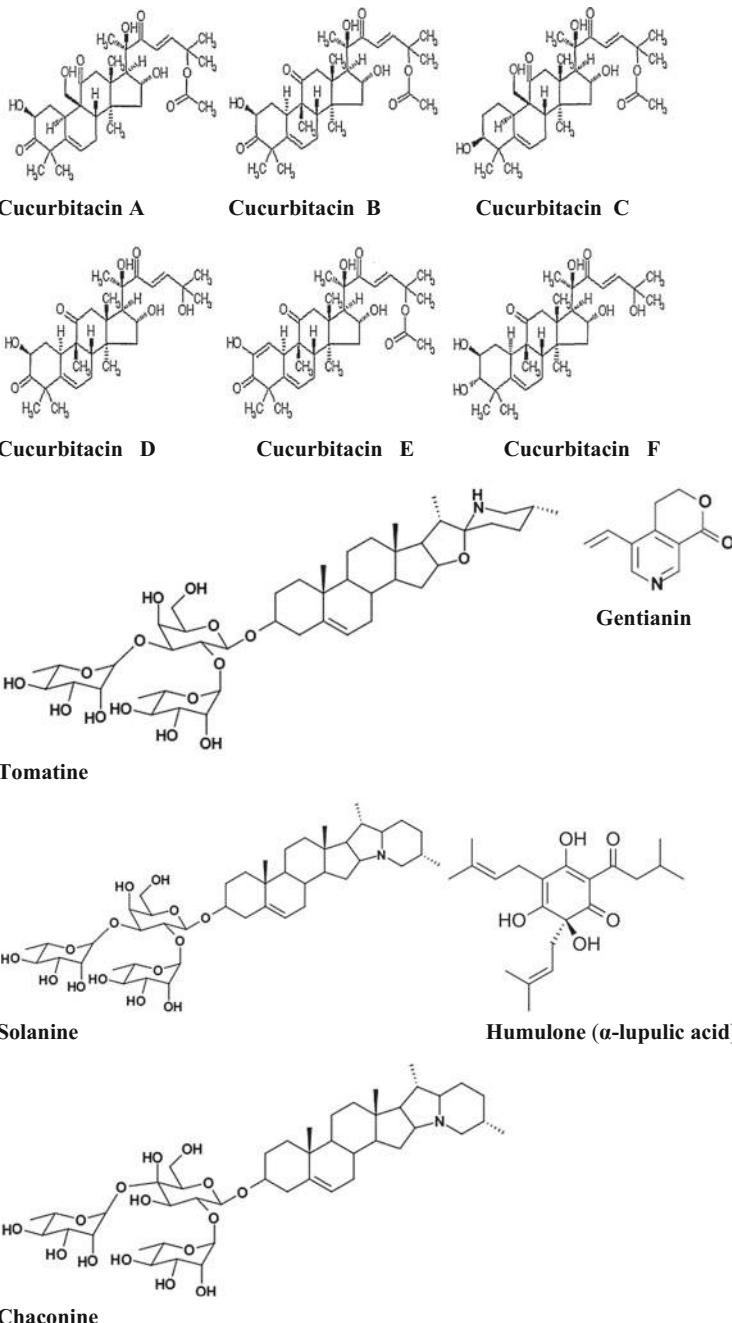


Fig. 3.48 Showing structures of different bitter principles

(chicory), dandelion or members of *Taxacum* genus of Asteraceae, *Valeriana officinalis* (valerian), wild lettuce (*Lactuca virosa*), and quassia bark (*Quassia amara*). Sesquiterpenes account for the bitter taste of the *Artemisia* wormwood (*Artemisiaabsinthium*), blessed thistle (*Cnicus benedictus*), and ginkgo (*Ginkgo biloba*). Other components which add to the bitterness are diterpene bitters, found in columbo root (*Jateorrhiza palmata*) and white horehound (*Marrubium vulgare*). Triterpenoids are the cause of bitterness in Cucurbitaceous plants including pumpkin, cucumber, colocynth, marrows, and the bryonies. Many genuses of Cucurbits such as *Trichosanthes*, *Cucurbita*, *Cucumis*, *Bryonia*, and *Citrullus* are affluent in cucurbitacins. Outside the Cucurbitaceae, Cucurbitacin-producing plants have also been identified in the members of Scrophulariaceae, Begoniaceae, Primulaceae, Liliaceae, Tropaeolaceae and Rosaceae. Cucurbitacins possess immense pharmacological potential. The structural composition of following cucurbitacins are known and on the basis of side chain derivatives have been designated by the letters: A, B, C, D, E, F, G, H, I, J, K, L, O, P, Q, R, and S (Kaushik et al. 2015). Many alkaloids also contribute to the bitter taste as in the protoberberine isoquinoline alkaloids of golden seal (*Hydrastis canadensis*), and Berberis of Berberidaceae, the morphine alkaloids, the quinoline alkaloids of quinine and angostura and the purine alkaloids (in coffee). In addition to this, many miscellaneous compounds like ketones and amino acids are responsible for the bitterness, as found in hops (*Humulus lupulus*). The bitter principles of hops are (i) lupamaric acid (humulone), (ii) lupamaric acid (lupulinic acid). Cucurbitacins, bitter principles of Cucurbitaceae, occur exclusively as glycosides and the alkaloid glycoside is tomatine. The bitter principle, known as gentianine, is a glucoside, soluble in water and alcohol.

The glycoalkaloid poisons α -solanine and α -chaconine are to be found in the nightshade family of plants, the (*Solanaceae*), in particular in potatoes (*Solanum tuberosum*), tomatoes (*Lycopersicon esculentum*), egg plant (*Solanum melongena*), Sweet and hot peppers (*Capsicum* species) Thorn-apple (*Datura stramonium*), Apple-of-Peru (*Nicandra physalodes*), Black Nightshade (*Solanum nigrum*), and Bittersweet (*Solanum dulcamara*). It is present in small quantities throughout potato tubers, especially in the sprouting shoots, but a lot more is synthesized by the potato if the tuber is exposed to sunlight, where the exposed parts become green (with harmless chlorophyll). It is in and near the green parts where the highest concentration of solanine is to be found. Solanine is not rendered safe by boiling, but deep frying at 170 °C does destroy most of the solanine. Normally, potatoes contain between 20 mg and 150 mg per kg of raw potato, but when turned green by exposure to sunlight may contain as much as 1000 mg/kg, mostly just under the skin (the shoots contain even higher amounts). Solanine adds an un-pleasant bitterness to the flavor of potatoes when its concentration exceeds 200 mg/kg, so potato poisoning is now rare, especially as cooks are now more aware of the dangers of greening or sprouting potatoes.

Bitter principles occupy a central place in herbal therapeutics beating the acrid constituents. A wide range of general actions are attributed to the bitter principle, including increasing saliva secretion, stimulating the appetite, bring about an

increase in the secretion of digestive juices, improving digestion, protect the tissues found in the digestive tract, boosts up the bile flow and strengthens the pancreas and liver detoxification, as well as some specific actions associated with a specific herb, e.g., valerian (*Valeriana officinalis*) of Caprifoliaceae and hops (*Humulus lupulus*) of Cannabaceae are relaxing nervines; white horehound (*Marrubium vulgare*) of mint family has pulmonary and expectorant actions, while bogbean (*Menyanthes trifoliata*) of Menyanthaceae and devil's claw (*Harpagophytum procumbens*) of Pedaliaceae are anti-inflammatory. Bitters are indispensable when it comes to counter a heavy meal. Sometimes, chicory and dandelion roots are mixed with coffee beans to produce a bitter drink usually taken after meals. The drink vermouth is the good example of an appetizer which gets its name from bitter herb wormwood.

Solanine has a choline esterase inhibitor function and thus affects the central nervous system. The symptoms of solanine poisoning are nausea, vomiting, diarrhea, stomach cramps, burning of the throat, heart arrhythmia, dizziness, and in severe cases hallucinations, loss of feeling, paralysis, jaundice, hypothermia and death. It causes apoptosis in cells; the cells commit suicide. Between 2 and 5 mg/kg of body weight will cause severe poisoning, possibly fatal. Tomatine is also a poisonous glycoalkaloid. It is found in green tomatoes and α -tomatine in tomatoes act as an antifungal, antibacterial and against insects and has been shown to display interesting pharmacological properties against bacteria, viruses, fungi and tumors.

Classification of bitter principles

According to the physiological, therapeutic or pharmacological activities

Laxatives glycosides are cathartic, purgative and aperients compounds or drugs that facilitate or increase bowel movements. Laxative glycosides include (a) sennoside A, B, C, D (from *Senna* leaves and fruits); (b) cascarioside A, B (from *Cascara* bark); (c) frangulin and glucofrangulin (from *Frangula* bark); (d) aloin and barbaloin (from *Aloe vera* and *Aloe barbadensis* juice) (Fig. 3.49). These are the examples of stimulant laxatives that induce bowel movements by increasing the contraction of muscles in the intestines. Cardiac glycosides, on the other hand include (a) digitalis glycosides such as digoxin, digitoxin, gitoxin (from Fox glove leaves); (b) ouabain or G-strophanthin (from *Strophanthus gratus* seeds); c-K-strophanthin (from *Strophanthus kombe* seeds); (d) scillaren A, B (from red and white Squill bulbs); (e) convolloside (from *Convallaria majalis*—lily of the valley). Cardiac glycosides act on the contractile force of the cardiac muscle and they are the cardiac muscles stimulators.

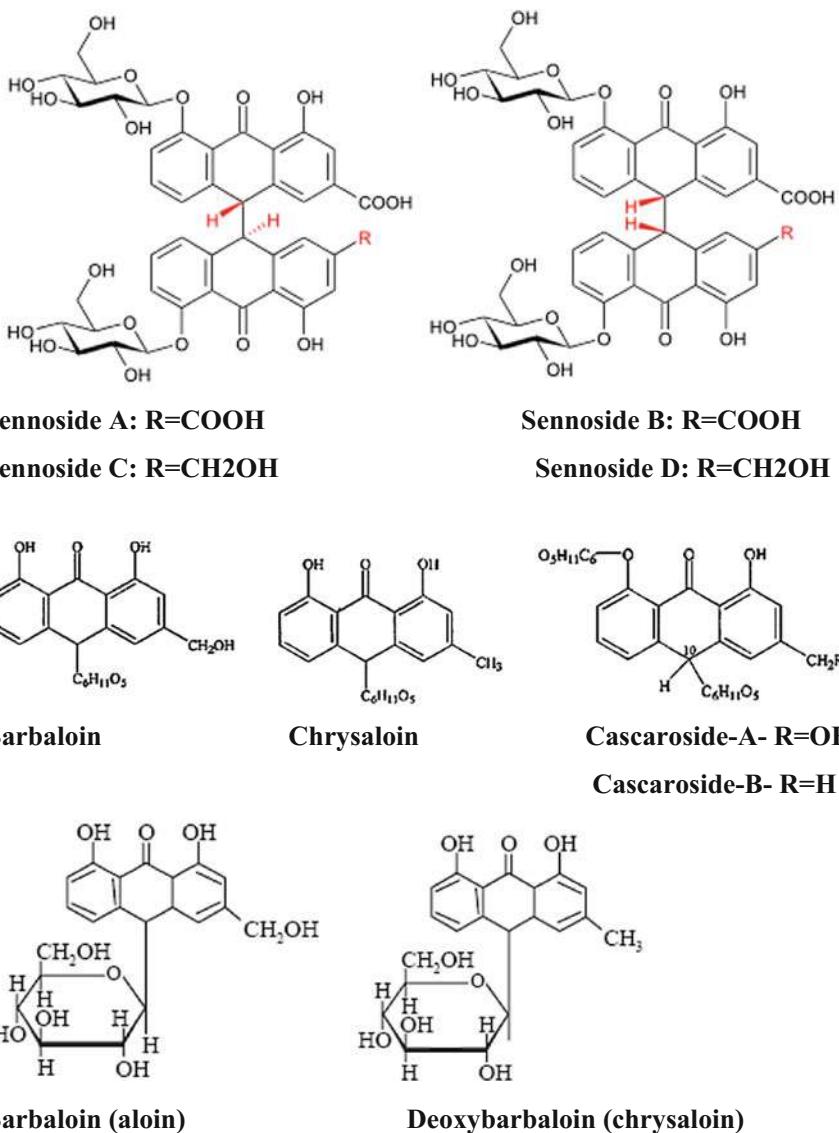


Fig. 3.49 Showing structures of different bitter principles—laxatives glycosides

According to the plant families

Coniferous glycosides

Coniferae is a large order of cone bearing plants. From this order we obtain the different varieties of pine, hemlock and spruce from which the various preparations of turpentine have been obtained. These are trees or shrubs, mostly evergreen, usually resinous. Leaves needle like or scale like. They are seen throughout the world, chiefly in cold regions.

Cathargyroside A and cathargyroside B are labdane diterpene glycosides; vernenone-10-O- β -D-glucopyranoside and vernenone-10-O- β -D-apiofuranosyl-(1"→6")- β -D-glucopyranoside are monoterpene glycosides; cedrusinin-4-O- α -L-rhamnopyranoside and (+)-cyclo-olivil-9'-O- β -D-xylopyranoside are lignan glycosides were obtained from the twigs and leaves of *Cathaya argyrophylla* of Conifer and some of these glycosides have antimicrobial and cytotoxicities. The antitumor activity of taxol, a diterpene alkaloid from several *Taxus* species, bark of *Taxus brevifolia*, the Pacific yew.

Liliaceous glycosides

The Liliaceae family are characterized as monocotyledonous, perennial, herbaceous, bulbous, or rhizomatous flowering plants with simple trichomes (root hairs) and contractile roots. The Liliaceae or lily family is composed of large number of plant with medicinal virtues. Most of these are herbs and rarely shrubs, e.g., Asphodel (*Asphodelus aestivus*), wild asparagus (*Asparagus aphyllus*), seaside squill (*Drimia maritima*), Mediterranean smilax (*Smilax aspera*), Greater butcher's broom (*Ruscus hypophyllum*), Butcher's broom (*Ruscus aculeatus*), Tassel hyacinth (*Muscaria comosum*), Madonna lily (*Lilium candidum*), Bluebell (*Hyacinthus orientalis*), Aloe (*Aloe vera*), Garlic (*Allium sativum*), Garden onion (*Allium cepa*), Mediterranean meadow saffron (*Colchium cupani*), Meadow saffron (*Colchium autumnale*)

Asphodel in folk medicine is used to reduce pigmentation of the skin and to stop wound bleeding. Wild asparagus is used as a diuretic, antispasmodic and sedative; it reduces high blood pressure and heart beat. Seaside squill yields a high quantity of glycosides that have various medicinal effects such as expectorant, diuretic and hair toning properties but red squill (*Urginea indica*) is less effective medicinally, however, used as to kill rodent pests. Mediterranean smilax yields bright red berries. It is used to reduce the blood sugar level, high blood pressure, as a diuretic and a treatment for hemorrhoids. Butcher's broom and greater butcher's broom's emerging shoots are similar to asparagus, and are edible. They are usually used in vascular disorders such as chilblains, varicose veins and haemorrhoids and these effects are attributed to steroid saponins. Additionally, these reduce the blood cholesterol levels. Tassel hyacinth is very similar to onion, in fact, the bulbs are boiled to remove the bitterness and are pickled in vinegar. Medicinally it has stimulant and diuretic effects. Madonna lily's medicinal constituents are found in the bulb and flowers. The bulb contains a high amount of mucilage ideal for skin conditions such as burns, boils and acne. The petals of the flowers, when soaked in oil yield an extract that is beneficial in eczema. Bluebell contains an essential oil that has antimicrobial activity. Aloe gel is widely used in several preparations such as skin and hair products. It has moisturizing and soothing effects especially in cases of sunburn, dermatitis, deep wounds where tissue regeneration is required. Aloe vera gel protects the skin from the ultraviolet irradiation and fights against cancer. It is used also for dry and itchy scalps. Garlic is used medicinally, in the fresh, dried or processed state. It contains an essential oil and alliin that is broken down into allicin as the tissue is disrupted on cutting or pressing. These constituents

make garlic strongly antiseptic, hypotensive and expectorant. Externally it can be applied to boils, insect bites and unbroken chilblains. Onion bulb is used for medicinal purposes. It is antiseptic, hypotensive, hypoglycemic and expectorant similar to garlic. Externally it is used for the treatment of boils and insect bites. Mediterranean meadow contains colchicine and its derivatives that have anticancer properties. Meadow saffron is traditionally used for the treatment of gout and skin cancer due to its colchicine content. It demecolcine is used in the treatment of leukemia.

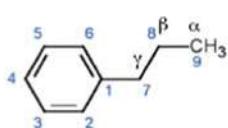
Based on correlation to the parent natural glycoside

Glycosides may be classified as Primary glycosides and Secondary glycosides on the basis of their in the source plant. For example, primary glycosides like amygdalin, purpurea glycoside A, stevioside, rebaudioside A, etc., are originally present in the plant while secondary glycosides like prunasin, digitoxin, etc., are formed from the primary glycosides by certain changes like removal of sugar as in the case of Digitoxin.

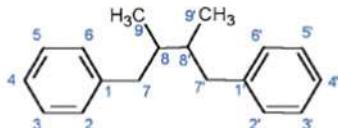
(xi) Miscellaneous glycosides

Lignans and phytoestrogens

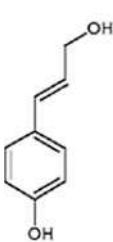
The lignan family is a large group of naturally abundant molecules and they are very common in the plant world. Plant lignans are plant-derived diphenoxy compounds (phenylpropanoids dimers) whose structure is the union of two units of phenylpropane (C_6C_3 —a propylbenzene skeleton) (1) are linked by their carbon



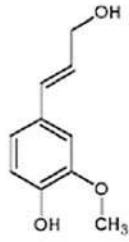
(1) Phenylpropane



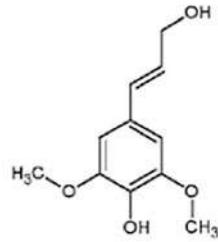
(2) Lignin



(3) p-Coumaryl alcohol



(4) Coniferyl alcohol



(5) Sinapyl alcohol

Fig. 3.50 Showing structures and carbon numbering of (1) phenylpropane and (2) lignin (β - β' or 8,8' link); structure of three common monolignols—(3) p-coumaryl alcohol (p-hydroxyphenyl alcohol); (4) coniferyl alcohol; and (5) sinapyl alcohol

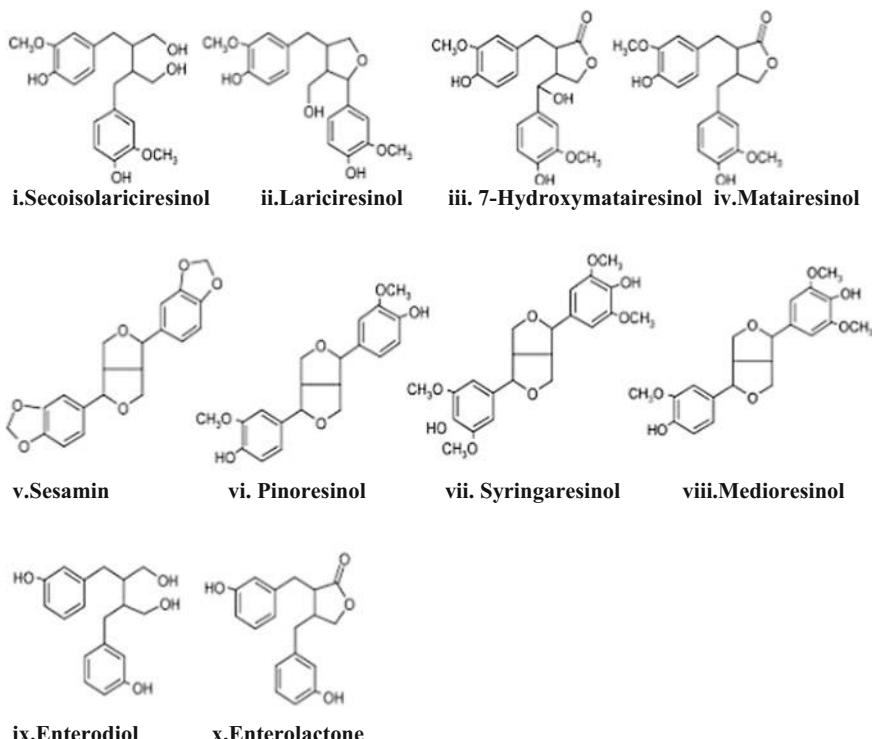


Fig. 3.51 Examples of eight lignans (i–viii) and two enterolignans (ix, x)

8,8' (β - β' link) (2) as represented in Fig. 3.50 along with three common monolignols. This definition is largely accepted although some authors prefer to describe lignans as “1,4-diarylbutane” compounds and subsequently the lignan family was extended to a series of compounds where the monomers are linked differently. A term neolignan is given when a structure is formed by joining the two propylbenzene residues at other than the β -carbon atom of the propyl side chain.

The enterolignans, enterodiol and enterolactone are formed by the action of intestinal bacteria on lignan precursors found in plants. Some examples of lignanprecursors are secoisolariciresinol, lariciresinol, 7-hydroxymatairesinol matairesino, sesamin, pinoresinol, medioresinol, podophyllotoxin, steganacin, syringaresinol, enterodiol and enterolactone (Fig. 3.51).

Lignans that can be metabolized to mammalian lignans are pinoresinol, lariciresinol, secoisolariciresinol, matairesinol, hydroxymatairesinol, syringaresinol and sesamin. Some plant lignans are metabolized by intestinal bacteria to mammalian lignans enterodiol and enterolactone. Lignans are one of the major classes of phytoestrogens, which are estrogen-like chemicals and act as antioxidants. The other classes of phytoestrogens are isoflavones and coumestans. Estrogens are signaling molecules (hormones) that exert their effects by binding to estrogen

receptors within cells. Flax seed and sesame seed contain higher levels of lignans than most other sources. The principal lignan precursor found in flaxseed is secoisolariciresinol diglucoside. Other sources of lignans include cereals (rye, wheat, oat and barley—rye being the richest source), soybeans, cruciferous vegetables such as broccoli and cabbage, and some fruits, particularly apricots and strawberries. Lariciresinol and pinoresinol contribute about 75% to the total lignan intake whereas secoisolariciresinol and matairesinol contribute only about 25%. This distribution of lignans in human diet may be changed on the availability of more data. Lignans are high in fiber content and have antiestrogenic effects as well as they have shown anti-inflammatory and antioxidant activity in basic research models of human diseases. There are several potential health benefits from flaxseed and other lignans, e.g., improve breast, prostate, colon, ovarian and uterine health; regulate hormone levels, diabetes, menopause symptoms; scavenge free radicals; support immune system, canine cushings treatment, hair growth, etc. Podophilotoxin is found in the podophylle rhizome (*Podophyllum peltatum*) and is the forerunner of two substances (etoposide and teniposide) used for antitumor therapy. Silymarin, a protector of the liver obtained from the Marian thistle (*Silybum marianum*). Diets rich in foods containing plant lignans (whole grains, nuts and seeds, legumes, fruits, and vegetables) have been consistently associated with reductions in risk of cardiovascular disease.

Phytoestrogens (dietary estrogens) are plant-derived xenoestrogens (foreign estrogens) not generated within the endocrine system but consumed by eating phytoestrogenic plants. Phytoestrogens include lignans, isoflavones, prenylflavonoids and coumestans, the last three are most active in estrogenic effects. Because of their structural similarity with estradiol (17- β -estradiol), they can cause estrogenic or/and antiestrogenic effects by fitting in and blocking receptor sites against estrogen (Fig. 3.52). Mycoestrogens have similar structures and effects.

In some countries, phytoestrogenic plants have been used for centuries in the treatment of menstrual, menopausal problems, fertility problems, etc. Plants used that have been shown to contain phytoestrogens include *Pueraria mirifica*, *P. Montana*, *Trifolium pratense* (Fabaceae), *Angelica sylvestris*, *Foeniculum vulgare*, *Pimpinella anisum* (Apiaceae), *Panax ginseng*, *P. quinquefolius* (Araliaceae), *Actaea racemosa* (Ranunculaceae) and others.

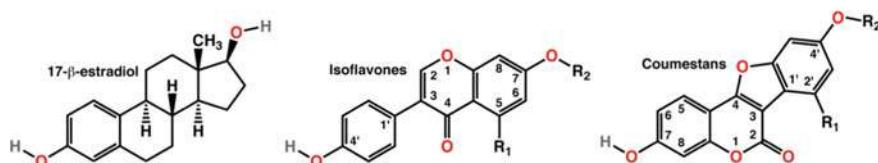


Fig. 3.52 Chemical structures of the most common phytoestrogens are isoflavones (e.g., daidzein—R₁=H, R₂=H, formonetin—R₁=H, R₂=CH₃, genistein—R₁=OH, R₂=H, biochanin A=R₁=OH, R₂=CH₃) and coumestans (e.g., coumestrol—R₁=H, R₂=H, 4-methoxycoumestrol—R₁=H, R₂=CH₃, repensol—R₁=OH, R₂=H, trifoliol—R₁=OH, R₂=CH₃) compared with estrogen (17- β -estradiol) found in animals

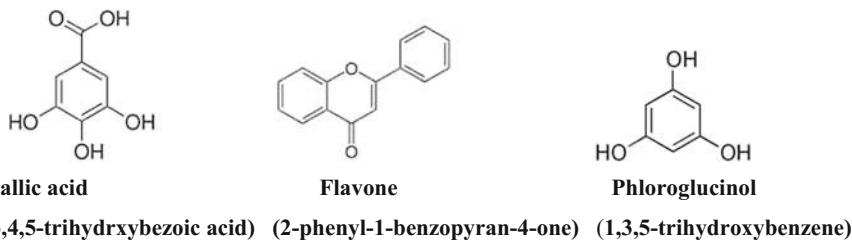


Fig. 3.53 Showing structures of monomer of hydrolyzable, non-hydrolyzable, or condensed tannins and phlorotannins

Tannins

Tannins are non-nitrogenous bitter plant polyphenolic compounds of vegetable origin having a molecular weight between 500 and 3000 (gallic acid esters) and up to 20,000 (proanthocyanidins). They are non-crystallisable colloidal compounds. There may be (a) hydrolyzable tannins, consist of gallic acid or related polyhydric compounds esterified with glucose and they are readily hydrolysed to yield the phenolic acids and the sugar; and (b) non-hydrolyzable or condensed tannins, contain only phenolic nuclei and most of such tannins are formed by the condensation of two or more flavanols, such as catechin. When condensed tannins are treated with hydrolytic agents they yield insoluble, red-colored products, known as phlobaphenes. Most of the time, they occur in glycosidic combinations with sugars. They bind and precipitate various organic compounds including proteins, amino acids, gelatin, alkaloids, heavy metals, etc., and form dark blue or greenish black compounds with ferric chloride. Tannins produce a deep red color with potassium ferricyanide and ammonia and are precipitated by salts of copper, lead and tin.

Base unit or monomer of the tannin includes gallic acid, flavone and phloroglucinol for hydrolyzable tannins, non-hydrolyzable or condensed tannins, and phlorotannins, respectively (Fig. 3.53). Hydrolyzable and non-hydrolyzable or condensed tannins may be derived from higher plants while phlorotannins are found in brown algae.

Most polyphenols contain repeating phenolic moieties of pyrocatechol, resorcinol, pyrogallol and phloroglucinol connected by esters (hydrolyzable tannins) or

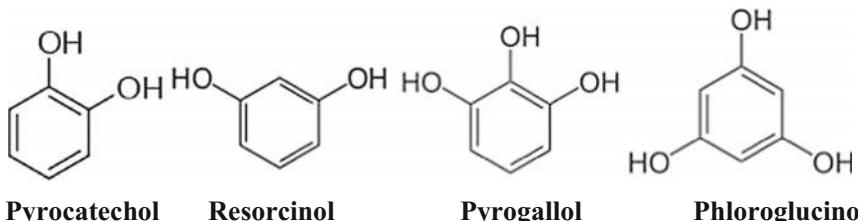


Fig. 3.54 Showing structures of phenolic moieties of pyrocatechol, resorcinol, pyrogallol, and phloroglucinol

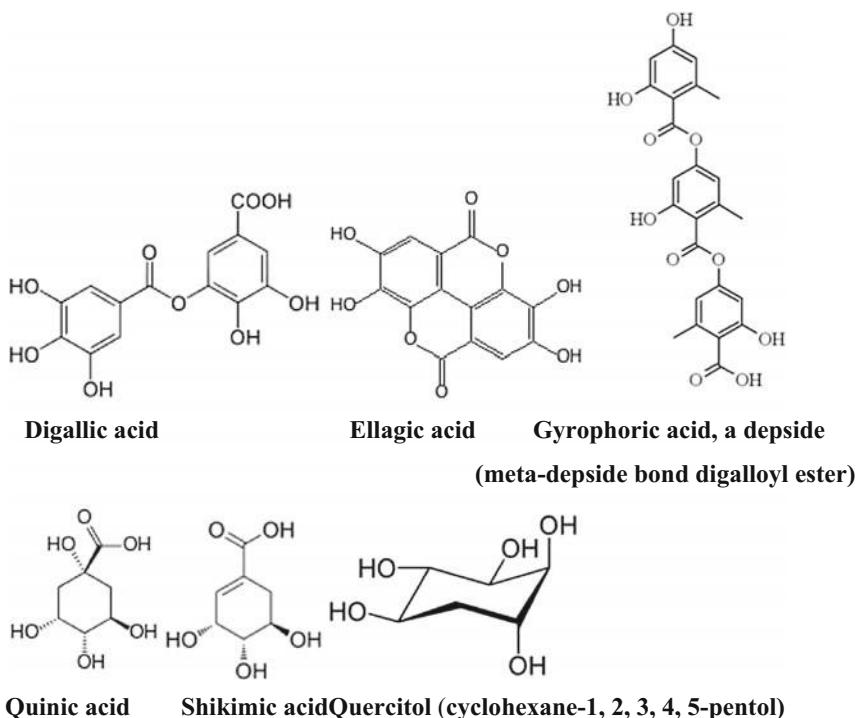


Fig. 3.55 Showing structures of gallotannin moieties

more stable C–C bonds (non-hydrolyzable condensed tannins) (Fig. 3.54). Proanthocyanidins are mostly polymeric units of catechin and epicatechin. Catechol- and resorcinol- (benzenediol-) types of polyphenols have two, and pyrogallol- and phloroglucinol-(benzenetriol-) types have three phenolic hydroxyl groups, respectively, though mixing of these types within polyphenols is also possible.

Gallotannins and ellagitannins are examples of hydrolyzable tannins and form when gallic or ellagic acids esterify and bind with the hydroxyl group of a polyol carbohydrate. Gallotannins are polymers formed when gallic acid, digallic acid (polyphenol monomer) esterifies and binds with the hydroxyl group of a polyol carbohydrate such as glucose, quinic acids, shikimic acids, and others—alloside, proto-quercitol, etc. (Figs. 3.55 and 3.56).

Ellagic acid is a natural phenol antiproliferative and antioxidant found in species like *Quercus alba* and *Quercus robur* and also macrophyte *Myriophyllum spicatum* and medicinal mushroom *Phellinus linteus*. Quinic acid is a cyclitol (cyclic polyol, cyclohexanecarboxylic acid), a crystalline acid obtained from cinchona bark, coffee beans, and other plant products. It is a constituent of the tara tannins. Quinic acid is used as an astringent and a medication for the treatment of influenza A and B strains. Shikimic acid (cyclitol, cyclohexanecarboxylic acid) is an

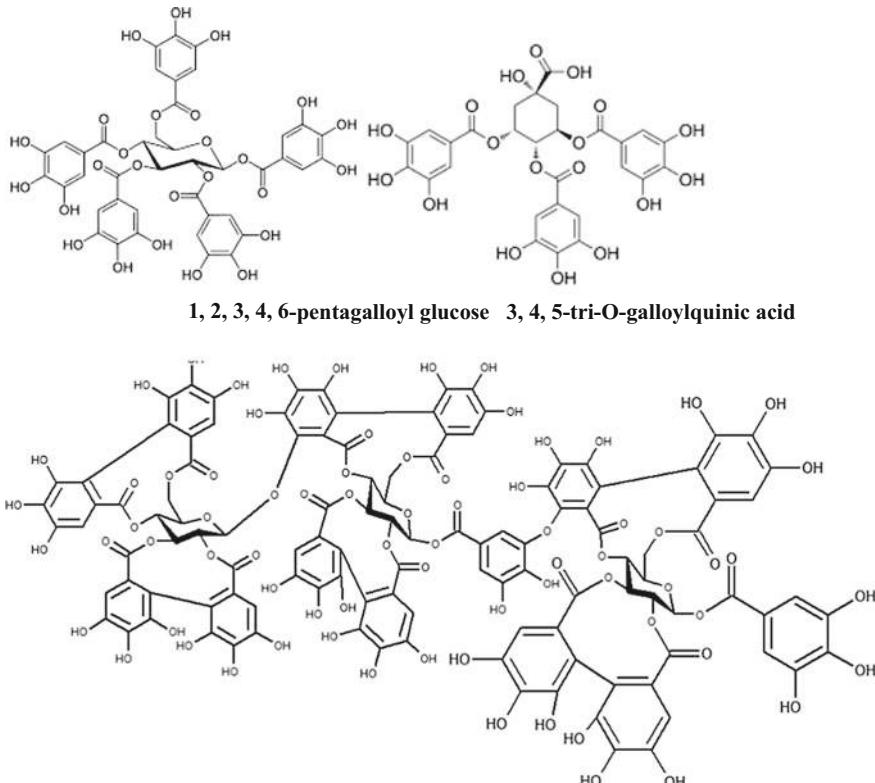


Fig. 3.56 Raspberry ellagitannin composed of 14 gallic acid units around a core of three units of glucose, with two gallic acids as simple esters, and the remaining 12 appearing in 6 ellagic acid-type units. Ester, ether, and biaryl linkages are present

important biochemical metabolite in plants (e.g., Chinese star anise—*Illicium verum*) and microorganisms. It appears in the list of Group 3 carcinogens (i.e., the agent is not classifiable as to its carcinogenicity to humans) of the International Agency for Research on Cancer. Shikimic acid is also the glycoside part of some hydrolyzable tannin. Quercitol (5-Deoxyinositol) is a cyclitol, found in *Quercus* sp., in *Gymnema sylvestre* and also in wines aged in oak wood barrels. A depside is a type of polyphenolic compound composed of two or more monocyclic aromatic units linked by an ester bond. Depsides are frequently found in lichens and in higher plant species of the family Ericaceae, Lamiaceae, Papaveraceae and Myrtaceae. Depsides have antibiotic, anti-HIV, antioxidant, and antiproliferative activity. As inhibitors of prostaglandin synthesis and leukotriene B4 biosynthesis, depsides are potent nonsteroidal anti-inflammatories. Gyrophoric acid of the lichen *Cryptothecia rubrocincta* is a depside.

Gallotannins are simple polygalloyl esters of glucose. The prototypical gallotannin is 1,2,3,4,6-pentagalloyl glucose (PGG), the pentahydroxy gallic acid ester of

glucose (β -1,2,3,4,6-pentagalloyl-O-D-glucopyranose). PGG has 5 identical ester linkages that involve aliphatic hydroxyl groups of the core sugar. It has many isomers with mol. wt. 940 g/mol. The polygalloyl ester chains found in gallotannins are formed by either *meta*—or *para*-depside bonds, involving a phenolic hydroxyl rather than an aliphatic hydroxyl group. The α -anomer of PGG is not common in nature. PGG is a common precursor of gallotannins and the related ellagitannins. Simple gallotannins with up to 12 esterified galloyl groups and a core glucose are found in pomegranate (*Punica granatum*), staghorn sumac (*Elaeocarpus sylvestris* or *Rhus typhina*) and also in tree peony (*Paeonia suffruticosa*). 3,4,5-tri-O-galloylquinic acid is hydrolyzable tannin found in *Lepidobotrys staudii*, *Guiera senegalensis* and in the resurrection plant *Myrothamnus flabellifolius*. It is classified as a natural product with anti-HIV activity and a DNA polymerase inhibitor. The raspberry ellagitannin is an ellagitannin found in raspberries. It is a polyphenol per se, containing 6 ellagic acid-type components and two additional monomeric phenolics, for a total of 14 gallic acid units. Red raspberry ellagitannins slow the growth of breast, pancreas, esophageal, skin, and prostate cancer cells and kill them (apoptosis). The ellagitannins also produce a breakdown in human leukemia cells. Ellagitannins act as scavengers to bind carcinogenous chemicals, making them inactive. Red Raspberry ellagitannins also protect DNA by blocking carcinogens from binding to the DNA, lower the incidence of birth defects, promote wound healing, reduce heart disease, and may reduce or reverse chemically induced liver fibrosis, show antibacterial and antiviral properties.

Gallotannins are hydrolyzable tannin; yield various water-soluble products, such as gallic acid and protocatechuic acid and sugars and other polyols. The ellagitannins are a diverse class of hydrolyzable tannins, a type of polyphenol formed primarily from the oxidative linkage of galloyl groups in 1,2,3,4,6-pentagalloyl glucose (Fig. 3.57). Ellagitannins differ from gallotannins, in that their galloyl

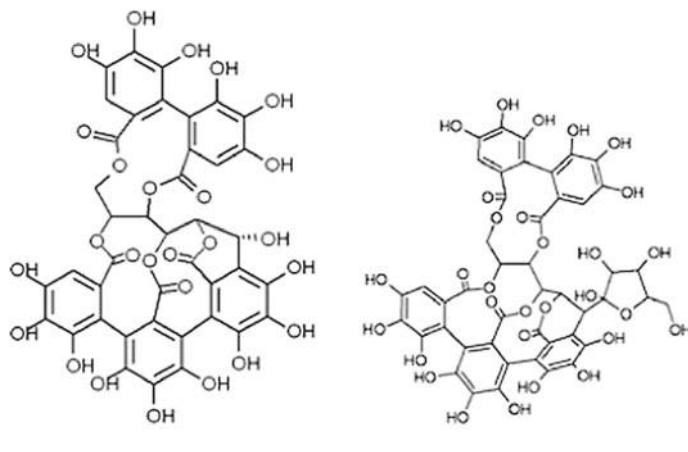
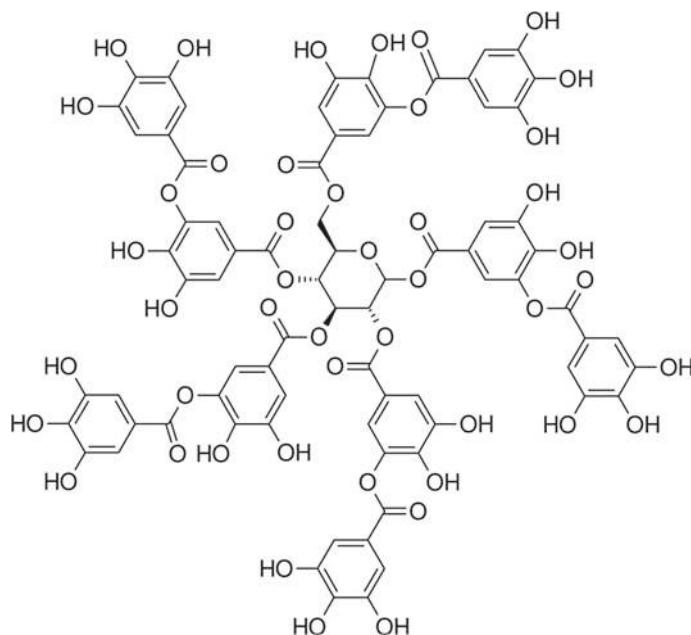


Fig. 3.57 Showing structures of ellagitannins (castalagin) and grandinin

groups are linked through C–C bonds, whereas the galloyl groups in gallotannins are linked by depside bonds. Condensed tannins, e.g., proanthocyanidins, polyflavonoid tannins, catechol-type tannins, pyrocatecollic-type tannins, or flavolans are polymers formed by the condensation of flavans. They do not contain sugar residues. Examples of some other condensed tannin are procyanidins, pro-pelargonidins, prodelphinidins, profisetinidins, proguibourtinidins or prorobinetidins, formed from flavonoids structures corresponding to the related anthocyanins. Procyanidins, condensed tannins found in grape, are polymers of 2 to >50 flavan-3-ol units joined by carbon–carbon bonds. These are not susceptible to being cleaved by hydrolysis. Castalagin (vescalagin) is an ellagitannin, a type of hydrolyzable tannin, found in oak and chestnut wood and in the stem barks of *Anogeissus leiocarpus* and *Terminalia avicennioides*.

Examples of ellagitannins are castalagin, castalin, casuarictin, grandinin, punicalagin, punicalin, roburin A, tellimagrandin II, terflavin B, vescalagin, etc.

Grandinin is an ellagitannin and a castalagin glycoside by binding of the pentose lyxose. It contains a nonahydroxytriphenolic acid moiety, can be found in *Melaleuca quinquenervia* leaves, in white (*Quercus alba*) and red (*Quercus robur*) oaks. It is an astringent compound and shows antioxidant activity. It suppresses the phosphorylation of the epidermal growth factor receptor in human colon carcinoma cells.



Polygalloyl glucoses

Fig. 3.58 Showing structures of polygalloyl glucoses—a tannic acid

Tannic acid is a type of polyphenol, a specific commercial form of tannin (Fig. 3.58). It is weak acidic (pK_a around 10) is due to the numerous phenol groups. The chemical formula, $C_{76}H_{52}O_{46}$, corresponds with decagalloyl glucose, but it is a mixture of polygalloyl glucoses or polygalloyl quinic acid esters with the number of galloyl moieties per molecule ranging from 2 to 12 depending on the plant source. Commercial tannic acid is usually extracted from Tara pods (*Caesalpinia spinosa*), gallnuts from (*Rhus semialata* or *Quercus infectoria*—source of Turkish and Chinese gallotannins) or Sicilian sumac leaves (*Rhus coriaria*) or Chinese sumac (*Rhus semialata*-source of Chinese and Korean gallotannins). Gallnut is a plant excretion produced when irritants are released by the larvae of gall insects/gall wasps of the Cynipidae family. A major commercial source of medicinal gallnuts is oak trees and Chinese sumac. The plant secretes the liquid gall that hardens to become the nut. Gallnuts are a native product of China, Turkey, India, Japan, and Korea. The annual yield of gallnuts in China is about 95% of the total world yield. Gallnuts from oak and sumac contain 50–75% tannin (gallotannin) and 2–4% each of gallic acid and ellagic acid. *Quercus infectoria* and *Rhus semialata* are sources of Turkish and Chinese gallotannins, respectively.

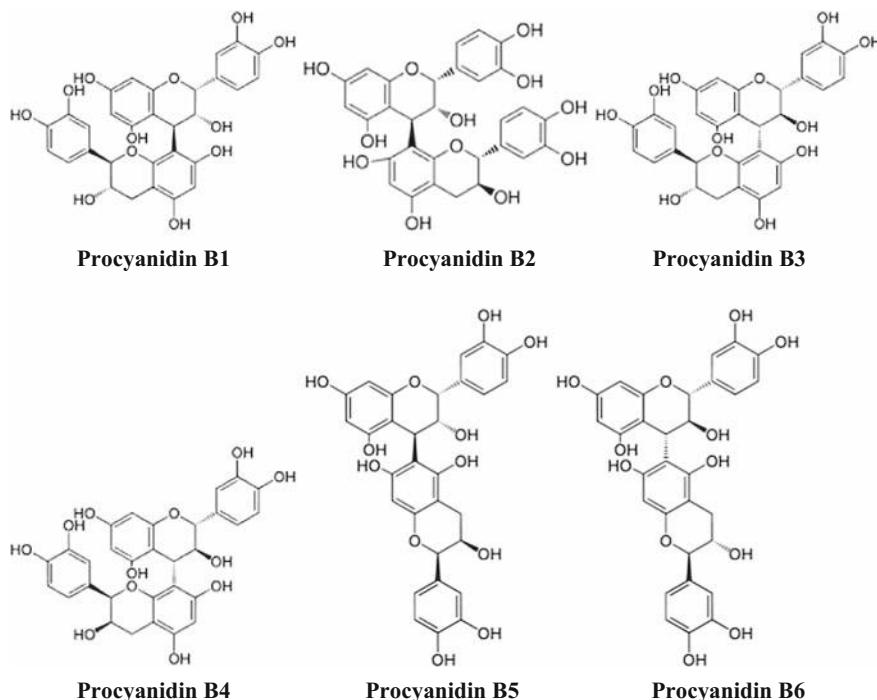
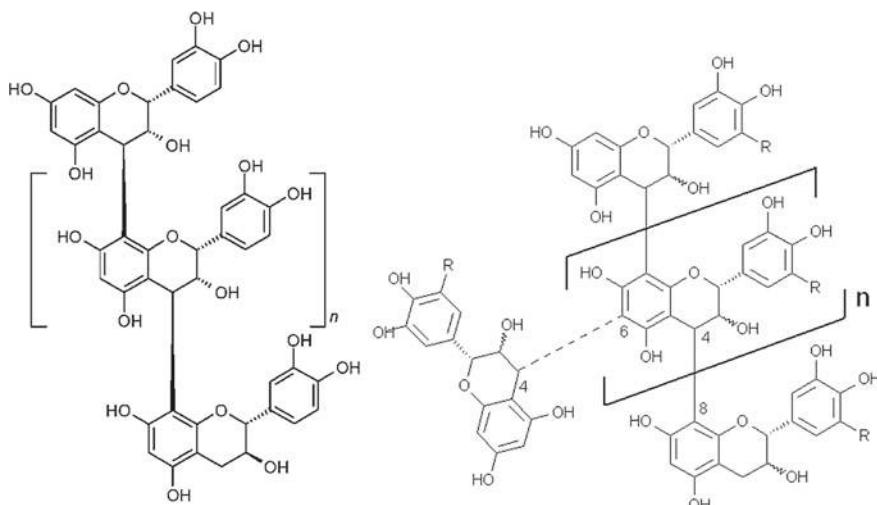


Fig. 3.59 Showing structures of the basic monomer of condensed tannins

Proanthocyanidins (condensed tannins) refer to a larger class of polyphenols, called flavanols, in which occur PCOs (proanthocyanidin oligomers) or OPCs (oligomeric proanthocyanidins), the simplest flavanols. The basic monomer of condensed tannins (proanthocyanidins) is epicatechin and catechin; the successive addition of similar phenol units then extends these to produce polymers (polyphenols) (Fig. 3.59). Traditionally, important commercial sources of condensed tannins are the heartwood of *Schinopsis* spp. (quebracho tannins), the bark and/or heartwood of *Acacia catechu* (catechu tannins) and *Acacia mollissima* (mimosa tannins), and the bark of *Rhizophora* (mangrove) and *Eucalyptus* species. The application of proanthocyanidins has health protective effect as antioxidants.

Procyanidin B1 is a procyanidin dimer. It is a molecule with a 4→8 bond (epicatechin-(4 β →8)-catechin). Proanthocyanidin-B1 can be found in *Cinnamomum verum* (Ceylon cinnamon, in the rind, bark or cortex), in *Uncaria guianensis* (cat's claw, in the root), and in *Vitis vinifera* (in the leaf) or in peach. Procyanidin B2 is a B type proanthocyanidin. Its structure is (-)-Epicatechin-(4 β →8)-(-)-epicatechin.

Procyanidin B2 can be found in *Cinchona pubescens* (in the rind, bark and cortex), in *Cinnamomum verum* (Ceylon cinnamon, in the rind, bark and cortex), in *Crataegus monogyna* (Common hawthorn, in the flower and blossom), in *Uncaria guianensis* (Cat's claw, in the root), in *Vitis vinifera* (Common grape vine, in the leaf), in *Litchi chinensis* (litchi, in the pericarp), in the apple, and in *Ecdysanthera utilis*. Procyanidin B3 is a B type proanthocyanidin. Procyanidin B3 is a catechin dimer (catechin-(4 α →8)-catechin). It can be found in red wine, in barley, in beer, in



Polyflavonoid condensed tannin molecules- linear with 4→8 carbon-carbon bonds and branched with 4→8 & 4→8 carbon-carbon bonds

Fig. 3.60 Showing structures of (schematic) of polyflavonoid condensed tannin molecules

peach or in *Jatropha macrantha*. It has been identified as a hair-growth stimulant. Procyanidin-B4 is a catechin-(4 α →8)-epicatechin dimer. It is found in the litchi pericarp, in grape seeds, and, along with 4-cis-isomer of procyanidin B4, in beer. Procyanidin B5 is a B type proanthocyanidin. Procyanidin B5 is an epicatechin-(4 β →6)-epicatechin dimers. It can be found in grape seeds and in *Hibiscus cannabinus* (kenaf) root and bark. Procyanidin B6 is a B type proanthocyanidin. Procyanidin B6 is a catechin-(4 α →6)-catechin dimer. It can be found in grape seeds and in beer. Figure 3.60 shows polyflavonoid condensed tannin molecules—linear with 4→8 carbon–carbon bonds and branched with 4→8 and 4→8 carbon–carbon bonds.

Condensed tannins are ubiquitous plant phenolics, and presented exceptional concentrations in the barks and heartwoods of a variety of tree species. They are oligomers or polymers of flavonoid units (flavan-3-ol) linked by carbon–carbon bonds not susceptible to hydrolysis. Condensed tannins can be linear (with 4→8 bounds) or branched (with 4→6 bounds—dotted line). Condensed tannins (proanthocyanidins, polyflavonoid tannins, catechol-type tannins, pyrocatecollic-type tannins, non-hydrolyzable tannins, or flavolans) are polymers formed by the condensation of flavans. They do not contain sugar residues. They are called proanthocyanidins as they yield anthocyanidins when depolymerized under oxidative conditions. Different types of condensed tannins exist, such as the procyanidins, propelargonidins, prodelphinidins, profisetinidins, proguibourtinidins or prorobinetidins, formed from flavonoids structures corresponding to the related anthocyanins. One condensed tannin, found in grape, are procyanidins, which are polymers of 2–50 (or more) flavan-3-ol units joined by carbon–carbon bonds. These are not susceptible to being cleaved by hydrolysis.

While many hydrolyzable tannins and most condensed tannins are water soluble, several tannins are also highly octanol soluble. Some large condensed tannins are insoluble. Differences in solubilities are likely to affect their biological functions. Tannins of tropical woods tend to be of a catechin nature rather than of the gallic type present in temperate woods. Condensed tannins can be recovered from *Lithocarpus glaber* or can be found in *Prunus* sp. The bark of *Commiphora angolensis* contains condensed tannins. Commercial sources of condensed tannins are plants such as quebracho wood (*Schinopsis lorentzii*), mimosa bark (*Acacia mollissima*), grapes seeds (*Vitis vinifera*), pine barks and spruce barks. Pycnogenol is a trademark for a French maritime pine bark extract. Condensed tannins are formed in tannosomes, specialized organelles, in Tracheophytes, i.e., vascular plants.

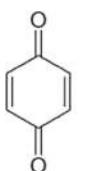
Both types of tannins, the hydrolyzable tannins have long been considered official medicinal agents in Europe and North America and they have been included in many pharmacopoeias as tannic acid. They were recommended for treatment of inflammation and ulceration, including topical application for skin diseases and internal use for intestinal ulceration and diarrhea. Now, the condensed tannins also have important medicinal roles, such as stable and potent antioxidants. In China, tannin-containing substances, such as galls, pomegranate rinds, and *terminalia* fruits, are used in several medicinal preparations.

Tannins are widely distributed in the plant kingdom and are found in leaf, bud, seed, root, bark and stem tissues, in grapes, persimmon, blueberry, tea, chocolate, legume forages, legume trees (*Acacia* spp., *Sesbania* spp.), grasses (sorghum, corn, etc.). They are found in plant families, especially in Aceraceae, Actinidiaceae, Anacardiaceae, Bixaceae, Burseraceae, Combretaceae, Dipterocarpaceae, Ericaceae, Fabaceae, Grossulariaceae, Myricaceae, Rhizophoraceae, Rosaceae, and Salicaceae for dicot, Najadaceae and Typhaceae in Monocot families and *Pinus* of Gymnosperm. About 73, 39, 6 and 4% of the species of Fagaceae, Mimosaceae, Solanaceae and Asteraceae, respectively, contain tannin. Some families like the Boraginaceae, Cucurbitaceae, and Papaveraceae contain no tannin-rich species. Tannins are also found in some brown algae.

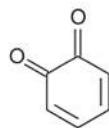
They are mostly used in the tanning industry for the conversion of animal hides to leather (as they form hydrogen bridges with the fibers of collagen in the skin), in the production of ink and as a laboratory reagent for the detection of proteins, alkaloids, and gelatin. Pharmaceutically, tannins have antibacterial, antiviral, antiparasitic, astringent and antiseptic properties, and may be used in the treatment of hemorrhages (constrict of blood vessels), burns (cicatrizing), diarrhea, and as an antidote for alkaloid poisoning because of their ability to precipitate alkaloids. Tannins of the stem bark of *Myracrodruon urundeuva* are effective against 6-hydroxydopamine-induced toxicity and also have anti-inflammatory and antiulcer activity (Souza et al. 2006; Nobre et al. 2007). Examples of natural drugs containing tannins include Hamamelis leaf and Nutgall.

Quinones

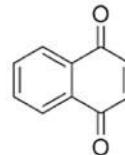
Quinones are a class of cyclic organic compounds containing two carbonyl groups (>C=O by conversion of an even number of -CH=groups) in a six-membered unsaturated ring. These are aromatic diketones which come from phenols through oxidation. In a few quinones, the carbonyl groups are located in different rings. The class representative is quinone (1,4-benzoquinone or cyclohexadienedione) and other important examples include 1,2-benzoquinone, 1,4-naphthoquinone and 9,10-anthraquinone (Fig. 3.61).



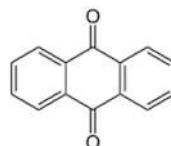
1, 4-Benzoquinone
(*p*-quinone)



1, 2-Benzoquinone
(*o*-benzoquinone)



1,4-Naphthoquinone
(*p*-naphthoquinone)



9,10-Anthraquinone
(Dioxoanthracene)

Fig. 3.61 Showing structures of class representative quinone (1,4-benzoquinone or cyclohexadienedione) and other important examples like 1,2-benzoquinone, 1,4-naphthoquinone, and 9,10-anthraquinone

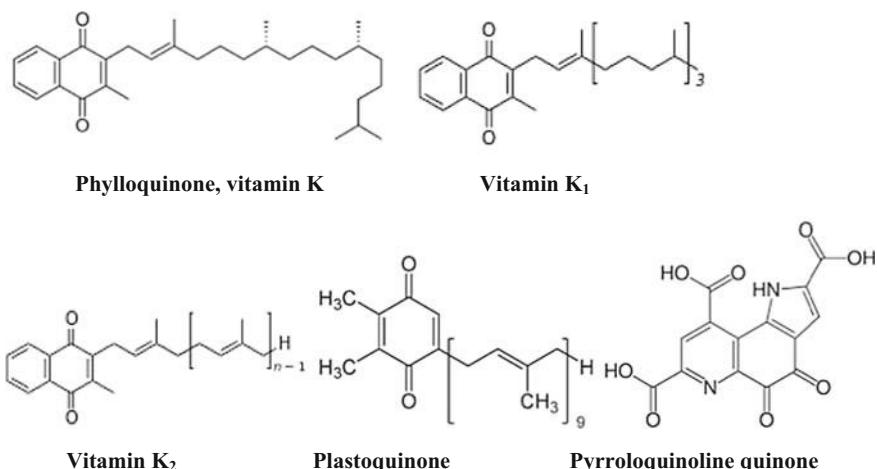


Fig. 3.62 Showing structures of vitamin K, vitamers (vitamin) K₁ and K₂; plastoquinone (PQ) and pyrroloquinoline quinone (PQQ)

Quinones occur as biological pigments, i.e., biochromes and include benzoquinones, naphthoquinones, anthraquinones and polycyclic quinones. The quinones are found in bacteria, in certain fungi, in various higher plant forms and in a few animals (e.g., sea urchins, aphids, lac insects, and certain scale insects). Animals obtain their quinone compounds from the plants they eat.

Parabenzoquinone is a pale yellow solid with a penetrating odor resembling that of chlorine. It is widely used in medicine, herbicides, chemical reagents, dyes, and tanning agents. P-benzoquinone is used in pharmaceutical industry for production of cortisone, in cosmetic industries, leather industries and also used in photographic chemicals. It is used as a chemical intermediate, a polymerization inhibitor and oxidizing agent. It is highly active anti-microbacterial, antifungal agent and highly toxic and fatal if swallowed, inhaled, or absorbed through the skin.

Phylloquinone, vitamin K, contain a functional naphthoquinone ring and an aliphatic phytyl (isoprenoid) side chain. Vitamin K family includes two natural vitamers: vitamin K₁ and K₂ (menaquinone), which consists of a number of related chemical subtypes differing in length of the side chain, made of isoprenoid residues. The most common number of these residues is four, since animal enzymes normally produce menaquinone-4 from plant phylloquinone. Figure 3.62 shows the structures of vitamin K, vitamers (vitamin) K₁ and K₂, plastoquinone (PQ) and pyrroloquinoline quinone (PQQ).

Plastoquinone (PQ), quinone of either plastid or chloroplast alluding to its location, is a 2,3-dimethyl-1,4-benzoquinone molecule with a side chain of nine isoprenyl units. PQ is involved in the electron transport chain in the light-dependent reactions of photosynthesis. Plastoquinone is reduced to plastoquinol on acceptance of two H⁺ from the stromal matrix of the chloroplast, coupled to two e⁻ from

photosystem II. It functions as an electron acceptor during photosynthesis, forming part of the electron transport chain of Photosystem I. It transports the protons to the lumen of thylakoid discs, while the electrons continue through the electron transport chain into the cytochrome b_6 protein complex. Pyrroloquinoline quinone (PQQ) is known as the third redox cofactor after nicotinamide and flavin in bacteria, protects mitochondria from oxidative stress, promotes the spontaneous generation of new mitochondria within aging cells, a neuroprotective—protects brain cells against oxidative damage, reduce heart damage following myocardial infarction.

It is a fat-soluble vitamin that is stable to air and moisture but decomposes in sunlight. It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system. Its best-known function in animals is as a cofactor in the formation of coagulation factors II (prothrombin), VII, IX, and X by the liver. It is also required for the formation of anticoagulant factors protein C and S. It is commonly used to treat warfarin toxicity, and as an antidote for coumatetralyl. Vitamin K is required for bone protein formation.

K vitamins found in alfalfa (*Medicago sativa* of Fabaceae) are antibacterial and antifungal agents; juglone from the walnut tree (*Juglans regia* of Juglandaceae) is antioxidant, antiproliferative, bone and cardio protective, lawsona, naphthoquinone, from henna (*Lawsonia inermis* of Lythraceae) has diverse activities: from body art (dye skin, hair, and fingernails), industry (dye fabrics including silk, wool, leather), toiletries (shampoo) to anticancer; plumbago, from the drosera (*Drosera rotundifolia* of Droseraceae), which is anti-expectorant; anthraciciflaxnes are nucleus of important antibiotics such as daunomicin and doxorubicin, as well as tetracyclines used in cancer chemotherapy derived from Streptomyces bacterium *Streptomyces peuceti* var. *caesius*; anthraquinones and fenanthraquinones act as laxatives and purgatives, when in heteroside form. Quinones show a wide range of pharmacological activities, e.g., they may be used as purgative (sennosides—anthraquinone derivatives named after their abundant occurrence in plants of the genus *Senna* of Caesalpiniaceae, Aloe-emodin—an anthraquinone from leaf exudates of *Aloe vera*), antibacterial (rhein—anthraquinone group found in rhubarb, *Rheum rhabarbarum* of Polygonaceae and saprorhoquinone—naphthoquinone from *Salvia montbretii* and *S. prionitis* of Lamiaceae), antitumor (emodin—trihydroxyanthroquinone from a Himalayan rhubarb—*Rheum emodi*, Japanese knotweed—*Fallopia japonica* of Polygonaceae and a number of other genus like *Senna*, *Cassia*, *Acalypha*, etc., and juglone—naphthoquinone from *Juglans regia* of Juglandaceae), inhibition of Prostaglandin E2, PGE2, biosynthesis (arnebinone and arnebifuranone—two quinonic compounds, monoterpenylbenzoquinones, from the roots of *Arnebia euchroma* of Boraginaceae) and anti-inflammatory, antioxidative, cytotoxic and anti-cardiovascular disease (tanshinone—phenanthrene—quinone derived from roots of *Salvia miltiorrhiza* of Lamiaceae) (Liu 2011).

3.8 Resins, Saponins, Cardioactive Drugs and Other Steroids

Resins Saponins, cardioactive drugs and other steroids

Saponins are amphiphilic glucoside molecules composed of hydrophilic glycoside glyccone and lipophilic triterpene or steroid aglycone. They have a high molecular weight and a high polarity; possess foaming characteristics in aqueous solutions and a high degree of structural diversity. Saponins are generally nonvolatile, surface-active compounds that are widely distributed in nature. A polycyclic aglycone becomes attached to one or more sugar side chains in saponin. The aglycone part (sapogenin) is either steroid (C_{27}) or a triterpene (C_{30}), i.e., saponins are glycosides of triterpenoids or steroids. Saponins on hydrolysis give on hydrolysis yield a triterpenoid or steroid sapogenin and one or more sugars (glucose, galactose, rhamnose or xylose, etc.). The aglycones or sapogenins are characterized by the presence of a spiroketal side chain. Because of amphipathic nature, they are largely used as emulsifying and detergents. Saponins have been used in medicine, foaming agents, in fire extinguishers and fish poisons. Dietary monosaccharides such as D-glucose and D-galactose are among the most common components of the attached chains. The steroidal saponins are called saraponins. Aglycone derivatives can also incorporate nitrogen, e.g., solanine, a monodesmosidic, branched-saccharide steroidal saponin. Their isolation in a state of purity presents some difficulties as they often occur as complex mixtures with the components differing only slightly from one another in the nature of the sugars present, or in the structure of the aglycone. Various chromatographic techniques have been employed for their isolation.

Distribution

Saponins are found in almost all groups of plants, but they have also been isolated from marine organisms such as marine invertebrate sea cucumber and star fish. Saponins derive their name from the soapwort plant genus *Saponaria* because the root of *Saponaria officinalis* of Caryophyllaceae was used historically as soap, the root of which was used historically as a soap. Beans and other legumes (e.g., kidney beans, navy beans and haricot beans, soybeans, and chickpeas) as well as garlic, asparagus, etc., are among the richest sources of saponins. Saponins are also found in different members of soapberry, soapnut, washnut (*Sapindus mukorossi*, *Sapindus trifoliatus*), licorice (*Glycyrrhiza glabra*), maples (*Acer*), genus *Sapindus*, *Glycyrrhiza*, *Acer*, etc., of Sapindaceae, soapbark tree (*Quillaja saponaria*) of Quillajaceae, Spanish dagger plant (*Yucca schidigera*) of Asparagaceae, licorice (*Glycyrrhiza glabra*) of Fabaceae and horse chestnuts (*Aesculus hippocastanum*) of Hippocastanaceae. Saponin is also found in *Gynostemma pentaphyllum* of Cucurbitaceae in a form called gypenosides, and ginseng or red ginseng (*Panax*) of Araliaceae in a form called ginsenosides. Saponin is found in various parts of the plant including leaves, stems, roots, bulbs, blossom and fruit. Commercial saponins are mainly extracted from the bark and root of desert plants *Quillaja saponaria* of

Chile and *Yucca schidigera* of Baja California, respectively. They are the two major commercial sources of saponins. *Chenopodium quinoa* of Amaranthaceae has a long history of use of edible seed (seed coat contains bitter saponin) in South America and is not harmful to humans. Toxic saponins are known as sapotoxins.

Application

Saponins are used in various ways such as liquid soap, jewelry polish, detergent, exzema, dermatitis cure, pesticide, insecticide, pet shampoo, human shampoo, household cleaner, laundry detergent, surfactant, wetting agent, nutrient uptake, spreader, sticker, antimicrobial agents, adjuvant (make other solutions work better), treat malaria, lower blood cholesterol, hypertension aid, kill nematodes, bone health, cancer fighter, support immune system (build it up), parasite remover (tick, flea), automobile cleaner. Because of their surfactant properties, saponins are also used industrially, in mining and ore separation, emulsions for photographic films and cosmetic products like lipstick and shampoo where their antifungal and antibacterial properties are important in addition to their emollient effects. Saponins when mixed with water reduce the surface tension of water, allowing the formation of small stable bubbles. Because of their surface-active properties, saponins are excellent foaming agents (very stable). Today, saponins are used in the manufacture of fire extinguisher foam, toothpaste, shampoos, detergents, liquid soaps, lipsticks, herbal skin balms, and cosmetics and to increase the foaming qualities of beer, beverages, and soft drinks. The soapy characteristics of saponins make them ideal for use as spray adjuvants and make sprays stick or spread better on leaf surfaces. They also allow nutrients to be absorbed better. Another important thing they do is to distribute water more evenly on hard-to-wet substrates. For these reasons saponins are often used in fertilizers, potting soils, and pesticides. Yucca root has high levels of saponin and Native Americans used it for years to make soap and shampoo. They used to wash their hair with Yucca to fight dandruff and hair loss and Yucca has been used to treat headaches, bleeding, gonorrhea, arthritis and rheumatism and many other ailments. Saponins come in powdered or liquid form and can be found in fertilizers (amendments) and soilless potting mixes and certain pesticides (insecticides), and many other things.

Biological activities and health benefits

Their biological and pharmacological activities range from antimicrobial, antifungal, anticancer, to immunomodulatory, etc. The most prominent feature of saponins is linked to their effects on cell membranes; they strongly affect cell membrane structure and integrity by different mechanisms depending on their chemical structure. The ability of saponins to increase membrane permeability can be used to facilitate the passage of drug molecules or other natural products through the cell membrane. The ability of saponins to affect cell membrane structure and integrity makes them interesting natural products in pharmacological and medical research and therapy, in particular, as agents for enhancing drug efficacy.

(i) Saponins have hemolytic, expectorant, anti-inflammatory and immune-stimulating activity; (ii) Saponins control blood cholesterol levels, bone

health, cancer, and building up the immune system; (ii) Saponins demonstrate antimicrobial properties particularly against fungi and additionally against bacteria and protozoa. (iv) Saponins form complex with cholesterol to develop pores in cell membrane bilayers, e.g., in erythrocyte membranes complexation leads to red cell lysis (hemolysis); (v) The amphipathic nature of saponin gives them activity as surfactants that can be used to enhance penetration of macromolecules such as proteins through cell membranes; (vi) Saponins improve function and pharmaceutical manufacturers often include saponins in vaccines as adjuvants to increase their effectiveness; (vii) Saponins may reduce the risk for high cholesterol, cancer and blood sugar, and saponins from the *Gypsophila paniculata* have been shown to very significantly augment the cytotoxicity of immunotoxins and other targeted toxins directed against human cancer cells (e.g., leukemia, lymphoma and other cancers). (viii) Saponin digitalis of the Foxglove plant is used in heart medicines.

There is tremendous, commercially driven promotion of saponins as dietary supplements and nutriceuticals. Saponins from oat and spinach may enhance nutrient absorption and aid in animal digestion. Saponins appear in beverages and cosmetics as emulsifiers or sweeteners. Yucca and quillaja saponins have both current and potential applications in animal and human nutrition. Yucca extracts are extensively used for ammonia and odor control in pig and poultry-raising facilities and in dog and cat foods. Yucca saponins and perhaps other components of yucca as well, have ammonia-binding activity. When added to the diet, yucca saponins pass through the digestive tract unabsorbed and are excreted in the feces. In the excreta, the yucca components bind to ammonia and certain other odiferous compounds and prevent them from being released into the air. In recent studies in England, feeding of yucca extract to dogs and cats was shown to reduce fecal odor and reduce emission of volatile compounds contributing to fecal odor. Many pet foods and kitty litter products now contain yucca extract to reduce these noxious odors. Saponins are often bitter in taste and so can reduce plant palatability and thus may serve as antifeedants (threatening animal toxicity) and protect the plant against microbes and fungi. Many saponins are used as fish poison. Oral saponins appear to be extremely safe but when injected intravenously saponin glycosides can cause hemolysis of red blood cells. The hemolytic effect seems to be due to increasing cell membrane permeability.

Classification of saponins

Saponins are a structurally diverse class of glycoside compounds occurring in many plant species. Traditionally, they are subdivided into triterpenoid and steroid glycosides, or into triterpenoid, spirostanol, and furostanol saponins (Vincken et al. 2007). Triterpenoid saponins (pentacyclic) are triterpenes to which various sugar molecules become attached. Triterpenes are synthesized from isoprene unit through mevalonate pathway to make a C₃₀ compound. Some triterpenes are steroidal in nature, e.g., cholesterol, phytosterols, phytoecdysteroids, etc. Steroidal saponins (commonly tetracyclic triterpenoids) are naturally occurring sugar conjugates of C₂₇ steroid compounds (tetracyclic molecules) that are synthesized from acetyl

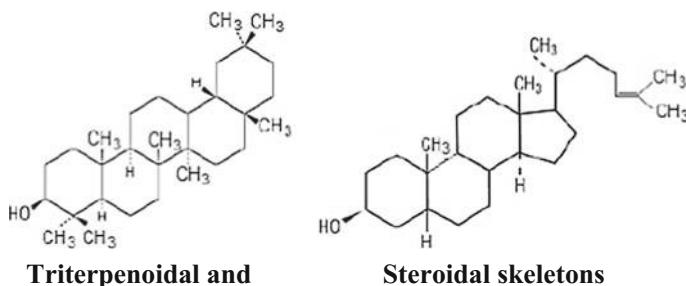


Fig. 3.63 Showing structures of triterpenoidal and steroid skeletons of saponin glycosides

coenzyme A (CoA). The aglycone of a steroid saponin is usually a spirostanol or a furostanol. The glycone parts of these compounds are mostly oligosaccharides, arranged either in a linear or branched fashion, attached to hydroxyl groups through an acetal linkage. Both have a glycosidic linkage at C₃ and have a common biogenetic origin. A distinct subgroup of the steroid saponins is that of the steroid alkaloids which characterize many members of the Solanaceae.

Triterpenoidal (pentacyclic) and Steroidal (tetracyclic) saponins are structurally distinct group of molecules but they have most properties in common (Fig. 3.63). Triterpenoids tend to be acidic in pH and occur more commonly in dicots while steroid saponins tend to be neutral in pH and occur more commonly in monocots. Triterpenoid saponins are rare in monocotyledons but they are abundant in many dicotyledonous families (e.g., Caryophyllaceae, Sapindaceae, Polygalaceae and Sapotaceae). Other dicotyledonous families in which triterpenoid saponins have been found are the Phytolaccaceae, Chenopodiaceae, Ranunculaceae, Berberidaceae, Papaveraceae, Linaceae, Zygophyllaceae, Rutaceae, Myrtaceae, Cucurbitaceae, Araliaceae, Umbelliferae, Primulaceae, Oleaceae, Lobeliaceae, Campanulaceae, Rubiaceae and Compositae. Altogether some 80 families are involved.

Commercial saponins extracted from *Yucca schidigera* are steroid saponins while *Quillaja saponaria* extract contains a triterpenoid saponin mixture. Some herbs rich in (i) Triterpenoid saponins are *Actaea racemosa* (black cohosh), *Azadirachta indica*(neem), *Centella asiatica* (gotu kola), *Canoderma lucidum* (reishi), *Glycyrrhiza glabra* (licorice), *Glycyrrhiza uralensis* (gan cao), *Panaxginseng* (Asian ginseng), *Panax quinquefolium* (American ginseng), *Zizyphus jujuba* (jujube), and (ii) Steroid saponin (phytosterols) are *Aesculus hippocastanum* (horse chestnut), *Asparagusracemosa* (shatavari), *Commiphora mukul* (guggul), *Dioscorea villosa* (wild yam), *Hedera helix* (ivy), *Ononis spinosa* (spiny restarrow), *Ruscus aculeatus* (butcher's broom), *Smilax officinalis* (sarsparilla), *Withania somnifera* (ashwagandha), *Yucca*. Some of the (i) tetracyclic triterpenoid saponins (steroidal) are diosgenin, dioscin digitonin, gitonin (Dioscorea bark, seed, etc.), solasodine (Solanum berries), sarsapogenin (Asparagus roots) and

Table 3.6 Showing plant sources of triterpenoidal and steroidal saponins

Name of the compounds	Plant source	
	Common name	Botanical name
α -hederin	Black cumin	<i>Nigella sativa</i>
Araloside A	Spikenard	<i>Aralia mandshurica</i>
Astragaloside	Huang qi	<i>Astragalus membranaceus</i>
Bacoside A	Brahmi	<i>Bacopa monniera</i>
Cucurbitacin	Bryonia	<i>Bryonia alba</i>
Eleutheroside	Siberian ginseng	<i>Eleutherococcus senticosus</i>
Ginsenoside, panaxoside	Ginseng	<i>Panax ginseng</i>
Gymnemic acid	Gurmar	<i>Gymnema sylvestre</i>
Gypenoside	Jiaogulan	<i>Gynostemma pentaphyllum</i>
20-hydroxyecdysone	Maral root	<i>Rhaponticum carthamoides</i>
Tangshenoside I	Bellflower	<i>Codonopsis pilosula</i>
Tinosporoside	Guruchi	<i>Tinospora cordifolia</i>
Withanolide	Ashwagandha	<i>Withania somnifera</i>
Aescin	Horse chest nut	<i>Aesculus hippocastanum</i>
Glycyrrhizin	Liquorice root	<i>Glycyrrhiza glabra</i>
Senegins	Senega	<i>Polygala senega</i>
Sarsaponin (Parillin)	Sarsparilla	<i>Smilax spp.</i>
Digitonin	Seed	<i>Digitalis purpurea, D. lanata</i>
Gitonin	Seed and leaves	<i>D. purpurea, D. lanata</i>
Dioscin	Wild yam	<i>Dioscorea spp.</i>

(ii) pentacyclic triterpenoid saponins are gingenoside (Ginseng), glycyrrhizin (Licorice), senegin-II (Senega), quillaia (Quillaja) and sarsapogenin (Sarsaparilla) (Table 3.6).

Pharmaceutically important triterpenoid saponins possess antimicrobial, haemolytic, hypolipidemic, immunomodulating and cytotoxic activities. Steroidal saponins are of great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisone, diuretic steroids, vitamin D and the cardiac glycosides. Some are used as starting materials for the synthesis of these compounds. Diosgenin is the principal sapogenin used by industry. Steroidal saponins (phytosterols) decrease cholesterol absorption from the gut, increase cholesterol excretion, and inhibit hepatic synthesis of cholesterol. All saponins tend to be immune modulating and have antineoplastic effects. Saponins of licorice root, (glycyrrhizin and aglycone glycyrrhetic acid) are anti-inflammatory and antiviral, inhibit cortisol catabolism, and have many other effects. Saponins can cause gastrointestinal distress through an unknown mechanism. Taking them with food tends to eliminate the problem. Saponin-rich herbs (e.g., *Hedera helix*) are consumed orally to cause an increase in the production of mucus in the lungs, as well as

coughing and the effect can be helpful for patients with coughs of all sorts, especially dry cough.

Cardioactive drugs and other steroids

Drugs that influence heart or drugs having an influence on the heart are cardioactive drugs.

- (i) Beta-adrenoceptor antagonists, (ii) Calcium channel blocking drugs and (iii) Cardiac glycosides are three major classes of cardioactive drugs.

Cardiac glycosides, a subgroup of cardioactive steroids (CAS) that also contain sugar residues, include a class of organic compounds, mostly secondary metabolites of plant origin, that increase the output force of the heart and decrease its rate of contractions by acting on the cellular sodium–potassium ATPase pump (Patel 2016). Cardioactive glycosides, like digoxin, digitalis, ouabain and related compounds, are drugs that inhibit Na⁺/K⁺-ATPase and have a strong inotropic effect on heart: they cause the Na⁺/Ca²⁺ exchanger to extrude Na⁺ in exchange with Ca²⁺ and therefore increase the [Ca²⁺] concentration and some of these drugs are currently used in the treatment of congestive heart failure and cardiac arrhythmias (Riganti et al. 2011). In addition to inotropic activity, cardiac glycosides are also important in the pathogenesis and therapy of different human diseases (e.g., stroke, diabetes, neurological diseases, cancer, etc.). Cardioactive steroids are a class of animal and plant-derived compounds with a steroid nucleus and a specific inotropic, chronotropic, and dromotropic effect.

Each molecule of this family consists of three distinct structural motifs such as (i) a steroid nucleus, (ii) a sugar moiety, and (iii) a lactone moiety (Fig. 3.64).

The sugar moiety defines the affinity for specific Na⁺/K⁺-ATPase isoforms and the lactone moiety defines the functional class of each compound.

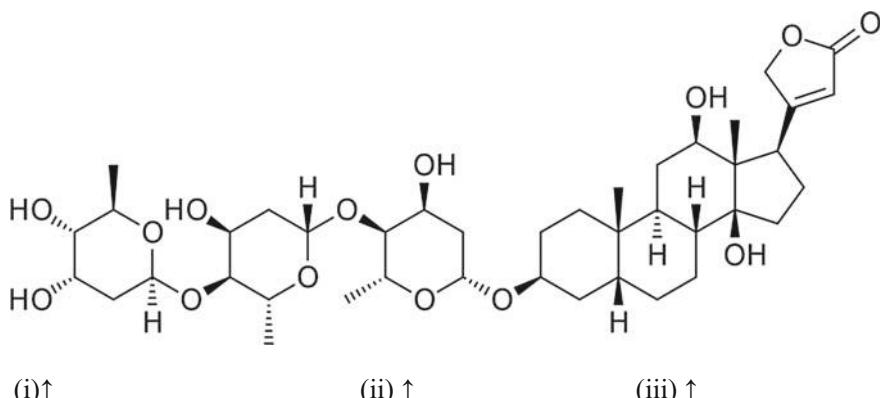


Fig. 3.64 (i) A sugar moiety (glycone), (ii) a steroid nucleus (aglycone genin), and (iii) a lactone moiety

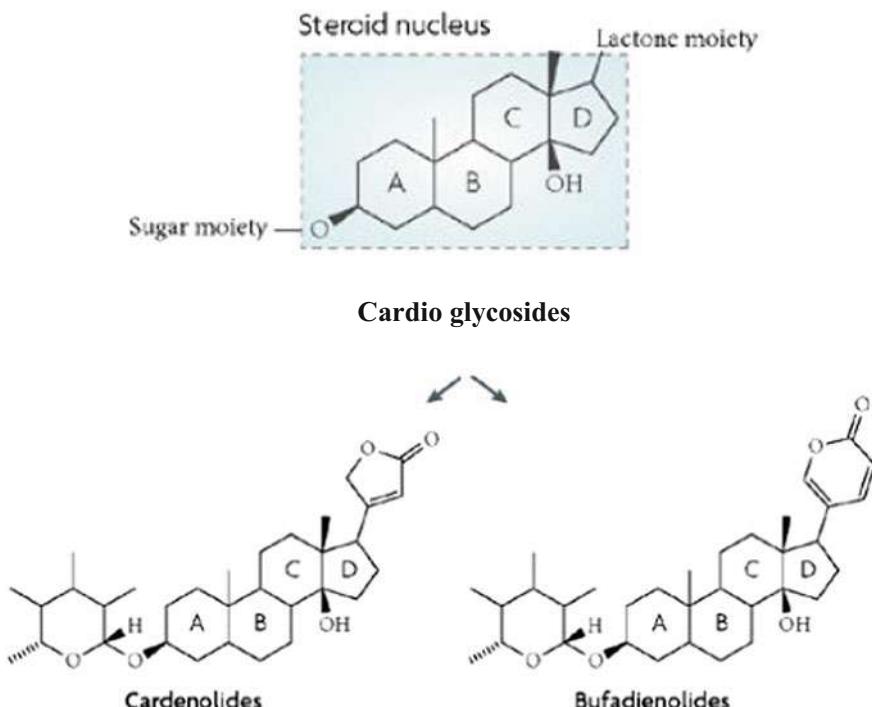


Fig. 3.65 Two groups of cardiac glycosides: a five-membered unsaturated butyrolactone ring—cardenolides and a six-membered unsaturated pyrone ring—bufadienolides

Cardenolides contain a five-membered unsaturated butyrolactone ring and bufadienolides contain a six-membered unsaturated pyrone ring (Fig. 3.65).

Cardioactive steroids (CAS) became the mainstay of treatment for congestive heart failure and to control the ventricular response rate in atrial tachydysrhythmias. The most commonly prescribed CAS in the United States is digoxin while digitoxin, ouabain, lanatoside C, deslanoside, and gitalin are other internationally available but much less commonly used preparations. The most common plant source of cardioactive steroid is *Digitalis lanata* (digoxin), which may be followed by *Digitalis purpura* (digitoxin), *Strophanthus kombe* (ouabain) and *Strophanthus gratus* (strophanthin). Other documented plant sources of CAS include oleander (*Nerium oleander*—oleandrin), yellow oleander (*Thevetia peruviana*—oleandrin), foxglove lily of the valley (*Convallaria majalis*—convallotoxin), dogbane (*Apocynum cannabinum*), milkweed (*Asclepias* sp.—oleandrin), kalanchoe plant (*Kalanchoe daigremontiana* and other *Kalanchoe* spp.—daigremontianin), moth-erwort (*Leonurus cardiac*—scillarenin) and red squill (*Urginea maritima*—proscillarin A). Some of the most remarkable poisonings occur with exposures to

plants such as *Digitalis purpurea* and *Nerium oleander* containing CAS, dried toad secretions (bufadienolides) from *Bufo* spp.

3.9 Antibiotics from Higher Plants

Antibiotics are the chemical substances produced by microorganisms and they have the capacity, in low concentration, to inhibit microorganisms through an anti-metabolic mechanism. Antibiotics differ from antiseptics and disinfectants; they vary in their mode of action, chemical and physical properties; they are affected differently by the composition of the substrate in which they act, vary in their toxicity to animals, and, also in their chemotherapeutic potentialities. Antibiotics are produced alone or in mixture by different groups of microorganisms, e.g., bacteria, fungi and actinomycetes; and in many cases by higher plants. The development of resistance among the microorganisms on prolonged contact with the drug is the present-day problems in the field of antibiotics.

About 25–50% of pharmaceuticals are derived directly or indirectly from higher plants but none of them are used as antimicrobials. The traditional healers have been using higher plants or their extracts to prevent or cure infectious conditions. In *in vitro* analysis, plants extracts containing secondary metabolites like phenolics including phenolic acids (caffeic acid, catechol, chrysins), quinines (hypericin), flavones and flavonoids (catechin, chrysins), coumarins (warfarin, 7-hydroxycoumarin) and tannins (pentagallayl glucose, procyanidin B-2); terpenoids (menthol, artemisinin, capsaicin) and essential oil; alkaloids (berberine, harmane), lectins and polypeptides; polyacetylenes, etc., have been found to show antimicrobial and antiviral properties (Batista et al. 1994; Estevez-Braun et al. 1994; Fujioka and Kashiwada 1994; Pengsuparp et al. 1995; Haslam 1996; Ivanovska et al. 1996; Meyer et al. 1997; Omulokoli et al. 1997; Zhang and Lewis 1997; Amaral et al. 1998; Cowan 1999; Alamgir et al. 2013). Structures of common antimicrobial plant chemicals are shown in the Fig. 3.66.

In vivo studies also support the results of many of these *in vitro* experiments (Cowan 1999). Mainstream medicine is increasingly accepting the use of antimicrobial drugs derived from higher plants because traditional antibiotics (antibiotics derived from microorganisms or synthesized derivatives) are become ineffective with time, and in addition, viral diseases remain intractable to this microbial drug. Cowan (1999), in a review article, mentioned more than 100 higher plants including *Achillea millefolium*, *Acorus calamus*, *Aegle marmelos*, *Agrostemma githago*, *Allium cepa*, *Allium sativum*, *Aloe barbadensis*, *Aloe vera*, *Aloysia triphylla*, *Anacardium pulsatilla*, *Anemone pulsatilla*, *Anethum graveolens*, *Arctium lappa*, *Armoracia rusticana*, *Arnica montana*, *Artemisia dracunculus*, *Barosma setulina*, *Berberis vulgaris*, *Calendula officinalis*, *Camellia sinensis*, *Cannabis sativa*, *Capsicum annuum*, *Carica papaya*, *Carum carvi*, *Cassia angustifolia*, *Centella*

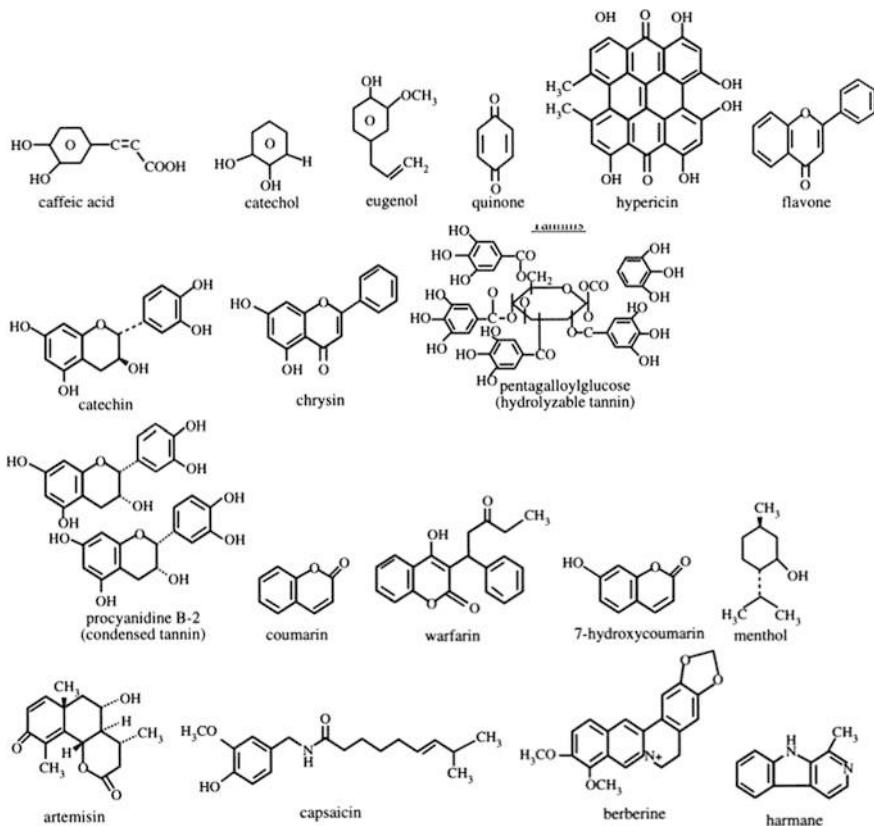


Fig. 3.66 Structure of some secondary metabolites from higher plants with antibiotic properties

asiatica, *Cinchona* sp., *Cinnamomum verum*, *Citrus paradisa*, *Citrus sinensis*, *Coriandrum sativum*, *Curcuma longa*, *Echinaceae angustifolia*, *Erythroxylum coca*, *Eucalyptus globulus*, *Euphorbia pulcherrima*, *Euphorbia tirucalli*, *Galium odoratum*, *Garcinia hanburyi*, *Gaultheria procumbens*, *Gloriosa superba*, *Glycyrrhiza glabra*, *Humulus lupulus*, *Hydrangea arborescens*, *Hydrastis canadensis*, *Hypericum perforatum*, *Hyssopus officinalis*, *Jatropha gossypiifolia*, *Lantana camara*, *Larrea tridentata*, *Laurus nobilis*, *Lawsonia inermis*, *Lophophora williamsii*, *Mahonia aquifolium*, *Malus sylvestris*, *Matricaria chamomilla*, *Medicago sativa*, *Melissa officinalis*, *Mentha piperita*, *Millettia thonningii*, *Momordica charantia*, *Myristica fragrans*, *Ocimum basilicum*, *Olea europaea*, *Onobrychis viciifolia*, *Panax notoginseng*, *Papaver somniferum*, *Peganum harmala*, *Petalostemum*, *Pimenta dioica*, *Piper betel*, *Piper nigrum*, *Podocarpus nagi*, *Polygonum aviculare*, *Prosopis juliflora*, *Quercus rubra*, *Rabdosia trichocarpa*, *Ranunculus bulbosus*, *Rauvolfia serpentina*, *Rhamnus purshiana*, *Ricinus communis*, *Rivea corymbosa*, *Rosmarinus officinalis*, *Rumex crispus*, *Salix alba*,

Santolina chamaecyparissus, Sassafras albidum, Satureja montana, Schinus terebinthifolius, Solanum tuberosum, Syzygium aromaticum, Tanacetum vulgare, Taraxacum officinale, Thevetia peruviana, Thymus vulgaris, Tussilago farfara, Vaccinium spp., Valeriana officinalis, Vicia faba Vinca minor, Withania somniferum, etc., for their toxic, antibiotic, and antiviral properties.

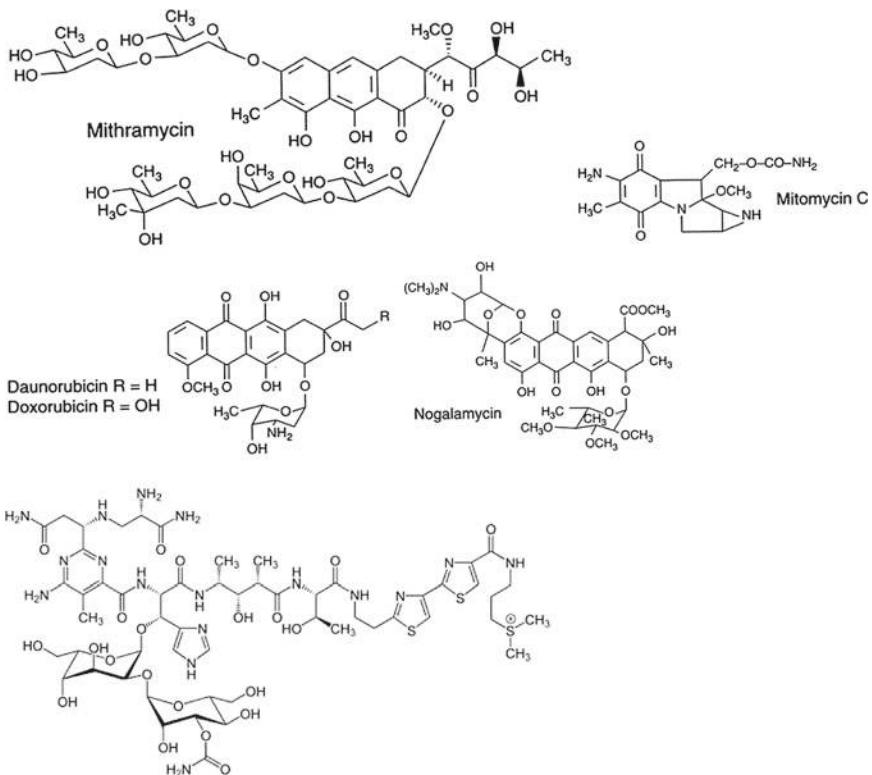
3.10 Tumor Inhibitors, Antiprotozoal, Antihepatotoxic, Antihyperglycemic, Antihypertensive, etc., Herbal Products

Tumor inhibitors

For over 40 years, natural products have served us well in combating cancer. The main sources of these successful compounds are microbes and plants. The microbial and plant sources from the terrestrial and marine environments are now providing natural products with antitumor activity. The microbial products were first discovered as antibiotics and higher plant derivatives were the secondary metabolites like alkaloids, taxoids and podophyllotoxins.

Microorganisms produce antitumor compounds or their derivatives, e.g., actinomycetes produces of a large number of natural products with antitumor and antimicrobial activities. One of the earliest applications of a microbial product was actinomycin D for Wilm's tumor in children that had resulted in a 90% survival rate (Chung 2009). Some of the most useful antitumor compounds of microbial origin include aromatic polyketides (daunorubicin, doxorubicin, epirubicin, pirarubicin, idarubicin, valrubicin, amrubicin as well as enediynes calicheamycin, macrolide lactones epothilones, ixebepilone); glycopeptides (bleomycin, phleomycin), non-ribosomal peptides (actinomycin D); anthracenones (mithramycin, streptozotocin, pentostatin); quinones (mitosanes mitomycin C); indolocarbazoles (glycosides rebeccamycin); nucleosides (2"-deoxycoformycin); halogenated compounds (salinosporamide A), etc. (Rawls 1998; Salas and Mendez 1998; Xue et al. 1999; Neumann et al. 2008) (Fig. 3.67).

Compounds with antitumor activity belong to several structural classes such as anthracyclines, enediynes, indolocarbazoles, isoprenoids, polyketide macrolides, non-ribosomal peptides including glycopeptides, and others. Most of the polyketides are produced by bacteria and fungi (Rawls 1998; Xue et al. 1999). Halogenated antitumor candidates include salinosporamide A and rebeccamycin (Neumann et al. 2008). The antitumor compounds act by several mechanisms such as inducing apoptosis through DNA cleavage mediated by topoisomerase I or II inhibition, mitochondrial permeabilization, inhibition of key enzymes involved in signal transduction (e.g., proteases), or cellular metabolism, and by inhibiting tumor-induced angiogenesis.



Bleomycin (nonribosomal peptide)

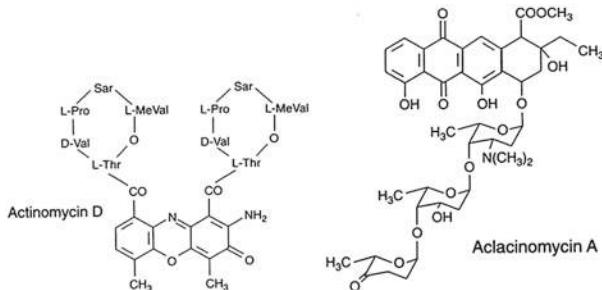


Fig. 3.67 Structures of some antitumor agents with clinical application

Plants have been a useful source of approved anticancer agents and vinblastine (velban), vincristine (oncovin), etoposide, teniposide, taxol (paclitaxel), navelbine (vinorelbine), taxotere (docetaxel), camptothecin (camptosar, campto), topotecan (hycamtin), irinotecan, etc., are some of the known land plant-derived antitumor

compounds (Dholwani et al. 2008). Antitumor compounds monoterpene indole alkaloids vinblastine and vincristine are derived from *Catharanthus roseus*, and now vinblastine is commonly used to treat Hodgkin's lymphoma. Serpentine produced by *C. roseus* have shown promise as anticancer agents and a wide range of halogenated alkaloids with antitumor activity are known (Duflos et al. 2000). Taxol is derived from *Taxus chinensis*, podophyllotoxin, an antimitotic metabolite of the roots of *Podophyllum peltatum*, etc., are antitumor agents. Cell culture products of the plant *Lithospermum erythrorhizon*, naphthoquinone pigment shikonin and two derivatives, were found to inhibit tumor growth in mice bearing Lewis Lung Carcinoma (Lee et al. 2008). Isoflavone genistin, indole-3-carbinol (I3C), 3,3'-diindolemethane, curcumin (−)-epigallocatechin-3-gallate, resveratrol, lycopene, etc., are other plant-derived products that inhibit the growth of cancer cells (Sarker et al. 2009).

Allixin from *Allium sativum*; 14 compounds including flavonoids and labdane diterpenoids from *Andrographis paniculata*; acetogenins from *Annona muricata*; polyacetylenes, flavonoids, terpenoids, phenylpropanoids, etc., from *Bidens pilosa*; a triterpenoid saponin Tubemimoside-V Chinese herb *Bolbostemma paniculatum*; components of marijuana from *Cannabis sativa*; mezerein compound from *Daphne mezereum*; seed oil of *Gossypium hirsutum*; Petroleum ether and ethyl acetate extracts of *Nervilia fordii*; tanshinone-I and IIA from *Salvia miltiorrhiza*; chebulinic acid, tannic acid, ellagic acid from *Terminalia chebula*; 6-gingerol from *Zingiber officinale*; a protein from honeybee *Apis mellifera*, etc., are considered to have a potential to inhibit the growth of cancer cells or chemotherapeutic potential (Dholwani et al. 2008; Bhutani and Gohil 2010; Poonam and Chandana 2015).

A list of anticancer plants with their active principles is available (Bhutani and Gohil 2010). It includes *Agapanthus africanus* (isoliquiritigenin-chalcone); *Aglaila sylvestre* (silvesterol); *Ailanthes altissima* (ailanthone, ailantenol—quassinoids); *Apium graveolens* (apigenin—flavonoid); *Bleckeria vitensis* (ellipticine—alkaloid); *Brucea antidyserterica* (bruceantin—quassinoid); *Bursera microphylla* (burseran—lignan); *Camptotheca acuminata* (campothecin—alkaloid); *Catharanthus roseus* (vincristine, vinblastine—alkaloid); *Centaurea montana* (montamine—alkaloid); *Centaurea schischkinii* (schischkinnin—alkaloid); *Cephalotaxus harringtonia* (homoharringtonine—alkaloid); *Cleistanthus collinus* (cleistanthin, collinusin—lignan); *Combretum caffrum* (combrestatins—stilbenes); *Croton lechleri* (taspine—alkaloid), *Daphne mezereum* (mezerein); *Diphyllia grayi* (diphyllin—lignan); *Dysoxylum binectariferum* (rohitukine—alkaloid); *Erythroxylum pervillei* (pervilleine—alkaloid); *Euphorbia semiperfoliata* (jatrophane—terpenoid); *Fritillaria thunbergii* (zhebeinone—alkaloid); *Gunnera perpensa* [{2-methyl-6(3-methyl 2-but enyl)}-quinone benzo 1–4 quinone]; *Hypericum perforatum* (hypericin—an-thraquinone); *Hypoxis colchicifolia* (hypoxoside, rooperol—glycoside); *Indigofera tinctoria* (indirubins—indigoids); *Justicia procumbens* (justicidin A, B-lignan); *Lantana camara* (verbascoside—glucoside); *Larrea tridentata* (terameprocol—lignan); *Linum album* (podophyllotoxin—lignan); *Lonicera japonica* (luteolin—

flavonoid); *Paris polyphylla* (polyphyllin—saponin); *Pestemon deustus* (liriodendrin—lignan); *Phaleria macrocarpa* (pinoresinol, larinocinesinol—lignan); *Podophyllum emodi* (epipodophyllotoxin—alkaloid); *Polygonum cuspidatum* (resveratrol—flavonoid); *Pteris multifida* (pterokaurane—terpenoid); *Pygeum africanum* (amygdalin—glycoside); *Vitex rotundifolia* (casticin—flavonoid); *Wikstroemia viridi* (wikstromol—caumarin).

Botanicals could possess effective anticancer compounds that may be used as adjuvants to existing chemotherapy to improve efficacy and/or reduce drug-induced toxicity. Herbal medicines, such as ginseng, potentiated the effects of chemotherapeutic agents via synergistic activities, supported by cell cycle evaluations, apoptotic observations, and computer-based docking analysis (Wang et al. 2012). Some natural compounds including plants induce apoptotic pathways that are blocked in cancer cells through various mechanisms in cancer cells. Multiple surveys reported that people with cancer commonly use herbs or herbal products. Vinca alkaloids, texans, podophyllotoxin, camptothecins, colchicine, ellipticine, lepachol, flavopiridol, colchicine, ellipticine, etoposide, rohitukine, etc., have been clinically used as plant-derived anticancer agents (Mukherjee et al. 2001; Safarzadeh et al. 2014).

Marine microorganisms such as coral reefs, mangroves and sea grass, have been targeted for bioprospecting because they host a high level of biodiversity. Marine sponge produce numerous bioactive compounds with promising anticancer properties, e.g., cytarabine (cytostar) used for non-Hodgkin's lymphoma was originally derived from a sponge (Rayl 1999). Other marine products with antitumor activity are pederin, theopederins, annamides, trabectedin (yondelis), aplidine, ecteinascidin 743 (ET743), etc.

Natural compounds appear to act by interference in multiple cellular signaling pathways, activating cell death signals, and bringing on apoptosis of cancer cells without negatively affecting normal cells. The antitumor agents induce apoptosis (programmed cell death) through DNA cleavage mediated by topoisomerase I or II inhibition; they may also induce mitochondrial permeabilization, inhibition of key enzymes involved in signal transduction (e.g., proteases), or cellular metabolism, inhibition of tumor-induced angiogenesis. Tumor cells secrete growth factors and trigger angiogenesis that allow tumor cells to obtain oxygen and nutrients, vascular endothelial growth factor (VEGF) is involved in angiogenesis and it could be a target for antiangiogenic drugs (Cao and Langer 2008). Fumagillin, product of *Aspergillus fumigatus*, its oxidation product ovalacin and the fumagillin analog TNP470 were antiangiogenesis compound.

Antiprotozoal medicinal plants

Protozoa are microscopic, eukaryotic one-celled animals and antiprotozoal principles are a class of pharmaceuticals used in treatment of protozoan infection. Antiprotozoal traditional medicinal plants, among others, include *Artemisia roxburghiana*, *Roylea cinerea*, *Leucas cephalotes*, *Nepeta hindostana*, *Viola canescens*, etc.; antiprotozoal principles of traditional medicinal plants include

bisbenzylisoquinoline, protoberberine, indole alkaloids, sesquiterpenes, quassinooids, limonoids, etc., and they destroy protozoa or inhibit their growth and ability to reproduce. Antiprotozoal principles effective against one pathogen may not be effective against another because protozoans are very much dissimilar and have little in common with each other. Examples of some of the protozoa of medical importance include *Plasmodium* (cause malaria); *Entamoeba histolytica* (cause amebiasis, amebic dysentery), *Trichomonas vaginalis* (cause vaginal infection); *Pneumocystis carinii* (cause pneumonia), etc.; and antiprotozoal drugs antimalarials aralen (chloroquine), daraprim (pyrimethamine), larium (mefloquine), plaquenil (hydroxychloroquine), flagyl (metronidazole), mepron (atovaquone), etc. Antiprotozoal drugs that are used to treat protozoal infections like amebiasis, giardiasis, cryptosporidiosis, microsporidiosis, malaria, babesiosis, trypanosomiasis, chaga's disease, leishmaniasis, toxoplasmosis, pneumocystis carinii pneumonia (PCP), African sleeping sickness, etc. (Khaw and Panosian 1995), but many of the treatments for these infections are limited by their toxicity (Graebin et al. 2009).

Protozoal diseases are a major threat to human and domestic animals and several antiprotozoal synthetic drugs have developed in this century, e.g., quinolines, diaminopyrimidines and triazenes (for malaria and toxoplasmosis); organometallic drugs and diamidines (for trypanosomiasis and leishmaniasis); 5 nitroimidazoles (for amoebiasis, giardiasis and trichomoniasis); and hydroxy-naphthoquinones (for theileriosis and malaria) (Croft 1997). Natural products have also made an important impact, e.g., quinine had been extensively used in malaria therapy since its discovery in 1820 from Cinchona bark, and now artemisinin of *Artemisia annua* is considered to be the most promising lead among the new antimalarial drugs. Similarly, emetine had been used in the treatment of amoebiasis since its discovery from the rhizome of *Cephaelis ipecacuanha* in 1828, but it has now been replaced by its derivative dehydroemetine.

Wild garlic, eucalyptus, thyme, etc., are some of the major plants which can annihilate the giarda cysts (Azadbakht and Azadbakht 2008). Ipecac, mango, papaya, etc., are some of the anti-amebic plants while myrtle, lavender, etc., are effective against *Trichomonas vaginalis*. Extracts of *Artemisia roxburghiana*, *Roylea cinerea*, *Leucas cephalotes*, *Nepeta hindostana* and *Viola canescens* possess good antiprotozoal activity ($IC_{50} < 5 \mu\text{g/ml}$) without any cytotoxicity against the protozoal parasites like *Plasmodium falciparum*; *Trypanosoma brucei rhodesiense*; *Leishmania donovani*; *Trypanosoma cruzi* (Dua et al. 2011). Extracts from preparations of *Salvia*, *Valeriana*, *Hypericum*, *Silybum*, *Arnica*, and *Curcuma* exhibited high activity ($IC_{50} < 2.5 \mu\text{g/mL}$) against parasites of the genera *Leishmania*, *Trypanosoma*, and *Plasmodium* (Llurba Montesino et al. 2015).

Antihepatotoxic herbal products

Liver plays major role in regulation of metabolism, storage, detoxification, and excretion of xenobiotics and liver injury may happen due to various toxic chemicals, e.g., antibiotic, chemotherapeutic agents, high doses of paracetamol, antitubercular drugs, carbon tetrachloride— CCl_4 , thioacetamide—TAA,

dimethylnitrosamine (DMN), D-galactosamine—GalN, lipopolysaccharide—LPS, excessive alcohol consumption, etc., and pathogen microbes. Chronic liver diseases like liver cirrhosis, viral hepatitis B and C, alcoholic liver disease, nonalcoholic fatty liver disease, and hepatocellular carcinoma represent a major health burden worldwide. Currently available therapies for liver ailments are not apposite and systemic toxicity inhibits their long-term use. The synthetic drugs available in the market to treat liver disorders in this condition also cause further damage to the liver and, under the circumstances; antihepatotoxic herbal products have become increasingly popular. Herbal products that prevent or cure the damage of liver and provide strength and stimulant for the functioning of liver are called antihepatotoxic (i.e., hepatoprotective) herbal products and medicinal plants have been traditionally used for treating liver diseases since antiquity as the toxicity factor appears to be on the lower side.

Hepatoprotective effects of different plants, their fruits, and plant products such as natural resin, main polysaccharides present in the cellular wall of yeasts, algae, and cereals are known; they are effective against different toxic compounds that cause hepatic damage; the principal mechanisms of action are their antioxidant potential (Madrigal-Santillán et al. 2014). Herbs containing such principles are antihepatotoxic herbs and they are claimed to restore bile flow and reduced total bilirubin and biliverdin concentration. They inhibit the increase in triglyceride, cholesterol, and total lipids in liver. Thus these plants can be used for the treatment of jaundice and hepatic failure. The hepatoprotective herbal agents generally have strong antioxidative potential and cause induction of antioxidant enzymes like superoxide dismutase, reduced glutathione and catalase; and other mechanisms of hepatoprotection may include stimulation of heme oxygenase-1 activity, inhibition of nitric oxide production, hepatocyte apoptosis and nuclear factor- κ B activation.

Plant tissues contain a wide variety of secondary metabolic compounds such as phenolic compounds (flavonoids and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), carotenoids, lignans, terpenes, etc., with antioxidant, hydrogen or electron donation, metal ion chelation, tumor inhibition properties, (Rice-Evans et al. 1996; Levy et al. 2004; Hall and Cuppett 1997). Phytoconstituents with different structure isolated from different plant species including flavonoids, alkaloids, glycosides and saponins obtained from various plant sources have been reported as potent hepatoprotective agents (Flora et al. 1996, 1998).

About 300 plants have been reported so far possessing antihepatotoxic activity. Hepatoprotective natural products in this category include *Abelmoschus moschatus*, *Acacia concina*, *Achillea millefolium*, *Aconitum heterophyllum*, *Adiantum capillus*, *Aegle marmelos*, *Alpinia galanga*, *Andrographis paniculata*, *Annona squamosa*, *Azadirachta indica*, *Cassia occidentalis*, *Cassia roxburghii*, *Chamomile capitula*, *Cinnamomum camphora*, *Coccinia grandis*, *Crocus sativus*, *Curcuma longa*, *Eclipta alba*, *Emblica officinalis*, *Ficus carica*, *Flacourtie indica*, *Foeniculum vulgare*, *Garcinia mangostana*, *Gentiana chirata*, *Gymnema sylvestre*, *Indigofera*

Table 3.7 Active compounds with source plant, hepatoprotective functions, and references

Name of the compounds	Source plant	Portection against	References
Asiaticoside	<i>Centella asiatica</i>	Lipopolysaccharide (LPS)/ d-galactosamine-induced	Zhang et al. (2010)
Cleomiscosins	<i>Cleome viscosa</i>	CCl ₄ -induced; thioacetamide-induced	Gupta and Dixit (2009)
Puerarin	<i>Pueraria lobata</i>	CCl ₄ -induced	Hwang et al. (2007)
Celosin A (1) and celosin B (2)	<i>Semen celosiae</i>	CCl ₄ -induced	Sun et al. (2010) and Xue et al. (2010)
α- and β-amyrin	<i>Protium heptaphyllum</i>	Paracetamol-induced	Oliveira et al. (2005)
Rubiadin	<i>Rubia cordifolia</i>	CCl ₄ -induced	Rao et al. (2006)
Dehydrocavidine	<i>Corydalis saxicola</i>	CCl ₄ -induced	Wang et al. (2008)
Wedelolactone	<i>Eclipta alba</i>	CCl ₄ -induced	Singh et al. (2001)
Cichotybosome	<i>Cichorium intybus</i>	CCl ₄ -induced	Ahmed et al. (2008)

tinctoria, Jatropha curcas, Lepidium sativum, Mimusops elengi, Morinda citrifolia, Mucuna pruriens, Myristica fragrans, Nigella sativa, Orthosiphon stamineus, Phyllanthus emblica, Picrorhiza kurroa, Pinus roxburghii, Piper cubeba, Plumbago zeylanica, Prostechea michuacana, Rauwolfia serpentina, Saraca asoca, Sargassum polycystum, Saussurea lappa, Solanum nigrum, Swertia chirata, Semecarpus anacardium, Silybum marianum, Solanum indicum, Strychnos nux-vomica, Swertia chirata, Symplocos racemosa, Tribulus terrestris, Trigonella foenum-graecum, Vetiveria zizanoides, Wedelia calendulacea, Woodfordia fruticosa, Zingiber officinalis, Ziziphus jujuba, etc. A summary note on active compounds, source plant, hepatoprotective functions and references is shown in Table 3.7.

Ghosh et al. (2011) noted plant sources as potential hepatoprotective agents for silymarin, andrographolide, neoandrographolide, curcumin, picroside, kutkoside, phyllanthin, hypophyllanthin, glycyrrhizin, etc. The hepatoprotective potential of several these herbal medicines has been clinically evaluated and among which significant efficacy has been found with silymarin, glycyrrhizin and Liv-52 in treatment of hepatitis, alcoholic liver disease and liver cirrhosis. Structure of some these hepatoprotective compounds are now available (Fig. 3.68). For example, silymarin component, a complex mixture of four flavonolignan: silybin; silychristin from *Silybum marianum*; glycyrrhizin, a triterpenoid saponin from *Glycyrrhiza glabra*; andrographolide, a labdane diterpene lactone and also neoandrographolide from *Andrographis paniculata*; picroside 1, an iridoid glycosides as well as kutkoside 1,10-vanillylcatalpol from *Picrorhiza kurroa*; curcumin, a phenolic

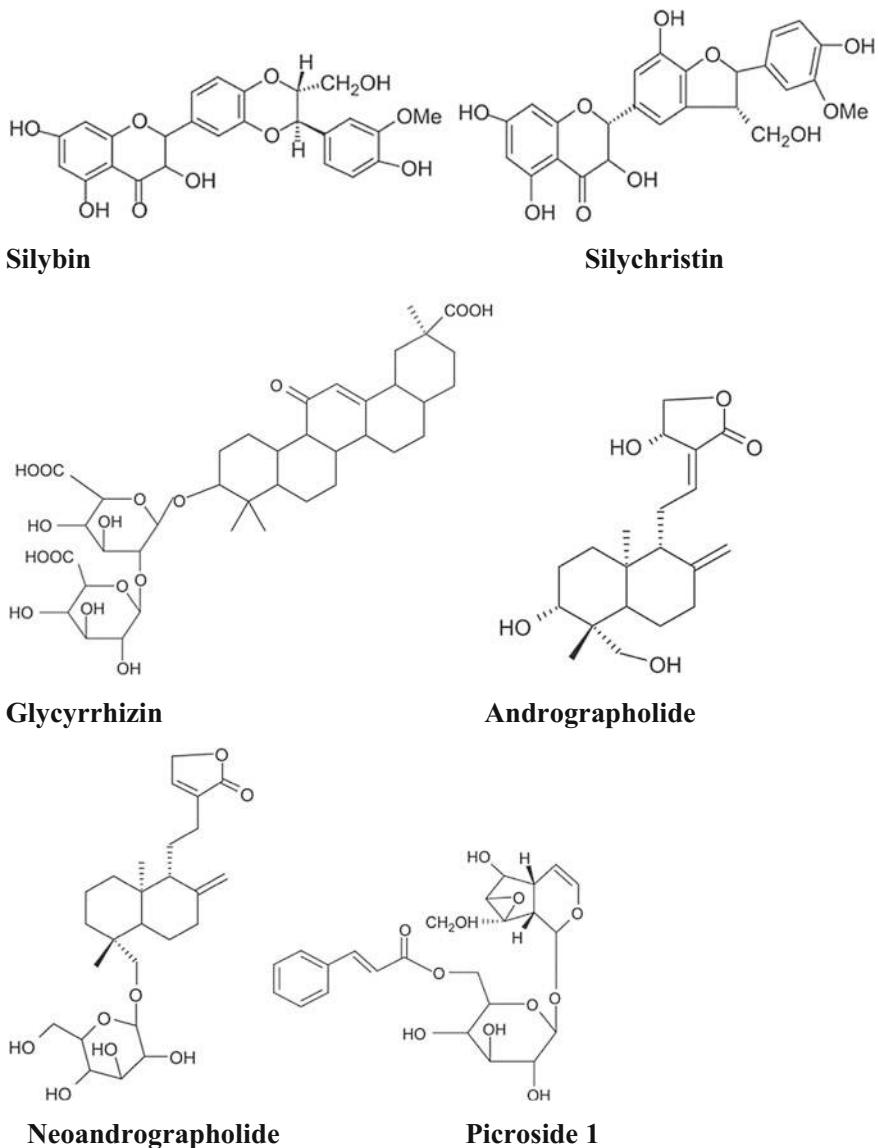
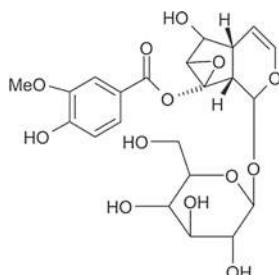
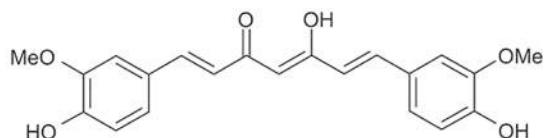
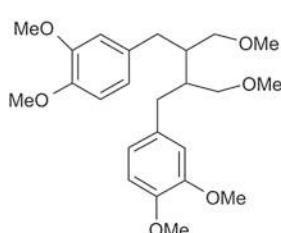
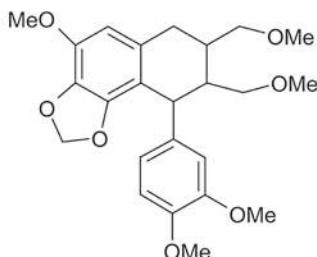
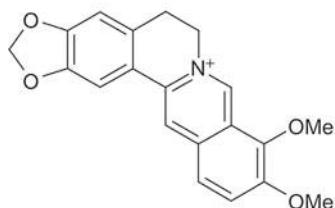
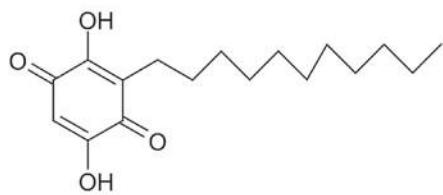
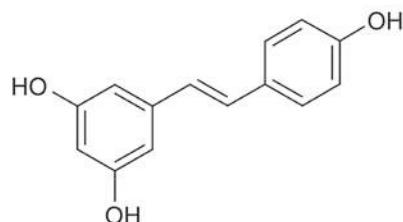
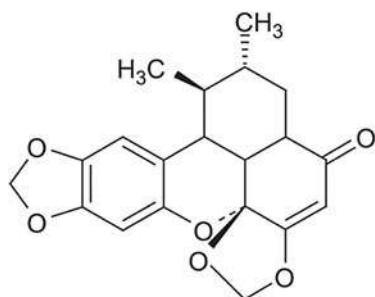
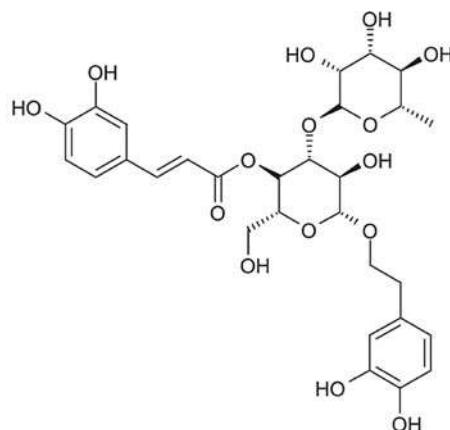
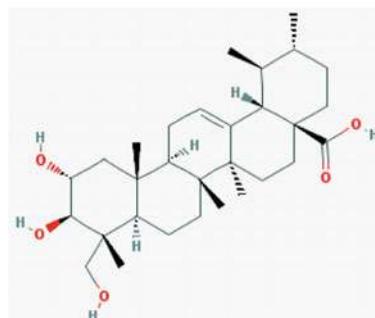
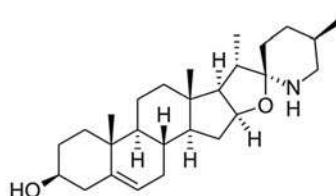
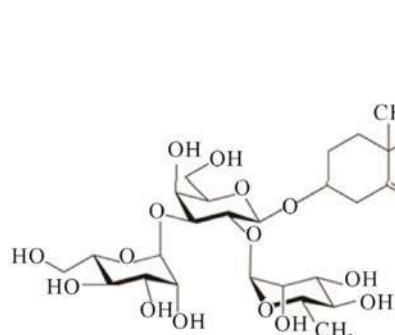


Fig. 3.68 Structure of some the hepatoprotective compounds

**Kutkoside 1, 10-vanillylcatalpol****Curcumin****Phyllanthin****Hypophyllanthin****Berberine****Embelin****Resveratrol****Fig. 3.68 (continued)**

**Acteoside****Sauchinone****Asiatic acid (AA)****Solasodine****Solasonine****Fig. 3.68 (continued)**

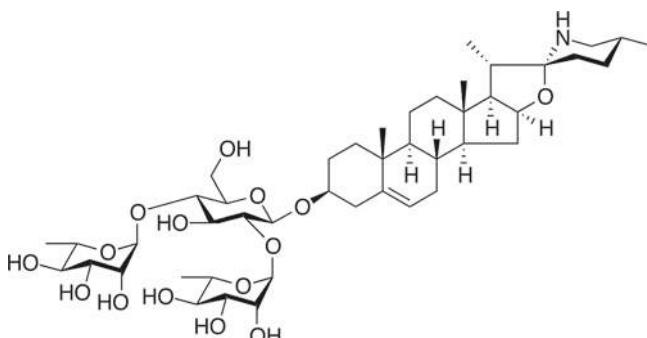


Fig. 3.68 (continued)

compound from *Curcuma longa*; phyllanthin and hypophyllanthin, lignans obtained from *Phyllanthus niruri*; berberine alkaloid obtained from *Berberis aristata*; embelin, 2,5-dihydroxy-3-undecyl-1,4-benzoquinone from *Embelia ribes*; resveratrol, a polyphenol (a phytoalexin) present in many plants and fruits, including red grapes, eucalyptus, spruce, blueberries, mulberries, peanuts, etc.; acteoside, a phenylethanoid glycoside obtained from *Cistanche tubulosa* and *Syringa vulgaris*; Sauchinone, a diastereomeric lignan from *Saururus chinensis*; asiatic acid (AA), a triterpenoid components of *Terminalia catappa*; Solasodine, a glycoalkaloid derived from the steroidal alkaloid, occurs in plants of the Solanaceae family. Solasonine and solamargine are glycoalkaloid derivatives of solasodine. Solamargine, a glycoalkaloid derived from the steroidal alkaloid solasodine from *Solanum nigrum* and other Solanaceae members such as potatoes, tomatoes, and eggplants.

Antidiabetic (hypoglycemic) herbal products

Diabetes mellitus (DM) has been recognized since antiquity and currently it affects as many as 285 million people worldwide and results in heavy personal and national economic burdens (Cicero et al. 2013). DM is a dreadful lifestyle related metabolic disorder of twenty-first century caused by insufficient or inefficient insulin secretion or insulin physiological unresponsiveness and characterized by increased blood glucose levels (hyperglycemia). Three key defects, e.g., increased hepatic glucose production, diminished insulin secretion, and impaired insulin action cause the onset of hyperglycemia in DM while in DM treatment; efforts are made to diabetes by improving insulin sensitivity, increasing insulin production and/or decreasing the amount of glucose in blood. *Ginseng*, *bitter melon*, *fenugreek*, *banaba*, *Gymnema sylvestre*, *Coptis chinensis*, etc., are most popular hypoglycemic herbs. These herbs act by increasing insulin secretion, enhancing glucose uptake by adipose and skeletal muscle tissues, inhibiting intestinal glucose absorption and inhibiting hepatic glucose production (Prabhakar and Doble 2011).

Conventional drugs treat diabetes by improving insulin sensitivity, increasing insulin production and/or decreasing the amount of glucose in blood. Several herbal preparations (hypoglycemic herbs) such as ginseng, bitter melon and *Coptis chinensis*. Several popular commercially available herbal preparations are also discussed, including ADHF (anti-diabetes herbal formulation), Jiangtangkeli, YGD (Yerbe Mate–Guarana–Damiana) and BN (Byakko-ka-ninjin-to). The efficacy of hypoglycemic herbs is achieved by increasing insulin secretion, enhancing glucose uptake by adipose and muscle tissues, inhibiting glucose absorption from intestine and inhibiting glucose production from hepatocytes.

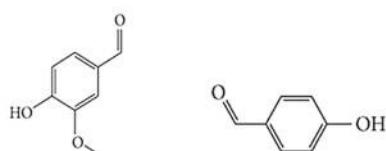
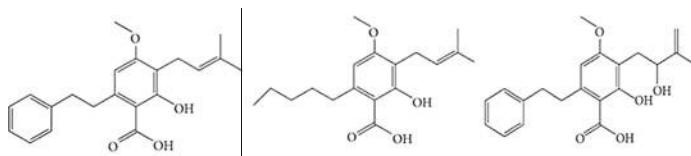
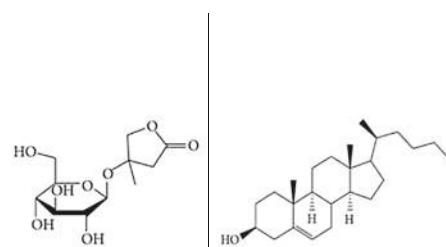
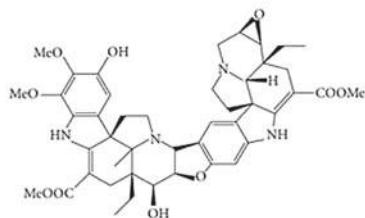
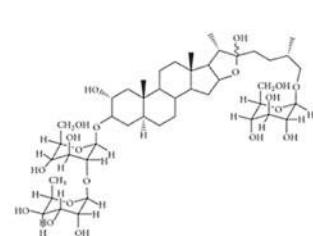
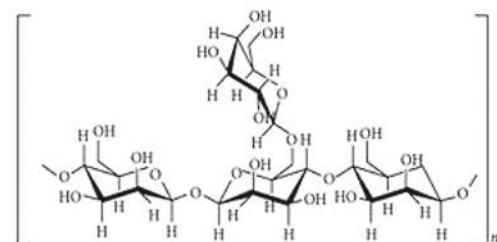
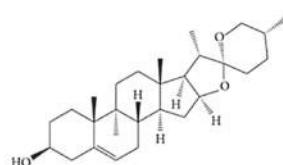
Myrcia Ericostemma littoralis, *Biophytum sensitivum*, *Ipomoea batatas*, *Tithonia diversifolia*, *Tithonia diversifolia*, Sangzhi, *Galega officinalis*, Fenugreek leaves, *Pterocarpus marsupium*, *Artemisia scoparia*, *Gymnema sylvestre*, Daio (Rhei rhizoma), *Lupinus termis*, Tea, *Coccinia indica* leaves, *Clausena anisata*, *Hovenia dulcis*, Aloes, Green Tea, Holy Basil, *Gymnema*, Fenugreek, Licorice, Sunflower, Ginseng, Garlic, Turmeric, Dandelion, Bilberry, Parsley, Sarsaparilla, Gentian Root and Olive Leaf, Ashwaghanda, Baical, Skullcap, Camu-Camu, Chamomile, Damiana, Ginseng, Gotu Kola, Hops, Kanna, Kava, Lavender, Linden, Liquorice, Milk Thistle, Motherwort, Oats, Parsnip, Passion Flower, Pumpkin, Rhodiola, Schisandra, StJohn'sWort, Siberian, Ginseng, Skullcap, Sweet Tea Vine, Valerian, Vervain, Yohimbe.

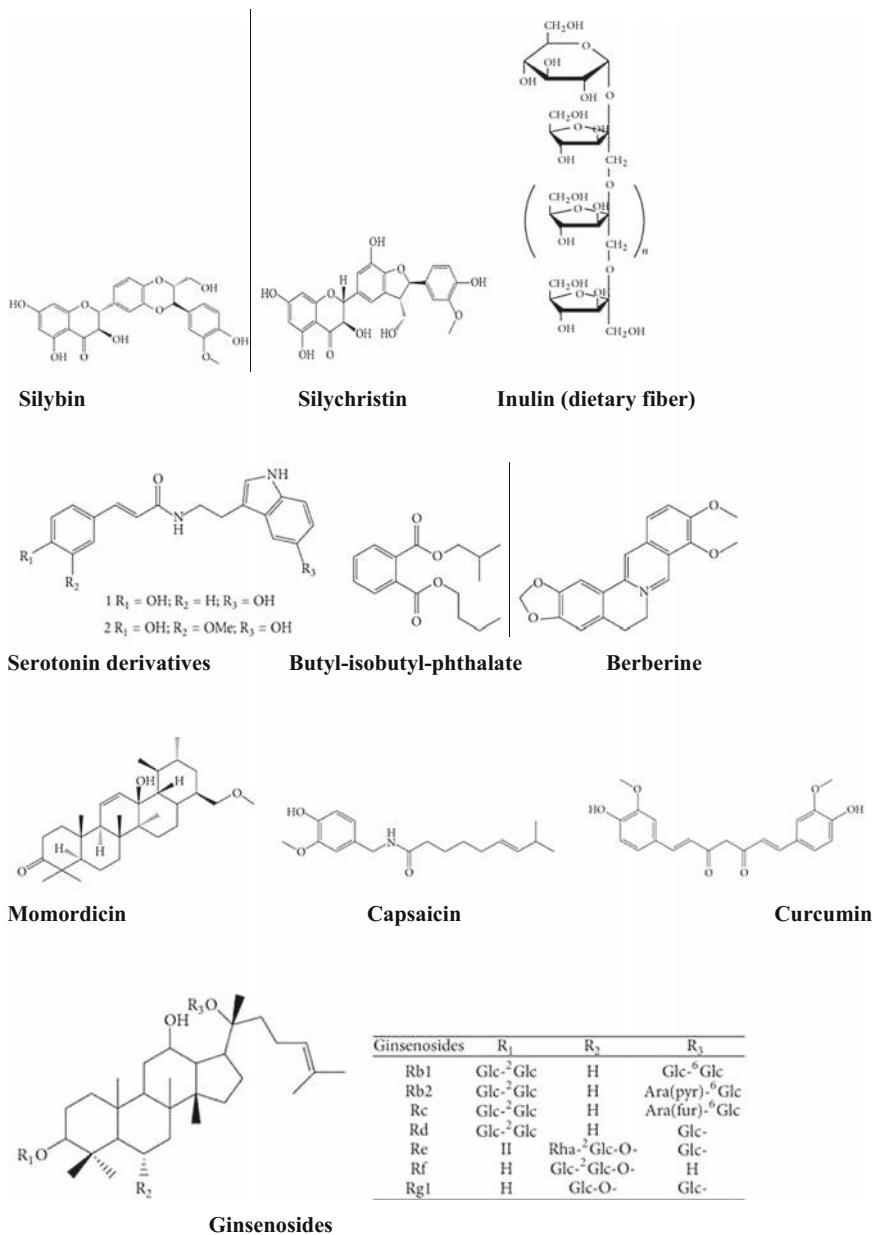
Different types of medicinal herbs can be classified based on their modes of action such as insulin resistance (type 1 herbs),—cell function (type 2 herbs), and GLP-1 (type 3 herbs) and glucose (re)absorption (type 4 herbs). (b) The selected plants and compounds exert their antihyperglycemic effect through targeting one single mechanism (insulin resistance (type 1 herbs),—cell function (type 2 herbs), GLP-1 (type 3 herbs), or glucose (re)absorption (type 4 herbs)) or multiple mechanisms.

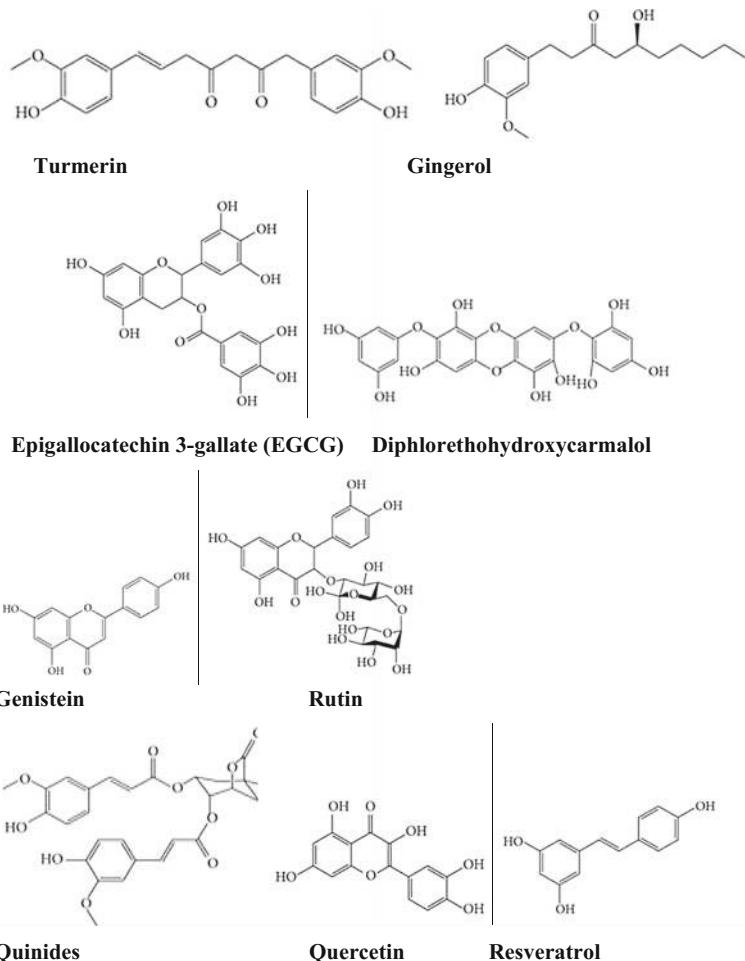
Medicinal herbs contain diverse bioactive compounds and can have multiple actions on insulin action, insulin production, or both. Some plant chemicals with the ability to control blood glucose are shown in Fig. 3.69. These antidiabetic plant chemicals include amorphitin 1–3 from *Glycyrrhiza uralensis* and vanillin 4-hydroxybenzaldehyde from *G. elata* to regulate insulin resistance; cinnamaldehyde from *Cinnamom* spp.; and diosgenin from *Trigonella foenum-graecum*. Serotonin derivatives, butyl-isobutyl-phthalate, and berberine regulate glucose absorption in the guts while momordicin, capsaicin, and curcumin regulate two or more pathways (lower blood glucose due to their insulin-like chemical structures); regulation of insulin resistance and probably β cells. Ginsenosides and turmerin regulate two or more pathways (regulate β -cell function, improvement of insulin resistance).

Antihypertensive herbal products

Hypertension is defined as having a systolic blood pressure (SBP) of ≥ 140 mmHg and a diastolic blood pressure (DBP) of ≥ 90 mmHg ($\geq 140/\geq 90$ mmHg). Every 20/10 (SBP/DBP) mmHg increase indicates a higher risk stage of hypertension:

**Cinnamaldehyde****Fig. 3.69** Structure of some antidiabetic (hypoglycemic) herbal principles

**Fig. 3.69** (continued)

**Fig. 3.69** (continued)

stage 1 (140–159/90–99 mmHg), stage 2 ($\geq 160/\geq 100$ mmHg). The American Society of Hypertension and the International Society of Hypertension (ISH) recommend that individuals with blood pressure of 120–139/80–89 mmHg be considered as pre-hypertensives (Weber et al. 2014). Hypertension (HTN) has several subclassifications including, HTN stage I, HTN stage II, and isolated systolic HTN. Isolated systolic HTN refers to elevated systolic pressure with normal diastolic pressure and is common in the elderly. Hypertension is by far the most prevalent trigger for cardiovascular diseases (CVDs) than other (diabetes, smoking, dyslipidemia, etc.); hypertension is responsible for around 16.5% of annual deaths worldwide and by 2030, the annual death toll is estimated to reach 23.5 million

people (WHO 2013). HTN besides increasing the risk of heart disease and stroke, HTN can also lead to other conditions such as congestive heart failure, atherosclerosis, peripheral artery disease, coronary artery disease, kidney damage, dementia, and blindness (August 2004; Freedman and Cohen 2016).

Drugs like diuretics (indapamide, furosemide, amiloride), sympathoplegic agents (clonidine, reserpine), renin inhibitor (aliskiren), angiotensin converting enzymes (ACE) inhibitors (enalapril, captopril, quinapril),—losartan, irbesartan, olmesartan), calcium channel blockers (nifedipine, verapamil, diltiazem), α -adrenergic blockers (prazosin, doxazosin), β -adrenergic blockers (nebivolol, atenolol), vasodilators (minoxidil, sodium nitroprusside), etc., are used to manage blood pressure levels in hypertensive patients (Archer 2000; Susalit et al. 2011). Sesquiterpene is the most active dandelion compound which provides the natural remedy with its beneficial diuretic properties. Parsley is rich in beneficial phytochemicals like carotene, lutein, potassium as well as a wide variety of vitamins and minerals. Like most of the other natural diuretics, parsley also works in eliminating water retention by increasing urination through inhibiting the potassium and ion pumps. Celery (*Apium graveolens*) seeds and flavored leaves (contain COX-2 inhibitors, vitamin C, potassium, etc.) are very useful as diuretic in the management of blood pressure.

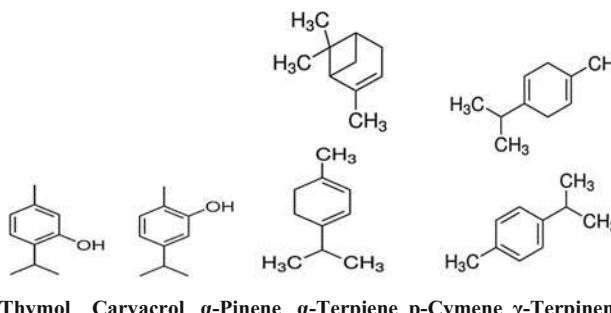
A large number of hypertensive population of the world use herbal medicines because of their low cost, better acceptability and lesser side effects. *Achillea wilhelmsii*, *Allium cepa*, *Allium sativum* (allicin), *Anethum graveolens*, *Apium graveolens*, *Avena sativa*, *Berberis vulgaris*, *Centaurea depressa*, *Cichorium intybus*, *Crataegus* sp., *Hypericum perforatum*, *Laurocerasus officinalis*, *Matricaria recutita*, *Nigella sativa*, *Panax quinquefolius*, *Passiflora edulis*, *Rumex acetosella*, *Viscum album*, *Ziziphus zizyphus*, etc., are some of the commonly used anti-hypertensive medicinal plants. Other plants used as hypotensive agents are *Agathosma betulina* (diureic and anti-inflammatory), *Annona muricata*, *Apium graveolens*, *Aristolochia manshuriensis* (aristolochic acid, aristoloside, magnoflorine, oleanolic acid, hederagenin, and tannins. magnoflorine has been found to possess hypotensive properties), *Artocarpus altilis*, *Avena sativa* (soluble fiber), *Blond psyllium*, *Camellia sinensis*, *Capparis cartilaginea*, *Carum copticum*, *Cassia absus*, *Cassia occidentalis*, *Castanospermum australe* (saponin fraction and medicogenic acid glucoside), *Coleus forskohlii*, *Commelina virginica*, *Crataegus pinnatifida*, *Crinum glaucum*, *Cuscuta reflexa*, *Daucus carota*, *Desmodium styracifolium*, *Fuchsia magellanica*, *Glycine max*, *Gossypium barbadense*, *Hibiscus sabdariffa*, *Lavandula stoechas*, *Lepidium latifolium*, *Linum usitatissimum*, *Lumnitzera racemosa*, *Lycopersicon esculentum*, *Moringa oleifera*, *Musanga cecropioides*, *Ocimum basilicum*, *Peganum harmala*, *Phyllanthus amarus*, *Pinus pinaster*, *Pueraria lobata*, *Punica granatum*, *Raphanus sativus*, *Rauvolfia serpentina*, *Rhaptophetalum coriaceum*, *Sesamum indicum*, *Solanum sisymbriifolium*, *Theobroma cacao*, *Triticum aestivum*, *Uncaria rhynchophylla*, *Urtica dioica*, *Viscum album*, *Vitex doniana*, *Zingiber officinale*, etc.

Secondary metabolites of herbs and spices exhibit antihypertensive effects. Commonly used antihypertensive plants with antioxidant activity are *Allium sativum* (garlic's hypotensive effects are based on garlic's organosulfur constituents

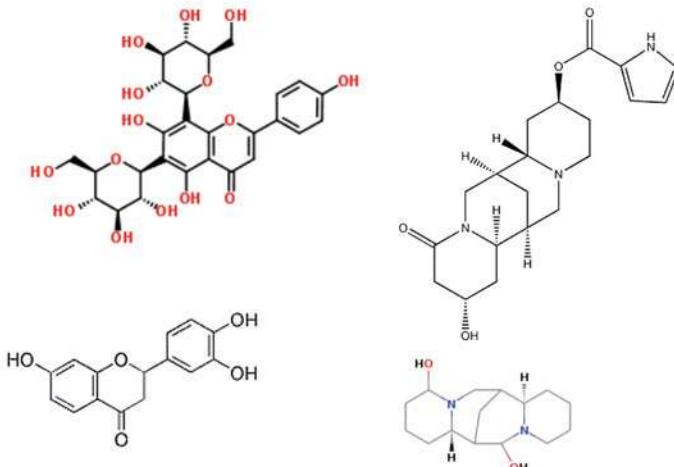
such as Allicin, and methyl thiosulfonate); *Andrographis paniculata* (several hypotensive labdane-type diterpenoid compounds include andrographolide, 14-deoxy-11,12-didehydroandrographolide and 14-deoxyandrographolide); *Apium graveolens* (Apigenin, a flavone isolate of *A. graveolens*); *Camellia sinensis* (catechins, the major flavonoids in tea, include (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin-3-gallate); *Coptis chinensis* (main component Berberine (BBR); *Coriandrum sativum*, *Crataegus* spp. (antihypertensive actions are credited to the plant's multiple components: flavonoids—hyperoside, quercetin, rutin, and vitexin, and oligomeric proanthocyanidins—OPCs, epicatechin, procyanidin, and procyanidin B-2); *Crocus sativus* (main components include crocin, picrocrocin, safranal, and crocetin); *Hibiscus sabdariffa*; *Panax* (heterogeneous triterpenoid saponins and steroid glycosides or ginsenosides or panaxosides are the active principle components); *Salviae miltiorrhizae* (most effective components include salvianolic acid A—SalA, salvianolic acid B—SalB, danshensu, and tanshinones); *Zingiber officinale* (6-gingerol and 6-shogaol); *Apium graveolens*, *Bidens pilosa*, *Cymbopogon citratus* (antihypertensive principle citral), *Nigella sativa* (thymoquinone—TQ as abundant bioactive component of seed); *Rauvolfia serpentina* (reserpine alkaloids). Figure 3.70 shows the structure of some antihypertensive (hypotensive) herbal principles.

Reserpine, an alkaloid found in the roots of *Rauwolfa serpentina* and *R. vomitoria*. Reserpine inhibits the uptake of norepinephrine into storage vesicles resulting in depletion of catecholamines and serotonin from central and peripheral axon terminals. It has been used as an antihypertensive and an antipsychotic as well as a research tool, but its adverse effects limit its clinical use.

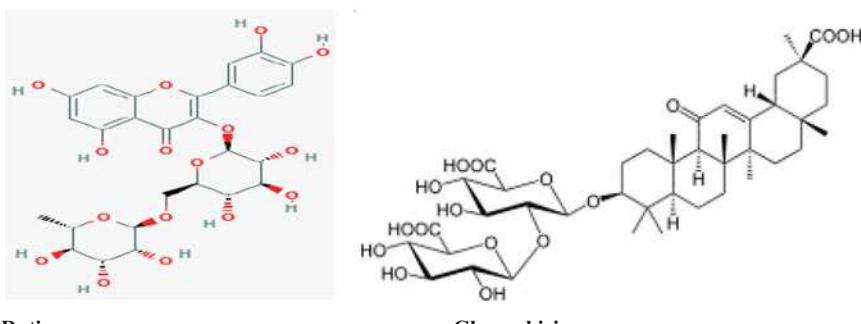
Commonly used antihypertensive plants possess vasorelaxant, anti-inflammatory, antiproliferative, diuretic, etc., activities. The mechanisms of action of antihypertensive herbal preparations are based on their function as antioxidant, diuretic agent, ROS scavenger, increases the bioavailability of endogenous signaling gases like NO and H₂S, inhibition of Angiotensin Converting Enzyme (ACE), decrease ACE and ROS activities, attenuation of NAPDH oxidase production, flow-mediated dilation (FMD), increase the antioxidant enzyme—SOD, decrease formation of endothelial microparticles (EMPs), prevent MI by inhibiting myofibrillar damage, up-regulate antioxidant enzymes (SOD, CAT) and augment the concentration of the reducing glutathione -GSH; regulation of voltage operated calcium channels (VOCCs), vasorelaxant pathways, inhibit cardiac hypertrophy and decrease heart rates, increase secretion of urea, calcium, sodium, and potassium in urine, etc.



Thymol Carvacrol α -Pinene α -Terpiene p-Cymene γ -Terpinene



Digitinine 4 β 13 α -dihydroxylupanine, quinolizidine alkaloids (the flavonoids vicenin-2, butin, 3'-hydroxydaidzein, etc.)



Rutin

Glycyrrhizin

Fig. 3.70 Structure of some antihypertensive (hypotensive) herbal principles

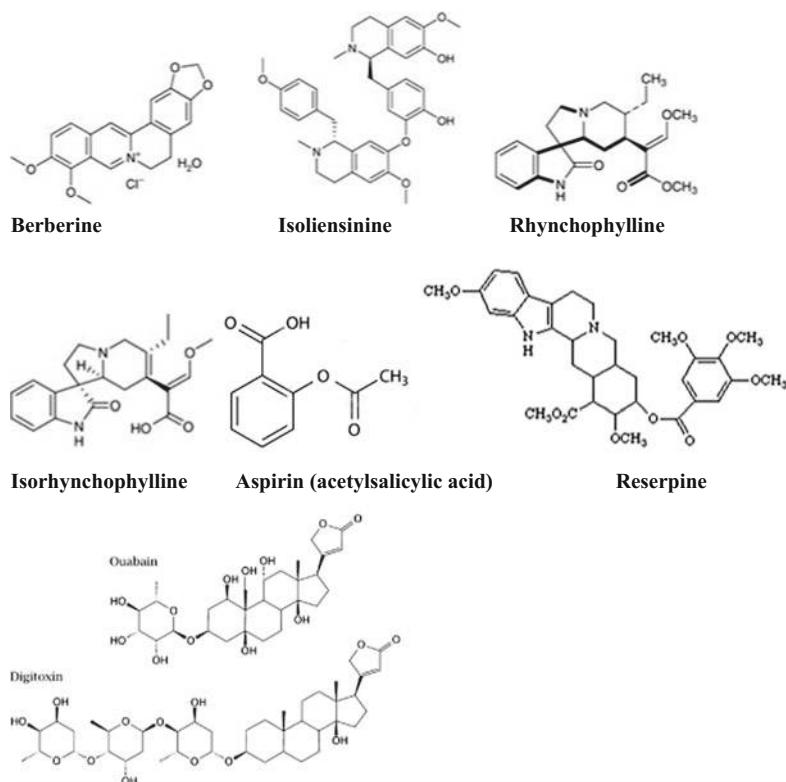


Fig. 3.70 (continued)

3.11 Sources, Chemistry, and Health Effects of the Bioactive Compounds of Secondary Metabolic Origin; Biotechnology of Bioactive Compounds

Bioactive compounds of secondary metabolic origin belonging to several groups of phenolics, terpenes and terpenoids, alkaloids, glycosides, etc., exhibit important health effects and play a central role in high-value pharmaceutical product development in the pharmaceutical industry. Bioactive compounds have been identified from diverse sources and their therapeutic benefits, nutritional value and protective effects in human and animal healthcare have underpinned their application as pharmaceuticals and functional food ingredients. The orderly study of biologically active products and the exploration of potential biological activities of these secondary metabolites, including their clinical applications, standardization, quality control, mode of action and potential biomolecular interactions, have emerged as one of the most exciting developments in modern natural medicine.

Biotechnology of bioactive compounds describes the current stage of knowledge on the production of bioactive compounds from microbial, algal and vegetable

sources. In addition, the molecular approach for screening bioactive compounds is also important, as well as examples of applications of these compounds on human health. The bioactive compounds profiled include compounds such as C-phycocyanins, glycosides, phytosterols and natural steroids. Importance of the usage of bioactive compounds as antioxidants and anti-inflammatory agents, anti-allergic compounds and in stem cell research, and also the medicinal applications of plant-derived compounds deserve special attention.

References

- Ahmed B, Khan S, Masood MH, Siddique AH (2008) Anti-hepatotoxic activity of cichotyboside, a sesquiterpene glycoside from the seeds of *Cichorium intybus*. *J Asian Nat Prod Res* 10:223–231
- Alamgir ANM, Rahman M, Rahman A (2013) Phytochemical characteristics, antimitotic, cytotoxic and antitumor activities of bark extract of *Streblus asper* Lour. *Bangladesh J Bot* 42(1):17–22
- Amaral JA, Ekins A, Richards SR, Knowles R (1998) Effect of selected monoterpenes on methane oxidation, denitrification, and aerobic metabolism by bacteria in pure culture. *Appl Environ Microbiol* 64:520–525
- Archer JS (2000) Evaluation and treatment of hypertension. *Prim Care Update Ob Gyns* 7:1–6
- August P (2004) Overview: mechanisms of hypertension: cells, hormones, and the kidney. *J Am Soc Nephrol* 15:1971–1973
- Azadbakht M, Azadbakht M (2008) Five prevalent antiprotozoal herbal drugs. *J Mazandaran Univ Med Sci* 18(67):118–132
- Bastida J, Lavilla R, Viladomat F (2006) Chemical and biological aspects of Narcissus alkaloids. In: Cordell GA (ed) *The Alkaloids*, 63:87–179. Elsevier Inc.
- Batista O, Duarte A, Nascimento J, Simones MF (1994) Structure and antimicrobial activity of diterpenes from the roots of *Plectranthus hereroensis*. *J Nat Prod* 57:858–861
- Bhutani KK, Gohil VM (2010) Natural product drug discovery research in India: status & appraisal. *Indian J Exp Biol* 48:199–207
- Bishayee A, Bhatia D, Thoppil RJ, Darvesh SA, Nevo E, Lansky EP (2011) Pomegranate-mediated chemoprevention of experimental hepatocarcinogenesis involves Nrf2-regulated antioxidant mechanisms. *Carcinogenesis* 32:888–896
- Buckingham J (2004) Dictionary of natural products, web version 2004. Chapman and Hall, London. Available at: <http://www.chemnetbase.com>
- Cao Y, Langer R (2008) A review of Judah Folkman's remarkable achievements in biomedicine. *PNAS* 105:13203–13205
- Chang CLT, Lin Y, Bartolome AP, Chen Y-C et al (2013) Herbal therapies for type 2 diabetes mellitus: chemistry, biology, and potential application of selected plants and compounds. *Evid Based Complement Alternat Med* 2013:33. Article ID 378657
- Chung KT (2009) H. Boyd Woodruff (b. 1917): antibiotics hunter and distinguished soil microbiologist. *SIM News* 59:178–185
- Cowan MM (1999) Plant products as antimicrobial agents. *Clin Microbiol Rev* 12(4):564–582
- Croft SL (1997) The current status of antiparasite chemotherapy. *Parasitology* 114:3–15
- Crowell PL (1999) Prevention and therapy of cancer by dietary monoterpenes. *J Nutr* 129 (3):775S–778S
- Davey MP, Bryant DN, Cummins I, Gates P, Ashenden TW, Baxter R et al (2004) Effects of elevated CO₂ on the vasculature and phenolic secondary metabolism of *Plantago maritima*. *Phytochemistry* 65:2197–2204

- Dholwani KK, Saluja AK, Gupta AR, Shah DR (2008) A review on plant-derived natural products and their analogs with antitumor activity. Indian J Pharmacol 40(2):49–58
- Downey RK (2003) Mustard. In: Katz SH and Weaver WW, Encyclopedia of Food and Culture. Gale virtual reference library. New York: Scribner. ISBN 0684314169
- Dua VK, Verma G, Agarwal DD, Kaiser M, Brun R (2011) Antiprotozoal activities of traditional medicinal plants from the Garhwal region of North West Himalaya. Indian J Ethnopharmacol 136(1):123–128
- Duflos A, Fahy J, Thillaye du Boulay V, Barret JM, Hill B (2000) Vinca alkaloid antimitotic halogenated derivatives. US Patent 6127377
- Ebad SS, Lin WH, Proksch P (2010) Bioactive sesterterpenes and triterpenes from marine sponges: occurrence and pharmacological significance. Mar Drugs 8(2):313–346
- Estevez-Braun A, Estevez-Reyes R, Moujir LM, Ravelo AG, Gonzalez AG (1994) Antibiotic activity and absolute configuration of 8S-heptadeca-2(Z),9(Z)-diene-4,6-diyne-1,8-diol from *Bupleurum salicifolium*. J Nat Prod 57:1178–1182
- Flora KD, Rosen HR, Benner KG (1996) The use of naturopathic remedies for chronic liver disease. Am J Gastroenterol 91:2654–2655
- Flora K, Hahn M, Rosen H, Benner K (1998) Milk thistle (*Silybum marianum*) for the therapy of liver disease. Am J Gastroenterol 93:139–143
- Freedman BI, Cohen AH (2016) Hypertension-attributed nephropathy: what's in a name? Nat Rev Nephrol 12(1):27–36
- Fujioka T, Kashiwada Y (1994) Anti-AIDS agents. 11. Betulinic acid and platanic acid as anti-HIV principles from *Syzygium claviflorum*, and the anti-HIV activity of structurally related triterpenoids. J Nat Prod 57:243–247
- Ghosh N, Ghosh R, Mandal V, Mandal SC (2011) Recent advances in herbal medicine for treatment of liver diseases. Pharm Biol 49:970–988
- Gould MN (1997) Cancer chemoprevention and therapy by monoterpenes. Environ Health Perspect 105:977–979
- Graebin C, Uchoa F, Bernardes L, Campo V, Carvalho I, Eifler-Lima V (2009) Antiprotozoal agents: an overview. AntiInfect Agents Med Chem 8(4):345–366
- Gupta NK, Dixit VK (2009) Evaluation of hepatoprotective activity of *Cleome viscosa* Linn. extract. Indian J Pharmacol 41:36–40
- Hall CA, Cuppett SL (1997) Structure-activities of natural antioxidants. In: Auroma OI, Cuppett SL (eds) Antioxidant methodology in vivo and in vitro concepts. AOCS Press, Champaign, IL, pp 141–172
- Haslam E (1996) Natural polyphenols (vegetable tannins) as drugs: possible modes of action. J Nat Prod 59:205–215
- Herbst ST (2001) The new food lover's companion: comprehensive definitions of nearly 6000 food, drink, and culinary terms. Barron's cooking guide. Barron's educational series, Hauppauge, NY. ISBN 0764112589
- Hwang YP, Choi CY, Chung YC, Jeon SS, Jeong HG (2007) Protective effects of puerarin on carbon tetrachloride-induced hepatotoxicity. Arch Pharm Res 30:1309–1317
- Ivanovska N, Philipov S, Istatkova R, Georgieva P (1996) Antimicrobial and immunological activity of ethanol extracts and fractions from *Isopyrum thalictroides*. J Ethnopharmacol 54:143–151
- Kaushik U, Aeri V, Mir SR (2015) Cucurbitacins—an insight into medicinal leads from nature. Pharmacogn Rev 9:12–18
- Khaw M, Panosian CB (1995) Human antiprotozoal therapy: past, present, and future. Clin Microbiol Rev 8(3):427–439
- Kim W, Seong KM, Youn B (2011) Phenylpropanoids in radioregulation: double edged sword. Exp Mol Med 43(6):323–333
- Lee HJ, Lee HJ, Magesh V, Nam D, Lee EO, Ahn KS et al (2008) Shikonin, acetylshikonin, and isobutyroylshikonin inhibit VEGF-induced angiogenesis and suppress tumor growth in Lewis lung carcinoma-bearing mice. Yakugaku Zasshi 128:1681–1688

- Levy C, Seeff LD, Lindor KD (2004) Use of herbal supplements for chronic liver disease. *Clin Gastroenterol Hepatol* 2:947–956
- Liby K, Royce DB, Williams CR, Risingsong R, Yore MM, Honda T et al (2007) The synthetic triterpenoids CDDO-methyl ester and CDDO-ethyl amide prevent lung cancer induced by vinyl carbamate in A/J mice. *Cancer Res* 67:2414–2419
- Liu ZJ (2011) Next generation sequencing and whole genome selection in aquaculture. Wiley-Blackwell
- Llurba Montesino N, Kaiser M, Brun R, Schmidt TJ (2015) Search for antiprotozoal activity in herbal medicinal preparations; new natural leads against neglected tropical diseases. *Molecules* 20(8):14118–14138
- Madrigal-Santillán E, Madrigal-Bujaidar E, Álvarez-González I, Sumaya-Martínez MT, Gutiérrez-Salinas J, Bautista M et al (2014) Review of natural products with hepatoprotective effects. *World J Gastroenterol* 20(40):14787–14804
- Meyer JJM, Afolayan AJ, Taylor MB, Erasmus D (1997) Antiviral activity of galangin from the aerial parts of *Helichrysum aureonitens*. *J Ethnopharmacol* 56:165–169
- Mukherjee AK, Basu S, Sarkar N, Ghosh AC (2001) Advances in cancer therapy with plant based natural products. *Curr Med Chem* 8:1467–1486
- Neumann CS, Fujimori DG, Walsh CT (2008) Halogenation strategies in natural product biosynthesis. *Chem Biol* 15:99–109
- Nobre HV Jr, Maia FD, de Oliveira RA, Bandeira MAM, do Ó Pessoa C, Moraesa MO et al (2007) Neuroprotective actions of tannins from myracrodruon urundeuva on 6-hydroxydopamine-induced neuronal cell death. *J Herbs Spices Med Plants* 13(2):41–57
- Oliveira FA, Chaves MH, Almeida FR, Lima RC Jr, Silva RM, Maia JL et al (2005) Protective effect of alpha- and beta-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice. *J Ethnopharmacol* 98:103–108
- Omulokoli E, Khan B, Chhabra SC (1997) Antiplasmodial activity of four Kenyan medicinal plants. *J Ethnopharmacol* 56:133–137
- Patel S (2016) Plant-derived cardiac glycosides: role in heart ailments and cancer management. *Biomed Pharmacother* 84:1036–1041
- Pengsuparp T, Cai L, Constant H, Fong HH, Lin Z, Kinghorn AD et al (1995) Mechanistic evaluation of new plant-derived compounds that inhibit HIV-1 reverse transcriptase. *J Nat Prod* 58:1024–1031
- Petronelli A, Pannitteri G, Testa U (2009) Triterpenoids as new promising anticancer drugs. *Anticancer Drugs* 20:880–892
- Poonam S, Chandana M (2015) A review on anticancer natural drugs. *Int J Pharm Tech Res* 8(7):131–141
- Prabhakar PK, Doble M (2011) Mechanism of action of natural products used in the treatment of diabetes mellitus. *Chin J Integr Med* 17(8):563–574
- Rabi T, Gupta S (2008) Dietary terpenoids and prostate cancer chemoprevention. *Front Biosci* 13:3457–3469
- Raffauf RF (1996) Plant alkaloids. A Guide to their discovery and distribution. The Haworth Press, New York
- Rao GM, Rao CV, Pushpangadan P, Shirwaikar A (2006) Hepatoprotective effects of rubiadin, a major constituent of *Rubia cordifolia* Linn. *J Ethnopharmacol* 103:484–490
- Rawls RL (1998) Modular enzymes. *Chem Eng News* 76:29–32
- Rayl AJS (1999) Oceans: medicine chests of the future? *Scientist* 13:1
- Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* 20:933–956
- Riganti C, Campia I, Kopecka J, Gazzano E, Doublier S, Aldieri E et al (2011) Pleotropic effects of cardiovascular glycosides. *Curr Med Chem* 18(6):872–885
- Safarzadeh E, Sandoghchian S, Baradaran B (2014) Herbal medicine as inducers of apoptosis in cancer treatment. *Adv Pharm Bull* 4:421–427

- Salas JA, Mendez C (1998) Genetic manipulation of antitumor-agent biosynthesis to produce novel drugs. *Trends Biotechnol* 16:475–482
- Sarker D, Reid AH, Yap TA, de Bono JS (2009) Targeting the PI3K/AKT pathway for the treatment of prostate cancer. *Clin Cancer Res* 15:4799–4805
- Setzer WN, Setzer MC (2003) Plant-derived triterpenoids as potential antineoplastic agents. *Mini Rev Med Chem* 3:540–556
- Singh B, Saxena AK, Chandan BK, Agarwal SG, Anand KK (2001) In vivo hepatoprotective activity of active fraction from ethanolic extract of *Eclipta alba* leaves. *Indian J Physiol Pharmacol* 45:435–441
- Souza SMC, Aquino LC, Milach AC Jr, Bandeira MA, Nobre ME, Viana GS et al (2006) Antiinflammatory and antiulcer properties of tannins from *Myracrodruon urundeuva* Allemão (Anacardiaceae) in Rodents. *Phytother Res* 21(3):220–225
- Sun ZL, Wang Y, Guo ML, Li YX (2010) Two new hepatoprotective saponins from *Semen celosiae*. *Fitoterapia* 81:375–380
- Susalit E, Agus N, Effendi I, Tjandrawinata RR, Nofiarny D, Perrinjaquet-Moccetti T et al (2011) Olive (*Olea europaea*) leaf extract effective in patients with stage-1 hypertension: comparison with Captopril. *Phytomedicine* 18:251–258
- Vincken JP, Heng L, Groot A, Gruppen H (2007) Saponins, classification and occurrence in the plant kingdom. *Phytochem* 68(3):275–297
- Wagner KH, Elmadaf I (2003) Biological relevance of terpenoids. Overview focusing on mono-, di- and tetraterpenes. *Ann Nutr Metab* 47(3–4):95–106
- Wang T, Sun NL, Zhang WD, Li HL, Lu GC, Yuan BJ et al (2008) Protective effects of dehydrocavidine on carbon tetrachloride-induced acute hepatotoxicity in rats. *J Ethnopharmacol* 117:300–308
- Wang CZ, Calway T, Yuan CS (2012) Herbal medicines as adjuvants for cancer therapeutics. *Am J Chin Med* 40:657–669
- Weber MA, Schiffrin EL, White WB, Mann S, Lindholm LH, Kenerson JG et al (2014) Clinical practice guidelines for the management of hypertension in the community a statement by the American society of hypertension and the international society of hypertension. *J Hypertens* 32:3–15
- WHO (2013) Cardiovascular diseases (CVDs). World Health Organization, Geneva. Fact sheet no 317
- Xue J, Duda L-C, Smith KE, Fedorov AV, Johnson PD, Hulbert SL et al (1999) Electronic structure near the Fermi surface in the quasi-one-dimensional conductor $\text{Li}_{0.9}\text{Mo}_6\text{O}_{17}$. *Phys Rev Lett* 83:1235–1238
- Xue Q, Sun ZL, Guo ML, Wang Y, Zhang G and Wang XK (2010) Two new compounds from *Semen celosiae* and their protective effects against $\text{CCl}(4)$ -induced hepatotoxicity. *Nat Prod Res* 1–8
- Zhang Y, Lewis K (1997) Fabatins: new antimicrobial plant peptides. *FEMS Microbiol Lett* 149:59–64
- Zhang L, Li HZ, Gong X, Luo FL, Wang B, Hu N et al (2010) Protective effects of Asiaticoside on acute liver injury induced by lipopolysaccharide/ β -galactosamine in mice. *Phytomedicine* 17:811–819

Chapter 4

Bioactive Compounds

and Pharmaceutical Excipients Derived from Animals, Marine Organisms, Microorganisms, Minerals, Synthesized Compounds, and Pharmaceutical Drugs



Abstract Many bioactive compounds and pharmaceutical excipients are derived from animals, marine organisms, microorganisms, minerals, etc. Glandular products, liver extract, fish liver oils, musk, bees' wax, certain hormones, enzymes, antitoxins, etc., are drugs obtained from animal sources. With the advent of Genentech, insulin, drugs for hemophilia and anemia, protein-based cancer drug, etc., are produced in genetically engineered organisms. The potent medicinal usage of the bioactive compounds, viz., steroids, terpenoids, isoprenoid, and non-isoprenoid compounds, quinones, brominated compounds, nitrogen heterocyclics, nitrogen-sulfur heterocyclics, antibiotics, etc., have been discovered in recent years from marine poriferans, cnidarians, annelids, arthropods, mollusks, echinoderms, vertebrates, seaweeds (marine macroalgae), seagrasses (submersed marine angiosperms), and microorganisms with therapeutic activities such as antioxidant, antibacterial, anti-inflammatory, anticarcinogenic properties. Bioactive compounds obtained from minerals include kaolin, calomel, iodine, iron, gold, sulfur, selenium, etc. Synthetic pharmaceutical drugs (bioactive compounds) of different categories including semisynthetic anticancer drug—Taxol, therapeutic peptides, and proteins—insulin and similar other protein products with the application of recombinant DNA technology and many other drugs are produced.

4.1 Bioactive Compounds and Excipients from Animal Sources

The potential for drug discovery from animal sources remains high as judged by the promise of numerous emerging new fields. Large-scale progress has been made during recent years to use the power of high-throughput screening (HTS) technologies to discover proof-of-principle molecules for many new targets, next-generation models, advantageous drug–drug combinations, use of automated whole-animal behavioral screening systems, advancement in understanding the role of epigenetics in drug addiction, the employment of organoid-level 3D test

platforms (tissue-chip or organs-on-chip), etc. Every animal (from hydra to man) makes antimicrobial peptides. They serve to protect us and to live in harmony with bacteria. There are some common animal-derived ingredients that are found in medications and several drugs that are commonly derived from animals. Some common drugs obtained from animals include glandular products (e.g., products of thyroid organ), liver extract, etc. Fish liver oils, musk, bees' wax, certain hormones, enzymes, and antitoxins are drugs obtained from animal sources. A number of medicines (including tablets, injections, capsules, creams, mixtures, and vaccines) contain animal products or are animal derived. For examples (i) carmine or natural red 4 (red dye), (ii) gelatin, (iii) glycerin, (iv) heparin, (v) insulin from pancreas, (vi) lactose, (vii) lanolin, (viii) magnesium stearate, (ix) premarin, (x) vaccines from animal blood, (xi) vitamin A and D from Cod fish liver, (xii) immunoglobulin G, (and also specific immunoglobulin hepatitis B immunoglobulin, rabies immunoglobulin, tetanus immunoglobulin), (xiii) human menopausal gonadotropin (hMG), (xiv) human chorionic gonadotropin (hCG) from urine of pregnant women, (xv) pituitary gonadotropins from anterior pituitary, (xvi) thyroxin from sheep thyroid, (xvii) enzymes pepsin and trypsin from stomach tissue, (xviii) venoms, (xix) toad alkaloids, (xx) proteins, etc., are derived from animal sources.

Many drugs are derived from animals, e.g., most of the insulin used by people with diabetes came from pigs or cows before the advent of Genentech, which introduced human genes that produce insulin into strains of bacteria. Genetically engineered mice are now used to produce some drug ingredients, researchers have created milk from genetically altered sheep and pigs that can treat a rare type of hemophilia, another strain of altered pigs secretes milk with a hormone to help anemia patients produce more red blood cells, newer protein-based drugs such as cancer drug Avastin and arthritis drug Enbrel, are produced in genetically engineered Chinese hamster ovary cells in culture, that are grown in huge stainless steel vats. All these developments also represent human reliance on animals for drugs. The following is a list of animals that have contributed to medicine.

Frog (*Hirudo medicinalis*) skin antibiotics is approved by the FDA for medical use; venom of Brazilian arrowhead viper, Brazilian pit viper, was the basis for developing one of the first ACE inhibitors, a group of drugs used to treat hypertension and congestive heart failure. Researchers isolated a molecule called bradykinin-potentiating factor from the viper venom and found it is related to a class of molecules that stop angiotensin-converting enzymes (ACE) from blocking bradykinins, a protein that causes blood vessels to dilate and lower blood pressure; in 2005, the Food and Drug Administration approved the drug Byetta, derived from Gila monster venom. The injectable medicine is effective at helping people with Type 2 diabetes to maintain healthy glucose levels. The drug also slowed the emptying of the stomach, decreasing appetite, and helping patients to lose weight.

Maggots, the creepy crawlers are voracious eaters that love to feast on diseased and dying flesh. But their nauseating idea of a great meal is a life-saving asset for those suffering from chronic wounds and infections. Cone snails are one of nature's most dangerous creatures and their toxic venom can be fatal to humans. But in the right doses, some of those compounds can be useful. In 2004, the FDA approved

the use of the pain medication ziconotide, marketed as Prialt, derived from one of the conopeptide proteins from cone snail venom. Other potential uses for compounds from the snail venom include drugs for neurological pain, epilepsy, heart disease, and stroke. Caribbean Sea sponge and coral might lead to the development of amazing future treatments including cancer and antibiotic resistant infections. This antibiotic strips bacteria of their protective biofilms, making them easier to kill. Scientists estimate that 65–80% of all bacterial infections are biofilm based.

Coho salmon fish have no thyroid glands, but they produce calcitonin hormones to regulate their own calcium levels from an endocrine gland in their neck. Humans secrete calcitonin hormone that inhibits bone loss in the thyroid gland. Calcitonin salmon is the generic name for a class of drugs, which include Miacalcin and Fortical, used to treat bone loss. Extra calcitonin can prevent such bone loss and promote bone density. The synthetic version of this calcitonin from the coho salmon, the calcitonin salmon, makes it into the final medical product for people with calcium regulation disorders.

Pygmy rattlesnake (found in the United States from North Carolina to Florida and west through Texas) venom has some startling properties; the venom leaves prey bleeding profusely, their blood unable to clot and could speed death for the prey of these small snakes; naturally occurring substances that can genuinely do harm, at different doses, could be drugs; this molecule from the rattlesnake venom was developed into eptifibatide, an antiplatelet drug that binds to platelets in the blood for a short time and prevents them from sticking together, or aggregating. Eptifibatide is used to treat people with advanced heart disease, particularly those at risk for sudden heart attack. The drug prevents blood clots, which can block arteries and cause heart attack and stroke, from forming. The horseshoe crab's blue blood (blue color due to copper) has its special properties that make the blood invaluable to modern medicine. A protein in the blood called Limulus Amebocyte Lysate (LAL) reacts to all kinds of microorganisms and can easily detect dangerous endotoxins that cause fever and can be fatal.

Farm animals became living pharmaceutical factories since February 2009 when the FDA approved a drug produced by a herd of bioengineered goats that had a human gene spliced into their DNA to produce antithrombin protein in the milk. Antithrombin is used to treat a rare human blood disease that can cause fatal blood clots during surgery or childbirth. A herd of 200 goats produce as much of the drug as 90,000 human blood donations. In other words, goats can produce more of the drug than previous methods (e.g., from donated human blood or genetically engineered cells) at a lower cost. A new set of bioengineered goats are expected to produce a new version of Rituxan, a cancer and arthritis drug now produced by Biogen Idec and Genentech.

The transgenic goats or spider-goats are very unique because they produce spider silk. Spider silk is referred to by many scientists as biosteel, spider silk has super tensile strength. Spider silk can be used to make artificial ligaments and tendons that support tissue, bone, and nerve cells, holding them steady while they grow. These artificial silk parts then fall apart gradually, after the cells have been given enough time to grow.

A good number of drugs are derived from porcine (swine, resembling a pig), bovine (includes domestic cattle, bison, African buffalo, the water buffalo, the yak, and the four-horned and spiral-horned antelopes), Chinese hamster ovary (CHO) cells (*Cricetulus griseus*, a small mouse originated from the deserts of northern China and Mongolia), murine (rats, mice), chicken, equine (horse), and many other domestic and wild animals. These are shown in Table 4.1.

Table 4.1 Medicines and pharmaceuticals of animal origin with source and functions

	Generic name	Functions
1. Porcine products		
Clexane	Enoxaparin	Anticoagulant, antithrombotics
Creon	Pancrelipase	Digestive supplements, cholelitholytics
Creon micro-enteric-coated granules	Pancrelipase	Digestive supplements, cholelitholytics
Curosurf	Poractant alfa	Respiratory agent
Ethical nutrients digestion plus	Herbal gastrointestinal preparations	–
Fragmin	Dalteparin	Anticoagulant
Heparin sodium injection	Heparin sodium	Anticoagulant
Heparinised saline	Heparin sodium	Anticoagulant
Heparinised saline injection	Heparin sodium	Anticoagulant
Organan	Danaparoid	Haemostatic agent
Panzytrat 25000	Amylase, lipase, pancrelipase, protease	Digestive supplement
Prothrombinex-VF	Antithrombin III, human; Factor II; V, VII, IX, X heparin, porcine	Haemostatic agent
Rotarix	Human rotavirus live, attenuated vaccine	Vaccine
Rota Teq	Rotavirus vaccine live oral pentavalent	Vaccine
Zostavax	Zoster virus vaccine live	Vaccine
2. Bovine products		
Blackmores immunodefence capsules	Immune supplement	
Calporo	Calporo	Herbal daily supplements
Cartilag	Cartilag	Herbal analgesics and anti-inflammatories
Ethical nutrients inner health plus capsules	Lactobacillus acidophilus, Bovine colostrum	Digestive supplements

(continued)

Table 4.1 (continued)

	Generic name	Functions
Ethical nutrients inner health plus powder	Lactobacillus acidophilus, Bovine colostrum	Digestive supplements
Gelofusine	Gelatin succinylated	
Haemaccel	Polygeline	Plasma volume expander
Hypurin isophane (NPH) injection	Insulin, isophane	Insulin preparations
Hypurin neutral injection	Insulin, neutral	Insulin preparations
Tisseel VH S/D solution	Aprotinin—Factor XIII—Fibrinogen, calcium chloride dihydrate—thrombin	Haemostatic agent
Travelan	Bovine colostrum	Anti-diarrhoeal
Varivax	Varicella zoster vaccine, live	Vaccines
Vivaxim	Hepatitis A vaccine; Salmonella typhi vaccine	Vaccines
Zyderm collagen implants	Collagen	Other dermatological preparations
Zyplast collagen implants	Collagen	Other dermatological preparations
Adacel	Pertussis vaccine, Diphtheria toxoid, Tetanus toxoid, Poliomyelitis vaccine.	Vaccine
Avaxim	Hepatitis A vaccine	Vaccine
Boostrix	Diphtheria toxoid, Tetanus toxoid, Pertussis vaccine	Vaccine
Boostrix—IPV suspension for injection	Diphtheria toxoid, Tetanus toxoid, Pertussis vaccine, Poliomyelitis vaccine	Vaccine
Engerix-B thiomersal free formulation suspension for injection	Hepatitis B vaccine	Vaccine
Havrix 1440	Hepatitis A vaccine	Vaccine
Havrix Junior	Hepatitis A vaccine	Vaccine
Hiberix	Haemophilus B conjugate vaccine	Vaccine
Merieux-inactivated rabies vaccine	Rabies vaccine	Vaccines
Prevenar	Pneumococcal vaccine	Vaccines
Priorix	Measles, mumps & rubella vaccine	Vaccines
Priorix-tetra	Varicella zoster vaccine, Rubella vaccine, mumps vaccine, measles vaccine	
Rabipur	Rabies vaccine	Vaccines

(continued)

Table 4.1 (continued)

	Generic name	Functions
Recombinate	Recombinant antihaemophilic factor	Haemostatic agents
Varivax	Varicella zoster vaccine, live	Vaccines
Fluarix	Influenza virus vaccine	Vaccine
ADT booster	Diphtheria toxoid	Vaccine
<i>3. Equine products</i>		
ATGAM	Antithymocyte globulin	Immunomodifier
Black snake antivenom	Black snake antivenom	Antivenom
Brown snake antivenom	Brown snake antivenom	Antivenom
Death adder antivenom	Death adder antivenom	Antivenom
Polyvalent snake antivenom	Antivenom of Brown, Death adder, King brown, Taipan, Tiger snakes	Antivenom
Premarin tablets	Oestrogens, conjugated	Gonadal hormone
Premarin for injection	Oestrogens, conjugated	Haemostatic agent
Premia	Medroxyprogesterone acetate	Gonadal hormone
Red back spider antivenom	Red back spider antivenom	Antivenom
Sea snake antivenom	Sea snake antivenom	Antivenom
Stonefish antivenom	Stonefish antivenom	Antivenom
Taipan antivenom	Taipan antivenom	Antivenom
Tiger snake antivenom	Tiger snake antivenom	Antivenom
Gonal-f	Follitropin alfa	Pituitary hormone
Granocyte	Lenograstim	Supportive therapy
Herceptin	Trastuzumab	Antineoplastic agent
Kogenate FS	Octocog alfa	Haemostatic agent
Luveris 75 IU	Lutropin alfa	Pituitary hormone
Mabcampath	Alemtuzumab	Antineoplastic agent
Mabthera	Rituximab	Antineoplastic agent
Metalyse	Tenecteplase	Fibrinolytic agent
Mircera	Methoxy polyethylene glycol-epoetin beta	Haemopoietic agent
NeoRecormon	Epoietin beta	Haemopoietic agent
Novicrit	Epoetin lambda	Haemopoietic agent
NovoSeven RT	Eptacog alfa	Haemostatic agent
Orencia	Abatacept	Immunomodifier
Ovidrel	Choriogonadotropin alfa	Pituitary hormone
Prolia	Denosumab	Affects calcium and bone metabolism
Puregon	Follitropin beta	Pituitary hormone
Pulmozyme	Dornase alfa	Respiratory agent
Rebif	Interferon beta-1a	Immunomodifier
Recombinate	Recombinate antihaemophilic factor	Haemostatic agent
Thyrogen	Thyrotrophin alfa	Diagnostic agent

(continued)

Table 4.1 (continued)

	Generic name	Functions
Vectibix	Panitumumab	Antineoplastic agents
Xgeva	Denosumab	Antineoplastic agent
Xolair	Omalizumab	Other respiratory agent
Xyntha	Moroctocog alfa	Haemostatic agent
<i>4. CHO cells products</i>		
Advate	Octocog alfa	Haemostatic agent
Aldurazyme	Laronidase	Enzyme replacement therapy
Aranesp	Darbepoietin	Haemopoietic agent
Avastin	Bevacizumab	Antineoplastic
Avonex	Interferon beta-1a	Immunomodifier
BeneFIX	Nonacog alfa	Haemostatic agent
Cerezyme	Imiglucerase	Enzyme replacement therapy
Elonva	Corifollitropin alfa	Pituitary hormones
Enbrel	Etanercept	Immunomodifier
Eprex	Epoietin-alfa	Haemopoietic agent
Eylea	Aflibercept	Ophthalmic medication
Fabrazyme	Agalsidase beta	Enzyme replacement therapy
Gonal-f	Follitropin alfa	Pituitary hormone
Granocyte	Lenograstim	Supportive therapy
Herceptin	Trastuzumab	Antineoplastic agent
Kogenate FS	Octocog alfa	Haemostatic agent
Luveris 75 IU	Lutropin alfa	Pituitary hormone
Mabcampath	Alemtuzumab	Antineoplastic agent
Mabthera	Rituximab	Antineoplastic agent
Metalyse	Tenecteplase	Fibrinolytic agent
Mircera	Methoxy polyethylene glycol-epoetin beta	Haemopoietic agent
NeoRecormon	Epoietin beta	Haemopoietic agent
Novicrit	Epoetin lambda	Haemopoietic agent
NovoSeven RT	Eptacog alfa	Haemostatic agent
Orencia	Abatacept	Immunomodifier
Ovidrel	Choriogonadotropin alfa	Pituitary hormone
Prolia	Denosumab	Affects calcium and bone metabolism
Puregon	Follitropin beta	Pituitary hormone

(continued)

Table 4.1 (continued)

	Generic name	Functions
Pulmozyme	Dornase alfa	Respiratory agent
Rebif	Interferon beta-1a	Immunomodifier
Recombinate	Recombinate antihaemophilic factor	Haemostatic agent
Thyrogen	Thyrotrophin alfa	Diagnostic agent
Vectibix	Panitumumab	Antineoplastic agents
Xgeva	Denosumab	Antineoplastic agent
Xolair	Omalizumab	Other respiratory agent
Xyntha	Moroctocog alfa	Haemostatic agent
<i>5. Murine products</i>		
Avastin	Bevacizumab	Antineoplastic agent
Erbitux	Cetuximab	Antineoplastic agent
Herceptin	Trastuzumab	Antineoplastic agent
Mabthera	Rituximab	Antineoplastic agent
Remicade	Infliximab	Immunomodifier
Reopro	Abciximab	Anticoagulant
Saizen	Somatropin	Pituitary hormone
Simponi prefilled syringe solution	Golimumab	Antirheumatoid agent
Simulect	Basiliximab	Immunomodifier
Synagis	Palivizumab	Immunomodifier
<i>6. Chicken eggs products</i>		
Agrippal	Influenza virus vaccine	Vaccine
Fluarix	Influenza virus vaccine	Vaccine
Fluvax	Influenza virus vaccine	Vaccine
Intanza	Influenza virus vaccine	Vaccine
Panvax H1N1 vaccine	H1N1 pandemic influenza vaccine	Vaccine
Q-Vax skin test	Coxiella burnetii vaccine	Vaccine
Rabipur	Rabies vaccine	Vaccine
Vaxigrip	Influenza virus vaccine	Vaccine
Influvac	Influenza virus vaccine	Vaccine
	Source	Use
<i>7. Other products</i>		
Bee pollen	Gathered by bees and collected from legs of bees	Has antimicrobial and antiviral properties; reduces inflammation, stimulates immune system; lowers cholesterol levels
Chitin	From insects and crustaceans	Antimicrobial, support healthy cholesterol levels and kidney function

(continued)

Table 4.1 (continued)

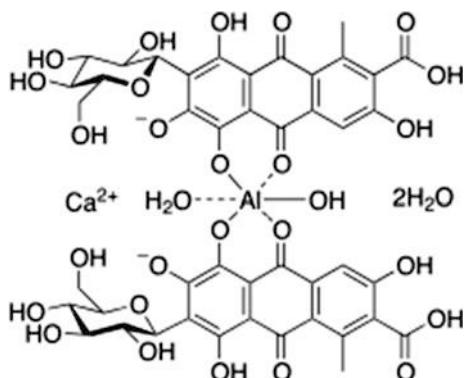
	Source	Use
Chymotrypsin Cochineal/ Carmine/Carminic acid	Ox pancreas	Chymotrypsin is taken by mouth to reduce liver damage in burn patients; assists in wound repair; inhaled or used topically to reduce pain, inflammation and infections
Cochineal/carmine/carmininc acid	Red pigment from crushed cochineal insects	Used for coloring food, fabric, and cosmetics
Disodium inosinate	From meat extract	Used as a food additive
Gelatin	From cows or pigs	Used for weight loss, for treating osteoarthritis, rheumatoid arthritis, etc.; for many capsules
Glycerol	May be derived from animal fats	Used in the pharmaceutical and toilet goods fields
Lactose	From cow's milk	Common filler in tablets
Lanolin	Fat extracted from sheep's wool	Used in medicine, cosmetics and toiletries; in the manufacture of vitamin D
Oleic oil and oleostearin	From pressed tallow	Used as an emulsifying agent, emollient, as an excipient in pharmaceuticals, and as an emulsifying or solubilizing agent in aerosol products; an insect pheromone
Propolis	Bee glue	Used for healing wounds
Shellac	Insect secretion	Used in the pharmaceutical industry as a tablet coating
Stearic acid	Fat from cows, sheep, dogs or cats Can be obtained from vegetable sources	Used in cosmetics and some medical applications
Trypsin	Enzyme from pork pancreas	Trypsin, an aggressive proteolytic enzyme. is used for the manufacture of human biological medicinal products

Help Source Guideline for the use of medicines/pharmaceuticals of animal origin, Department of Health, Queensland Government; Effective from: 11/01/2013, Document Number # QH-GDL-954:2013, pp 1–12. https://www.health.qld.gov.au/_data/assets/pdf_file/0024/147507/qh-gdl-954.p

4.1.1 Carmine

Carmine (crushed female cochineal insects) is also called cochineal, cochineal extract, crimson lake, carmine lake, C.I. 75470, E120, or natural red 4 (Dapson

Fig. 4.1 Carmine—the aluminium salt of carminic acid



et al. 2007). Carmine is a pigment of a bright red color obtained from some scale insects (e.g., Cochineal scale) and certain *Porphyrophora* species (Armenian cochineal and Polish cochineal). Chemically, it is an aluminum salt or the aluminum salt of the carminic acid (Fig. 4.1).

For preparation of carmine, the dried or powdered scale insect bodies are boiled in an ammonia or sodium carbonate solution or in water, the insoluble matter is removed by filtering, and alum is added to the clear salt solution of carminic acid to precipitate the red aluminium salt, called carmine lake or crimson lake (the term lake was from the word lac indicating a resinous secretion). The purity of color is ensured by the absence of iron. Aluminum gives the traditional crimson color and this color may be degraded by the presence of iron salts; the addition of lime (calcium) can give carminic acid lakes a purple cast.

Carmine was used in dyeing textiles and in painting since antiquity. Carmine is used in the manufacture of artificial flowers, paints, crimson ink, rouge and other cosmetics, and some medications (Greenhawt et al. 2009). Carmine is used in foods, pharmaceuticals, toiletries, etc., as a dye, and also has use as a microscopic stain and biological marker. The pharmaceutical industry uses cochineal to color pills and ointments. It can be used as a staining agent in histology (to stain glycogen, acidic mucopolysaccharides, and cell nuclei). It is routinely added to food products such as yogurt, candy, and certain brands of juice, the most notable ones being those of the ruby-red variety. As a food dye, it has been known to cause severe allergic reactions and anaphylactic shock in some people (Tabar et al. 2003; Greig 2012), but the FDA has not banned the use of carmine and stated that it found no evidence of a significant hazard to the general population (Anonymous 2006); it is also included in the list of EU-approved food additives (Anonymous 2012).

4.1.2 Gelatin

Gelatin (or gelatine) is an animal protein prepared by the thermal denaturation (partial hydrolysis) of collagen from animal skin, ligaments, tendons, and bones with very dilute acid. It can also be extracted from fish skins. It is a heterogeneous mixture of protein fractions consisting of single or multi-stranded polypeptides (Fig. 4.2). Gelatin, the partially hydrolyzed collagen is usually bovine (beef) or porcine (pig) in origin. Type A gelatin is derived from pig skin by means of acid hydrolysis and type B gelatine from alkaline hydrolysis of cattle hides and bones.

Many prescription medications are prepared in capsule form and gelatin is used in making capsule shells or pharmaceutical excipients. Due to religious belief, patients may have a strict choice for one or another type of gelatin source, bovine (beef) or porcine (pig) origin. For this, they could seek product information (PI) or consumer medicine information (CMI) from their pharmacist or doctor. The PI gives details on the composition of the medicine, e.g., active and inactive constituents and provides a brief description on how the medicine is produced, e.g., whether the manufacture of the product included exposure to animal-derived materials. Consumer medicine information (CMI) leaflets are available for most prescription medicines which enable patients to check the medicine's ingredients. Some gelling agents of plant origin, often called vegetable gelatins such as pectin, are carbohydrate-based and not chemically related to animal gelatins.

4.1.3 Glycerol

Glycerol, glycerin, or glycerine may be obtained from cow or pig tallow. Plant-derived glycerin may be an alternative and the typical plant sources include soybeans, palm, or seaweed. Glycerol is a simple polyol (trihydric alcohol), colorless, odorless, viscous, water-soluble (due to hydroxyl groups) nontoxic liquid,

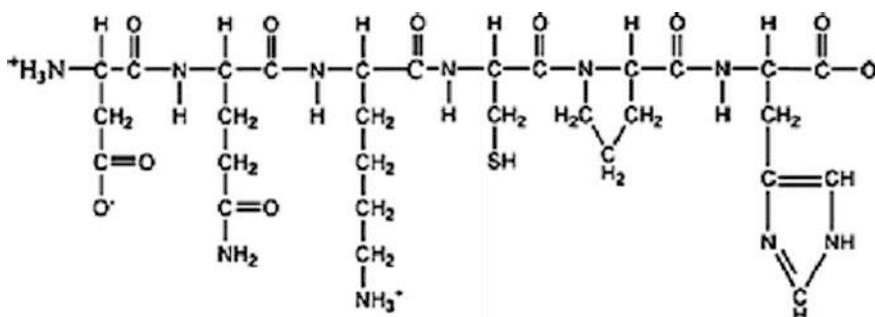
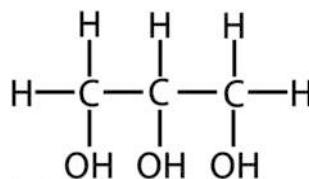


Fig. 4.2 Gelatin—chain of polypeptides

Fig. 4.3 Structure of glycerol

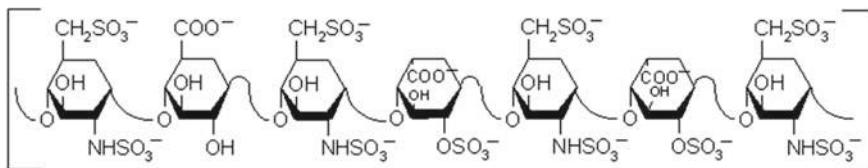


and sweet in taste (Fig. 4.3). The glycerol backbone is found in all lipids known as triglycerides. It is widely used in the food industry as a sweetener and humectant (hygroscopic) and in pharmaceutical formulations. Glycerol has three hydroxyl groups that are responsible for its solubility in water and its hygroscopic nature. Glycerol is used in medical, pharmaceutical, toiletries, and cosmetic industries mainly as a means of improving smoothness, providing lubrication, and as a humectant. It is found in allergen immunotherapies, cough syrups, elixirs and expectorants, toothpaste, mouthwashes, skin care products, shaving cream, hair care products, soaps, and water-based personal lubricants. In solid dosage forms like tablets, glycerol is used as a tablet-holding agent. For human consumption, glycerol is classified by the U.S. FDA among the sugar alcohols as a caloric macronutrient.

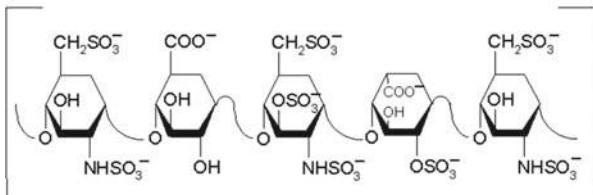
4.1.4 Heparin

Heparin, an injectable anticoagulant medication, is derived from bovine (lungs) and porcine (intestines) sources. Heparin chains are composed of long fully sulfated segments interrupted with undersulfated domains (Fig. 4.4). Heparin is a mucopolysaccharide with a molecular weight ranging from 6000 to 40,000 Da; the average molecular weight of most commercial heparin preparations is being in the range of 12,000–15,000. The polymeric chain is composed of repeating disaccharide unit of D-glucosamine and uronic acid linked by 1→4 interglycosidic bond. The uronic acid residue could be either D-glucuronic acid or L-iduronic acid. The sulfated hydroxyl groups on each of these monosaccharide residues give rise to highly negatively charged polymer. The average negative charge of individual saccharide residues is about 2.3. Because of its highly acidic sulfate groups, heparin exists as the anion at physiologic pH and is usually administered as the sodium salt. Heparin is relatively nontoxic. Composition in terms of disaccharidic sequences is considerably heterogeneous.

The key structural unit of heparin is a unique pentasaccharide sequence (below). This sequence consists of three D-glucosamine and two uronic acid residues. The central D-glucosamine residue contains a unique 3-O-sulfate moiety that is rare outside of this sequence. Four sulfate groups on the D-glucosamines are found to be critical for retaining high anticoagulant activity, partial elimination (any one of them) reduces the anticoagulant activity while removal of the unique 3-O-sulfate



Fully sulfated heparin



Partially desulfated heparin

Fig. 4.4 Structure of fully sulfated (top) and partially desulfated (bottom) heparin

group results in complete loss of the anticoagulant activity. Removal of sulfate groups other than the critical ones seems to not affect the anticoagulant activity.

Heparin is partially metabolized in the liver by heparinase to uroheparin, which has only slight antithrombin activity. Twenty to fifty percent is excreted unchanged. The heparin polysaccharide chain is degraded in the gastric acid and must, therefore, be administered intravenously or subcutaneously. Heparin should not be given intramuscularly because of the danger of hematoma formation. It is generally given to postoperative patients and to those with acute infarctions requiring immediate anticoagulant action. Heparin overdose or hypersensitivity may result in excessive bleeding. Protamines, highly positively charged low-molecular weight proteins, are used as antidote for excessive bleeding complications.

4.1.5 Insulin

Insulin (*from the Latin, insula meaning island*) is a 51-amino acid peptide hormone produced by beta cells of the pancreatic islets, and it is considered to be the main anabolic hormone of the body. Insulin is composed of two long amino acid chains or polypeptide chains referred to as the A chain of 21 amino acids and B chain of 30 amino acids (Fig. 4.5). Both chains contain alpha helices and there are 3 conserved disulfide bridges which keep the two chains together; A and B chains are linked together covalently by two disulfide bonds (residues A7 to B7, and A20 to B19) and an additional internal disulfide bond or third cysteine bridge is formed within the A chain (residues A6 to A11). These joints are similar in all mammalian forms of insulin. The structure of insulin is different among different species of animals.

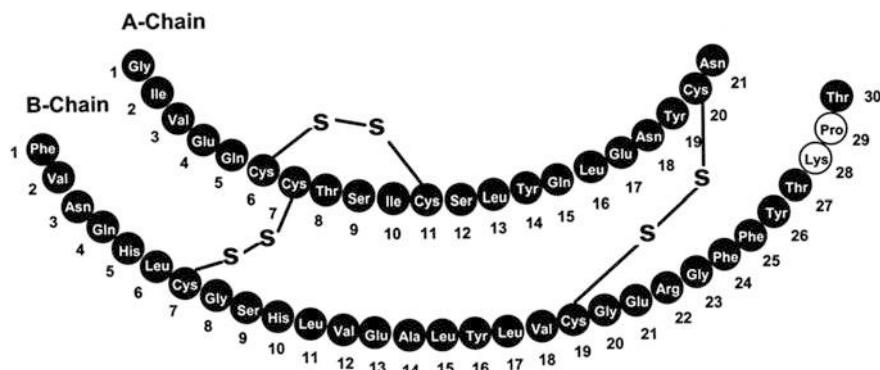


Fig. 4.5 Insulin structure. Source <https://pharmafactz.com/pharmacology-of-insulin-analogues/>

Human insulin is closest in structure and function with bovine or porcine insulin. Bovine insulin differs from human in only three amino acid residues, and porcine insulin in one. Recombinant DNA technology scientists inserted human insulin gene into the plasmid of a bacterial strain (genetically transformed), which could now produce the commercial peptide hormone (insulin).

Insulin regulates the metabolism of carbohydrates, fats, and protein by promoting the absorption of, especially, glucose from the blood into fat, liver, and skeletal muscle cells. Insulin from some invertebrates and even fish can be clinically useful in humans as they possess several similarities.

Biologically active is monomeric or exists as a single molecule. Insulin can form dimers (two molecules) in solution due to the hydrogen bonding between the B chains (shown as white lines). The dimers can further interact to form hexamers (three molecules) due to the interaction between hydrophobic surfaces. This scene highlights the hydrophobic and polar parts of an insulin monomer at a pH of 7. Figure 4.6 shows the structures of proinsulin (a), human insulin monomer (b), dimer (c), and hexamer (d). In b-d: green, A chain(s); magenta: B chain(s). In a, the C-peptide within proinsulin is indicated in blue.

Regular insulin has a nature of self-association and regular insulin is found in a self-associated hexameric form (Fig. 4.7). To be absorbed by the capillary the hexameric form must dissociate to dimer and then monomer. This dissociation process delays the onset of action from 0.5 to 1 h, may not be peak for 4 h and duration of action of 8 h. So they should be taken 30 min before meal. As their duration of action is prolonged, it reaches peak concentration 2 h after injection (in many cases and depending on the dose it may peak 4–6 h after injection) when blood glucose level already may be low.

Glucose production and secretion by the liver is strongly inhibited by high concentrations of insulin in the blood. Circulating insulin also affects the synthesis of proteins in a wide variety of tissues, an anabolic hormone that promotes the conversion of small molecules in the blood into large molecules inside the cells. Low insulin levels in the blood have the opposite effect by promoting widespread

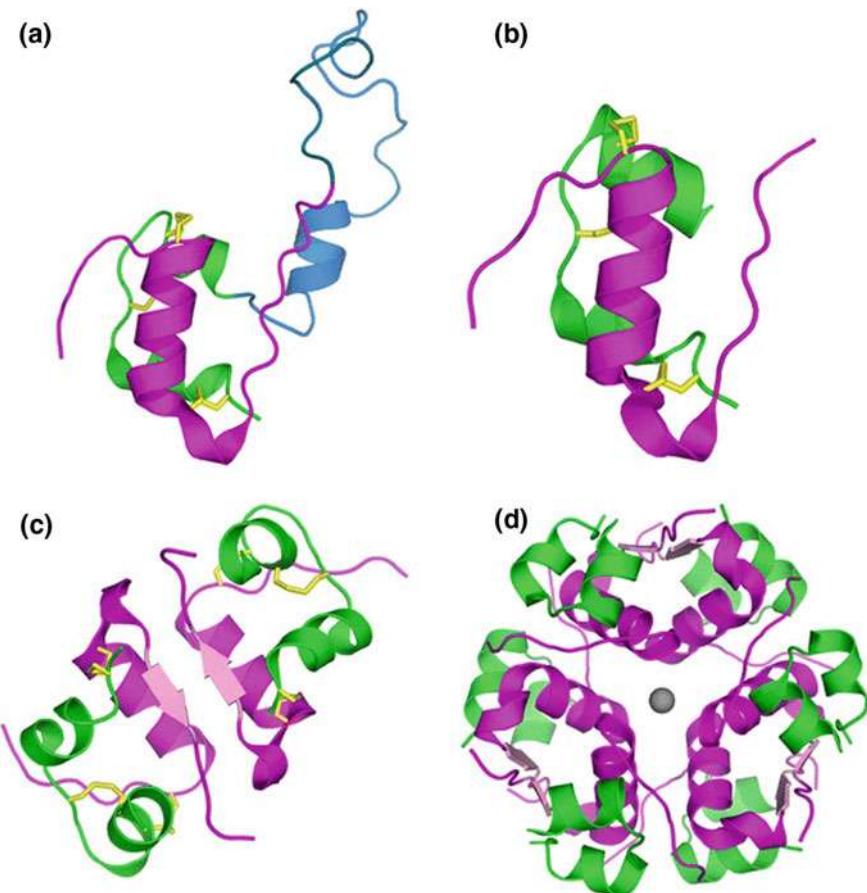


Fig. 4.6 Structures of proinsulin (a), human insulin monomer (b), dimer (c), and hexamer (d). In b–d: green, A chain(s); magenta: B chain(s). In a, the C-peptide within proinsulin is indicated in blue. Source https://www.researchgate.net/publication/262681370_The_Evolution_of_Insulin_Glargine_and_its_Continuing_Contribution_to_Diabetes_Care/figures?lo=1

catabolism. Beta cells are sensitive to glucose concentrations, also known as blood sugar levels. When the glucose level is high, the beta cells secrete insulin into the blood; when glucose levels are low, secretion of insulin is inhibited. Their neighboring alpha cells, by taking their cues from the beta cells, secrete glucagon into the blood in the opposite manner: increased secretion when blood glucose is low and decreased secretion when glucose concentrations are high. The secretion of insulin and glucagon into the blood in response to the blood glucose concentration is the primary mechanism of glucose homeostasis (Fig. 4.8).

In type 1 diabetes, the pancreatic cells do not release insulin, resulting in high blood sugar levels and increased fat metabolism. Consequently, there is “spillover” of glucose into the urine, and weight loss due to the loss of body fat stores.

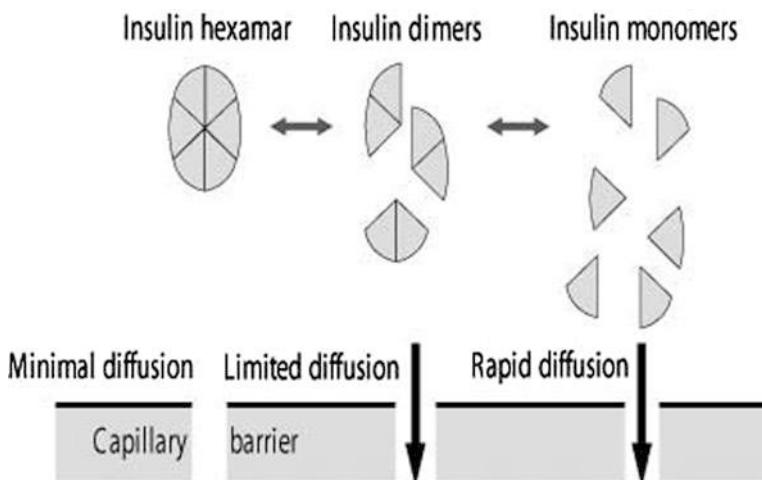


Fig. 4.7 Regular insulin in hexameric form. This form needs to become the monomeric form for diffusion and absorption from the subcutaneous or intramuscular area. Source http://www.orion-group.net/journals/Journals/vol28_01_September2007/498.htm

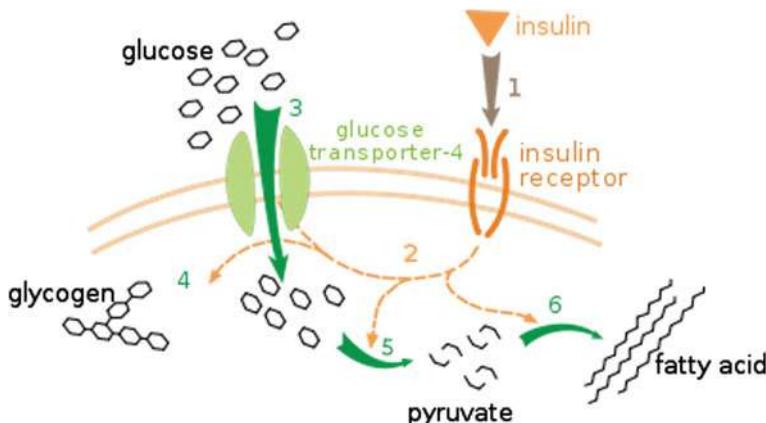
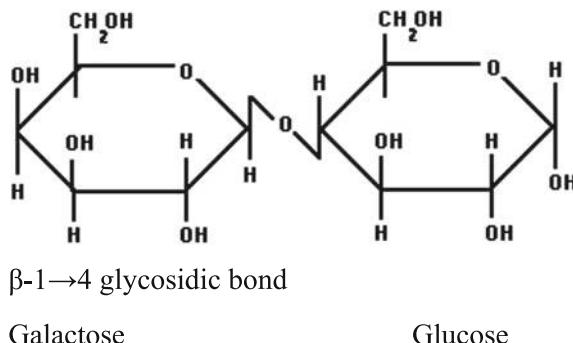


Fig. 4.8 Effect of insulin on glucose uptake and metabolism: Insulin binds to its receptor (1), which starts many protein activation cascades (2). These include translocation of the Glut-4 transporter to the plasma membrane and influx of glucose (3), glycogen synthesis (4), glycolysis (5), and triglyceride synthesis (6)

4.1.6 Lactose

Lactose or milk sugar is a disaccharide sugar composed of D-galactose and D-glucose (Fig. 4.9). Lactose makes up around 2–8% of milk, although the amount varies among species and individuals, and milk with a reduced amount of lactose

Fig. 4.9 Lactose (β -D-galactopyranosyl-(1 \rightarrow 4)-D-glucose)



also exists. It is an extremely common sugar of mammal milk; an alternative is plant milk sugars. The glucose and galactose are then absorbed by the cells lining the small intestine. The enzyme lactase, located on the surface of the cells lining the small intestine, splits lactose into glucose and galactose and the split lactose is absorbed from the intestine into the body in the form of glucose and galactose.

Deficiency (or absence) of lactase, when the intestine is partially or totally incapable of splitting lactose for digestion, causes lactose intolerance, a common medical condition that results in diarrhea, abdominal pain, and gas (flatulence) and is caused by the reduced or absent activity of enzyme lactase. Lactose is found mainly in milk and milk products but not in all dairy products such as cheese. Live culture yogurts, which contain lactose, also contain organisms that break it down, and hence neither hard cheeses nor live culture yogurts are likely to evoke the symptoms of lactose intolerance.

Lactose is used in food, beverage, and pharmaceutical industries as a carrier and stabilizer of aromas. In pharmaceutical industry, lactose is widely used as a filler, filler-binder, running powder, and diluent in tablets and capsules, and to a more limited extent in lyophilized products, infant feed formulas, and a diluent in dry-powder inhalations. Lactose is added to pills as a filler because of its physical compressibility property and low price, and can be used to dilute heroin. Lactose is a widely used as an excipient because of its cost-effectiveness, easy availability, bland taste, low hygroscopicity, compatibility with active ingredients and other excipients, excellent physical and chemical stability, and water solubility.

4.1.7 Lanolin

Lanolin, wool wax or wool grease, is a yellow waxy substance secreted by the sebaceous glands of wool-bearing animals. The oil glands of sheep are the source of this ingredient. Historically, many pharmacopeias have referred to lanolin as wool fat; but it is not a true fat because lanolin lacks glycerides (glycerol esters), instead, it primarily consists of sterol esters. A typical high purity grade of lanolin is

composed predominantly of long chain waxy esters, hydroxy esters, diesters (approximately 97% by weight), the remainders are lanolin alcohols, lanolin acids, and lanolin hydrocarbons.

Lanolin used by humans comes from domestic sheep breeds that are raised specifically for their wool. Crude lanolin constitutes about 5–25% of the weight of freshly shorn wool. Lanolin is extracted by scouring the wool in hot water with a detergent to remove dirt, wool grease (crude lanolin), suint (sweat salts), and anything else stuck to the wool. The wool grease is continuously removed during this washing process by centrifugal separators, which concentrate it into a wax-like substance melting at approximately 38 °C.

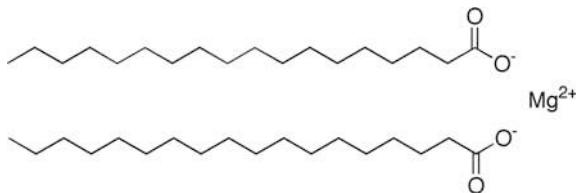
Lanolin and its many derivatives are used extensively in both the personal care (e.g., in high-value cosmetics, facial cosmetics, lip products, etc.) and healthcare sectors, e.g., topical liniments and in the protection, treatment, and beautification of human skin. This medication is used as an emollient (softener and moisturizer) to treat or prevent dry, rough, scaly, itchy, and flaking skin and minor skin irritations (e.g., diaper rash, skin burns from radiation therapy, and others). It is frequently used in protective baby skin treatment and to treat sore and cracked nipples caused by breast feeding in breast-feeding mothers. It is an ingredient in some ophthalmic drugs and is used as a carrier in certain drugs that are given by injection. Lanolin is also found in eye drops because it has antibacterial properties and can protect against dry eyes (plant oils are an alternative). Lanolin is often used as a raw material for producing Vitamin D₃ using irradiation.

Lanolin is widely used in ointment bases, burns dressings, and wound sprays; as an emulsifier, stabilizer, and emollient; to support the wound healing process; to deliver active ingredients through the skin (transdermal); pigmented medications (e.g., zinc oxide), as a dispersing agent; topical products for cutaneous infections (e.g., acne) and in deodorizing toiletries, as an antimicrobial and disinfectant; ophthalmic ointments, as an emollient with high physiological compatibility and low irritation potential; suppositories substantial base, as a carrier for active ingredients; surgical adhesive tapes, as an impregnating agent, plasticiser, and skin-suited stack enhancer; chewing gum bases as a food additive (physiologically compatible emollient); pre-blended combinations for specific purposes, such as absorption bases, etc. Lanolin is also found in lubricants, rust-preventive coatings, shoe polish, and other commercial products.

4.1.8 *Magnesium Stearate*

Magnesium stearate, a chemical compound with the formula: Mg(C₁₈H₃₅O₂)₂, is a solid, white powder at room temperature. It is a soap, consisting of salt containing two equivalents of stearate (the anion of stearic acid) and one magnesium cation (Mg²⁺) (Fig. 4.10). The stearate portion of magnesium stearate is a form of stearic acid, which is a saturated fat found in beef, pork, coconut oil, cocoa butter, butter, chicken, fish, milk, cottonseed, grains, and other foods. Depending on the source of

Fig. 4.10 Structure of magnesium stearate



stearate, this medication ingredient may be animal or plant derived. Magnesium stearate is prepared from stearic acid, a fatty acid, usually. Its applications exploit its softness, insolubility in many solvents, and low toxicity. It is used as a release agent and as a component or lubricant in the production of pharmaceuticals and cosmetics. Magnesium stearate is recognized as safe by the FDA for use in the pharmaceutical industry as a coating of supplements and diluents (filler) for the manufacture of tablet, capsule, and powder dosage forms.

Medications are usually manufactured using magnesium stearate to prevent them from sticking together and from sticking to the equipment. Magnesium stearate is often used as an anti-adherent in the manufacture of medical tablets, capsules, and powders, most commonly used lubricant for tablets as a functional excipient used to ensure efficient ejection of tablets. Magnesium stearate can also be used efficiently in dry coating processes. Magnesium stearate is also used to bind sugar in hard candies like mints, and is a common ingredient in baby formulas. As a lubricant, magnesium stearate is useful for capsules and tablets in industry (prevents pharmaceutical ingredients from adhering to industry equipment).

4.1.9 Premarin

Premarin, a conjugated estrogen product, is the brand name for an estrogen medication (Fig. 4.11). This comes from the urine of pregnant mares. Conjugated estrogens or conjugated equine estrogens (CEEs) are composed of a mixture of the water-soluble salts of sulfate esters from estrone (52–62%), equilin (22–30%), 17 α -dihydro equilin, 17- α -estradiol, and 17- β -dihydro equilin, etc., steroids that are purified from pregnant horse urine. The potency of the preparation is expressed in terms of an equivalent quantity of sodium estrone sulfate. Estrogens are important in the development and maintenance of the female reproductive system and secondary sex characteristics.

Premarin is a form of hormone replacement therapy (HRT). It is useful for the treatment of menopausal symptoms, most commonly in postmenopausal women who have had a hysterectomy to treat hot flashes, and burning, itching, and dryness of the vagina and surrounding areas. Other uses include prevention of osteoporosis in postmenopausal women, and replacement of estrogen in women with ovarian failure or other conditions that cause a lack of natural estrogen in the body.

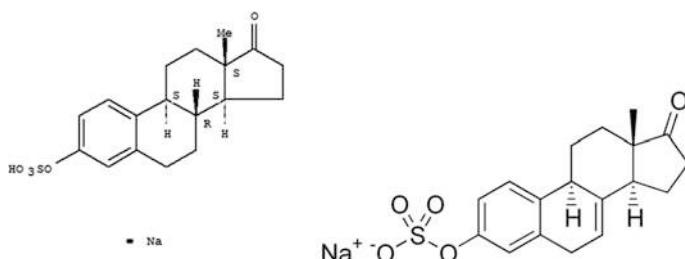


Fig. 4.11 Chemical structure of the sodium salt of estrone and equilin sulfate, two constituents of premarin

Premarin is sometimes used as part of cancer treatment in women and men. Premarin is available in oral (0.3, 0.625, 0.9, 1.25, or 2.5 mg pills), intravenous, and vaginal (cream) forms. Use of premarin includes some risk factors.

4.1.10 Vaccines

Vaccine is a biological preparation that produces immunity (acquired immunity) to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism (e.g., bacteria or viruses, or components of these) and is often made from weakened or killed forms of the microbe, its toxins, or one of its surface proteins. It stimulates the body's immune system to recognize the agent as a threat, destroy it, recognize and destroy any of these microorganisms that it later encounters. Vaccines can be prophylactic, or therapeutic (Frazier 2014; Brotherton 2015; Melief et al. 2015; Bol et al. 2016). Vaccine can be administered through needle injections, by mouth, or by aerosol and administration of vaccines is called vaccination. Vaccination is the most effective method of preventing infectious diseases; the effectiveness of vaccination has been widely studied and verified, e.g., the influenza vaccine, the HPV vaccine, and the chicken pox vaccine (Chang et al. 2009; Fiore et al. 2009; Liesegang 2009). The World Health Organization (WHO 2012) reports that licensed vaccines are currently available for 25 different preventable infections (Table 4.2). Animal products in vaccine include gelatin, chicken embryo, guinea pig embryo cells, bovine serum albumin or fetal calf serum and serums.

Vaccination is one of the most successful and cost-effective public health interventions of all. It has eradicated smallpox, lowered the global incidence of polio by 99% since 1988 and achieved dramatic reductions in diseases such as measles, diphtheria, whooping cough (pertussis), tetanus, and hepatitis B. There are different types of vaccines based on what works best to prevent the disease and how the vaccine is made, e.g., live, attenuated vaccines, inactivated vaccines, subunit vaccines, toxoid vaccines, conjugate vaccines, DNA vaccines, recombinant vector

Table 4.2 Types of vaccines for different preventable infections

Vaccine type	Vaccines for immunization against diseases
Live, attenuated	Measles, mumps, rubella (MMR combined vaccine) Varicella (chickenpox) Influenza (nasal spray) Rotavirus Zoster (shingles) Yellow fever
Inactivated/killed	Polio (IPV) Hepatitis A Rabies
Toxoid (inactivated toxin)	Diphtheria, tetanus (part of DTaP combined immunization)
Subunit/conjugate	Hepatitis B Influenza (injection) <i>Haemophilus influenza</i> type b (Hib) Pertussis (part of DTaP combined immunization) Pneumococcal Meningococcal Human papillomavirus (HPV)

vaccines, etc. Vaccines are made using several different processes. They may contain live viruses that have been attenuated (weakened or altered so as not to cause illness), inactivated or killed organisms or viruses, inactivated toxins (for bacterial diseases where toxins generated by the bacteria, and not the bacteria themselves, cause illness); or merely segments of the pathogen (this includes both subunit and conjugate vaccines).

The injected cholera vaccines are effective for people living where cholera is common. Oral vaccines were first introduced in the 1990s, they are generally safe and two or three doses are typically recommended (WHO 2017). Two distinct types of oral cholera vaccine have been developed: those consisting of live, attenuated bacteria and those consisting of killed (inactivated) bacterial cells (bacterium *Vibrio cholerae*). The vaccine that the FDA recommends is an oral-attenuated live vaccine that is effective as a single dose (FDA 2016). In some cases, the latter are combined with the purified recombinant DNA-derived B subunit of the cholera toxin. The cholera toxin (CTX or CT) is an oligomeric complex made up of six protein subunits: a single copy of the A subunit (part A) and five copies of the B subunit (part B), connected by a disulfide bond.

Some vaccines contain inactivated microorganisms that have been destroyed with chemicals, heat, or radiation (e.g., polio vaccine, hepatitis A vaccine, rabies vaccine, and some influenza vaccines); some vaccines contain live, attenuated microorganisms (viruses with disabled virulent properties, or closely related less dangerous organisms) to produce a broad immune response. Most attenuated vaccines are viral (e.g., yellow fever, measles, mumps, and rubella,), some are bacterial in nature (e.g., typhoid). A toxoid (anatoxin or anatoxin) is a bacterial toxin (an exotoxin) whose toxicity has been inactivated or suppressed either by

chemical (formalin) or heat treatment, while immunogenicity is maintained. Toxoid-based vaccines include tetanus, diphtheria, and botulism. Not all toxoids are for microorganisms, e.g., *Crotalus atrox* toxoid is used to vaccinate dogs against rattlesnake bites. Subunit vaccine includes a protein fragment to create an immune response, e.g., the subunit vaccine against Hepatitis B virus is composed of only surface proteins of the virus, the virus-like particle (VLP) vaccine against human papillomavirus (HPV) that is composed of the viral major capsid protein, and the hemagglutinin and neuraminidase subunits of the influenza virus. Subunit vaccine is being used for plague immunization. A conjugate vaccine is created by covalently attaching a poor antigen (bacterial polysaccharide outer coats) to a strong antigen (protein toxins) thereby eliciting a stronger immunological response to the poor antigen. This approach is used in the *Haemophilus influenzae* type B vaccine. Once the genes from a microbe have been analyzed, scientists could attempt to create a DNA vaccine against it. It could not cause the disease because it would not contain the microbe, just copies of a few of its genes that code for those all-important antigens; this is relatively easy and inexpensive to design and produce. DNA vaccines take immunization to a new technological level. Recombinant vector vaccines are experimental vaccines similar to DNA vaccines, but they use an attenuated virus or bacterium to introduce microbial DNA to cells of the body.

Influenza vaccines prevent or mitigate infections. They are designed to induce a protective immune response in the body against the viruses represented in the vaccine. Antivirals are drugs that can treat people who have already been infected by a virus. Antibiotics are medicines that interfere with the reproduction of bacteria and are, therefore, only useful for treating bacterial infections.

Vaccines contain an active component (the antigen) which induces the immune response (a modified form of the pathogen, e.g., virus, bacterium or their part or toxin that no longer causes the disease or replicates but elicits an immune response from the body is used as antigen). In addition, they may also contain additional components such as preservatives (thiomersal, phenoxyethanol, phenol), additives (neomycin and/or polymyxin B), diluents (sterile water or sterile saline water), adjuvants (various aluminium salts), stabilizers (lactose and sucrose, glycine and monosodium glutamate, human or bovine serum albumin, gelatin of bovine or porcine), and traces of other components (cell culture fluids, egg proteins, yeast, antibiotics, or inactivating agents).

Prior to the advent of cell culture, viruses could be propagated only on whole organisms, animal, or plants. Whole organisms could include the natural host and laboratory animals such as chicken embryonated eggs, rabbits, mice, rats, and others. The development of cell culture techniques in the 1950s opened the door to the manufacturing of a wide range of biological pharmaceutical products at industrial scale. Continuous cell lines (CCLs) have been used for the production of safe and effective biotherapeutics and vaccines since the 1970s. Vaccines for polio, measles, mumps, rubella, chickenpox, Rotavirus, and HPV are currently manufactured using cell cultures.

4.1.11 Chitosan

Chitosan is a linear polysaccharide composed of randomly distributed β -(1 \rightarrow 4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) (Fig. 4.12).

It is made by treating the chitin shells of shrimp and other crustaceans with an alkaline substance, like sodium hydroxide. Chitosan is produced commercially by deacetylation of chitin, the structural element in the exoskeleton of crustaceans (e.g., crabs and shrimp) and cell walls of fungi. The degree of deacetylation (%DD) in commercial chitosans ranges from 60 to 100%.

Chitosan has a number of commercial and possible biomedical uses. It can be used in agriculture as a seed treatment and biopesticide, helping plants to fight off fungal infections. In winemaking, it can be used as a fining agent, also helping to prevent spoilage. In industry, it can be used in a self-healing polyurethane paint coating. In medicine, it may be useful in bandages to reduce bleeding and as an antibacterial agent; it can also be used to help deliver drugs through the skin.

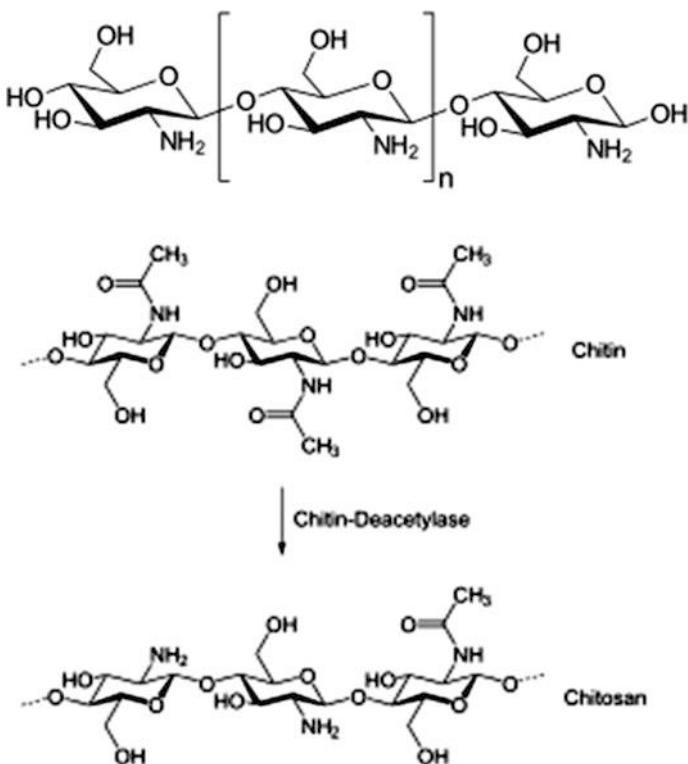


Fig. 4.12 Showing structure of chitosan (above) and its formation by partial deacetylation of chitin (below)

Chitosan properties allow it to rapidly clot blood, and it has recently gained approval in the United States and Europe for use in bandages and other hemostatic agents (Ducheyne et al. 2011; Zhang et al. 2015). Chitosan hemostatic products have been shown in testing by the U.S. Marine Corps to quickly stop bleeding and to reduce blood loss, and result in 100% survival of otherwise lethal arterial wounds in swine (Brown Mark et al. 2009). Chitosan hemostatic products reduce blood loss in comparison to gauze dressings and increase patient survival (Pusateri Anthony et al. 2003). The hemostatic agent works by an interaction between the cell membrane of erythrocytes (negative charge) and the protonated chitosan (positive charge) leading to the involvement of platelets and rapid thrombus formation (Brotherton 2015). Burns are similar to other wounds but are problematic because they are associated with membrane destabilization, energy depletion, and hypoxia, all of which can cause severe tissue necrosis if not treated properly or quickly enough. Chitosan-gelation bandages using nanofibrin have been shown to be more durable than ointments, while still allowing gas exchange at the cell surface (Sudeesh Kumar et al. 2014). Chitosan is hypoallergenic and has natural antibacterial properties, which further support its use in field bandages. Chitosan's hemostatic properties also allow it to reduce pain by blocking nerve endings. Chitosan has been the subject of interest for its use as a polymeric drug carrier material in dosage form design due to its appealing properties such as biocompatibility, biodegradability, low toxicity, and relatively low production cost from abundant natural sources. Chitosan's properties also allow it to be used in transdermal drug delivery; it is mucoadhesive in nature, reactive (so it can be produced in many different forms), and most importantly, has a positive charge under acidic conditions. So, chitosan can be used to transport a drug to an acidic environment, where the chitosan packaging will then degrade, releasing the drug to the desired environment (Sadigh-Eteghad et al. 2013). One example of this drug delivery has been the transport of insulin (Agnihotri et al. 2004). Chitosan can also be combined with other materials, e.g., a composite with hydroxyapatite was effective as temporary post-operation bone filler, which was gradually biodegraded and replaced by native bone tissue (Danilchenko et al. 2009).

4.2 Bioactive Compounds and Excipients from Marine Organisms

Nature has been the traditional source of new pharmaceuticals. Representatives of every phylum are found in the sea; 12 phyla are exclusively marine. Marine environment represents an ecological resource comprising of a diverse group of aquatic plant and animal resources for new drug development including cancer or malaria. Environmental pressures, competition for space, nutrition, and self-defense have led to the production of a diverse array of secondary metabolites for inter-communication within their environment. Many of them have been screened for

antibacterial, immunomodulator, antifungal, anti-inflammatory, anticancer, antimicrobial, neuroprotective, analgesic, and antimalarial properties. Marine pharmacology offers the scope for research on these drugs of marine origin. Many different marine organisms have been explored for bioactive compounds such as vertebrates (fish, sharks, snakes, mammals, etc.); invertebrates (sponges, coelenterates, echinoderms, corals tunicates, mollusks, bryozoans, etc.); plants including algae and microorganisms including bacteria, fungi, *cyanobacteria*, etc. (Chakraborty et al. 2009). Seagrasses are also potential source of natural antibacterial, antioxidant, and anti-inflammatory agents (Yuvaraj et al. 2012). The share of marine biota as well as their contribution to life-saving drugs production (in %) may be as sponges (37%), coelenterates (21%), and microorganisms (18%) are the major sources of biomedical compounds followed by algae (9%), echinoderms (6%), tunicates (6%), mollusks (2%), bryozoans (1%), etc. (Blunt et al. 2004). The marine organisms produce an innumerable number of diverse bioactive metabolites (Donia and Hamann 2003; Haefner 2003) and search for new metabolites from marine organisms has resulted in the isolation of more or less 10,000 metabolites (Fusetani 2000), many of which are endowed with pharmacodynamic properties (Jha and Zi-rong 2004). Marine sediments are also now recognized as a rich source of microbial taxonomic diversity and new biologically active compounds. The potent medicinal usage of the bioactive compounds, viz., steroids, terpenoids, isoprenoid and non-isoprenoid compounds, quinones, brominated compounds, nitrogen heterocyclics, and nitrogen-sulfur heterocyclics from marine non-chordates, chordates, seaweeds, etc., have been discovered in recent years. Marine poriferans, cnidarians, annelids, arthropods, mollusks, and echinoderms could be rich sources of therapeutic agents having antibacterial, anti-inflammatory, anticarcinogenic properties.

The successes to date in the discovery of novel chemicals from marine organisms that have demonstrated potential as new treatments for cancer, infectious diseases, and inflammation, suggest that there needs to be a greater focus on the development of drugs from marine sources for the twenty-first century.

4.2.1 Major Marine Invertebrates and Their Bioactive Compounds

The anticancer drug, Ara-C, is used to treat acute myelocytic leukemia and non-Hodgkin's lymphoma. The antiviral drug, Ara-A, is used for the treatment of herpes infections (McConnell et al. 1994). Both are derived from nucleosides isolated from a shallow-water marine sponge collected off the coast of Florida, the only two marine-derived pharmaceuticals that are clinically available today. Bryostatin, isolated from the bryozoan *Bugula neritina*, is a polyketide with both anticancer and immune-modulating activity (Kalechman et al. 1992; Suffness et al. 1989; Philip et al. 1993; Pettit et al. 1982).

(i) Marine sponges are among the most prolific sources of diverse chemical compounds with therapeutic potential and out of the >5000 chemical compounds derived from marine organisms, more than 30% have been isolated from sponges (Ireland et al. 1993). Porifera (e.g., sponges *Cryptothecaa crypt*, *Discodermia dissolute*, *Hemiasterella minor*) contain manzamine, phenolic or quinoid, alkaloids, terpenoids, tryptamines, etc., and these are effective as antimalarial, antiviral especially AIDS, antibacterial, antifungal, anticancer, etc. (McConnell et al. 1994; Edrada et al. 1996; Ang et al. 2000; Ravichandran et al. 2007; Hardoim and Costa 2014); (ii) Coelenterata (the term now encompassing the phyla Cnidaria—hydra, coral animals, true jellies, sea anemones, sea pens, and their allies, and Ctenophora—comb jellies) contain postaglandins, proteins, enzymes, steroids, terpenoids, brominated alkaloids, macrolides, and ceramides and these are effective as antibacterial, antifungal, antialgal, cardiac and nerve musclerelaxation, antitumor, anticancer, antineoplastic, etc. (Groveiss et al. 1985; Zhang et al. 2006a, b; Sima and Vettika 2011; Su and Wen 2011); (iii) Annelida (*Arenicola marina*, *Nereis diversicolor*, *Perinereis aibuhitensis*) contain peptides, arenecins, hedistins, antimicrobial peptide (AMP), and these are effective against arthritis, osteoporosis, bone cancer, as well as antimicrobial, antibacterial, antifungal, etc. (Mynderse et al. 1997; Pan et al. 2004; Tasiemski et al. 2007; Elayaraja et al. 2010); (iv) Arthropoda (scorpion, shrimp, fiddler crab species, *Uca rosea*, horseshoe crabs *Limulus polyphemus*, *Carcinoscorpius rotundacauda*) contain lectin, viz., limulin and carcinoscorpin, thiol ester protein, fatty acids, triglycerides, carotenoids, and lipids, and these are effective as antibacterial, anticancer, antioxidant, antiproliferative, antimutagenic, anti-inflammatory, immune response, etc. (Hurley et al. 1991; Schwartz et al. 2012; López-Saiz et al. 2013); (v) Mollusca (*Anisodoris nobilis*, *Conus spp.*, *Babylonia japonica*, *Dollabella auricularia*, *Elysia rufescens*, *Aplysia dactylomela*, *Spisula polynyma*, *Trochus tentorium*) contain dolostatin, lectin, steroid, terpenoids, acetylenic compounds, dollstains, polysaccharides and these are effective in antileukemic, immune response, hypotension, relaxation smooth muscle, antinicoticin activity, antiviral especially HIV virus inhibiting compound, etc. (Hanmann et al. 1993; Poncet 1999; Luesch et al. 2002; Sivasubramanian et al. 2011); (vi) Bryozoa (Polyzoa, Ectoprocta, or commonly as moss animals) are a phylum of aquatic invertebrate animals; typically about 0.5 mm (0.020 in.) long and they are filter feeders, i.e., sieve food particles out of the water. They are not rich in bioactive compounds and most of the extracted products are alkaloids (Blunt et al. 2004). *Flustra foliacea* collected in the southern North Sea yielded deformylflustrabromine, which displayed moderate cytotoxicity against the HCT-116 cell-line (Lysek et al. 2002). *Amathia convoluta* from the east coast of Tasmania was the source of the tribrominated alkaloids convolutindole-B and convolutindole-A. The compounds displayed potent and selective activity against *Haemonchus contortus*, a parasitic nematode of ruminants (Narkowicz et al. 2002). *Watersipora subtorquata* from Tsutsumi Island, Japan, was the source of bryoanthrathiophene that exhibited potent anti-angiogenic activity on bovine aorta endothelial cell (BAEC) proliferation (Jeong et al. 2002). Bryostatin, a potent anticancer compound from *B. neritina* (Lilles 1996) shows remarkable selectivity

against human leukemia, renal cancer, melanoma, and non-small cell lung cancer cell lines. The major metabolite convolutamide-A from *Anthia convoluta* exhibits in vitro cytotoxicity against L1210 murine leukemia cells and KB human epidermoid carcinoma cells (Zhang et al. 1994). *Cribricellina cibraria* has yielded β-carboline alkaloid, which exhibited cytotoxic, antibacterial, antifungal, and antiviral activities (Princep et al. 1991). Indole alkaloids isolated from *F. foliacea* have shown strong antimicrobial activity (Holst et al. 1994); (v) Echinodermata (sea-urchins, starfishes, sea cucumber, etc.) contains saponins and sterol derivatives, tarpenoids, glycoproteins, cerebrosides, pyrimidine nucleosides, thymine deoxyriboside and uracil deoxyribose, polysaccharides, β-carotene and these are effective as hemolytic, antibacterial, antifungal, antineoplastic, antitumor, antiviral especially anti-HIV activity, anti-inflammatory, anticancer, anti-allergic, etc. (Komori et al. 1980; Linhardt et al. 1990; Chen 2003; Zhang et al. 2006a; Rahman et al. 2014).

Marine drugs can be broadly classified based on their actions such as (i) Antibacterial and antimicrobial, (ii) Anti-inflammatory, (iii), (iv) Antiparasitic, (v) Antiviral agents, (vi) Anticancer, (vii) Analgesic, and (viii) Antimalarial agent.

(i) Antibacterial and antimicrobial compounds: Eicosapentaenoic acid (EPA), a polyunsaturated fatty acid (PUFA), was isolated from marine diatom *Phaeodactylum tricornutum*. It is also found in fish oil of cod liver, herring, mackerel, salmon, menhaden, and sardine, and various types of edible seaweed and phytoplankton. EPA is a carboxylic acid with a 20-carbon chain and five *cis* double bonds; the first double bond is located at the third carbon from the omega end. This compound showed activity against an array of gram-positive and gram-negative bacteria, and a multidrug-resistant variety of *Staphylococcus aureus* (Desbois et al. 2009). It also lowers inflammation and triglycerides level. The cephalosporins, a class of β-lactam antibiotics, have a marine fungal source of origin. Cephalosporin C was first extracted and purified from a marine fungus (*Cephalosporium acremonium*, nowadays *Acremonium*) (Murti and Agarwal 2010). Vidarabine or 9-β-D-arabinofuranosyladenine (ara-A), a purine nucleoside was isolated from the Caribbean sponge *Tectitethya crypta* and is currently obtained from *Streptomyces antibioticus*. It is an antiviral drug active against herpes simplex and varicella zoster viruses. Vidarabine works by interfering with the synthesis of viral DNA (Anonymous 1999). It is approved by FDA for use in recurrent epithelial keratitis caused by HSV type 1 and 2, acute kerato-conjunctivitis, and also for superficial keratitis (Mayer et al. 2010). Figure 4.13 shows the structure of eicosapentaenoic acid (EPA), cephalosporins and vidarabine.

(ii) Anti-inflammatory compounds: Mediterranean sponge *Spongia officinalis* extract showed anti-inflammatory function in the in vivo study on rat model of carrageenan-induced paw edema assay (Dellai et al. 2010). (iii) Neuroprotective compounds: Green seaweed *Ulva reticulata* showed neuroprotection activity by inhibiting acetyl- and butyryl-cholinesterases and the efficacy would be comparable to agents currently approved for Alzheimer's disease treatment (Suganthy et al. 2010). Until date, the only symptomatic treatment for this disease is based on the cholinergic hypothesis where the drugs enhance acetylcholine levels in the brain by

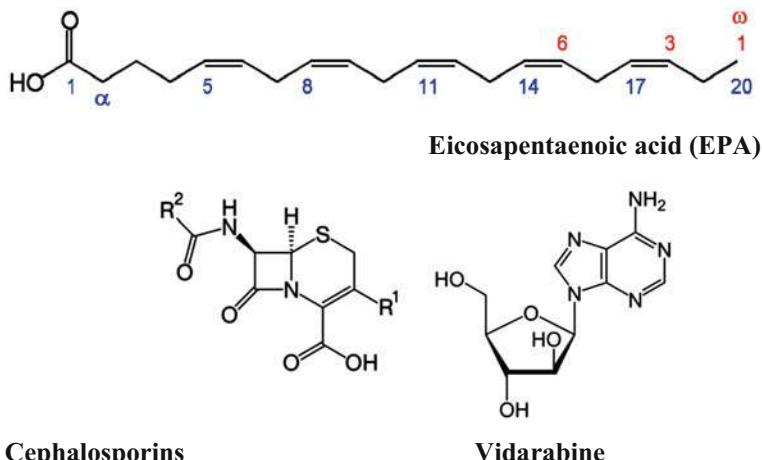


Fig. 4.13 Eicosapentaenoic acid (EPA), cephalosporins, and vidarabine

inhibiting acetylcholinesterase (AChE). An acetylcholinesterase inhibitor (AChEI) or anti-cholinesterase is a drug that inhibits the acetylcholinesterase enzyme from breaking down acetylcholine, thereby increasing both the level and duration of action of the neurotransmitter acetylcholine. (iv) Antiparasitic compounds: Extracts of *Sarcotragus* sp. known as Tunisian sponge in dichloromethane have shown in vitro anti-leishmanial activity (Ben Kahla-Nakbi et al. 2010). (v) Antiviral agents: Anti-herpes simplex virus-1 (HSV) activity was found in high molecular weight (~ 800 kDa) exo-polysaccharides (EPS) extracted from the *Celtdoryx girardae* (French marine sponge) and its associated symbiotic bacteria and the sulphated groups of EPS interact with the glycoproteins on the surface of the virus' membrane (Rashid et al. 2009). About 40% of the biomass of sponges can be from microorganisms and it may not be surprising that some compounds may actually be produced by symbiotic microorganisms rather than the host. Figure 4.14 shows the structure of acetylcholine and its enzymatic breakdown into acetate ion and choline.

(vi) Anticancer compounds: Bryostatins, a group of macrolide lactones, may be obtained from the Bryozoan (*B. neritina*) as well as from sponges and tunicates, Sorbicillin-derived alkaloids sorbicillactone A and its 2', 3'-dihydro analog

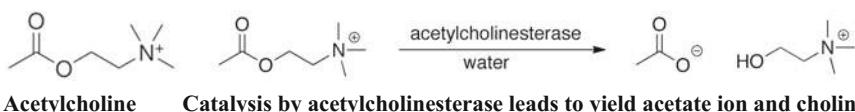
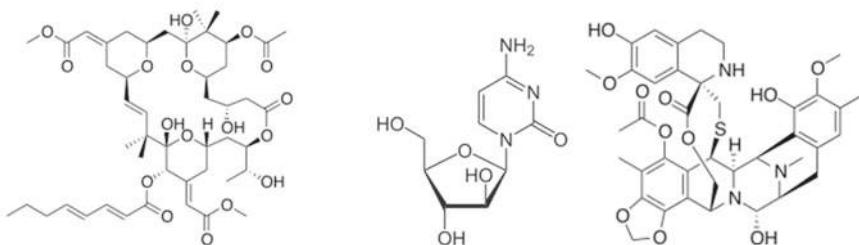


Fig. 4.14 Shows the structure of acetylcholine and its enzymatic breakdown into acetate ion and choline

sorbicillactone-B have shown activity against leukemia cells free from any noteworthy cytotoxicity. Sorbicillactone-B has been derived from a salt-water culture of a bacterial strain *Penicillium chrysogenum* associated with a Mediterranean sponge (*Ircinia fasciculata*) (Bringmann et al. 2007). Bryostatins are potent modulators of protein kinase C. They have been studied in clinical trials as anticancer agents, as anti-AIDS/HIV agents and in people with Alzheimer's disease. Bryostatin 1 is a potent modulator of protein kinase C (PKC) (Kollár et al. 2014). Cytarabine, also known as cytosine arabinoside (ara-C), is a pyrimidine nucleoside derived from spongothymidine and primarily isolated from a Caribbean sponge species *Tethya crypta*. It is a chemotherapy medication used to treat acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML), and non-Hodgkin's lymphoma while FDA approved and mainly used in different types of leukemia, including acute myelocytic leukemia, lymphocytic leukemia, meningeal leukemia, and blast crisis phase of chronic myelogenous leukemia (Mayer et al. 2010). A marine natural product extracted from a tunicate species *Ecteinascidia turbinata* generally inhabitant of the Mediterranean and Caribbean Sea. Trabectedin molecule, an antitumor chemotherapy drug, is an alkaloid of tetrahydroisoquinoline class, and it was the first anticancer molecule of marine origin got approval in EU for use in the treatment of soft-tissue sarcoma and in relapsed cases of platinum-sensitive ovarian cancer (Mayer et al. 2010). It is also undergoing clinical trials for the treatment of breast, prostate, and pediatric sarcomas. Recently, it has been shown that Trabectedin blocks DNA binding of the oncogenic transcription factor FUS-CHOP and reverses the transcriptional program in myxoid liposarcoma. By reversing the genetic program created by this transcription factor, Trabectedin promotes differentiation and reverses the oncogenic phenotype in these cells (Grohar et al. 2011). Figure 4.15 shows the structure of anticancer compounds bryostatin1, cytarabine, and trabectedin.

(vii) Analgesic compounds: FDA in 2004, for the first time approved ziconotide as a drug of marine origin to use as painkiller (Prialt). Ziconotide is an atypical analgesic agent for the amelioration of severe and chronic pain. It was originally extracted from the marine fish-hunting cone snail (*Conus magus*), it is the synthetic



Bryostatin 1

Cytarabine

Trabectedin

Fig. 4.15 Shows the structure of anticancer compounds bryostatin1, cytarabine, and trabectedin

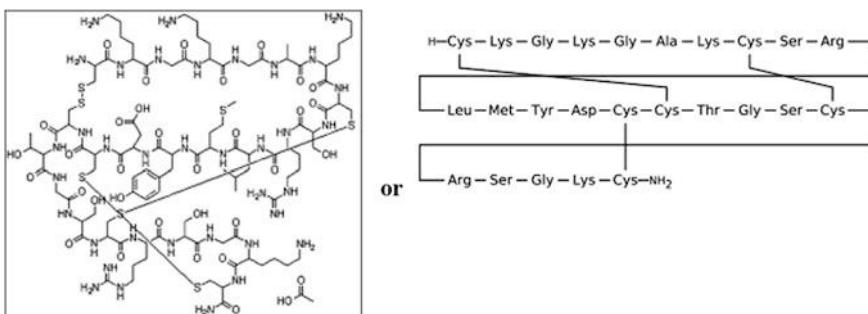


Fig. 4.16 Shows the structure of analgesic compound ziconotide peptide

form of an ω -conotoxin peptide (Skov et al. 2007). Ziconotide is equivalent to a natural 25-amino acid peptide with the amino acid sequence H-Cys-Lys-Gly-Lys-Gly-Ala-Lys-Cys-Ser-Arg-Leu-Met-Tyr-Asp-Cys-Cys-Thr-Gly-Ser-Cys-Arg-Ser-Gly-Lys-Cys-NH₂ (Fig. 4.16). Ziconotide has shown potential as an analgesic with a novel mechanism of action (Mayer et al. 2010). It is approved as an analgesic by FDA. Pharmacological results from animal studies suggest that ziconotide play role in blocking of N-type calcium channels on the primary nociceptive nerves of the spinal cord (Skov et al. 2007).

(viii) Isonitrile-containing antimalarial molecules have been extracted from the Japanese sponge *Acanthella* sp. The isolated molecules belong to kalihinane diterpenoids class, which also contains antifungal, anthelmintic, and antifouling compounds (Miyaoka et al. 1998).

(viii) Antimalarial agent: The discovery of antimalarial drug artemisinin, an endoperoxide cadinene sesquiterpene lactone from Chinese herb *Artemisia annua* (Asteraceae) leaves was breakthrough in the treatment of chloroquine-resistant malaria (Klayman et al. 1984; Klayman 1985). Totally synthetic routes to artemisinin have been developed now (Avery et al. 1992). Similar to artemisinin activity, ~60 secondary metabolites produced by marine organisms have been reported recently for their antimalarial activities and grouped into three structural types into three different classes according to their chemical structures: (i) isonitrile-containing derivatives; (ii) alkaloids; (iii) endoperoxides (Fattorusso and Taglialatela-Scafati 2009). The parent compound of the class of isonitrile-(Fig. 4.17) containing marine secondary metabolites is axisonitrile-1 (3), isolated in 1973 from the marine sponge *Axinella cannabina*, where it co-occurred with the strictly related axisothiocyanate-1 (4) (Cafieri et al. 1973). Axisonitrile-1 was soon followed by other isonitrile-, isothiocyanate-, and formamide-containing sesquiterpenoids from the same source, namely axamide-1 (5), axisonitrile-2, (6) (Fattorusso et al. 1974), axisothiocyanate-2 (7), axamide-2 (8) (Fattorusso et al. 1975), axisonitrile-3 (9), axisothiocyanate-3 (10), and axamide-3 (11) (Di Blasio 1976). Figure 4.18 shows representative isonitrile- and isothiocyanate-containing diterpenoids (12–19) isolated from the marine sponge *Cymbastela hooperi*, representative kalihinane

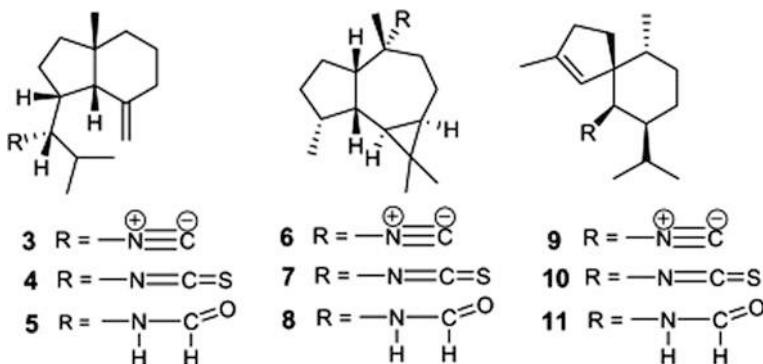


Fig. 4.17 Showing isonitrile-, isothiocyanate-, and formamide-containing sesquiterpenoids from the sponge *Axinella cannabina*

diterpenoids isolated from the Japanese marine sponge *Acanthella* sp. (20, 21) and chemical structure of alkaloids manzamine A (22) and manzamine F (23).

Manzamines are undoubtedly the most important and potent antimalarial alkaloids isolated from marine sources, and they are an Okinawan sponge belonging to the genus *Haliclona* (Sakai et al. 1986; Peng et al. 2008). They are very complex polycyclic (7–8 rings or more) alkaloids first reported by Higa and coworkers in 1986. These molecules are characterized by an intricate pentacyclic heterocyclic system attached to a β-carboline moiety. Since the first report of manzamine A (22) at least 60 additional manzamine-type alkaloids have been reported from taxonomically unrelated sponges belonging to different genera (e.g., *Xestospongia*, *Ircinia*, and *Amphimedon*) and different orders. Manzamines have also been reported to be anti-inflammatory, antifungal, antibacterial and antituberculosis agents, and to exhibit activity against AIDS opportunistic pathogens (e.g., *Toxoplasma gondii*) (El Sayed et al. 2001; Rao et al. 2003; Yousaf et al. 2004).

Marine Plakinidae sponges contain a series of simple endoperoxide derivatives that have been identified as polyketide metabolites possessing six- or five-membered 1,2-dioxygenated rings (1,2-dioxane or 1,2-dioxolane, respectively). A further variation is represented, in some cases, by the presence of a 3-methoxy substitution, building a peroxyketal group.

Plakortin (36) was isolated more than 25 years ago from *Plakortis halichondroides* (Higgs and Faulkner 1978) and recently re-isolated in remarkable amounts from the Caribbean sponge *Plakortis simplex* (Cafieri et al. 1999). In the latter study, the absolute configuration of the four stereogenic carbons of plakortin has been determined by means of chemical derivatization and reaction with chiral auxiliaries. The plakortin analogs, dihydroplakortin (37), 3-epiplakortin (38), plakotide Q (39) have been obtained from the same sponge (Cafieri et al. 1999; Campagnuolo et al. 2005) (Fig. 4.19).

Different bioactive secondary metabolites of marine organisms are known and some of their structures are shown in Fig. 4.20. Marine secondary metabolites

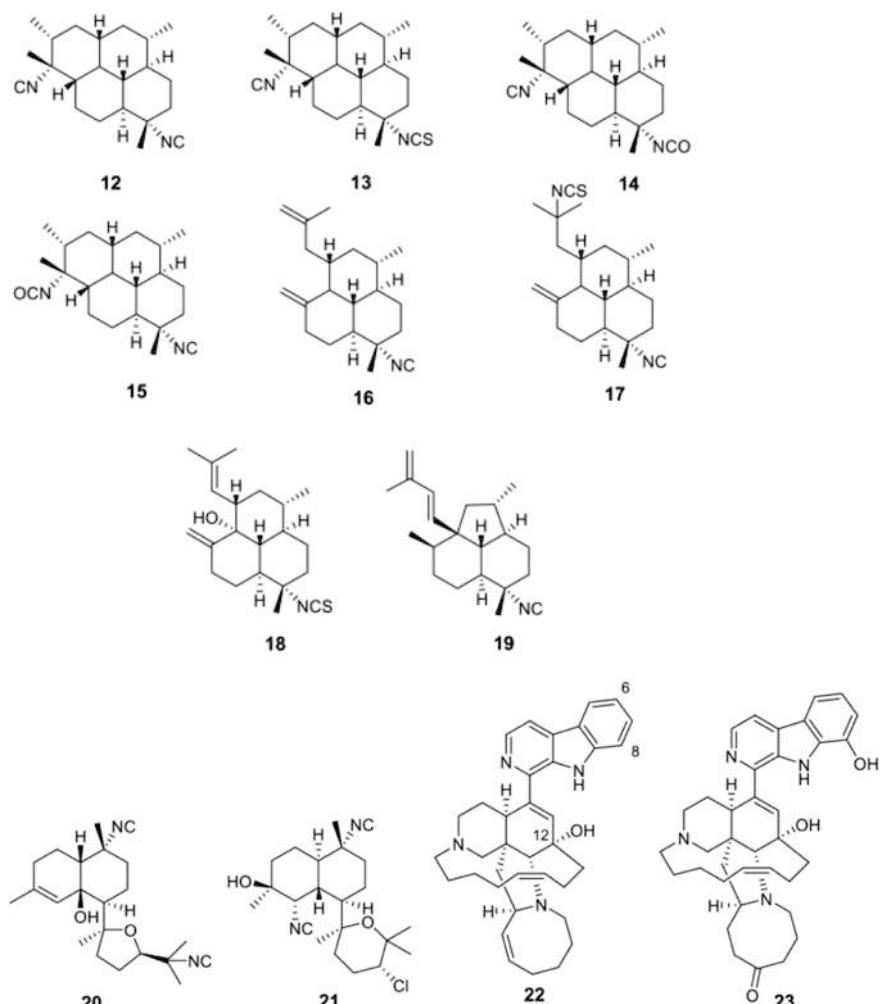


Fig. 4.18 Representative isonitrile- and isothiocyanate-containing diterpenoids (12–19), Representative kalihinane diterpenoids (20, 21) and chemical structure of alkaloids manzamine A (22) and manzamine F (23)

endowed with antimalarial activity may further be met in two structural categories, e.g., quinones and phenols, and peptides. Antimalarial potentials of xestoquinone (51), a protein kinase inhibitor isolated from marine sponge *Xestospongia* sp. (Nakamura et al. 1985) and ilimaquinone (52) from the Australian marine sponge *Dactylospongia elegans* have been reported (Nakamura et al. 1985; Goclik et al. 2000; Laurent et al. 2006). Other quinone derivatives are different alisiaquinones (e.g., alisiaquinone A, 53 and C, 54) from Caledonian sponge (Desoubzdanne et al. 2008).

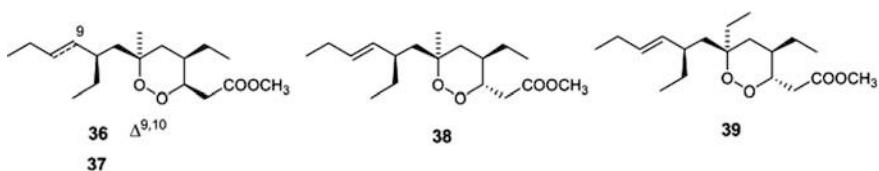


Fig. 4.19 Chemical structures of plakortin (36) dihydropakortin (37), 3-epiplakortin (38), and plakotide Q (39)

(S)-Curcuphenol (55) is a sesquiterpene phenol isolated from different marine sponges belonging to the genus *Didiscus* (El Sayed et al. 2002). Another phenol-containing antimalarial marine metabolite is 15-oxopuupehenol (56), isolated from sponges of the genus *Hyrtios*. Antimalarial peptides from marine sources are known, e.g., cyclic hexapeptides venturamides (venturamide A, 57) from the marine cyanobacterium *Oscillatoria* sp., alkynoic lipopeptide dragomabin (58) from a Panamanian strain of the marine cyanobacterium *Lyngbya majuscula* (McPhail et al. 2007), as well as nonaromatic dragonamide B (59) from the same source, and *cyanobacterial* peptide derivative gallinamide A (60) from *Schizothrix* sp. Figure 4.20 shows chemical structures of xestoquinone (51) and ilimaquinone (52), alisiaquinones A (53) and C (54), cucurphenol (55) and 15-oxopuupehenol (56), venturamide A (57), dragomabin (58), dragonamide B (59), and gallinamide A (60).

4.2.2 Major Marine Vertebrates and Their Bioactive Compounds

A few bioactive metabolites have been extracted from fish, sharks, sea snakes, mammals, and other aquatic vertebrates. Several compounds have been extracted from fish and these are employed as remedies in the official medicine (Hamada and Nagai 1995) and some of these compounds are important as tools for biochemical research or as new leads for the development of anticancer and antiviral drugs. Oil extracted from different marine fish species (tuna, salmon, herring, mackerel, anchovies, sardines, codfish, shark, etc.) are rich in omega-3 fatty acids. Among the many other (>12) omega-3 fatty acids (polyunsaturated fatty acids—PUFAs), three essential PUFAs are important in human physiology, e.g., α -linolenic acid (lipid name—18:3, *n*-3 ALA), eicosapentaenoic acid (lipid name—20:5, *n*-3; EPA), and docosahexaenoic acid (lipid name—22:6, *n*-3; DHA) (Fig. 4.21). These three polyunsaturates have either 3, 5, or 6 double bonds in a carbon chain of 18, 20, or 22 carbon atoms, respectively; from the *n* end (i.e., diagram right), the first double bond appears as the third carbon–carbon bond and hence the name *n*-3. These omega-3 fatty acids are used in the preparation of various drugs substances for the remedies of human ailments like arthritis, inflammation, and for the treatment of

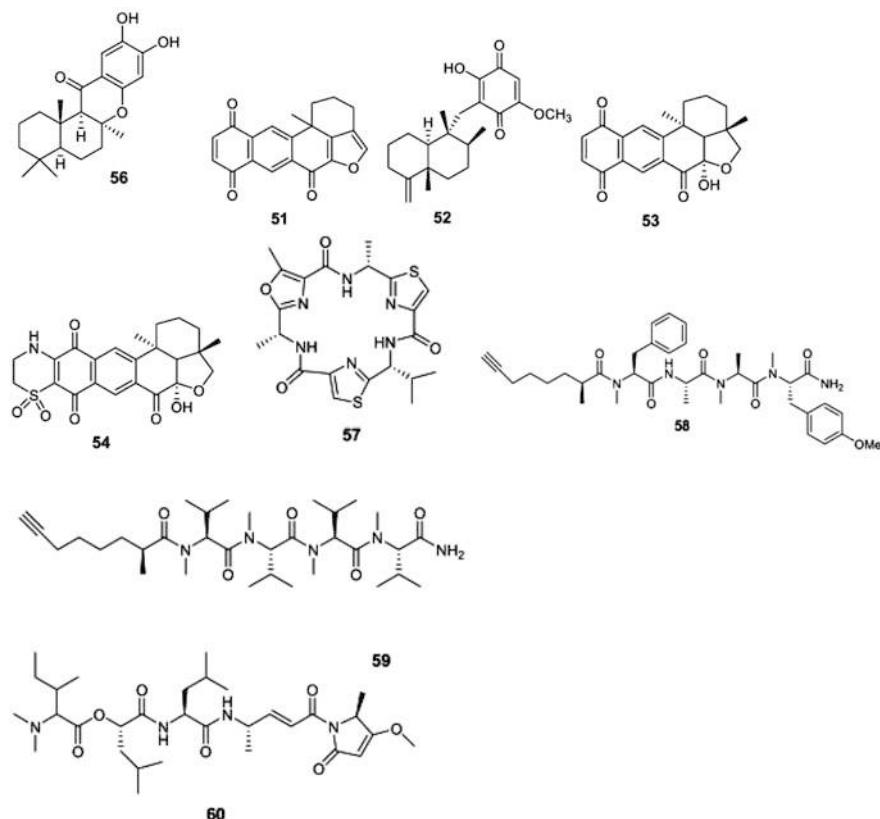


Fig. 4.20 Showing chemical structures of xestoquinone (51) and ilimaquinone (52); alisiquinones A (53) and C (54); 15-oxopuupehenol (56); venturamide A (57), dragomabin (58), dragonamide B (59), and gallinamide A (60)

many others (hypertriglyceridemia, heart attacks or strokes clinical depression, anxiety, cancer, and macular degeneration). Fish oils also have found roles in external use, as emollients or as general ointments as well as in body art, or for alleged insulation against cold. Although fish is a dietary source of omega-3 oils, fish do not synthesize them; they obtain them from the algae (microalgae in particular) or plankton in their diets (Falk-Petersen et al. 1998). Toxins extracted from marine fish species with pharmacological importance include tetrodotoxin (TTX), ciguatoxin, etc. TTX from puffer fish (Tetraodontiformes) is a potent neurotoxin and sodium channel blocker, but it has become a useful tool for researchers studying the voltage-gated sodium channel and it also plays an important role in many biological experiments (Auyoung 1999). TTX, a water-soluble guanidinium derivative and resembles procaine in its ability to inhibit transmission of nerve cells (Colwell 1997), acts as an extraordinary narcotic and analgesic after dilution (Bisset 1991). TTX has been investigated as a possible treatment for cancer-associated

pain. Ciguatoxin (CTX), the puffer or fugu poison, from electric rays is a potent antidote for pesticide poisoning (Oliviera et al. 2003). Ciguatoxins are a class (ciguatoxin 1,2,3,4, etc.) of toxic polycyclic polyethers found in fish that cause ciguatera. Some ciguatoxins lower the threshold for opening voltage-gated sodium channels in synapses of the nervous system causing depolarization, which could sequentially cause paralysis, heart contraction, and changing the senses of heat and cold. Such poisoning from ciguatoxins is known as ciguatera. Ciguatoxins are lipophilic, able to cross the blood–brain barrier, and can cause both central and peripheral neurologic symptoms. Ciguatoxin is produced by *Gambierdiscus toxicus*, a type of dinoflagellate and they are usually accumulated in the skin, head, viscera, and roe of big reef fish like grouper, wrasse, triggerfish, lionfish, and amberjack. Ciguatoxin cannot be destroyed by cooking (Swift and Swift 2008). Squalamine, a sterol linked to a polyamine, is a new class of water-soluble broad spectrum antibiotics isolated from the stomach extracts of dogfish, *Squalus acanthias* (Moore et al. 1993). It exhibits potent activity in vitro and in vivo against gram-negative and gram-positive bacteria, fungi, protozoa, and many viruses (Alhanout et al. 2010a, b; Zasloff et al. 2011). Sea snake venom contains potent neurotoxin (Acott and Williamson 1996) but it also contains several bioactive compounds such as anticoagulant and anticancer agent (Singla and Garg 2013). The sea snakes or coral reef snakes (Hydrophiidae) derived “Fu-anntai”, anticancerous drug, has antiblastic effects on cervical carcinoma, stomach cancer, rhinocarcinoma and leukemia cells (Sci-Edu 2000). A group of scientists in Australia have extracted a novel drug from rat snake (Anonymous 2004). Damotharan et al. (2015) suggested that the sea snake *Enhydrina schistosa* venom (a peptide of 44 kDa) could be a feasible source for potential antibiotics agents against drug-resistant human pathogenic bacteria. Figure 4.21 shows structures of different bioactive compounds from marine vertebrates.

Marine mammals rely on the ocean and other marine ecosystems for their existence. They include animals like seals (*Phoca vitulina*), sea lions (*Zalophus californianus*), dolphin (Delphinidae), whales (*Balaenoptera acutorostrata*), sea cows or manatees (*Trichechus manatus*), sea otters (*Enhydra lutris*), polar bears (*Ursus maritimus*), etc. Because of their dependence on the sea ice, polar bears are classified as marine mammals. Marine mammals have a polyphyletic relation and so do not represent a distinct taxon or systematic taxonomic grouping; they are unified by their reliance on the marine environment for feeding and other life activities. Some of them are obligate water dwellers or aquatic (cetaceans—dolphin, whales, and sirenians—sea cows), some are semiaquatic (pinnipeds—seals and sea lions), i.e., they spend the majority of their time in the water and need to return to land for mating, breeding, and molting. Both sea otter and polar bear are much less adapted to aquatic living. Marine mammals are major consumers of production at most trophic levels of the food chain from primary production (i.e., sirenians) to predatory fish and even to other marine mammals (killer whales *Orcinus orca*). The global use of aquatic mammals in traditional folk medicine is known since ancient times. Blubber and intramuscular lipids of seal meat contain a large proportion of highly unsaturated fatty acids (HUFA) of ω -3 type. The content of eicosapentaenoic

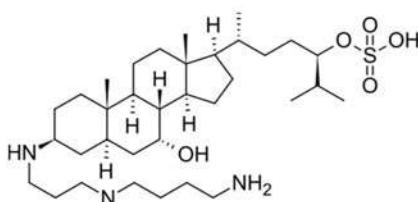
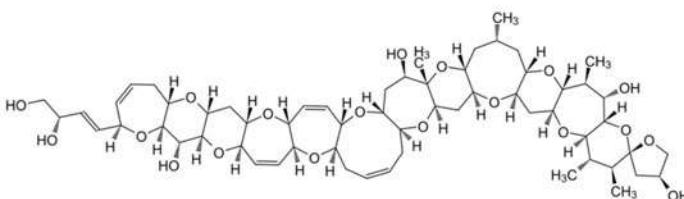
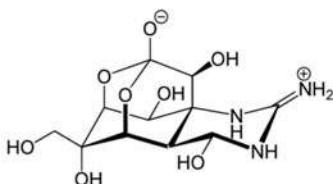
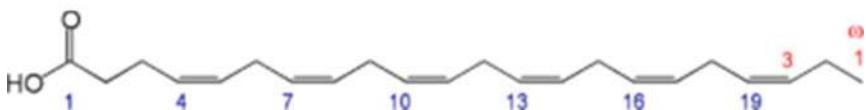
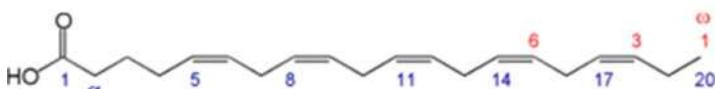
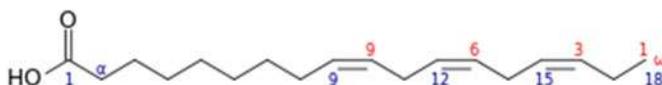


Fig. 4.21 Shows structures of different bioactive compounds from marine vertebrates

acid (EPA, 20:5), docosapentaenoic acid (DPA, 22:5), and docosahexaenoic acid (DHA, 22:6) in mechanically separated seal meat (MSSM) was 6.9, 5.6, and 10.1%, respectively (Shahidi et al. 1994). Seal is valued as the natural penicillin of the north and raw seal fat is often used to treat skin ailments. Applied in thin slices or rubbed in, it is effective on cuts, burns, wounds, and impetigo, and helps stop bleeding. Dolphins have been cited as having special capabilities that enhance healing potential in people with disabilities, and perhaps the most profound changes from dolphin therapy are in children with autism and similar challenges. Dolphin therapy (dolphin–human interaction) as alternative treatment aids in reducing stress and increasing relaxation, alleviating depression, boosting production of infection-fighting t-cells, stimulating production of endorphins and hormones, enhancing recovery, and reducing pain, the emerging field of psychoneuroimmunology, which include study of the interactions between the nervous, the endocrine and the immune system. Ambergris, the sperm whale vomit, can be found floating on the sea or washed up on the coast. It is a solid, waxy, flammable substance of a dull gray or blackish in colour with a sweet, earthy, marine, and animalic smell which has been generally described as a vastly richer and smoother version of isopropanol without its stinging harshness. The key component in fresh ambergris is a triterpene alcohol called Ambrein. Oxidative breakdown of the relatively scentless ambrein produces Ambroxan and Ambrinol, the main odor components of ambergris. Ambroxan is now produced synthetically and used extensively in the perfume industry. Ambergris has been adopted for a number of uses such as incense in perfumes and cologne and as a medication for headaches, colds, epilepsy, and other ailments; to protect individuals from a plague. It has also been used historically as a flavoring for food and as an aphrodisiac in many cultures.

Although varies with the species, whale oil is mainly composed of triglycerides, oil from sperm whale contains a substantial amount of wax esters, most of the fatty acids are unsaturated and common fatty acids are oleic acid and its isomers (18:1 carbon chains) (Bottino 1971; Chakrabarty 2009; Rice 2009). Presence of different types of healthy fatty acids, e.g., long-chained highly unsaturated fatty acids (LC-HUFA), long-chained mono and polyunsaturated fatty acids (LC-MUFA, LC-PUFA); short-chained saturated fatty acids, SC-SAFA, short-chained mono- and polyunsaturated fatty acids (SC-MUFA, SC-PUFA) was reported in marine mammals (seals, whales, and dolphins) (Dahl et al. 2000; Olsen and Grahl-Nielsen 2003; Thiemann and Iverson 2008). LC-HUFA includes both eicosapentaenoic acid (EPA) and docosahexaenoic acids (DHA). Marine mammal products (meat and oil) are rich in minerals, proteins with a well-balanced amino acid composition, high trace elements (iron and zinc), vitamins (especially A, D₃ and B₁₂) (Brunborg et al. 2006; Kuhnlein et al. 2006; Hidiroglou et al. 2008). Marine mammal fat was superior to that of fish for red blood cell w-3 LC-PUFA levels (Lucas et al. 2010). Consumption (20 g/day) of seal oil decreases of the omega-6/omega-3 ratio and the coagulant inhibitor, protein C, and an increase of EPA, DHA, and DPA, EPA/AA, (AA: arachidonic acid) DHA/AA and NEFA (nonesterified fatty acids) in serum phospholipids (Conquer et al. 1999). Eicosanoids derived from omega-3 fatty acids

are mainly anti-inflammatory, anticarcinogenic and inhibit platelet aggregation; while saturated FA and omega-6 FA may promote cancer development (Prener et al. 1996). EPA and DHA are also shown to have a beneficial influence on ventricular arrhythmias, thrombosis, triglyceride, apolipoprotein B, high-density lipoprotein, adhesion molecule expression in plaque, platelet-derived growth factor, nitric oxide-induced endothelial relaxation, and blood pressure reduction (DeFilippis and Sperling 2005). Intake of EPA or a combination of EPA and DHA is an efficacious adjunctive therapy for several psychiatric disorders, including mood disorders, schizophrenia, and ADHD (Freeman et al. 2006). Moreover, EPA and DHA supplementation delays cognitive decline in patients with Alzheimer's disease (Freund-Levi et al. 2006). There is a growing body of facts indicating that functional deficiencies or imbalances of omega-3 and omega-6 HUFA may play a role in dyslexia, dyspraxia, autism, and ADHD (Cyhlarova et al. 2007). From a double-blind clinical, it was reported that whale and seal oil significantly reduced joint pain, back pain, and the indexes of inflammatory bowel disease (IBD) and both of the marine mammal oils improved quality of life (Bjørkjkær et al. 2009; Brunborg et al. 2008). Polar bear materials have historically been used by native people of the Arctic for fur, meat, and medicines. Figure 4.22 shows some structures of different bioactive lipid compounds from marine vertebrates.

4.2.3 *Bioactive Compounds from Seagrass*

Seagrasses are submerged marine angiosperms, which grow in fully saline environments in shallow coastal and estuarine waters and provide critical habitat for numerous finfish, shellfish, waterfowl, and herbivorous mammals. Seagrasses are angiospermic flowering plants of different families such as Posidoniaceae, Zosteraceae, Hydrocharitaceae, and Cymodoceaceae of Alismatales order of Monocotyledons. There are 12 genera with >60 species known. Though seagrasses are well known for their bioactive secondary metabolites, only a few have been explored for their natural products and their determinant role. The most important of the secondary metabolic pathways are those with shikimic acid, acetyl-CoA, and mevalonic acid intermediates. In shikimate pathway, products like phenylpropanoids, simple phenolic compounds, phenylmethane and phenylethane derivatives, flavonoid derivatives, etc., are synthesized while acetate-polyketide pathways are involved in the synthesis of sterols and volatile derivatives, lipids, and fatty acids, etc. Heglmeier and Zidorn (2010) reported about 51 bioactive secondary metabolic compounds including phenols, phenylmethane, phenylethane, phenylpropane derivatives and their esters, chalk ones, and flavonoids in *Posidonia oceanica*. Seagrasses are a rich source of phenolic substances including phenolic acids, sulfated phenolic acids, flavones, condensed tannins, and lignins, but not hydrolyzable tannins (Vergeer et al. 1995; Arnold and Targett 2002). The phenolic acids such as coumaric acid, caffeic acid, ferulic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, gentisic acid, and gallic acid occur

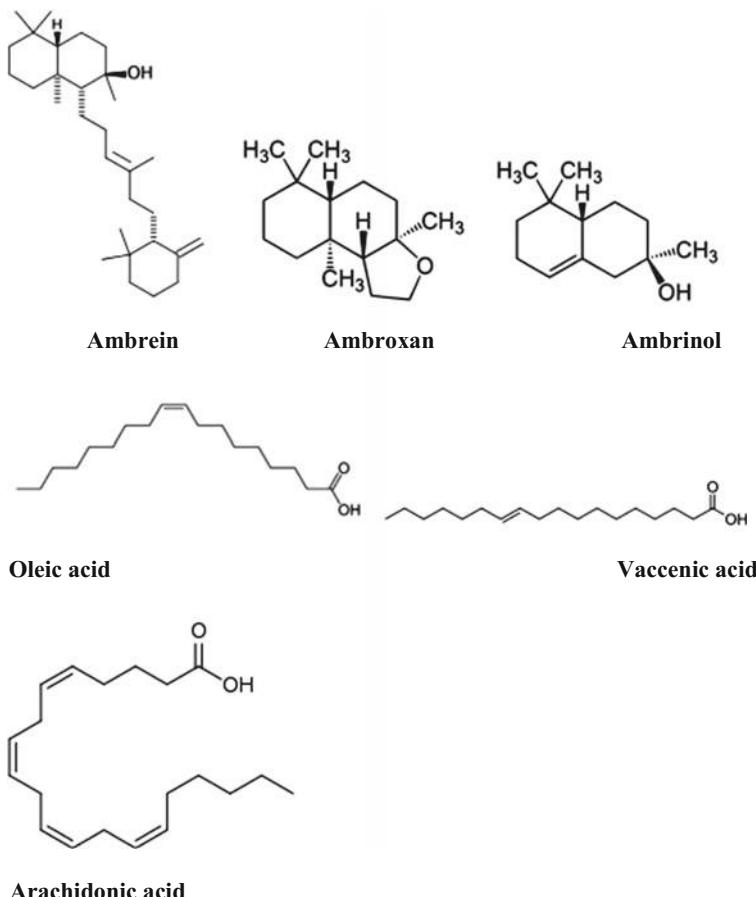


Fig. 4.22 Shows structures of different bioactive lipoid compounds from marine vertebrates

predominantly in *Halophila ovalis*, *Thalassia hemprichii*, *Halodule* spp., *Cymodocea* spp., *Enhalus acoroides*, *Syringodium isoetifolium*, and other seagrass species (Zapata and McMillan 1979; Ravn et al. 1994). Figure 4.23 shows chemical structures of few phenolic compounds from different seagrasses.

Different metabolites reported from seagrasses such as sulfated flavones from *Zostera marina* (McMillan et al. 1980) and flavonoids from *H. ovalis*, *H. minor* (McMillan 1986); malonyl derivatives from *Halophila stipulacea* (Bitam et al. 2010); flavone glycosides from *Halophila johnsonii* (Meng et al. 2008), *Thalassia testudinum* (Jensen et al. 1998); condensed tannins (Dawes 1998) and thalassiolins from *T. testudinum* (Rowley et al. 2002); L-chiro-inositol from *Syringodium filiforme* (Nuissier et al. 2008); diarylheptanoids from *Cymodocea nodosa* (Kontiza et al. 2008); etc.

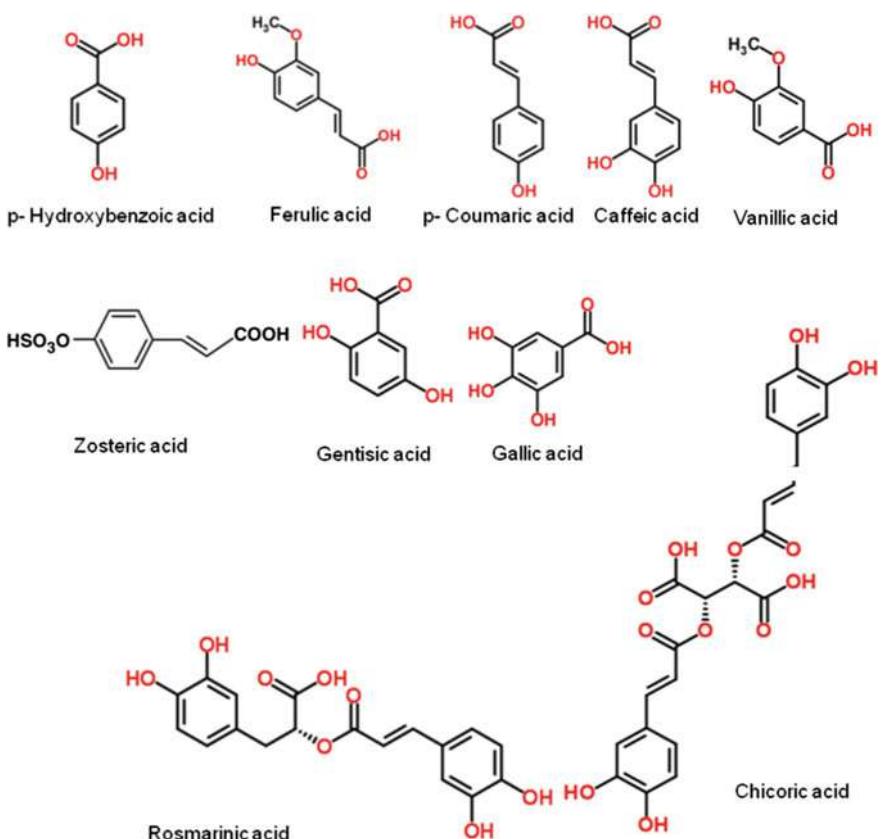


Fig. 4.23 Showing chemical structures of phenolic compounds from different seagrasses

Flavonoids, a class of polyphenolic compounds, occur in any of the five chemical structures like flavones, flavonols, flavanones, flavanols, and anthocyanidins. The presence of sulfated flavones was indicated in *Halophila*, *Thalassia* and *Zostera* species (McMillan et al. 1980). Figure 4.24 shows chemical structures of different flavonoid compounds from seagrasses.

The sterols of *P. oceanica* and *C. nodosa* are essentially β -sitosterol and stigmasterol, with minor sterols including cholesterol, campesterol and avenasterol (Iatrides et al. 1983; Sica et al. 1984). Figure 4.25 shows chemical structures of different thalassiolins (A-C) identified from the seagrass *T. testudinum*; four new metabolites (1–4) isolated from seagrass *C. nodosa*: 1 and 2 belong to the structural class of diarylheptanoids, compound 3 is a meroterpenoid, compound 4 is a briarane diterpene with a tricyclic skeleton (Kontiza et al. 2008); structure of zosterin ($C_{25}H_{28}O_{12}$).

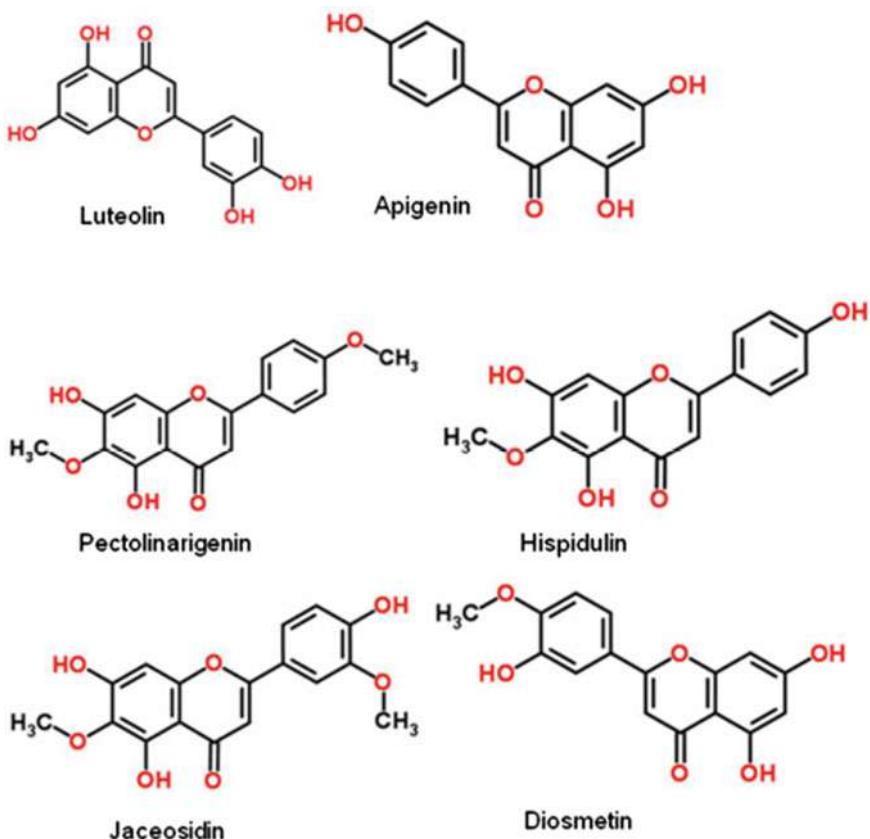


Fig. 4.24 Showing chemical structures of different flavonoid compounds from seagrasses

Subhashini et al. (2013) described a wide array of secondary compounds including phenols, flavonoids, sterols, lipids, fatty acids, etc., from different seagrass species and their biological applications. Seagrasses are nutraceutical in nature and, therefore, of importance as food supplements. *Halophila* spp. is a strong medicine against malaria and skin diseases and is found to be very effective in early stages of leprosy and *H. ovalis* was reported to exhibit appreciable antibacterial, antioxidant, and anti-inflammatory activities (Yuvaraj et al. 2012; Vijayakumar and Amirthanathan 2014) and seagrass thus could be used as a potential source for nutraceuticals and natural health products. Unique nature of seagrass such as heavy metal chelating peptides and proteins and DMSC establish its nutraceutical application (Bharathi et al. 2016). One of the rare real applications seems to be Zosterin, bioactive pectin from *Zostera asiatica*, which decreases the toxicity of antitumor drugs and purges heavy metals from human organisms (Yuri et al. 2012).

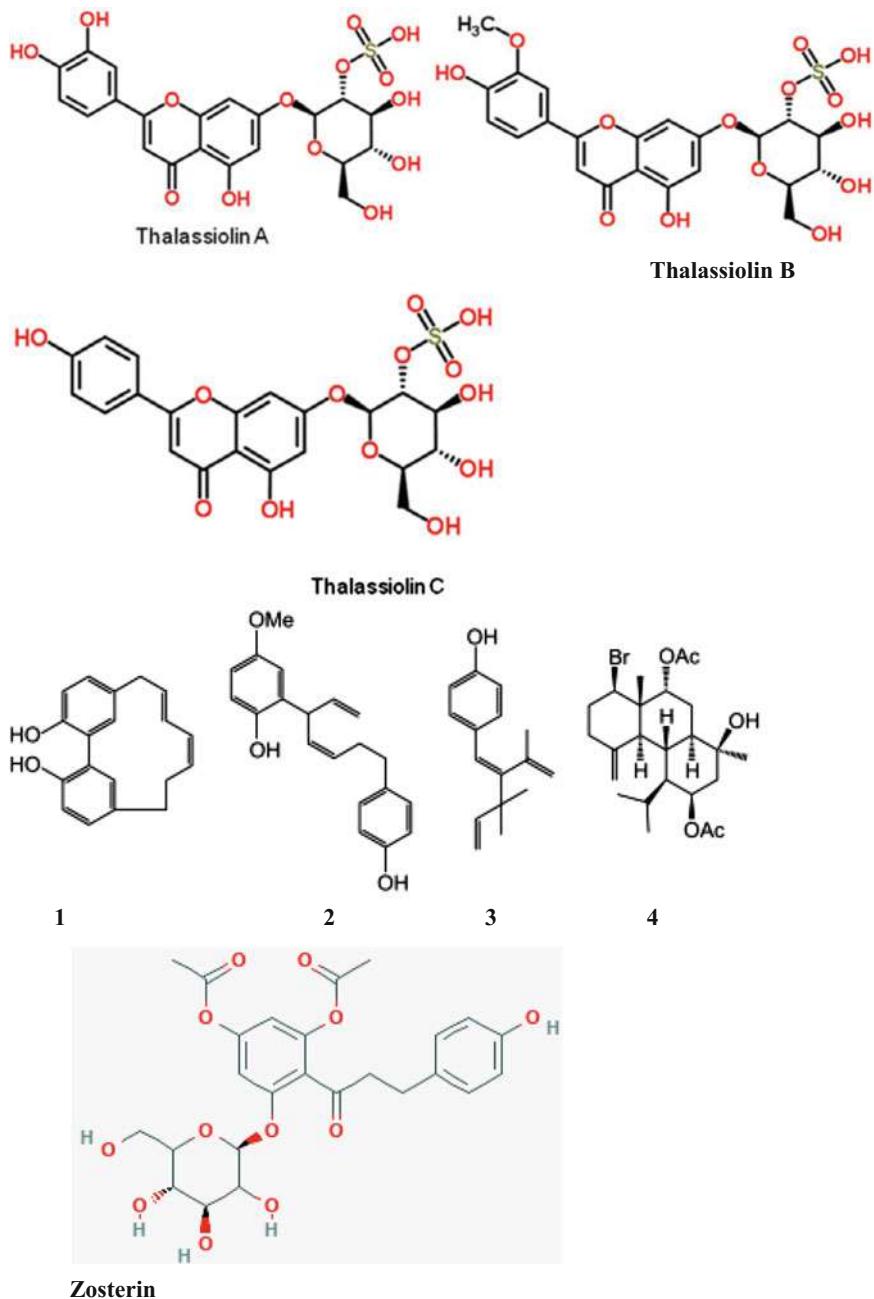
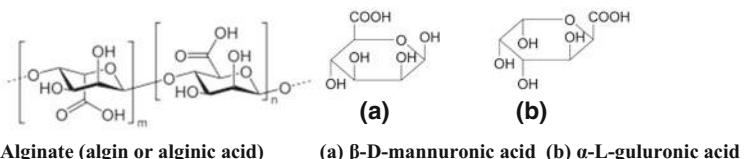


Fig. 4.25 Showing chemical structures of different thalassiolins (A-C); four new metabolites (1-4): 1 and 2 belong to the structural class of diarylheptanoids, 3 is a meroterpenoid, 4 is a briarane diterpene with a tricyclic skeleton; and structure of zosterin ($C_{25}H_{28}O_{12}$)

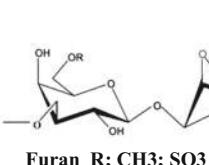
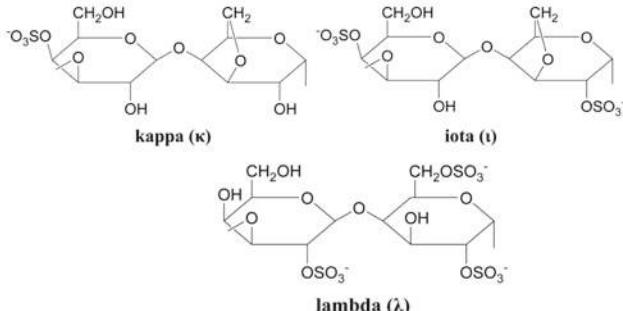
4.2.4 Bioactive Compounds from Seaweeds

Seaweeds (marine macroalgae) are rich in dietary fibers and unique bioactive compounds like unsaturated fatty acids, polyphenols, carotenoids, vitamins, phy-cobilins, phycocyanins, and polysaccharides, iodine, among others, and many of these are known to possess beneficial applications in human health (Kadam and Prabhasankar 2010). Edible seaweeds including *Himanthalia elongata*, *Ascophyllum nodosum*, *Laminaria digitata*, and *Palmaria palmata* contain a higher percentage of total dietary fiber and lower soluble carbohydrate compared with brown rice and bananas (Lahaye 1991; MacArtain et al. 2007). Edible seaweeds contain 33–50% total fibers on a dry weight basis, which is higher than the levels found in higher plants, and these fibers are rich in soluble fractions (Lahaye 1991). The dietary fibers of marine algae are of two types: (i) insoluble (e.g., cellulose, mannans and xylan), and (ii) soluble (e.g., agars, alginic acid, furan, laminaran and porphyran). Total dietary fibers, on dry weight basis, was reported to be 58% in *Undaria*, 50% in *Fucus*, 30% in *Porphyra*, and 29% in *Saccharina* (Murata and Nakazoe 2001). *Fucus* and *Laminaria* have the highest content of insoluble dietary fibers, 40 and 27%, respectively, and *Undaria pinnatifida*, *Chondrus*, and *Porphyra* have the highest content (15–22%) of soluble dietary fibers (Fleury and Lahaye 1991). Commercial varieties of alginate are extracted from seaweed, including the giant kelp *Macrocystis pyrifera*, *A. nodosum*, and various types of *Laminaria*. The marine algae-soluble dietary fibers are primarily consisting of indigestible viscous polysaccharides such as alginates (anionic polyuronide polysaccharide), carrageenans (sulfated galactans polysaccharides), funorans (highly sulfated agarose), etc., and they are known to decrease serum cholesterol level as well as decrease the risk of coronary heart disease, mainly due to their characteristics of dispersibility in water (water-holding capacity), viscosity, binding ability, absorptive capacity, inhibition of lipid absorption in the gastrointestinal tract, fecal bulking capacity, and fermentability in the alimentary canal (Jiménez-Escrig and Sánchez-Muniz 2000).

In Traditional Chinese Medicine (TCM) and Japanese Folk Medicine (JFM), seaweeds are used to treat, diabetes, tumors, etc. Bioactive compounds from brown seaweeds including phloroglucinol, fucoxanthin, and fucoidan are potential anti-breast cancer agents; antioxidant, antiproliferative, and proapoptotic, the main anticancer mechanisms and potentiate the activity of cytotoxic drugs as well as synergism (Pádua et al. 2015). Seaweed compounds are also reported to be anti-inflammatory, inducing hepatic antioxidant enzymes' activities, and stimulating glucose transport and incretin hormones release, as well as β -cell cytoprotection (Sharifuddin et al. 2015). Various edible seaweeds including *U. pinnatifida*, *H. elongata*, and *Laminaria ochroleuca*, contain higher percentage of unsaturated fatty acids such as monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) compared with saturated fatty acids; *U. pinnatifida* contains almost 70% of fatty acids as PUFA (Sanchez-Machado et al. 2004). Many seaweed species from the Phaeophyta and Rhodophyta contain higher concentrations of unsaturated fatty acids compared with those from Chlorophyta, with the exception of *Ulva*



Alginate (algin or alginic acid)

(a) β -D-mannuronic acid (b) α -L-guluronic acidFuran R; CH_3 ; SO_3^-

The diagram shows the repeating unit of an agarose polymer, which is a linear chain of two glucose units linked by an oxygen atom. The linkage is between the C1 carbon of one glucose unit and the C6 carbon of the other.

An agarose polymer

The diagram shows the repeating units of fucosidic and fucoidan polysaccharides. It includes a branched structure where a glucose unit is linked to a fucose unit, and a linear structure of a fucosidic unit.

Fucoidan: branched polysaccharide sulfate ester Laminarin: β -(1, 3) and β -(1,6) linked glucose

The diagram shows the repeating units of Laminarin, which consists of two types of beta-linked glucose units: one linked at the C1 carbon and another linked at the C6 carbon.

The diagram shows the chemical structures of β -D-galactose and α -L-galactose, illustrating their anomeric differences.

Agar constituents: (R=H or CH_3)

◀Fig. 4.26 Showing chemical structures of alginic acid and its components: **a** β -D-mannuronic acid, **b** α -L-guluronic acid; structure of kappa-, iota-, and lambda-carrageenan; fucoidan: Branched polysaccharide sulfate ester with L-fucose building blocks as the major component with predominantly α -(1,2) linkages; basic chemical units of laminarin: β -(1,3) and β -(1,6) linked glucose; Agar constituents: (R=H or CH₃) β -D-galactose and α -L-galactose

sp. (marine green alga), which possesses high concentrations of omega-3 fatty acids (Kumari et al. 2010; Pereira et al. 2012).

Extract of different seaweeds such as *Jania rubens*, *Corallina mediterranea*, and *Pterocladia capillacea* contained alkaloids, phenols, flavonoids, steroids, terpenoids, tannins, phlobatannins pigments, and mineral and showed antioxidant as well as antibacterial activity against human pathogenic bacteria (El-Din 2016). Seaweeds dietary fibers, unsaturated fatty acids, and polyphenolic compounds are beneficial to human health and also in the management of Type II diabetes mellitus (T2DM). High intake of dietary fiber is consistently correlated to a markedly reduced incidence of T2DM (Schulze et al. 2007) and omega-6 PUFA from plant sources exhibit positive effects on insulin sensitivity (Summers et al. 2002) and are linked to lower risk of developing T2DM (Feskens 2001; Hu et al. 2001; Marshall and Bessesen 2002). Almost all marine macroalgae (seaweeds) have been regarded as a good source of dietary PUFA as their omega-6 and omega-3 fatty acids ratio range was within the recommended value (Pereira et al. 2012).

Many of the marine algal bioactive compounds are potentially useful in inhibiting enzymes activities such as α -glucosidase, α -amylase, lipase, aldose reductase, protein tyrosine phosphatase 1B (PTP1B) and dipeptidyl-peptidase-4 (DPP-4). Bioactive compounds from seaweed species like *Caulerpa racemosa* and *Spatoglossum schroederi*, *Halimeda macroloba*, *Padina sulcata*, *Sargassum binderi*, and *Turbinaria conoides* demonstrated the inhibition of α -amylase and α -glucosidase activities (Chin et al. 2014). Good α -amylase inhibitory activity by several phloroglucinol derivatives from *Eisenia bicyclis* (Okada et al. 2004). Inhibitory potential of fucofuroeckol A from *E. bicyclis* is more potent against α -amylase compared with (Eom et al. 2012). Several bromophenols isolated from red seaweed *Odonthalia corymbifera* showed strong inhibition against *Saccharomyces cerevisiae* α -glucosidase activity (Kurihara et al. 1999). Bromophenols from *Symplocladia latiuscula* also strongly inhibited α -glucosidase of *S. cerevisiae*, (Kurihara et al. 1999). The two bromophenols from *S. latiuscula* also inhibited the activities of rat intestinal maltase and sucrase but with lower inhibitory potencies. Figure 4.26 shows chemical structures of alginic acid and other bioactive compounds from different seaweeds.

Fucoidans are a complex series of sulfated polysaccharides found both intercellularly and in the cell wall of brown algae (5–20% fucoidan). Fucoidans are reported to display physiological and biological activities, including anticoagulant, antithrombotic, antiviral, antitumor, immunomodulatory, antioxidant, and anti-inflammatory (Li et al. 2008), with therapeutic potential increasing with the degree of sulfation (Rinaudo 2007). Laminarin is a relatively low molecular weight storage polysaccharide of brown algae. It has been identified as a modulator of

intestinal metabolism through its effects on mucus composition, intestinal pH, and short-chain fatty acid (SCFA) production, especially butyrate (Deville et al. 2004; 2007). Agars (consisting of agarose and agarpectin), the gel-forming polysaccharides of *Rhodophyta*, are linear polymers with a sugar skeleton consisting of alternating 3-linked β -D-galactopyranosyl and 4-linked 3,6-anhydro- α -L-galactopyranosyl units. Carrageenans or carrageenins are a family of linear sulfated polysaccharides made up of galactose units, β -D-galactose alternates with α -D-galactose, not α -L-galactose as in agars. The degree of sulfation in carrageenans is generally much greater than that in agars. Based on their degree of sulphation (1, 2 or 3) per disaccharide, three types of carrageenans are kappa (1), iota (2), and lambda (3) carrageenan and such sulphation affects the physical (gelling) properties of carrageenans as kappa forms strong rigid gels, iota soft elastic gels, and lambda-thickening polymer (Rinaudo 2007). The red seaweed *Chondrus crispus* (Irish moss) seaweed has been used to extract Carrageenans food additives since long back. Other red seaweed sources are *Eucheuma cottonii* (k), *Eucheuma spinosum* (i), *Olgartina acicularis* (l), *Glgartina stellata* (k + i + l), *Irldea* sp. Kappa carrageenan today is almost exclusively obtained from farmed *Kappaphycus alvarezii* and iota from farmed *Eucheuma denticulatum* (Rasmussen and Morrissey 2007). The Carrageenan market is worth US\$527 m with most Carrageenan used as human foodgrade semi-refined carrageenan (90%) and the rest going into pet food as semi-refined carrageenan. *C. crispus* and *Kappaphycus* sp. are species containing up to 71 and 88% of carrageenan, respectively (Chopin et al. 1999; Rodriguez and Montaño 2007).

Alginic acid is a polyuronide made up of a sequence of two hexuronic acid residues: 3-D-mannuronic acid unit and α -L-guluronic acid. Alginates are essentially extracted from the commercial sources of phaeophytes such as *L. digitata*, *Laminaria hyperboreana*, *A. nodosum*, *Fucus serratus*, *Mycrocystis*, etc., and the global alginate production is ca. 26,500 tons and valued at US\$318 million annually from the wild. Other minor sources include Sargassum, Durvillea, Eklonia, Lessonia, and *Turbinaria* (Bixler and Porse 2010). The rich source of polysaccharides (fiber components) from seaweeds, in addition to their therapeutic importance, could potentially be exploited as prebiotic functional ingredients for both human and animal health applications as prebiotics (non-digestible, selectively fermented compounds) stimulate the growth and/or activity of beneficial gut microbiota which, in turn, confer health benefits on the host (O'Sullivan et al. 2010).

4.2.5 Bioactive Compounds from Marine Bacteria

Marine bacteria are capable of producing unusual bioactive compounds that are not observed in terrestrial sources (Fenical 1993; Fenical and Jensen 1993).

Thermostable proteases, lipases, esterases, and starch and xylan degrading enzymes have been actively sought and in many cases are found in bacterial and archaeal hyperthermophilic marine microorganisms (Bertoldo and Antranikian 2002). Gram-positive bacteria from deep-sea sediment was reported to produce a series of new natural products, e.g., macrolactin A-F (Gustafson et al. 1989). Macrolactin A inhibits B16-F10 murine melanoma cells in *in vitro* assays, significantly inhibits mammalian herpes simplex virus (type I and II) and protects T lymphocytes against human immunodeficiency virus (HIV) replication (Carte 1996). Some *Vibrio* species have been found to produce a variety of extracellular proteases, e.g., *V. algicolyticus* produces six proteases including an unusual detergent-resistant, alkaline serine exoprotease, and also collagenase (Fenical and Jensen 1993). Sodium channel blocker toxins such as tetrodotoxin, saxitoxin, ciguatoxins, and brevetoxins are produced by marine bacteria (Kodama et al. 1988, 1990; Simudu et al. 1990). The ciguatoxins (CTX) are marine algal neurotoxins that act on voltage-gated Nav channels. Both cholinergic and α -adrenergic receptors of the Nav channel are affected by ciguatoxins. Figure 4.27 shows chemical structures of Macrolactin A-C and some other bioactive compounds from marine bacteria.

4.2.6 Bioactive Compounds from Marine Cyanobacteria

The marine *cyanobacteria* have emerged as an important source of secondary metabolites of therapeutic importance. They have proven to be an excellent source of bioactive metabolites and many of these biologicactive compounds such as oligopeptides (cyclic peptides), terpenes, alkaloids, unusual amino acids, etc., are potent toxins including dermatoxins, neurotoxins, hepatotoxins, cytotoxins, and endotoxins. Cyanotoxins are not cyanides despite the similarity in name and the chemical structure of cyanotoxins falls into several broad groups such as (i) cyclic peptides (microcystins and nodularins), (ii) alkaloids (anatoxin-*a*, cylindrospermopsin, lyngbyatoxin-*a*, saxitoxin), (iii) lipopolysaccharides (lipoglycans or endotoxins), (iv) polyketides (geldanamycin, doxycycline, erythromycin, aflatoxin B1), (v) unusual amino acids (β -*N*-methylamino-L-alanine- BMAA), (vi) fatty acids, (vii) macrolides, (viii) amides, etc. Kim and Tan (2013) highlighted the pharmaceutical potential of more than 70 marine *cyanobacterial* metabolites. Their role as anticancer, antitumor, anti-inflammatory, neuromodulating, immunosuppressive, anti-infective, antiviral, antibacterial, anti-HIV, antiprotozoal, anti-helminthic, antimarial, antimycotics, multidrug resistance reversers, antifeedant, herbicides, and a food additive have been well established including the specific cellular targets such as actin, microtubule filaments, proteasome, and histone deacetylase enzymes of certain bioactive compounds (Burja et al. 2001; Singh et al. 2005; Kim and Tan (2013). The level of the total cholesterol, LDL, and VLDL cholesterol in rat serum was reduced by *cyanobacteria* due to the activity of its lipoprotein lipase (Iwata et al. 1990). *Cyanobacteria* are gram-negative prokaryotes (oxygenic photoautotrophic), distributed from the Arctic to Antarctic regions,

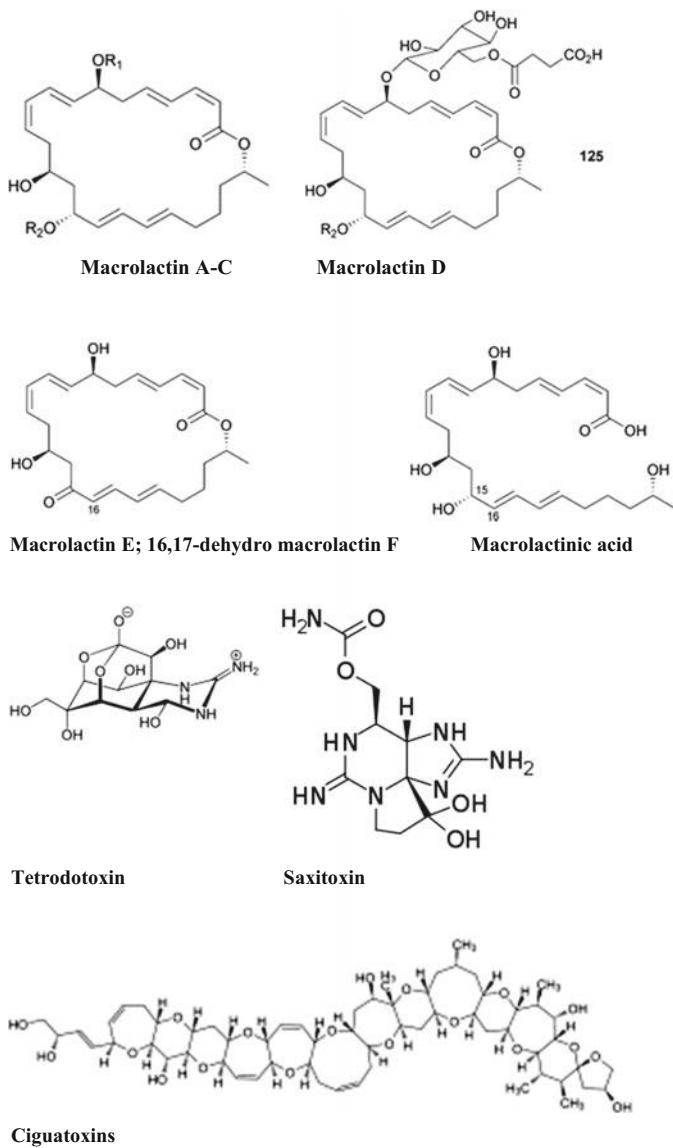


Fig. 4.27 Showing chemical structures of Macrolactin A-C (A: R₁=R₂=H; B: R₁=β-glucosyl, R₂=H; C=R₁=H, R₂=β-glucosyl); Macrolactin D; Macrolactin E; 16,17-dehydro macrolactin F; Macrolactic acid, 15-keto-16,17-dehydromacrolactic acid; Tetrodotoxin; Saxitoxin, contains a reduced purine ring system; Ciguatoxins, a class of toxic polycyclic polyethers responsible for ciguatera; Brevetoxins A; Brevetoxins B, cyclic ether compounds

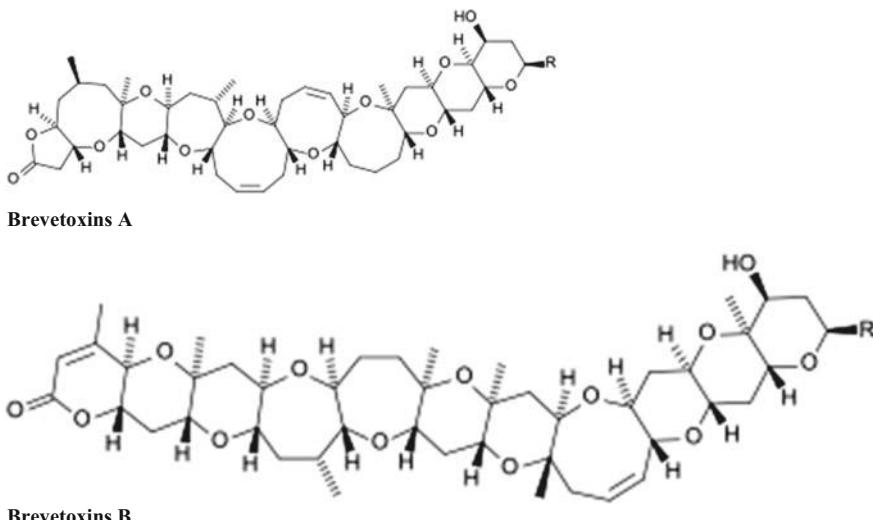


Fig. 4.27 (continued)

important biomass producers in aquatic (freshwater and marine) and terrestrial ecosystems, and three *Cyanophyta* orders, e.g., *Chroococcales*, *Oscillatoriales*, and *Nostocales* and among them, five types (e.g., two strains of *Anabaena flos-aquae*, *Aphanizomenon flos-aquae*, *Microcystis aeruginosa*, and *Nodularia* species) produce the majority of cyanotoxins (Burja et al. 2001). Cyanotoxins like microcystins are produced by *Microcystis*, *Anabaena*, *Planktothrix*, *Nostoc*, *Hapalosiphon*, and *Anabaenopsis*; nodularins are produced by *Nodularia*; anatoxin-a is produced by *Anabaena*, *Planktothrix*, *Aphanizomenon*; cylindrospermopsins are by *Cylindrospermopsis*, *Aphanizomenon*, *Umezakia*; lyngbyatoxin-a is produced by *Lyngbya*; saxitoxin by *Anabaena*, *Aphanizomenon*, *Lyngbya*, *Cylindrospermopsis*; polyketides aplsiatoxins by *Lyngbya*, *Schizothrix*, *Planktothrix*; lipopolysaccharides, and neurotoxin amino acid β -N-methylamino-L-alanine (BMAA) are produced by all members of the *cyanobacteria*. *Lyngbya majuscule*, abundant in the tropical marine, produces ~30% of all the natural products isolated from marine *cyanobacteria* and the list includes nitrogen-containing compounds, polyketides, cyclic peptides, lipopeptides, and many others with very diverse biological activities such as potent protein kinase C activators and tumor promoters (lyngbyatoxins and aplsiatoxins—microlides), inhibitor of microtubulin assembly (Curacin A), etc. *Cyanobacterial* novel bioactive compounds including toxins have wide pharmaceutical applications.

Microcystins (consisting of seven amino acids) and nodularin (consisting of five amino acids) are cyclic peptide toxins account for most of the toxic *cyanobacterial* blooms in fresh and brackish waters. Microcystins family and nodularins are potent hepatotoxins and marine bivalves were the likely source of hepatotoxic shellfish

poisoning and confirmed the cause of marine mammals (a number of sea otters) death from ingesting a cyanotoxin.

Anatoxin-a (or very fast death factor—VFDF), is a secondary, bicyclic amine alkaloid and cyanotoxin with acute neurotoxicity. Toxic effects from anatoxin-a progress very rapidly because as a neurotoxin it acts directly on the nerve cells or neurons. Its molecular shape so fits it to mimic the natural neurotransmitter to permanently occupy the acetylcholine receptor. Cylindrospermopsin (CYN, or CYL), a cyanotoxin, is a polycyclic uracil derivative containing guanidino and sulfate groups produced by different freshwater *cyanobacteria*; toxic to liver and kidney tissue. Saxitoxin (STX) is a potent neurotoxin (acts as a selective sodium channel blocker) and the best-known paralytic shellfish toxin (PST) for the human illness known as paralytic shellfish poisoning (PSP). It is found in marine pufferfish species and its presence in bivalve shellfish (mussels, clams, oysters, and scallops) frequently leads to bans on commercial shellfish harvesting in many temperate coastal waters around the world. Anatoxin-a(S), a cyanotoxin from *cyanobacteria* (*Anabaena*), causes excess salivation in mammals via inhibition of acetylcholinesterase. Structurally, it is a cyclic N-hydroxyguanine organophosphate with a phosphate ester moiety. Figure 4.28 shows chemical structures of microcystin-LR, nodularin-R, anatoxin-a, cylindrospermopsin, saxitoxin, and other bioactive compounds from marine *cyanobacteria*.

Structurally, polyketides are complex and often highly active (biologically) compounds, e.g., geldanamycin, doxycycline, and erythromycin are antibiotics while aflatoxin B1 is one of the most carcinogenic compounds ever known. Many pharmaceuticals are derived from or inspired by polyketides. Polyketides as antibiotics, antifungals, cytostatics, anticholesteremic, antiparasitics, coccidiostats, animal growth promoters, and natural insecticides are in commercial use. Aplysiatoxin, a cyanotoxin, is a defensive secretion to protect *cyanobacteria* from predation by fish as a potent irritant and carcinogen by acting as a powerful activator of protein kinase C. It has tumor-promoting effect and protein kinase C activation can be medically beneficial for anticancer effects.

Lipopolysaccharides (LPS), lipoglycans or endotoxins, are large molecules consisting of a lipid and a polysaccharide composed of O-antigen, outer core, and inner core joined by a covalent bond; they are found in the outer membrane of gram-negative bacteria, and elicit strong immune responses in animals. β -Methylamino-L-alanine, or BMAA, is a non-proteinogenic amino acid produced by *cyanobacteria*. BMAA is a neurotoxin and its potential role in various neurodegenerative disorders is the subject of scientific research. BMAA is a derivative of the amino acid alanine with a methylamino group on the side chain. This non-proteinogenic amino acid is classified as a polar base. BMAA is produced by *cyanobacteria* in marine, freshwater, and terrestrial environments. A high concentration of BMAA is present in shark fins and, as BMAA is a neurotoxin, consumption of shark fin soup and cartilage pills may, therefore, pose a health risk.

The saccharolipid Kdo₂-Lipid A: Kdo residues in red (core), glucosamine residues in blue, acyl chains in black and phosphate groups in green marine alga *L. majuscula* (formerly *Lyngbya mausculata*) produces cyanotoxins Lyngbyatoxin-A

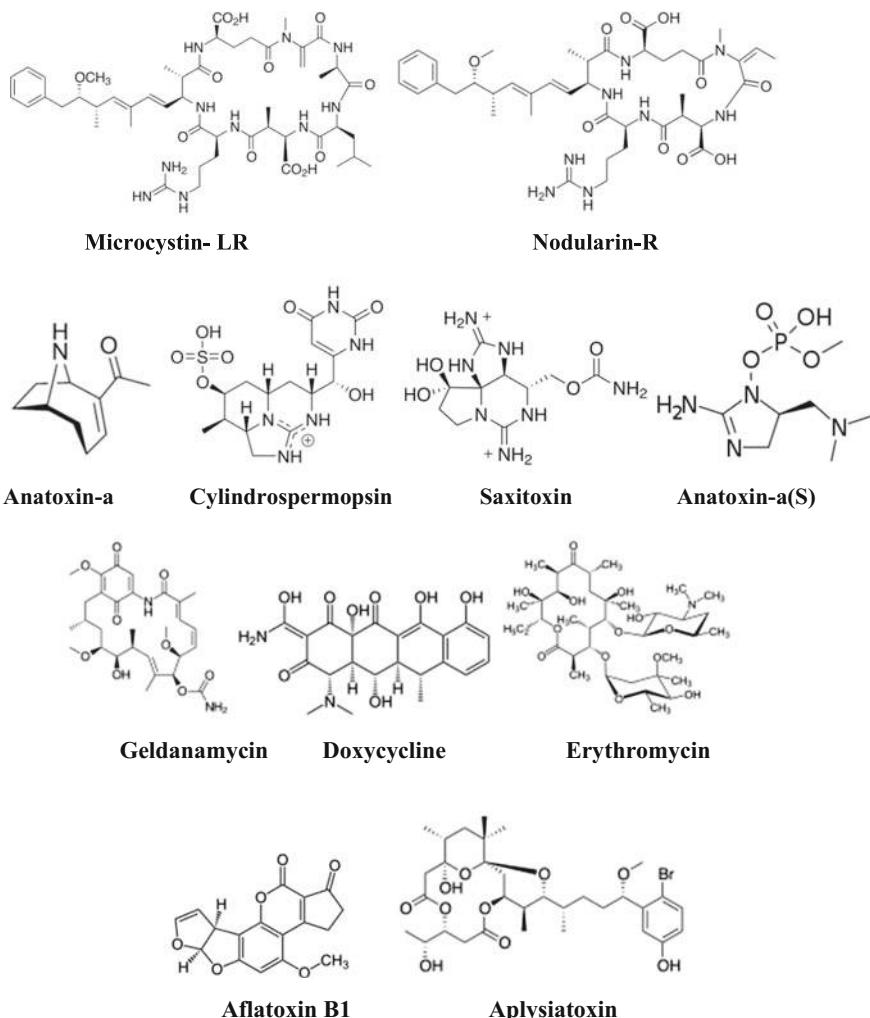


Fig. 4.28 Showing chemical structures of Microcystin-LR, Nodularin-R, Anatoxin-a, Cylindrospermopsin, Saxitoxin, Anatoxin-a(S), Geldanamycin, Doxycycline, Erythromycin, Aflatoxin B1, Aplysiatoxin, The saccharolipid Kdo₂-Lipid A, β-Methylamino-L-alanine, or BMAA, Lyngbyatoxin-A, Debromoaplysiatoxin, Cryptophycin, Borophycin, etc., bioactive compounds from marine *cyanobacteria*

and debromoaplysiatoxin, two highly inflammatory metabolites, causes dermatitis in seaweed red alga *Gracilaria coronopifolia* (Cardellina et al. 1979). These tumor promoters but has an antiproliferative activity against various cancer cell lines in mice, e.g., against P-388 mouse lymphatic leukemia (Osborne et al. 2001; Ito et al. 2002). Cyanotoxins, malyngamide, and floridamide were isolated from *Moorea producens* (Cardillina et al. 1979; Sabry et al. 2017) and anatoxin-a cyanotoxin

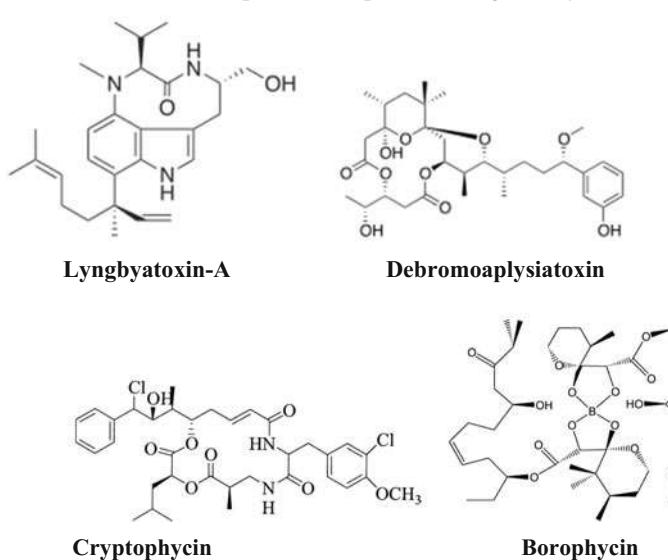
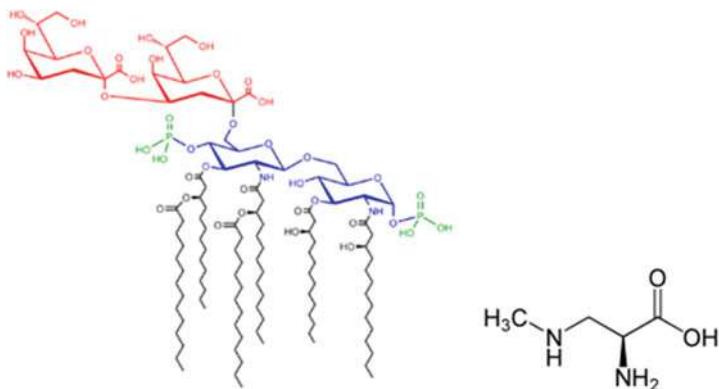


Fig. 4.28 (continued)

from *Anabaena ciecinialis* (Beltron and Nielan 2000). Some marine *cyanobacteria* are potentially viable sources for large-scale commercial production of vitamins such as vitamins of the B complex group and vitamin E (Plavsic et al. 2004). The carotenoids and phycobiliprotein pigments of *cyanobacteria* are natural food coloring agents, feed additives, enhancer of egg yolks color, cattle health, and fertility; they are also used in pharmaceutical and cosmetic industries. Some bioactive compounds from *Lyngbya lagerhaimanii* and *Phormidium tenue* are anti-HIV, some other kill cancer cells by inducing apoptotic death and some other affect cell signaling through activation of the members of protein kinase C family enzymes (Wender et al. 1986; Fujiki and Sugimura 1987). Cultured *Fusarium*

chlamydosporum from the Japanese marine red alga *Carpopeltis affinis* source produced fusaperazines A and B, two new sulfur-containing dioxopiperazine derivatives; cultured the *Leptosphaeria* spp. from the Japanese brown alga *Sargassum tortile* source produced four new epipolysulphanyldioxopiperazines (Yamada et al. 2002).

Cryptophycin from *Nostoc sp.* is a potent fungicide and also exhibited potent cytotoxicity against human tumor cell lines, showed good activity against a broad spectrum drug-sensitive and drug-resistant murine and human solid tumors (Burja et al. 2001). Borophycin, a boron-containing cyanotoxin from marine *Nostoc linckia* and *N. spongiaeforme var. tenuie*, exhibits potent cytotoxicity against human epidermoid carcinoma and human colorectal adenocarcinoma cell lines and has antimicrobial activity (Burja et al. 2001). Figure 4.29 shows chemical structures of Curacin A Hapalosin, Phycoerythrobilin, and Phycocyanobilin from marine cyanobacteria.

Lipopeptide Hapalosin, a cyclic depsipeptide from *cyanobacteria Hapalosiphon welwitschii*, has a reversing activity against MDR (multidrug resistance) (Kashihiara et al. 2000). Lipopeptides are biochemically active, having cytotoxic, anticancer, antibiotic, enzyme inhibitor, antiviral and antifungal activities (Burja et al. 2001).

Scytonemin, an extracellular sheath pigment isolated from the cyanobacterium *Stigonema* spp., showed anti-inflammatory and antiproliferative properties (Proteau et al. 1993; Stevenson et al. 2002a, b). Goniodomin-A, an antifungal polyether macrolide from the dinoflagellate *Goniodoma pseudogoniaulax* (Murakami et al. 1988), showed antiangiogenesis by the inhibition of endothelial cell migration and basic fibroblast growth factor (bFGF)-induced tube formation (Abe et al. 2002). An

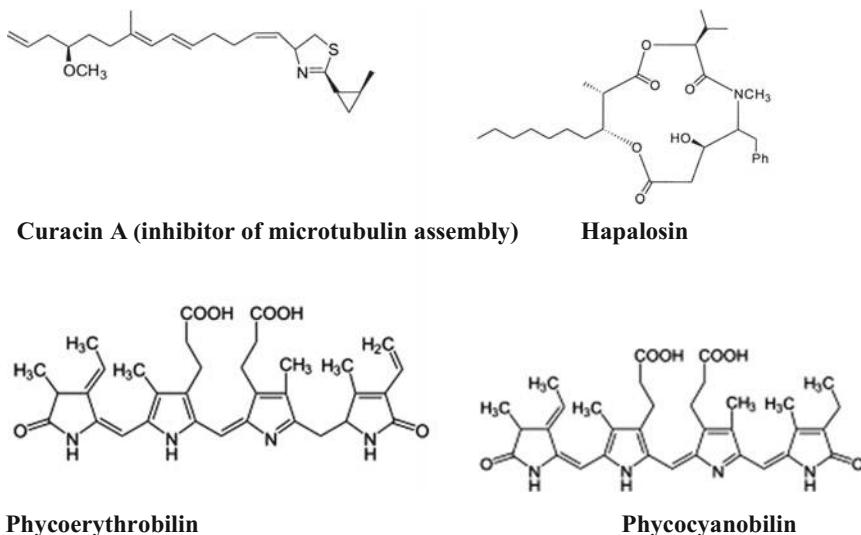


Fig. 4.29 Showing chemical structures of Curacin A (inhibitor of microtubulin assembly), Hapalosin, Phycoerythrobilin, and Phycocyanobilin from marine *cyanobacteria*

immunosuppressive linear peptide microcolin-A, isolated from *L. majusculata*, at nanomolar concentrations has been reported to suppress the two-way murine mixed lymphocyte reaction has been (Koehn et al. 1992). Curacin- A, a thiazoline-containing compound, has been purified from the organic extract of a Curacao collection of *L. majuscula* (Gerwick et al. 1994). Curacin- A has been found to be an exceptionally potent antiproliferative agent and inhibits the polymerization of tubulin having some selectivity for colon, renal, and breast cancer-derived cell lines (Carte 1996). A series of novel antibiotics agents have been isolated from dianoflagellates and antifungal agents from *G. toxicus* (Nagai et al. 1992) as well as brevitoxins from *Ptychodiscus brevis*. Okadaic acid (a polyether fatty acid), a selective protein phosphatase inhibitor produced by *Prorocentrum* spp., has been a key molecule in studying signal transduction pathways in eukaryotic cells (Cohen et al. 1990).

Phycobiliproteins are deep-colored, water-soluble proteins that are present mainly in *cyanobacteria* and *Rhodophyta*. Phycoerythrobilin is the typical chromophore in phycoerythrin. It is similar to porphyrin of chlorophyll for example, but tetrapyrrole is linear, not closed into the ring with a metal ion in the middle.

4.3 Bioactive Compounds and Pharmaceutical Excipients from Microorganisms

Microorganisms or microbes are microscopic organisms are found all around, on the earth surface, in air and in water, at the bottom of deep sea and even inside living bodies. There are some unicellular microbes that are visible to the naked eye, and some multicellular organisms that are microscopic. An organism about 100 micrometers (μm) is visible without a microscope, but most microorganisms are many times smaller than that. A typical animal cell measures roughly 10 μm across but is still microscopic, bacterial cells are typically about 1 μm , and viruses can be 10 times smaller than bacteria. Viruses are visible only under electron microscope (0.1 nm–1 μm), but not under light microscope (100 nm to >1 mm). So, microorganisms differ from each other in size, and also in structure, habitat, metabolism, and many other characteristics. They may be unicellular, multicellular organisms, or acellular such as viruses. Microorganisms are found in each of the three domains of life: Archaea, Bacteria, and Eukarya. Bacteria, *cyanobacteria*, and Archaea are prokaryotes; microbes in the domain Eukarya are eukaryotes while some microorganisms such as viruses do not fall within any of the three domains of life. The domain Eukarya includes all uni- or multicellular eukaryotes such as protists, fungi, plants, and animals. Protists are unicellular eukaryotes like algae and protozoa (but not plants, animals, or fungi).

Microorganisms include a massive range of organisms including archaea, viruses, actinomycetes bacteria, fungi, algae, and protozoa. A diverse groups of bioactive compounds are produced by these organisms, e.g., (i) actinomycin,

amphotericin, chloramphenicol, erythromycin, kanamycin, neomycin, gentamicin, streptomycin, tetracycline, etc., are produced by actinomycetes; (ii) polymyxin B, bacitracin, etc., are produced by *Bacillus* bacteria; (iii) aminoglycosides like gentamicin, tobramycin, etc., are produced by *Streptomyces*; (iv) penicillin, griseofulvin, cephalosporin, etc., produced by the aspergillate group of fungi; (v) apicidin, a broad spectrum antiprotozoal principles from the fungus *Fusarium pallidoroseum*, and so on.

4.3.1 Bioactive Compounds from Prokaryotes: Bacteria, Cyanobacteri, and Actinomycetes

Demonstration of the safety and therapeutic value of penicillin coupled with the discoveries of tyrothricin, actinomycin, and streptothricin initiated the halcyon era of antibiotic discovery (1940–1959). During this period, the prototypes of virtually all families of antibacterial antibiotics now important in medicine were discovered. Examination of previously little studied genera of microorganisms for antibiotic elaboration, use of new culturing and detection techniques, and testing for more diverse types of biological activity will also provide significant new discoveries. Antibiotics are the chemical substances produced by microorganisms and they have the capacity, in low concentration, to inhibit microorganisms growth through an antimetabolic mechanism. Antibiotics differ from antisepsics and disinfectants; they vary in their mode of action, chemical and physical properties; they are affected differently by the composition of the substrate in which they act, vary in their toxicity to animals, and, also in their chemotherapeutic potentialities.

The modern antibiotic era was opened on February 12 of 1941 with the first clinical trial of penicillin, and within a very short time, antibiotic substances occupied the mainstay of clinical therapy of infectious diseases and more than 2000 individual antibiotics known up to this day. Antibiotics are produced alone or in mixture by different groups of microorganisms, e.g., bacteria, fungi, and actinomycetes. The spore-forming aerobes are the most important among the bacteria, the *Penicillium* and *Aspergillus* groups are most important among the fungi, and the genus *Streptomyces* is most important among the actinomycetes. Penicillin G, obtained from a strain of *P. chrysogenum*, is an agent acting against many pathogenic gram-positive bacteria and used to treat syphilis. Cloxacillin, dicloxacillin, methicillin, nafcillin, and oxacillin are semisynthetic penicillins which are used for treatment of staphylococcal infections. Ampicillin has special clinical value for the treatment of infections caused by *H. influenzae*, *Salmonella* species and *Shigella* species. Clavulanic acid is a fermentation product of *Streptomyces clauuligerus* and it controls many infectious diseases. Other antibiotics are cephalosporins (from *Cephalosporium acremonium*), chloramphenicol (from *Streptomyces venezuelae*), and lincomycin (from *S. lincolnensis*), cycloserine (from *S. orchidaceus*), dractinomycin (from *S. parvullus*), vidarabine (from *S. antibioticus*), polymyxin B

(*Bacillus polymyxa*), colistin (*B. polymyxa*), tyrothricin (*B. brevis*), vancomycin (*S. orientalis*), bleamycin (*S. uerticillus*), tetracyclines (*S. aweofaciens*), mitomycin (*S. caespitosus*), erythromycin (*S. erythreus*), amphotericin B (*S. nodosus*), navamycin (*S. natalensis*), griseofulvin (*Penicillium griseofulvum*), rifampin (*S. mediterranei*), novbbiooln (*S. niveus* and *S. sphaeroides*), stgreetamycin (*S. griseus*) neomycin, and paromomycin (*Streptomyces fradiae* and *S. rimosus* var, paromomycinus), kanamycin (*S. kanamyceticus*), gentamicin (*Micromonospora purpurea*), tobramycin or nebramycin factor 6 or nebrarius), amikacin (semisynthetic antibiotic-derived from kanamycin A by acylation), netilmicin (*Micromonospora inyoensis*) and spectinomycin (*Streptomyces specidblis* and *S. flavopersicus*). The development of resistance among the microorganisms on prolonged contact with the drug is the present-day problems in the field of antibiotics.

4.3.2 Bioactive Compounds from Protists: Microalgae (Unicellular Algae) and Protozoa

Microalgae are small-sized (~5 µm, *Chlorella* to >100 µm, *Spirulina*), unicellular to filamentous or planktonic organisms and found in fresh and saline water in both the water column and sediment. Microalgae are comprised of photoautotrophic small-sized algal species from different phyla such as *Cyanophyta*, *Chlorophyta*, *Rhodophyta*, *Cryptophyta*, *Haptophyta*, *Pyrrophyta*, *Streptophyta*, *Heterokontophyta*, etc., including phytoplanktonic species *Dunaliella*, *Chlorella*, *Isocrysis*, *Nannochloropsis*, *Nannochloris*, *Chlamydomonas*, *Haematococcus*, and *Spirulina*, among others. Microalgae are generally eukaryotic organisms, although *Spirulina* (*cyanobacteria*), is included under microalgae due to their photosynthetic (oxygenic) and reproductive properties. Microalgae have been explored for their bioactive compounds with promising applications as antibacterial, antiviral, anti-tumor, anti-HIV antifungal, and antialgal agents including carotenoids, antioxidants, fatty acids, enzymes, polymers, peptides, toxins, and sterols. Microalgae are rich in high polyunsaturated fatty acids—PUFAs (DHA-C22:6, EPA-C20:5, ARA-C20:4, GAL-C18:3), vitamins (A, B₁, B₆, B₁₂, C, E, biotin, riboflavin, nicotinic acid, pantothenate, folic acid), antioxidant (catalases, polyphenols, superoxide dismutase, tocopherols) pigments (β-carotene, astaxanthin, lutein, zeaxanthin, canthaxanthin, chlorophyll, phycocyanin, phycoerythrin, fucoxanthin), polysaccharides (sulphated extracellular polysaccharide) and many other bioactive agents (antimicrobial, antifungal, antiviral agents, toxins, aminoacids, proteins, sterols, MAAs). Many of these biomolecules such as fatty acids, carotenoids, proteins, polysaccharides, polyphenols, and phenolic compounds, polyunsaturated fatty acids (PUFA), or phytosterols among others, and they could be used in some biological applications related with health benefits as antioxidant, anti-inflammatory, or anticarcinogenic agents.

The immense chemical diversity of microalgae provides numerous applications in the food, feed and pharmaceutical industries. Microalgae are cultivated for the production of whole biomass and valuable substances such as nutraceuticals, food additives, carotenoids, phycocyanin and polyunsaturated fatty acids (PUFAs), which are utilized in the food and feed (aquaculture) industry. As evident from different research works (Pulz and Gross 2004; Spolaore et al. 2006; Casal et al. 2011; Guedes et al. 2011; Batista et al. 2013; Sørensen et al. 2013) that some microalgae species very important economically because they produce different high-value bioactive compounds that are useful as health food, food supplement, pharmaceuticals, nutrition, cosmetics, etc., e.g., (i) *Chlorella vulgaris* is important for biomass and pigments useful as health food and food supplement; (ii) *Chlorella* spp., *Chlorella ellipsoidea* and *Coccomyxa acidiphila* produce lutein and β-carotene which are useful as pharmaceuticals and nutrition; (iii) *Coelastrella striolata* var. *multistriata* produces canthaxanthin, astaxanthin and β-carotene are useful as pharmaceuticals, nutrition, and cosmetics; (iv) *Cryptothecodium conhi* produces docosahexaenoic acid useful as pharmaceuticals, nutrition; (v) *Diacronema vikianum* produces fatty acids which are useful as pharmaceuticals and nutrition; (vi) *Dunaliella salina* is source of carotenoids and β-carotene which are useful as health food, food supplement, and feed; (vii) *Galdiera sulphuraria* is a source of phycocyanin which is useful as pharmaceuticals and nutrition; (viii) *Haematococcus pluvialis* is a source of carotenoids, astaxanthin, cantaxanthin, and lutein which are useful as health food, pharmaceuticals, and feed additives; (ix) *Isochrysis galbana* produces fatty acids, carotenoids, and fucoxanthin useful as pharmaceuticals, nutrition, cosmetics, and animal nutrition; (x) *L. majuscule* produces immune modulators which are useful as pharmaceuticals, nutrition; (xi) *Muriellopsis* sp. produces lutein which is useful as pharmaceuticals, and nutrition; (xii) *Nannochloropsis gaditana* produces eicosapentaenoic acid which is useful as pharmaceuticals, and nutrition; (xiii) *Nannochloropsis* sp., *Odontella aurita* produce fatty acids which are useful as pharmaceuticals, cosmetics, and baby food; (xiv) *Parietochloris incise* is a source of arachidonic acid which are useful as nutritional supplement; (xv) *Phaedactylum tricornutum* is a source of lipids, and eicosapentaenoic acid and fatty acids which are useful as nutrition and fuel production; (xvi) *Porphyridium cruentum* is a source of arachidonic acid and polysaccharides which are useful as pharmaceuticals, cosmetics, and nutrition; (xvii) *Scenedesmus almeriensis* is a source of lutein and β-carotene useful as pharmaceuticals, nutrition, and cosmetics; (xviii) *Schizochytrium* sp. produces docosahexaenoic acid which are useful as pharmaceuticals and nutrition; (xix) *Spirulina platensis* produces phycocyanin, γ-linolenic acid, biomass and protein which are useful as health food and cosmetics; (xx) *Ulkenia* spp. produce docosahexaenoic acid which are useful as pharmaceuticals, nutrition; etc.

Bioactive compounds obtained from microalgae show different biological activities such as (i) Carotenoids like β-carotene from *D. salina*, *Haematococcus* sp., etc., functions as antioxidant, pro-vitamin A, and anti-inflammatory anticancer agents (Ramos et al. 2011; Davidi et al. 2014; Markou et al. 2015); astaxanthin from *H. pluvialis*, *Chlorella zofigiensis*, *Chlorococcum* sp., etc., functions as

antioxidant, anti-inflammatory, and anticancer agents (Yuan et al. 2011; Liu et al. 2014); lutein from *D. salina Chlorella sorokiniana Chlorella prothecoide*, etc., functions as antioxidant, anti-inflammatory, anticancer (Shi et al. 2002; Cordero et al. 2011; Fu et al. 2013); violaxanthin from *Dunaliella tertiolecta Chlorella ellipsoidea*, etc., functions as anti-inflammatory anticancer (Pasquet et al. 2011; Soontornchaiboon et al. 2012); zeaxanthin from *synechocystis* sp. *chlorella saccharophila*, etc., functions as antioxidant anti-inflammatory (Lagarde et al. 2000; Singh et al. 2013); fucoxanthin from *P. tricornutum*, *Isochrysis* sp., etc., functions as anticancer (Kim et al. 2011; Crupi et al. 2013). Figure 4.30 shows the chemical structures of bioactive compounds such as β -Carotene, Lutein, Fucoxanthin, Violaxanthin, Zeaxanthin, etc., from protists.

(ii) Fatty acid like eicosapentaenoic acid (EPA) from *Tetraselmis* sp. functions as anti-inflammatory anti-angiogenic (Mobraten et al. 2013; Adarme-Vega et al. 2014); docosahexaenic acid (DHA) from *tetraselmis* sp., etc., functions as anti-inflammatory anti-angiogenic (Spencer et al. 2009; Lee and Han 2015); docosapentaenoic acid (DPA) from *Nannochloropsis oculata*, etc., functions as anti-inflammatory (Spencer et al. 2009; Nauroth et al. 2010); Fig. 4.31 shows chemical structures of fatty acid compounds.

Fatty acids like eicosapentaenoic acid (EPA), docosahexaneoic acid (DHA), and α -linolenic acid (ALA) are important omega-3 fatty acids and omega-3 fatty acids are important constituents of the cell membrane. Dietary supplementation with omega-3 fatty acids can yield many health benefits including reduces the risk for cardiac arrhythmias improves memory, Alzheimer disease, reduces symptoms of

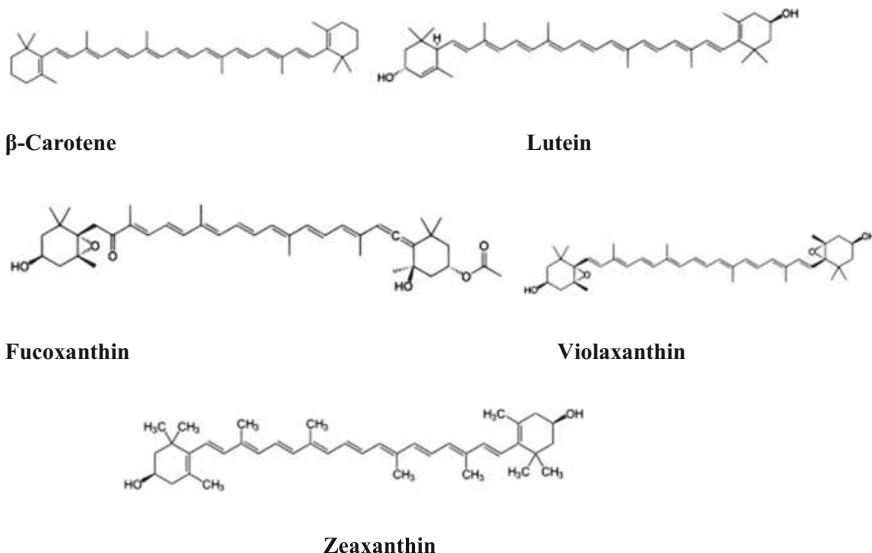


Fig. 4.30 Showing chemical structures of bioactive compounds such as β -Carotene, Lutein, Fucoxanthin, Violaxanthin, Zeaxanthin, etc., from protists

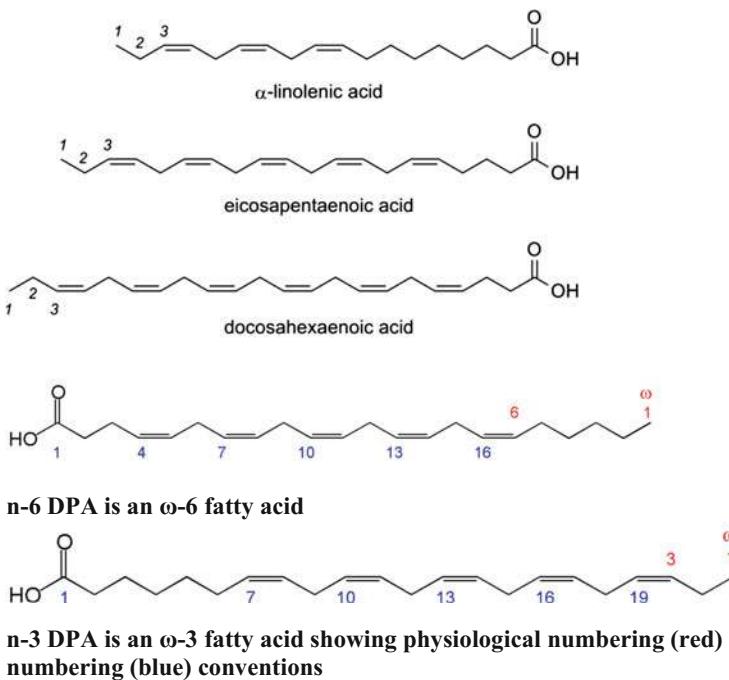


Fig. 4.31 Showing chemical structures of fatty acid compounds

depression, decrease in triglyceride levels and blood pressure, improves fetal development, etc. (Covington 2004; Swanson et al. 2012).

Docosapentaenoic acid (DPA), straight chain of 22 carbons and 5 double bonds (22:5) polyunsaturated fatty acid (PUFA), is primarily used to designate two isomers, commonly termed *n*-6 DPA and *n*-3 DPA; these designations describe the position of the double bond being 6 or 3 carbons closest to the (omega) carbon at the methyl end of the molecule. They are dietary-essential PUFA for mammals, including humans, must be supplied from outside obtain fatty acids from both classes in order to maintain normal health (anti-inflammatory) (Edwards and O'Flaherty 2008; Spector and Kim 2015; Dalli et al. 2015).

(iii) Glycolipids like monogalactosyl diacylglycerol (MGDG) from *Gymnodinium mikimotoi*, *Stephanodiscus* sp., *Pavlova lutheri*, *Stephanodiscus* sp., etc., functions as anticancer antioxidant (Maeda et al. 2008; Mizushina et al. 2012); digalactosyldiacylglycerol (DGDG) from *Stephanodiscus* sp., etc., functions as anticancer antioxidant (Hossain et al. 2005; Maeda et al. 2009); sulfo-quinovosyl-acyl-glycerol (SQAG) from *Stephanodiscus* sp., etc., functions as anticancer antioxidant (Hossain et al. 2005; Maeda et al. 2013). Figure 4.32 shows chemical structures of Glycolipids.

Galactolipids are glycolipids with galactose sugar, absence of nitrogen in the molecule differ them from glycosphingolipid. Of the total lipid content of the

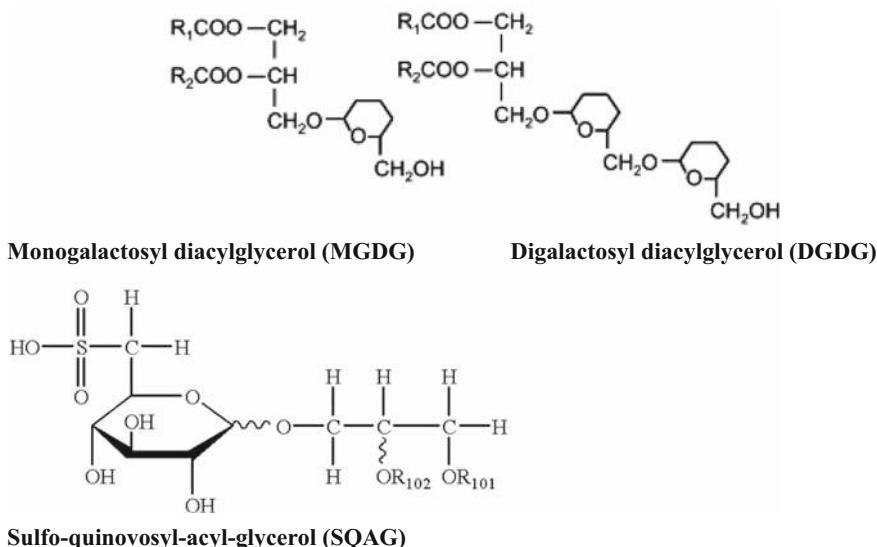
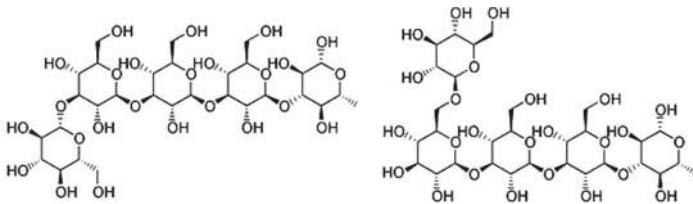


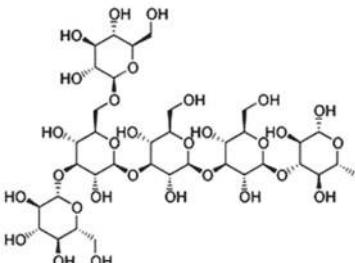
Fig. 4.32 Showing chemical structures of Glycolipids—mono- and di-galactosyl diacylglycerol (MGDG, DGDG; R^1 and R^2 are fatty chains); Sulfo-quinovosyl-acyl-glycerol (SQAG) (R_{101} and R_{102} represent an acyl residue and a hydrogen atom or an acyl residue, respectively of a higher fatty acid)

thylakoid membrane of higher plant chloroplasts, these two nonionic lipids, MGDG and DGDG, are present at 56 and 29%, respectively. DGDG is a bilayer-forming lipid, while MGDG alone only forms hexagonal-II structures. The plant galactolipids MGDG and DGDG have been linked to the anti-inflammatory and cancer benefits due to their ability to regulate the levels of free radicals like nitric oxide (NO) (Gao et al. 2014). It has been reported that a class of sulfolipids, sulfo-quinovosyl-acyl-glycerols (SQAGs) from ferns and algae, are potent inhibitors of eukaryotic DNA polymerase alpha and beta and effective antineoplastic agents, while the inhibitory effect could be influenced by the chain size of fatty acids in the SQAGs as well as the sulfonyl group in quinovose was also needed to inhibit the enzymes (Mizushina et al. 2005).

(iv) Polysaccharides like sulphated extracellular polysaccharide from diatom *P. tricornutum*, etc., functions as anti-inflammatory immunomodulating (Guzmán et al. 2003); sulphated polysaccharide β -(1,3)-glucan from Chlorophyte *Chlorella stigmatophora*, *C. vulgaris*, etc., functions as anti-inflammatory immunomodulating anticancer (Nomoto et al. 1983; Guzmán et al. 2003); sulphated polysaccharide from Prasinophyte *Tetraselmis suecica*, etc., functions as anti-inflammatory (Jo et al. 2010); sulphated polysaccharide from haptophyte *I. galbana*, etc., functions as anticancer (Sadovskaya et al. 2014); sulphated polysaccharide from rhodophyte *Porphydium* sp., etc., functions as anti-inflammatory immunomodulating anticancer (Matsui et al. 2003); sulphated polysaccharide from dinoflagellate *Gyrodinium impudicum*, etc., functions as anti-inflammatory immunomodulating anticancer (Bae



Polysaccharide β -(1-3-glycosidic linkage)-glucan Polysaccharide β -(1-6-glycosidic linkage)- β -glucan



Polysaccharide β -(1, 3: 1-6-glycosidic linkages)- β -glucan

Fig. 4.33 Showing chemical structures of various polysaccharides with various β -glucan glycosidic linkages

et al. 2006); extracellular polysaccharide s-spirulan from *Cyanobacteria* *Arthrospira platensis*, etc., functions as anticancer (Challouf et al. 2011). Figure 4.33 shows chemical structures of various polysaccharides with various β -glucan glycosidic linkages

β -Glucans (beta-glucans) comprise a group of β -D-glucose polysaccharides naturally occurring in the cell walls of cereals, bacteria, fungi, algae, etc., with significantly differing physicochemical properties dependent on source. Typically, β -glucans form a linear backbone with 1-3 β -glycosidic bonds but vary with respect to molecular mass, solubility, viscosity, branching structure, and gelation properties, causing diverse physiological effects in animals. β -Glucan decreases the levels of saturated fats in the blood, reduces the risk of heart disease, shows immunomodulatory properties. β -Glucans are also used in various nutraceutical and cosmetic products, as texturing agents, and as soluble fiber supplements, but can be problematic in the process of brewing.

Marine microalgae biomass and the compounds, especially the exo- or extracellular polysaccharides (EPS) and sulphated polysaccharides (sPS) or their derivatives, they produce have been shown to possess several biological applications with numerous health benefits as antioxidant, antilipidaemic (hypolipidaemic), and hypoglycaemic (antidiabetic), anticoagulant and/or antithrombotic, immunomodulatory ability, anti-inflammatory, antitumor and cancer preventive, antibiotics or as biolubricant agents and drug reducers. Potentials of sPS from marine microalgae may

be used as nutraceuticals, therapeutic agents, cosmetics, or in engineering (de Jesus Raposo et al. 2013, 2015).

(v) Protein and peptides like phycobiliproteins from *S. platensis*, *Porphyridium* sp. etc., functions as antioxidant anti-inflammatory anticancer (Romay et al. 2003; Zheng et al. 2011); peptides from *Chlorella pyrenoidosa*, *Cyanobacteria*, etc., functions as antioxidant anti-inflammatory anticancer (Piplani et al. 2012; Wang and Zhang 2013);

Phycobiliproteins are complexes formed between proteins and covalently bound phycobilins that act as chromophores that capture light energy in microalgae and phycobiliproteins are classified into Phycoerythrin (PE), a red pigment, phycocyanin (PC), a blue pigment, and allophycocyanin (APC). They are water-soluble proteins present in *cyanobacteria* and algae (e.g., rhodophytes, cryptomonads, glaucocystophytes). The major phycobiliproteins are R-Phycoerythrin (R-PE) (mol. wt. 240 kDa), B-Phycoerythrin (B-PE) (mol.wt. 240 kDa), C-Phycocyanin (CPC) (mol.wt. 232 kDa), Allophycocyanin (APC) (mol.wt. 105 kDa), etc. R-PE, B-PE, CPC, and APC have health-promoting properties and a wide range of potential industrial applications, mainly in the food and feed industries, as well as in the pharmaceutical and the cosmetic industries.

Phycobiliproteins can be used in various foods, such as dairy products, candies, ice-creams, beverages, etc. (Christaki et al. 2015; Manirafasha et al. 2016). Because of the fluorescent properties, phycobiliproteins are used in immunoassays as biochemical tracers, especially phycoerythrin for its intense fluorescence is used in the pharmaceutical industry as sensitive indicator, and in the cosmetic industry as a skin cream to stimulate collagen synthesis (Christaki et al. 2011, 2015; Manirafasha et al. 2016). Phycobiliproteins have antioxidant activities for reducing the oxidative stress (Rodriguez-Sanchez et al. 2012) and have shown the ability to protect organisms against various chronic disorders as cancer, diabetes, coronary disease, neurodegenerative diseases or to ameliorate the cognitive functions (Christaki et al. 2015; Manirafasha et al. 2016) and in particular, phycocyanin could be used as a nephroprotector or a protector of human pancreatic cells (Chu 2012; Rodriguez-Sanchez et al. 2012). Other compounds such as amides from *L. majuscula*, etc., functions as anticancer (Kwan et al. 2010), quinones from *Calothrix* sp. etc., functions as anticancer (Hatae et al. 2014); phenolic compounds from *Spirulina maxima*, *Chlorella ellipoidea*, *Nannochloropsis* sp., etc., functions as antioxidant (Abd El-Baky et al. 2010; Cha et al. 2011); tocopherols from *Porphyridium* sp., etc., functions as antioxidant (Mendiola et al. 2008).

Protozoa comprise a diverse group of microscopic single-celled nonfilamentous eukaryotic animals or organisms with animal-like behaviors including motility and predation. They include unicellular heterotrophic protists like ciliates, amoebae, and flagellates (e.g., dinoflagellates, amoebas, paramecia, Plasmodium, etc.). They are confined to moist or aquatic habitats (ubiquitous in such environments worldwide), many are symbionts, and some species are parasites. Some of the common pathogenic protozoan parasites are *Cryptosporidium parvum*, *Entamoeba histolytica*, *Giardia intestinalis*, *Leishmania braziliensis*, *Leishmania donovani*,

Leishmania major, *Naegleria gruberi*, *Plasmodium falciparum*, *Plasmodium vivax*, *T. gondii*, *Trichomonas vaginalis*, *Trypanosoma brucei*, and *Trypanosoma cruzi*.

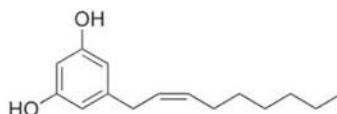
Climacostol, a defense toxin produced by the freshwater ciliated protozoan *Climacostomum virens* for chemical defense against predators, is one of the resorcinolic lipids and shows antimicrobial, antiparasitic, and cytotoxic activities. Resorcinolic lipids have been widely detected in prokaryotes and eukaryotes and attracted attention for their antimicrobial, antiparasitic, antitumor, and genotoxic activities (Buonanno et al. 2008). Climacostol appears to inhibit the growth of tumor cells and induce apoptosis in vitro (Buonanno et al. 2008; Fiorini et al. 2010, Perrotta et al. 2016). Petrelli et al. (2012) demonstrated the effective antimicrobial activity of this compound against gram-positive bacteria but no significant effect against gram-negative species. Figure 4.34 shows chemical structures of protozoan metabolites Climacostol, Paramylon, etc.

Researchers have found a protozoan species may one day offer a source of healthy nutrients. Protozoan like Euglena (unicellular flagellate eukaryote without cell wall around the outer cell membrane, behaves like photoautotroph in sunlight as plant heterotroph like an animal in darkness) may be an excellent source for nutritional supplements may provide several bioactive compounds including antioxidants, vitamins C and E, and also omega-3 fatty acids nutrients they feed on algal species and pick up these compounds from algae. Euglena produces paramylon, a bioactive compound useful for health improvement. Paramylon starch is a β -1,3 polymer of glucose made in the pyrenoids of *Euglena* (Calvayrac et al. 1981; Monfils et al. 2011).

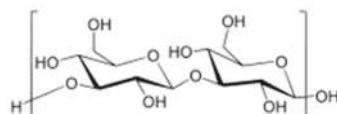
4.4 Bioactive Compounds Obtained from Minerals

4.4.1 Kaolin

The name “kaolin” (china clay) was derived from Chinese “Gaoling”, chemical composition rocks that are rich in kaolinite $[Al_2Si_2O_5(OH)_4]$ are known as kaolin.



Climacostol (1,3-dihydroxy-5-[(Z) -non-2'-enyl]benzene)



Paramylon (a storage carbohydrate of *Euglena* similar to starch)

Fig. 4.34 Showing chemical structures of protozoan metabolites Climacostol, Paramylon, etc

Kaolin is also known for its capabilities to induce and accelerate blood clotting. Activated charcoal or activated carbon is a form of carbon processed to have small, low-volume pores high degree of microporosity that increase the surface area available for adsorption or chemical reactions, just one gram of activated carbon has a surface area in excess of 3000 m^2 ($32,000\text{ ft}^2$) (Dillon et al. 1989). Activated charcoal is used to treat poisonings and overdoses following oral ingestion and as an over-the-counter drug is used to treat diarrhea, indigestion, and flatulence.

4.4.2 *Calomel*

Mercury is a chemical element with symbol Hg and atomic number 80. Calomel is a Mercury(I) chloride mineral, a colorless solid is really the compound with the formula Hg_2Cl_2 , with the connectivity Cl–Hg–Hg–Cl, it occurs as a secondary mineral which forms as an alteration product in mercury deposits. Calomel is a laxative and once was a common medicine, especially on the American frontier. It fell out of use at the end of the nineteenth century due to its toxicity.

4.4.3 *Iodine*

Iodine is a chemical element, with atomic number 53. It is an essential element for human body, iodine must come from the diet with salt or through seafood, especially seaweed. The thyroid gland needs iodine to make hormones, iodine deficiency causes the thyroid to work harder and this can cause an enlarged thyroid gland (goiter), which becomes evident as a swollen neck. Iodine deficiency and the resulting low levels of thyroid hormone can cause women to stop ovulating, leading to infertility, deficiency can also lead to an autoimmune disease of the thyroid and may increase the risk of getting thyroid cancer and also increases the risk of prostate, breast, endometrial, and ovarian cancer. Iodine can kill fungus, bacteria, and protozoa such as amoebas. Iodine as potassium iodide is also used to treat (but not prevent) the effects of a radioactive accident.

4.4.4 *Iron*

Iron, with atomic number 26, is an essential element for the proper growth and development of the human body and must be supplied through diet such as legumes, lentils, soy beans, whole grains, green leafy vegetables, cereals, bread, spinach, turnip, fish, meat, sprouts, broccoli, and dry fruits. It helps to metabolize proteins and plays a role in the production of hemoglobin and red blood cells. Iron deficiency can lead to conditions like anemia, chronic anemia, cough, and

pre-dialysis anemia. Iron helps to eradicate different causes of fatigue, plays a key role in strengthening the immune system, builds concentration, treats insomnia, and regulates body temperature.

4.4.5 Gold

Gold with atomic number 79 is an aesthetic memorable metal that occupies a special place in the human mind. However, it is used as a drug to treat a number of ailments such as auranofin, sodium aurothiomalate (gold sodium thiomalate), aurothioglucose (gold thioglucose), three gold compounds, are used (in one or another form) as drugs to treat rheumatoid arthritis in many developed countries including USA and UK; particles of a radioactive gold isotope are implanted in tissues to serve as a radiation source in the treatment of certain cancer; small amounts of gold are used (as implant in the upper eyelid) to treat lagophthalmos (an inability of a person to close their eyes completely) and the implanted gold “weights” the eyelid, and the force of gravity helps the eyelid close fully; gold is used in dentistry for fillings, crowns, bridges, and orthodontic appliances because it is chemically inert, nonallergenic, and also because of its superior performance and aesthetic appeal. Radioactive gold is used in diagnosis. Many surgical instruments, electronic equipment, and life-support devices are made using small amounts of gold. Gold is nonreactive in the instruments and is highly reliable in the electronic equipment and life-support devices. Figure 4.35 shows chemical structures of auranofin, sodium aurothiomalate, and aurothioglucose.

4.4.6 Sulfur

Sulfur, atomic number 16, is an essential element of human nutrition and must get it through diet supplement containing garlic, onions, broccoli, and other members of Brassicaceae. Sulfur is used in the treatment for shortness of breath, allergies, swelling in the back of the throat (pharyngitis), high cholesterol, clogged arteries, menopause, and upper respiratory tract infections like the common cold. Sulfur has antibacterial effects and cream, lotion, ointment, and bar soap containing sulfur are used to treat many different skin and other ailments such as acne or seborrheic dermatitis (scaly and red skin patches), and scabies (an itchy skin infection caused by mites), skin redness (rosacea), dandruff, lice, cold sores, warts, and sumac infections. Sulfonamides, thioethers, sulfones, penicillin, etc. are common sulfur-containing drugs and many sulfur-containing drugs possess antiviral, antibacterial, antiallergic, antimarial, and cytotoxic properties among others.

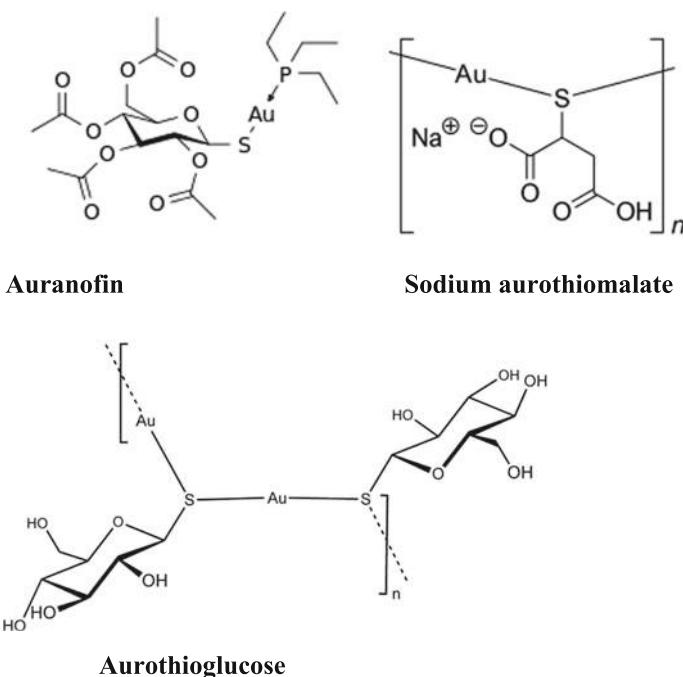


Fig. 4.35 Showing chemical structures of auranofin, sodium aurothiomalate, and aurothioglucose

4.4.7 Aluminum Hydroxide

Aluminium, with atomic number 13, is a light white metal, ductile and malleable, and good conductor of electricity. It occurs widely in nature in clay. Aluminium hydroxide, $\text{Al}(\text{OH})_3$, is found in nature as the mineral gibbsite (hydrargillite). Aluminium hydroxide is amphoteric in nature, it has both basic and acidic properties. Due to amphoteric property, it acts as a Brønsted–Lowry base in acid, picks up hydrogen ions, neutralizes the acid, and yields a salt:

$3\text{HCl} + \text{Al}(\text{OH})_3 \rightarrow \text{AlCl}_3 + 3\text{H}_2\text{O}$, while in bases, it acts as a Lewis acid by taking an electron pair from the hydroxide ions: $\text{Al}(\text{OH})_3 + \text{OH}^- \rightarrow \text{Al}(\text{OH})_4^-$.

4.4.8 Magnesium Hydroxide

Magnesium, with atomic number 12, is a naturally occurring mineral. Magnesium is important for many systems in the body especially the muscles and nerves. Magnesium hydroxide, $\text{Mg}(\text{OH})_2$, reduces stomach acid, and increases water in the intestines which may induce defecation. It is used as a laxative to relieve occasional



Fig. 4.36 Showing chemical reactions between an aluminum hydroxide containing antacid; a magnesium hydroxide containing antacid

constipation (irregularity) and as an antacid to relieve indigestion, sour stomach, and heartburn.

Most antacids contain aluminum hydroxide and/or magnesium hydroxide. Some antacids contain calcium carbonate; sodium bicarbonate (baking soda) is used also as an antacid. The duration of action of sodium bicarbonate is less than that of many antacids because sodium bicarbonate reacts rapidly with HCl and the mixture disappears (empties) quickly from the stomach. Figure 4.36 shows how chemical equations illustrating the reaction between an aluminum hydroxide [Al(OH)_3]-containing antacid or a magnesium hydroxide [Mg(OH)_2]-containing antacid takes place.

4.4.9 Magnesium Trisilicate

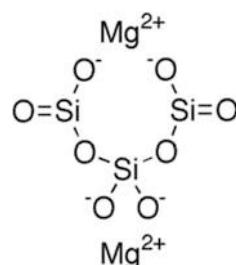
Magnesium trisilicate (Fig. 4.37) is an inorganic compound that is used as a food additive and it is often employed by fast food chains (e.g., KFC) to absorb fatty acids and remove impurities that form in edible oils during the frying process.

Aluminum hydroxide/Magnesium trisilicate is an antacid. It is used to relieve the symptoms of indigestion, heartburn, or gastroesophageal reflux disorder (GERD).

4.4.10 Magnesium Sulfate

Magnesium sulfate, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, as a medication is used to treat and prevent low blood magnesium. It is the primary treatment and preventative measure in women with eclampsia and seizures in women with eclampsia. It lowers systolic blood

Fig. 4.37 Showing chemical structure of magnesium trisilicate



pressure while maintaining diastolic blood pressure, thus leaving blood flow to the fetus uncompromised. It is also used in the treatment of torsades de pointes, severe asthma exacerbations, constipation, and barium poisoning. As Epsom salts, it is also used for mineral baths.

4.4.11 Mercurial Salts

The metal mercury or quicksilver, with atomic number 80, was used very extensively as a medicine, chiefly as compounds of mercurial salts, although they are much less common today because the toxic effects of mercury and its compounds now are more widely understood. Inorganic mercurial salt such as mercury(I) chloride was used to treat syphilis, many mercurial salts including organomercury compound mersalyl acid, chlormerodrin, meralluride, etc., were previously used as diuretics, but are hardly used anymore (Fig. 4.38). All salts of mercury are extremely poisonous.

4.4.12 Zinc and Zinc Oxide

Zinc, with atomic number 30, is an essential trace element for human health and should be supplied through diet. Zinc is found in various foods, including lean red meats, seafood (herring, oysters, etc.), peas, and beans, whole grains; and zinc may be added to the diet through treated (galvanized) cookware. Zinc deficiency consequences include stunted growth and acute diarrhea in children, and slow wound healing. It is used for boosting the immune system, treating the common cold and recurrent ear infections, and preventing lower respiratory infections. It is also used for malaria and other diseases caused by parasites. Zinc is also used for eye disease macular degeneration, night blindness, and cataracts. It is also used for asthma; diabetes; high blood pressure; acquired immunodeficiency syndrome (AIDS); and skin conditions such as psoriasis, eczema, acne, aging skin, herpes simplex infections, and to speed wound healing.

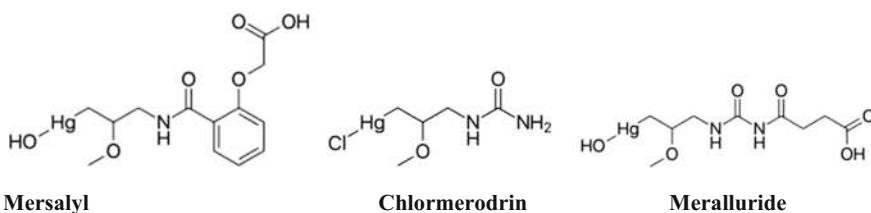


Fig. 4.38 Showing chemical structure of the organomercury diuretic compound

Zinc oxide is an inorganic compound with the formula ZnO. Zinc oxide is an amphoteric oxide. It is nearly insoluble in water, but it will dissolve in most acids, such as acid HCl to produce zinc chloride: ZnCl₂ and; also in alkali NaOH to give soluble zincates: Na₂[Zn(OH)₄]. Zinc oxide as a mixture with about 0.5% iron (III) oxide (Fe₂O₃) is called calamine and is used in calamine lotion. ZnO is deodorizing and antibacterial and its nanoparticles can enhance the antibacterial activity of ciprofloxacin. It is used in many products such as baby powder and barrier creams to treat diaper rashes, calamine cream, antidandruff shampoos, and antiseptic ointments. Zinc oxide is also used in ointments, creams, and lotions to protect against sunburn and other damage to the skin caused by ultraviolet light.

4.4.13 *Flourine*

Fluorine, with atomic number 9, is a univalent element of halogen group, and the most chemically reactive and electronegative of all the elements. Fluorine is the 13th most abundant element in the Earth's crust (950 ppm), while soils contain approximatively 330 ppm of fluorine, ranging from 150 to 400 ppm. Fluorine, in aqueous solution, occurs as the fluoride ion F⁻, and it readily combines with some positively charged counterpart to forms fluoride compounds. Fluorides are released into the air in the wind-blown soil.

Fluorine is naturally present in water, air, plants, and animals in traces and thus humans are exposed to fluorine through food and drinking water and by breathing air. Large quantities of fluorine are present in tea and shellfish. Fluorine increases the bioavailability of calcium and helps to buffer acids present in the mouth. It is essential for the maintenance of solidity of bones, protect spleen and dental decay. But too frequent use or excess fluorine can cause teeth decay (dental fluorosis), osteoporosis, and harm to kidneys, bones, nerves, and muscles. Deficiency may cause tooth decay, poor eyesight, curvature of the spine and susceptibility to infection. Green and colored vegetables as well as nuts are the dietary source of fluorine.

Application of fluorine to a number of cutting-edge technologies is evident, e.g., perfluorocarbons (PFCs) are capable of holding enough oxygen to support human liquid breathing, organofluorine in the form of its radioisotope ¹⁸F is used in positron emission tomography (PET), a modern medical imaging technique; and a PET scan produces three-dimensional-colored images of parts of the body that use a lot of sugar, particularly the brain or tumors. Fluorine added to drug molecules (even a single atom) changes the chemical properties of the molecule in desirable ways., e.g., many drugs are fluorinated to delay their metabolism (i.e., prolongs their half-lives), increases a drug's bioavailability by increasing the drugs lipophilicity and cell membrane penetration, protects the drug's aromatic ring and prevents the formation of epoxide. Fluorine is used in dexamethasone, triamcinolone, and fludrocortisone (mineralocorticoids) to increase both their medical

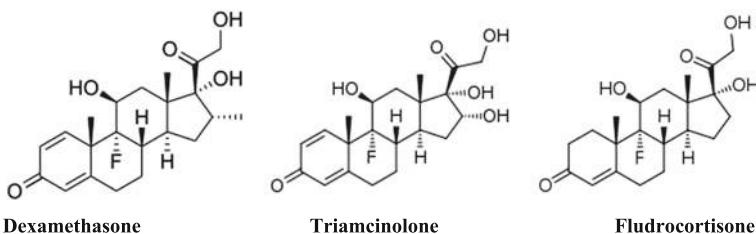


Fig. 4.39 Showing chemical structure of different mineralocorticoids

power and anti-inflammatory effects. Many other fluorinated pharmaceutical drugs are available and about 20 % of commercialized pharmaceuticals contain fluorine in their molecule (Emsley 2011). Figure 4.39 shows chemical structure of different mineralocorticoids.

4.4.14 Borax

Boron, with atomic number 5, is brown amorphous powder, and occurs as borax and boric acid. Borax, sodium pyroborate $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ or better as $\text{Na}_2[\text{B}_4\text{O}_5(\text{OH})_4] \cdot 8\text{H}_2\text{O}$, since borax contains the $[\text{B}_4\text{O}_5(\text{OH})_4]^{2-}$ ion), salt of boric acid, sodium borate, is an important boron compound. Borax is a component of many detergents, cosmetics, and it is used to make buffer solutions in biochemistry, an antifungal compound, a texturing agent in cooking, and is useful as an insecticide. Borax is a natural substance, mined from the earth, or collected as evaporative deposits from seasonal lakes beds, just like salt. Mines are located in countries including the United States, Bolivia, Chile, China, Ukraine, Turkey, India, Tibet, etc. Borax contains boron, a trace mineral, which can be lacking in modern diets. Restoring healthy boron levels can improve a number of health conditions.

Borax is a natural cleaning agent and is simple and an extremely inexpensive and amazing remedy used for conditions like arthritis, osteoporosis, parathyroid issues, balancing calcium and magnesium, and as a natural aphrodisiac. Borax is used to treat overall chronic illness, including autoimmune diseases, hormone problems, and chronic pain. Sodium borate is also effective for treating a variety of specific ailments as an anti-inflammatory agent; borax effectively treats gout, swollen gums, and other inflammatory diseases. Additionally, the substance eliminates infection such as bladder infection, urinary tract infection and others. It has also been used to treat cancer, obesity, high blood pressure and arterial disease. Additionally, borax is quite alkaline and suitable to fight acidity as many health problems arise because the body is too acidic.

4.4.15 Selenium and Selenium Sulfide

Selenium, with atomic number 34, is a nonmetal with properties that are intermediate between the elements above and below in the periodic table, sulfur, and tellurium, and also has similarities to arsenic. Selenium is an essential micronutrient for animals but it is toxic in large doses. Selenium is a component of the unusual amino acids selenocysteine and selenomethionine; selenium is a trace element nutrient and dietary selenium comes from nuts, cereals and mushrooms. Marine fishes and vertebrate thyroid glands have the highest concentration of selenium, and iodine. In humans, selenium functions as cofactor for reduction of antioxidant enzymes, such as glutathione peroxidases and certain forms of thioredoxin reductase found in animals and some plants. The glutathione peroxidase family of enzymes (GSH-Px) catalyzes certain reactions that remove reactive oxygen species such as hydrogen peroxide and organic hydroperoxides. The thyroid gland and every cell that uses thyroid hormone use selenium, which activate and then deactivate various thyroid hormones and their metabolites. Increased dietary selenium reduces the effects of mercury toxicity.

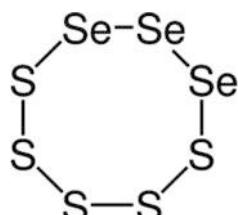
Selenium disulfide, composition that approximates to SeS_2 and sometimes called selenium sulfide (SeS)—antifungal and cytostatic agents, is a medication used to treat pityriasis versicolor, seborrhoeic dermatitis, and dandruff (Fig. 4.40). It is applied to the affected area as a lotion or shampoo. At the 2.5% strength, selenium disulfide is also used on the body to treat tinea versicolor, a type of fungal skin infection caused by a different species of *Malassezia*.

Selenium disulfide is not a pure chemical compound but a mixture where the overall Se: S ratio is 1:2. The compounds are Se–S rings containing a variable number of S and Se atoms, $\text{Se}_n\text{S}_{8-n}$.

4.4.16 Petroleum

Petroleum, crude or fossil oil is a thick and black liquid. It is a natural material mainly made of hydrocarbons. It is a complex mixture of hydrocarbons that occur in the Earth in liquid, gaseous, or solid forms, the liquid, gaseous, and viscous or solid forms are commonly designated as crude oil, natural gas, and bitumen, respectively. The liquid and gaseous phases of petroleum constitute the most

Fig. 4.40 Showing structure of $1,2,3\text{-Se}_3\text{S}_5$, illustrative of selenium sulfide



important of the primary fossil fuels. Almost all crude oil ranges from 82 to 87% carbon by weight and 12–15% hydrogen and viscous bitumens generally vary from 80 to 85% carbon and from 8 to 11% hydrogen. Hydrocarbons of crude oil can be grouped into three basic chemical series: paraffins (C_nH_{2n+2}), naphthenes (C_nH_{2n}), and aromatics (C_nH_{2n-6}). Most crude oils are mixtures of these three series in various and seemingly endless proportions. Petroleum jelly is a semi-solid mixture of hydrocarbons (mixture of mineral oils and waxes, a semisolid jelly-like substance) used as a topical ointment for its healing properties. Petroleum jelly's benefits come from its main ingredient petroleum, which helps seal skin with a water-protective barrier to retain moisture. As an occlusive moisturizer, it protects skin, hand, face, lips, nose, cracked heels from drying out. Also prevents diaper rash and skin stains from hair dye or nail polish, save split ends of hair, removes eye makeup, preserve perfume scents, etc. In the pharmaceutical and cosmetic industries, petroleum jelly is mainly used as a base material and to increase consistency.

Petroleum jelly is made up of a partial solid mix of hydrocarbons that comes from mines while vaseline is made up of pure petroleum jelly, which contains minerals and microcrystalline wax (so it is smoother). Petroleum jelly is colorless translucent or pale yellow color (when not highly distilled). Petroleum jelly is moisturizer for topical use, when containing phenol, it gives the jelly additional antibacterial effect, it acts as a sunscreen and provides protection against ultraviolet rays, as an ingredient in skin lotions and cosmetics it functions as a grooming aid, with pure beeswax (50/50), it makes an effective mustache wax, provide heat insulation and keeps swimmers warm in water, prevents chilling of the face due to evaporation of skin moisture during cold weather, reduce the friction between skin and clothing during various sport activities (prevent chafing of the seat region of cyclists, the nipples of long distance runners wearing loose T-shirts, commonly used in the crotch area of wrestlers and footballers), used as a personal lubricant because it has a distinctive "feel" and does not dry out like water-based lubricants, not recommended for use with condoms because it swells latex and thus increases the chance of rupture, and also not for vaginal intercourse because of the risk of yeast infection and bacterial vaginosis.

4.5 Bioactive Synthesized Compounds and Pharmaceutical Drugs

Since antiquity, native healers, midwives, herbalists, witches, and many other people were the health caregivers and until the mid-nineteenth century medicinal drugs came from natural sources (nature's pharmaceuticals) in the form of herbs, plants, roots, vines and fungi for use against different ailments, e.g., the bark extract of the white willow tree had been used for centuries to treat various fevers and inflammation. By 1865, the discipline of scientific medicine was imported from Europe, particularly from Germany. The germ theory of disease, specific disease

etiology, and the discovery of the tubercle bacillus (*Mycobacterium tuberculosis*) by Robert Koch, Rudolf Virchow, and Louis Pasteur helped to develop the opinion of a specific cure for a specific ailment and it worked as a basis as well as impetus for the production of synthetic drugs. The first synthetic drug chloral hydrate was discovered in 1869, and it was introduced as a sedative-hypnotic; and the first analgesics and antipyretics, e.g., phenacetin and acetanilide (both were byproducts from coal-tar) were simple chemical derivatives of aniline and p-nitrophenol. Salicin or salicylic acid, the active principle in white willow, had a bitter taste and irritated the gastric mucosa, but much more palatable acetylsalicylic acid or better known as Aspirin (the first blockbuster drug), came from a simple chemical modification of salicin. Beginning in the 1930s, synthetic drugs gradually replaced the herbals. Synthetic penicillin ushered in the synthetic drug revolution and subsequently, synthesized drugs were given a boost by different national acts. Herb medicines made their way to food supplement stores. Figure 4.41 shows structure of some bioactive compounds—chloral hydrate, phenacetin, acetanilide, Aspirin, and benzylpenicillin (penicillin G).

There exists an urge for new drugs either from natural, semisynthetic, or synthetic sources in response to the appearance of new diseases and replacement of existing drugs due to the development of virulence in different pathogens. Whether human made or natural, the most important criteria for a drug molecule are its safety, effectiveness and quality including identity, purity, potency, and stability. Semi-synthetic drugs produced by treating the natural drug chemically to modify or isolate its active ingredient for better performance, e.g., morphine, codeine, heroin, etc., are semi-synthetic drugs produced from opium while cocaine is a semi-synthetic drug produced from coca plant. Synthetic drugs (pharmaceuticals) are synthesized chemically in the laboratory to produce drugs not found in nature by extracting the active ingredient from a plant, replicating its structure in the laboratory and mass-producing it, e.g., opiates, digitalis, Taxol, etc., are

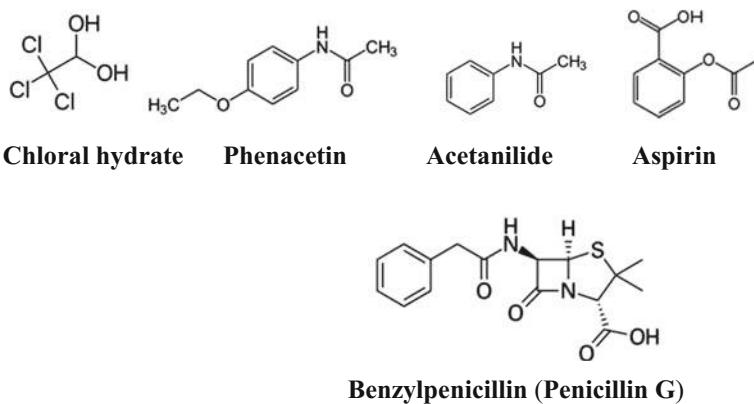


Fig. 4.41 Showing structure of some bioactive compounds—Chloral hydrate, Phenacetin, Acetanilide, Aspirin, and Benzylpenicillin (Penicillin G)

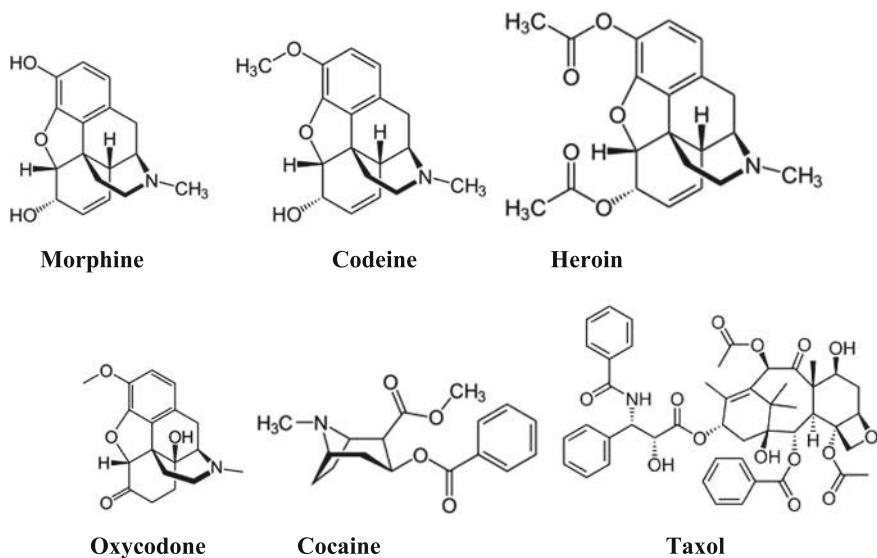


Fig. 4.42 Structure of semisynthetic drugs—morphine, codeine, heroin oxycodone, cocaine, and taxol

plant-derived drugs used in the developed countries including USA. Chemical synthesis of drugs includes many things such as minor modification of existing molecular frameworks or making new materials. Figure 4.42 shows structure of semisynthetic drugs—morphine, codeine, heroin oxycodone, cocaine, and taxol.

Medicines commonly used by the late 1920s included aspirin, codeine, and morphine for pain; digitalis, nitroglycerin, and quinine for heart disorders, and insulin for diabetes. Other drugs included antitoxins, a few biological vaccines, and a few synthetic drugs. At the start of the twentieth century, the first synthetic drugs of the barbiturate family (an old class of synthetic drugs working on the central nervous system) entered the pharmacopoeia. The effects range from mild sedation to coma and they may be used as anticonvulsants, anxiolytics, sedatives, hypnotics, or as part of anesthesia. Barbiturates used to be regularly prescribed to treat insomnia, depression and anxiety. Some barbiturates are used to relieve tension or anxiety prior to surgery. Barbiturate drugs are derived from barbituric acid (malonylurea or 6-hydroxyuracil), an organic compound based on a pyrimidine heterocyclic skeleton, which was first synthesized in 1864 by German chemist Adolf von Baeyer by condensing urea (an animal waste product) with diethyl malonate (an ester derived from the acid of apples). This parent compound (barbituric acid) of barbiturate drugs is not pharmacologically active is not pharmacologically active. Examples of barbiturates by time to act, e.g., quick and intermediate acting—methohexitol (Brevital), thiethyl (Surital), thiopental (Pentothal); short and intermediate acting—amobarbital (Amytal), pentobarbital (Nembutal, Secobarbital (Seconal), butalbital (Fiorina), butabarbital (Butisol); long-acting barbiturates—

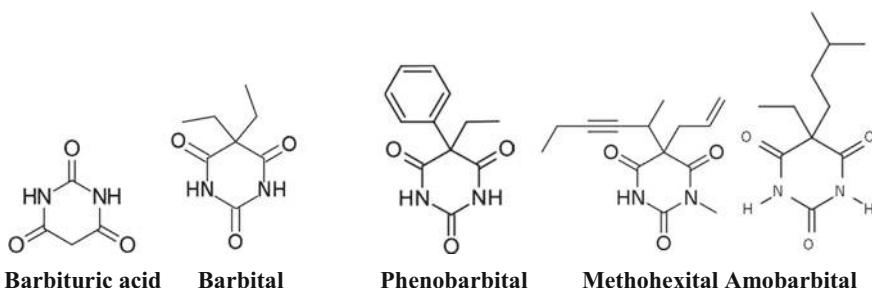


Fig. 4.43 Showing structure of barbituric acid (an organic compound based on a pyrimidine heterocyclic skeleton) and some early synthesized barbiturates

phenobarbital (Luminal), mephobarbital (Mebaral), etc. Phenobarbital, one of the earliest barbiturates to be synthesized, has distinctive anticonvulsant properties useful in the treatment of epilepsy. Around 2500 different types of barbiturates have been synthesized since 1881 (barbital was the first synthesized pharmacologically active) form, but only ~ 50 of these agents have ever been used in medicine and many barbiturates have been replaced with safer medicines. Figure 4.43 shows the structure of barbituric acid (an organic compound based on a pyrimidine heterocyclic skeleton) and some early synthesized barbiturates.

Synthetic pharmaceuticals are organic compounds, which are often divided into broad classes of small organic molecules (e.g., atorvastatin, fluticasone, clopidogrel) and biologics (e.g., infliximab, erythropoietin, insulin glargine). Biologics are most often medicinal preparations of proteins (natural and recombinant antibodies, hormones, etc.). Inorganic and organometallic compounds are also useful as drugs (e.g., lithium and platinum-based agents such as lithium carbonate and cisplatin as well as gallium). Figure 4.44 show structure of synthetic pharmaceuticals of small molecules, e.g., atorvastatin (Lipitor statin), fluticasone (Glucocorticoid), clopidogrel, biologics insulin glargine, and cisplatin. Insulin glargine is a basal insulin analog, where a substitution of glycine residue was at position 21 of the A chain for asparagine and the addition of two arginine residues to the B chain at position 30 of the regular insulin molecule. Cisplatin cisplatinum, cis-diamminedichloroplatinum (II) (CDDP) compound, is a platinum-based chemotherapy medication to treat various cancers.

Synthetic pharmaceutical drugs of different categories include (i) antipyretics (substances that reduce fever—ibuprofen and aspirin, the nonsteroidal anti-inflammatory drugs NSAIDs); (ii) analgesics (drugs act in various ways on the peripheral and central nervous system and reduce pain, i.e., painkillers—paracetamol, NSAIDs such as the salicylates, aspirin, ibuprofen, and naproxen, and opioid drugs such as morphine and oxycodone; and tricyclic antidepressants (TCAs), tetracyclic antidepressant (TeCA), and anticonvulsants as nonconventional analgesics for neuropathic pain); (iii) antimalarial drugs (drugs that prevent or cure malaria—chloroquine and hydroxychloroquine, amodiaquine, pyrimethamine, proguanil, sulfadoxine and sulfamethoxypyridazine, mefloquine, atovaquone,

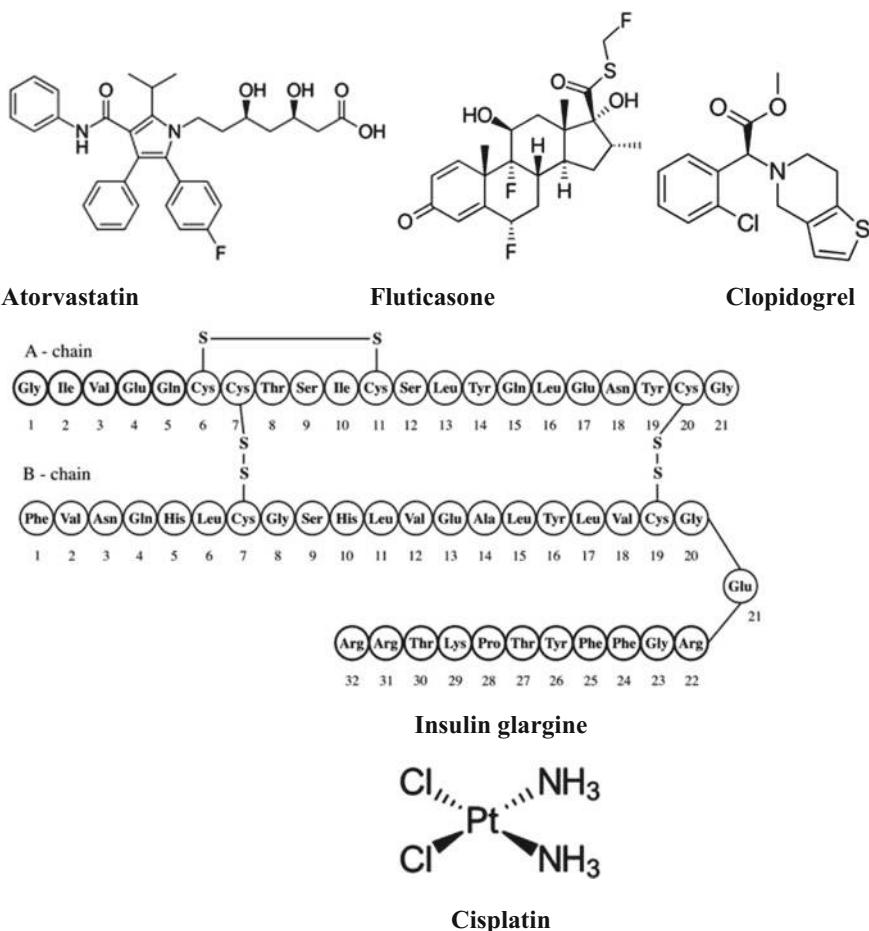


Fig. 4.44 Structure of synthetic pharmaceuticals of small molecules- atorvastatin, fluticasone, clopidogrel insulin glargine and cisplatin

primaquine, artemisinin and derivatives (artemether, artesunate), halofantrine, doxycycline); (iv) antibiotics also called antibacterials (drugs to treat and prevent bacterial infections, inhibit germ growth, and include Penicillins—penicillin and amoxicillin; Cephalosporins—cephalexin; Macrolides—erythromycin, clarithromycin, and azithromycin; Fluoroquinolones—ciprofloxacin, levofloxacin, and ofloxacin; Sulfonylurea or sulfonamide—acetazolamide, hydrochlorothiazide, furosemide and trimethoprim; Tetracyclines—tetracycline and doxycycline; Aminoglycosides—streptomycin, deoxystreptamine—kanamycin, tobramycin, gentamicin, neomycin; Glycopeptide antibiotics—vancomycin, teicoplanin, telavancin, ramoplanin, and daptomycin, and the antitumor antibiotic bleomycin; Lipopeptide antibiotics—daptomycin, a cyclic lipopeptide antibiotic; and many other including metronidazole); (v) antiseptics (antimicrobial substances that are

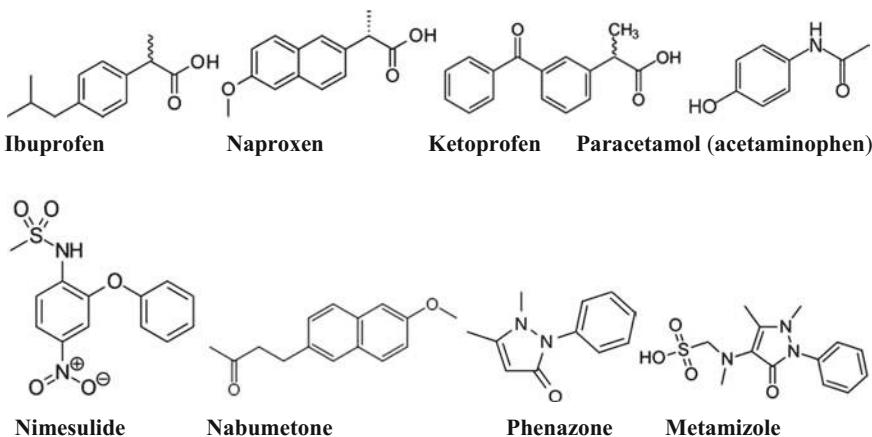


Fig. 4.45 Showing structure of nonsteroidal anti-inflammatory drugs (NSAID) with analgesic and antipyretic effects—ibuprofen, naproxen, ketoprofen, paracetamol (acetaminophen), nimesulide, nabumetone, phenazone, and metamizole

applied to living tissue or skin to reduce infection, sepsis, putrefaction, and prevention of germ growth near burns, cuts and wounds—alcohols including ethanol and 2-propanol or surgical spirit, chlorhexidine gluconate, hydrogen peroxide-6%, alcoholic solution of iodine-tincture iodine, octenidine dihydrochloride, polyhexanide); (vi) mood stabilizers (psychiatric pharmaceuticals to treat mood disorders—mineral—lithium; anticonvulsant—valpromide, valproic acid, valproate semisodium, and sodium valproate, lamotrigine, carbamazepine, oxcarbazepine, sultiamine—the anticonvulsant sulfonamide; and antipsychotics—aripiprazole, risperidone, olanzapine, quetiapine, asenapine, paliperidone, ziprasidone, and lurasidone (vii) hormone replacements therapy (HRT) (hormone therapy either to supplement natural hormone or to substitute other hormones—estrogens, progesterone, or progestins, and testosterone hormone replacement therapy for menopause; HRT for transgender people—testosterone for trans men and estrogen for trans women; androgen replacement therapy to counter the effects of male hypogonadism; conjugated estrogens premarin); (viii) oral contraceptives or OCPs, (birth control pills taken by mouth, available only female OCPs contraceptive pill—estrogen (estradiol) and a progestogen (progesterin), ormeloxifene; emergency contraception pills—levonorgestrel, ulipristal acetate, mifepristone, and misoprostol, enovid—estrogen mestranol, and progestin norethynodrel combined oral contraceptive pill (COC), available in monophasic, biphasic, triphasic, etc., preparations and the number refers to different doses of progestogen they contain); (ix) stimulants (psychostimulants, cover drugs that increase activity of the body, pleasurable and invigorating, or drugs with sympathomimetic effects—lisdexamfetamine, methylphenidate, and amphetamine; stimulants have been used in medicine for many conditions including obesity, sleep disorders, mood disorders, impulse control disorders, asthma, nasal congestion, and as anesthetics); (x) tranquilizers (drugs

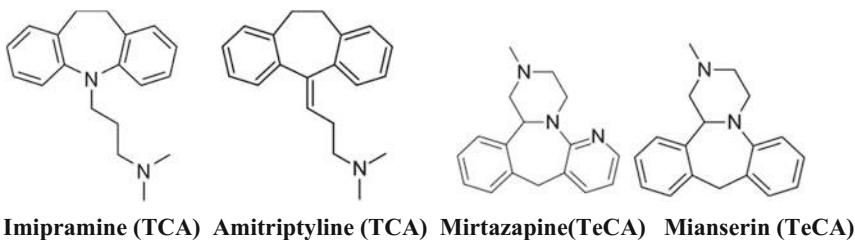


Fig. 4.46 Showing structure of tricyclic (TCAs) and tetracyclic antidepressant (TeCA)

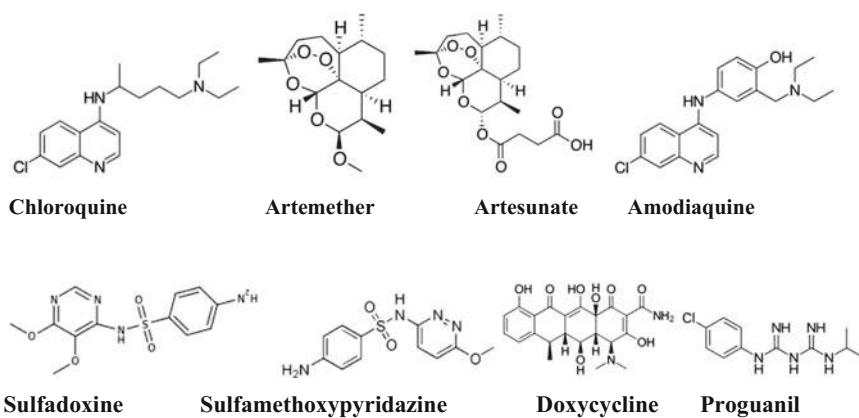
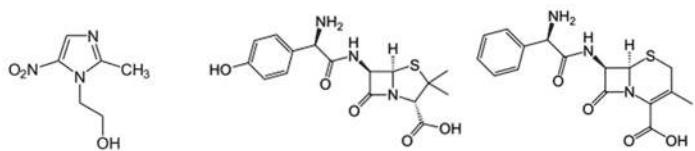


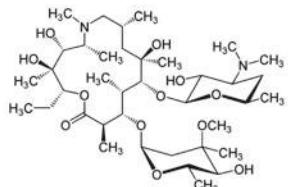
Fig. 4.47 Showing structure of antimalarial drugs—chloroquine, artemether, artesunate, amodiaquine, sulfadoxine, sulfamethoxypyridazine, doxycycline, and proguanil

for the treatment of anxiety, fear, tension, agitation, and disturbances of the mind—meprobamate, chlorpromazine, reserpine, chlordiazepoxide, diazepam, and alprazolam; (xi) statins (lipid-lowering medications, also reduce cardiovascular disease (CVD), side effects include muscle pain, increased risk of diabetes mellitus, and abnormalities in liver enzyme tests—atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin); (xii) semisynthetic anticancer drug—Taxol, a modified anticancer drug from Beccatin III from yew tree (*Taxus baccata*); (xiii) therapeutic peptides and proteins—insulin and similar other protein products with the application of recombinant DNA technology; and many other drugs. Figures 4.45, 4.46, 4.47, 4.48, 4.49, 4.50, 4.51, 4.52, 4.53, 4.54 and 4.55 show structures of nonsteroidal anti-inflammatory drugs (NSAID) with analgesic and antipyretic effects such as ibuprofen, naproxen, ketoprofen, paracetamol (acetaminophen), nimesulide, nabumetone, phenazone, metamizole, etc. and other bioactive compounds pharmaceutical drug principles.

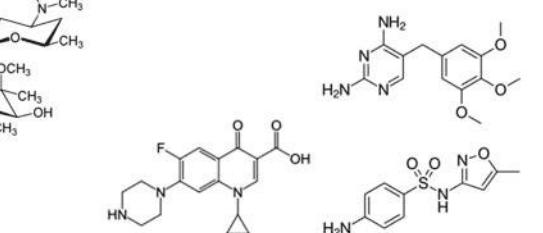


Metronidazole (nitroimidazole group) Amoxicillin

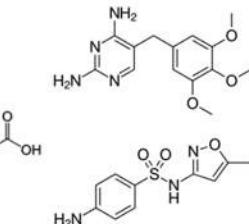
Cephalexin



Azithromycin

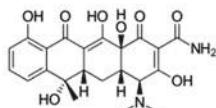


Ciprofloxacin

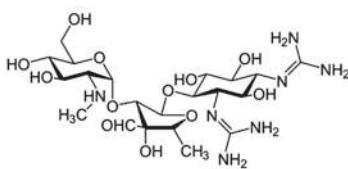


TMP (top) & SMX (bottom)

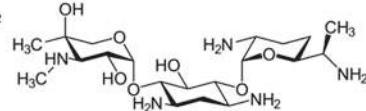
Trimethoprim/Sulfamethoxazole (TMP/SMX), the co-trimoxazole consists of one part trimethoprim to five parts sulfamethoxazole



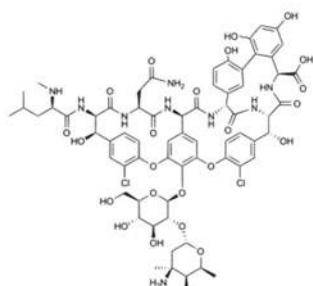
Tetracycline



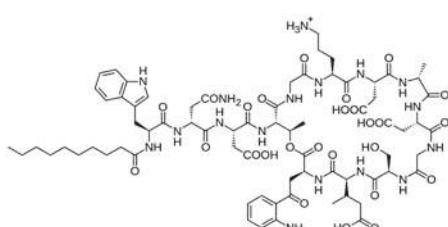
Streptomycin



Gentamicin



Vancomycin



Daptomycin

Fig. 4.48 Showing structure of antibiotics: penicillins (amoxicillin), cephalosporins (cephalexin), macrolides (azithromycin), fluoroquinolones (ciprofloxacin), sulfonamides (co-trimoxazole), tetracyclines (tetracycline), aminoglycosides (streptomycin), deoxystreptamine (gentamicin), glycopeptide (vancomycin), lipopeptide (daptomycin), and metronidazole

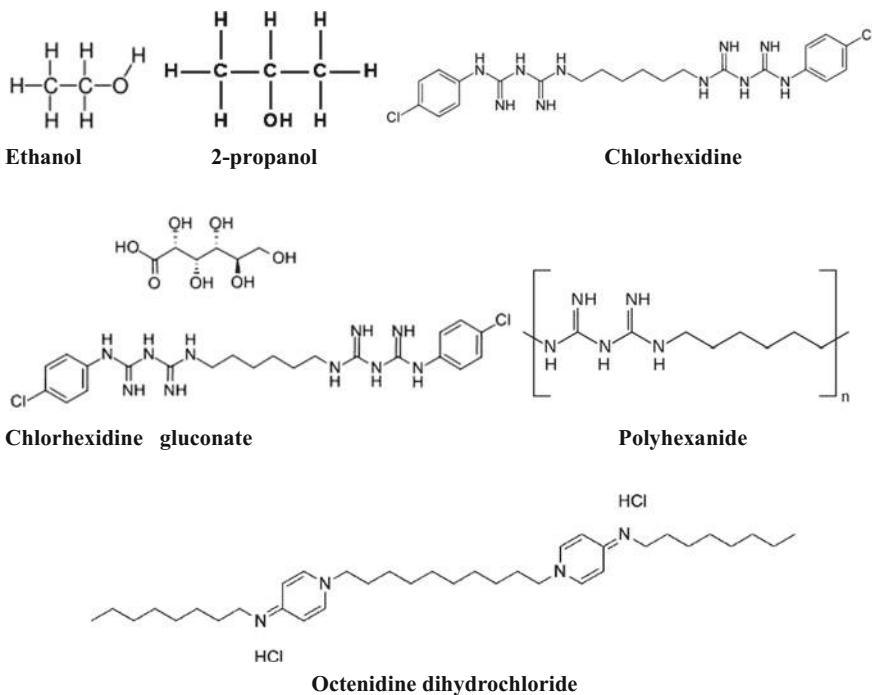


Fig. 4.49 Showing structure of antiseptics: ethanol, 2-propanol, chlorhexidine, chlorhexidine gluconate, octenidine, polyhexanide and dihydrochloride

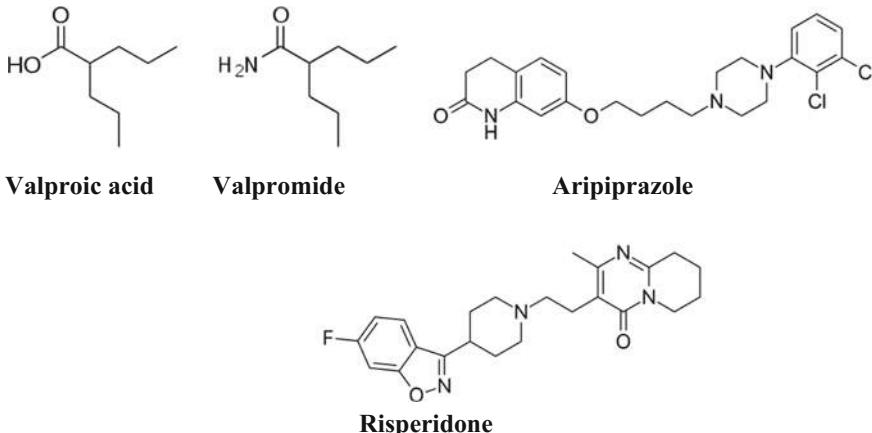


Fig. 4.50 Showing structure of mood stabilizers: anticonvulsant (valproic acid, valpromide) and antipsychotics (aripiprazole, risperidone)

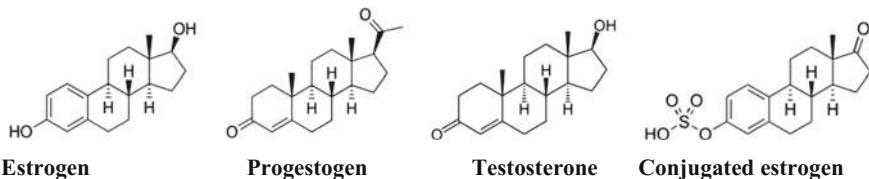


Fig. 4.51 Showing structure of hormones for HRT: estrogen, progestogen, testosterone, and conjugated estrogens

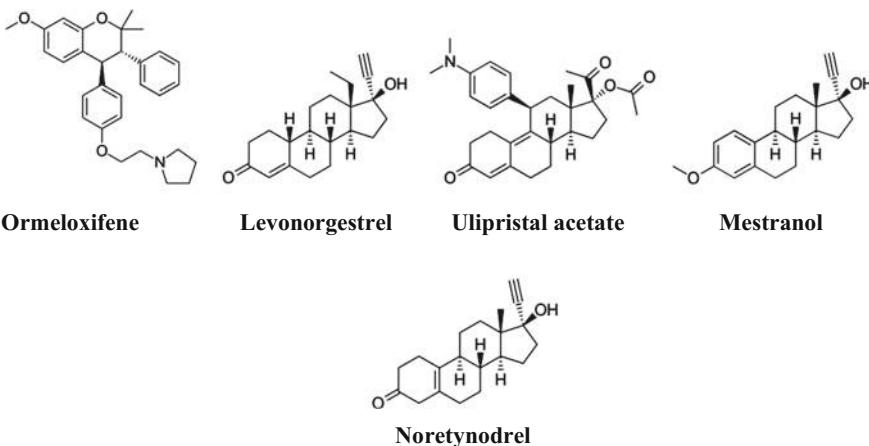


Fig. 4.52 Showing structure of some principles of OCP and COCP

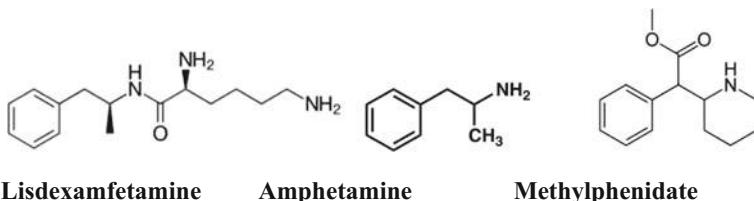


Fig. 4.53 Showing structure of sympathomimetic stimulants—lisdexamfetamine, amphetamine, and methylphenidate

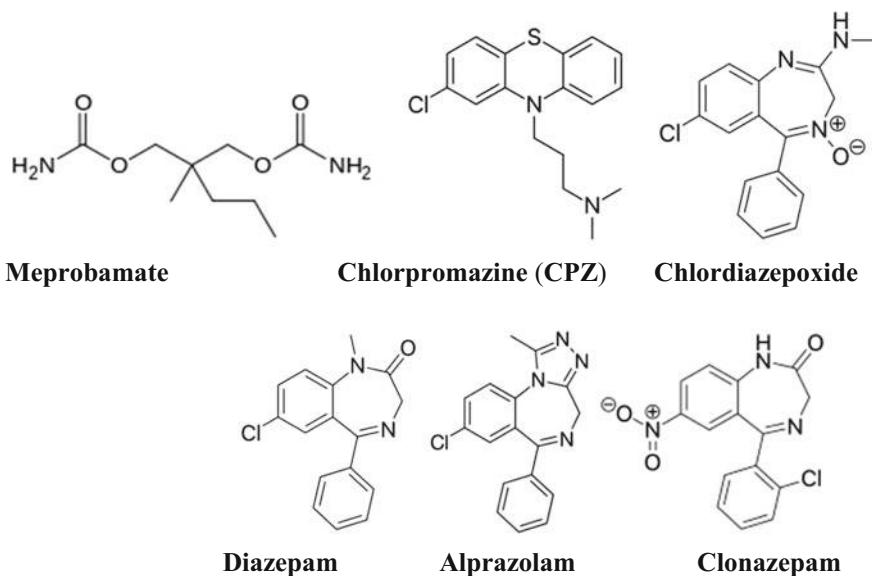


Fig. 4.54 Showing structure of tranquilizers— meprobamate, chlorpromazine, chlordiazepoxide, diazepam, alprazolam, and clonazepam

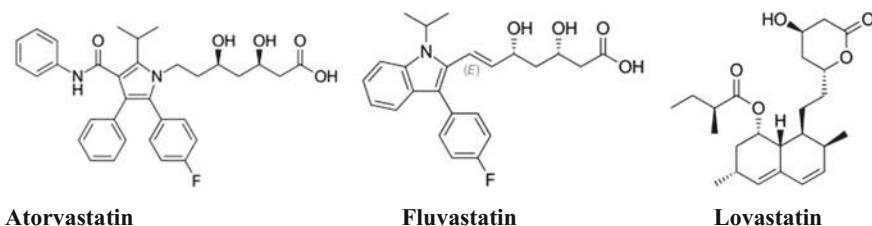


Fig. 4.55 Showing structure of statins—atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin

In the 1930s, synthetic antibiotics emerged first as sulfa drugs, then penicillin, and followed by other antibiotics and synthetic drugs increasingly became the center of medical practice. In the 1950s, other drugs like corticosteroids for inflammation, rauvolfia alkaloids as tranquilizers and antihypertensives, antihistamines for nasal allergies, xanthines for asthma, and chlorpromazine antipsychotics for psychosis emerged. As a continuous process, thousands of approved pharmacopoeial drugs have been developed successively in the following years and in the twenty-first century, multidisciplinary approaches including biotechnology have been increasingly playing different key roles in the discovery of pharmaceuticals.

References

- Abd El-Baky HH, El-Baz FK, El Baroty GS (2010) Enhancing antioxidant availability in wheat grains from plants grown under seawater stress in response to microalgae extract treatments. *J Sci Food Agric* 90:299–303
- Abe M, Inoue D, Matsunaga K, Ohizumi Y, Ueda H, Asano T et al (2002) Goniodomin A, an antifungal polyether macrolide, exhibits antiangiogenic activities via inhibition of actin reorganization in endothelial cells. *J Cell Physiol* 190:109–116
- Acott C, Williamson J (1996) Sea snake. In: Williamson JA, Fenner PJ, Burnett JW, Rifkin JF (eds) *Venomous and poisonous marine animals: a medical and biological handbook*. University of New South Wales Press, Sydney, pp 396–402
- Adarme-Vega TC, Thomas-Hall SR, Lim DK, Schenk PM (2014) Effects of long chain fatty acid synthesis and associated gene expression in microalga *Tetraselmis* sp. *Mar Drugs* 12:3381–3398
- Agnihotri SA, Mallikarjuna NN, Aminabhavi TM (2004) Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *J Controlled Release* 100(1):5–28
- Alhanout K, Brunel JM, Ranque S, Rolain JM (2010a) In vitro antifungal activity of aminosterols against moulds isolated from cystic fibrosis patients. *J Antimicrob Chemother* 65(6):1307–1309
- Alhanout K, Rolain JM, Brunel JM (2010b) Squalamine as an example of a new potent antimicrobial agents class: a critical review. *Curr Med Chem* 17(32):3909–3917
- Ang KK, Holmes MJ, Higa T, Hamann MT, Kara UAK (2000) In vivo antimalarial activity of the beta-carboline alkaloid manzamine A. *Antimicrob Agents Chemother* 44(6):1645–1649
- Anonymous (1999) Merck manual of diagnosis and therapy. In: Berkow R, Beers MH (eds) 17th ed. Whitehouse station, NJ: Merck Company, Chapter 154, pp 1127–1128
- Anonymous (2004) United Kingdom. National Prescribing Centre. Task force on medicines partnership. Drugs of porcine origin and their clinical alternatives. An introductory guide. March 2004. Available from: <http://www.mcb.org.uk/uploads/PBEnglish.pdf>
- Anonymous (2006) FDA: You're eating crushed bug juice. Cochineal extract, carmine should be listed on labels, officials say. Friday, January 27, 2006; Posted: 9:14 p.m. EST (02:14 GMT)
- Anonymous (2012) Food Standards Agency—Current EU approved additives and their E Numbers. Food.gov.uk. 14 March 2012
- Arnold TM, Targett NM (2002) Marine tannins: the importance of mechanistic framework for predicting ecological roles. *J Chem Ecol* 28(10):1919–1934
- Auyoung E (1999) A brief history and overview of Tetrodotoxin (TTX) MCB165-molecular neurobiology and neurochemistry, pp 1–2. www.sulcus.berkeley.edu/mcb/165-001/index.html
- Avery MA, Chong WKM, Jennings-White C (1992) Stereoselective total synthesis of (+)-artemisinin, the antimalarial constituent of *Artemisia annua* L. *J Am Chem Soc* 114:974–979
- Bae SY, Yim JH, Lee HK, Pyo S (2006) Activation of murine peritoneal macrophages by sulphated exopolysaccharide from marine microalga *Gyrodinium impudicum* (strain KG03): involvement of the NF-kappa B and JNK pathway. *Int Immunopharmacol* 6:473–484
- Batista AP, Gouveia L, Bandarra-Narcisa M, Franco JM, Raymundo A (2013) Comparison of microalgal biomass profiles as novel functional ingredient for food products. *Algal Res* 2:164–173
- Beltron EC, Nielan BA (2000) Geographical segregation of neurotoxin-producing cyanobacterium *Anabaena circinalis*. *Appl Environ Microbiol* 66(10):4468–4474
- Ben Kahla-Nakbi A, Haouas N, El Ouaer A, Guerbej H, Ben Mustapha K, Babba H (2010) Screening of antileishmanial activity from marine sponge extracts collected off the Tunisian coast. *Parasitol Res* 106:1281–1286
- Bertoldo C, Antranikian G (2002) Starch hydrolyzing enzymes from thermophilic archea and bacteria. *Curr Opin Chem Biol* 6:151–160
- Bharathi NP, Amudha P, Vanitha V (2016) Seagrasses—novel marine nutraceuticals. *Int J Pharm Bio Sci* 7(4):567–573

- Bisset NG (1991) One man's poison, another man's medicine? *J Ethnopharm* 32:71–81
- Bitam F, Ciavatta ML, Carbone M, Manzo E, Mollo E, Gavagnin M (2010) Chemical analysis of flavonoid constituents of the seagrass *Halophila stipulacea*: first finding of malonylated derivatives in marine phanerogams. *Biochem Syst Ecol* 38:686–690
- Bixler HJ, Porse H (2010) A decade of change in the seaweed hydrocolloids industry. *J Appl Phycol* 23:321–335
- Bjørkkjær T, Araujo P, Madland TM, Berstad A, Frøyland L (2009) A randomized double blind comparison of short-term duodenally administrated whale and seal blubber oils in patients with inflammatory bowel disease and joint pain. *Prostaglandins, Leukot Essent Fat Acids* 81:425–432
- Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR (2004) Marine natural products. *Nat Prod Rep* 21:1–49
- Bol KF, Aarntzen EH, Pots JM, Olde Nordkamp MA, van de Rakt MW, Scharenborg NM et al (2016) Prophylactic vaccines are potent activators of monocyte-derived dendritic cells and drive effective anti-tumor responses in melanoma patients at the cost of toxicity. *Cancer Immunol Immunother* 65(3):327–339
- Bottino NR (1971) The composition of marine-oil triglycerides as determined by silver ion-thin-layer chromatography. *J Lipid Res* 12:24–30
- Bringmann G, Gulder TA, Lang G, Schmitt S, Stöhr R, Wiese J et al (2007) Large-scale biotechnological production of the antileukemic marine natural product sorbicillactone A. *Mar Drugs* 5:23–30
- Brotherton J (2015) HPV prophylactic vaccines: lessons learned from 10 years experience. *Future Med* 10(8):999–1009
- Brown Mark A, Daya Mohamud R, Worley Joseph A (2009) Experience with Chitosan dressings in a civilian EMS system. *The J Emerg Med* 37(1):1–7
- Brunborg LA, Julshamn K, Nortvedt R, Frøyland L (2006) Nutritional composition of blubber and meat of hooded seal (*Cystophora cristata*) and harp seal (*Phagophilus groenlandicus*) from Greenland. *Food Chem* 96:524–531
- Brunborg LA, Madland TM, Lind RA, Arslan G, Berstad A, Frøyland L (2008) Effects of short-term oral administration of dietary marine oils in patients with inflammatory bowel disease and joint pain: a pilot study comparing seal oil and cod liver oil. *Clin Nutr* 27:614–622
- Buonanno F, Quassinti L, Bramucci M, Amantini C, Lucchiarini R, Santoni G et al (2008) The protozoan toxin climacostol inhibits growth and induces apoptosis of human tumor cell lines. *Chem Biol Interact* 176:151–164
- Burja AM, Banaigs EB, Mansour A, Burgess JG, Wright PC (2001) Marine *cyanobacteria*-a prolific source of natural products. *Tetrahedron* 57:9347–9377
- Cafieri F, Fattorusso E, Magno S, Santacroce C, Sica D (1973) Isolation and structure of axisonitrile 1 and axisothiocyanate 1, two unusual sesquiterpenoids from the marine sponge *Axinella cannabina*. *Tetrahedron* 29:4259–4262
- Cafieri F, Fattorusso E, Taglialatela-Scafati O, Ianaro A (1999) Metabolites from the sponge Plakortis simplex. Determination of absolute stereochemistry of plakortin. Isolation and stereostructure of three plakortin related compounds. *Tetrahedron* 55:7045–7056
- Calvayrac R, Laval-Martin D, Briand J, Farineau J (1981) Paramylon synthesis by *Euglena gracilis* photoheterotrophically grown under low O₂ pressure. *Planta* 153(1):6–13
- Campagnuolo C, Fattorusso E, Romano A, Taglialatela-Scafati O, Basilico N, Parapini S, Taramelli D (2005) Antimalarial polyketide cycloperoxides from the marine sponge Plakortis simplex. *Eur J Org Chem* 23:5077–5083
- Cardellina JH 2nd, Marner FJ and Moore RE (1979) Seaweed dermatitis: structure of lyngbyatoxin A. *Science* 13; 204(4389):193–195
- Cardellina JHII, Marner FJ, Moore RE (1979) Seaweed dermatitis: structure of lyngbyatoxin A. *Science* 204:193–195
- Carte BK (1996) Biomedical potential of marine natural products. *Bioscience* 46:271–286
- Casal C, Cuaresma M, Vega JM, Vilchez C (2011) Enhanced productivity of a lutein-enriched novel acidophile microalga grown on urea. *Mar Drugs* 9:29–42

- Cha TS, Chen CF, Yee W, Aziz A, Loh SH (2011) Cinnamic acid, coumarin and vanillin: alternative phenolic compounds for efficient agrobacterium-mediated transformation of the unicellular green alga, *Nannochloropsis* sp. J Microbiol Methods 84:430–434
- Chakrabarty MM (2009) Chemistry and technology of oils and fats, p 183
- Chakraborty C, Hsu CH, Wen ZH, Lin CS (2009) Anticancer drugs discovery and development from marine organism. Curr Top Med Chem 9(16):1536–1545
- Challouf R, Trabelsi L, Dhibe RB, El Abed O, Yahia A, Ghozzi K et al (2011) Evaluation of cytotoxicity and biological activities in extracellular polysaccharides released by cyanobacterium *Arthrospira platensis*. Braz Arch Biol Technol 54:831–838
- Chang Y, Brewer NT, Rinas AC, Schmitt K, Smith JS (2009) Evaluating the impact of human papillomavirus vaccines. Vaccine 27(32):4355–4362
- Chen J (2003) Overview of sea cucumber farming and sea ranching practices in China. SPC Beche-de-mer Inf Bull 18:18–23
- Chin YX, Lim PE, Maggs CA, Phang SM, Sharifuddin Y, Green BD (2014) Anti-diabetic potential of selected Malaysian seaweeds. J Appl Phycol. <https://doi.org/10.1007/s10811-014-0462-8>
- Chopin T, Sharp G, Belyea E, Semple R, Jones D (1999) Open-water aquaculture of the red alga *Chondrus crispus* in Prince Edward Island, Canada. Hydrobiologia 398/399:417–425
- Christaki E, Bonos E, Florou-Paneri P (2015) Innovative microalgae pigments as functional ingredients in nutrition. In: Kim SK (ed) Handbook of marine microalgae: biotechnology advances. Elsevier Academic Press, London, UK, pp 233–243
- Christaki E, Florou-Paneri P, Bonos E (2011) Microalgae: a novel ingredient in nutrition. Int J Food Sci Nutr 62:794–799
- Chu WL (2012) Biotechnological applications of microalgae. Int e-J Sci Med Educ 6:S24–S37
- Cohen P, Holmes C, Tsukitani Y (1990) Okadaic acid: a new probe for the study of cellular regulation. Trends Biochem Sci 15:98–102
- Colwell RR (1997) Microbial biodiversity and biotechnology. In: Reaka-kudla ML et al (eds) Biodiversity II: understanding and protecting our biological resources. Joseph Henry Press, Washington, DC, pp 77–78
- Conquer JA, Cheryk LA, Chan E, Gentry PA, Holub BJ (1999) Effect of supplementation with dietary seal oil on selected cardiovascular risk factor and hemostatic variables in healthy male subjects. Thrombosis Res 96:239–250
- Cordero BF, Obraztsova I, Couso I, Leon R, Vargas MA, Rodríguez H (2011) Enhancement of lutein production in *Chlorella sorokiniana* (chlorophyta) by improvement of culture conditions and random mutagenesis. Mar Drugs 9:1607–1624
- Covington MB (2004) Omega-3 fatty acids. Am Fam Phys 70(1):133–140
- Gerwick WH, Proteau PJ, Nagh DG, Hamel E, Blobbin A, Slate DL (1994) Structure of cruentin A, a novel antimitotic, antiproliferative and brine shrimp toxic natural product from the marine cyanobacterium *Lyngbya majuscula*. J Org Chem 59:1243–1245
- Crupi P, Toci AT, Mangini S, Wrubl F, Rodolfi L, Tredici MR et al (2013) Determination of fucoxanthin isomers in microalgae (*Isochrysis* sp.) by high-performance liquid chromatography coupled with diode-array detector multistage mass spectrometry coupled with positive electrospray ionization. Rapid Commun Mass Spectrom 27:1027–1035
- Cyhalova E, Bell JG, Dick JR, MacKinlay EE, Stein JF, Richardson AJ (2007) Membrane fatty acids, reading and spelling in dyslexic and nondyslexic adults. Eur Neuropsychopharmacology 17:116–121
- Dahl TM, Lydersen C, Kovacs KM, Falk-Petersen S, Sargent J, Gjertz I et al (2000) Fatty acid composition of the blubber in white whales (*Delphinapterus leucas*). Polar Biol 23:401–409
- Dalli J, Chiang N, Serhan CN (2015) Elucidation of novel 13-series resolvins that increase with atorvastatin and clear infections. Nat Med 21(9):1071–1075
- Damotharan P, Veeruraj A, Arumugam M, Balasubramanian T (2015) In vitro antibacterial activity of venom protein isolated from sea snake *Enhydrina schistosa* against drugresistant human pathogenic bacterial strains. J Coast Life Med 3(6):453–458

- Danilchenko SN, Kalinkevich OV, Pogorelov MV (2009) Chitosan–hydroxyapatite composite biomaterials made by a one step co-precipitation method: preparation, characterization and *in vivo* tests. *J Biol Phys Chem* 9(3):119–126
- Dapson RW, Frank M, Penney DP, Kiernan JA (2007) Revised procedures for the certification of carmine (C.I. 75470, Natural red 4) as a biological stain. *Biotech Histochem* 82(1):13–15
- Davidi L, Shimon E, Khozin-Goldberg I, Zamir A, Pick U (2014) Origin of β-carotene-rich plastoglobuli in Dunaliella bardawil. *Plant Physiol* 164:2139–2156
- Dawes CJ (1998) Marine botany, 2nd edn. Wiley, New York, pp 1–7
- de Jesus Raposo MF, de Moraes AMB, de Moraes RMSC (2015) Review: marine polysaccharides from algae with potential biomedical applications. *Mar Drugs* 13(5):2967–3028
- de Jesus Raposo MF, de Moraes RMSC, de Moraes AMMB (2013) Review: bioactivity and applications of sulphated polysaccharides from marine microalgae. *Mar Drugs* 11:233–252
- DeFilippis AP, Sperling LS (2005) Understanding omega-3's. *Am Heart J* 151:564–570
- Dellai A, Laroche-Clary A, Mhadhebi L, Robert J, Bouraoui A (2010) Anti-inflammatory and antiproliferative activities of crude extract and its fractions of the defensive secretion from the mediterranean sponge. *Spongia officinalis*. *Drug Dev Res* 71:412–418
- Desbois AP, Mearns-Spragg A, Smith VJ (2009) A fatty acid from the diatom *Phaeodactylum tricornutum* is antibacterial against diverse bacteria including multi-resistant *Staphylococcus aureus* (MRSA). *Mar Biotechnol (NY)* 11:45–52
- Desoubzdanne D, Marcourt L, Raux R, Chevalley S, Dorin D, Doerig C et al (2008) Alisiaquinones and alisiaquinol, dual inhibitors of *Plasmodium falciparum* enzyme targets from a New Caledonian deep water sponge. *J Nat Prod* 71:1189–1192
- Deville C, Damas J, Forget P, Dandrifosse G, Peulen O (2004) Laminarin in the dietary fiber concept. *J Sci Food Agric* 84:1030–1038
- Deville C, Gharbi M, Dandrifosse G, Peulen O (2007) Study on the effects of laminarin, a polysaccharide from seaweed, on gut characteristics. *J Sci Food Agric* 87:1717–1725
- Di Blasio B, Fattorusso E, Magno S, Mayol L, Pedone C, Santacroce C et al (1976) Axisonitrile-3, axisothiocyanate-3 and axamide-3. Sesquiterpenes with a novel spiro [4,5] decane skeleton from the sponge *Axinella cannabina*. *Tetrahedron* 32:473–478
- Dillon EC, Wilton JH, Barlow JC, Watson WA (1989) Large surface area activated charcoal and the inhibition of aspirin absorption. *Ann Emerg Med* 18(5):547–552
- Donia M, Hamann MT (2003) Marine natural products and their potential applications as antiinfective agents. *The Lancet* 3:338–348
- Ducheyne P, Healy K, Hutmacher DE, Grainger DW, James Kirkpatrick C (eds) (2011) Comprehensive biomaterials. Elsevier, Amsterdam, p 229
- Edrada RA, Proksch P, Wray V, Witte L, Müller WE, Van Soest RW (1996) Four new bioactive manzamine-type alkaloids from the Philippine marine sponge *Xestospongia ashmorica*. *J Nat Prod* 59(11):1056–1060
- Edwards IJ, O'Flaherty JT (2008) Omega-3 fatty acids and PPARgamma in cancer. *PPAR Res* 2008:358052. <https://doi.org/10.1155/2008/358052>
- El Sayed KA, Kelly M, Kara UAK, Ang KKH, Katsuyama I, Dunbar DC et al (2001) New manzamine alkaloids with potent activity against infectious diseases. *J Am Chem Soc* 123:1804–1808
- El Sayed KA, Yousaf M, Hamann MT, Avery MA, Kelly M, Wipf P (2002) Microbial and chemical transformation studies of the bioactive marine sesquiterpenes (S)-(+)–Curcuphenol and -Curcudiol isolated from a deep reef collection of the Jamaican sponge *Didiscus oxeata*. *J Nat Prod* 65:1547–1553
- Elayaraja S, Murugesan P, Vijayalakshmi S, Balasubramanian T (2010) Antibacterial and antifungal activities of polychaete *Perinereis cultrifera*. *Indian J Mar Sci* 39(2):257–261
- El-Din SMM (2016) Bioactivity and phytochemical constituents of marine red seaweeds (*Jania rubens*, *Corallina mediterranea* and *Pterocladia capillacea*). *J Taibah Univ Sci* 10(4):471–484
- Emsley J (2011) Nature's building blocks: an A-Z guide to the elements, 2nd edn. Oxford University Press, Oxford, p 178

- Eom SH, Lee SH, Yoon NY, Jung WK, Jeon YJ, Kim SK et al (2012) α -Glucosidase and α -amylase-inhibitory activities of phlorotannins from *Eisenia bicyclis*. J Sci Food Agric 92:2084–2090
- Falk-Petersen S, Sargent JR, Henderson J, Hegseth EN, Hop H, Okolodkov YB (1998) Lipids and fatty acids in ice algae and phytoplankton from the marginal ice zone in the Barents Sea. Polar Biol 20(1):41–47
- Fattorusso E, Taglialatela-Scafati O (2009) Marine antimarials. Mar Drugs 7(2):130–152
- Fattorusso E, Magno S, Mayol L, Santacroce C, Sica D (1974) Isolation and structure of axisonitrile 2. New sesquiterpenoid isonitrile from the sponge *Axinella cannabina*. Tetrahedron 30:3911–3913
- Fattorusso E, Magno S, Mayol L, Santacroce C, Sica D (1975) New sesquiterpenoids from the sponge *Axinella cannabina*. Tetrahedron 31:269–270
- FDA (2016) Vaxchora (Cholera vaccine, Live, Oral). U.S. Food and Drug Administration. (<http://www.immunize.org/fda/>)
- Fenical W (1993) Chemical studies of marine bacteria: developing a new resource. Chem Rev 93 (5):1673–1683
- Fenical W, Jensen PR (1993) Marine micro-organisms. A new biomedical resource. In: Attaway D, Zaborsky O (eds) Marine biotechnology, vol 1. Plenum Press, New York, pp 419–457
- Feskens EJ (2001) Can diabetes be prevented by vegetable fat? Diabetes Care 24:1517–1518
- Fiore AE, Bridges CB, Cox NJ (2009) Seasonal influenza vaccines. Curr Top Microbiol Immunol 333:43–82
- Fiorini D, Giuli S, Marcantoni E, Quassinti L, Bramucci M, Amantini C et al (2010) A straight forward diastereoselective synthesis and evaluation of climacostol, a natural product with anticancer activities. Synthesis (tuttg) 9:1550–1556
- Fleury N, Lahaye M (1991) Chemical and physicochemical characterization of fibers from *Laminaria digitata* (Kombu Breton): a physiological approach. J Sci Food Agric 35:389–400
- Frazier I (2014) Development and implementation of papillomavirus prophylactic vaccines. J Immunol 192(9):4007–4011
- Freeman MP, Hibbels JR, Wisner KL, Davis JM, Mischoulon D, Peet M et al (2006) Omega-3 fatty acids: evidence basis for treatment and future research in psychiatry. J Clin Psychiatry 67:1954–1967
- Freund-Levi Y, Eriksdotter-Jonhagen M, Cederhol T, Basun H, Faxen-Irving G, Garlind A (2006) Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study: a randomized double-blind trial. Arch Neurol 63(10):1402–1408
- Fu W, Gudmundsson O, Paglia G, Herjolfsson G, Andrésson OS, Palsson BØ et al (2013) Enhancement of carotenoid biosynthesis in the green microalga *Dunaliella salina* with light-emitting diodes and adaptive laboratory evolution. Appl Microbiol Biotechnol 97:2395–2403
- Fusetani N (2000) Drugs from the sea. In: Fusetani M (ed), Karger Publishers: Basel, Switzerland, Volume Chapter 1, pp 1–5
- Fujiki H, Sugimura T (1987) New classes of tumor promoters: telocin, aplysiatoxin and palytoxin. Adv Cancer Res 59:223–264
- Goclik E, Konig GM, Wright AD, Kaminsky R (2000) Pelorol from the tropical marine sponge *Dactylospongia elegans*. J Nat Prod 63:1150–1154
- Greenhawt M, McMorris M, Baldwin J (2009) Carmine hypersensitivity masquerading as azithromycin hypersensitivity. Allergy Asthma Proc 30(1):95–101
- Greig JB (2012) WHO food additives series 46: cochineal extract, carmine, and carminic acid. Food Standards Agency
- Grohar PJ, Griffin LB, Yeung C, Chen Q-R, Pommier Y, Khanna C et al (2011) Ecteinascidin 743 interferes with the activity of EWS-FLI1 in Ewing Sarcoma cells. Neoplasia 13(2):145–153
- Groveiss A, Fenical W, Cun-Heng H, Clardy J, Zhongde W, Zhongnian Y et al (1985) Subergorgia acid, a novel tricyclopentanoid cardiotoxin from the Pacific gorgonian coral *Subergorgia suberosa*. Tetrahedron Lett 26:2379–2386

- Guedes AC, Amaro HM, Malcata FX (2011) Microalgae as sources of carotenoids. *Mar Drugs* 9(4):625–644
- Gustafson K, Roman M, Fenical W (1989) The microlactins, a novel class of antiviral and cytotoxic macrolides from deep-sea marine bacterium. *J Am Chem Soc* 111:7519–7524
- Guzmán S, Gato A, Lamela M, Freire-Garabal M, Calleja JM (2003) Anti-inflammatory and immunomodulatory activities of polysaccharide from *Chlorella stigmatophora* and *Phaeodactylum tricornutum*. *Phytoether Res* 17:665–670
- Haefner B (2003) Drugs from the deep. *Drug Discov Today* 8:536–544
- Hanmann MT, Scheuer PJ, Kahalide F (1993) A Bioactive Depsipeptide from the Sacoglossan Mollusk *Elisia refescens* and the Green Alga *Byopsis sp.* *J American Chem Soc* 115: 5825–5826
- Hamada M, Nagai T (1995) Inorganic components of bones of fish and their advanced utilization. *J Shimonoseki Univ Fish* 43:185–194
- Hardoim CC, Costa R (2014) Microbial communities and bioactive compounds in marine sponges of the family Irciniidae—a review. *Mar Drugs* 12(10):5089–5122
- Hatae N, Satoh R, Chiba H, Osaki T, Nishiyama T, Ishikura M et al (2014) N-substituted calothrixin B derivatives inhibited the proliferation of HL-60 promyelocytic leukemia cells. *Med Chem Res* 23:4956–4961
- Heglmeier A, Zidorn C (2010) Secondary metabolites of *Posidonia oceanica* (Posidoniaceae). *Biochem Syst Ecol* 38:964–970
- Hidiroglou N, Peace RW, Jee P, Leggee D, Kuhnlein H (2008) Levels of folate, pyridoxine, niacin and riboflavin in traditional foods of Canadian Arctic Indigenous peoples. *J Food Compos Anal* 21:474–480
- Higgs MD, Faulkner DJ (1978) Plakortin, an antibiotic from Plakortis halichondrioides. *J Org Chem* 43:3454–3457
- Holst PB, Anthoni U, Christoffersen C, Neilson PN (1994) Marine alkaloids, two alkaloids, flustramine E and debromoflustramine B, from the marine bryozoan *Flustra foliacea*. *J Nat Prod* 57:997–1000
- Hossain Z, Kurihara H, Hosokawa M, Takahashi K (2005) Growth inhibition and induction of differentiation and apoptosis mediated by sodium butyrate in Caco-2 cells with algal glycolipids. *Vitro Cell Dev Biol Anim* 41(5–6):154–159
- Hu FB, van Dam RM, Liu S (2001) Diet and risk of type II diabetes: the role of types of fat and carbohydrate. *Diabetologia* 44:805–817
- Hurley JC, Tosolini FA, Louis WJ (1991) Quantitative limulus lysate assay for endotoxin and the effects of plasma. *J Clin Pathol* 44(10):849–854
- Iatrides MC, Artaud J, Vicente N (1983) Sterol composition of Mediterranean marine plants. *Oceanol Acta* 6:73–77
- Ireland CM, Copp BR, Foster MP, McDonald LA, Radisky DC, Swersey JC (1993) Biomedical. In: Attaway DH, Zaborsky OR (eds) *Marine biotechnology*. Vol. 1: Pharmaceutical and Bioactive Natural Products. New York, NY: Plenum Press, pp 1–43
- Ito E, Satake M, Yasumoto T (2002) Pathological effects of lyngbyatoxin A upon mice. *Toxicon* 40(5):551–556
- Ikeda K, Inayama T, Kato T (1990) Effects of *Spirulina platensis* on plasma lipoprotein lipase activity in fructose-induced hyperlipidemic rats. *J Nutr Sci Vitaminol (Tokyo)* 36:165–171
- Jensen PR, Jenkins KM, Porter D, Fenical W (1998) Evidence that a new antibiotic flavones glycoside chemically defends the seagrass *Thalassia testudinum* against zoosporic fungi. *Appl Environ Microbiol* 64(4):1490–1496
- Jeong SJ, Higuchi R, Miyamoto T, Ono M, Kuwano M, Mawatari SF (2002) Bryoanthrathiophene, a new antiangiogenic constituent from the bryozoan *Watersipora subtorquata* (d'Orbigny, 1852). *J Nat Prod* 65(9):1344–1345
- Jha RK, Zi-rong X (2004) Review biomedical compounds from marine organisms. *Marine Drugs* 2(3):123–146

- Jiménez-Escríg A, Sánchez-Muniz FJ (2000) Dietary fibre from edible seaweeds: chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutr Res* 20(4):585–598
- Jo WS, Cho YJ, Kim HJ, Nam BY, Hong SH, Lee GA et al (2010) Anti-inflammatory effect of microalgal extracts from *Tetraselmis suecica*. *Food Sci Biotechnol* 19:1519–1528
- Kadam SU, Prabhansankar P (2010) Marine foods as functional ingredients in bakery and pasta products. *Food Res Int* 43:1975–1980
- Kalechman Y, Albeck M, Sredni B (1992) In vivo synergistic effect of the immunomodulator As 101 and the PKC inducer bryostatin. *Cell Immunol* 143(1):143–153
- Kim SK, Tan LT (2013) Marine cyanobacteria: a prolific source of bioactive natural products as drug leads. In: *Marine microbiology: bioactive compounds and biotechnological applications*. Pukyong National University, Marine Bioprocess Research Center Daeyeon-Dong, Nam-Gu 599-1, Busan 608-737, Republic of Korea; Published Online: 8 Jul 2013 DOI:<https://doi.org/10.1002/9783527665259.ch05>
- Kashihara N, Toe S, Nakamura K, Umezawa K, Yamamura S, Nishiyama S (2000) Synthesis and biological activities of hapalosin derivatives with modification at C12 position. *Bioorg Med Chem Lett* 10:101–103
- Kim Y, Seo JH, Kim H (2011) Beta-carotene and lutein inhibit hydrogen peroxide-induced activation of NF- κ B and IL-8 expression in gastric epithelial AGS cells. *J Nutr Sci* 57:216–223
- Klayman DL (1985) Qinghaosu (artemisinin): an antimalarial drug from China. *Science* 228:1049–1055
- Klayman DL, Lin AJ, Acton N, Scovill JP, Hoch JM, Milhous WK, Theoharides AD, Dobek AS (1984) Isolation of artemisinin (qinghaosu) from *Artemisia annua* growing in the United States. *J Nat Prod* 47:715–717
- Kodama M, Ogata T, Sato S (1988) Bacterial production of saxitoxin. *Agric Biol Chem* 52:1075–1077
- Kodama M, Ogata T, Sato T, Sakamoto S (1990) Possible association of marine bacteria with paralytic shellfish toxicity of bivalves. *Mar Ecol Prog Ser* 61:203–206
- Koehn FE, Longley R, Reed JK (1992) Microcolin A and B, new immunosuppressive peptides from the blue green alga *Lyngbya majuscula*. *J Nat Prod* 55(5):613–619
- Kollár P, Rajchard J, Balounová Z, Pazourek J (2014) Marine natural products: bryostatins in preclinical and clinical studies. *Pharm Biol* 52(2):237–242
- Komori T, Sanechika Y, Ito Y, Matsuo J, Nohara T, Kawasaki T et al (1980) Biologically active glycosides from asteroidea, structures of a new cerebroside mixture and of two nucleosides from the starfish *Acanthaster planci*. *Liebigs Ann Chem* 653–668
- Kontiza I, Stavri M, Zloh M, Vagias C, Gibbons S, Roussis V (2008) New metabolites with antibacterial activity from the marine angiosperm *Cymodocea nodosa*. *Tetrahedron* 64(8):1696–1702
- Kuhnlein HV, Barthet Farren A, Falahi E, Leggee D, Receveur O, Berti P (2006) Vitamins A, D, and E in Canadian Arctic traditional food and adult diets. *J Food Compos Anal* 19:495–506
- Kumari P, Kumar M, Gupta V, Reddy CRK, Jha B (2010) Tropical marine macroalgae as potential sources of nutritionally important PUFAs. *Food Chem* 120:749–757
- Kurihara H, Mitani T, Kawabata J, Takahashi K (1999) Inhibitory potencies of bromophenols from Rhodomelaceae algae against α -glucosidase activity. *Fish Sci* 65:300–303
- Kwan JC, Teplitski M, Gunasekera SP, Paul VJ, Luesch H (2010) Isolation and biological evaluation of 8-epi-malyngamide C from the Floridian marine cyanobacterium *Lyngbya majuscula*. *J Nat Prod* 73:463–466
- Lagarde D, Beuf L, Vermaas W (2000) Increased production of zeaxanthin and other pigments by application of genetic engineering techniques to *Synechocystis* sp. strain PCC 6803. *Appl Environ Microbiol* 66(1):64–72
- Lahaye M (1991) Marine-algae as sources of fibers—determination of soluble and insoluble dietary fiber contents in some sea vegetables. *J Sci Food Agric* 54(4):587–594

- Laurent D, Jullian V, Parenty A, Knibiehler M, Dorin D, Schmitt S et al (2006) Antimalarial potential of xestoquinone, a protein kinase inhibitor isolated from a Vanuatu marine sponge *Xestospongia* sp. *Bioorg Med Chem* 14:4477–4482
- Lee I, Han JI (2015) Hydrothermal-acid treatment for effectual extraction of eicosapentaenoic acid (EPA)-abundant lipids from *Nannochloropsis salina*. *Bioresour Technol* 191:1–6
- Li B, Lu F, Wei XJ, Zhao RX (2008) Fucoidan: structure and bioactivity. *Molecules* 13:1671–1695
- Liesegang TJ (2009) Varicella zoster virus vaccines: effective, but concerns linger. *Can J Ophthalmol* 44(4):379–384
- Lilies G (1996) Gambling on marine biotechnology. *Bioscience* 46:250–253
- Linhardt RJ, Loganathan D, Al-Hakim A, Wang HM, Walenga JM, Hoppenstedt D et al (1990) Oligosaccharide mapping of low molecular weight heparins: structure and activity differences. *J Med Chem* 33:1639–1645
- Liu J, Sun Z, Gerken H, Liu Z, Jiang Y, Chen F (2014) *Chlorella zofingiensis* as an alternative microalgal producer of astaxanthin: biology and industrial potential. *Mar Drugs* 12:3487–3515
- López-Saiz CM, Suárez-Jiménez GM, Plascencia-Jatomea M, Burgos-Hernández A (2013) Shrimp Lipids: a source of cancer chemopreventive compounds. *Mar Drugs* 11(10):3926–3950
- Lucas M, Proust F, Blanchet C, Ferland A, Déry S, Abdous B et al (2010) Is marine mammal fat or fish intake most strongly associated with omega-3 blood levels among the Nunavik Inuit? Prostaglandins, Leukot Essent Fat Acids 83:143–150
- Luesch H, Harrigan GG, Goetz G, Horgen FD (2002) The cyanobacterial origin of potent anticancer agents originally isolated from sea hares. *Curr Med Chem* 9:1791–1806
- Lysek N, Rachor E, Lindel T (2002) Isolation and structure elucidation of deformylflustrabromine from the North Sea bryozoan *Flustra foliacea*. *Z Naturforsch, C: J Biosci* 57(11–12):1056–1061
- MacArtain P, Gill CIR, Brooks M, Campbell R, Rowland IR (2007) Nutritional value of edible seaweeds. *Nutr Rev* 65:535–543
- Maeda N, Kokai Y, Hada T, Yoshida H, Mizushina Y (2013) Oral administration of monogalactosyl diacylglycerol from spinach inhibits colon tumor growth in mice. *Exp Ther Med* 5:17–22
- Maeda N, Kokai Y, Ohtani S, Hada T, Yoshida H, Mizushina Y (2009) Inhibitory effects of preventive and curative orally administered spinach glycoglycerolipid fraction on the tumor growth of sarcoma and colon in mouse graft models. *Food Chem* 112:205–210
- Maeda N, Kokai Y, Ohtani S, Sahara H, Kumamoto-Yonezawa Y et al (2008) Anti-tumor effect of orally administered spinach glycolipid fraction on implanted cancer cells, colon-26, in mice. *Lipids* 43:741–748
- Manirafasha E, Ndikubwimana T, Zeng X, Lu Y (2016) Phycobiliproteins: potential microalgae derived pharmaceutical and biological reagent. *Biochem Eng J* 109:282–296
- Markou G, Iconomou D, Sotirodhis T, Israilides C, Muylaert K (2015) Exploration of using stripped ammonia and ash from poultry litter for the cultivation of the cyanobacterium *Arthrospira platensis* and the green microalga *Chlorella vulgaris*. *Bioresour Technol* 196:459–468
- Marshall JA, Bessesen DH (2002) Dietary fat and the development of type 2 diabetes. *Diabetes Care* 25:620–622
- Matsui SM, Muizzudin N, Arad SM, Marenus K (2003) Sulfated polysaccharides from red microalgae anti-inflammatory properties in vitro and in vivo. *Appl Biochem Biotechnol* 104:13–22
- Mayer AM, Glaser KB, Cuevas C, Jacobs RS, Kem W, Little RD et al (2010) The odyssey of marine pharmaceuticals: a current pipeline perspective. *Trends Pharmacol Sci* 31:255–265
- McConnell OJ, Longley RE, Koehn EE (1994) The discovery of marine natural products with therapeutic potential. In: Gullo VP (ed) *The discovery natural products with therapeutic potential*. Butterworth-Heinemann, Boston, pp 109–174
- McMillan C (1986) Sulfated flavonoids and leaf morphology in the *Halophila ovalis*-H. minor complex (Hydrocharitaceae) of the Indo-Pacific ocean. *Aquat Bot* 25:63–72

- McMillan C, Zapata O, Escobar L (1980) Sulfated phenolic compounds in seagrass. *Aquat Bot* 8:267–278
- McPhail KL, Correa J, Linington RG, Gonzalez J, Ortega-Barria E, Capson TL et al (2007) Antimalarial linear lipopeptides from a panamanian strain of the marine cyanobacterium *Lyngbya majuscula*. *J Nat Prod* 70:984–988
- Melief C, van Hall T, Arens R, Ossendorp F, van der Burg S (2015) Therapeutic cancer vaccines. *J Clin Invest* 125(9):3401–3412
- Mendiola JA, García-Martínez D, Ruperez FJ, Martín-Álvarez PJ, Reglero G, Cifuentes A et al (2008) Enrichment of vitamin E from *Spirulina platensis* microalga by SFE. *J Supercrit Fluid* 43:484–489
- Meng Y, Krzysiak AJ, Durako MJ, Kunzelman JI, Wright JLC (2008) Flavones and flavone glycosides from *Halophila johnsonii*. *Phytochemistry* 69:2603–2608
- Myaoka H, Shimomura M, Kimura H, Yamada Y, Kim HS, Yusuke W (1998) Antimalarial activity of kalihinol A and new relative diterpenoids from the Okinawan sponge, *Acanthella* sp. *Tetrahedron* 54:13467–13474
- Mizushina Y, Hada T, Yoshida H (2012) In vivo antitumor effect of liposomes with sialyl Lewis X including monogalactosyl diacylglycerol, a replicative DNA polymerase inhibitor, from spinach. *Oncol Rep* 28:821–828
- Mizushina Y, Kasai N, Iijima H, Sugawara F, Yoshida H, Sakaguchi K (2005) Sulfo-quinovosyl-acyl-glycerol (SQAG), a eukaryotic DNA polymerase inhibitor and anti-cancer agent. *Curr Med Chem Anticancer Agents* 5(6):613–625
- Mobraten K, Haug TM, Kleiveland CR, Lea T (2013) Omega-3 and omega-6 PUFAs induce the same GPR120-mediated signalling events, but with different kinetics and intensity in Caco-2 cells. *Lipids Health Dis* 12:101–107
- Monfils AK, Triemer RE, Bellairs EF (2011) Characterization of paramylon morphological diversity in photosynthetic euglenoids (Euglenales, Euglenophyta). *Phycologia* 50(2):156–169
- Moore KS, Wehrli S, Roder H, Rogers M, Forrest JN, McCrimmon D et al (1993) Squalamine: an aminosterol antibiotic from the shark. *Proc Natl Acad Sci USA* 90:1354–1358
- Mynderse JK, Moore M, Kashiwagi M, Norton T (1997) Antileukemia activity in the Osillatoriaceae: isolation of debromoaplysiatoxin from lyngbya. *Sci* 196:538–540
- Murakami M, Makabe K, Yamaguchi K, Konosu S, Walchli MR (1988) Goniodomin A, a novel polyether macrolide from the dinoflagellate *Goniodoma pseudogoniaulax*. *Tetrahedron Lett* 29:1149–1152
- Murata M, Nakazoe J (2001) Production and use of marine algae in Japan. *Jarq Jpn Agr Res Q* 35(4):281–290
- Murti Y, Agarwal T (2010) Marine derived pharmaceuticals-development of natural health products from marine biodiversity. *Int J ChemTech Res* 2:2198–2217
- Nagai H, Murata M, Torigoe K, Satake M, Yasumoto T (1992) Gambieric acids, new potent. Antifungal substance with unprecedented polyether structures from a marine dinoflagellate *Gambierdiscus toxicus*. *J Org Chem Commun* 57:5448–5453
- Nakamura H, Kobayashi J, Kobayashi M, Ohizumi Y, Hirata Y (1985) Physiologically active marine natural products from Porifera. VII. Xestoquinone. A novel cardiotonic marine natural product isolated from the Okinawan sea sponge *Xestospongia sapra*. *Chem Lett* 6:713–716
- Narkowicz CK, Blackman AJ, Lacey E, Gill JH, Heiland K (2002) Convolutindole A and convolutamine H, new nematocidal brominated alkaloids from the marine bryozoan *Amathia convoluta*. *J Nat Prod* 65(6):938–941
- Nauroth JM, Liu YC, Van Elswyk M, Bell R, Hal EB, Chung G et al (2010) Docosahexaenoic acid (DHA) and docosapentaenoic acid (DPAn-6) algal oils reduce inflammatory mediators in human peripheral mononuclear cells in vitro and paw edema in vivo. *Lipids* 45:375–384
- Nomoto K, Yokokura T, Satoh H, Mutai M (1983) Anti-tumor effect by oral administration of *Chlorella* extract, PCM-4 by oral admission. *Gan To Kagaku Zasshi* 10:781–785 (in Japanese)
- Nuissier G, Diaba F, Dubois MG (2008) Bioactive agents from beach waste: Syringodium flotsem evaluation as a new source of L-chiro-inositol. *Innov Food Sci Emerg Tech* 9(3):396–400

- O'Sullivan L, Murphy B, McLoughlin P, Duggan P, Peadar G, Lawlor PG et al (2010) Review: prebiotics from marine macroalgae for human and animal health applications. *Mar Drugs* 8 (7):2038–2064
- Okada Y, Ishimaru A, Suzuki R, Okuyama T (2004) A new phloroglucinol derivative from the brown alga *Eisenia bicyclis*: potential for the effective treatment of diabetic complications. *J Nat Prod* 67:103–105
- Oliviera JS, Pires JOR, Morales RAV, Bloch JC, Schwartz CA, Freitas JS (2003) Toxicity of Puffer fish-two species (*Lagocephalus Laevigatus*, Linnaeus 1766 and *Sphoeroides Spengleri*, Bloch 1785) from the southeren Brazilian coast. *J Venom Anim Toxins Incl Trop* 9:76–82
- Olsen E, Grahl-Nielsen O (2003) Blubber fatty acids of minke whales: stratification, population identification and relation to diet. *Mar Biol* 142:13–24
- Osborne NJ, Webb PM, Shaw GR (2001) The toxins of *Lyngbya majuscula* and their human and ecological health effects. *Environ Int* 27(5):381–392
- Pádua D, Rocha E, Gargiulo D, Ramos AA (2015) Bioactive compounds from brown seaweeds: phloroglucinol, fucoxanthin and fucoidan as promising therapeutic agents against breast cancer. *Phytochem Lett* 14:91–98
- Pan W, Liu X, Ge F, Han J, Zheng T (2004) Perinerin, a novel antimicrobial peptide purified from the clamworm *Perinereis aibuhitensis* Grube and its partial characterization. *J Biochem* 135:297–304
- Pasquet V, Morisset P, Ihannouine S, Chepied A, Aumailley L, Berard JB et al (2011) Antiproliferative activity of violaxanthin isolated from bioguided fractionation of *Dunaliella tertiolecta* extracts. *Mar Drugs* 9:819–831
- Peng J, Rao Karumanchi V, Choo YM, Hamann MT (2008) Modern alkaloids. In: Fattorusso E, Taglialatela-Scafati O (eds), Wiley-VCH Pub, Weinheim, Germany, pp 189–232
- Pereira H, Barreira L, Figueiredo F, Custodio L, Vizotto-Duarte C, Polo C et al (2012) Polyunsaturated fatty acids of marine macroalgae: potential for nutritional and pharmaceutical applications. *Mar Drugs* 10:1920–1935
- Perrotta C, Buonanno F, Zecchini S, Giavazzi A, Serafini FP, Catalani E et al (2016) Climacostol reduces tumour progression in a mouse model of melanoma via the p53-dependent intrinsic apoptotic programme. *Sci Rep* 6:27281
- Pettit GR, Herald CL, Doubek DL, Herald DL (1982) Isolation and structure of bryostatin 1. *J Am Chem Soc* 104:6846–6848
- Petrelli D, Buonanno F, Vital LA, Ortenz C (2012) Antimicrobial activity of the protozoan toxin climacostol and its derivatives. *Biology* 67(3):525–552
- Philip PA, Rea D, Thavasu P, Carmichael J, Stuart NS, Rockett H et al (1993) Phase I study of bryostatin 1: assessment of interleukin 6 and tumor necrosis factor alpha induction in vivo. The cancer research campaign phase i committee. *J Natl Cancer Inst* 85(22):1812–1818
- Piplani H, Vaish V, Sanyal SN (2012) Dolastatin 15, a mollusk linear peptide, and Celecoxib, a selective cyclooxygenase-2 inhibitor, prevent preneoplastic colonic lesions and induce apoptosis through inhibition of the regulatory transcription factor NF-κB and an inflammatory protein, iNOS. *Eur J Cancer Prev* 21:511–522
- Plavsic M, Terzic S, Ahel M, van den Berg CMG (2004) Folic acid in coastal waters of the Adriatic Sea Mar Freshw Res 53:1245–1252
- Poncet J (1999) The dolastatins, a family of promising antineoplastic agents. *Curr Pharm Des* 5(3):139–162
- Prener A, Storm HH, Nielsen NH (1996) Cancer of the male genital tract in Circumpolar Inuit. *Acta Oncol Stockh Swed* 35:589–593
- Princep MR, Blunt JW, Munro MHG (1991) New cytotoxic B-carbolines alkaloids from the marine bryozoans *Cribicellina cribraria*. *J Nat Prod* 54:1068–1076
- Proteau PJ, Gerwick WH, Garcia-Pichel F, Castenholz R (1993) The structure of scytonemin, an ultraviolet sunscreen pigment from the sheaths of *cyanobacteria*. *Experientia* 49:825–829
- Pulz O, Gross W (2004) Valuable products from biotechnology of microalgae. *Appl Microbiol Biotechnol* 65:635–648

- Pusateri Anthony E, McCarthy Simon J, Gregory Kenton W, Harris Richard A, Cardenas Luis McManus Albert T et al (2003) Effect of a Chitosan-based hemostatic dressing on blood loss and survival in a model of severe venous hemorrhage and hepatic injury in swine. *The J Trauma: Inj, Infect Crit Care* 54(1):177–182
- Gao Q, Yu K, Ye YeXia, Shine MB, Wang C, Navarre DR, Kachroo A, Kachroo P (2014) Mono- and digalactosyldiacylglycerol lipids function nonredundantly to regulate systemic acquired resistance in plants. *Cell Rep* 9(5):1681–1691
- Rahman MA, Arshad A, Md. Yusoff F (2014) Sea Urchins (Echinodermata: Echinoidea): their biology, culture and bioactive compounds. In: Proceedings of the International Conference on Agricultural, Ecological and Medical Sciences (AEMS-2014) London (UK). DOI: [10.15242/IICBE.C714075](https://doi.org/10.15242/IICBE.C714075)
- Ramos AA, Polle JJ, Tran D, Cushman JC, Jin E, Varela JC (2011) The unicellular green alga *Dunaliella salina* Teod as a model for abiotic stress tolerance: genetic advances and future perspectives. *Algae* 26:3–20
- Rao KV, Santarsiero BD, Mesecar AD, Schinazi RF, Tekwani BL, Hamann MT (2003) New manzamine alkaloids with activity against infectious and tropical parasitic diseases from an Indonesian sponge. *J Nat Prod* 66:823–828
- Rashid ZM, Lahaye E, Defer D, Douzenel P, Perrin B, Bourgougnon N et al (2009) Isolation of a sulphated polysaccharide from a recently discovered sponge species (*Celtodoryx girardae*) and determination of its anti-herpetic activity. *Int J Biol Macromol* 44:286–293
- Rasmussen RS, Morrissey MT (2007) Marine biotechnology for production of food ingredients. In Taylor SL (ed) Advances in food and nutrition research, 52, Elsevier, New York, pp 237–292
- Ravichandran S, Kathiresan K, Hemalatha B (2007) Anti-malarials from marine sponges. *Biotechnol Mol Biol Rev* 2(2):33–38
- Ravn H, Pedersen MF, Borum J, Andary C, Anthoni U, Christopen C et al (1994) Seasonal variation and distribution of two phenolic compounds, rosmarinic acid and caffeic acid, in leaves and root-rhizomes of eelgrass (*Zostera marina L.*). *Ophelia* 40(1):51–61
- Rice DW (2009) Spermaceti. Encyclopedia of marine mammals (2nd ed.), pp 1098–1099
- Rinaudo M (2007) Comprehensive glycoscience. In: Kamerling JP (ed) Seaweed polysaccharides, vol 2. Elsevier, Amsterdam, The Netherland, pp 691–735
- Rodriguez-Sanchez R, Ortiz-Butron R, Blas-Valdivia V, Hernandez-Garcia A, Cano-Europa E (2012) Phycobiliproteins or C-phycocyanin of *Arthrosira* (*Spirulina*) maxima protect against HgCl₂-caused oxidative stress and renal damage. *Food Chem* 135:2359–2365
- Romay Ch, González R, Ledón N, Remirez D, Rimbau V (2003) C-phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. *Curr Protein Pept Sci* 4:207–216
- Rowley DC, Hansen MST, Rhodes D, Sottriffer CA, Ni HH, McCammon JA et al (2002) Thalassiolins A-C: new marine-derived inhibitors of HIV cDNA integrase. *Bioorg Med Chem* 10:3619–3625
- Sabry OM, Goeger DE, Gerwick WH (2017) Biologically active new metabolites from a Florida collection of *Moorea producens*. *Nat Prod Res* 31(5):555–561
- Sadigh-Eteghad S, Talebi M, Farhoudi M, Mahmoudi J, Reyhani B (2013) Effects of Levodopa loaded chitosan nanoparticles on cell viability and caspase-3 expression in PC12 neural like cells. *Neurosci (Riyadh)* 18(3):281–283
- Sadovskaya I, Souissi A, Souissi S, Grard T, Lencel P, Greene CM et al (2014) Chemical structure and biological activity of a highly branched (1→3,1→6)-β-D-glucan from *Isochrysis galbana*. *Carbohydr Polym* 111:139–148
- Sakai R, Higa T, Jefford CW, Bernardinelli G (1986) Manzamine A, a novel antitumor alkaloid from a sponge. *J Am Chem Soc* 108:6404–6405
- Sanchez-Machado DI, Lopez-Hernandez J, Paseiro-Losada P, Lopez-Cervantes J (2004) Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chem* 85:439–444
- Schwartz EF, Mourao CB, Moreira KG, Camargos TS, Mortari MR (2012) Arthropod venoms: a vast arsenal of insecticidal neuropeptides. *Biopolym* 98(4):385–405

- Schulze MB, Schulz M, Heidemann C, Schienkiewitz A, Hoffmann K, Boeing H (2007) Fiber and magnesium intake and incidence of type 2 diabetes: a prospective study and meta-analysis. *Arch Intern Med* 167:956–965
- Sci-Edu (2000) New cancer drug extracted from marine organism. People's Daily. 2000. pp 1–4. www.fpeng.peopledaily.com.cn/200012/05/eng
- Shahidi F, Synowiecki J, Amarowicz R and Wanasaundara U (1994) Omega-3 fatty acid composition and stability of seal lipids. In: Ho CT, Hartman TG (eds) *Lipids in food flavors*. Copyright © 1994 American Chemical Society, ACS Symposium Series, vol 558, Chapter 16, pp 233–243
- Sharifuddin Y, Chin YX, Lim PE, Phang SM (2015) Potential bioactive compounds from seaweed for diabetes management. *Mar Drugs* 13:5447–5491
- Shi XM, Jiang Y, Chen F (2002) High-yield production of lutein by the green microalga *Chlorella protothecoides* in heterotrophic fed-batch culture. *Biotechnol Prog* 18:723–727
- Sica D, Picialli V, Masullo A (1984) Configuration at C-24 of sterols from the marine phanerogams Posidonia oceanica and *Cymodocea nodosa*. *Phytochemistry* 23(11):2609–2611
- Sima P, Vetvicka V (2011) Bioactive substances with anti-neoplastic efficacy from marine invertebrates: porifera and coelenterata. *World J Clin Oncol* 2(11):355–361
- Simudu U, Kita-Tsukamoto K, Yasumoto T, Yotsu M (1990) Taxonomy of four marine bacterial strains that produce tetrodotoxin. *Int J Syst Bacteriol* 40:331–336
- Singh D, Puri M, Wilkens S, Mathur AS, Tuli DK, Barrow CJ (2013) Characterization of a new zeaxanthin producing strain of *Chlorella saccharophila* isolated from New Zealand marine waters. *Bioresour Technol* 143:308–314
- Singh S, Kate BN, Banerjee UC (2005) Bioactive compounds from *cyanobacteria* and *microalgae*: an overview. *Critical Rev Biotechnol* 25:73–95
- Singla S, Garg R (2013) Therapeutic potential of snake venom. *Int Res J Pharm* 4(11):9–16
- Sivasubramanian K, Ravichandran S, Kumaresan M (2011) Preliminary studies for a new antibiotic from the marine mollusk Melo melo (Lightfoot, 1786) *Asian Pacif J Tropic Med* 4(4):310–314
- Skov MJ, Beck JC, de Kater AW, Shopp GM (2007) Nonclinical safety of ziconotide: an intrathecal analgesic of a new pharmaceutical class. *Int J Toxicol* 26(5):411–421
- Soontornchaiboon W, Joo SS, Kim SM (2012) Anti-inflammatory effects of violaxanthin isolated from microalga *Chlorella ellipsoidea* in RAW 264.7 macrophages. *Biol Pharm Bull* 35:1137–1144
- Sørensen L, Hantke A, Eriksen NT (2013) Purification of the photosynthetic pigment C-phycocyanin from heterotrophic *Galdieria sulphuraria*. *J Sci Food Agric* 93:2933–2938
- Spector AA, Kim HY (2015) Discovery of essential fatty acids. *J Lipid Res* 56(1):11–21
- Spencer L, Mann C, Metcalfe M, Webb M, Pollard C, Spencer D et al (2009) The effect of omega-3 FAs on tumour angiogenesis and their therapeutic potential. *Eur J Cancer* 45:2077–2086
- Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. *J Biosci Bioeng* 101:87–96
- Stevenson CS, Capper EA, Roshak AK, Marquez B, Grace K, Gerwick WH et al (2002a) Scytomenin-a marine natural product inhibitor of kinases key in hyperproliferative inflammatory diseases. *Inflammation Res* 51:112–118
- Stevenson CS, Capper EA, Roshak AK, Marquez B, Eichman C, Jackson JR (2002b) The identification and characterization of the marine natural product scytomemin as a novel antiproliferative pharmacophore. *J Pharmacol Exp Ther* 303:858–866
- Su JH, Wen ZH (2011) Bioactive membrane-based diterpenoids from the soft coral *Sinularia triangularis*. *Mar Drugs* 9:944–951
- Subhashini P, Dilipan E, Thangaradjou T, Papenbrock J (2013) Bioactive natural products from marine angiosperms: abundance and functions. *Nat Prod Bioprospect* 3:129–136
- Sudeesh Kumar PT, Praveen G, Raj M, Chennazhi KP, Jayakumar R (2014) Flexible, micro-porous chitosan–gelatin hydrogel/nanofibrin composite bandages for treating burn wounds. *Royal Soc Chem RSC Adv* 4:65081–65087

- Suffness M, Newman DJ, Snader K (1989) Bioorganic marine chemistry. In: Scheuer PJ (ed) Springer Verlag, New York 3:131–168
- Suganthy N, Karutha Pandian S, Pandima Devi K (2010) Neuroprotective effect of seaweeds inhabiting South Indian coastal area (Hare Island, Gulf of Mannar Marine Biosphere Reserve): cholinesterase inhibitory effect of *Hypnea valentiae* and *Ulva reticulata*. *Neurosci Lett* 468:216–219
- Summers LK, Fielding BA, Bradshaw HA, Ilic V, Beysen C, Clark ML et al (2002) Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. *Diabetologia* 45:369–377
- Swanson D, Block R, Mousa SA (2012) Omega -3 fatty acids EPA and DHA: health benefits throughout life. *Adv Nutr* 3(1):1–7
- Swift AE, Swift TR (2008) Ciguatera. *J Toxicol Clin Toxicol* 31(1):1–29
- Tabar AI, Acero S, ArreguiC Urdánoz M, Quirce S (2003) Asma y alergia por el colorante carmín [Asthma and allergy due to carmine dye]. *Anales Del Sistema Sanitario De Navarra* 26(Suppl 2):65–73 (in Spanish)
- Tasiemski A, Schikorski D, Le Marrec-Croq F, Camp CPV, Boidin-Wichlacz U, Sautiere PE (2007) Hedistin: a novel antimicrobial peptide containing bromotryptophan constitutively the marine annelid, expressed in the NK cells-like of *Nereis diversicolor*. *Dev Comp Immunol* 31:749–762
- Thiemann GW, Iverson SJ (2008) Variation in blubber fatty acid composition amon marine mammals in the Canadian Arctic. *Mar Mamm Sci* 24(1):91–111
- Vergeer LHT, Aarts TL, De Groot JD (1995) The ‘wasting disease’ and the effect of abiotic factors (light intensity, temperature salinity) and infection with *Labyrinthula zosterae* on the phenolic content of *Zostera marina* shoots. *Aquat Bot* 52(1–2):35–44
- Vijayakumar S, Amirthanath A (2014) Bioactivity of sea grass against the malarial fever mosquito *Culex quinquefasciatus*. *Asian Pac J Trop Dis* 4(4):287–291
- Wang X, Zhang X (2013) Separation, antitumor activities, and encapsulation of polypeptide from *Chlorella pyrenoidosa*. *Biotechnol Prog* 29:681–687
- Wender PA, Koehler KE, Sharkey NA, Dell'Aquila HL, Blumberg PM (1986) Analysis of phorbol ester pharmacophor on protein kinase C as a guide to the retional design of new classes of analogs. *Proc Natl Acad Sci USA* 83:4214–4218
- WHO (2012) World Health Organization, Global Vaccine Action Plan 2011–2020. Geneva
- WHO (2017) Cholera vaccines: WHO position paper No. 34, August 2017. *Wkly Epidemiol Rec* 92:477–500
- Yamada T, Iwamoto C, Yamagaki N, Yamanouchi T, Minoura K, Yamori T et al (2002) Leptosins M-N1, cytotoxic metabolites from a Leptosphaeria species separated from a marine alga. Structure determination and biological activities. *Tetrahedron* 58:479–487
- Yousaf M, Hammond NL, Peng J, Wayhuono S, McIntosh KA, Charman WN, Mayer AMS, Hamann MT (2004) New manzamine alkaloids from an Indo-Pacific sponge. Pharmacokinetics, oral availability, and the significant activity of several manzamines against HIV-I, AIDS opportunistic infections, and inflammatory diseases. *J Med Chem* 47:3512–3517
- Yuan JP, Peng J, Yin K, Wang JH (2011) Potential health-promoting effects of astaxanthin: a high-value carotenoid mostly from microalgae. *Mol Nutr Food Res* 55:150–165
- Yuri K, Elena K, Valeri K, Maxim K (2012) Cerium binding activity of pectins isolated from the Seagrasses *Zostera marina* and *Phyllospadix iwatensis*. *J Mar Drugs* 10:834–848
- Yuvaraj N, Kanmani P, Satishkumar R, Paari A, Pattukumar V, Arul V (2012) Seagrass as a potential source of natural antioxidant and anti-inflammatory agents. *Pharm Biol* 50(4):458–467
- Zapata O, McMillan C (1979) Phenolic acids in seagrass. *Aquat Bot* 7:307–317
- Zasloff M, Adams AP, Beckerman B, Campbell A, Han Z, Luijten E et al (2011) Squalamine as a broad-spectrum systemic antiviral agent with therapeutic potential. *Proc Natl Acad Sci* 108 (38):15978–15983
- Zhang W, Guo YW, Gu YC (2006a) Secondary metabolites from the South China Sea invertebrates: chemistry and biological activity. *Curr Med Chem* 13:2041–2090

- Zhang SY, Yi YH, Tang HF (2006b) Bioactive triterpene glycosides from the sea cucumber *Holothuria fuscocinerea*. *J Nat Prod* 69(10):1492–1495
- Zhang H, Shigemori H, Ichibashi M, Kosaka T, Pettit GR et al (1994) Convolutamides A-F, novel γ -lactam alkaloids from the marine bryozoan *Amathia convoluta*. *Tetrahedron* 50:10201–10206
- Zhang Y-J, Gao Bo, Liu X-W (2015) Topical and effective hemostatic medicines in the battlefield. *Int J Clin Exp Med* 8(1):10–19
- Zheng LH, Wang YJ, Sheng J, Wang F, Zheng Y, Lin XK et al (2011) Antitumor peptides from marine organisms. *Mar Drugs* 9:1840–1859

Chapter 5

Vitamins, Nutraceuticals, Food Additives, Enzymes, Anesthetic Aids, and Cosmetics



Abstract Vitamins are low-molecular-weight organic compounds, indispensable for life activity in trace amounts for essential metabolic reactions, where deficiency causes specific disease symptom and do not include other essential nutrients such as dietary minerals, essential fatty acids, or essential amino acids, nor does it encompass the large number of other nutrients that promote health and do not provide cellular structural material and energy. Animals derived vitamins from plants and microorganisms. Vitaminoids are compounds with “vitamin-like” activity. Vitamins fall into two main groups: fat-soluble (e.g., A, D, E) and water-soluble (e.g., B, C, P) vitamins. Nutraceuticals include a number of substances ranging from natural diets, herbal products, biofortified crops, genetically modified, and processed food products. Nutraceuticals beyond basic nutrition provide health benefit, modulate immunity, and/or prevent and cure specific diseases. Functional foods are whole, fortified, enriched, or enhanced foods that provide health benefits beyond the provision of essential nutrients and classified into several groups on the basis of food group, the diseases it prevents or alleviates, physiological effects, etc. Food additives are antioxidants, food preservatives, food coloring agents, flavoring agents, anti-infective agents, excipients, and other similar substances used in the processing or storage of foods or animal feed. Excipients have little or no therapeutic value, but contribute largely to the performance of the active pharmaceutical ingredient (API) and maintain the quality, efficacy, safety, etc., of the formulation and include solvents, diluting, suspending, and emulsifying agents as well as antioxidants, preservatives, pharmaceutical, coloring agents, flavoring agents, vehicles, excipients, ointment bases, etc. Proteins and peptides allow the development of antibodies and different fermentations, purification processes, and recombination technology produced potential protein drugs at acceptable cost which can be useful in various diseases through various routes like oral, transdermal, nasal, pulmonary, ocular, buccal, and rectal. Many protein pharmaceuticals are available for treating rheumatoid arthritis, coronary artery thrombosis, multiple sclerosis, and chronic lymphocytic leukemia. Papain, a plant enzyme, is very helpful for the prevention of atherosclerosis and diabetic heart disease. Natural anesthetics, e.g., cocaine, methyl salicylate, capsaicin, piperine, opium, etc., have

been in use since antiquity. Cosmeceuticals are cosmetic-pharmaceutical hybrid products intended to improve the health and beauty of the skin by providing a specific result, ranging from acne-control and anti-wrinkle effects to sun protection.

5.1 Natural Sources, Classification, Chemistry and Therapeutic Use of Vitamins

Introduction

The discovery of vitamins was linked to the studies of nutrients and their role in the vital activity of the living organism. In 1880, the Russian physician N. I. Lunin was the first to demonstrate that, alongside the known elementary components (proteins, fats, carbohydrates, water, and minerals), some other accessory factors were needed for the normal growth and maintenance of the organism. In 1905, the English physician W. Fletcher while researching the causes of Beriberi discovered that, instead of polished rice, eating unpolished rice prevented Beriberi and he postulated the existence some special nutrients in the rice husk. In 1912, Polish biochemist C. Funk named the special nutritional parts of food as a “vitamine” after the Latin word “vita” meaning life and “amine” from compounds found in the thiamine he isolated from rice bran. Vitamine was later shortened to vitamin.

Vitamins are low-molecular-weight organic compounds, indispensable for life activity of the organism, required in trace amounts for essential metabolic reactions in the body and deficiency causes specific disease symptom. The term vitamin does not include other essential nutrients such as dietary minerals, essential fatty acids, or essential amino acids, nor does it encompass the large number of other nutrients that promote health. As distinct from other organic nutrients, which provide cellular structural material and energy, vitamins either participate in the production of coenzymes or act as regulators of biochemical processes. Deficiency of a particular vitamin develops in the organism-specific symptoms which may not be overcome by other vitamins. Animal world derived vitamins from plants and microorganisms.

Vitaminoids are compounds with “vitamin-like” activity, considered by some to be vitamins or partially to replace vitamins, including flavonoids, inositol, carnitine, choline, lipoic acid, and the essential fatty acids. With the exception of the essential fatty acids, there is little evidence that any of them is a dietary essential. With exceptions of vitamin B₆ and B₁₂, they are readily excreted in urine without appreciable storage, so frequent consumption becomes necessary. They are generally nontoxic when present in excess of needs, although symptoms may be reported in people taking megadoses of niacin, vitamin C, or pyridoxine (B₆). All the B vitamins function as coenzymes or cofactors, assisting in the activity of important enzymes and allowing energy producing reactions to proceed normally. Water-soluble vitamins are easily lost with overcooking. Vitamins K and certain B complex vitamins are synthesized in the human body by bacteria in the small intestine; D can be synthesized by the skin when exposed to sunlight. The same compound can serve as a vitamin for some organisms, while being an ordinary

substance for the other, e.g., ascorbic acid is a vitamin for man and for guinea pig, since it is not synthesized in their tissues, while for rat, rabbit, and dog, ascorbic acid is no vitamin, since it is synthesized in their tissues. In humans, the vitamin sources are food and intestinal bacteria.

Avitaminosis arises due to chronic or long-term vitamin deficiency (beriberi, scurvy, rickets, and pellagra). Avitaminoses: due to the deficiency vitamin A—xerophthalmia or night blindness, thiamine (B_1)—beriberi, niacin (B_3)—pellagra, B_{12} —megaloblastic anemia, vitamin C—scurvy, vitamin D—ricketsia, and vitamin K—impaired blood coagulation. Hypovitaminosis is any of several diseases caused by deficiency of one or more vitamins. Antivitamins are substance that destroy or inhibit the metabolic action of a vitamin. Antivitamins include vitamin decomposing enzymes (thiaminase and ascorbase), compounds forming nonactive complexes with vitamins (avidin) or structurally similar to vitamins (sulphonamides), etc. Hypervitaminosis or vitamin intoxication is a condition resulting from the chronic excessive intake of vitamins or vitamin supplements with associated side effects (nausea, diarrhea, and vomiting). Hypervitaminosis, or vitamin intoxication, is manifested by general symptoms, i.e., loss of appetite, disorder in the motor function of gastrointestinal tract, strong headache, high excitability of the nervous system, hair shedding, skin desquamation, and other signs. Hypervitaminosis may lead to a fatal outcome. Hypervitaminosis can be called forth by an excessive intake of food rich in a fat-soluble vitamin (e.g., the liver of polar bear or whale, which is rich in vitamin A), or by a prescription of large vitamin doses.

Multivitamin

An over-the-counter (OTC) and self-prescribed diet/nutritional supplement containing lipid-soluble vitamins (A, D, E, and K) and water-soluble vitamins (thiamin (B_1), riboflavin (B_2), B_6 , B_{12} , C, folic acid, niacin, pantothenic acid, and biotin). Multivitamins may also contain minerals—e.g., calcium, phosphorus, iron, iodine, magnesium, manganese, copper, and zinc.

Classification

Vitamins, by their physicochemical properties, fall into two main groups: fat-soluble vitamins, and water-soluble vitamins. A vitamin of either group is assigned a letter of the Latin alphabet, as well as a chemical or physiologic name (Table 5.1).

The fat-soluble vitamins are usually absorbed in fat globules (chylomicrons) and stored in body tissues. Intake of too much fat-soluble vitamin may lead to high accumulation and hypervitaminosis.

Fat-soluble vitamins and vitaminoids: A, D, E, KF, K, Coenzyme Q

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin A

Source: For humans, all foodstuffs of animal origin serve as the source of vitamin A. The fish liver, especially which of cod and banded sea perch, is rich in vitamin

Table 5.1 Classification of vitamins and their derivatives

Letter label	Chemical name	Chemical forms		Coenzymes	Physiological name
		Biologically inactive Derivatives	Biological Active Derivatives		
I. Fat-soluble vitamins					
A	Retinol	Retinyl acetate, retinylpalmitate	Retinol, retinal, retinoic acid		Antixerophthalmic
D	Calciferols	Ergocalciferol (D ₂), cholecalciferol (D ₂), cholecalciferol (D ₃)	1,25-dihydroxy-calciferol		Antirachitic
E	Tocoferols		$\alpha, \beta, \gamma, \delta$ -tocoferols, tocotrienols and their esters Phylloquinone (K ₁), menaquinone (K ₂)		Antisterile Antihemorrhagic
K	Naphthoquinone				
II. Fat-soluble vitaminoids					
F	Essential fatty acids (EFA), ubiquinone (coenzyme Q)			Ubiquinone (CoQ), ubiquinol (CoQ, H ₂)	
III. Water-soluble vitamins					
B ₁	Thiamine	Thiamine Riboflavin	Ascorbic acid	Thiamine diphosphate, thiaminetriphosphate FMN, FMN, H ₂ , FAD, FAD, H ₂ , pantetheine 4 phosphate, coA, depospho—CoA, NAD ⁺ , NAD, H ₂ , NADP ⁺ , NADP, H ₂ PALP, PAMP	Antineuritic Growth vitamin Antipellagraic Growth factor Antianemic
B ₂	Riboflavin				
B ₃	Pantothenic acid	Nicotinamide, nicotinic acid			
B ₅		Pyridoxine			
(PP)		Folin (folic acid)			
B ₆		Cyanocobalamin			
B ₉		Ascorbic acid (B _c)			

(continued)

Table 5.1 (continued)

Letter label	Chemical name	Chemical forms	Biologically inactive Derivatives	Biologically Active Derivatives	Coenzymes	Physiological name
B ₁₂					Tetrahydrofolic acid and its derivatives with one-carbon radicals Methylcobalamin, deoxyadenosylcobalamin	
IV. Water-soluble vitaminoids						
H	Biotin	Biotin	Phosphocholine Flavones, rutin, quercetin, flavonones, hesperidin, caeohol, complex Inositol, <i>meso</i> -inositol, <i>myo</i> -inositol, diphosphoinositol, diccephalin	Carboxybiotin		Antiseborrheic
B ₄	Choline	Choline				
P	Bioflavonoids					
B ₅	Inositol					
N	Lipoic acid	Lipoic acid		Lipamide (oxidized and reduced forms)		
B _T	Carnitine		Carnitine, acylcarnitine			
	Orotic acid	Orotic acid	Orotidin 5-phosphate			Growth factor
B ₁₃	Pangamic acid		Pangamic acids, methylmethionine, methylthioninesulphonium folic acid			Antianoxic
B ₁₅	S-Methylmethionine					Antilulcerous
U	<i>para</i> -Aminobenzoic acid (PABA)	<i>para</i> -Aminobenzoic acid				Microbial vitamin

A. Vitamin A is abundant in pork and beef liver, egg yolk, sour cream, and whole milk. Vegetable products (asparagus, beet, celery, carrots cabbage, dandelion, lettuce, endive, orange, turnip leaf, tomato, prune, parsley, spinach, and watercress) contain carotenoids which are provitamins A. Therefore, a partial supply of the human organism with vitamin A is provided by the vegetables, if the conversion of alimentary carotenoids to vitamin A is not impaired. The daily requirement in vitamin A for the adult human is 1.5 mg.

Chemical nature and biologically active forms of vitamin A

Vitamin A is a diterpenoid alcohol (an unsaturated monobasic alcohol) and includes retinol, retinal, and retinoic acid, as well as several provitamin A carotenoids like α - and β -carotenes, β -cryptoxanthin, etc. Retinol and its derivatives are collectively called retinoids. Retinyl palmitate (vitamin A palmitate), ester of retinol (vitamin A) and palmitic acid, is a common synthetic vitamin A supplement. Chemically retinol or vitamin A is an isoprenoid and derived most notably from β -carotene having a β -ionone ring and a side chain of two isoprene residues with a primary carbinol group at the end. Vitamin A has at least six vitamer (vitamin with similar molecular structure) chemicals (e.g., retinol, retinal, and four carotenoids: α , β , γ , and δ carotenes; and β -cryptoxanthin) that all qualify as "vitamin A", but each with slightly different properties. A vitamer of a particular vitamin is any of a number of chemical compounds having similar molecular structure and physiological function. Four vitamers (three carotenes and one xanthophyll) are found naturally in plant origin foods and retinol (alcohol) and retinal (aldehyde) forms occur in animal-based foods (e.g., fish). The retinoids (retinol, retinal, retinoic acid, isotretinoin, alitretinoin, etc.) are pharmaceutical forms of vitamin A. In the organism, retinol (vitamin A alcohol) is converted to retinal (vitamin A aldehyde) and retinoic acid (vitamin A acid). In the organism tissues, the vitamin A ester derivatives such as retinyl palmitate, retinyl acetate, and retinyl are formed (Fig. 5.1).

Three precursor or provitamins A are known such as α -, β -, and γ -carotenes, which differ in the chemical structure and biological activity (Fig. 5.2). Of these, the most active is β -carotene which is subjected, in the intestinal mucosa, to oxidation at the central double bond with the participation of the enzyme carotene dihydroxygenase. Two molecules of active retinal are formed. The degradation of α - and γ -carotenes, each containing, unlike β -carotene, only one β -ionone ring, leads in either case to only one vitamin A molecule. Similar is the case with β -cryptoxanthin only one molecule of vitamin A (retinol). Hence, a lesser activity of both α - and γ -carotenes as well as β -cryptoxanthin as compared to β -carotene. All of the vitamin A forms (retinol, retinal, retinoic acid, and their esterified derivatives) exhibit biological activity.

Biochemical functions

Retinoids, pharmaceutical forms of vitamin A (retinal, retinol, retinoic acid, and their derivatives), have many important functions throughout the body including roles in vision; regulation of cell proliferation and differentiation of a developing

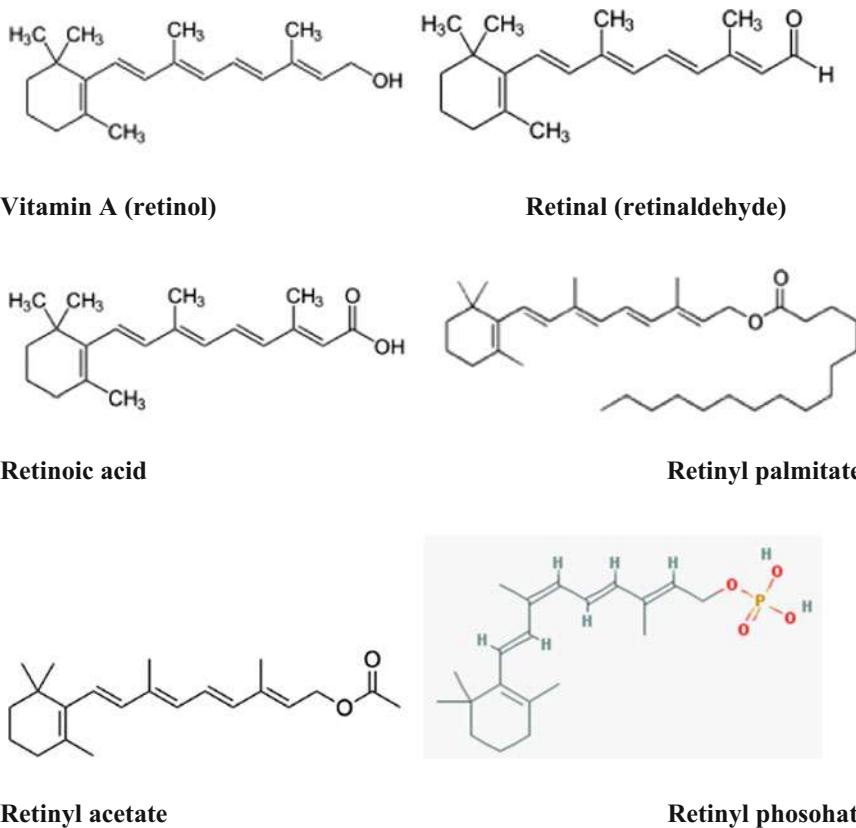
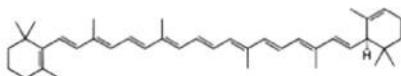


Fig. 5.1 Structure of some fat-soluble vitamin A (retinol and its derivatives retinal, retinoic acid, retinyl palmitate, retinyl acetate, retinyl phosphate)

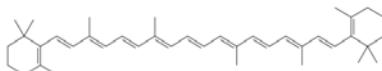
organism (embryo, juvenile organism); differentiation of rapidly proliferating tissues such as cartilage and bone tissue, spermatogenic epithelium and placenta, skin epithelium, and mucosae; immune function; and activation of tumor suppressor genes.

Deficiency of vitamin A

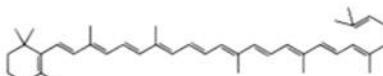
The earliest symptom of vitamin A deficiency is the dark adaptation disorder and night blindness. Moreover, possible disturbances are the juvenile growth retardation, follicular hyperkeratosis (excessive keratinization of the skin caused by a delayed renewal of epithelium), mucosal dryness (also due to delayed epithelial renewal), xerophthalmia (dryness of the conjunctiva and cornea), keratomalacia (opacification of the cornea and its softening), and disordered reproductive function (failure of the spermatozoa to fertilize).



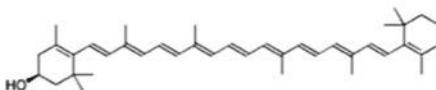
α -Carotene (a β -ionone and an α -ionone ring at 2 ends)



β -Carotene (2 β -ionone rings at both ends)



γ -Carotene (1 β -ionone ring at one end)



β -Cryptoxanthin (related to β -carotene with addition of a OH group)

Fig. 5.2 Structure of provitamins A (α -, β -, γ -Carotenes, and β -Cryptoxanthin)

Practical applications

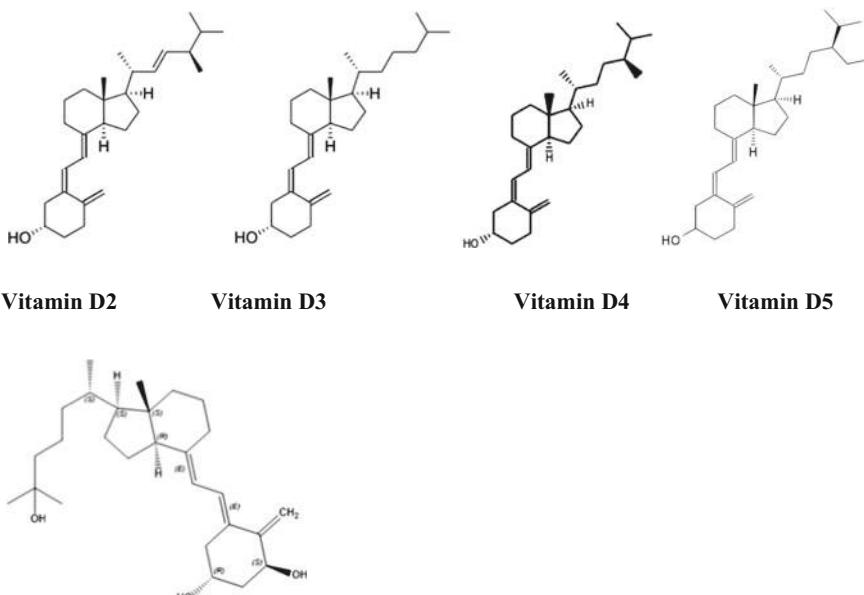
Preparation of natural vitamin A (containing a mixture of its biological forms) and its synthetic analogs (retinol acetate and retinol palmitate) are used for medical purposes. They are applied prophylactically to the medication of hypovitaminosis in persons whose professional occupation requires visual exertion and to stimulation of growth and development in children. In addition, vitamin A preparations are used as regeneration stimulants for treating poorly healable tissues, for increasing the resistibility to infection, and in prophylactical treatment of sterility.

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin D

Source: Vitamin D is mainly found in certain products of animal origin such as liver, butter, milk, yeast, and vegetable oils but not in vegetables, fruits, or cereals. Cod liver is particularly rich in vitamin D. For children, the recommended daily dose of vitamin D varies from 12 to 25 μ g; for adult humans, the daily requirement is 10 times as less.

Chemical nature and biologically active forms

Vitamin D includes a group of compounds chemically related to steroids—the fat-soluble secosteroids (a type of steroid with a “broken” ring) that are derived from cholesterol (Fig. 5.3). Cholecalciferol (vitamin D₃) is an example of a 9,10-seco steroid. Vitamin D has several vitamers including calcitriol. Among different D vitamins, the most active are D₂ (ergocalciferol) and D₃ (cholecalciferol). They structurally differ as the side chain of D₂ contains a double bond between



Vitemer Calcitriol

Fig. 5.3 Structure of Vitamin D (D_2 —ergocalciferol, D_3 —cholecalciferol, D_4 —22 dihydroergocalciferol, D_5 —sitocalciferol, vitemer calcitriol)

carbons 22 and 23, and a methyl group on carbon 24. Ergocalciferol (D_2) is made from its plant precursor ergosterol (provitamin D). Vitamin D_3 (cholecalciferol) is generated in the skin of animals when UV light energy is absorbed by a precursor molecule 7-dehydrocholesterol (present in the skin of humans and animals). Vitamin D_4 is 22 dihydroergocalciferol. The less active vitamers D_4 , D_5 , D_6 , and D_7 are produced by UV irradiation from their respective plant precursor dihydroergosterol, 7-dehydrositosterol, 7-dehydrostigmasterol, and 7-dehydrocampesterol, respectively. However, neither ergo- nor cholecalciferols are biologically active and are incapable of performing regulatory functions. Their biologically active forms that act like steroid hormones are produced in the course of metabolism.

Biochemical functions

The biological activity of 1,25-dihydroxycalciferols is tenfold superior over the activity of parent calciferols. Vitamin D has a significant role in calcium homeostasis and metabolism. Vitamin D controls the transport of calcium and phosphate ions across the cell membranes and therefore acts as a regulator for the level of these ions in the blood. This control includes at least three processes involving vitamin D: Transport of calcium and phosphate ions across the epithelium of small intestinal mucosa on their absorption; mobilization of calcium from the bone tissue; and reabsorption of calcium and phosphate in the tubules of the kidney.

The mechanism of vitamin D action on these processes is envisioned in the following manner. The absorption of calcium in the small intestine proceeds by facilitated diffusion with the involvement of a special calcium-binding protein (CaBP) and by active transport with the aid of Ca^{2+} -ATPase. Presumably, 1,25-dihydroxycholecalciferols elicit formation of CaBP and protein components of Ca^{2+} -ATPase by acting on the genetic cellular apparatus of small intestinal mucosa. Apparently, the vitamin D-induced stimulation of Ca^{2+} -ATPase, contained in the membranes of renal tubules, leads to the reabsorption of calcium ions in the tubules. However, the mechanisms of vitamin D involvement in the transmembrane transfer of phosphate in the intestine and kidneys and the calcium mobilization from the bone tissue remain yet to be elucidated. On the whole, the action of vitamin D is reflected in increased concentrations of calcium and phosphate in the blood.

Deficiency of vitamin D

The lack of vitamin D manifests itself in the disease known as osteomalacia or rickets when it occurs in children, which is a softening of the bones. Vitamin D deficient diet in conjunction with inadequate sun exposure UV irradiation (for production of endogenous vitamin D) causes this disease. A relatively lesser sensitivity of the tissues responsive to calciferols (apparently, due to the lack of calciferol-binding receptors) may also be the cause. In rickets, all vitamin D-controlled processes are inhibited, namely, the intestinal uptake of calcium ions and phosphate (even if their dietary supply to the infant in dairy products is sufficient) and their reabsorption in the kidneys. Because of this, the level of calcium and phosphorous in the blood is lowered, and the bone mineralization becomes impaired, that is no deposition of mineral materials on the newly formed collagen matrix of growing bones occurs. Therefore, in children suffering from rickets, the deformation of skeletal bones of limbs, skull, and thorax is observed. The relative deficiency in vitamin D may also develop when its supply to the organism is normal. This may be evoked by diseased liver and, especially, kidney, since these organs are involved in the production of the active forms of vitamin D.

Practical applications

In medical practice, natural preparations of vitamin D (cod liver oil) and synthetic preparations (ergocalciferol or cholecalciferol) are used. Vitamin D preparations are used in the prophylaxy (preventive measures to disease or health problems) and therapy of rickets and in the medication of other diseases (tuberculosis of bones and joints, and tuberculosis of skin).

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin E

Sources: The sources of tocopherol for humans are wheat germ, celery, lettuce or other green leafy vegetables, parsley, spinach, turnip leaf, watercress, and vegetable oils, especially sunflower oil, corn oil, cottonseed oil, and olive oil (Bieri et al. 1974; Reboul et al. 2006). Abundant tocopherol is in wheat seedling oil. Products

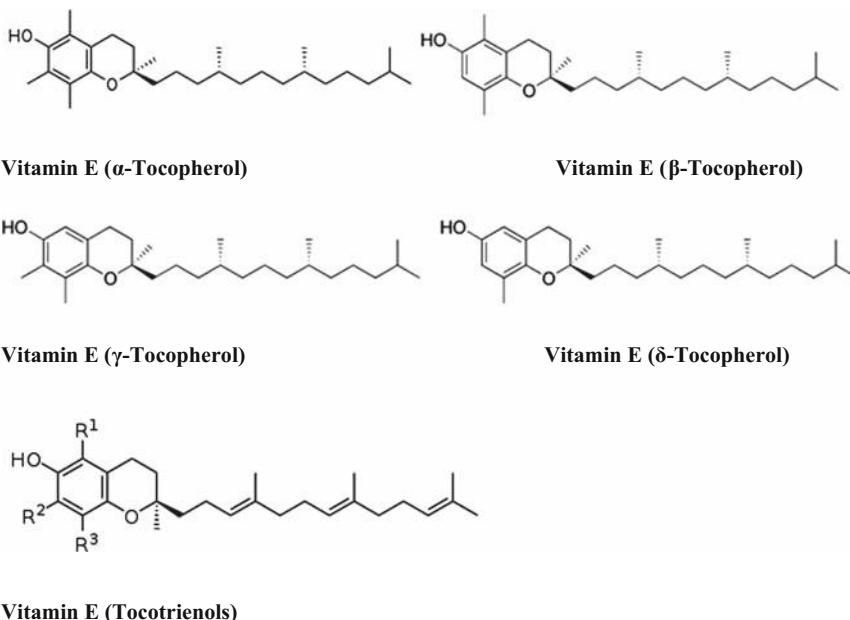
of animal origin, including those of dairy produce, are poor in tocopherol. For adult humans, the recommended daily intake of tocopherol is 20–50 mg.

Chemical nature and biologically active forms of vitamin E

Vitamin E refers to a group of methylated derivative compounds that include eight different forms, four of each tocopherol and tocotrienol. Both the tocopherols and tocotrienols occur in α (alpha), β (beta), γ (gamma), and δ (delta) forms, determined by the number and position of methyl groups on the chromanol ring. Structurally, tocopherols and tocotrienols are closely related; the tocotrienols have the same methyl structure at the ring and the same Greek letter—methyl—notation and an isoprenoid side chain; tocopherols have saturated isoprenoid side chains, whereas tocotrienols differ from the analogous tocopherols by the presence of unsaturated hydrophobic isoprenoid side chains with three double bonds (farnesyl isoprenoid tails) (Fig. 5.4).

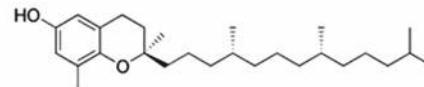
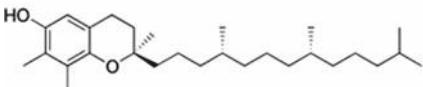
Biochemical functions

Tocopherol controls the rate of free radical reactions in living cells by inhibiting spontaneous chain reactions of peroxide oxidation of unsaturated lipids in



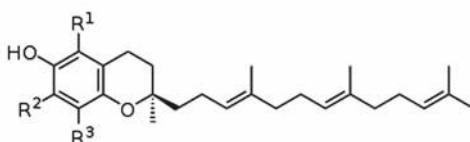
Vitamin E (Tocopherol)

Vitamin E (β -Tocopherol)



Vitamin E (γ -Tocopherol)

Vitamin E (δ -Tocopherol)



Vitamin E (Tocotrienols)

where α -Tocotrienol: R¹ = Me, R² = Me, R³ = Me; β -Tocotrienol: R¹ = Me, R² = H, R³ = Me; γ -Tocotrienol: R¹ = H, R² = Me, R³ = Me; δ -Tocotrienol: R¹ = H, R² = H, R³ = Me

Fig. 5.4 Structure of some fat-soluble vitamins. Vitamin E: Tocopherol (α , β , γ , and δ forms of tocopherol) and Tocotrienols (α , β , γ , and δ forms of tocotrienols); vitamin K: K1 (phylloquinone) and K2 (menaquinones—MK-4, MK-7)

biomembranes and viewed mechanistically, tocopherol is a biological antioxidant which provides for the stability of cell biomembranes in the organism (Bracco and Oral 2011; Traber and Stevens 2011). A close relationship between tocopherol and selenium in the lipid peroxide oxidation control has been ascertained, since selenium acts as a cofactor for glutathione peroxidase which inactivates lipid hydroperoxides. Tocopherol increases the biological activity of vitamin A by protecting the unsaturated side chain of this vitamin from peroxide oxidation. Probably, tocopherol and its derivatives are involved in other, yet to be discovered regulatory processes.

Deficiency in tocopherol

In adult humans, vitamin E hypovitaminosis has not been recorded. Occasionally, the hypovitaminosis symptom is observed in premature infants in which the vitamin deficiency leads to hemolytic anemia (because of a low stability of the erythrocyte membranes and their breakdown); in experimental animals, tocopherol deficiency shows up as a specific membrane pathology: the resistance of membranes to peroxide attack is reduced, and their increased permeability leads to a loss of intracellular components, for example, proteins which are normally incapable of passing across the membrane. The tissue membrane pathology under E hypovitaminosis may be presumed to be the cause of a diversity of clinical symptoms: susceptibility of erythrocytes to peroxide hemolysis; atrophy of the testes (conducive to male sterility); death of the embryo in pregnant females; muscular dystrophy and loss of intracellular nitrogenous components and muscle proteins; hepatic necrosis; and local encephalomalacia, especially cerebromalacia. Vitamin E deficiency can cause spinocerebellar ataxia (Traber and Atkinson 2007), myopathies (Brigelius-Flohé and Traber 1999), peripheral neuropathy, ataxia, skeletal myopathy, retinopathy, impairment of the immune response, etc. (Kowdley et al. 1992; Anonymous 2000), red blood cell destruction (Whitney and Sharon 2011).

Practical applications

Commercially available are preparations of synthetic D,L- α -tocopherol acetate in vegetable oil and concentrated oil extracts of tocopherol mixtures from wheat seedlings. Tocopherolic preparations are used as antioxidants to prevent the eventual hazard of excessive lipid peroxide accumulation; they are also used in the prophylaxy (preventive measures) of sterility and imminent abortion, liver diseases, muscular atrophy, in treating congenital disturbances of erythrocyte membranes in neonates, premature infants, etc.

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin F

Vitamin F, essential fatty acids

Source: Vitamin F consisted of essential fatty acids (EFAs), especially omega-3 and omega-6 fatty acids, and so they have to be derived only from food. Omega-3 and omega-6 fatty acids are found, respectively, in fish, canola oil, and walnut oil;

and in raw nuts, seeds, legumes, grape seed oil, and flaxseed oil; and also present in hemp seed, olive oil, soy oil, canola (rapeseed) oil, chia seeds, pumpkin seeds, sunflower seeds, leafy vegetables, walnuts, avocados, all kinds of sprouts, as well as meat, shellfish, salmon, trout, mackerel, and tuna. Vegetable oils may be a major source of vitamin F. The daily requirement in vitamin F for adult humans amounts to 5–10 g.

Chemical nature and biologically active forms of vitamin F

Vitamin F is a fat-soluble vitamin consisting of the unsaturated fatty acids, usually come in the form of liquid vegetable oils, while saturated fatty acids are found in animal fat. Vitamin F is the sum total of unsaturated fatty acids that cannot be synthesized in the tissues which are essential for the normal activity of the organism (Fig. 5.5). Only two fatty acids are known to be essential for humans: α -linoleic acid—ALA (an omega-3 fatty acid) and linoleic acid—LA (an omega-6 fatty acid) (Whitney and Rolfs 2008). Omega-6 fatty acid also includes γ -linoleic acid. They are polyunsaturated fatty acids (PUFA) and α -linolenic acid (ALA) is a carboxylic acid with an 18-carbon chain and three *cis* double bonds, while linoleic acid (LA) is a carboxylic acid with an 18-carbon chain with two double bonds in *cis* configuration.

Biochemical functions

Vitamin F is functionally involved in the production of prostaglandins that control metabolism. Vitamin F acts to maintain vitamin A reserve and to facilitate the vitamin A activity in the tissue metabolism. Vitamin F works in tandem with vitamin D in the body in making calcium available to the tissues, assisting in the assimilation of phosphorus, and stimulating the conversion of carotene into vitamin A. It is essential for the normal functioning of reproductive system; it nourishes skin

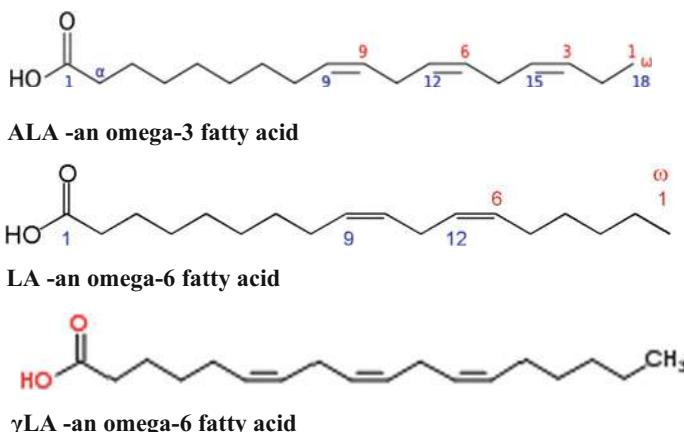


Fig. 5.5 Structure of vitamin F. α -linolenic acid-ALA—an omega-3 fatty acid and linoleic acid-LA—an omega-6 fatty acid

cells and is needed for healthy mucous membranes and nerves. Vitamin F reduces the cholesterol content in blood and thus helps lower risk of heart disease.

Deficiency of vitamin F

In humans, unequivocal deficiency symptoms have not been described. It is commonly believed that F hypovitaminosis is accompanied by follicular hyperkeratosis, i.e., excessive keratinization of skin epithelium around the hair follicles. These symptoms resemble the vitamin A deficiency symptoms. In animals, deficiency in vitamin F may lead to sterility.

Practical applications

Arachidonic acid is obviously an authentically essential fatty acid, which is the only one to eliminate all the deficiency symptoms. Of clinical use are the essential fatty acid preparations, xxlinetol and linol, chiefly in the prophylaxy (preventive measures) of cholesterol deposition in the vascular walls under atherosclerosis: they are also used in local treatment of skin diseases.

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin K

Source: Phylloquinones (K1) and their derivatives are found in plants (e.g., cabbage, spinach, also root crop and fruits) and animal (liver) products and are supplied to the organism in food, while menaquinones (K2) are produced by the small intestinal bacterioflora or are derived from naphthoquinone metabolism in the tissues of the organism. For adult humans, the daily requirement in vitamin K is about 2 mg.

Chemical nature and biologically active forms of vitamin K

Vitamin K is a quinone with an isoprenoid side chain and includes a group of compounds which are chemically 2-methyl-1,4-naphthoquinone (3-) derivatives. All K vitamins share a “quinone” ring, but differ in length, degree of saturation, and the number of side chains. This fat-soluble vitamin includes two naphthoquinone series such as phylloquinones (K1-series) and menaquinones (K2-series). Menaquinones (MQ or MK-*n*) include several related compounds, which are subdivided into the short chain (e.g., MK-4 with isoprene units) and the long chain (e.g., MK-7, MK-8, and MK-9 with 7, 8, 9 isoprene units, respectively) menaquinones depending on isoprenoid chain length. Synthetic preparations of vitamin K (menadione, vicasol, and synkayvite) are derivatives of 2-methyl-1,4-naphthoquinone. In the organism, they are converted into biologically active menaquinones. Menadione is an analog of 1,4-naphthoquinone with a methyl group in the 2-position, occasionally used as a nutritional supplement because of its vitamin K activity or as a provitamin because it is metabolized by the human body into K₂ (Fig. 5.6).

Biochemical functions

In the organism, vitamin K controls the blood coagulation via participation in the buildup of the several blood clotting system components such as factor II

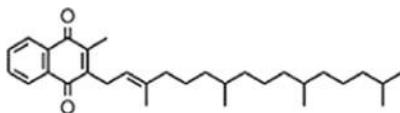
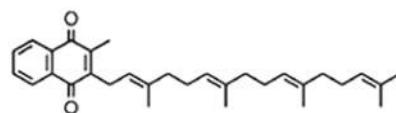
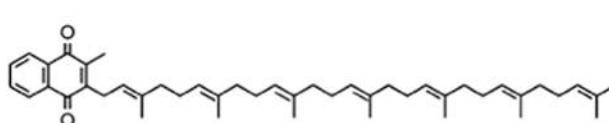
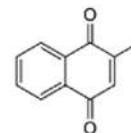
**Vitamin K1 (Phylloquinone)****Vitamin K2 (MK-4)****Vitamin K2 (MK-7)****Menadione**

Fig. 5.6 Structure of some fat-soluble vitamins. Vitamin K: K1 (phylloquinone), K2 (menaquinones—MK-4, MK-7), and menadione

(prothrombin), factor VII (proconvertin), factor IX (Christmas factor), and factor X (Stewart factor). Vitamin K is involved in the conversion of a prothrombin precursor, called preprothrombin, into prothrombin. This process takes place in the liver. Vitamin K stimulates the γ -carboxylation of glutamic acid residues in the prothrombin molecule by activating the microsomal carboxylase. The prothrombin thus formed becomes bound to phospholipids through Ca^{2+} ions and is subjected to enzymic cleavage to produce thrombin. The latter triggers the blood coagulation system to generate a fibrin clot.

Deficiency in vitamin K

The symptom of vitamin K deficiency is a distinct predisposition to hemorrhagic disease, especially manifest in traumas. In adult humans, the intestinal flora provides for a complete supply of the organism with vitamin K. In infants (with as yet not fully developed intestinal flora), the cause of K hypovitaminosis may be an alimentary deficit of vitamin K. Major reasons for K hypovitaminosis are suppression of the intestinal flora by drugs and diseases of liver and gallbladder leading to a diminished production of bile acids (which are needed for the vitamin uptake). Moreover, the liver is a producer of the active forms of vitamin K and is involved in the synthesis of a number of blood coagulation factors and in preprothrombin-to-thrombin conversion.

Practical applications

In medical practice, preparations of vitamin K1 or its synthetic analog vicasol are used. They are applied in the treatment of hemorrhagic disease or hemophilic bleeding. Vitamin K₂ (menaquinone) has been shown to safely *suppress* growth and invasion of human hepatocellular carcinoma, a common and deadly form of liver cancer. It exerts multiple effects on these tumors, modifying growth factors and their receptor molecules in a way that makes them less able to stimulate tumor

growth and progression. It freezes the cell cycle, blocking further replication. And it triggers programmed cell death by apoptosis through several distinctive mechanisms. Three of vitamin K's synergistic anticancer mechanisms have recently been identified. Vitamin K₃ inhibits DNA-building enzymes. Vitamins K₂ and K₃ block new blood vessel formation essential to support the rapid growth of tumor tissue. And vitamin K₃ disrupts crucial intracellular communications networks composed of microtubules, preventing the cells from proliferating in a coordinated fashion. Table 5.2 gives data on sources and functions of fat-soluble vitamins in summary.

Table 5.2 Data on sources and functions of fat-soluble vitamins in summary

Fat-soluble vitamins		
Name	Sources	Function
Vitamin A and its precursor, beta-carotene	Vitamin A from animal sources (retinol): fortified milk, cheese, cream, butter, fortified margarine, eggs, liver Beta-carotene: Leafy, dark green vegetables; dark orange fruits (apricots, cantaloupe) and vegetables (carrots, winter squash, sweet potatoes, pumpkin)	Needed for vision, healthy skin and mucous membranes, bone and toothgrowth, immune system health
Vitamin D	Egg yolks, liver, fatty fish, fortified milk, and fortified margarine. When exposed to sunlight, the skin can make vitamin D	Needed for proper absorption of calcium; stored in bones
Vitamin E	Polyunsaturated plant oils (soybean, corn, cottonseed, safflower); leafy green vegetables; wheat germ; whole-grain products; liver; egg yolks; nuts and seeds	Antioxidant; protects cell walls
Vitamin F	Omega-3 and omega-6 fatty acids are found, respectively, in fish, canola oil and walnut oil; and in raw nuts, seeds, legumes, grape seed oil, and flaxseed oil	Involved in the production of prostaglandins, maintains vitamin A reserve, facilitate the vitamin A activity, works in tandem with vitamin D in the body in making calcium available to the tissues, assisting in the assimilation of phosphorus, it nourishes skin cells and reduces the cholesterol content in blood and thus helps slower risk of heart disease
Vitamin K	Leafy green vegetables and vegetables in the cabbage family; milk; also produced in intestinal tract by bacteria	Needed for proper blood clotting

Fat-soluble vitaminoids

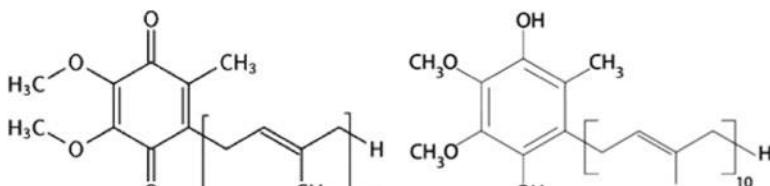
Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitaminoid Ubiquinone

Ubiquinone, Coenzyme Q (CoQ), and Coenzyme Q₁₀ (CoQ₁₀)

Source: A natural antioxidant synthesized by the body cells is found in many foods and available as a supplement. CoQ₁₀ is naturally found in high levels in spinach, broccoli, and cauliflower, beef, sardines, mackerel, etc., especially in the organs with the highest energy requirements such as heart, liver, and kidney (Okamoto et al. 1989; Aberg et al. 1992; Shindo et al. 1994). Fruits and berries are poor in CoQ₁₀, but avocados have a relatively high CoQ₁₀ content (Pravst et al. 2010). It comes in two forms: ubiquinol, the reduced antioxidant form, and ubiquinone, the oxidized form. Ubiquinone is present in different foods such as beef (shoulder), beef (liver, kidney), pork (shoulder), pork (thigh), chicken (breast), mackerel, tuna (canned), yellowtail, broccoli, parsley, and orange along with a varying amounts of ubiquinol that accounted for 46% of the total CoQ₁₀ intake in the Japanese diet (Hosoe et al. 2007; Kubo et al. 2008). Ubiquinol is not essential as the body can synthesize it and not classed as a vitamin (Banerjee 2007), but it is vitamin-like and a coenzyme of the respiratory chain. CoQ₁₀ levels decrease with age and diseases like cancer, certain genetic disorders, diabetes, heart conditions, HIV/AIDS, muscular dystrophies, Parkinson's disease, etc., and also may lower its level. Recommend 50–100 mg and from 100 to 200 up to 300 mg daily for people under 60 and over 60 or on a statin drug, respectively.

Chemical nature, biologically active forms

Ubiquinone is a coenzyme, synthesized in the human tissues from mevalonic acid and metabolites of phenylalanine and tyrosine. It is ubiquitous in animals and most bacteria (hence, the name ubiquinone). Ubiquinone is a 1,4-benzoquinone, where Q refers to the quinone chemical group and 10 refers to the number of isoprenyl chemical subunits in its tail. There are three redox states of CoQ₁₀: fully oxidized (ubiquinone), semiquinone (ubisemiquinone), and fully reduced (ubiquinol) (Fig. 5.7). No symptoms of ubiquinone deficiency in humans have been reported. The requirement in ubiquinone for humans is unknown.



Coenzyme Q₁₀ or ubiquinone (fully oxidized) Ubiquinol (fully reduced)

Fig. 5.7 Showing the structure of Coenzyme Q₁₀ or ubiquinone (fully oxidized) Ubiquinol (fully reduced)

Coenzyme Q₁₀ exists in three redox states, fully oxidized (ubiquinone), partially reduced (semiquinone or ubisemiquinone), and fully reduced (ubiquinol). Complete reduction of ubiquinone to ubiquinol leads to the conversion of two ketone groups (fully oxidized state) into hydroxyl groups (fully reduced state) on the active portion of the molecule causing an increase in the polarity of the CoQ₁₀ molecule and may be a significant factor behind the observed enhanced bioavailability of ubiquinol.

Biochemical functions

This fat-soluble substance, which resembles a vitamin, is present in all respiring eukaryotic cells, primarily in the mitochondria as a component of the electron transport chain of aerobic respiration that generates energy in the form of ATP and human organs with the highest energy requirements such as the heart, liver, and kidney have the highest CoQ₁₀ concentrations (Okamoto et al. 1989; Aberg et al. 1992; Shindo et al. 1994). The redox functions of ubiquinol in cellular energy production and antioxidant protection are based on the ability to exchange two electrons in a redox cycle between ubiquinol (reduced) and the ubiquinone (oxidized) form (Mellors and Tappel 1966a, b). Body cell makes CoQ₁₀ and uses it to produce energy and it also functions as an antioxidant. It assists in maintaining the normal oxidative state of LDL cholesterol, helps assure circulatory health, and supports optimal functioning of the heart muscle; and may also help support the health of vessel walls, may play a role in reducing the number and severity of migraine headaches, and improve sperm motility in men and many neural diseases including Parkinson's disease.

Q10 deficiency

Studies in both animals and humans have associated significantly decreased levels of CoQ10 with a wide variety of diseases. Since this enzyme is found in high concentration in heart muscle cells, deficiency has been associated with cardiovascular problems including angina, arrhythmia, heart failure, and high blood pressure. Problems with blood sugar regulation, gingival (gum) health, and stomach ulcers have also been associated with CoQ10 deficiency.

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical applications of water-soluble vitamins, and vitaminoids: B₁, B₂, B₃, B₇, B₉, B₁₂ (B complex), and C

Water-soluble vitamins are vitamin B₁ (thiamin), vitamin B₂ (riboflavin), vitamin B₃ (niacin), vitamin B₅ (pantothenic acid), vitamin B₆ (pyridoxine), vitamin B₇ (biotin), vitamin B₉ (folic acid), vitamin B₁₂ (cobalamin), vitamin C (ascorbic acid), etc. All eight vitamins of B groups (B₁, B₂, B₃, B₅, B₆, B₇, B₉, and B₁₂) are collectively referred to as a vitamin B complex. Structurally, they show a very wide diversity and each B vitamin is either a cofactor (generally a coenzyme for key metabolic processes or is a precursor needed to make one. Vitamin C is a cofactor in many enzymatic activities, and it can also act as a strong antioxidant (a strong

reducing agent) against oxidative stress. Vitamin C is purely the L-enantiomer of ascorbate; the D-enantiomer has no physiological significance. The majority of water-soluble vitamins, supplied in food or synthesized by the intestinal bacterial flora, exhibit a biological activity with the corresponding metabolically preformed coenzymes.

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin B₁

Source: Vitamin B₁ or thiamine is an essential nutrient for all organisms, but it is synthesized only in bacteria, fungi, and plants. Vitamin B₁ is derived from dietary sources like whole grains, meat, and fish is abundant in coarse bread, pea, beans, pineapple, asparagus, cabbage, carrot, celery, grapefruit, coconut, lemon, parsley, pomegranate, radish, watercress, turnip leaf, and meat products but not polished rice. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system. The daily requirement in thiamine for adult humans is about 1–3 mg.

Chemical nature and biologically active forms of vitamin B₁

Vitamin B₁ or thiamine, a colorless organosulfur compound, is structurally consisted of an aminopyrimidine and a thiazole ring linked by a methylene bridge. The thiazole is substituted with methyl and hydroxyethyl side chains. It is soluble in water and organic solvents like methanol, and glycerol, stable at acidic pH, unstable in alkaline solutions (Tanphaichitr 1999; Mahan and Escott-Stump 2000), unstable to heat, stable during frozen storage and unstable to ultraviolet light (Tanphaichitr 1999), and gamma irradiation exposure. Thiamine is an *N*-heterocyclic carbene and can be used in place of cyanide as a catalyst for benzoin condensation. Thiamine reacts strongly in Maillard-type reactions (Mahan and Escott-Stump 2000). The salt thiamine mononitrate, rather than thiamine hydrochloride, is used for food fortification, as the mononitrate is more stable and does not absorb water from natural humidity (is nonhygroscopic), whereas thiamine hydrochloride is hygroscopic. Its phosphate derivatives are involved in many cellular processes, e.g., thiamine pyrophosphate (TPP), a coenzyme in the catabolism of sugars and amino acids. There are five known natural thiamine phosphate derivatives as thiamine monophosphate (ThMP), thiamine diphosphate (ThDP), also sometimes called thiamine pyrophosphate (TPP), thiamine triphosphate (ThTP), and the recently discovered adenosine thiamine triphosphate (AThTP), and adenosine thiamine diphosphate (AThDP). While the coenzyme role of thiamine diphosphate is well-known and extensively characterized, the non-coenzyme action of thiamine and derivatives may be realized through binding to a number of recently identified proteins which do not use the catalytic action of thiamine diphosphate. Figure 5.8 shows structures of water-soluble vitamins—vitamin B₁ (thiamin) and derivative.

Biochemical functions

Participation of thiamine in the tissue metabolism regulation is defined by thiamine diphosphate (TOP) which makes part of pyruvate dehydrogenase or 2-oxoglutarate dehydrogenase complexes and of transketolase. Owing to this, TOP facilitates the

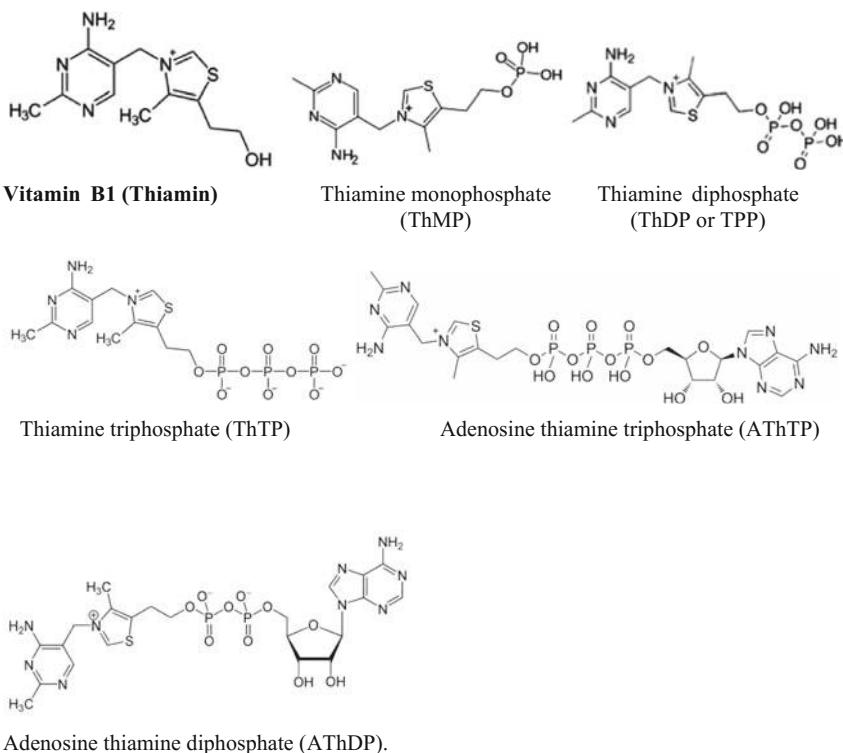


Fig. 5.8 Showing the structure of water-soluble vitamins—vitamin B₁(thiamin) and derivative

mitochondrial oxidation of pyruvate and 2-oxoglutarato and consequently, the energy generation from carbohydrates and amino acids. Transketolase is known to ensure the activity of the nonoxidative stage in the pentose phosphate cycle which is a major source of NADP-H, and the only source of ribose 5-phosphate in the cells. Thiamine is involved in other, even noncoenzymic, functions. In particular, thiamine triphosphate which is present in noticeable amounts in the nerve cells is implicated, directly or indirectly, in the synaptic transmission of nervous impulses.

Thiamine deficiency

The deficiency in thiamine is commonly encountered in places where people consume polished rice containing but traces of thiamine. Thiamine deficiency can prove fatal and in less severe cases, deficiency is manifested by a sudden loss of appetite, reduced secretion of gastric juice and hydrochloric acid, atony (lack of normal tone or strength), diarrhea, malaise, weight loss, irritability, and confusion. The characteristic sign is the sharp atrophy of muscular tissue, with ensuing contractile reduction of skeletal muscles (distinct myasthenia or muscular debility), heart (reduced cardiac contractility, dilatation of the right heart, tachycardia, acute cardiac insufficiency), and smooth muscles (reduced muscular tension of intestinal

smooth muscles). Well-known syndromes caused by thiamine deficiency include beriberi, Wernicke–Korsakoff syndrome, and optic neuropathy. Beriberi is manifested in metabolism disturbances and impaired functions of digestive, cardiovascular, and nervous systems. Disturbances of the nervous system show up in a gradual decrease of peripheral sensibility, lack of certain peripheral reflexes, paroxysmal pain extending along the course of nerves (neuralgia), impaired higher nervous activity (phobia, mental depression), and convulsions.

Practical applications

A variety of medicinal forms based on free thiamine and thiamine diphosphate (cocarboxylase) are used in medicine. In the blood, thiamine diphosphate is liable to hydrolysis, and at present it remains unclear whether it is precisely this enzyme form that is supplied to the cells, or it subserves only as a source of free thiamine. The thiamine-based preparations are used to facilitate carbohydrate assimilation in diabetes mellitus, in hypovitaminosis, in dystrophies of heart and skeletal muscles, in inflammation of peripheral nerves, and in treating the affected nervous system (including alcoholism).

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin B₂

Sources: The riboflavin sources for humans are dietary products and, in part, intestinal bacterioflora. Food and beverages that provide riboflavin without fortification are milk, cheese, curd, egg yolk, liver, kidney, mushrooms, and almonds, as well as grapefruit, apple, apricot, cabbage, carrot, coconut, dandelion, prune, spinach, turnip leaf, watercress, legumes, etc. Animal and dairy products are rich in riboflavin but less abundant in vegetable products. Milk, milk products, and foods of animal origin contain high amounts of (free) riboflavin with good bioavailability. In foods of plant origin, the majority of riboflavin is protein-bound and therefore less bioavailable. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system. The recommended daily intake of riboflavin for the adult human is 1–3 mg.

Chemical nature, biologically active forms, biochemical functions

It is required by the body for cellular respiration. Vitamin B₂ or riboflavin is chemically defined as 7,8-dimethyl-10-(1'Y-D-ribityl)isoalloxazine. Figure 5.9 shows the oxidized and reduced form of the vitamin. The ending “flavin” (from the Latin word *flavus* = yellow) refers to its yellowish color. Riboflavin is a yellow-orange solid substance with poor solubility in water compared to other B vitamins. Visually, it imparts color to vitamin supplements (and bright yellow color to the urine of persons taking a lot of it). Riboflavin functions as a coenzyme, meaning that it is required for enzymes (proteins) to perform normal physiological actions. Specifically, the active forms of riboflavin flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) function as cofactors for a variety of flavoprotein enzyme reactions (Fig. 5.9).

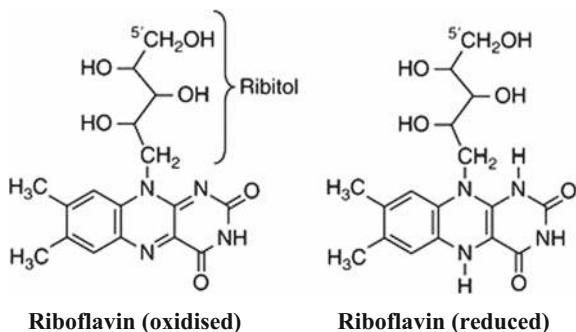


Fig. 5.9 Showing the structure of water-soluble vitamins—riboflavin (oxidized) and riboflavin (reduced)

Riboflavin is heat stable in the absence of light but extremely photosensitive. It has a high degree of natural fluorescence when excited by UV light. This property can be used for detection and determination. Two coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are derived from riboflavin (Fig. 5.10).

Biochemical functions

Flavin coenzymes take part in numerous reactions of substrate-linked oxidation in the cells, i.e., transfer of electrons and protons in the respiratory chain, mitochondrial oxidation of pyruvate, succinate, 2-oxoglutarate, α -glycerol phosphate, fatty acids, oxidation of biogenic amines, aldehydes, etc.

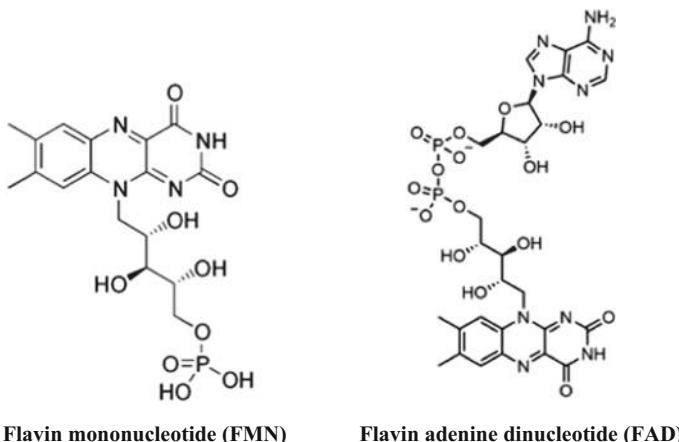


Fig. 5.10 Showing structure of water-soluble vitamins— B_2 (riboflavin) and derivative coenzymes—flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)

Deficiency in riboflavin

Riboflavin deficiency (also called ariboflavinosis) results in stomatitis including painful red tongue with sore throat, chapped and fissured lips (cheilosis), and inflammation of the corners of the mouth (angular stomatitis). There can be oily scaly skin rashes on the scrotum, vulva, philtrum of the lip, or the nasolabial folds. The eyes can become itchy, watery, bloodshot, and sensitive to light (Sebrell and Butler 1939). Riboflavin deficiency is manifested by lowered concentrations of coenzymic riboflavin forms, primarily FMN, in the tissues; the relevant symptoms are also lesions of the epithelium of cutaneous mucosa and cornea. The dryness of labial mucosa and oral cavity is observed. The labial mucosa is of red color, and the lips and the angles of the mouth are often cracked. The skin, especially facial, is desquamative because of a reduced epithelium renewal. The conjunctiva is dry and inflamed, the cornea is vascularized and prone to keratoleucoma, and the patients suffer from photophobia. Since riboflavin participates in oxidative processes, many of which are energy-generating, one will have easily perceived the reason because of which the vitamin deficiency affects mainly the regenerative tissues. The vascularization facilitates the oxygen supply to the central, nonvascular zone of the cornea, to compensate for the insufficiency of corneal respiratory function, produced by a deficit of flavoproteins involved in the redox processes.

Practical applications

Riboflavin is added to baby foods, breakfast cereals, pastas, and vitamin-enriched meal replacement products. It is difficult to incorporate riboflavin into liquid products because it has poor solubility in water, hence the requirement for riboflavin-5'-phosphate, a more soluble form of riboflavin. Riboflavin is also used as a food coloring. Riboflavin, coenzymic FMN and FAD (Flavin mono- and dinucleotide), preparations are used in medical practice in a variety of formulations. They are used clinically in treating hyporiboflavinosis and in the medication of skin and eye diseases caused not by riboflavin deficiency but rather by an excessive riboflavin requirement: in treating dermatitides (skin inflammation), poorly healable wounds and ulcers, keratitis (inflammation of the cornea), and conjunctivitis (inflammation of the conjunctiva). In addition, they are applied in treating intoxication by respiratory poisons (carbon monoxide CO), in the medication of affected liver, for muscular alleviation after prolonged physical exertion, etc.

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin B₃ Niacin

Source: Vitamin B₃, niacin or nicotinic acid, is one of the 20–80 essential human nutrients. Niacin naturally occurs as nicotinic acid and nicotinamide; these are nutritionally equivalent and are supplied to the human organism in food. The sources of alimentary niacin are meat products (especially liver) and many vegetable products. In milk and egg yolk, niacin is contained in trace amounts. Niacin is found in a variety of whole and processed foods, including fortified packaged

foods, meat from various animal sources, seafoods, and spices. Fortified whole-grain flours, such as from wheat, rice, barley or corn, and pasta, have niacin contents in a range of 3–10 mg per 100 grams. However, in distinction from other vitamins, niacin can be synthesized in the human organism from tryptophan; therefore, niacin should not be regarded as an essential food component if tryptophan is not in short supply. For this reason, the foodstuffs that are poor in niacin but rich in tryptophan (for example, milk and egg yolk) replenish a deficit of this vitamin in the organism. The daily requirement in niacin is dependent on tryptophan consumption. Niacin is provided in the diet from a variety of whole and processed foods, with highest contents in fortified packaged foods and meat from various animal sources. The recommended intake for the adult human is about 25 mg.

Chemical nature, biologically active forms

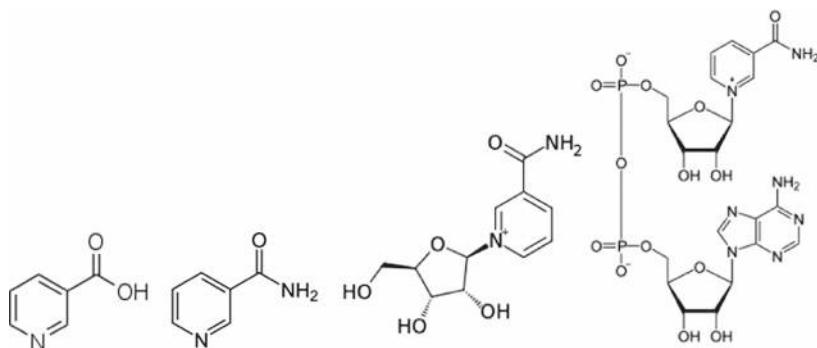
Vitamin B₃ or niacin is an organic compound called nicotinic acid or pyridine carboxylic acids. It is solid, colorless, and water-soluble solid and is a derivative of pyridine with a carboxyl group (COOH) at the 3-position (pyridine-3-carboxylic acid). Together with nicotinamide, it makes up the group known as vitamin B₃ complex, which is a family of vitamins that includes nicotinamide (niacinamide), niacin (nicotinic acid), and nicotinamide riboside (Krutmann and Humbert 2010). Niacin and nicotinamides are precursors of the dehydrogenase coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). NAD is important in catabolism of fat, carbohydrate, protein, and alcohol, as well as cell signaling and DNA repair, and NADP mostly in anabolism reactions such as fatty acid and cholesterol synthesis (Wan et al. 2011). Figure 5.11 shows the structure of water-soluble vitamins—vitamin B₃ (niacin) and derivatives, nicotinamide riboside, nicotinamide adenine dinucleotide (NAD), and nicotinamide adenine dinucleotide phosphate (NADP).

Biochemical functions

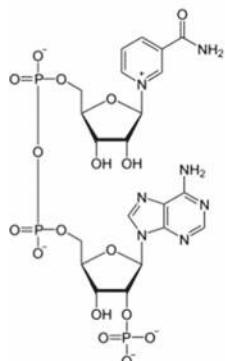
It is a water-soluble vitamin belonging to the vitamin B family, which occurs in many animal and plant tissues, with antihyperlipidemic activity. Niacin is sometimes used in addition to other lipid-lowering medication. Niacin alone appears to reduce the rate of cardiovascular events as well as coronary or cardiovascular deaths (Bruckert et al. 2010; Duggal et al. 2010). Function of niacin the coenzymic forms such as NAD and NADP may be divided into (i) function associated with hydrogen transfer in redox reactions; (ii) function as that of a substrate for synthetic reactions; and (iii) regulatory function, in the capacity of an allosteric effector.

Deficiency in niacin

Mild niacin deficiency has been shown to slow metabolism, causing decreased tolerance to cold. Severe deficiency of niacin in the diet causes the disease pellagra, which is characterized by diarrhea, dermatitis, and dementia, as well as Casal's necklace lesions on the lower neck, hyperpigmentation, thickening of the skin, inflammation of the mouth and tongue, digestive disturbances, amnesia, delirium, and eventually death, if left untreated; psychiatric symptoms of niacin deficiency



B3(Niacin) Nicotinamide Nicotinamide riboside Nicotinamide adenine dinucleotide (NAD)



Nicotinamide adenine dinucleotide phosphate (NADP)

Fig. 5.11 Showing structure of water-soluble vitamins—vitamin B₃ (niacin) and derivatives, nicotinamide riboside, nicotinamide adenine dinucleotide (NAD), and nicotinamide adenine dinucleotide phosphate (NADP)

include irritability, poor concentration, anxiety, fatigue, restlessness, apathy, and depression; and the Hartnup disease is a hereditary nutritional disorder resulting in niacin deficiency (Prakash et al. 2008). Absorption of dietary tryptophan is impaired due to niacin deficiency. Most commonly, niacin hypovitaminosis is accompanied by riboflavin and pyridoxine hypovitaminosis, since riboflavin and pyridoxine coenzymes are needed for the production of nicotinic acid from tryptophan.

Practical applications

Nicotinic acid and nicotinamide as well as other compounds structurally incorporating nicotinic acid are used in medical practice. Apparently, NAD and NADP should not be recommended for use as coenzymic preparations because of their low

plasmic membrane permeability. Any other, i.e., noncoenzymic, functions of NAD and NADP are as yet unknown. Nicotinamide and nicotinic acid are used in treating pellagra as well as dermatitides evoked by other causes, in the therapy of affected peripheral nerves, cardiac muscle dystrophy, etc. In addition, nicotinic acid exerts a vasodilative action, which is used for clinical purposes. This property of nicotinic acid bears no relation to its biochemical functions.

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin B₅ pantothenic acid

Source: The sources of Vitamin B₅ or pantothenic acid for the human organism are intestinal bacteria and foodstuffs like yeast, liver, hen eggs, fish, meat, milk, leguminous plants, etc. Small quantities of pantothenic acid are found in nearly every food, with high amounts in fortified whole-grain cereals, egg yolks, liver, and dried mushrooms. It is a water-soluble vitamin and is an essential nutrient as required for the synthesis of coenzyme A (CoA). The adult human requires about 10 g of this vitamin daily.

Chemical nature, biologically active forms

Vitamin B₅ or pantothenic acid is a water-soluble vitamin that is an essential nutrient as cell requires pantothenic acid to synthesize coenzyme A (CoA). Pantothenate is anionic form of the vitamin B₅. In chemical structure, pantothenic acid is the amide between D-pantoic acid and β-alanine. It is the beta-alanine derivative of pantoic acid (Bender and Bender 2005), with the chemical formula C₉H₁₇NO₅, a light-yellow, water-soluble, viscous compound. It is commonly found as its alcohol analog, the provitamin panthenol (panthenol), and as calcium pantothenate. It has two isomers: D and L. Only the dextrorotatory (D) isomer of pantothenic acid possesses biologic activity (Gregory 2011) and the levorotatory (L) form may antagonize the effects of the dextrorotatory isomer (Kimura et al. 1980). Coenzyme A is synthesized from pantothenate and others such as cysteine, and four molecules of ATP (Matthew et al. 2002) in five steps (Leonardi et al. 2005) (Fig. 5.12).

Biochemical functions

The importance of pantothenic acid is defined by the participation of its coenzyme A in biochemical reactions. CoA is a major enzyme in the cells. Pantothenic acid in the form of CoA is also required for acylation and acetylation, which, for example, are involved in signal transduction and enzyme activation and deactivation, respectively (Gropper et al. 2009). Pantothenic acid is critical in the metabolism and synthesis of carbohydrates, proteins, fats, etc. In addition, pantothenic acid is important in antibody formation, conversion of cholesterol to hormones that deal with stress, production of red blood cells, and production of the neurotransmitter acetylcholine. 4-Phosphopantetheine is a coenzyme for the acyl-transferring protein of fatty acid synthetase; dephospho-CoA is a coenzyme for citrate lyase and acts, in part, as a coenzyme in numerous reactions of acyl conversion.

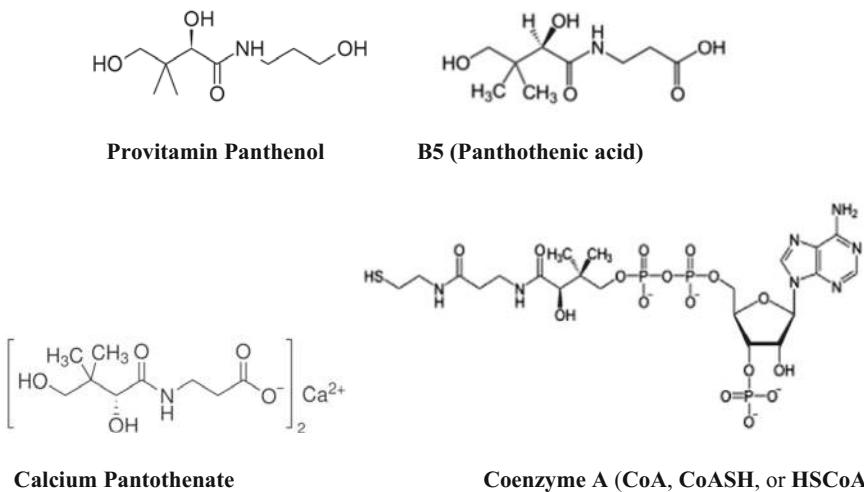


Fig. 5.12 Showing structure of water-soluble vitamins—vitamin (pantothenic acid) and coenzymes

Deficiency in pantothenate

Since pantothenic acid participates in a wide array of key biological roles, it is essential to all forms of life and as such, deficiencies in pantothenic acid may have numerous wide-ranging effects. Symptoms of deficiency are similar to other vitamin B deficiencies. There is impaired energy production, due to low CoA levels, which could cause symptoms of irritability, fatigue, and apathy (Gropper et al. 2009). Acetylcholine synthesis is also impaired; therefore, neurological symptoms can also appear in deficiency including numbness, paresthesia, and muscle cramps (Otten et al. 2008). Deficiency in pantothenic acid can also cause hypoglycemia, or an increased sensitivity to insulin (Gropper et al. 2009). Insulin receptors are acylated with palmitic acid when they do not want to bind with insulin (Trumbo 2006). Therefore, more insulin will bind to receptors when acylation decreases, causing hypoglycemia (Voet et al. 2006). Additional symptoms could include restlessness, malaise, sleep disturbances, nausea, vomiting, and abdominal cramps (Otten et al. 2008). In a few rare circumstances, more serious (but reversible) conditions have been seen, such as adrenal insufficiency and hepatic encephalopathy. Deficiency symptoms in other nonruminant animals include disorders of the nervous, gastrointestinal, immune systems, reduced growth rate, decreased food intake, skin lesions and changes in hair coat, and alterations in lipid and carbohydrate metabolism.

Practical applications

In practical medicine, calcium pantothenate, pantetheine, and CoA preparations are used in a variety of pharmacologic formulations. Most commonly, they are used in treating skin and hair diseases, as well as in the medication of affected liver, cardiac muscle dystrophy, etc. Some formulations are also used in perfumery.

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin B₆ pyridoxine

Source: The sources of vitamin B₆ (pyridoxine, pyridoxal, and pyridoxamine) for humans are intestinal bacteria and food. Vitamin B₆ is part of the vitamin B group of essential nutrients. Cereal and leguminous plants, meat, and fish are rich in vitamin B₆. The recommended daily intake for the adult human is 2–3 mg.

Chemical nature and biologically active forms

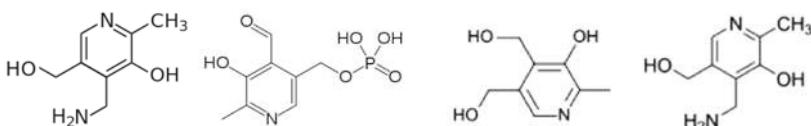
Vitamin B₆ refers to a group of chemically similar compounds which can be interconverted in biological systems. Several forms (vitamers) of vitamin B₆ are known such as pyridoxine (PN), the form most commonly given as vitamin B₆ supplement, pyridoxine 5'-phosphate (P5P), pyridoxal (PL), pyridoxal 5'-phosphate (PLP), the metabolically active form (sold as P-5-P vitamin supplement), pyridoxamine (PM), pyridoxamine 5'-phosphate (PMP), 4-pyridoxic acid (PA), the catabolite which is excreted in urine, pyritinol, a semisynthetic derivative of pyridoxine, where two pyridoxine moieties are bound by a disulfide bridge, etc. (Fig. 5.13).

All forms except pyridoxic acid and pyritinol can be interconverted. Absorbed pyridoxamine is converted to PMP by pyridoxal kinase, which is further converted to PLP by pyridoxamine phosphate transaminase or pyridoxine 5'-phosphate oxidase which also catalyzes the conversion of PNP to PLP. Pyridoxine 5'-phosphate oxidase is dependent on flavin mononucleotide (FMN) as a cofactor which is produced from riboflavin (vitamin B₂), i.e., in this biochemical pathway, dietary vitamin B₆ cannot be used without vitamin B₂.

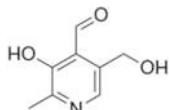
Its active form, pyridoxal 5'-phosphate, serves as a coenzyme in some 100 enzyme reactions in amino acid, glucose, and lipid metabolism.

Biochemical functions

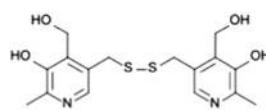
PLP, the metabolically active form of vitamin B₆, is involved in many aspects of macronutrient metabolism, neurotransmitter synthesis, histamine synthesis, hemoglobin synthesis and function, and gene expression. PLP generally serves as a



B6 (Pyridoxine) Pyridoxal 5'-phosphate (PLP/P5P) Pyridoxine (PN) Pyridoxamine (PM)



Pyridoxal (PL)



Pyritinol

Fig. 5.13 Showing structure of water-soluble vitamins—vitamin B₆ (pyridoxine), pyridoxal 5'-phosphate (PLP/P5P), pyridoxine (PN), pyridoxamine (PM), pyridoxal (PL), and pyritinol

coenzyme (cofactor) for many reactions including decarboxylation, transamination, racemization, elimination, replacement, and beta-group interconversion. The liver is the site for vitamin B₆ metabolism. In the organism tissues, the major coenzymic form of vitamin B₆ is pyridoxal 5-phosphate. It makes part of the enzymes of nearly all classes including oxide reductases, transferases, hydrolases, lyases, and isomerases.

Deficiency in pyridoxine

Pyridoxine deficiency has been described in children. It is concomitant with hyperexcitability of the central nervous system and recurrent convulsions, which presumably can be attributed to an insufficient production of γ -aminobutyric acid, which is the inhibition mediator for cerebral neurons. In adult humans, pyridoxine deficiency symptoms have been observed under a long-term therapy with the tuberculostatic isoniazid which is an antagonist to pyridoxal. This indisposition is also attended by hyperexcitability of the nervous system, polyneuritides, and skin lesions, i.e., the characteristic signs of niacin deficiency. The classic clinical syndrome for vitamin B₆ deficiency is a seborrhoeic dermatitis-like eruption, atrophic glossitis with ulceration, angular cheilitis, conjunctivitis, intertrigo, and neurologic symptoms of somnolence, confusion, and neuropathy (due to impaired sphingosine synthesis) and sideroblastic anemia (due to impaired heme synthesis) (William et al. 2006).

Less severe cases present with metabolic disease associated with insufficient activities of the coenzyme PLP. The most prominent of the lesions is due to impaired tryptophan–niacin conversion. This can be detected based on urinary excretion of xanthurenic acid after an oral tryptophan load. Vitamin B₆ deficiency can also result in impaired transsulfuration of methionine to cysteine. The PLP-dependent transaminases and glycogen phosphorylase provide the vitamin with its role in gluconeogenesis, so deprivation of vitamin B₆ results in impaired glucose tolerance (Combs 2008).

Practical applications

Clinically, pyridoxine is applied in a variety of medicinal forms; of late, its coenzyme, pyridoxal phosphate, has gained acceptance. These agents are used in medication of B₆ hypovitaminosis, in prophylaxy and therapy of isoniazid side effects, in treatment of polyneuritides, dermatitides, gestational toxicosis (assistance in biogenic amine detoxification), impaired hepatic function, congenital pyridoxine-dependent anemia in children, etc.

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin B₉, B_C, vitamin M, or Folacin

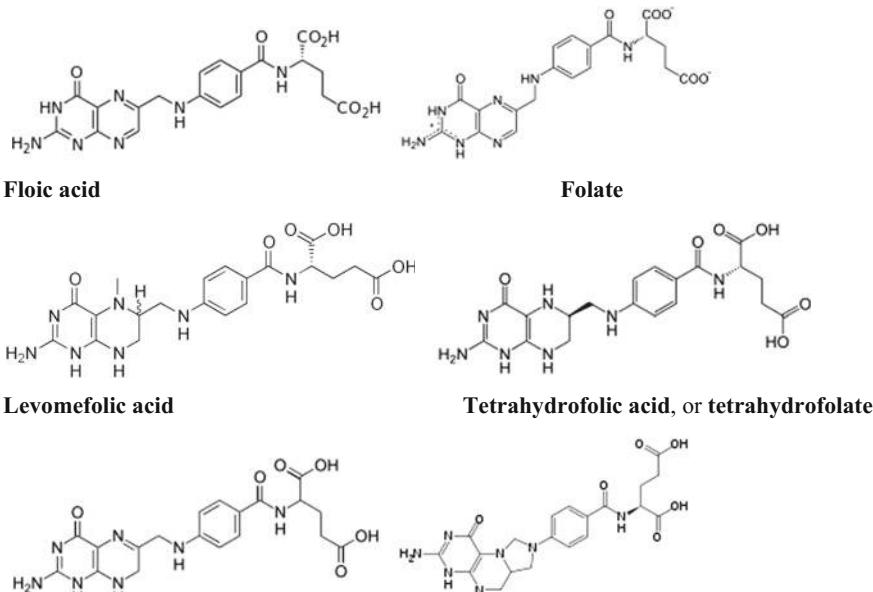
Source: The term “folic” is from the Latin word *folium*, which means leaf (Anonymous 2004). Folates occur naturally in many foods especially dark green leafy vegetables and liver. Folate naturally occurs in a wide variety of foods,

including vegetables (particularly dark green leafy vegetables), fruits and fruit juices, nuts, beans, peas, dairy products, poultry and meat, eggs, seafood, grains, and some beers. Avocado, beetroot, spinach, liver, yeast, asparagus, and Brussels sprouts are among the foods with the highest levels of folate. Folate naturally found in food is susceptible to high heat and ultraviolet light, and is soluble in water. It is heat labile in acidic environments and may also be subject to oxidation. Folic acid is added to grain products in many countries, and these fortified products make up a significant source of the population's folate intake (Dietrich et al. 2005).

Food is the major source of folacin (B_9). Folacin is abundant in the foodstuffs of vegetable origin (lettuce, cabbage, tomato, strawberry, and spinach) and animal origin (liver, meat, and egg yolk). The recommended daily intake for the adult human is about 400 μg , and twice as large for pregnant human females.

Chemical nature, biologically active forms

Vitamin B_9 includes folic acid, folinic acid, levomefolic acid, or 5-methyltetrahydrofolate (Fig. 5.14). Folic acid or vitamin B_c , a member of the B -complex group of vitamins, is synthesized by microorganisms in the mammalian gut and also required in the normal diet. Folic acid is involved in the synthesis of nucleic acids as well as red blood cells, and a deficiency causes reduced growth and anemia. Folacin and folate are the forms of the vitamin B_9 that are found naturally



Dihydrofolic acid (DHF) is a folic acid derivative 5,10-Methylenetetrahydrofolate

Fig. 5.14 Showing structure of water-soluble vitamin B_9 —floic acid and derivatives folat, levomefolic acid, tetrahydrofolic acid, or tetrahydrofolate, dihydrofolic acid (DHF), 5,10-methylenetetrahydrofolate

in food, while folic acid is the synthetic form that is found in vitamin supplement. Folic acid is very stable and gets converted into folate in the body. Folate is the term used to name the many forms of the vitamin, viz., folic acid and its congeners, including tetrahydrofolic acid, methyltetrahydrofolate, methenyltetrahydrofolate, and folinic acid. Other names include vitamin B₉, vitamin B_c, vitamin M (Darby and Jones 1945), folacin, and pteroyl-L-glutamate (Darby and Jones 1945; Anonymous 2005; Fenech 2012). Tetrahydrofolic acid is a cofactor in many reactions, especially in the synthesis (or anabolism) of amino acids and nucleic acids. It is water-soluble and most of it gets stored in the liver, which contains around half the body's total amount of folate. The nutrient is destroyed by boiling and heating.

Biochemical functions

Folic acid is essential for the body to make DNA, RNA, and metabolize amino acids which are required for cell division (Anonymous 1998). Folate is necessary for the production and maintenance of new cells, for DNA synthesis and RNA synthesis through methylation, and for preventing changes to DNA, and, thus, for preventing cancer (Kamen 1997). As humans cannot make folic acid, it is required from the diet, making it an essential vitamin (Jeffrey 2009). Levomefolic acid (INN) (also known as L-5-MTHF, L-methylfolate and L-5-methyltetrahydrofolate and (6S)-5-methyltetrahydrofolate, and (6S)-5-MTHF) is the primary biologically active form of folate used at the cellular level for DNA reproduction, the cysteine cycle, and the regulation of homocysteine. Functions of folacin are defined by its coenzymes: N⁵-formyl-THFA, N¹⁰-formyl-THFA, N⁵,N¹⁰-methenyl-THFA, N⁵, N¹⁰-methylene THFA, and N⁵-methyl-THFA. The active one-carbon moiety can be transferred from one coenzymic form onto another one and can be used in a variety of reactions for the synthesis of purines, pyrimidines, and certain amino acids (glycine from serine, or methionine from homocysteine). The folacin coenzymes participate in the biosynthesis of carbon atoms at the positions 2 and 8 of the purine ring as well as in the formation of dTMP from dUMP. Because of this, folacin plays a decisive role in nucleic acid biosynthesis and in the cell division.

Deficiency in folacin

Folate deficiency in children may develop within a month of poor dietary intake (Marino and Fine 2009). In adults normal total body folate is between 10,000 and 30,000 micrograms (μg) with blood levels of greater than 7 nmol/L (3 ng/mL) (Anonymous 1998). Folate deficiency may result in a type of anemia in which low numbers of large red blood cells occur (Anonymous (1998)). Symptoms may include feeling tired, heart palpitations, shortness of breath, open sores on the tongue, and changes in the color of the skin or hair. Folate deficiency may lead to glossitis, diarrhea, depression, confusion, anemia, and fetal neural tube defects and brain defects (during pregnancy). Other symptoms include fatigue, gray hair, mouth sores, poor growth, and swollen tongue. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system (WHO 2015). A shortage in tetrahydrofolic acid (FH4) can cause megaloblastic anemia.

Short supply of folic acid or disturbances in its uptake lead to megaloblastic anemia (a disorder of abnormally large red blood cells that results from inhibition of DNA synthesis in red blood cells, deficient in enough healthy red blood cells). This disease is caused by impaired biosynthesis of purine bases and deoxythymidine phosphate, with the resultant inhibition of DNA synthesis and proliferation (mitosis) of hemopoietic cells. In megaloblastic anemia, the concentrations of erythrocytes and hemoglobin in the blood are reduced; large cells, megaloblasts, are formed in peripheral blood and marrow. The quantity of leucocytes is also reduced (leucopenia), since for their formation in the marrow; the normal synthesis of DNA is prerequisite.

Practical applications

In medical practice, folic acid preparations are employed in treating megaloblastic anemia, for stimulation of cell proliferation, etc.

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin B₁₂ cobalamins

Source: Vitamin B₁₂ or cobalamins are only produced in bacteria (Le Blanc et al. 2013) and also archaea because they have the enzymes needed for its synthesis and no fungi, plants, humans, or animals are capable of producing vitamin B₁₂. Vitamin B₁₂ is present in animal products, such as meat, poultry, fish (shellfish), and to a lesser extent dairy products and eggs (Brody 1999). Liver and kidney are rich in cobalamins; vegetable products are rather poor in them. Recent analyses revealed that some plant source foods, such as certain fermented beans and vegetables and edible algae and mushrooms, contain substantial amounts of bioactive vitamin B₁₂ (Watanabe et al. 2013). Fresh pasteurized milk contains 0.9 µg per cup and is an important source of vitamin B₁₂ for some vegetarians (Anonymous 1998). Some substantial sources of B₁₂ include fortified food products and dietary supplements. Partly, vitamin B₁₂ is synthesized by the intestinal bacteria. The recommended daily intake of vitamin B₁₂ for adult humans is about 2 µg. Together with B vitamin fortified food and supplements, these foods may contribute, though modestly, to prevent vitamin B₁₂ deficiency in individuals consuming vegetarian diets.

Chemical nature, biologically active forms

Vitamin B₁₂ has the largest and most complex chemical structure, it contains a metal ion, cobalt, and so named as cobalamin. The structure of B₁₂ is based on a corrin ring, which is similar to the porphyrin ring found in heme, chlorophyll, and cytochrome. The central metal ion is cobalt. Four of the six coordination sites are provided by the corrin ring, and a fifth by a dimethylbenzimidazole group. The sixth coordination site, the center of reactivity, is variable, being a cyano group (–CN), a hydroxyl group (–OH), a methyl group (–CH₃), or a 5'-deoxyadenosyl group (here the C5' atom of the deoxyribose forms the covalent bond with cobalt), respectively, to yield the four B₁₂ forms, e.g., (i) Cyanocobalamin is one such form, i.e., “vitamer”, of B₁₂, (ii) Hydroxocobalamin is another form of B₁₂ commonly

encountered in pharmacology, which is not normally present in the human body. Hydroxocobalamin is sometimes denoted B_{12a} , (iii) Adenosylcobalamin ($adoB_{12}$), and (iv) Methylcobalamin (MeB_{12}) are the two enzymatically active cofactor forms of B_{12} that naturally occur in the body (Fig. 5.15). Methylcobalamin and 5-deoxyadenosylcobalamin are the forms of vitamin B_{12} used in the human body

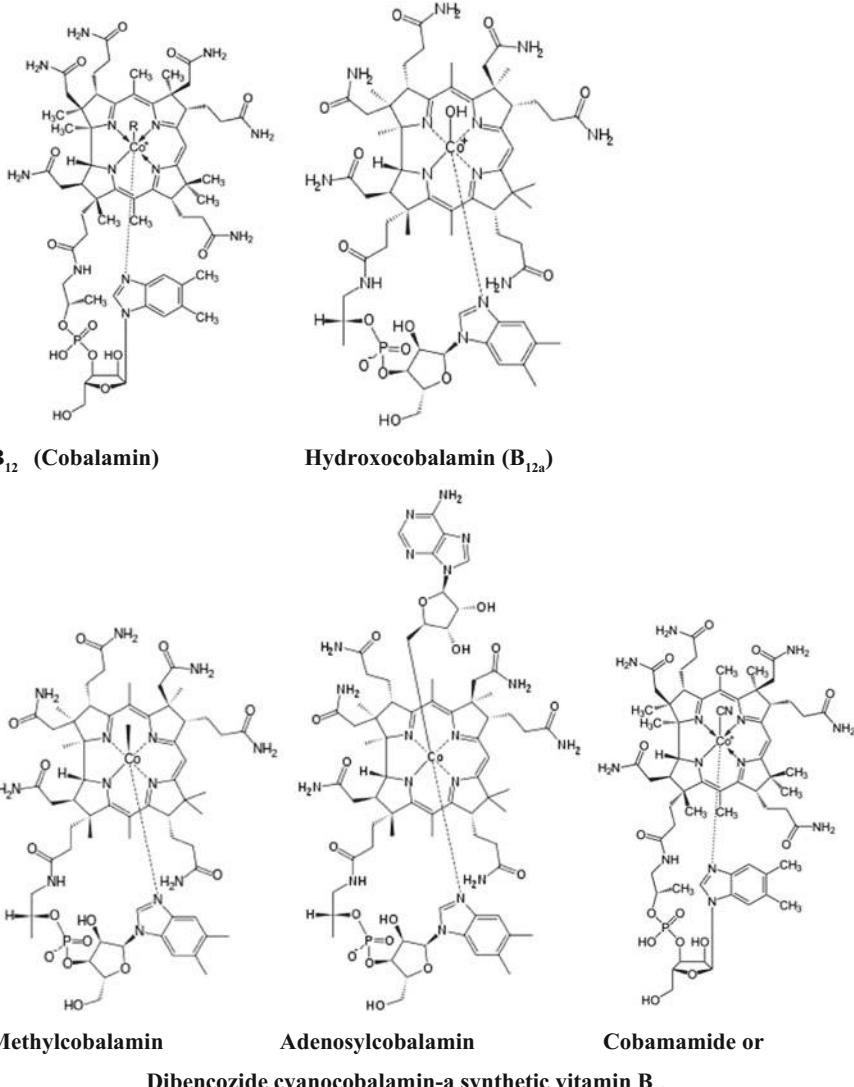


Fig. 5.15 Showing structure of water-soluble vitamin B_{12} —cobalamin (B_{12}), hydroxocobalamin (B_{12a}), methylcobalamin, adenosylcobalamin, cobamamide, or dibencozide cyanocobalamin—a synthetic vitamin B_{12}

(Brody 1999). Cyanocobalamin is used in most nutritional supplements and fortified foods, and it is readily converted to 5-deoxyadenosylcobalamin and methylcobalamin in the body.

Biochemical functions

Vitamin B₁₂ or cobalamin is one of eight B vitamins that has a key role in the normal functioning of the brain and nervous system via the synthesis of myelin (myelinogenesis) (Miller et al. 2005), and the formation of red blood cells. It is involved in the metabolism of every cell of the human body, especially affecting DNA synthesis, fatty acid, and amino acid metabolism (Yamada 2013). Cobalamin is a cofactor for only two enzymes, methionine synthase and L-methylmalonyl-coenzyme A mutase in mammals (Carmel 2006).

Cofactor for methionine synthase: Methylcobalamin is required for the function of the folate-dependent enzyme, methionine synthase. This enzyme is required for the synthesis of the amino acid, methionine, from homocysteine. Methionine in turn is required for the synthesis of S-adenosylmethionine, a methyl group donor used in many biological methylation reactions, including the methylation of a number of sites within DNA, RNA, and proteins (Shane 2000).

Cofactor for L-methylmalonyl-coenzyme A mutase: 5-Deoxyadenosylcobalamin is required by the enzyme that catalyzes the conversion of L-methylmalonyl-coenzyme A to succinyl-coenzyme A (succinyl-CoA), which then enters the citric acid cycle. Succinyl-CoA plays an important role in the production of energy from lipids and proteins and is also required for the synthesis of hemoglobin, the oxygen-carrying pigment in red blood cells (Shane 2000).

Deficiency in cobalamins

Vitamin B₁₂ deficiency is commonly associated with chronic stomach inflammation leading to pernicious anemia and to a food-bound vitamin B₁₂ malabsorption syndrome. Impairment of vitamin B₁₂ absorption can cause megaloblastic anemia and neurologic disorders in deficient subjects. Vitamin B₁₂ deficiency can potentially cause severe and irreversible damage to brain and nervous system (Van der Put et al. 2001). At mild deficiency levels, a range of symptoms such as fatigue, lethargy, depression, poor memory, breathlessness, headaches, and pale skin may develop among others, especially in elderly people (age >60) mainly because of impaired intestinal absorption due to less stomach acid as the age. Vitamin B₁₂ deficiency can also cause symptoms of mania and psychosis (Masalha et al. 2001; Sethi et al. 2005). Both depression and osteoporosis have been linked to diminished vitamin B₁₂ status and high homocysteine levels. Vitamin B₁₂ is rare from plant sources, so vegetarians are more likely to suffer from vitamin B₁₂ deficiency.

Practical applications

In medical practice, cyanocobalamin and, recently, deoxyadenosylcobalamin are of common use. These agents are employed in treating megaloblastic anemia, damages of spinal cord and peripheral nerves, congenital disturbances of vitamin B₁₂ metabolism, etc. Joint application of cobalamin with folic acid and iron appears to

be expedient, since the latter two are necessary for the synthesis of hemoglobin in the hemopoietic cells.

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin Ascorbic acid

Source: Vitamin C or ascorbic acid is found in food and used as a dietary supplement. Fresh fruits and vegetables, e.g., cabbage, cucumber, grapefruit, orange, lemon, lime, papaya, parsley, pineapple, radish, potato skins, spinach, tomato, turnip, carrot, rhubarb, etc., are the major source of vitamin C for humans. Wild-rose fruits are especially abundant in vitamin C. The daily requirement in vitamin C for the adult human is 50–100 mg.

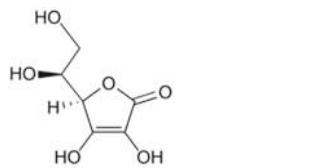
Vitamin C decomposes chemically under certain conditions, e.g., during the cooking of food around 60% possibly partly due to increased enzymatic destruction at sub-boiling temperatures, longer cooking times also add to this effect and copper food vessels (as Cu catalyze the decomposition), length of storage time and temperature at which foods are stored (Allen and Burgess 1950; Roig et al. 1995). Leaching is also a cause of vitamin C loss from cut vegetables as water-soluble vitamin dissolves into the cooking water though not at the same rate and broccoli, seems to retain more than any other (Combs 2001). Freshly cut fruits do not lose significant nutrients when stored in the refrigerator for a few days.

Chemical nature, biologically active forms

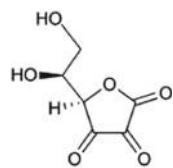
Vitamin C, also known as ascorbic acid and L-ascorbic acid always refers to the L-enantiomer of ascorbic acid and its oxidized forms. Ascorbic acid is a weak sugar acid, structurally related to glucose. In biological systems, ascorbic acid can be found only at low pH, but in neutral solutions above pH 5 is predominantly found in the ionized form, ascorbate. All of these molecules have vitamin C activity. Ascorbic acid has no coenzyme form, and the active form of vitamin C is ascorbate acid itself. Figure 5.16 shows structure of water-soluble vitamin C: vitamin C(ascorbic acid), L-ascorbic acid (reduced form), Vitamin C, dehydroascorbic acid (oxidized form), calcium ascorbate, calcium salt of ascorbic acid, and sodium L-ascorbate.

Biochemical functions

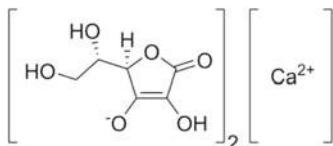
The main function of vitamin C (ascorbate) is as a reducing agent in a number of different reactions and it has the potential to reduce cytochromes a and c of the respiratory chain as well as molecular oxygen. Ascorbic acid acts as a hydrogen donor in enzymic redox reactions. It forms a redox pair with dehydroascorbic acid. The reduction of dehydroascorbic acid to ascorbic acid in the tissues is carried out by ascorbatereductase with the participation of the reductantglutathione. Hydroxylation of proline and lysine residues in collagen synthesis appears to be the most important reaction where ascorbate functions as a cofactor. Thus, vitamin C is important for the maintenance of normal connective tissue as well as for wound healing. It is also involved in the hydroxylation of tryptophan to 5-hydroxytryptophan (in serotonin biosynthesis); conversion of 3,4-dihydroxyphenylethylamine to noradrenalin;



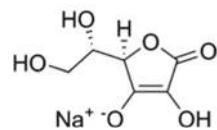
Vitamin C, L-Ascorbic acid (reduced form)



Vitamin C, dehydroascorbic acid (oxidized form)



Calcium ascorbate, calcium salt of ascorbic acid



Sodium L-ascorbate

Fig. 5.16 Showing structure of water-soluble vitamin C: vitamin C (ascorbic acid), L-ascorbic acid (reduced form), citamin C, dehydroascorbic acid (oxidized form), calcium ascorbate, calcium salt of ascorbic acid sodium L-ascorbate

hydroxylation of *p*-hydroxyphenylpyruvate to homogentisic acid; hydroxylation of steroids during the biosynthesis of adrenocortical hormones from cholesterol; hydroxylation of 5-butyrobetain in carnitine biosynthesis; intestinal reduction of Fe^{3+} ions to Fe^{2+} to provide for the bivalent iron uptake; release of iron from its binding with the transport protein transferrin to facilitate the supply of iron to tissues; conversion of folic acid to its coenzymic forms. Vitamin C has a definitive role in treating scurvy, which is a disease caused by vitamin C deficiency. Beyond that, a role for vitamin C as prevention or treatment for various diseases is contentious, with individual clinical trials and even reviews, systemic reviews, and meta-analyses reporting conflicting results.

Ascorbic acid deficiency

Deficiency in ascorbic acid results in the long-known disease called scurvy, or scorbutus. Scurvy is an avitaminosis resulting from lack of vitamin C, since without this vitamin, collagen made by the body is too unstable to perform its function. This results in an increased permeability and fragility of the blood capillaries, which leads to subcutaneous hemorrhages. Scurvy leads to the formation of brown spots on the skin, spongy gums, and bleeding from all mucous membranes. Acute forms of this disease manifest signs of disturbed biochemical functions of ascorbic acid, e.g., the possibility of using the stored iron for the marrow cell hemoglobin synthesis and the participation of folic acid in hemopoietic cell proliferation is reduced leading to the development of anemia. The evoked biochemical disturbances are concomitant with the outward scorbutic symptoms: the loosening and shedding of teeth (dedentition), hemorrhage from the gingivae, dolorous and edemic joints, pallor (anemia) of the skin, hemorrhages, affected bones, and impaired wound healing.

Practical applications

In medicinal practice, ascorbic acid is used in treating hypovitaminosis, in stimulating the hemopoiesis (alongside folic acid, vitamin B₁₂, and iron), in strengthening the ruptured inner wall of blood capillaries, in stimulating the regenerative processes, and in medicating afflicted connective tissues and acutely diseased respiratory ducts.

Water-soluble vitamins

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitaminoid, bioflavonoids, or P-vitamins

Source: Bioflavonoids or P-vitamins are active ingredients in many herbal remedies and they are found in abundance in the rind of citrus fruits, rose hips, black currants, blackberries, cranberries, raspberries, elderberries, and foods containing vitamin C, soybeans, and root vegetables. Other sources of bioflavonoids include tea, vegetables such as broccoli radishes, endives, eggplant, red cabbage, onionskins, leeks, kale, green beans, tomatoes, potatoes, lettuce, flaxseed, whole grains, apricots, buckwheat, cherries, prunes, and core of green peppers. Fresh fruits and berries, especially black chokeberry, apple, grapes, lemon as well as tea leaves and sweet briar fruits, are rich in P-vitamins. The dietary intake of these foodstuffs provides for the human requirement in biflavonoids whose recommended daily intake for the adult organism is 25–50 mg.

Flavonoids are polyphenolic compounds in the human diet and are found ubiquitously in plants (Spencer 2008). Flavonols, the original bioflavonoids such as quercetin, are also found ubiquitously, but in lesser quantities. The widespread distribution of flavonoids, their variety, and their relatively low toxicity compared to other active plant compounds (for instance, alkaloids) mean that many animals, including humans, ingest significant quantities in their diet. Foods with a high flavonoid content include parsley, onions, blueberries and other berries, black tea, green tea and oolong tea bananas, all citrus fruits, *Ginkgo biloba*, red wine, sea buckthorns, buckwheat, and dark chocolate (with a cocoa content of 70% or greater). Blueberries are a dietary source of anthocyanidins (Ayoub et al. 2016; Oomah and Mazza 2016; Anonymous 2017). Further information on dietary sources of flavonoids can be obtained from the USDA flavonoid database, updated on February 7, 2017 contains values for 500 food items for five subclasses of flavonoids (flavonols, flavones, flavanones, flava-3-ols.anthocyanadins) (Anonymous 2017).

Chemical nature of bioflavonoids

Flavonoids or bioflavonoids are a class of secondary metabolites of plant and fungal origin. Chemically, they have a general 15-carbon skeletal backbone consisting of two phenyl rings (A and B) and heterocyclic ring (C), which may be written as C6-C3-C6. Ring A usually shows a phloroglucinol substitution pattern. Bioflavonoids are classified in a variety of ways, sometimes with overlapping as (i) flavonoids or bioflavonoids, (ii) isoflavonoids, derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure, and (iii) neoflavonoids, derived from 4-phenylcoumarine

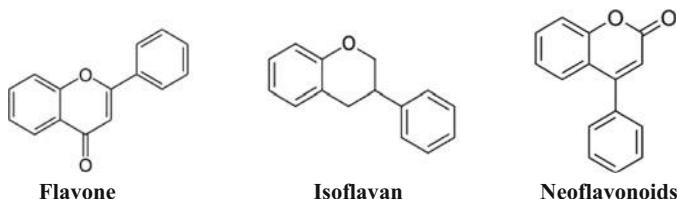


Fig. 5.17 Showing structure of flavone (2-phenyl-1,4-benzopyrone), isoflavan, and neoflavanoids backbone

(4-phenyl-1,2-benzopyrone) structure (Fig. 5.17). These three flavonoid classes are anthoxanthins (flavones and flavonols) as they are ketone-containing compounds. The non-ketone polyhydroxy polyphenol compounds termed flavonoids are also included in the group of compounds flavonoid and bioflavonoid.

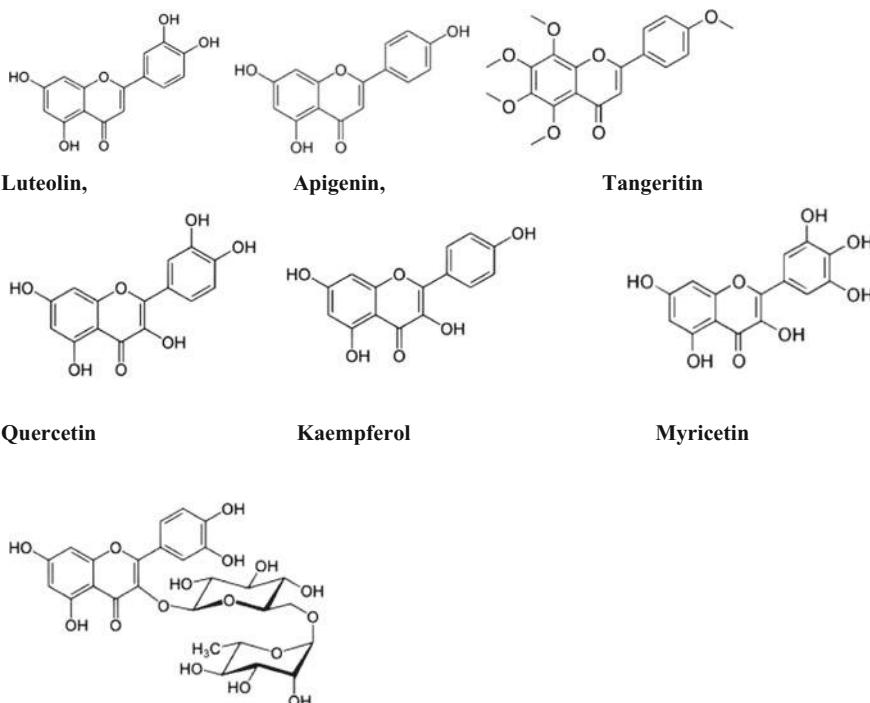
Over 8000 naturally occurring flavonoids have been characterized from various plants by the year 2000 (Pietta 2000). They have been classified according to their chemical structure, and are usually subdivided into several subgroups (Ververidis et al. 2007).

Anthoxanthins include flavones (e.g., luteolin, apigenin, tangeritin) and flavonols (e.g., flavonoid class of polyphenolic compounds—quercetin, kaempferol, myricetin, fisetin, galangin,isorhamnetin, pachypodol, rhamnazin, pyranoflavonols, and furanoflavonols), and two natural water-soluble flavonoid pigments of plants, which range in color from white or colorless to a creamy to yellow, often on petals of flowers, important in nutrition, also used as food additives and exhibit antioxidant properties (Fig. 5.18).

The flavanones, a type of flavonoids, are various aromatic, colorless ketones derived from flavone that often occur in plants as glycosides (e.g., Hesperetin, Naringenin, Eriodictyol, Homoeriodictyol) and flavanonols include taxifolin (or dihydroquercetin), dihydrokaempferol, etc. (Fig. 5.19).

Hesperidin is a flavonon glycoside of hesperetin aglycone, the main flavonoid found in citrus fruits, e.g., lemons and sweet oranges. The flavonoid naringenin occurs naturally in citrus fruits, especially in grapefruit and naringin is responsible for its bitter taste. Eriodictyol is a bitter-masking flavanone and eriocitrin or eriodictyol glycoside is commonly found in lemons and rose hips (*Rosa canina*). Taxifolin or dihydroquercetin is a flavanonol-type flavonoid. Dihydrokaempferol, aromadendrin, or aromodedin is also flavanonol-type flavonoid. It can be found in the wood of *Pinus*.

Flavans include flavan-3-ols (flavanols), flavan-4-ols, and flavan-3,4-diols. Flavan-3-ols (also called flavanols) are derivatives of flavans that use the 2-phenyl-3,4-dihydro-2H-chromen-3-ol skeleton. These compounds include catechin, gallicatechol or gallicatechin, epicatechin gallate, epigallicatechin, epigallocatechin gallate, proanthocyanidins, theaflavins, thearubigins while anthocyanidins include cyanidin, delphinidin, malvidin, pelargonidin, peonidin,



Rutin or rutoside- a quercetin glycoside

Fig. 5.18 Showing structure of anthoxanthins: flavones (e.g., luteolin, apigenin, and tangeritin) and flavonols (e.g., quercetin, rutin, kaempferol, and myricetin)

petunidin, etc. Cyanidin and delphinidin are anthocyanidins—primary plant pigments, and also antioxidant. The flavan-4-ols (3-deoxyflavonoids) are flavone-derived alcohols and a family of flavonoids and include apiforol and luteoforol for example. Figure 5.20 shows structure of flavans: Flavan-3-ols, Flavan-4-ols, and Flavan-3,4-diols.

Catechin is a type of plant secondary metabolite, natural phenol, and antioxidant; gallocatechin is one of the antioxidant chemicals found in food; theaflavin (TF) and its derivatives (collectively called theaflavins) are antioxidant polyphenols. The flavan-3,4-diols or leucoanthocyanidins are colorless chemical compounds related to anthocyanidins and anthocyanins, e.g., leucocyanidin, leucodelphinidin, leucocisetinidin, leucomalvidin, leucopelargonidin, leucopeonidin, leucorobinetinidin, melacacidin, etc. Anthocyanidins are the aglycones of anthocyanins, and six common aglycones are cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin; anthocyanins are the pigments and exhibit red, purple, and blue coloring of plants and acting as antioxidants, anthocyanins may offer anti-inflammatory, antiviral, and anticancer benefits as well as protection against ischemic heart

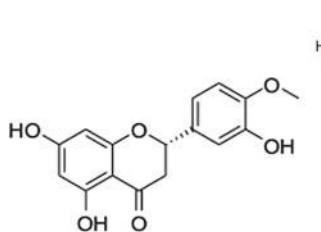
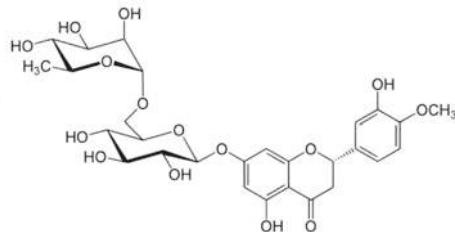
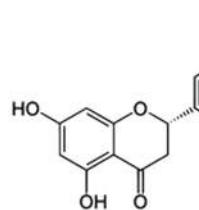
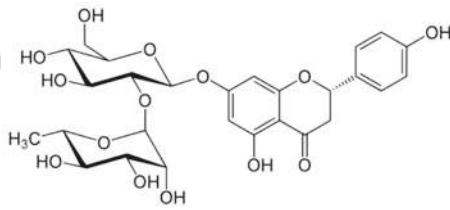
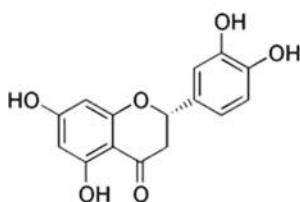
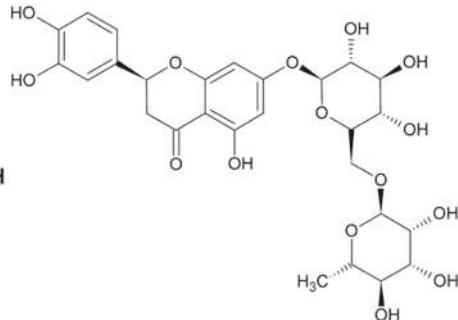
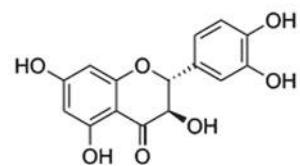
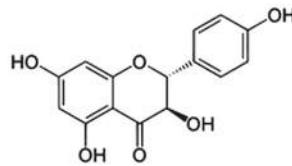
**Hesperetin,****Hesperidin, a glycoside of Hesperetin****Naringenin,****Naringin or naringoside, a glycoside of Naringenin****Eriodictyol****Eriocitrin, a eriodictyol glycoside****Taxifolin****Dihydrokaempferol**

Fig. 5.19 Showing structure of flavanones: hesperetin, naringenin, eriodictyol, homoeriodictyol and Flavanonols: Taxifolin or dihydroquercetin and dihydrokaempferol

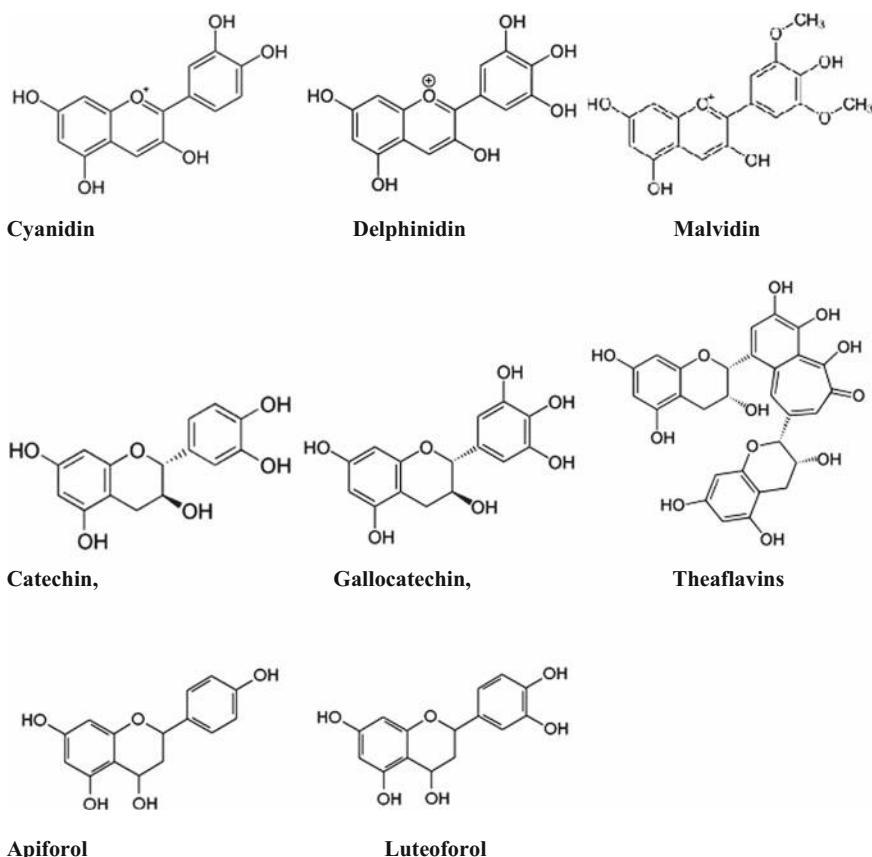


Fig. 5.20 Showing structure of Flavans: Flavan-3-ols, Flavan-4-ols, and Flavan-3,4-diols

disease, diabetes, allergy, and mutagenesis (Kähkönen and Heinonen 2003; Galvano et al. 2004; Ghosh 2005).

Isoflavonoids are a class of flavonoid phenolic compounds, and many of them are biologically active (Fig. 5.21). Isoflavonoids and their derivatives are sometimes referred to as phytoestrogens, as many isoflavonoid compounds have biological effects via the estrogen receptor. The isoflavonoid group is broad and includes many structurally similar groups, e.g., isoflavones (genistein, daidzein, glycitein); isoflavanes (equol); isoflavones (glabrene, 2-methoxyjudaicin, haginin D); coumestan (coumestrol, wedelolactone, plicadin); pterocarpans (bitucarpin A and B, erybraedin A and B, glyceollin I, II, III and IV, gycyrhizol A, medicarpin, phaseolin, etc.); rotenoids (deguelin, dehydrodeguelin, rotenol, rotenone, tephrosin, etc.); etc.

Genistein is an isoflavone that is described as an angiogenesis inhibitor and a phytoestrogen. Daidzein and other isoflavones, found exclusively in soybeans and

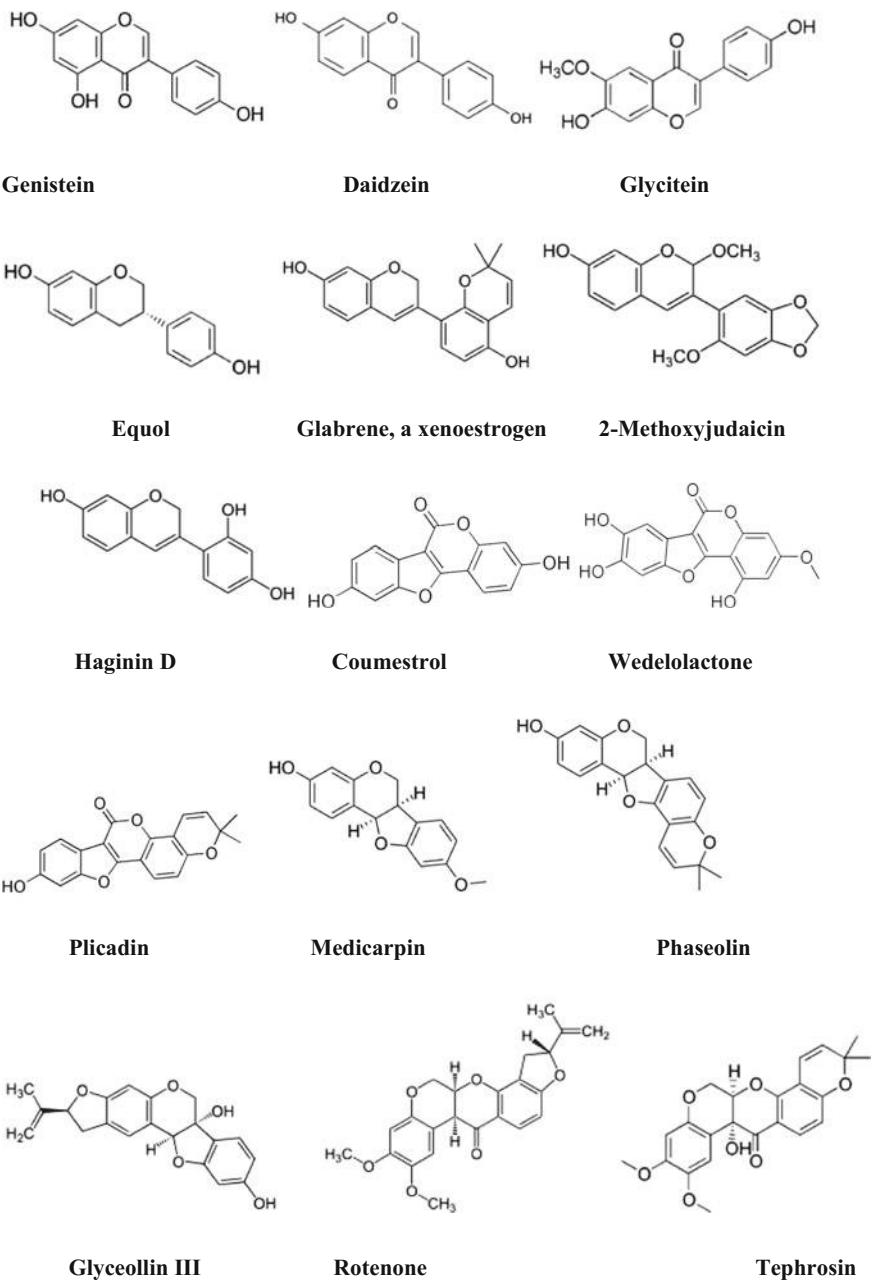


Fig. 5.21 Showing structure of Isoflavonoids: isoflavones, isoflavanones, isoflavanones, coumestan, pterocarpans, and rotenoids

other legumes, are produced in plants through the phenylpropanoid pathway of secondary metabolism and are used as signal carriers, and defense responses to pathogenic attacks. In humans, recent research has shown the viability of using daidzein in medicine for menopausal relief, osteoporosis, blood cholesterol, and lowering the risk of some hormone-related cancers, and heart disease. Glycitein is an *O*-methylated isoflavone which accounts for 5–10% of the total isoflavones in soy food products. Glycitein is a phytoestrogen with weak estrogenic activity, comparable to that of the other soy isoflavones. Equol is an isoflavandiol metabolized from daidzein, a type of isoflavone found in soybeans and other plant sources, by bacterial flora in the intestines. Equol is a nonsteroidal estrogen. Coumestan is a heterocyclic organic compound. Coumestan forms the central core of a variety of natural compounds known collectively as coumestans. Coumestans are oxidation products of pterocarpan that are similar to coumarin. Coumestans, including coumestrol, a phytoestrogen, are found in a variety of plants. Food sources high in coumestans include split peas, pinto beans, lima beans, and especially alfalfa and clover sprouts. Glabrene, found in the roots of liquorice, is also a xenoestrogen. 2-Methoxyjudaicin found in the roots of *Cicer bijugum*. Haginin D is derived from the heartwood of *lespedeza cyrtobotrya*, trailing vines of Fabaceae. Pterocarpans are derivatives of isoflavonoids found in the Fabaceae family. Rotenoids are related to the isoflavones containing a *cis*-fused tetrahydrochromeno [3,4-b] chromene nucleus; many have insecticidal and nonselective piscicide activity including rotenone. Tephrosin is rotenoid. It is a natural fish poison found in the leaves and seeds of *Tephrosia purpurea* and *T. vogelii*.

The consumption of isoflavones-rich food or dietary supplements is under preliminary research for its potential association with lower rates of postmenopausal cancer and osteoporosis in women (Wei et al. 2012; Varinska et al. 2015). Use of soy isoflavone dietary supplements may be associated with reduction of hot flashes in postmenopausal women. Isoflavonoids dietary antioxidants have a variety of bioprotective effects including antioxidant, antimutagenic, anticarcinogenic, and antiproliferative activities (Birt et al. 2001; Miadokova et al. 2002; Ryan-Borchers et al. 2006; Iwasaki et al. 2008; Scarpato et al. 2008; Miadoková 2009).

Biochemical functions and use

Bioflavonoids or flavonoids are a large class of naturally occurring antioxidant compounds. The active principle in bioflavonoids is flavones structurally based on the flavone ring system. In plants, some >8000 flavonoid and related compounds have been identified. Among these, anthoxanthins, anthocyanins, and catechols exhibit P-vitaminic properties. Bioflavonoids have been used in alternative medicine as an aid to enhance the action of vitamin C, to support blood circulation, as an antioxidant, and to treat allergies, viruses, or arthritis and other inflammatory conditions. Bioflavonoids are active ingredients in many herbal remedies and often sold as an herbal supplement.

In their natural state, bioflavonoids are usually found in close association with vitamin C. In treating conditions, vitamin C and bioflavonoids each enhance the action of the other compound. Therefore, when taken as supplements, they often

should be used in combination to increase effectiveness. In general, all bioflavonoids are potentially useful as antioxidants, antivirals, and anti-inflammatories. Rutin can be used to treat chronic venous insufficiency, glaucoma, hay fever, hemorrhoids, varicose veins, poor circulation, oral herpes, cirrhosis, stress, low serum calcium, cataracts, can relieve the pain from bumps and bruises, to help reduce serum cholesterol, and diseases (gout, arthritis, systemic lupus erythematosus, and ankylosing spondylitis). Bioflavonoids like anthocyanins and proanthocyanidins can be used to treat a number of eye conditions such as cataracts, night blindness, diabetic retinopathy, macular degeneration, osteoporosis by stabilizing collagen (the major protein in bone), reduce cholesterol deposits in arteries, and prevent damage to the artery walls. Hesperidin is useful in treating the complaints of menopause and in dealing with the viruses that cause herpes. Quercetin is a good antihistamine. It can help reduce the inflammation that results from hay fever, allergies, bursitis, gout, arthritis, and asthma. Kaempferols stimulate liver detoxification and strengthen the blood vessels. They may also inhibit tumor formation. In addition, bioflavonoids are used to prevent nosebleeds, miscarriages, postpartum bleeding, and other types of hemorrhages; prevent menstrual disorders; protect against cancer and heart disease; prevent blood clotting (anticoagulant activity); reduce the occurrence of easy bruising; decrease the cholesterol level; improve symptoms related to aging; protect against infections; counteract the effects of pollution, pesticides, rancid fats, and alcohol; reduce pain, macular degeneration; improve circulation; improve liver function, vision, and eye diseases; strengthen the walls of the blood vessels; etc.

Bioflavonoid deficiency

A deficiency of bioflavonoids in the organism is revealed by the symptoms of increased fragility and permeability of blood capillaries, punctate hemorrhages (a capillary hemorrhage into the skin that forms petechiae or spot), and odontorrhagia (painful swallowing, in the mouth (oropharynx) or esophagus).

Practical applications

In medical practice, complex P-vitaminic preparations (sum total of tea leave catechols, sum total of black chokeberry flavonoids) as well as individual flavonoids (rutin, quercitol, hesperidin and their derivatives) are used. Of practical utility are also preparations combined with vitamin C: ascorutin, galascorbin, and tea catechols with ascorbic acid. These preparations are recommended in the therapy of diseases associated with a damage and increased permeability of the capillary walls (capillarotoxicosis, allergic vasculitis, toxin poisoning, and radiation sickness) and reduced blood coagulability.

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin Biotin

Source: Biotin, an enzyme cofactor, is present in minute amounts in every living cell and occurs mainly bound to proteins or polypeptides and is abundant in liver, kidney, pancreas, yeast, and milk. The human requirement in biotin is chiefly

supplied through its biosynthesis by intestinal bacteria. Some biotin is supplied in food, e.g., pea, lentil, soya, peanut, walnuts, sunflower seeds, cauliflower, mushrooms, pecans, egg's yolk, beef liver, butter, brewer's yeast, etc., are rich in biotin. The biotin content of cancerous tissue is higher than that of normal tissue. It is stable at room temperature and not destroyed by cooking. The recommended daily intake of biotin for the adult human is about 150–200 µg.

Chemical nature and biologically active form

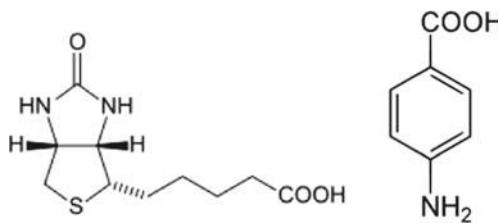
Biotin or B₇ is a water-soluble vitamin and formerly it was known as vitamin H or coenzyme R (Fig. 5.22). It is composed of an ureido ring fused with a tetrahydrothiophene ring. A valeric acid substituent is attached to one of the carbon atoms of the tetrahydrothiophene ring. In cellular activities, biotin acts as coenzyme for carboxylase enzymes.

Biochemical functions of B₇ (Biotin)

Biotin functions as coenzyme for carboxylase enzymes (e.g., acetyl-CoA carboxylase alpha, acetyl-CoA carboxylase beta, methylcrotonyl-CoA carboxylase, propionyl-CoA carboxylase, pyruvate carboxylase, etc.) that are involved in the synthesis of fatty acids, isoleucine, and valine, and in gluconeogenesis. Biotin is necessary for cell growth, the production of fatty acids, and the metabolism of fats and amino acids. Biotin assists in various metabolic reactions involving the transfer of carbon dioxide. It may also be helpful in maintaining a steady blood sugar level. Biotin is often recommended as a dietary supplement for strengthening hair and nails, though scientific data supporting this outcome are weak. Nevertheless, biotin is found in many cosmetics and health products for the hair and skin.

Deficiency

Biotin deficiency is rare because it is needed in very small amount, a wide range of foods contain biotin, and intestinal bacteria synthesize biotin usable by the host animal. However, biotin deficiency can be caused by inadequate dietary intake or genetic disorders that affect biotin metabolism. Subclinical deficiency can cause mild symptoms, such as hair thinning or skin rash typically on the face. A number of rare metabolic disorders exist such as deficiency in the holocarboxylase synthetase due to abnormal metabolism of biotin (Zempleni et al. 2008). Biotin is needed for healthy metabolic, nerve, digestive, and cardiovascular functions.



B7 (Biotin)

Paraaminobenzoic acid (PABA)

Fig. 5.22 Showing structure of water-soluble vitamin: B₇ (Biotin) and Para-aminobenzoic acid (PABA)

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin para-aminobenzoic acid (PABA)

Source: Vitamin para-aminobenzoic acid (PABA) is practically ubiquitous in all foodstuffs (Fig. 5.22). It is especially abundant in liver, milk, hen's egg, and brewer's yeast as well as in wheat germ, whole grains such as rice and molasses. Human intestinal bacteria, including *E. coli*, generate PABA. Plants produce PABA in their chloroplasts and store it as a glucose ester (*p*A_BA-Glc) in their tissues. In humans, PABA, also a member of the B vitamins—B_x, is considered nonessential because human colon bacteria generate PABA, and as such it is no longer recognized as a vitamin. The compound occurs naturally. There is no recommended dietary allowance (RDA) for PABA. It is available in supplements of 50–1000 mg capsule; about 50–100 mg, three times daily is recommended for common treatment but it should be increased if taken with sulfa antibiotics.

Chemical nature and biologically active forms

Para-aminobenzoic acid (PABA) or 4-aminobenzoic acid consists of a benzene ring substituted with amino and a carboxyl groups. It so named because the number 4 carbon (the *para* position) of the benzoic acid bears an amino group (−NH₂). PABA is an intermediate in the synthesis of folate by bacteria, plants, and fungi, and it is part of the folic acid molecule. Many bacteria including human intestinal bacteria generate PABA from chorismate by the combined action of the enzymes 4-amino-4-deoxychorismate synthase and 4-amino-4-deoxychorismate lyase. PABA, being a B vitamin, is a cofactor and precursor in the synthesis of folic acid, purines, and thymine in most species of bacteria. PABA is a white solid, although commercial samples can appear gray. It is partially soluble in water.

Medicinal use/Functions

PABA is an antioxidant that is considered by some as a B complex vitamin, and sometimes called vitamin B_x. However, it is not really a vitamin for the human organism, but actually an amino acid that is part of folic acid. It serves as a vitamin for microorganisms (except the tuberculous bacilli, which are capable of its synthesis) and stimulates the growth of microorganisms. It is known specifically for its nourishment to hair and its usefulness as a sunscreen. The potassium salt of PABA is used as a drug against fibrotic skin disorders, such as Peyronie's disease, under the trade name Potaba. PABA is also occasionally used in pill form by sufferers of irritable bowel syndrome to treat its associated gastrointestinal symptoms, and in nutritional epidemiological studies to assess the completeness of 24-hour urine collection for the determination of urinary sodium, potassium, or nitrogen levels.

Despite the lack of any recognized syndromes of PABA deficiency in humans, except for a few people who lack the bacteria that generate PABA in their colons, many claims of benefit for fatigue, irritability, depression, weeping eczema (moist eczema), scleroderma (premature hardening of skin), patchy pigment loss in skin (vitiligo), and premature gray hair are made by commercial suppliers of PABA as a nutritional supplement. No coenzyme for PABA has been reported. PABA makes

part of the folacin structure, which explains its importance for the vital activity of microorganisms. PABA has also been shown to be a growth factor for birds; it is essential for the production of melanin pigments in rodents. In humans, PABA is presumably used by the intestinal bacteria for generating folacin in the organism. PABA is important to skin, hair pigment, and intestinal health. In practice, PABA is used as a cosmetic ointment ingredient in the prophylaxy of skin sunburns. Used as a sunscreen, it also can protect against the development of sunburn and skin cancer from excess ultraviolet light exposure. It may also be recommended for restoration of the normal intestinal microflora. PABA, as part of the coenzyme tetrahydrofolic acid, aids in the metabolism and utilization of amino acids and is also supportive of blood cells, particularly the red blood cells. PABA supports folic acid production by the intestinal bacteria.

Deficiency and toxicity of PABA

Deficiency problems are not very common; they occur more frequently with the use of sulfa or other antibiotics that alter the functioning of intestinal bacteria and, therefore, the production of PABA. General fatigue, irritability, depression, nervousness, graying hair, headache, and constipation or other digestive symptoms may occur. PABA, at high doses, can be somewhat irritating to the liver; in addition, nausea and vomiting have occurred, as have anorexia, fever, skin rash, and even vitiligo.

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin inositol

Source: Inositol, a pseudovitamin or vitamin like organic substance is naturally found in plants and animals. It is widely spread in foodstuffs of animal and vegetable origin, particularly abundant in lecithin granules, beef heart, liver, meat products, brain, egg yolk as well as bread, potato, green pea, green leaf vegetables, wheat germ, citrus fruits, nuts, brown rice, cereals, whole-grain bread, soy flour, molasses, and mushrooms. *Myo*-inositol was once considered a member of the vitamin B complex, called vitamin B₈ in this context, but it is not an essential nutrient because it is produced by the human body from glucose. The exact human requirement in inositol is unknown; it may amount to 1.0 or 1.5 g per day.

Chemical nature and biologically active forms

By its chemical structure, inositol is a hexabasic cyclic alcohol, i.e., cyclitol—alicyclic polyhydroxy compound or cyclohexane-1,2,3,4,5,6-hexol with formula C₆H₁₂O₆ or (-CHOH-)₆. It is a derivative of cyclohexane with six hydroxyl groups, making it a polyol. It exists in nine possible stereoisomers, of which *myo*-inositol (*cis*-1,2,3,5-*trans*-4,6-cyclohexanehexol, or former *meso*-inositol or *i*-inositol), is the most widely occurring form in nature. Inositol is a sugar alcohol with half the sweetness of sucrose. Figure 5.23 shows structure of water-soluble vitamin: *myo*-inositol and *myo*-inositol isomer.

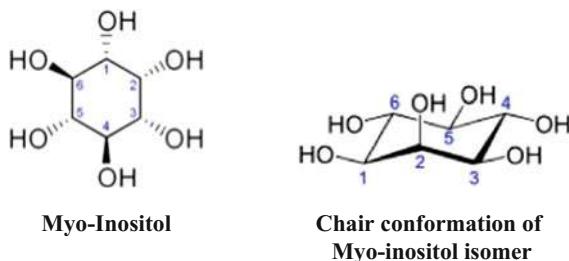


Fig. 5.23 Showing structure of water-soluble vitamin: *myo*-inositol and *myo*-inositol isomer

In its most stable conformation, the *myo*-inositol isomer assumes the chair conformation, which moves the maximum number of hydroxyls to the equatorial position, where they are farthest apart from each other. In this conformation, the natural *myo*-isomer has a structure in which five of the six hydroxyls (the first, third, fourth, fifth, and sixth) are equatorial, whereas the second hydroxyl group is axial. Naturally occurring stereoisomers of *myo*-inositol are scyllo-, muco-, D-chiro-, and neo-inositol, as well as other possible isomers such as L-chiro-, allo-, epi-, and cis-inositol are included (Fig. 5.24). These isomers occur in minimal quantities in nature. L- and D-chiro inositols are the only pair of inositol enantiomers and are enantiomers of each other, but not of *myo*-inositol.

Biochemical functions

Only one of the optical cyclitol forms, *myo*-inositol, exhibits biological activity. Inositol makes part of inositol phosphatides which are present in all tissues. Inositol phosphatides are especially abundant in the nervous tissue. Biological functions of inositol are, presumably, determinative of its involvement in the buildup of inositol

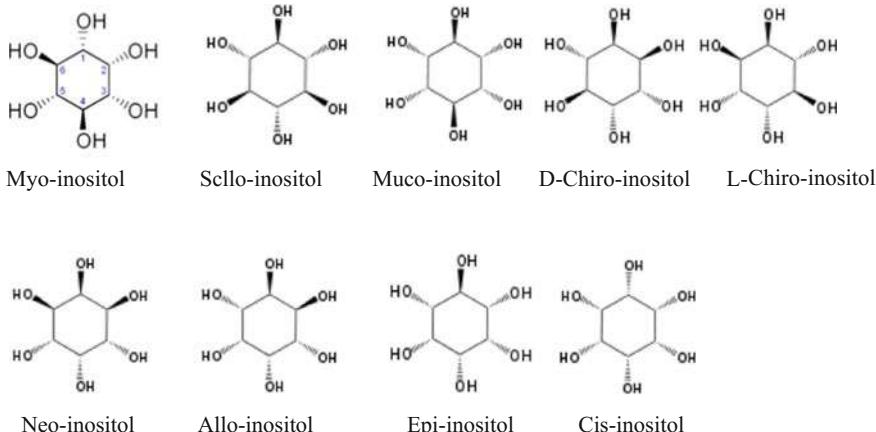


Fig. 5.24 Showing structure of naturally occurring stereoisomers of *myo*-inositol and other possible isomers such as L-chiro-, allo-, epi-, and cis-inositol

phosphatides. Inositol plays the role of a lipotropic factor. The alimentary deficiency of inositol in experimental animals leads to the accumulation of triacylglycerides and to a drop in phospholipid concentrations in the liver, which is ultimately conducive to hepatic adipose degeneration. Addition of inositol to the diet prevents these deleterious alterations. However, the lipotropic effect due to inositol is inferior to that of choline.

Myo-inositol plays an important role as the structural basis for a number of secondary messengers in eukaryotic cells, inositol phosphates, and inositol serves as an important component of the structural lipids, phosphatidylinositol (PI), and its various phosphates, phosphatidylinositol phosphate (PIP) lipids. Inositol or its phosphates and associated lipids are found in many foods, in particular fruit, especially cantaloupe and oranges. In plants, the hexaphosphate of inositol, phytic acid or its salts, the phytates, serve as phosphate stores in nut and bean seeds. Phytic acid also occurs in cereals with high bran content. Phytate is not digestible and not directly bioavailable to humans from diet. Some food preparation techniques partly break down phytates to change this and inositol in the form of glycerophospholipids found in certain plant-derived substances such as lecithin is well-absorbed and relatively bioavailable.

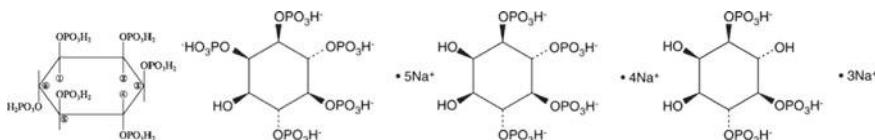
Health benefit of inositol

Inositol functions closely with choline as one of the primary components of cell membranes. It is important for growth of cells in the bone marrow, eye membranes, and intestines. Like choline, it is a lipotropic agent or fat emulsifier, though milder. It helps to metabolize fat and cholesterol by breaking down fats into smaller particles that are easier to remove and reduces fatty buildup in the body organs, especially the liver. Inositol has a calming effect as it is involved in the production and action of neurotransmitters (chemicals that transmit messages between nerve cells) like serotonin and acetylcholine. People who are depressed have been found to have lower levels of inositol than normal. Inositol has been tested as a treatment for depression, and initial evidence is encouraging. In a small double-blind study, those on the supplement who took 12 g of inositol daily for 4 weeks showed significant improvement compared to the placebo group. Preliminary findings from double-blind studies also suggest that inositol may help alleviate polycystic ovary syndrome, including infertility.

Phytic acid (inositolhexaphosphate)

Phytic acid ($C_6H_{18}O_{24}P_6$), inositolhexaphosphate (IP6), inositol phosphate, is the principal storage form of phosphorus in many plant tissues, especially bran and seed (Fig. 5.25).

Phosphorus and inositol in phytate form are not generally bioavailable to non-ruminant animals because they lack the digestive enzyme phytase required to remove the phosphate group but ruminant animals can because of the presence of the digestive enzyme (Klopfenstein et al. 2002). Phytic acid chelates important minerals such as calcium, magnesium, iron, and zinc, making them unabsorbable,



Phytates: (Na salts of inositol penta- (IP5), tetra- (IP4), and triphosphate (IP3)

Fig. 5.25 Showing structure of phytic acid (inositolhexaphosphate) and its isomers

and contributing to mineral deficiencies in people whose diets rely highly on bran and seeds for their mineral intake, such as occurred in developing countries (Anonymous 1973; Hurrell 2003). Inositol penta- (IP5), tetra- (IP4), and triphosphate (IP3) are also called phytates.

Inositol, phosphatidylinositol, and some of their mono- and polyphosphates function as secondary messengers in a number of intracellular signal transduction pathways and they are involved in a number of biological processes such as insulin signal transduction (Lerner 2002), cytoskeleton assembly, nerve guidance (epsin), intracellular calcium (Ca^{2+}) concentration control (Gerasimenko et al. 2006), cell membrane potential maintenance (Kukuljan et al. 1997), breakdown of fats (Rapiejko et al. 1986), gene expression (Shen et al. 2003; Steger et al. 2003), etc.

Phytic acid has been considered as an anti-nutritional component in cereals, seeds and beans because of its the ability to bind minerals, proteins and starch resulting in lower absorption of these cellular components. In spite of that, phytic acid has many health benefits including antioxidant, anticancer, hypocholesterolemic, and hypolipidemic effects. Phytic acid acts as anticancer agent by reversing the proliferative effects of carcinogens. Phytic acid lowers blood glucose response by reducing the rate of starch digestion and slowing the gastric emptying and thus provides health benefits to diabetes patients.

Deficiency of inositol

Deficiency is rare as the body manufactures inositol and it is present in a wide variety of foods. However, long-term use of antibiotics increases the need for inositol, so does regular consumption of more than 2 cups of coffee daily as coffee destroys this nutrient. Extremely high coffee intake can therefore produce a deficiency. Inositol deficiency produces a retardation of juvenile growth, hair shedding, and anemia in rats and mice. Disturbances of the nervous system have been observed in pigeons. No inositol avitaminosis has been reported in humans. In practice, inositol is used as a lipotropic agent; it is also used in the therapy of muscular dystrophy. Inositol deficiency symptoms include eye abnormalities, hair loss or alopecia or patchy baldness, memory loss, eczema, constipation, higher cholesterol level, liver excess fat, hardening and narrowing of arteries (atherosclerosis), etc. Lower levels of inositol have been found in the nerves of people with multiple sclerosis and diabetic nerve disorders; supplementation may help as inositol benefits nerve transmission. In cosmetics, inositol is utilized as an ingredient of nutritive liquids for hair.

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin Orotic acid

Source: Orotic acid (OA) is not a vitamin, but once it was believed to be part of the vitamin B complex and was called vitamin B₁₃ (Fig. 5.26). It is widely spread in animal foodstuffs (especially the beef). Good sources are root vegetables and whey. It is not essential as is synthesized within body by the intestinal natural micro-flora.

Chemical nature and biologically active forms

Orotic acid, pyrimidinecarboxylic acid, is a heterocyclic compound. The body can produce it via a mitochondrial enzyme, dihydroorotate dehydrogenase, or a cytoplasmic enzyme of pyrimidine synthesis pathway. It is sometimes used as a mineral carrier in some dietary supplements (to increase their bioavailability), most commonly for lithium orotate.

Biochemical functions

Orotic acid is used in the synthesis of orotirelin, and it is a precursor in the biosynthesis of pyrimidine bases (uracil, thymine, and cytosine) and nucleotides. The biologically active form of orotate, orotidine 5'-monophosphate, is involved in the synthesis of nucleotides and nucleic acids as well as stimulates protein biosynthesis, cell division, growth, and development of animals and plants.

Orotic acid can partially compensate for B₁₂ deficiency. An orotic acid with mineral forms an insoluble organic salt, orotate. Minerals such as calcium, potassium, and magnesium are available in the orotate form. Mineral orotates are easily absorbed and utilized by the body. Minerals are actively transported from the digestive system into the bloodstream by orotate salts, and in the bloodstream, the mineral separates from the orotate. The minerals are no longer bound to the orotate salt and are now free to exert their therapeutic action.

Health benefit and toxicity

Many of the vitamin-like effects of orotic acid are undoubtedly due to its role in RNA and DNA synthesis. Orotic acid appears to have a direct effect on folate metabolism. Orotic acid and folate are also involved in DNA synthesis. It is primarily used for metabolism of folic acid and vitamin B₁₂. Orotic acid is used as a cosmetic ingredient, in medicine, as a feed supplement and in biochemical research. Orotic acid may improve myocardial purine and pyrimidine levels by stimulating hepatic release of uridine into the bloodstream, which in turn augments depleted

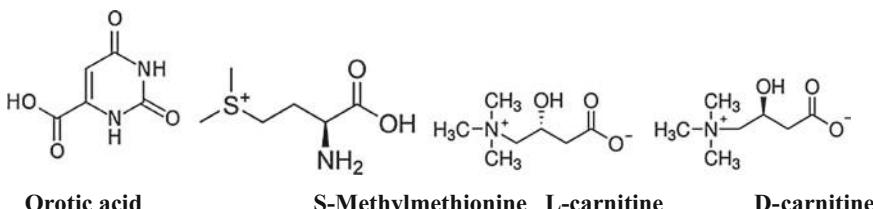


Fig. 5.26 Showing structure of orotic acid, S-methylmethionine, L-carnitine, and D-carnitine

myocardial pyrimidines and purines (rat heart). Orotic acid can improve the energy status of the recently infarcted myocardium (rat hearts) and improves the tolerance of the recently infarcted heart to global ischemia (rats). Orotic acid is recognized as a treatment for multiple sclerosis and is dispensed under the name of calcium orotate. Magnesium orotate may reduce the severity of chronic myocardial dysfunction and structural damage in cardiomyopathy. Magnesium orotate may improve exercise tolerance in patients with coronary artery disease and in trained athletes. Magnesium orotate has only a weak inotropic effect, if any, on normal hearts. In medicinal practice, orotic acid is used as a growth factor for premature infants, for stimulating the hemopoiesis under certain anemias and for accelerating regenerative processes in afflicted tissues. Orotic acid provides health to the skin as well to the hairs and nails as well as it prevents from premature aging.

Orotic acid is a chemical overproduced in an alternative pathway when there is a block in the urea cycle. A buildup of orotic acid can lead to orotic aciduria and academia. It may be a symptom of an increased ammonia load due to a metabolic disorder, such as a urea cycle disorder. Orotic aciduria is a cause of megaloblastic anemia. Orotic acid can be mutagenic in mammalian somatic cells. It is also mutagenic for bacteria and yeast.

Deficiency of orotic acid

No orotic acid deficiency occurs in humans; however, the growing organism or certain tissues in their regeneration periods may stand in increased need of this compound. Although the deficiency of orotic acid is rare but in case of its severe deficiency there arises different signs and symptoms including appearance of crystals in the urine, degeneration of cells, impaired immunity, anemia, low vitamin B₁₂ levels, problems related to heart, weight gain, large and abnormal sized blood cells, skin problems, liver disorders, mental retardation, complaints of premature aging, etc.

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin methylmethionine

Source: S-methylmethionine is particularly abundant in plants, being more abundant than methionine (Bourgis 1999). S-Methylmethionine is sometimes referred to as vitamin U (from Latin *ulcus* to ulcer), but it is not considered a true vitamin. It is an anti-ulcerogenic (Cheney 1950) factor in raw cabbage juice that may help speed healing of peptic ulcers. S-Methylmethionine is found in raw vegetables, especially abundant in cabbage.

Chemical nature and biologically active forms

S-Methylmethionine (SMM) is a derivative of methionine with the chemical formula $[\text{CH}_3]_2\text{S}^+\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_3^+)\text{CO}_2^-$ (Fig. 5.26). This cation is an intermediate in many biosynthetic pathways owing to the sulfonium functional group. The natural derivative S-methylmethionine is biosynthesized from L-methionine which is first converted to S-adenosylmethionine. The subsequent conversion, involving

replacement of the adenyl group by a methyl group, is catalyzed by the enzyme methionine *S*-methyltransferase.

As it is a methylated derivative of methionine by its chemical structure, vitamin U is similar to methionine for an active donor of methyl groups; and thus, it facilitates the synthesis of choline, choline phosphatides, creatine, and other methylated compounds in the organism tissues.

Biochemical functions

The biological roles of *S*-methylmethionine are not well understood, but vitamin U is profitably employed in the therapy of gastric ulcer, duodenal ulcer, and various gastritides. A lyotropic action of this vitamin is also possible. Speculated roles include methionine storage, use as a methyl donor, regulation of methylsulfoniopropionate (SAM) (Bourgis 1999). A few plants use *S*-methylmethionine as a precursor to the osmolyte dimethylsulfoniopropionate (DMSP). Intermediates include dimethylsulfoniumpropylamine and dimethylsulfoniumpropionaldehyde (McNeil 1999). The active principle of cabbage juice exhibits the property to inhibit the development of experimental gastric ulcer, and for this reason it has been named the antiulcer factor, or vitamin U (from Latin *ulcus* to ulcer). *S*-Methylmethionine is claimed to have protective effects in the gastrointestinal mucosa and in the liver (Patel and Prajapati 2012).

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin Carnitine

Source: Carnitine (vitamin B_T) is a substance of wide occurrence in different food products of animal origin, but it is not a vitamin for mammals because they can synthesize it from γ -butyrobetaine. Carnitine is a true vitamin for insects (e.g., mealworm). Animal products like meat, fish, poultry, and milk are the best sources, especially red meat—the redder the meat, the higher its carnitine content. Dairy products contain carnitine primarily in the whey fraction, in small amounts relative to red meat. The daily human requirement in carnitine is about 500 mg.

Chemical nature and biologically active forms

Carnitine (β -hydroxy- γ -*N*-trimethylaminobutyric acid, 3-hydroxy-4-*N,N,N*-trimethylaminobutyrate) is a quaternary ammonium compound (Karlic and Lohninger 2004) involved in metabolism in most mammals, plants, and some bacteria (Bremer 1983). Carnitine may exist in two isomers, labeled *D*-carnitine and *L*-carnitine, as they are optically active (Fig. 5.26). At room temperature, pure carnitine is a white powder, and a water-soluble zwitterion with low toxicity. Carnitine only exists in animals as the *L*-enantiomer, and *D*-carnitine is toxic because it inhibits the activity of *L*-carnitine. Carnitine, discovered in 1905, was originally labeled vitamin B_T; as carnitine is synthesized in the human body, it is no longer considered a vitamin (Bremer 1983). Carnitine can be synthesized by most humans; about 1 in 350 males is unable to synthesize it due to genetic causes on the X chromosome (Patrícia et al. 2012). Carnitine is involved in the oxidation of fatty acids, and involved in systemic

primary carnitine deficiency. It has been studied for preventing and treating other conditions, and is used as a purported performance-enhancing drug (Karlic and Lohninger 2004). Figure 5.26 shows structure of orotic acid, S-methylmethionine, L-carnitine, and D-carnitine.

Biochemical functions

Carnitine is involved in transporting fatty acids across the mitochondrial membrane, by forming a long chain acetylcarnitine ester and being transported by carnitine palmitoyltransferase I and carnitine palmitoyltransferase II (Flanagan et al. 2010). Carnitine also plays a role in stabilizing acetyl-CoA and coenzyme A levels through the ability to receive or give an acetyl group. Some research has been carried out on carnitine supplementation in athletes, given its role in fatty acid metabolism; the carnitine content of seminal fluid is directly related to sperm count and motility, suggesting that the compound might be of value in treating male infertility.

Carnitine biosynthesis is chiefly accomplished in the liver. The initial step is methylation of proteinic lysine producing e-N-trimethyllysine. Then, the lysine carbon chain is shortened by cleaving off the first and the second carbon atoms to form y-butyrobetain. In the hepatic cell sap, the hydroxylation of y-butyrobetain with the participation of y-butyrobetainhydroxy-lase leads to carnitine.

L-Carnitine is the biologically active agent. Carnitine takes part in the transfer of fatty acid, long-chained acyls and acetyl groups across the membrane lipid layer of mitochondria and, possibly, other organelles. For this reason, it exerts a pronounced influence on fatty acid oxidation and energy generation as well as on the utilization of mitochondrial acetyl residues in biochemical cytoplasmic reactions. Other instances of carnitine activity may also be envisioned. There has been evidence that carnitine stimulates the exocrine function of pancreas and exerts a favorable effect on spermatogenesis and mobility of spermatozoa. When carnitine is administered to animals, it enhances the energy generation in the respiratory chain of mitochondria of various organs and stimulates regenerative processes in the affected myocardium and in the spermatogenic epithelium of small seminiferous tubules.

There have been described cases of carnitine deficiency in humans associated with affected skeletal muscles. A distinct decrease in carnitine concentrations in the muscles has been observed, concomitant with muscular debility, dystrophy, and thinning of nerve fibers. Large doses of carnitine alleviate the course of this disease. Dietary deficiency of lysine depletes the supply of organism with carnitine.

In medical practice, carnitine is a novel medicament, and in many instances its therapeutic effects await further elucidation. It is used for stimulating muscular activity, exocrine secretion of pancreas, and in treating myocardial dystrophic processes.

Deficiency

Carnitine deficiency caused by a genetic defect in carnitine transport occurs in roughly 1 in 50,000 in the US. Systemic primary carnitine deficiency (SPCD) is characterized by various cardiological, metabolic, and musculoskeletal symptoms that vary widely in age of onset and presentation. Prognosis is generally good with

carnitine supplementation (Magoulas and El-Hattab 2012). Secondary carnitine deficiency may occur due to conditions such as malnutrition, poor absorption, or access to only vegetables (Flanagan et al. 2010).

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin lipoic acid

Source: Lipoic acid (vitamin N) is supplied to the organism with food. α -Lipoic acid (LA) is also synthesized in small amounts by humans. Lipoic acid is present in almost all foods, but meat, kidney, heart, liver, milk, spinach, broccoli, and yeast are relatively rich in lipoic acid (Lodge et al. 1997; Durrani et al. 2010). Naturally occurring lipoic acid is always covalently bound and not readily available from dietary sources. The human requirement in lipoic acid is as yet unknown. LA appears physically as a yellow solid and structurally contains a terminal carboxylic acid and a terminal dithiolane ring. For use in dietary supplement materials and compounding pharmacies, the USP has established an official monograph for a racemic mixture (*R/S*)-lipoic acid (*R/S*-LA).

Chemical nature and biologically active forms

Lipoic acid (LA), also known as α -lipoic acid and alpha-lipoic acid (ALA) and thioctic acid is an organosulfur compound derived from caprylic acid (octenoic acid) (Fig. 5.27). Lipoic acid (LA), also known as α -lipoic acid (Shay et al. 2008) and alpha-lipoic acid (ALA) and thioctic acid (Reljanovic et al. 1999), is an organosulfur compound derived from octanoic acid. LA contains two sulfur atoms (at C6 and C8) connected by a disulfide bond and is thus considered to be oxidized although either sulfur atom can exist in higher oxidation states. Lipoate is the conjugate base of lipoic acid, and the most prevalent form of LA under physiologic conditions. The carbon atom at C6 is chiral, and the molecule exists as two enantiomers (*R*)-(+)-lipoic acid (RLA) and (*S*)-(−)-lipoic acid (SLA) and as a racemic mixture (*R/S*)-lipoic acid (*R/S*-LA).

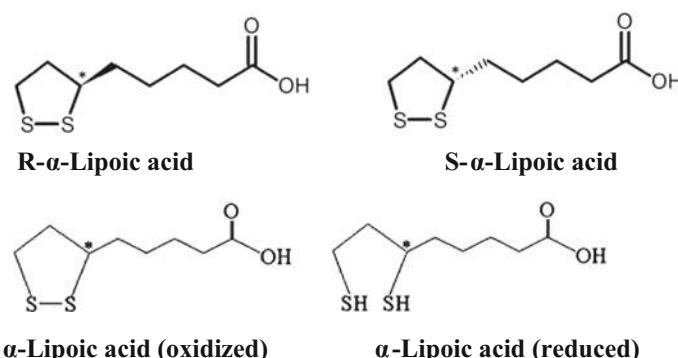


Fig. 5.27 Showing structure of R- α -lipoic acid (RLA) and S- α -Lipoic acid (SLA) and structure of oxidized and reduced forms of R- α -lipoic acid (RLA); their chiral centers with asterisk (*)

Only the (*R*)-(+)-enantiomer (RLA) exists in nature and is essential for aerobic metabolism because RLA is an essential cofactor of many enzyme complexes (Raddatz and Bisswanger 1997). LA contains two thiol (sulfur) groups, which may be oxidized or reduced (Fig. 5.27). The reduced form is known as dihydrolipoic acid (DHLA), while the oxidized form is known as LA (Kramer and Packer 2001).

Biochemical functions

ALA is made in animals normally and is essential for aerobic metabolism. Lipoic acid is cofactor for at least five enzyme systems, two of these are in the citric acid cycle. One of the most studied roles of RLA is as a cofactor of the pyruvate dehydrogenase complex (PDC or PDHC), though it is a cofactor in other enzymatic systems. It is also manufactured and is available as a dietary supplement in some countries where it is marketed as an antioxidant, and is available as a pharmaceutical drug in other countries. Lipoic acid when supplied to the tissues becomes covalently bound to the lysine NH₂ group of an active center of apoenzymes of lipoic enzymes. These include multienzyme complexes involved in the oxidative decarboxylation of pyruvate, 2-oxoglutarate, and other α -keto acids. Lipoic acid deficiency in humans has never been reported.

In addition to protein-bound LA, there is increasing scientific and medical interest in potential therapeutic uses of pharmacological doses of free LA (Smith et al. 2004). In medical practice, preparations of lipoic acid or its amide are used in the therapy of affected liver, diabetes mellitus, heavy metal poisoning, etc. The application of lipoic acid is commonly believed to favor the oxidation of carbon metabolism intermediates and improves the cell energetics. Although LA is a potent antioxidant in the test tube, LA supplementation may affect health by stimulating glutathione synthesis, enhancing insulin signaling, and modulating the activity of other cell signaling molecules and transcription factors.

Deficiency symptoms and practical application

LA deficiency has not been described, suggesting that humans are able to synthesize enough to meet their needs for enzyme cofactors (Biewenga et al. 1997).

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin pangamic acid

Source: Pangamic acid (vitamin B₁₅) occurs in many food sources including seeds, nuts, brown rice, brewer's yeast, pumpkin seeds, and sesame seeds. However, its human requirement is unknown and there is much confusion surrounding vitamin B₁₅ as it is not a true vitamin, has no nutritional value, has no known use in the treatment of any disease although a number of compounds labeled "pangamic acid" have been studied or sold as if a "quack remedy." Its status as vitamin is embroiled in controversy.

Chemical nature, biologically active forms

The Krebses derived the term “pangamic” from *pan*—universal and *gamic*—seed to describe the compound ubiquitous in seeds (Herbert and Herbert 1981). The chemical structure of pangamic acid has not been elucidated unambiguously (Fig. 5.28). Pangamic acid or pangamate is the name given to the chemical compound with the empirical formula $C_{10}H_{19}O_8N$ and a molecular weight of 281 which appeared to be an ester derived from D-gluconic acid and *N*-dimethylglycine (D-gluconodimethylamino acetic acid).

Biochemical functions

Pangamic acid, similar to methionine, serves as a source of transferable methyl groups. It participates in the biosynthesis of methylated species (choline, choline phosphatides, creatine, and others). In medical practice, pangamic acid preparations are used as a lipotropic agent in the therapy of adipose infiltration of the liver, atherosclerosis, and certain other diseases (including cancer, heart disease, schizophrenia, tolerance to hypoxia, etc.); however, basic biochemical indications for the application of this drug are not always clear. The Krebses’ original patent claimed pangamic acid or vitamin B₁₅ could be used for detoxification as well as treatment of asthma, skin conditions, joint pain, and nerve pain; but none of these claims was supported by clinical evidence. There is no evidence that it meets the definition of a vitamin as there is no evidence it is a nutrient needed by the body (Herbert 1979). Early promotion for pangamic acid included use by racehorses as well as humans.

Deficiency symptoms and practical application

There is no evidence to suggest that a lack of pangamic acid has any adverse effects. It has been called a quack remedy (Herbert 1979).

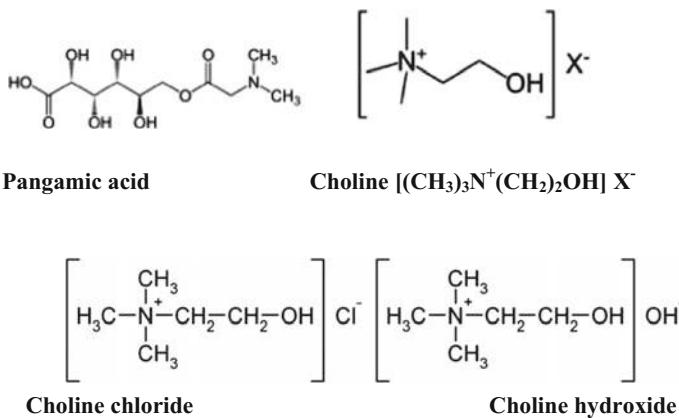


Fig. 5.28 Showing structure of pangamic acid, choline, choline chloride, and choline hydroxide

Source, chemical nature, biologically active forms, biochemical functions, deficiency symptoms, and practical application of vitamin choline

Source: Choline, also named as vitamin B₄, is a water-soluble vitamin-like essential nutrient (Zeisel and da Costa 2009). Choline is supplied to the human organism in food, e.g., meat, beef liver, chicken, egg, codfish, milk, firm tofu, spinach, wheat germ, soybean, sunflower seeds, kidney beans, peanuts, and almonds, and food-stuffs produced from cereals are rich in choline. Cruciferous vegetables including cauliflower other may also be good sources of choline (Gossell-Williams et al. 2005). Some choline is produced by the intestinal microflora. The recommended daily intake of choline for the adult human is 250–600 mg.

Chemical nature and biologically active forms

Choline refers to the class of quaternary ammonium salt containing the *N,N,N*-trimethylethanolammonium cation with the chemical formula $[(CH_3)_3N^+(CH_2)_2OH] X^-$, where X^- on the right denotes an undefined counterion such as chloride in choline chloride, hydroxide in choline hydroxide or tartrate in choline tartrate, etc. (Figure 5.28) It is a basic constituent of lecithin, which is present in many plants and animal organs.

Biochemical functions

In the cells, choline participates in the synthesis of phosphatides and acetylcholine and acts as a methyl group donor in transmethylation reactions. The choline cation appears in the head groups of cell membrane phospholipids such as phosphatidylcholine and sphingomyelin. Choline and its metabolites play structural integrity and signaling roles for cell membranes, cholinergic neurotransmission (acetylcholine synthesis), and acetylcholine is involved in many functions including memory and muscle control. Choline is a major source for methyl groups via its metabolite, trimethylglycine (betaine) that participates in the S-adenosylmethionine (SAMe) synthesis pathways (Anthony et al. 1993; Glier et al. 2014).

Humans make choline in the liver but some animals get it through diet to remain healthy as they cannot produce choline. Whether dietary or supplemental choline is beneficial or harmful to humans has not been determined (Leermakers et al. 2015), but as claim goes, the benefits include reducing the risk of neural tube defects and fatty liver disease. It has also been found that intake of choline during pregnancy can have long-term beneficial effects on memory for the child (Zeisel and da Costa 2009).

Deficiency symptoms and practical application

In humans, choline deficiency has never been recorded. In experimental animals, choline deficiency manifests itself as adipose infiltration of the liver and other disturbances of lipid synthesis. In medical practice, choline-based preparations are used in the therapy of the liver affected by various diseases and intoxications. Table 5.3 summarizes the information on sources and functions of water-soluble vitamins, and Table 5.4 gives information on coenzyme and cofactor' function, additional component, chemical group (s) transferred, and distribution of water-soluble vitamins and derivatives.

Table 5.3 The table summarises the information on sources and functions of water-soluble vitamins

Water-soluble vitamins		
Name	Sources	Function
Thiamine (vitamin B ₁)	Found in all nutritious foods in moderate amounts: pork, whole-grain or enriched breads and cereals, legumes, nuts and seeds	Part of an enzyme needed for energy metabolism; important to nerve function
Riboflavin (vitamin B ₂)	milk and milk products; leafy green vegetables; whole-grain, enriched breads and cereals	Part of an enzyme needed for energy metabolism; important for normal vision and skin health
Niacin (vitamin B ₃)	Meat, poultry, fish, whole-grain or enriched breads and cereals, vegetables (especially mushrooms, asparagus, and leafy green vegetables), peanut butter	Part of an enzyme needed for energy metabolism; important for nervous system, digestive system, and skin health
Pantothenic acid	Widespread in foods	Part of an enzyme needed for energy metabolism
Biotin	Widespread in foods; also produced in intestinal tract by bacteria	Part of an enzyme needed for energy metabolism
Pyridoxine (vitamin B ₆)	Meat, fish, poultry, vegetables, fruits	Part of an enzyme needed for protein metabolism; helps make red blood cells
Folic acid	Leafy green vegetables and legumes, seeds, orange juice, and liver; now added to most refined grains	Part of an enzyme needed for making DNA and new cells, especially red blood cells
Cobalamin (vitamin B ₁₂)	Meat, poultry, fish, seafood, eggs, milk and milk products; not found in plant foods	Part of an enzyme needed for making new cells; important to nerve function
Ascorbic acid (vitamin C)	Found only in fruits and vegetables, especially citrus fruits, vegetables in the cabbage family, cantaloupe, strawberries, peppers, tomatoes, potatoes, lettuce, papayas, mangoes, kiwifruit	Antioxidant; part of an enzyme needed for protein metabolism; important for immune system health; aids in iron absorption

Antivitamins and their mechanism of action

Antivitamins are a term applied to vitamin analogs that act as anticoenzymes (Table 5.5). Antivitamins replace coenzymes (vitamin derivatives) but cannot perform the functions of the latter in enzymic reactions. The concept of antivitamins was proposed in 1940 by Wood upon his discovery of the ability of *p*-aminobenzoic acid to counteract the bacteriostatic effect of sulphanilamide. In a broader sense, the term antivitamins were proposed for any agents capable of inactivating or limiting the action of vitamins in the organism. However, the involvement of certain vitamins in biochemical interactions may evoke a deficit of other vitamins. Viewed from the standpoint of broader sense of the term, many vitamins may formally be characterized as antivitamins, which will inevitably result in confusion of notions.

Table 5.4 Coenzyme and cofactor's function, additional component, chemical group (s) transferred and distribution of water-soluble vitamins and derivatives

Coenzyme & cofactor	Vitamin	Additional component	Chemical group (s) transferred	Distribution
Thiamine pyrophosphate, TPP	Thiamine (B ₁)	None	Aldehyde group transfer, α cleavage	Bacteria, archaea, and eukaryotes
Thiamine pyrophosphate, TPP	Thiamine (B ₁)	None	Aldehyde group transfer, α cleavage	Bacteria, archaea, and eukaryotes
NAD ⁺ and NADP ⁺	Niacin (B ₃)	ADP	oxidation or hydrogen transfer, electron–proton transfer	Bacteria, archaea, and eukaryotes
Pyridoxal phosphate	Pyridoxine (B ₆)	None	Amino and carboxyl groups	Bacteria, archaea, and eukaryotes
Lipoamide	Lipoic acid	None	electrons, acyl groups	Bacteria, archaea, and eukaryotes
Methylcobalamin	Vitamin B ₁₂	Methyl group	acyl groups	Bacteria, archaea, and eukaryotes
Cobalamine	Cobalamine (B ₁₂)	None	hydrogen, alkyl groups	Bacteria, archaea, and eukaryotes
Biotin	Biotin (H)	None	CO ₂	Bacteria, archaea, and eukaryotes
Coenzyme A	Pantothenic acid (B ₅)	ADP	Acetyl and acyl groups	Bacteria, archaea, and eukaryotes
Tetrahydrofolic acid	Folic acid (B ₉)	Glutamate residues	Methyl, formyl, methylene and formimino groups	Bacteria, archaea, and eukaryotes
Menaquinone	Vitamin K	None	Carbonyl group and electrons	Bacteria, archaea, and eukaryotes
Ascorbic acid	Vitamin C	None	Electrons	Bacteria, archaea, and eukaryotes
Flavinmononucleotide, FMN	Riboflavin (B ₂)	None	oxidation or hydrogen transfer, electron–proton transfer	Bacteria, archaea, and eukaryotes

(continued)

Table 5.4 (continued)

Coenzyme & cofactor	Vitamin	Additional component	Chemical group(s) transferred	Distribution
Flavin adenine dinucleotide, FAD	Riboflavin (B_2)	None	oxidation or hydrogen transfer, electron–proton transfer	Bacteria, archaea and eukaryotes
Coenzyme F420	Riboflavin (B_2)	Amino acids	Electrons	Methanogens and some bacteria

The specific anticoenzymic action of antivitamins has enabled their wide use in practice for eliciting experimental avitaminoses in animals and for treating bacterial infections and tumoral diseases.

Therapeutic uses of vitamins

Vitamins are a group of organic compounds occurring naturally in food and are necessary for good health. Lack of a vitamin may lead to a specific deficiency syndrome, which may be primary (due to inadequate diet) or secondary (due to malabsorption or to increased metabolic need), and it is rational to use high-dose vitamin supplementation in situations where these clinical conditions exist. However, pharmacological doses of vitamins are claimed to be of value in a wide variety of conditions which have no, or only a superficial, resemblance to the classic vitamin deficiency syndromes. The enormous literature on which these claims are based consists mainly of uncontrolled clinical trials or anecdotal reports (a report of a single case of a disease/incomplete description). Only a few studies have made use of the techniques of randomization and double blinding. Evidence from such studies reveals a beneficial therapeutic effect of vitamin E in intermittent claudication (impairment in walking, or pain, discomfort or tiredness in the legs) and fibrocystic breast disease and of vitamin C in pressure sores, but the use of vitamin A in acne vulgaris (cystic acne or simply acne is a common human skin disease), vitamin E in angina pectoris, hyperlipidaemia and enhancement of athletic capacity, of vitamin C in advanced cancer, and niacin in schizophrenia has been rejected. Evidence is conflicting or inconclusive as to the use of vitamin C in the common cold, asthma and enhancement of athletic capacity, of pantothenic acid in osteoarthritis, and folic acid (folacin) in neural tube defects. Most of the vitamins have been reported to cause adverse effects when ingested in excessive doses. It is therefore worthwhile to consider the risk–benefit ratio before embarking upon the use of high-dose vitamin supplementation for disorders where proof of efficacy is lacking.

Table 5.5 Antivitamins, their mechanism of action, and practical application of Antivitamins

Vitamins	Antivitamins	Mechanism of antivitaminic action	Practical application of Antivitamins
Naphthoquinones, vitamin K	Coumarins (dicumarol, warfarin, etc.)	Antivitamins replace Naphthoquinones in biochemical processes and block formation of prothrombin, proconvertin, and other blood clotting factors in liver by exerting anticoagulative action in blood	Used in prophylaxis and medication of thrombosis in various diseases
Niacin, B ₅ , PP	Isoniazid (nicotinic acid hydrazide) and its derivatives	Antivitamins are substituted for nicotinamide into NAD and NADP structures to form pseudovitamins incapable of participating in redox and other reactions (replication and repair). This action is manifested in cells accessible to invasion of antivitamins, e.g., tubercle bacillus	Used in treatment of tuberculosis because of tuberculostatic effect
Folacin (folic acid)	Pteridines (aminopterin, methotrexate or methotrexate)	Antivitamins replace folic acid in folatedependent enzymatic reactions, blocking thereby synthesis of nucleotides and nucleic acids, which shows up in cell division inhibition. Most pronounced action is observed on proliferating cells	Used in treatment of leucoses (inhibition of extensive formation of affected leucocytes in marrow) and tumors (inhibition of tumoral cell division)
p-Aminobenzoic acid (PABA)	Sulphanilamides and their derivatives (sujphathiamide, phthalsulphathiazole, sulphamethoxypyridazine, and others)	Antivitamins are substituted for PABA into the folic acid structure synthesized in microorganisms, and block the function of folic acid coenzymes, i.e., inhibiting ultimately proliferation of sulphanilamide sensitive microorganisms	Used in treatment of infectious diseases
Thiamine (B ₁) Riboflavin (B ₂)	Hydroxythiamine, pyritthiamine Dichlororiboflavin	Antivitamins replace thiamine coenzymes in enzymic reactions and,	Used experimentally to initiate thiamine insufficiency

(continued)

Table 5.5 (continued)

Vitamins	Antivitamins	Mechanism of antvitaminic action	Practical application of Antvitamins
		presumably, in neuromediatory processes Antvitamins replace riboflavin coenzymes in enzymic reactions conducive to riboflavin deficiency	Used experimentally to initiate hypo and ariboflavinoles
Pyridoxine (B ₆)	Deoxypyridoxine	Antivitamins replace pyridoxal coenzymes in enzymic reactions and produces pyridoxine deficiency states	Used experimentally to produce pyridoxine insufficiency
Pantothenic acid (B ₅)	Homopantothenic acid, w-methylpant-tothenic acid	Antivitamins replace pantothenic coenzymes in enzymic reactions and initiate pantothenic acid deficiency in the organism	Used experimentally to produce pantothenic insufficiency

5.2 Natural Sources, Classification, Chemistry, and Therapeutic Use of Nutraceuticals, Food Additives and Excipients (e.g., Coloring, Flavoring, Emulsifying and Suspending Agents, Diluents, Bulking or Filler Agents, Disintegrants, Sweeteners, Binders, Adhesives, Solidifiers, etc.)

Natural sources, classification, chemistry, and therapeutic use of nutraceuticals

The term “nutraceutical” was coined from “nutrition” and “pharmaceutical” in 1989 by Stephen L. De Felice (Jack 1995, Mannion 1998) and was originally defined as “a food (or part of the food) that provides medical or health benefits, including the prevention and/or treatment of a disease” (Brower 1998, Kalra 2003, Trottier 2010). A nutraceutical is a pharmaceutical-grade and standardized natural nutrient-rich food (e.g., spirulina, garlic, soy, etc.) or a specific component of a food (e.g., omega-3 fatty acids, lycopene, saponins, etc.). They are also known as medical foods, “designer” foods, nutritional supplements, and dietary supplements, and include a number of substances ranging from natural diets, herbal products, bio-fortified crops, genetically modified and processed food products such as cereals, soups, beverages, etc. Human diet offers a greater and more diverse group of plant bioactives compared to drugs, and many drugs have been derived from the plant food compounds. Nutraceuticals can deliver benefits beyond basic nutrition and provide health benefit, modulate immunity and/or prevent, and cure specific diseases. The ability of nutraceuticals to influence chronic diseases (e.g., diabetes,

cancers, etc.) has been recognized and they will play important role in future therapeutic development. In addition, it is also claimed that nutraceuticals delay the aging process, increase life expectancy, or support the structure or function of the body. Herbal nutraceutical is useful in maintaining health, and it works against nutritionally induced acute and chronic diseases, promotes optimal health, longevity, and quality of life (Chauhan et al. 2013).

Nutraceuticals have received considerable interest because of their presumed safety and potential nutritional and therapeutic effects (Rajasekaran et al. 2008). Nutraceuticals include both functional foods and dietary supplements as two major classes. People can improve their health by supplementation and by consuming foods that have been formulated or fortified. The vitamin B-enriched flour protects pellagra and vitamin D-enriched milk are effective in eliminating rickets and iodine-fortified salt decreases incidences of goitre. Commercial nutraceuticals have to pass through strict regulatory controls for quality and positive health impact.

The term functional foods, first introduced in Japan, include processed foods containing nutritious ingredients that support healthy body functions. A functional food with new ingredients gives an additional function or enhanced benefit to human health beyond the basic nutritional value. Functional foods differ from medical foods and prescription drugs. Functional foods could be consumed freely as part of everyday life, whereas medical foods and prescription drugs are consumed when recommended by medical professionals. Functional foods are used in energy enhancement, weight management, bolster gut, bone or heart health, disease risk reduction, memory improvement, etc.; medical foods are used in dietary management of a disease or condition with distinctive nutritional requirements, while prescription drugs are used in the treatment of disease, symptom, or condition.

Sources of nutraceuticals

Plant, animal, and microbial sources are important for nutraceuticals. Fenugreek, garlic, soybeans, and other legumes, citrus fruit, pepper fruit, tomatoes, cruciferous vegetables, oat bran, whole grains, berries, turmeric, strawberries, tree nuts, olive oil, cocoa, flax, *Cordyceps militaris* fungus, etc., and fish oils, beef, yogurt and other dairy products, etc., constitute important plants and animal sources, respectively, for nutraceutical bio-metabolites with therapeutic importance. Conjugated linoleic acid (CLA) was first isolated from beef which is an anticarcinogenic fatty acid. Bioactive compounds in nutraceuticals of plant origin include α -glucan, ascorbic acid, γ -tocotrienol, quercetin, gallic acid, indole-3-carbonol, monounsaturated fatty acids (MUFAs), pectin, daidzein, glutathione, allicin, δ -limonene, genistein, luteolin, lycopene, β -carotene, β -ionone, zeaxanthin, capsaicin, geraniol, α -tocopherol, nordihydrocapsaicin, hemicelluloses, lignin, minerals including potassium and selenium; bioactive compounds in nutraceuticals of animal sources include polyunsaturated fatty acids (PUFAs), conjugated linoleic acid (CLA), Coenzyme Q10, eicosapentaenoic acid (EPA), docosahexenoic acid (DHA), sphingolipids, choline, creatine, minerals including calcium, selenium, zinc, etc., and dairy products or fermented dairy products (probiotics); while microbial sources

include *Saccharomyces boulardii* (yeast), *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Streptococcus salarius* and many other prebiotic, probiotic, and symbiotic organisms. Probiotics include both microorganisms and functional foods (e.g., yogurt). Probiotic microorganisms provide health benefits to the host animal by improving its intestinal microorganisms, while probiotic functional foods can alter and modify preexisting intestinal flora, and these probiotics are known for their anticarcinogenic, hypocholesterolemic, and antagonistic actions against gut pathogens. Probiotics are used in colon cancer risk reduction as they (e.g., lactic acid bacteria) are able to alter the activity of fecal enzymes, e.g., β -glucuronidase, azoreductase, nitroreductase, etc., which plays a role in the development of colon cancer. Prebiotics are “nondigestible food ingredients” including starches, dietary fibers, sugar alcohols, oligosaccharide, etc., which beneficially affect the host by selectively stimulating the growth and activity of one or a few number of bacteria in the colon to improve host health. Prebiotics as food additives are valuable for functional foods and also helps in preventing diet-related diseases. Prebiotics oligosaccharides found naturally in many fruits and vegetables and have received great attention for their health benefits. A mixture of pro- and prebiotics develops the prebiotic concept of the symbiotics. Fermented dairy products are considered as functional foods as they are rich in calcium and many other components (probiotics) and can be useful in preventing osteoporosis, hypertension, colon cancer, etc. Probiotics are important for the improvement and modification of intestinal microflora of the host organisms.

Classification of nutraceuticals

Nutraceuticals are classified as traditional or natural nutraceuticals (e.g., nutrients, herbals, phytochemicals, probiotic microorganisms, nutraceutical enzymes, etc.) and nontraditional or artificial nutraceuticals (e.g., fortified and recombinant nutraceuticals).

1. Traditional Nutraceuticals

Traditional nutraceuticals are simply natural with no changes to the food. Food contains several natural components that deliver benefits beyond basic nutrition, such as lycopene in tomatoes, omega-3 fatty acids in salmon or saponins in soy. Traditional nutraceuticals are grouped on the basis of (a) Chemical constituents: (i) Nutrients, (ii) Herbals, (iii) Phytochemicals; (b) Probiotic microorganisms; (c) Nutraceutical enzymes.

(a) Chemical constituents

(i) Nutrients

Substances such as vitamins, minerals, amino acids (cysteine), and fatty acids with established nutritional functions. Most vegetables, whole-grain cereals, millets, fruits, as well as animal products such as dairy products, fish, meat, poultry, etc., contain vitamins and other bioactive compounds of

therapeutic value are helpful in curing heart diseases, stroke, cataracts, osteoporosis, diabetes, cancer, and many other ailments. Minerals like Zn, Cu, Fe, Mn, Ca, etc., found in plant, animal, and dairy products, are useful in osteoporosis, anemia and build strong bones, teeth, muscles, improve nerve impulses and heart rhythm. Flax seed and salmon contain fatty acids omega-3 PUFAs, and are potent controllers of the inflammatory processes, maintenance of brain function, and reduce cholesterol deposition.

(ii) **Herbals**

Nutraceuticals holds a great promise to improve health and prevent chronic diseases with the help of herbals (Fig. 5.29). Some examples are willow bark (*Salix nigra*), having active component as salicin, which is anti-inflammatory, analgesic, antipyretic, astringent, and anti-arthritis (Ehrlich 2008). *Brassica* vegetables including cabbage (cabbage, Chinese cabbage, broccoli), watercress, horseradish, capers, and radishes are rich sources of glucosinolates, flavonoids, vitamins, and mineral nutrients, and they appear to be useful in protecting humans against cancer, skin diseases, and health-promoting purposes (Moreno et al. 2006). Glucosinolates, a large group of sulfur-containing glucosides, occur as secondary metabolites of almost all plants of the order Brassicales. Black kale was recently identified as one of the greatest sources of glucosinolates (Becerra-Moreno et al. 2014; De Nicola et al. 2014). Like glucosinolates, isothiocyanates are also sulfur-containing phytochemicals (general formula R-NCS) with the strongest anticancer effects (e.g., phenylethylisothiocyanate, benzylisothiocyanate, and 3-phenylpropylisothiocyanate). Isothiocyanates occur naturally as glucosinolate conjugates in cruciferous vegetables such as broccoli, cauliflower, kale, turnips, collards, Brussels sprouts, cabbage, radish, turnip, and watercress. Glucosinolates are precursors of isothiocyanates and enzyme myrosinase hydrolyses the glucosinolates into glucose and isothiocyanates. Parsley (*Petroselinum crispum*) contains flavonoids (apiole, psoralen) and is diuretic, carminative, and antipyretic. Peppermint (*Mentha piperita*) contains menthol as an active component and cures cold and flu (Ehrlich 2009). Lavender (*Lavandula angustifolia*) contains tannin which is helpful in curing depression, hypertension, stress, cold, cough, and asthma. Cranberries (*Vaccinium erythrocarpum*) contain proanthocyanadin and are found to be useful in cancer, ulcers, and urinary tract infections. Strawberries, a rich source of phytochemicals flavonoids, antioxidants, and vitamins, are considered as functional food for their preventive and therapeutic health benefits anti-inflammatory, antihyperlipidemic, antihypertensive, or antiproliferative effects principally via downregulation of NF- κ B (nuclear factor kappa-light chain enhancer of activated B cells) activity. NF- κ B is a protein complex that controls transcription of DNA, cytokine production, and cell survival.

Isothiocyanates such as sulforaphane (SFN), phenethyl isothiocyanate (PEITC), and benzyl isothiocyanate (BITC) were found highly effective in

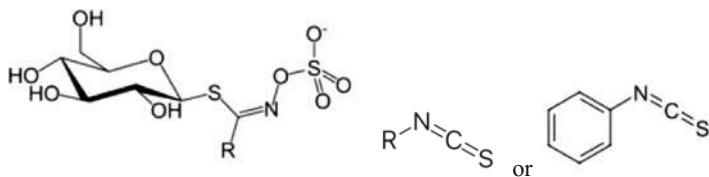
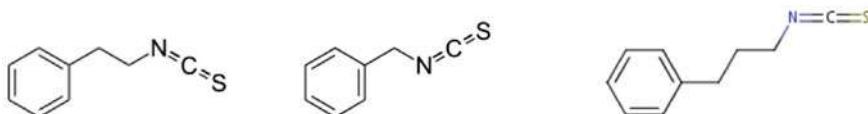
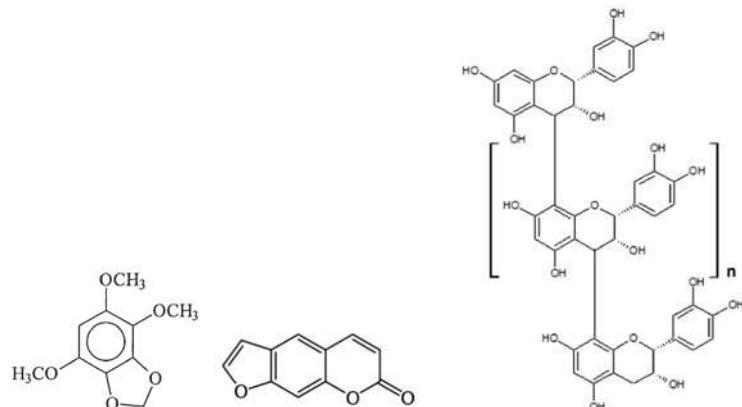
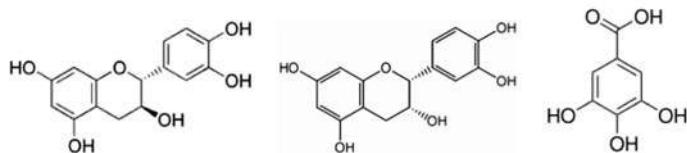
**Glucosinolate****Isothiocyanate; side group *R* varies****Phenylethylisothiocyanate Benzylisothiocyanate 3-Phenylpropyl isothiocyanate****Apiol****Psoralen****Proanthocyanidins****Catechin****Epicatechin****Gallic acid**

Fig. 5.29 Showing structure of herbal nutraceuticals—glucosinolate, Isothiocyanate (side group *R* varies), phenylethylisothiocyanate, benzylisothiocyanate, 3-phenylpropyl isothiocyanate, apiol, psoralen, proanthocyanidins, catechin, epicatechin, and gallic acid

preventing the risk of cancer induced by carcinogens in animal models; and both BITC and PEITC induced apoptosis through ROS, caspase-3, and mitochondrial, and NO signaling pathways (Wu et al. 2011).

Apitol is used for the treatment of menstrual disorders, but it is an irritant and in high doses it is toxic and can cause liver and kidney damage. Psoralen is a derivative of furocoumarins and widely used in PUVA (psoralen plus UVA) therapy that can be used to treat hyperproliferative skin disorders (psoriasis), eczema, vitiligo, and cutaneous T-cell lymphoma (Wu et al. 2005).

Proanthocyanidins (PC), a class of polyphenols, are oligomeric flavonoids (OPC)—oligomers of catechin and epicatechin (building blocks of proanthocyanidins) and their gallic acid esters (oligomeric procyanidins: $n = 0\text{--}5$; polymeric procyanidins: $n > 5$).

(iii) Phytochemicals

Phytochemicals constitute a class of nutraceuticals (Fig. 5.30). Nutraceutical substances of plant-based diet include phytoestrogens, diosgenin, allyl sulfur compounds, isoflavones, quercetin, capsaicinoids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), β -carotene, lycopene, isothiocyanates, β -glucan, catechins, adenosine, curcumin, ellagic acid, cellulose, monounsaturated fatty acids (MUFA), tocopherols, omega-3 and omega-6 fatty acids, inulin, fructooligosaccharides, catechins, lignans, ginseng, cyclic peptides, cordycepin, cephalosporolides C, E and F, pyridine-2,6-dicarboxylic acid, dipicolinic acid, etc. Epidemiological studies and clinical data indicated that a plant-based diet can reduce the risk of various chronic diseases, e.g., cancer and they are very good source of nutraceuticals like proteins, minerals, vitamins, etc., as well as dietary fiber (Block et al. 1992; Charalampopoulos et al. 2002; Truswell 2002).

Phytochemicals are classified on the basis of chemical name given

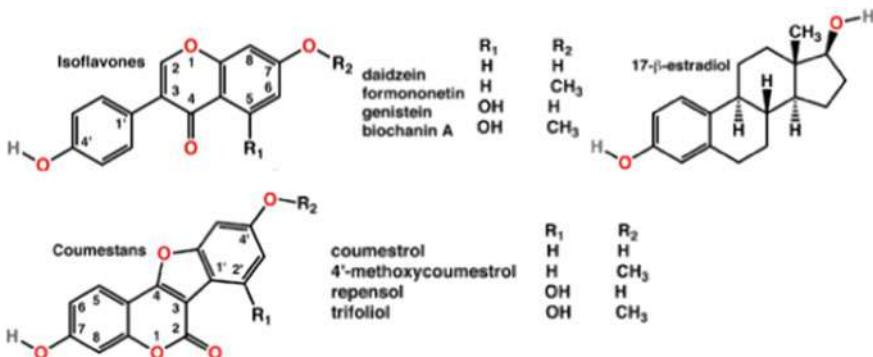


Fig. 5.30 Showing structure of phytochemicals phytoestrogens -daidzenin (left top), formononetin, genistein, biochanin A, and bottom-coumestrol, 4-methoxycoumestrol, repensol, trifolios) and estrogen found in animals: right top-17- β -estradiol

according to their phytochemical properties. For example, Carotenoids (isoprenoids) found in various fruits, vegetables, and egg yolk are anti-carcinogenic, boost natural killer immune cells, and protect cornea against UV light. Legumes (chickpeas and soybeans), grains, and palm oil contain non-carotenoids, which remove cholesterol and are anticarcinogenic. Flavonoid polyphenolics are found in berries, fruits, vegetables, and legumes, which are potent antioxidants, phytoestrogens, prevent breast cancer, prostate cancer, and control diabetes. Non-flavonoid polyphenolics are present in dark grapes, raisins, berries, peanuts, and turmeric roots are strong anti-inflammatory, antioxidants, and effective anticoagulant agents and reduce cholesterol. Phenolic acids, found in blueberries, tomatoes and bell peppers having antioxidant activity, reduce mutagenicity of polycyclic aromatic hydrocarbons. Seeds of Barbarea vulgaris, broccoli contain isothiocyanates (glucosinolates) and have antitumorigenesis activity.

Phytoestrogens or dietary estrogens are a diverse group of nonsteroidal xenoestrogens (xenohormone) derived by eating phytoestrogenic plants and because of their structural similarity with estrogen found in animals, e.g., 17- β -estradiol, they have the ability to cause estrogenic or/and antiestrogenic effects, by fitting in and blocking receptor sites against estrogen (Theresa and James 1996; Yildiz 2005).

Diosgenin is a phytosteroid sapogenin, and diosgenin is precursor of cortisone, pregnenolone, progesterone, and other steroid products. Diosgenin has estrogenic activity (Liu et al. 2005) and can reduce the level of serum cholesterol (Cayen and Dvornik 1979) and may behave as a prodrug to progesterone (Tucci and Benghuzzi 2003; Hajirahimkhan et al. 2013). Allyl sulfur compounds play a major role in the chemoprevention against carcinogenesis and allyl sulfur compounds suppress carcinogen bioactivation. Allyl sulfur components, e.g., diallyl disulfide (DADS), and S-allylcysteine (SAC), have many of the health benefits of garlic. Diallyl disulfide is an efficient agent for detoxification of the cells as well as antimicrobial, insecticidal and larvicidal properties. Garlic can prevent the colorectal cancer and diallyl disulfide is a major component responsible for this action. S-Allyl cysteine (SAC), a derivative cysteine, is a natural constituent of fresh garlic, a potent cholesterol lowering agent and also a chemopreventive. Capsaicinoids, the chemicals which give rise to the heat of chillies, include capsaicin and dihydrocapsaicin as major capsaicinoids (~80–90% of the total capsaicinoid concentration), the rest being made up by nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin. Capsaicin is a vanilloid, and it is a potent inhibitor of substance P, a neuropeptide associated with inflammatory processes. Other health benefits include its ability to provide pain relief, diminish the frequency of cluster headaches, treat psoriasis, manage diabetes, and help you lose weight. Figure 5.31 shows structure of phytochemicals—diosgenin, diallyl disulfide (DADS), S-allylcysteine (SAC), Capsaicin and dihydrocapsaicin.

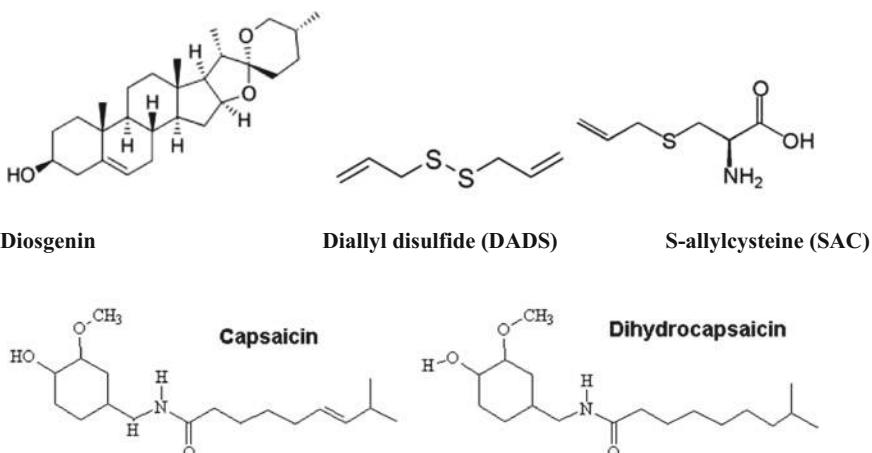


Fig. 5.31 Showing structure of phytochemicals—diosgenin, diallyl disulfide (DADS), *S*-allylcysteine (SAC), capsaicin, and dihydrocapsaicin

EPA and DHA fatty acids are long chain omega-3 fatty acids of cold-water fish such as mackerel, sardines, salmon, herring, halibut, trout, anchovies, and tuna, they are highly unsaturated as they contain six and five double bonds on their long structural chains. EPA and DHA have been associated with fetal development, cardiovascular function, and Alzheimer's disease. EPA and DHA are incorporated in many parts of the body including cell membranes, and play a role in anti-inflammatory processes and in the viscosity of cell membranes.

Cyclic peptides are polypeptide chains (2 to >100 amino-acid residues) containing a circular sequence of bonds such as through (i) a connection between the amino and carboxyl ends of the peptide (e.g., cyclosporin); (ii) a connection between the amino end and a side chain (bacitracin); (iii) the carboxyl end and a side chain (colistin); or (iv) two side chains or more complicated arrangements (amanitin). Many cyclic peptides have been discovered in nature and many others have been synthesized in the laboratory. In nature, they are frequently antimicrobial or toxic; in medicine, they have various applications, for example, as antibiotics and immunosuppressive agents. Figure 5.32 shows structure of phytochemicals fatty acids—EPA (20:5n-3) and DHA (22:6n-3); cyclic peptides—cyclosporin, bacitracin, colistin, and amanitin.

Monounsaturated fatty acids (MUFAs) have one double bond, e.g., palmitoleic acid (16:1*n*-7), *cis*-vaccenic acid (18:1*n*-7), oleic acid (18:1*n*-9), etc. (Figure 5.33) Palmitoleic acid has 16, *cis*-vaccenic acid and oleic acids have 18 carbon atoms with the first double bond at 7 carbon atom in both palmitoleic acid and *cis*-vaccenic acid and at 9 carbon atom in oleic acid. Monounsaturated fats protect against cardiovascular disease by

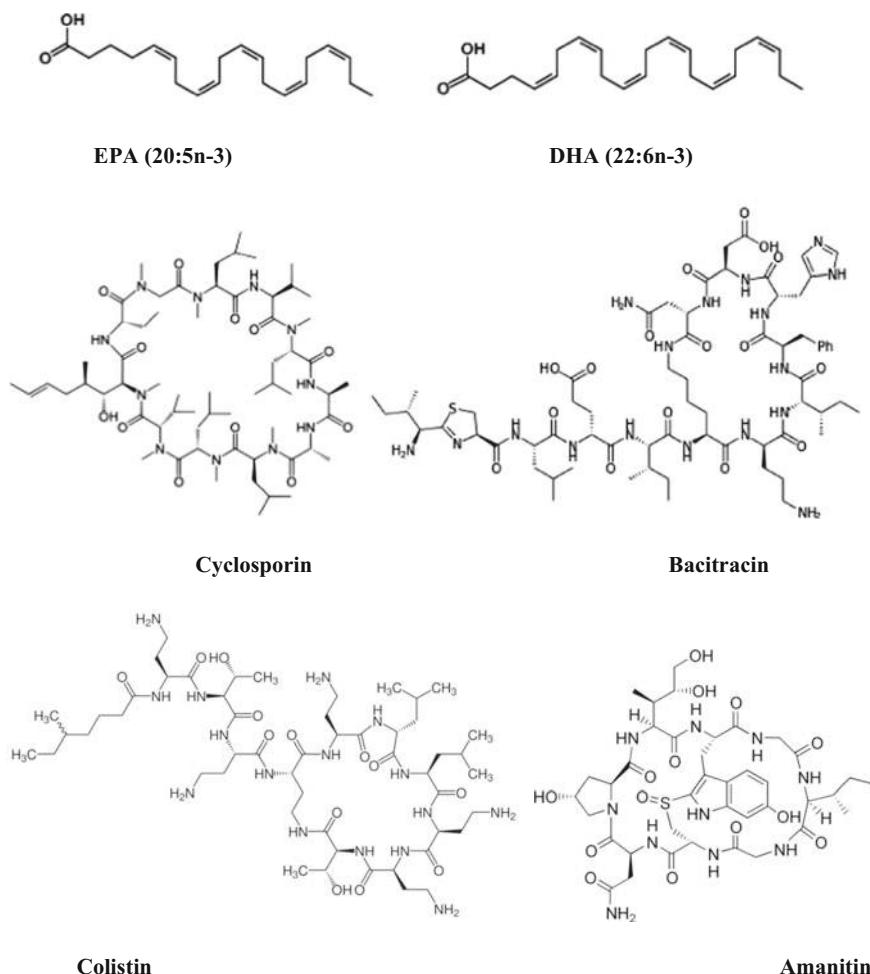


Fig. 5.32 Showing structure of phytochemicals fatty acids—EPA (20:5n-3) and DHA (22:6n-3); cyclic peptides—Cyclosporin, bacitracin, colistin, and amanitin

providing more membrane fluidity than saturated fats, but they are more vulnerable to lipid peroxidation (rancidity). Foods containing monounsaturated fats reduce low-density lipoprotein (LDL) cholesterol, while possibly increasing high-density lipoprotein (HDL) cholesterol. Monounsaturated fats are found in animal flesh (red meat), whole milk products, nuts, and high fat fruits such as avocados, olives, etc., as well as olive oil, avocado oil, macadamia nut oil, grapeseed oil, peanut oil, sesame oil, corn oil, almond oil, sunflower oil, hemp oil, popcorn, and whole grains (wheat, cereal, oatmeal).

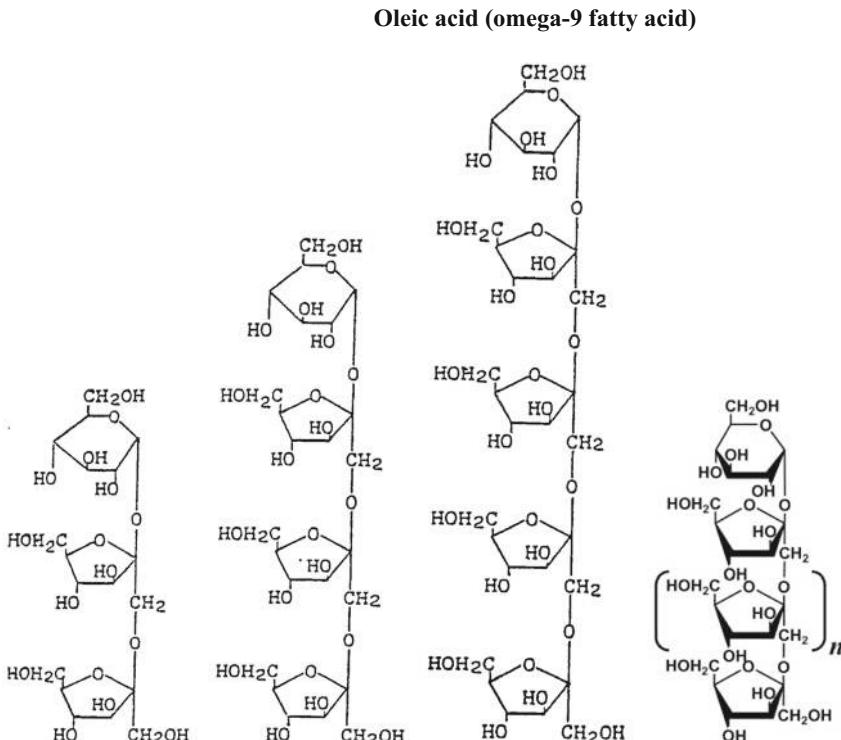
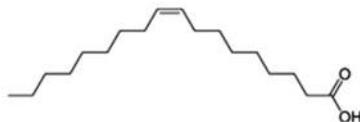
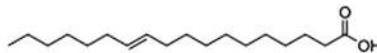
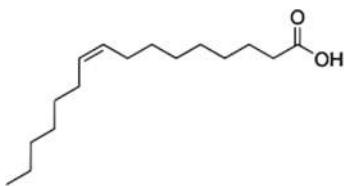


Fig. 5.33 Showing structure of phytochemicals monounsaturated fatty acids—palmitoleic acid (16:1 n -), *cis*-vaccenic acid (18:1 n -7), oleic acid (18:1 n -9), and fructooligosaccharides—Kestose (GF2), Nystose (GF3), Fructofuranosyl nystose (GF4) and Inulin (a fructose polymer). First unit in every polymeric molecule is glucose (G) and the rests are fructoses (F2, F3, F4, Fn, etc.)

Fructooligosaccharides (FOS) are oligosaccharide fructans as shown in Fig. 5.33. An oligosaccharide is a saccharide polymer containing a small number, typically 3–10. Short chains of galactose, mannan molecules, etc., constitute galactooligosaccharides (GOS), mannan oligosaccharides (MOS), etc., respectively. FOSs are used as an alternative low-calorie sweetener and in commercially prepared syrups, sweetness levels vary between 30 and 50% of sugar. Based on inulin degradation or transfructosylation processes, two different classes of fructooligosaccharide (FOS) mixtures are produced commercially. Natural sources include blue Agave, bananas, onions, chicory root, garlic, asparagus, jicama, leeks, etc. Some grains and cereals (wheat, barley) also contain FOSs. The Jerusalem artichoke together with the blue Agave plant has been found to have the highest concentrations of FOS. The main components of commercial products are kestose (GF2), nystose (GF3), fructosylnystose (GF4), bifurcose (GF3), inulobiose (F2), inulotriose (F3), and inulotetraose (F4). Fructooligosaccharides (FOSs) are popular as low-calorie sweetener and have prebiotic effects. FOS are also used for constipation, traveler's diarrhea, and high cholesterol levels. Oligosaccharides are prebiotics that feed the probiotics in intestines and improve gastrointestinal health. Inulin consists of a mixture of oligomers and polymers that belong to the group of glucofructans and occur in plants such as garlic, onion, artichoke, and chicory. The inulin molecules contain from two to more than 60 fructose molecules linked by β -2→1-bonds. Inulin is resistant to digestion in the upper gastrointestinal tract, but is degraded by colonic microflora. Inulin with a high degree of polymerisation was used to prepare biodegradable colon-specific films.

Both catechins and lignans are a large group of plant polyphenols. Plant lignans include enterolignans (e.g., enterodiol, enterolactone), secoisolariciresinol, lariciresinol, 7-hydroxymatairesinol, matairesinol, sesamin, pinoresinol, syringaresinol, medioresinol, etc. Figure 5.34 shows structure of phytochemicals polyphenols catechins and lignans—catechin, enterodiol, enterolactone, secoisolariciresinol, lariciresinol, 7-hydroxymatairesinol, matairesinol, sesamin, pinoresinol, syringaresinol, and medioresinol; and ellagic acid, dipicolinic acid, butyric acid, and butarate, a conjugate base (=butanoic acid).

Tea contains a number of bioactive chemicals and it is rich in catechins (e.g., epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate—EGCG). As natural polyphenols, catechins and derivatives function as antioxidant and provide a variety of health benefits, including the maintenance of cardiovascular health, the reduction of cancer risk and weight loss. Lignans are secondary metabolites present and flax seed and sesame seed contain higher levels of lignans than most other foods. Lignans are one of the major classes of phytoestrogens, which are estrogen-like

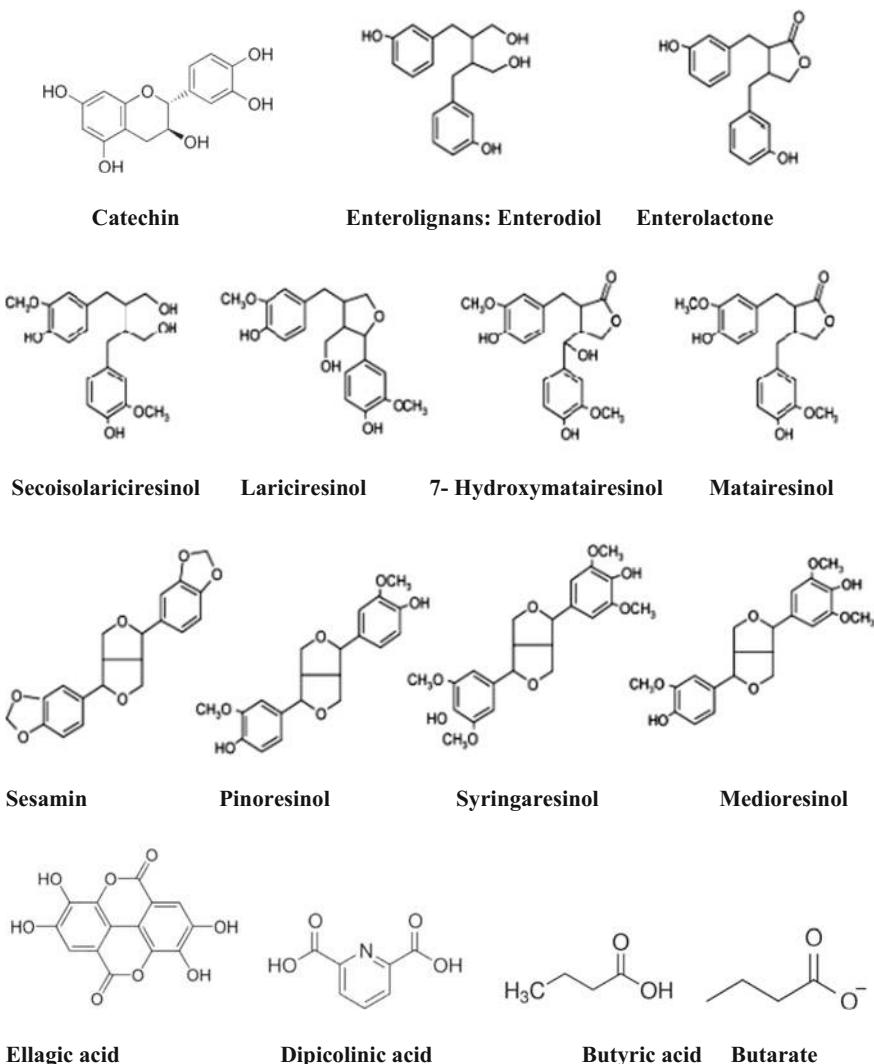


Fig. 5.34 Showing structure of phytochemicals polyphenols catechins and lignans—catechin, enterodiol, enterolactone, secoisolariciresinol, lariciresinol, 7-hydroxymatairesinol, matairesinol, sesamin, pinoresinol, syringaresinol, and medioresinol; and ellagic acid, dipicolinic acid, butyric acid, and butarate

chemicals and also act as antioxidant and anti-inflammatory agents. Cereals (rye, wheat, oat, and barley—rye being the richest source), soybeans, cruciferous vegetables such as broccoli and cabbage, particularly apricots and strawberries are some other sources of lignans.

Ellagic acid is a natural phenol antiproliferative and antioxidant, found in numerous fruits and vegetables (e.g., walnuts, pecans, cranberries, raspberries, strawberries, grapes, peaches, pomegranates, medicinal mushroom *Phellinus linteus*). Dipicolinic acid (pyridine-2,6-dicarboxylic acid or PDC and DPA) is a chemical compound, and composes 5–15% of the dry weight of bacterial spores.

(b) Probiotic microorganisms

Probiotics, for life, are defined as live microorganisms, which when consumed in adequate amounts, confer a health effect on the host (Michail 2006). The scientific interest in probiotics was boosted from the work of Metchnikoff to transform the toxic flora of the large intestine into a host-friendly colony of *Bacillus bulgaricus* (Hord 2008). Health begins in gut and probiotics protect gut health, improve gut microflora (friendly bacteria including *Lactobacillus acidophilus*, *L. bulgaricus*, *L. reuteri*, *Streptococcus thermophilus*, *Saccharomyces boulardii*, *Bifidobacterium bifidum*, *Bacillus subtilis*, etc.), and promote healthy digestion and absorption of nutrients. They act to crowd out pathogens including yeasts, bacteria, and viruses that may otherwise cause disease and develop a mutually advantageous symbiosis with the human gastrointestinal tract (Holzapfel 2001). Probiotics are useful in boosting immune system, preventing and treating urinary tract infections, improving digestive function, healing inflammatory bowel conditions like IBS, managing and preventing eczema in children, fighting food-borne illnesses, etc. Alcohol ethanol and organic acid lactic acid, butanoic acid (butyrate), etc., are some of the fermentation products of probiotic activity in the gut. Nutritional factors including several B vitamins including vitamin B₁₂, vitamin K, folate, and short chain fatty acids, enzymes that destroy harmful bacteria are produced by probiotics. Probiotics improve eczema and psoriasis, reduce cold and flu, heal leaky gut, destroy candida and good gut bacteria stimulate secretion of IgA and regulatory T-cells. They have an antimicrobial effect through modifying the microflora, preventing adhesion of pathogens to the intestinal epithelium, competing for nutrients necessary for pathogen survival, producing an antitoxin effect and reversing some of the consequences of infection on the intestinal epithelium, such as secretory changes and neutrophil migration. Probiotics can cure lactose intolerance by the production of the specific enzyme (β -galactosidase) that can hydrolyze the offending lactose into its component sugars (Pineiro and Stanton 2007). Probiotic-rich foods include high-quality goat milk yogurt, kefir or coconut kefir, raw cheese, fermented vegetables (cabbage and other), fermented soybeans, kvass, apple cider vinegar, brine-cultured olives, salted gherkin pickles, etc., and consumption of probiotic-rich foods is essential to boosting and increasing gut probiotics and gut health. Some sources of probiotic microorganisms are mentioned in Table 5.6.

(c) Nutraceutical enzymes

Enzymes are an essential part of life, without which our bodies would cease to function. Those people who are suffering from medical conditions such as

Table 5.6 Sources of probiotic microorganisms

Milk	Yogurt	Fermented products	Human breast milk	GI tract	Vegetables/ grains/fruits
<i>Lactobacillus</i> <i>acidophilus</i> , <i>L. lactis</i>	<i>L. delbrueckii</i> subsp. <i>bulgaricus</i>	<i>L. casei</i> , <i>L. cellobiosus</i> , <i>L. curvatus</i> , <i>L. fermentum</i> , <i>L. helviticus</i> , <i>L. farcininis</i>	<i>L. reuteri</i> , <i>L. salivarius</i>	<i>L. gasseri</i> <i>L. johnsonii</i>	<i>L. brevis</i> <i>L. plantarum</i>
	<i>Bifidobacterium</i> <i>adolescentis</i>	<i>B. thermophilum</i> , <i>B. animalis</i>	<i>B. infantis</i> , <i>B. longum</i> , <i>B. breve</i> , <i>B. lactis</i>	<i>Escherichia coli</i> , <i>Nissle 1917</i>	<i>Leuconostoc</i> <i>mesenteroides</i>
<i>Propionibacterium</i> <i>freudenreichii</i>	<i>Streptococcus</i> <i>thermophilus</i>	<i>Enterococcus faecium</i> , <i>Pediococcus acidilactici</i>			<i>S. cerevisiae</i> , <i>S. boulardii</i> Mushrooms

Source Holzapfel et al. (2001)

Table 5.7 List of nutraceutical enzymes from microbes, plants, and animals

Microbial enzymes/source	Plant enzymes	Animal enzymes/source
Hemicellulase (microorganisms, mushrooms, and <i>Trichoderma</i> sp.)	Hemicellulase (plant walls)	Ox bile (ox)
Catalase	Pectinase (cell wall)	Pancreolipase (pancreatic juice)
Amyloglucosidase (ascomycetes)	α -Galactosidase (beans, cabbage, Brussels sprouts, broccoli, asparagus, other vegetables, and whole grains)	Trypsin (pancreatic juice)
Diastase, Glucoamylase (<i>A. niger</i> , <i>Saccharomyces fibuligera</i>)	Diastase, β -amylase (higher plants)	Chymotrypsin (all classes of vertebrates)
Cellulase (all living cells, <i>Trichoderma</i> sp.)	Bromelain (pineapple)	pepsin (animals tracheal secretions)
Invertase—sucrase (yeast)	Biodiastase (soybean)	Lysozyme (saliva, tears, egg white, and many animal fluids)
Lactase— β -Galactosidase (bacteria)	Glucoamylase (callus and suspension cultures of sugar beets as well as in mature roots)	Diastase, α -amylase (saliva)
Xylanase (<i>Trichoderma</i> sp.)	Papain is (protease) (Papaya fruit)	Phytase
Neutral protease (<i>Bacillus</i> sp.)	Phytase	—
Lipase (<i>Rhizopus</i> sp.)	Pectinase	—
Lactase (<i>Aspergillus</i> sp.)	—	—

hyperglycemia, blood sugar disorders, digestive problems and obesity, eliminate the symptoms by enzyme supplements to their diet. Nutritional enzymes have risen to prominence during the past several years. These enzymes are derived from microbial, plant, and animal sources as given in Table 5.7.

2. Nontraditional Nutraceuticals

Nontraditional nutraceuticals are artificial foods prepared with the help of biotechnology. Food samples contain bioactive components which are engineered to produce products for human wellness. They are arranged into (i) Fortified nutraceuticals, and (ii) Recombinant nutraceuticals

(i) Fortified nutraceuticals

It constitutes fortified food from agricultural breeding or added nutrients and/or ingredients, e.g., orange juice fortified with calcium, cereals with added vitamins or minerals and flour with added folic acid. Some examples are milk

fortified with cholecalciferol used in vitamin D deficiency (Casey et al. 2010). Prebiotic and probiotic fortified milk with *bifidobacteriumlactis HN019* used in diarrhea, respiratory infections, and severe illnesses, in children (Sazawal et al. 2010). Banana fortified using soybean ferritin gene in iron deficiency was discovered by Kumar et al. (2011).

(ii) Recombinant nutraceuticals

Energy-providing foods, such as bread, alcohol, fermented starch, yogurt, cheese, vinegar, and others, are produced with the help of biotechnology. The production of probiotics and the extraction of bioactive components by enzyme/fermentation technologies as well as genetic engineering technology are achieved through biotechnology. Some of the products of recombinant organisms are shown in Table 5.8.

Commercial nutraceuticals

Many pharmaceutical companies are now trying to manufacture nutraceutical because there is undoubtedly a very huge and growing market. Nutraceuticals cover most of the therapeutic areas, such as anti-arthritis, cold and cough, sleeping disorders, digestion and prevention of certain cancers, osteoporosis, blood pressure, cholesterol control, painkillers, depression, and diabetes. Recognition of health benefits from consumption of omega-3 rich seafoods is one of the most promising developments in human nutrition and disease prevention research in the past three decades (Pandey et al. 2010). Examples of some of the commercial nutraceutical manufacturers and their products name with expected claim are given below.

Bio Serae Laboratories of France manufactures “Serenzo certified Organic” which constitutes citrus extract and acts as an anti-stress product and “Resveravine” which constitutes resveratrol and helps in cardiovascular protection and antiaging properties [www.bioserae.com/]. Shotz Health of UK prepares “Big Shotz” from ginseng, prebiotics rich in MEG-3 brand omega-3 EPA/DHA [<http://www.nutraceuticalsworld.com/>]. Guangzhou Lohas Biological Technology Co. Ltd. of China produces “Ginseng KianpiPil” using reishi extract, ginseng extracts, and rhodiolarosea extract as dietary supplement. [www.company.indiatradepage.com/]. An Indian company, for example, La Casa Agrotech Private Ltd., manufactures “Smruthihills” from Brahmi, Mandukparnee as nervine tonic for mind and memory. SAB Herbals and Nutraceuticals assemble “Methoxsalen Xanthotoxin Calcium Sennoside” from phytochemicals used in treating psoriasis, eczema, and vitiligo [www.hotfrog.in/Companies/].

Isha Agro Developers Pvt. Ltd. produces “Imunohills” from amla, guduchi, and gokshura, which promotes cellular and humoral immunity. Bio Bodyfuelz Ltd. prepares “PWR Sports” from *Sida cordifolia* extract which helps to boost endurance and refreshes muscles and “Fat Burner” from cocoa beans extract (6% theobromine). Lifestyle care produces “Arctic Sea Super Omega” from olive oil and fish oil and is a rich source of omega-3 fatty acids and “Forever Absorbent C” from bioflavonoids of oranges and papayas. Essential’z Energize Your Health manufacturers “Muscle Juice from protein blend (whey protein isolate, whey protein

Table 5.8 Product produced by recombinant organisms (microorganisms, plants, and animals)

Product produced by recombinant microorganisms, plants, and animals			
A. Recombinant microorganisms			
Source	Enzyme	Products	References
<i>Acetobacter xylinum</i>	β -glucuronidase	Kombucha beverage	Malbasa et al. (2011)
<i>Escherichia coli K-12</i>	Chymosin	milk-coagulated products	El-Sohaimy et al. (2010)
<i>Fusarium venenatum</i>	Xylanase	Increased bran solubilization	Sibbesen and Sorensen (2010)
<i>Aspergillus oryzae</i>	Esterase–Lipase, Aspartic proteinase, Glucose oxidase, Laccase, Lipase, Pectin esterase,	Alcoholic beverages (Sake, koji)	Ghorai et al. (2009)
<i>Saccharomyces cerevisiae</i>	Stilbene synthase and 4-coumaroyl-CoA	resveratrol	White (2009)
<i>Spirulina pacifica</i>	Indoleamine 2,3-dioxygenase (IDO)	Increased hemoglobin	www.nutraceuticalsworld.com/
B. Recombinant plant			
Recombinant	Deficiency	Gene for recombination	References
Gold kiwifruit	Iron	High level of ascorbic acid, carotenoids lutein and zeaxanthin	Beck et al. (2011)
Potatoes	Protein	Tuber-specific expression of a seed protein, <i>AmA1(Amaranth Albumin 1)</i>	Chakraborty et al. (2010)
Golden mustard	Vitamin A	Soybean <i>ferritin</i> gene	Chow et al. (2010)
Multivitamin corn	Multivitamin	Vitamins β -carotene corn (<i>Zea mays</i>) phytoene synthase (<i>psyI</i>) cDNA), ascorbate (rice dehydroascorbate reductase (<i>dhar</i>) cDNA), and Folate (<i>E. coli folE</i> gene encoding GTP cyclohydrolase (<i>GCH1</i>))	Naqvi et al. (2009)
Maize	Vitamin A (Retinol)	Bacterial genes <i>crtB</i> and <i>crtI</i>	Aluru et al. (2008)
Tomato	Folate	Aminodeoxychorismate synthase (<i>AtADCS</i>)	de la Garza et al. (2007)
Golden rice	Vitamin A (Retinol)	Two daffodil genes and one bacterial gene	Rockefeller Foundation/ www.rockfound.org

(continued)

Table 5.8 (continued)

B. Recombinant plant			
Recombinant	Deficiency	Gene for recombination	References
Iron rice	Iron deficiency	Soybean <i>ferritin</i> gene	www.biotechnature.com
C. Recombinant animals			
Cattle	human lysozyme	<i>rHLZ</i> expression vector <i>pBC2-HLY-NEOR</i>	Yang et al. (2011)
yogurt	probiotics microorganism	<i>Bifidobacterium lactis Bb-12</i> and <i>Lactobacillus acidophilus LA-5</i>	Allgeyer et al. (2010)
Cows	Lactoferrin deficiency	Recombinant human lactoferrin (rhLf)	Hyvonen et al. (2006)

concentrate, calcium caseinate and egg white albumin)" which feeds and nourishes the muscles. Amrutam Life Care Private Ltd. prepares "Obexi" from *Boerhaavia diffusa* and is an anti-obesity drug [www.trade.indiamart.com/].

Regulations

Food regulation is aimed at protecting the consumer's health, increasing economic viability, harmonizing well-being, and engendering fair trade on foods within and between nations. Commercial nutraceuticals have to pass through strict regulatory controls to provide a positive impact on an individual's health.

Functional foods

There is no universally accepted definition of functional foods. Conventional foods generally satisfy nutritional function and hedonistic or sensory function of an individual, while functional foods fulfill specific physiological function beyond adequate nutritional effects in a way that is relevant to either an improved state of health and/or reduction of risk of disease. Functional foods can be considered to be those whole, fortified, enriched, or enhanced foods that provide health benefits beyond the provision of essential nutrients (Hasler 2002). Functional foods must remain foods, contain an ingredient (or fortified with an ingredient) like micronutrient or chemical with a beneficial effect on one or more target functions in the body beyond adequate nutritional effects (well-being or disease prevention), they must demonstrate their effects (or at least claim), and these effects can be expected to materialize when the food is consumed in normal amounts as part of the usual diet (but not be pills or capsules). A functional food can be a natural food, a fortified food, or a food from which a component has been removed by technological or biotechnological means, a food where the nature or bioavailability of one or more components has been modified, or any combination of these possibilities. A functional food might be functional for all members of a population or for particular groups of the population on the basis of age or genetic constitution.

There exists a direct relationship of diet to disease and according to Willett (2002) over 60 percent of the risk for chronic diseases (e.g., heart disease, stroke, colon cancer, type II diabetes, etc.) is potentially preventable by lifestyle

modifications, including changes in diet. Based on decades of scientific inquiry, the World Health Organization (WHO 2003) states that diet plays an important role in affecting the risk for a variety of chronic diseases and disorders (e.g., cancer, heart disease, type II diabetes, and obesity). There is continued interest in characterizing the contribution of diet to bone, joint, and eye health as well as to cognitive function. Increased consumption of fruits/vegetables is associated with a lowering of risk for a variety of cancers (Steinmetz and Potter 1996). For these and several other reasons (high healthcare costs, aging, health-conscious population, desire for healthy eating and lifestyle habits, changes in food regulations, numerous technological advances, growing marketplace for health-promoting products, etc.), interest in functional foods is growing worldwide. It is, however, not a new concept as the Ministry of Health, Labour and Welfare of Japan has been regulating Foods for Specified Health Uses (FOSHU) since 1080s with documented health benefits (Arai 1996; Nakajima 2004).

The foods might provide a therapeutic benefit is also not a new concept. The tenet “Let food be thy medicine and medicine be thy food” was embraced ~2500 years ago by Hippocrates, the father of medicine. According to Roberfroid (2002), functional foods are generally part of a diet that provides health benefits beyond traditional nutritional effects. Functional foods have a potentially positive effect on health beyond basic nutrition. Functional foods promote optimal health and help reduce the risk of disease. For example, oatmeal is functional food because it contains soluble fiber that can help lower cholesterol levels and thereby reduce the risk of heart attack. Some foods are modified to have health benefits, e.g., orange juice that has been fortified with calcium for bone health. Health benefit is most likely due to the collective presence of many nutrient and non-nutrient plant components (a cocktail of phytochemicals). Glucosamine, calcium, and anti-inflammatory and antioxidant nutrients and phytochemicals are suggested for the improvement of joints, muscles, and bones. Role of xanthophylls (lutein) in eye health, conjugated linoleic acid (CLA) and tea phenolics in weight maintenance and the balance between muscle mass and fatty tissue are suggested.

Consumption of refined and processed foods may trigger an immune response leading to inflammation which may contribute too many diseases and disorders from atherosclerosis to Alzheimer’s disease. Some functional foods (e.g., fatty fish, whole grains, dark leafy greens, nuts, peppers, tomatoes, beets, ginger and turmeric, onions and garlic, berries, etc.) with the help of their important minerals, fiber, vitamins, and other contents can reduce the risk of inflammation and many other diseases. Fatty or oily fish (e.g., salmon, tuna, sardines, and mackerel) is high in omega-3 fatty acids; whole grains (brown rice, steel cut oats, buckwheat, and bulgur wheat) have more fiber than refined grains (white bread, white rice, and degermed cornmeal); dark leafy greens (e.g., broccoli, spinach, kale, collard greens, rainbow chard, etc.) are a low-glycemic food, full of vitamin E, and have very high concentrations of minerals (Mg) and phytochemicals; almonds are full of fiber, vitamin E, calcium, omega-3s, and heart-healthy fat; bell peppers have capsaicin in them that are also full of important antioxidants; tomatoes are high in antioxidant and lycopene; beet root juice is high in antioxidants, fiber, vitamin C, and betalains;

the anti-inflammatory compound gingerol of ginger provides free radical protection and curcumin of turmeric is a powerful antioxidant; and berries (strawberries, blueberries, cranberries, etc.) are high in anthocyanins, the powerful antioxidants. They reduce the risk of inflammation, blood pressure, insulin spike, and many other diseases. Food products enriched for soy protein, plant sterols and stanols, omega-3 fatty acids, antioxidants, and fiber are being formulated and offered to the consumer (Meister 2002). The delivery of health benefits through functional food is a relatively new concept and it is gaining in popularity in the society including producers (food industries) and consumers. However, the legal status with respect to food law is not yet well documented. Functional foods represent one of the most intensively investigated and widely promoted areas in the food and nutrition sciences today but these are not magic bullets or panaceas for poor health habits (but diet) and linking the consumption of functional foods with health claims should be based on sound scientific evidence (Hasler 2002).

Scientists have identified the great majority of different physiologically active components in foods from plants (phytochemicals) and a few from animals (zoochemicals) or microbes that potentially could reduce risk for a variety of chronic diseases. Characteristics of some functional foods available on the US market are shown in Table 5.9.

Like functional food, there are more terms for dietary products such as food supplements (or dietary supplements) and nutraceuticals (or nutriceuticals) that directly link nutrition with health.

Food supplements are concentrated sources of nutrients, dietary ingredients (including vitamins, minerals, amino acids, enzymes, glandulars, metabolites, organ, tissues, herbs, or other botanicals), or other substances with a nutritional or physiological effect whose purpose is to supplement the normal diet, available in the market in dose form (e.g., as pills, tablets, capsules, softgels, gelcaps, powders, extracts or liquids in measured doses, etc.) taken by mouth and must not represent the product as a conventional food or a sole item of a meal or diet (EC 2007, FDA 2007).

Functional foods are similar in appearance to conventional foods and are consumed as part of a normal diet (Zeisel 1999), whereas the food supplements are not considered to be proper food. For nutraceuticals, the concept is less clear. A nutraceutical is a food or naturally occurring food supplement or part of a food that allegedly provides medicinal or health benefits, including the prevention and treatment of disease. A nutraceutical may be a naturally nutrient-rich or medicinally active food, such as garlic or soybeans, or it may be a specific component of a food, such as the omega-3 fish oil that can be derived from salmon and other cold-water fish.

A nutraceutical may be a product isolated or purified from foods, provides physiological benefit or protection against chronic disease, and is generally sold in medicinal forms not usually associated with foods (Canada 2007). Zeisel (1999) defines nutraceuticals as those diet supplements that deliver a concentrated form of a presumed bioactive agent from a food, presented in a nonfood matrix, and used to enhance health in dosages that exceed those that could be obtained from normal foods. The definition of nutraceuticals may be limited to natural, bioactive chemical compounds that have health promoting, disease preventing or medicinal properties

Table 5.9 Strength of evidence for functional foods currently on the U.S. market

Functional food	Bioactive component	Health benefit	Type of evidence	Strength of evidence	Recommended amount or frequency of intake	Regulatory status
1. Plant origin (phytochemicals)						
Fortified margarines	Plant sterol and stanol esters	Reduce total and LDL cholesterol	Clinical trials	Very strong	1.3 g/d for sterols 1.7 g/d for stanols	Health claim
Psyllium	Soluble fiber	Reduce total and LDL cholesterol	Clinical trials	Very strong	1 g/d	Health claim
Soy	Protein	Reduce total and LDL cholesterol	Clinical trials	Very strong	25 g/d	Health claim
Whole oat products	Glucan	Reduce total and LDL cholesterol	Clinical trials	Very strong	3 g/d	Health claim
Cranberry juice	Proanthocyanidins	Reduce urinary tract infections	Small number of clinical trials	Moderate	300 mL/d	Conventional food
Garlic	Organosulfur compounds	Reduce total and LDL cholesterol	Clinical trials	Moderate	600–900 mg/d	Conventional food or dietary supplement
Spinach, Kale, collard greens	Lutein/zeaxanthin	Reduce risk of age-related macular degeneration	Epidemiological	Weak to moderate	6 mg/d	Conventional food or dietary supplement
Tomatoes and processed tomato products	Lycopene	Reduce risk prostate cancer	Epidemiological	Weak to moderate	Daily	Conventional food

(continued)

Table 5.9 (continued)

Functional food	Bioactive component	Health benefit	Type of evidence	Strength of evidence	Recommended amount or frequency of intake	Regulatory status
Cruciferous, vegetables	glucosinolates, indoles	Reduce risk of certain types of cancer	Epidemiological	Weak	3 or more servings/wk	Conventional food
Green tea	Catechins	Reduce risk of certain types of cancer	Epidemiological	Weak to moderate	Unknown	Conventional food
<i>2. Animal origin (zoochemicals)</i>						
Fatty fish	(n-3) Fatty acids	Reduce TG, reduce heart disease cardiac deaths and fatal and nonfatal myocardial infarction	Clinical trials; epidemiological studies	Strong	2/wk	Qualified health claim for dietary supplements
Lamb, turkey, beef, dairy	CLA (Conjugated linoleic acid)	Reduce breast cancer	In vivo and in vitro studies	Weak	Unknown	Conventional food
Fermented dairy products	Probiotics	Support GI (gastrointestinal) health, boost immunity	In vivo and in vitro studies, limited clinical data	Weak	Daily	Conventional food or dietary supplement

Source Hasler (2002)

Note Foods that have an FDA-approved health claim (sterol/stanol esters, oats, psyllium, soy) generally are supported by two dozen or more well-designed published clinical trials

or the concept may be extended by adding the category of medicinal foods (e.g., transgenic plants for oral vaccination against infectious diseases) to the other two nutraceutical categories of dietary supplements (e.g., vitamins, minerals, and plant extracts) and functional foods (e.g., omega-3 milk, cholesterol reducing oils, and fats). According to these definitions, there is a clear distinction between functional food and food supplements, while nutraceuticals can cover functional food and food supplements, i.e., both functional food and food supplements could be considered nutraceuticals—as long as they can be derived from natural sources.

Classification of functional foods

Functional food may be classified into several groups on the basis of (i) food group it belongs to (e.g., dairy products, beverages, cereal products, confectionary, oils, and fats); (ii) the diseases it is expected to prevent or alleviate (e.g., diabetes, osteoporosis, and colon cancer), (iii) its physiological effects (e.g., immunology, digestibility, and antitumor activity), (iv) the category of its specific biologically active ingredients (e.g., minerals, antioxidants, lipids, and probiotics), (v) its physicochemical and organoleptic properties (e.g., color, solubility, and texture), or (vi) the processes that are used in its production (e.g., chromatography, encapsulation, and freezing) (Juvan et al. 2005).

The future of functional foods

According to the Department of Health and Human Services, diet plays a role in 5 of 10 of the leading causes of death, including coronary heart disease (CHD), certain types of cancer, stroke, diabetes (noninsulin dependent or type 2), and atherosclerosis. The dietary pattern characterized by high total and saturated fat, cholesterol, sodium and refined sugars and low unsaturated fat, grains, legumes, fruits, and vegetables has been linked with these major causes of death in many developed countries including the USA. It has been suggested that consumption of certain foods or their associated physiologically active components may be linked to disease risk reduction (Hasler 1998).

Extensive research activities by academic, government, and private research institutes across the world are currently going on to understand and explore the mechanism of action of functional foods against chronic diseases of consumers. Nutrigenomics, following the results of human genome sequence, will have a profound effect on future functional foods research and development and also on future disease prevention efforts including the future of the functional foods industry (Fogg-Johnson and Meroli 2000, Anonymous 2001). Biotechnology will also influence the future of functional foods (Gura 1999). For examples, development of genetically engineered iron-enriched rice and golden rice help prevent iron deficiency anemia and vitamin A deficiency-related blindness of millions of worldwide (Anonymous 2000). In the future, other foods enhanced with other nutritive or nonnutritive substances may even help to prevent chronic diseases such as heart disease, osteoporosis, or cancer (Falk et al. 2002; Pande et al. 2010).

Natural sources, classification, chemistry and use of nutraceuticals, food additives, flavors, and excipients: Diluents, bulking, or filler agents

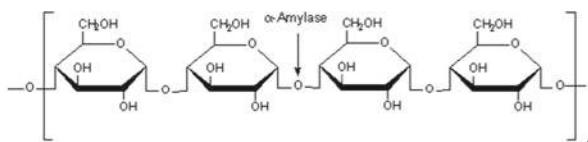
Food additives are substances used in the processing or storage of foods or animal feed including antioxidants, food preservatives, food coloring agents, flavoring agents, anti-infective agents, excipients, and other similarly used substances. Natural flavors derived from herbs, spices, blossoms, etc. offer a wide range of herbal and spice flavors and include the classical flavors peppermint, fennel, lemon, ginger, cardamom, cilantro, cinnamon, cumin, coriander, hot peppers, black pepper, cloves, thyme, parsley, bay leaf, and blossom flavors from elder, orange mallow, jasmine, etc. A natural flavor derived from these edible biological sources is consisted of essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, etc., while a synthetic or artificial flavor is a petroleum product and can cause a host of health problems. Many of these substances are used as pharmaceutic aids or excipients. Excipients are substances which are of little or no therapeutic value, but are necessary for the manufacture, compounding, storage, etc., of pharmaceutical preparations or drug dosage forms. Excipients contribute largely to the performance of the active pharmaceutical ingredient (API) and maintain the quality, efficacy, safety, etc. of the formulation (Pifferi et al. 1999). Excipients include solvents as well as diluting, suspending and emulsifying agents, antioxidants, preservatives, pharmaceutical, coloring agents, flavoring agents, vehicles, excipients, ointment bases, etc. Excipients are usually inert substances added to a prescription in order to provide suitable consistency to the dosage form and primarily function as diluents, binders, disintegrants, adhesives, glidants, inert vehicles, and sweeteners in conventional dosage forms like tablets and capsules as well as base or diluent in pills, tablets, creams, salves, etc. Excipients affect the behavior and effectiveness of the drug product more and more functionality and significantly. They often improve the stability, release, and bioavailability of the active ingredient, enhancement of patient acceptability, and performance of technological functions that ensure ease of manufacture (Hamman and Tarirai 2006). These natural excipients are nontoxic, less expensive, and freely available but often associated with heavy metals and microbial contamination. Coloring agents are chemicals and substances that impart color including soluble dyes and insoluble pigments. They are used in inks, paints, and as indicators and reagents.

Natural polysaccharides are extensively used excipients for the development of solid dosage forms. They are inexpensive, highly stable, safe, nontoxic, and hydrophilic and gel forming in nature. They are available in a variety of structures with a variety of properties, e.g., starch, pectins, guar gum, amylase, and karaya gum are a few examples of polysaccharides commonly used in dosage forms.

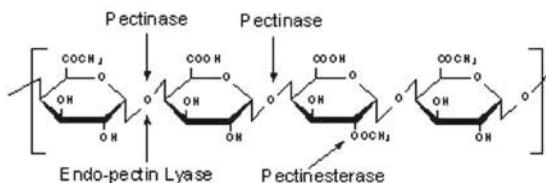
Starch, a biopolymeric carbohydrate material made out of glucose units linked by α -1→4 glycosidic linkage, is found in abundance in seeds, leaves, roots, fruits, tubers, and stem piths of plants. It is a white, amorphous, tasteless powder made up of amylose and amylopectin. Starches can be obtained from maize, cassava, yam, rice, potatoes, wheat, tiger nuts, and several other sources. In the, starches have been employed in pharmaceutical industry as binders, disintegrants, diluents (fillers), lubricants, glidants, coating, and dusting media for tablet coating, production

of microparticles for drug delivery and delivery of orally and intramuscularly administered vaccines, etc. (Illum et al. 2001; Hauschild and Pickr-Freyer 2004; Monek et al. 2012). Figure 5.35 shows the structure of natural polysaccharides excipients—amylose, homogalacturonan, rhamnogalacturonan; alginic acid, (A) amylopectin or α -amylase and (B) β -amylase, guar gum, locust bean gum, xanthan gum; cellulose, agarose, chitin, and chitosan.

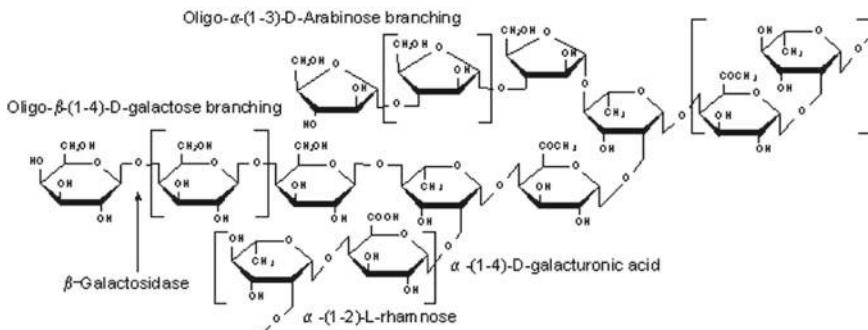
Pectins like cellulose and hemicelluloses are non-starch, linear polysaccharides of the plant cell wall. Pectins are complex branched heteropolysaccharides. The linear backbone of pectin is composed of α -1 \rightarrow 4-linked D-galacturonic acid units interrupted with highly branched regions and the polygalacturonic acid backbone which



Amylose (polymer of α -1 \rightarrow 4-D-glycopyranosyl units) showing amylase specificity

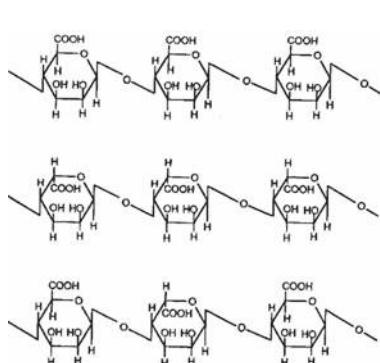


Homogalacturonan showing pectinase and pectinesterase specific sites

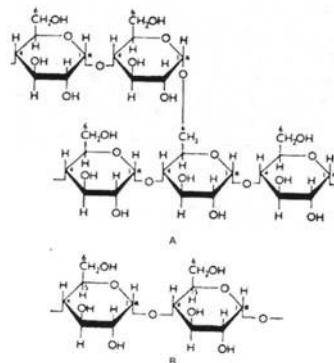
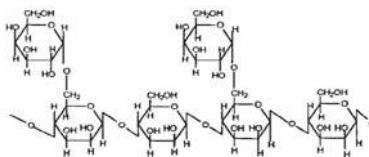


Rhamnogalacturonan I showing pectinase and pectinesterase specific sites and contains alternating α -(1 \rightarrow 4)-D-galacturonosyl and α -(1 \rightarrow 2)-L- rhamnosyl backbone with two types of branching composed of α -(1 \rightarrow 3) arabinose and β -(1 \rightarrow 4) galactose oligomers

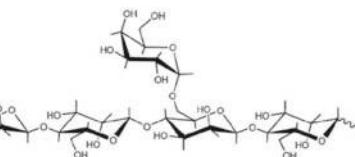
Fig. 5.35 Showing structure of natural polysaccharides excipients—amylose, homogalacturonan, rhamnogalacturonan, alginic acid, (A) amylopectin or α -amylase and (B) β -amylase, guar gum, locust bean gum, and xanthan gum; cellulose, agarose, chitin, and chitosan



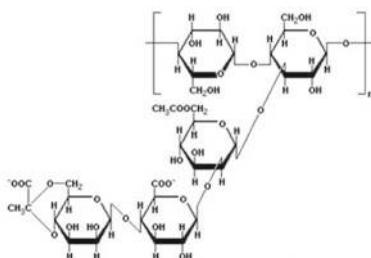
Alginic acid

(A) amylopectin or α - amylase and (B) β -amylose

Guar gum



Locust bean gum



Xanthan gum

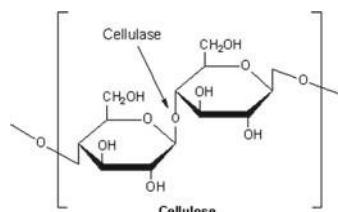
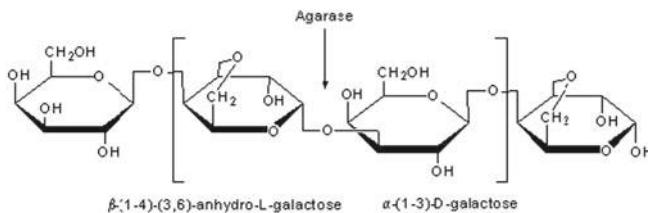
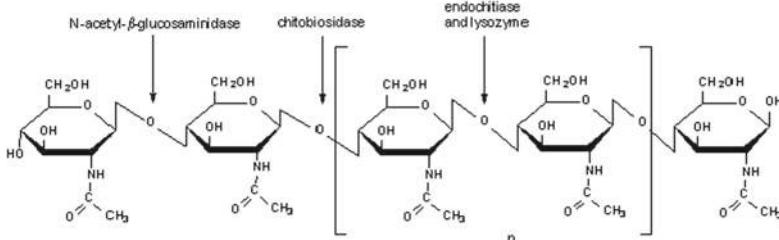
Cellulose (polymer of β -1 \rightarrow 4-D-glycosidic units) showing cellulase specificity

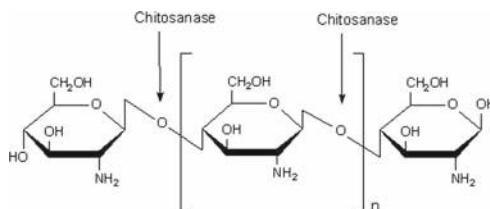
Fig. 5.35 (continued)



Agarose (polymer of alternating $\alpha\text{-}1\rightarrow3\text{-D-galactosyl}\text{-}\beta\text{-}1\rightarrow4\text{-anhydro-L-galactosyl}$ units) showing agarase specificity. Agarose is the principal neutral gelling component of agar



Chitin (polymer of $\beta\text{-}1\rightarrow4\text{-N-Acetyl-D-glucosamine}$) showing chitase specificity



Chitosan (polymer of $\beta\text{-}1\rightarrow4\text{-D-glucosamine}$) showing chitosanase specificity

Fig. 5.35 (continued)

can be randomly acetylated and methylated. The galacturonic acid polysaccharides are rich in neutral sugars such as rhamnose, arabinose, galactose, xylose, and glucose. The composition of pectin can vary based on the botanical source, e.g., pectin from citrus contains less neutral sugars and has a smaller molecular size compared to pectin obtained from apples. Three different pectins have been isolated from plant cell walls such as (i) Homogalacturonans are composed of $\alpha\text{-}(1\rightarrow4)$ polygalacturonic acid backbone with random-partial methylation and acetylation; (ii) Substituted homogalacturonans are modifications of this backbone with $\beta\text{-D-xylose}$ branching at C3, or apofuranose substitutions in the backbone with $\beta\text{-D-Apisyl-(1,3')-}\beta\text{-D-Apiose}$ branching; (iii) Rhamnogalacturonan I contains alternating $\alpha\text{-}(1\rightarrow4)$ galacturonyl and $\alpha\text{-}(1\rightarrow2)$ rhamnosyl residues, with primarily oligo $\alpha\text{-}(1\rightarrow3)$ arabinose and oligo $\beta\text{-}(1\rightarrow4)$ galactose branching; and (iiib) Rhamnogalacturonan II is composed of the simple $\alpha\text{-}(1\rightarrow4)$ polygalacturonic acid backbone with complex branching with composed of up to 11 different monosaccharide types.

Non-starch, linear polysaccharides remain unchanged in the physiological environment of the stomach and the small intestine, but they are degraded by the colon probiotics which make them potentially useful in targeted delivery systems to the colon. Polymeric hydrogels (high-methoxy pectin) are widely used as controlled-release matrix tablets. Alginic acid is a linear polymer consisting of β -(1 \rightarrow 4) linked D-mannuronic acid and α -L-guluronic acid units. Alginate has regions rich in sequential mannuronic acid units, guluronic acid units, and regions in which both monomers are equally prevalent. Alginate, a natural anionic polysaccharide found in seaweed, is most important for its use in biomedicine as a cell-compatible hydrogel and for its various applications in drug delivery, such as in matrix-type alginate gel beads, in liposomes, in modulating gastrointestinal transit time, for local applications and to deliver the biomolecules in tissue engineering applications. A number of starches (amylopectin or α -amylose, and β -amylose) are recognized for pharmaceutical use. These include maize, rice, wheat, and potato.

Natural gums may be obtained from different sources such as (i) seaweed (e.g., agar, carrageenans, alginic acid, and laminarin); (ii) land plant exudates (e.g., gum arabic, gum ghatti, gum karaya, gum tragacanth, and khaya and albizia gums), and seed (e.g., guar gum, locust bean gum, starch, amylose, and cellulose); extracts (e.g., pectin, larch gum); tuber and roots (potato starch); (iii) animal (e.g., chitin and chitosan, chondroitin sulfate, and hyaluronic acid); and (iv) microbes bacteria and fungi (e.g., xanthan, dextran, curdian, pullulan, zanflo, emulsan, Baker's yeast glycan, schizophyllan, lentinan, krestin, and scleroglucan). Plant gums (exudates, seed gums, extracts, etc.) are composed mostly of complex polysaccharides. Gums such as guar gum, locust bean gum, karaya gum, xanthan gum, tragacanth, etc. are translucent and amorphous products of plants, usually pathological products. They are commonly used as a dietary fiber, thickening agent, foaming agent, film, emulsifier, stabilizer, and drug delivery agent. Gums are hydrocolloids and may be anionic or non ionic polysaccharides and on hydrolysis, gums yield sugar and salts of uronic acid. Guar gum derived from the seeds of *Cyamopsis tetragonolobus* is a naturally occurring galactomannan polysaccharide, made up of a linear chain of β -D-mannopyranose joined by β -(1 \rightarrow 4) linkage with α -D-galactopyranosyl units attached by 1,6-links in the ratio of 1:2². Gum acacia or gum arabic is the dried gummy exudate obtained from the stem and branches of *Acacia senegal* and it is as an acidic polysaccharide containing D-galactose, L-arabinose, L-rhamnose, and D-glucuronic acid and is mainly used in oral and topical pharmaceutical formulations as a suspending and emulsifying agent, often in combination with tragacanth. It is also used in the preparation of pastilles and lozenges and as a tablet binder. Locust bean gum is a polysaccharide of galactomannans and extracted from the seeds of the carob tree (*Ceratonia siliqua*). It is a linear polysaccharide (1 \rightarrow 4) linked backbone of mannose units with single (1 \rightarrow 6) D-galactose units. Karaya gum (gum sterculia, or Indian gum tragacanth) is obtained from *Sterculia urens* which is a partially acetylated polymer of galactose, rhamnose, and glucuronic acid (i.e., acid polysaccharide sugars). It is used as a thickener and emulsifier in foods, as a laxative, and as a denture adhesive. Xanthan gum is a high molecular weight extracellular polysaccharide produced by the fermentation of the gram-negative

bacterium *Xanthomonas campestris*. The primary structure of this naturally produced cellulose derivative contains a cellulosic backbone (β -D-glucose residues) and a trisaccharide side chain of β -D-mannose- β -D-glucuronic acid- α -D-mannose attached with alternate glucose residues of the main chain. Tragacanth gum is obtained from the branches of *Astragalus gummifer*. Tragacanth when used as the carrier in the formulation of 1- and 3-layer matrices produced satisfactory release prolongation either alone or in combination with other polymers.

Flavors can be used to mask unpleasant-tasting active ingredients and improve the acceptance that the patient will complete a course of medication. Pharmaceutical flavoring agents or excipients improve the desirable characteristics of taste, texture, and overall palatability of the formulations and include peppermint oil, methyl salicylate, thymol, vanillin, etc. Flavors improve a bitter product (mint, cherry, or anise); a salty product (peach, apricot, or liquorice); a sour product (raspberry or liquorice), and excessively sweet product (vanilla). Flavorings may be natural (e.g., fruit extract) or artificial. A natural flavor is the essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, or any product of roasting, heating or enzymolysis, which contains the flavoring constituents derived from a spice, fruit or fruit juice, vegetable, or vegetable juice, edible yeast, herb, bark, bud, root, leaf or similar plant material, meat, seafood, poultry, eggs, dairy products, or fermentation products thereof, whose significant function in is flavoring rather than nutritional, etc. Artificial flavors are those that are made from components that do not meet this definition. Both artificial and natural flavors contain chemicals, and the distinction between natural and artificial flavorings is the source of chemicals, e.g., natural (animals and vegetables sources) or created synthetically. Most artificial flavors are specific and often complex mixtures of singular naturally occurring flavor compounds combined together to either imitate or enhance a natural flavor. Here are given names of some artificial chemicals with their respective odor given within parenthesis: diacetyl, acetylpropionyl, acetoin (buttery); isoamyl acetate (banana); benzaldehyde (bitter almond, cherry); cinnamaldehyde (cinnamon); ethyl propionate (fruity); methyl anthranilate (grape); limonene (orange); ethyl decadienoate (pear); allyl hexanoate (pineapple); ethyl maltol (sugar, cotton candy); ethylvanillin (vanilla); methyl salicylate (wintergreen); manzanate (apple); etc. The compounds used to produce artificial flavors are almost identical to those that occur naturally. It has been suggested that artificial flavors may be safer to consume than natural flavors due to the standards of purity and mixture consistency that are enforced either by the company or by law (Smitha et al. 2005). Most flavors represent a mixture of aroma compounds, the raw material that is produced by flavor companies. In rare cases, a single synthetic compound is used in pure form. Artificial vanilla flavors vanillin and ethylvanillin are a notable exception, as well as the artificial strawberry flavor (ethyl methylphenylglycidate). The ubiquitous “green apple” aroma is based on hexyl acetate. Figure 5.36 shows structure of different flavor excipients—vanillin, eugenol, menthol, cineole, carvone, limonene, diacetyl, hexyl acetate, isoamyl acetate, benzaldehyde, cinnamaldehyde, ethyl propionate, methyl anthranilate, ethyl decadienoate, allyl hexanoate, ethyl maltol, ethylvanillin, methyl salicylate, manzanate, and thymol.

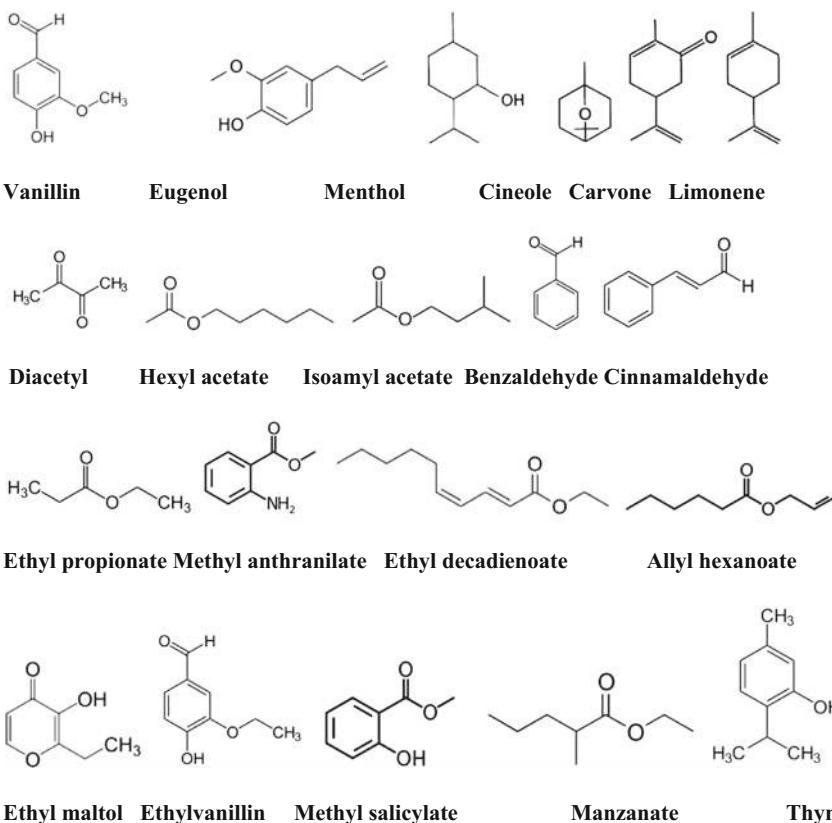


Fig. 5.36 Showing structure of different flavor excipients—vanillin, eugenol, menthol, cineole, carvone, limonene, diacetyl, hexyl acetate, isoamyl acetate, benzaldehyde, cinnamaldehyde, ethyl propionate, methyl anthranilate, ethyl decadienoate, allyl hexanoate, ethyl maltol, ethylvanillin, methyl salicylate, manzanate, and thymol

Volatile oils such as menthol, cineole, ketone carvone, limonene, and propylene glycol (PG) are terpenoid in origin, i.e., some are aromatic derivatives mixed with terpenes (e.g., cinnamon, and clove), a few are terpenoid in origin and aromatic in structure (e.g., thymol and carvacrol), etc. Menthol of *Mentha piperita* functions as penetration enhancer in membrane-moderated transdermal therapeutic system (TTS) of nimodipine. Clove oil is an essential oil extracted from *Syzygium aromaticum*. Eugenol is used in perfumes, flavorings, and essential oils and also as a local antiseptic and anesthetic. Vanillin (phenolic aldehyde) is a food flavoring agent. The volatile oil of dry caraway fruit (*Carum carvi*) consists of the ketone carvone and the terpene limonene. Preservatives include antibacterial and antioxidants agents and are added to pharmaceutical preparations for protection against chemical change or microbial action. Tragacanth, a suspending agent from *Astragalus gummifer* and related plants, forms gelatinous mass in water and is used as a

bulk-forming laxative as well as suspending agent, excipient or emulsifier in foods, cosmetics, and pharmaceuticals. Adjuvants are pharmaceutic agents that aid or increase the action of the principle drug (drug synergism) or that affects the absorption, mechanism of action, metabolism, or excretion of the primary drug (pharmacokinetics) in such a way as to enhance its effects.

Pharmaceutical suspension is a coarse dispersion in which the internal phase (therapeutically active ingredient) is dispersed uniformly throughout the external phase. Like other disperse systems, suspensions are thermodynamically unstable and the development of a stable suspension over the shelf life of the drug product continues to be a challenge on many fronts. The selection of the proper excipients (surfactants, viscosity imparting agents, etc.) is essential in the development of a suitable pharmaceutical suspension because the physical stability of a suspension is mainly dependent on the type of suspending agent rather than the physical properties or characteristics of the polysaccharide. Starch is a good suspending agent.

Disintegrants, sweeteners, binders, adhesives, and solidifiers

Disintegrating agents are substances included in tablet formulations and in some hard shell capsule formulations to promote moisture penetration and dispersion of the matrix of the dosage form in dissolution fluids. An oral solid dosage form should ideally disperse into the primary particles from which it was prepared. Although various compounds have been proposed and evaluated as disintegrants, relatively few are in common usage today. Traditionally, starch has been the disintegrant of choice in tablet formulations, and it is still widely used. For instance, starch generally has to be present at levels greater than 5% to adversely affect compactibility, especially in direct compression. Moreover, intragranular starch in wet granulations is not as effective as dry starch. The ideal disintegrant should have the characteristics including poor solubility, poor gel formation, good hydration capacity, good compressibility and flow properties, no tendency to form complexes with the drugs, etc. Examples of disintegrants include (i) crosslinked polymers such as crosslinked polyvinylpyrrolidone (crospovidone), crosslinked sodium carboxymethyl cellulose (croscarmellose sodium), etc., and (ii) the modified starch sodium starch glycolate.

Disintegrants may be grouped into three classes such as (i) modified starches: sodium carboxymethyl starch; the carboxymethyl groups induce hydrophilicity and cross-linking reduces solubility; (ii) modified cellulose: sodium carboxymethyl cellulose which has been cross-linked to render the material insoluble; and (iii) cross-linked polyvinyl pyrrolidone: cross-linked polyvinyl pyrrolidone; cross-linking renders the material insoluble in water.

Binders or adhesives are one of an important excipient to be added in tablet formulation. Binders or adhesives provide and promote cohesive strength to powdered materials and are used as binding agent in tablets. Binders are added in both dry and wet forms for converting powder into granules through a process known as granulation by which small powdery particles are agglomerated into granules. Binders ensure that tablets and granules can be formed with required mechanical strength, and give volume to low active dose tablets. Binders may be

classified into natural as well as synthetic. Natural binders include substances such as saccharides (glucose, sucrose, lactose), polysaccharides (starch, cellulose), modified cellulose (microcrystalline cellulose and cellulose ethers such as hydroxypropyl cellulose), sugar alcohols (xylitol, sorbitol, or maltitol), gum (Acacia, tragacanth), protein (gelatin), pregelatinized starch, alginic acid, etc., and synthetic or semisynthetic binders include methyl cellulose, ethyl cellulose, hydroxy propyl methyl cellulose (hpmc), hydroxy propyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidone (pvp), polyethylene glycol (peg), polyvinyl alcohols, polymethacrylates, etc. glucose, etc., are considered as sugar binders of natural origin. Binders may also be classified according to their application, such as (i) solution binders, which are dissolved in water or alcohol and can be used in wet granulation processes (e.g., gelatin, cellulose, cellulose derivatives, polyvinylpyrrolidone, starch, sucrose, polyethylene glycol, etc.); (ii) dry binders are added to the powder blend, either after a wet granulation step or as part of a direct powder compression (DC) formula (e.g., cellulose, methyl cellulose, polyvinylpyrrolidone, and polyethylene glycol).

Tablet coatings protect tablet ingredients from deterioration by moisture in the air and make large or unpleasant-tasting tablets easier to swallow. For most coated tablets, a cellulose ether hydroxypropyl methylcellulose (HPMC) or in short hypromellose (a semisynthetic, inert, viscoelastic polymer used) film coating is used which is free of sugar and potential allergens. Occasionally, other coating materials are used, for example, synthetic polymers, shellac, corn protein zein, or other polysaccharides. Capsules are coated with gelatin. Enterics control the rate of drug release and determine where the drug will be released in the digestive tract. Materials used for enteric coatings include fatty acids, waxes, shellac, plastics, and plant fibers.

Natural dyes or colorants are derived from plants, invertebrates, or minerals. The majority of natural dyes are vegetable dyes from plant sources—roots, berries, bark, leaves, and wood—and other biological sources such as fungi and lichens. Plant-based dyes such as woad (*Isatis tinctoria*), indigo, saffron, and madder were raised commercially and were important trade goods in the economies of Asia and Europe. Commercial dye or colorants such as insect (red), cow urine (Indian yellow), lac insect (red, violet), murex snail (purple), octopus/cuttlefish (sepia brown), etc. are animal origin; and catechu (brown) from *Acacia catechu*, gamboge tree (genus *Garcinia*) resin (dark mustard yellow), Himalayan rubhada root (*Rheum emodi*) (yellow), indigofera plant (blue), kamala tree (red), plant (yellow), alizarin, and purpurin constituents in madder root (*Rubia tinctorum*) create rich colors ranging from orange, pink to bright red, fruit (yellow, green, black), pomegranate peel (yellow), weld herb (yellow), etc., are of plant origin. Colors are added to improve the appearance of formulation. Color consistency is important as it allows easy identification of a medication. Furthermore, colors often improve the aesthetic look and feel of medications. Titanium oxide is commonly used as a coloring agent to produce the popular opaque colors along with azo dyes for other colors. By increasing these organoleptic properties, a patient is more likely to adhere to their

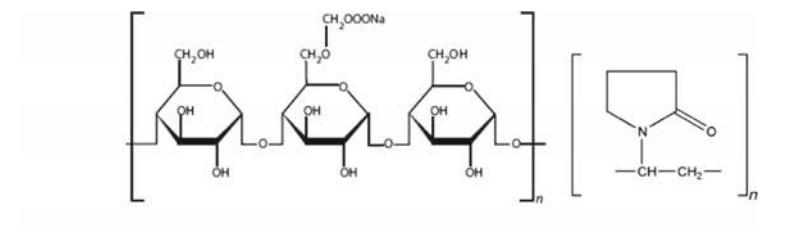
schedule and therapeutic objectives will also have a better outcome for the patient, especially children.

Gelling agents are hydrocolloidal substances that give thixotropic consistency to the gel. These are also known as solidifiers, stabilizer, and thickening agents. They are widely used as excipients for both conventional and novel dosage forms. Glycerol monostearate (GMS) is a monoglyceride commonly used as an emulsifier in foods. It takes the form of a white, odorless, and sweet-tasting flaky powder that is hygroscopic. Chemically, it is the glycerol ester of stearic acid.

As a food additive, GMS is used as a thickening, emulsifying, anticaking, and preservative agent; an emulsifying agent for oils, waxes, and solvents; a protective coating for hygroscopic powders; a solidifier and control release agent in pharmaceuticals; and a resin lubricant. It is responsible for giving ice cream and whipped cream their smooth texture. It is sometimes used as an anti-stealing agent in bread. It is also used in cosmetics and hair care products. Figure 5.37 shows the structure of sodium carboxymethyl starch, crospovidone or cross-linked povidone, carboxymethyl cellulose, hydroxy propyl cellulose, xylitol, sorbitol, maltitol, polyvinylpyrrolidone, polyethylene glycol, hydroxypropyl methylcellulose, alizarin, purpurin, indigo blue dye, indigo white, catechin, and glycerol monostearate

Sweetening agents are substances that sweeten food, beverages, medications, etc., such as sugar, saccharine, or other low-calorie synthetic products. Sweetening agents include nonnutritive sweeteners, nutritive sweeteners, dietary sucrose, high fructose corn syrup, small or large molecules, etc. Sweeteners are divided into (i) nutritive sweeteners and (ii) nonnutritive sweeteners. Sweeteners that add sweet taste and also some energy value to food are nutritive sweeteners. They include natural sugars such as sucrose, fructose, and galactose, and certain sugar alcohols. Sweeteners or chemical additives that add sweet taste only to food and not any energy to food are nonnutritive sweeteners, e.g., aspartame, saccharin, sucralose, etc.; they give a sweet taste to foods without contributing significant calories or promoting tooth decay. They are generally much sweeter than sucrose. Stevia, a plant genus of the family Asteraceae, members contain stevioside and other sweet diterpene glycosides; the leaf is used for sweetening (sweetening agents).

Sucrose (table sugar) has 4 calories per gram. Sugar substitutes are used to limit food energy during dieting, to reduce the formation of dental plaque, and to help regulate blood sugar levels in diabetic individuals. Artificial sweetener, sugar substitute, is a food additive that duplicates the effect of sugar in taste, usually with less food energy. Some sugar substitutes are natural (steviol, mogroside v, and brazzein), and some are synthetic or artificial sweeteners (saccharin, aspartame, acesulfame K, sucralose, etc.). People use artificial sweeteners because they suffer from diseases such as diabetes mellitus, because they are concerned about dental caries and periodontal disease, or because they wish to lose or to avoid gaining weight. Artificial sweeteners in very small quantities give foods sweetness, and most are not metabolized, so the artificial sweeteners themselves furnish zero dietary calories. In the US, FDA has approved several artificial, synthetic, or noncaloric sweeteners, e.g., saccharin, aspartame, sucralose, neotame, and acesulfame potassium (Ace-K), advantame, etc. Figure 5.38 shows structure of different



Sodium carboxy methyl starch

Crospovidone or cross linked

povidone

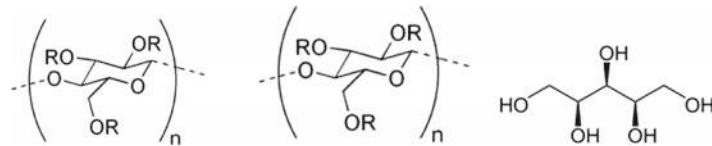
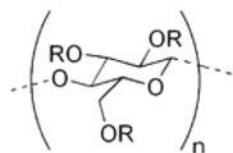
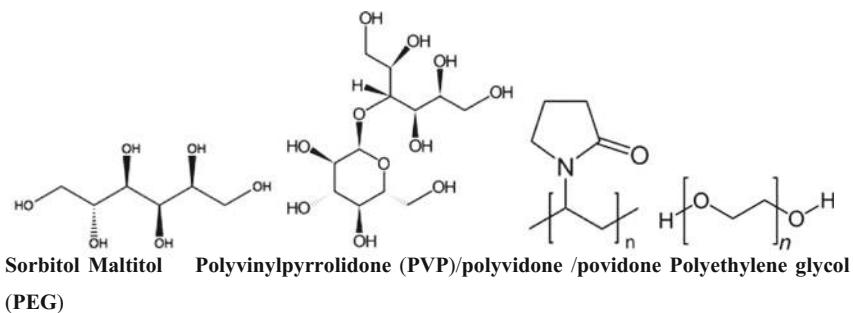
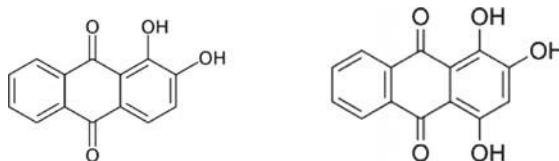
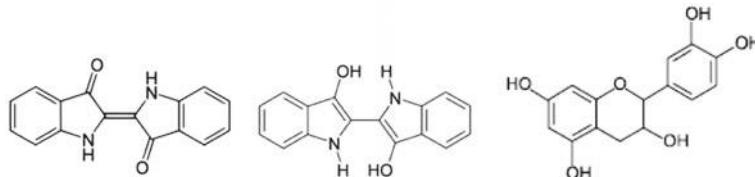
Carboxy methyl cellulose ($\text{R}=\text{H}$ or $\text{CH}_2\text{CO}_2\text{H}$), Hydroxy propyl cellulose ($\text{R}=\text{H}$ or $\text{CH}_2\text{CH}(\text{OH})\text{CH}_3$), XylitolHydroxypropyl methylcellulose ($\text{R}=\text{H}$ or CH_3 or $\text{CH}_2\text{CH}(\text{OH})\text{CH}_3$)

Fig. 5.37 Showing structure of sodium carboxymethyl starch, crospovidone or cross-linked povidone, carboxymethyl cellulose, hydroxy propyl cellulose, xylitol, sorbitol, maltitol, polyvinylpyrrolidone, polyethylene glycol, hydroxypropyl methylcellulose, alizarin, purpurin, indigo bluedye, indigo white, catechin, and glycerol monostearate



Alizarin (1,2-dihydroxyanthraquinone) red dye **Purpurin (1,2,4-trihydroxyanthraquinone)** red/yellow dye



Indigo blue dye

Indigo white (leuco-indigo)

Catechin



Glycerol monostearate (GMS)

Fig. 5.37 (continued)

sweetening agents such as sugar and sugar alcohols—sucrose, lactose, sorbitol, xylitol, erythritol, natural non-sugar substances—steviol, stevioside, rebaudioside A, mogroside V (esgoside), and artificial sweeteners—saccharin, aspartame, sucralose, neotame, acesulfame potassium (Ace-K), advantame, etc.

Sucrose is common table sugar a disaccharide consisting of two unit—glucose and fructose. Lactose is a disaccharide milk sugar composed of galactose and glucose units. Sugar alcohols like sorbitol, xylitol, and erythritol are natural sugar alcohols found in fruits and vegetables. They can be made commercially by catalytic hydrogenation from the corresponding sugars such as xylitol from xylose, sorbitol from glucose, erythritol by fermentation of glucose with the yeast *Moniliella pollinis*. Sugar alcohols contain fewer calories than pure sugar and honey, but more than stevia.

Stevia (*Stevia rebaudiana* of Asteraceae) leaves are used to produce extracts containing steviol, a diterpene compound, having up to 300 times the sweetness of sugar. Stevioside and rebaudioside A are two main sweet steviol glycosides out of 7 steviol glycosides in the stevia leaf. A commercial steviol glycoside mixture extracted from the plant was found to have about 80% stevioside, 8% rebaudioside A, and 0.6% rebaudioside C. Mogroside V (esgoside), a sugar alcohol, is the main sweetening compound of monk fruit (*Siraitia grosvenorii* of Cucurbitaceae), extract along with other four different mogrosides. It is approximately 300 times sweeter

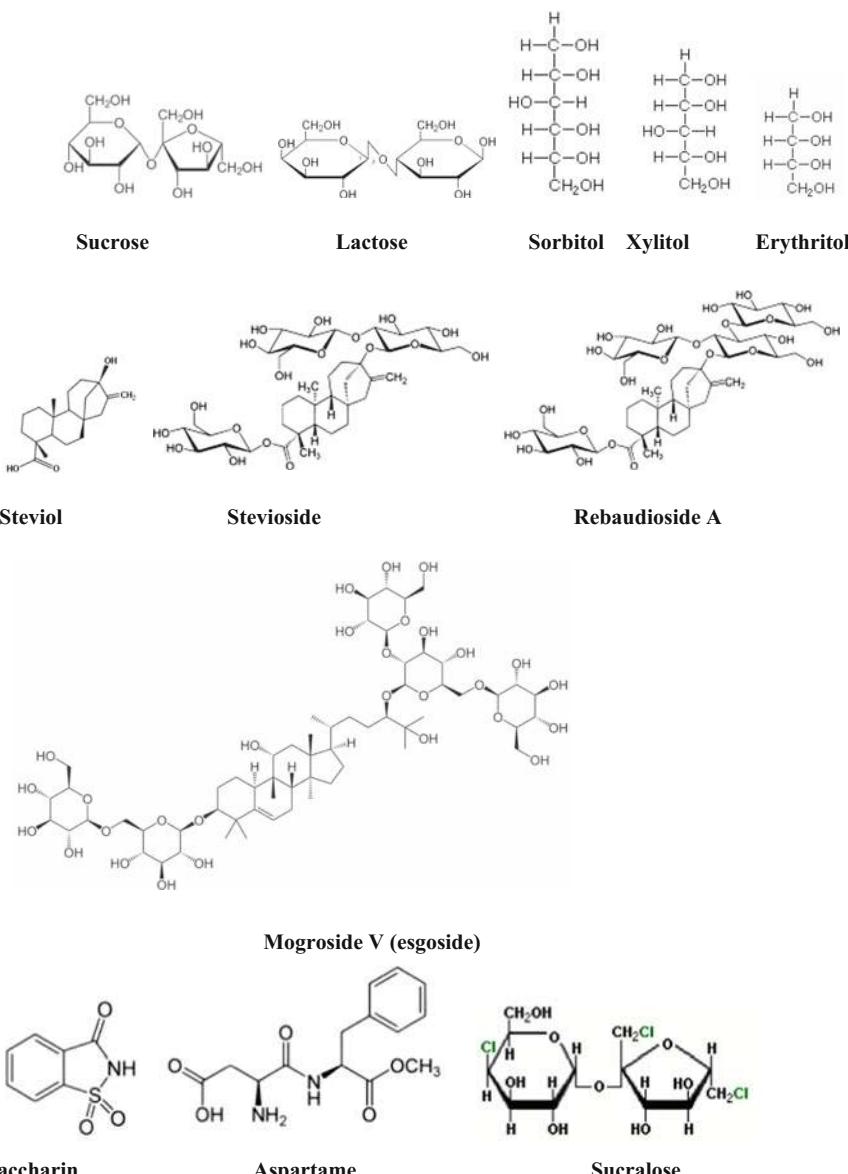
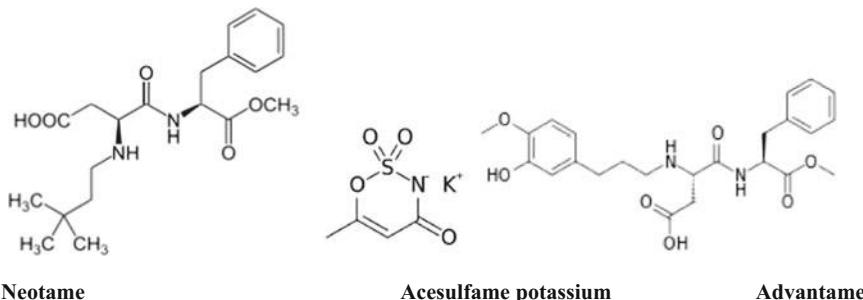


Fig. 5.38 Showing structure of different sweetening agents—sucrose, lactose, sorbitol, xylitol, erythritol; steviol, stevioside, rebaudioside A, mogroside V (esgoside); saccharin aspartame, sucralose, neotame, acesulfame potassium, and advantame

**Fig. 5.38** (continued)

than sugar and has been used as a natural sweetener in Chinese traditional medicine for about 1000 years. Brazzein is a sweet-tasting protein extracted from the fruit of a West African wild vine Oubli (*Pentadiplandra brazzeana* of Pentadiplandraceae). The brazzein molecule consists of 54 amino acid residues and four disulfide bonds arranged in one alpha-helix and three strands of antiparallel betasheets. On weight basis, brazzein is about 1000 times sweeter than sugar. Brazzein is stable over a broad pH range from 2.5 to 8, and it is heat stable at 98 °C for 2 h. Large-scale extraction of the sweetener from its natural source is not feasible, but brazzein has been produced from genetically engineered corn. The protein from the modified corn contains 4% brazzein, which when purified is up to 1200 times sweeter than sucrose on a weight basis.

Saccharin (benzoic sulfimide) is the oldest nonnutritive sweetener, 300 times sweeter than sucrose but has a bitter aftertaste. Saccharin is used to sweeten drinks, candies, medicines, and toothpaste. Saccharin is not used for baking because it is unstable when heated. Aspartame is 200 times sweeter than sugar. It is marketed under the brand names NutraSweet and Equal. Aspartame is the methyl ester of the dipeptide of the amino acids aspartic acid and phenylalanine (aspartyl-phenylalanine-1-methyl ester). Aspartame is used as a tabletop sweetener, and it is added to a wide variety of foods, including breakfast cereals, soft drinks, desserts, candy, and chewing gum. Aspartame loses its sweetness when heated and it is usually not suitable for baking. There's conflicting evidence regarding the safety of aspartame, a common chemical sweetener used in diet soda and other low-cal foods. Neotame is between 8000 and 13,000 times sweeter than table sugar. Neotame is chemically similar to aspartame, but sweeter and more stable. Acesulfame Potassium (Acesulfame K) is a nonnutritive sweetener 200 times sweeter than table sugar and its chemical structure is the potassium salt of 6-methyl-1,2,3-oxathiazine-4(3H)-one 2,2-dioxide. Sucralose is marketed as Splenda, about 600 times sweeter than table sugar, stable at hot and cold temperatures and can be used in cold and hot drinks, as well as baked goods. It is a mixture of dextrose, maltodextrin, and sucralose, though marketed as a no-calorie sweetener. Cyclamate is 30–50 times sweeter than sugar and is sold under the trade names Sucaryl and Sugar Twin; it is sodium or calcium salt of cyclamic acid

(cyclohexanesulfamic acid). Cyclamate was banned in the United States in 1970 because large doses caused bladder cancer in rats, but it is still approved as a sweetener in more than 55 countries. Aspartame is relatively easy to make and is approximately 200 times sweeter than sucrose. It is most commonly sold as Nutra Sweet and Equal.

Most noncaloric sweeteners are mixed with dextrose and maltodextrin to provide bulk, but unfortunately, these bulking agents are digestible carbohydrates that add calories.

5.3 Natural Sources, Classification, Chemistry, and Therapeutic Use of Enzymes and Anesthetic Aids

Proteins, peptides, and enzymes as drugs

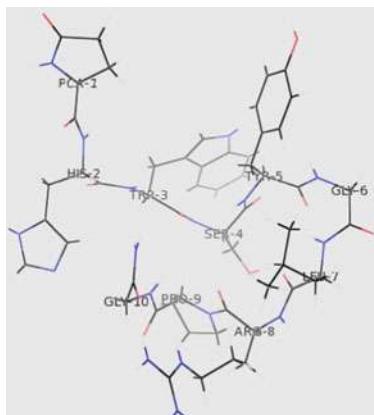
Peptides and proteins are complex molecules of naturally occurring 20 different α -amino acid monomers, which joined with each other by peptide bonds. Proteins are consisted of one or more peptide chains, and peptides are small chains of amino acid monomers and distinguished from proteins by their size (peptide contains <50 monomer units and molecular weight <5 kD, while a protein possesses ≥ 50 amino acids and its molecular weight lies >5 kD). Peptides allow the development of antibodies of a very specific region of a protein, useful for the identification of proteins of interest based on peptide masses and sequence and peptides are used in clinical research to examine the inhibition of different diseases including cancer. Different fermentations, purification processes, and recombination technology produced potential protein drugs at acceptable cost which can be useful in various diseases through various routes like oral, transdermal, nasal, pulmonary, ocular, buccal, and rectal. Availability and therapeutic application of proteins and peptides as drugs will replace many existing organic-based pharmaceuticals.

Ailments that might be treated with this type of therapeutics include auto-immune diseases, cancer, mental disorder, hypertension, and certain cardiovascular and metabolic diseases. Recombinant technology has allowed the production of many potential protein drugs at an acceptable cost, allowing the treatment of severe, chronic, and life-threatening diseases, such as diabetes, rheumatoid arthritis, hepatitis, etc. The first genetically engineered drug was insulin (a peptide hormone). The second recombinant enzyme drug was Activase1 (alteplase; recombinant human tissue plasminogen activator). It was approved by the Food and Drug Administration (FDA) for the treatment of heart attacks caused by the blockage of a coronary artery by a clot. Currently, over 160 protein drugs are available on the world market, and several hundreds more are in clinical trials. The total protein drug market already exceeds 30 billion and is expected to rise by at least 10% a year. One of the biggest opportunity areas in the Protein Therapeutics Market will be in the field of Biogenerics, which is expected to create a multi-billion dollar market in future.

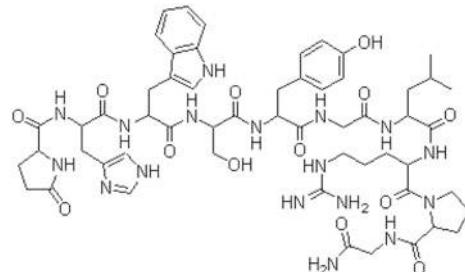
Many protein pharmaceuticals are available for treating rheumatoid arthritis, coronary artery thrombosis, multiple sclerosis, and chronic lymphocytic leukemia (Sheremata et al. 2005; Lequerré et al. 2008; Zhou et al. 2009). Some isolated proteins have been approved by the Food and Drug Administration (FDA) of the United States for clinical use or clinical trials. Some protein pharmaceuticals from Chinese medicine have been developed to treat cardiovascular diseases, genetic diseases, and cancer. Luteinizing hormone-releasing hormone (LHRH) agonist (also known as Gonadotropin-Releasing Hormone—GnRH or Luteinizing Hormone-Releasing Factor—LRF) is a naturally occurring decapeptide (Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂) hormone with a molecular weight of 1.182kD. It is used in the treatment of prostate carcinoma. Insulin is a peptide molecule, with a molecular weight of 6kD. It is used in the treatment of diabetes. Vasopressin (antidiuretic hormone—ADH or arginine vasopressin—AVP) is a nonapeptide (an oligopeptide) with a molecular weight of 1.084kD. It is used as an antidiuretic hormone. Figure 5.39 shows the structure of proteins, peptides, and enzymes drugs—gonadotropin-releasing hormone—GnRH, gonadorelin—synthetic GnRH, vasopressin with labeled amino acids, and glutathione. Figure 5.39 shows the structure of proteins, peptides, and enzymes drugs—gonadotropin-releasing hormone—GnRH, gonadorelin—synthetic GnRH, vasopressin with labeled amino acids, glutathione, thyrotropin-releasing hormone (TRH), oxytocin with labeled amino acids, somatostatin, melittin, endothelin, angiotensin II, and glucagon below.

Examples of common natural therapeutic peptides include (i) Glutathione (a tripeptide consisting of amino acids L-cysteine, L-glutamic acid, and glycine), common non-ribosomal peptide and a component of the antioxidant defenses of most aerobic organisms, present in most living cells and stimulates tissue growth; (ii) Thyrotropin-releasing hormone (TRH) (a tripeptide of Glu-His-Pro-NH₂) hypothalamic neurohormone and governs release of thyrotropin; (iii) Angiotensin II (octapeptide composed of Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) pressor agent and acts on the adrenal gland, bradykinin (nonapeptide) hypotensive vasodilator, and acts on smooth muscle; (iv) Oxytocin (nonapeptide) uterus-contracting hormone stimulates lactation, (v) Somatostatin (consisting of 14 amino acid residues) inhibits growth hormone release and used to treat ulcers, (vi) Endothelin (consisting of 21 amino acid residues) potent vasoconstrictor and structurally similar to some snake venoms, (vii) Melittin (consisting of 26 amino acid residues) honey bee venom and used to treat rheumatism, (viii) Glucagon (consisting of 29 amino acid residues) hyperglycemic factor and used as an antidiabetic, and (ix) Insulin (consisting of 51 amino acid residues) pancreatic hormone and used in treatment of diabetes.

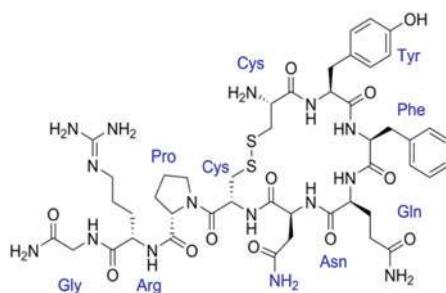
Bioactive proteins and peptides with various pharmacological properties have been successfully isolated from different Chinese herbs including (i) medicinal fungi such as *Cordyceps militaris*—lectin designated as Chlorophyllum molybdites lectin (CML) (31 kDa), an immunomodulatory protein—Ling Zhi-8 or LZ8 (12.4 kDa protein); *Ganoderma* spp.—Lectin (a 18-kDa protein); *Poria cocos*—*P. cocos* immunomodulatory protein(PCP) (35.6 kDa); (ii) medicinal plants such as *Viola tricolor*—Cyclotides; *Momordica cochinchinensis* seeds—Cochinin



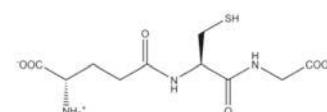
Gonadotropin-Releasing Hormone –GnRH



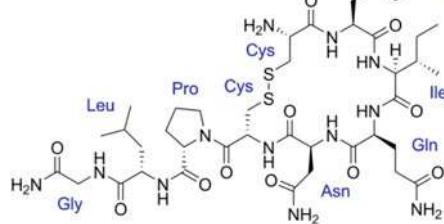
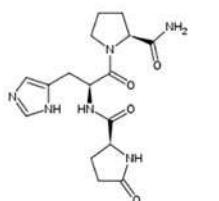
Gonadorelin- synthetic GnRH



Vasopressin with labeled amino acids

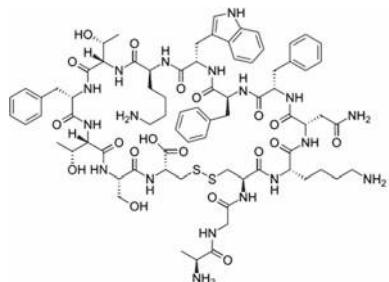
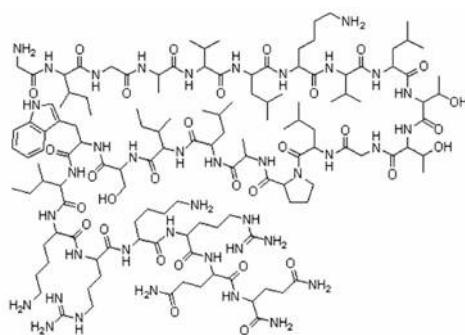
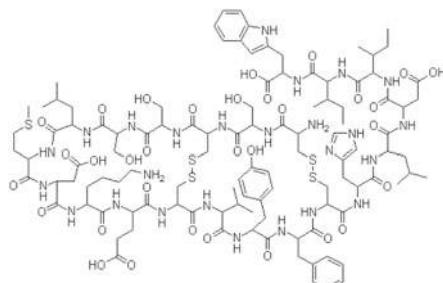
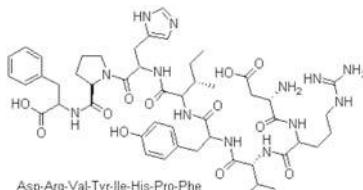
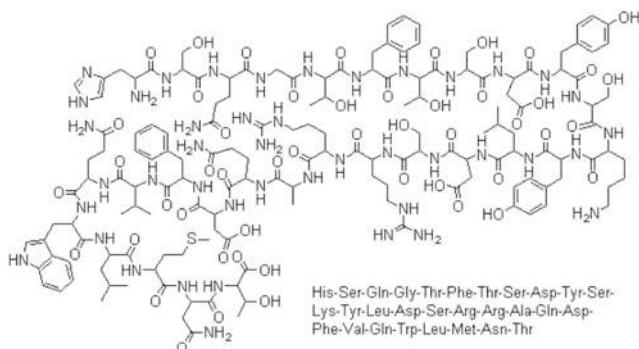


Glutathione



Thyrotropin-releasing hormone (TRH) Oxytocin with labeled amino acids

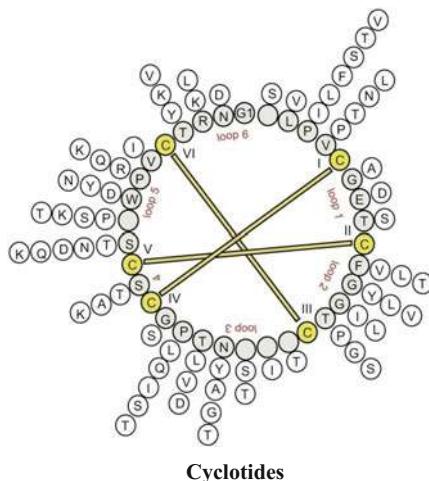
Fig. 5.39 Showing structure of proteins, peptides and enzymes drugs—gonadotropin-releasing hormone –GnRH, gonadorelin—synthetic GnRH, vasopressin with labeled amino acids, glutathione, thyrotropin-releasing hormone (TRH), oxytocin with labeled amino acids, somatostatin, melittin, endothelin, angiotensin II, and glucagon

**Somatostatin****Melittin****Endothelin****Angiotensin II****Glucagon****Fig. 5.39** (continued)

B, ribosome-inactivating protein (RIP) (28 kDa), MCoCC-1 (a 33 amino acid long peptide), Chymotrypsin inhibitor designated as MCoCI (7.5 kDa); *Viscum album*—lectin designated as CM-1 (55 kDa), lectin designated as ACML-55; the seeds of *Senna obtusifolia*—novel protein (19.7 kDa); *Narcissus tazetta* var. chinensis—*Narcissus tazetta* lectin (26 kDa); *Smilax glabra* rhizomes—smilaxin (30 kDa); *Ginkgo biloba* seeds—Ginkobilobin (13 kDa); *Dioscorea batata*—Dioscorin (32 kDa); *Trichosanthes kirilowii*—trichosanthin (247 amino acid long peptide); and (iii) medicinal animals such as *Hirudo* spp. (Leeches)—hirudin; *Eisenia fetida* (earthworm)—earthworm fibrinolytic enzyme and *Mesobuthus martensii*—anti-epilepsy protein (8.3 kDa). These therapeutic proteins, peptides, and enzymes, in vitro and/or in vivo experiments, were found active against different types of cancer, oxidative stress, cholesterol biosynthesis, and some showed antiviral, antibacterial, antifungal, anticoagulant, antiepileptic, anti-HIV, as well as immunomodulatory (Byers and Baldwin 1991; Rydel et al. 1991; Wang and Ng 2000; Tsoi et al. 2005; Chu and Ng 2006; Ricotti and Delanty 2006; Chen et al. 2007; Li et al. 2008; Ma et al. 2008; Ooi et al. 2010; Tang et al. 2010). Lectins are carbohydrate-binding protein (glycoproteins) macromolecules that can bind to cell membranes. Lectins perform recognition on the cellular and molecular level and play numerous roles in biological recognition phenomena involving cells, carbohydrates, and proteins. Gelatin is obtained from animal collagen and is a pharmaceutical aid. Other protein-based drugs are absorbable gelatin sponge and film, microfibrillar collagen surgical sutures, penicillamine, heparin sodium, heparin calcium, protamine sulfate, and levodopa (Fig. 5.40).

Natural sources, classification, and chemistry of enzymes and anesthetic aids
Proteins and certain RNA with catalytic activities are known as ferments or enzymes. These groups of compounds are capable of instituting chemic changes without apparently entering into the reaction or forming a part of the end products. Their activity is very persistent, but not unlimited. They are unstable bodies and are nearly all destroyed at a temperature of about 60 °C. Examples are invertase, which transforms cane sugar into fructose and glucose; lactase, which changes sugar-of-milk into glucose and galactose; maltase, which converts maltose into glucose; emulsion and myrosin, of whose reactions with certain glucosides we have spoken, and pepsin, trypsin, and the other enzymes of the digestive tract. A number of enzymes have a reversible action, i.e., can, under certain circumstances, bring about changes just the reverse of the usual. The metabolic changes going on in the plant or animal body are brought about by enzymes. The oxidases, for example, are concerned in the oxidation processes of the tissues. There are six major classes of enzymes such as (1) Oxidoreductases, (2) Transferases, (3) Hydrolases, (4) Lyases, (5) Isomerases, and (6) Ligases or synthetases.

Enzymes are sometimes called organic catalysts which are produced by plants and animals having high molecular weight ranging from 13,000 to 8,40,000 Da. At ordinary temperatures, they bring about chemical changes by lowering quantity of energy required for substrate activation. Most enzymes are insoluble in alcohol, ether, and other organic solvents, but are soluble in water. In some cases, the



Cyclotides

The circular structure, three conserved disulfide bonds (yellow), and the amino acid variation at all other positions shown for a collection of 18 cyclotides from a single plant species, *Oldenlandia affinis*.

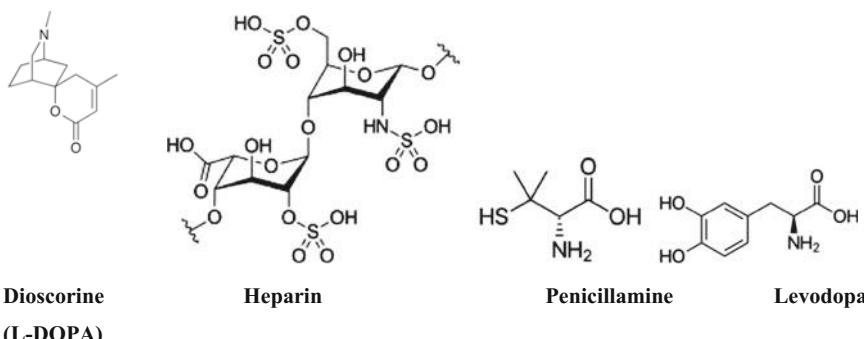


Fig. 5.40 Showing structure of circular cyclotides, dioscorine (alkaloid toxin), heparin (unfractionated heparin -UFH), penicillamine (cuprimine), and levodopa (L-DOPA)

enzymes are combined with the protoplasm which must be killed by an organic solvent (e.g., CHCl₃, toluene) or by mechanical means before extraction of the enzyme. Some enzymes do not preexist in the tissues, but are formed from substances termed zymogens. In nature, decomposition of the zymogen is carried out by a complex substance, known as kinase, to form the enzyme when needed.

The substances on which enzyme acts or with which enzyme reacts are termed as substrates. In nature, enzyme and substrate are sometimes present in the same cell and the reaction may take place continuously. In other cases, enzymes and substrate are found in different cells. The reaction starts on diffusion of one of the substances.

Some enzymes are combined with the protoplasm, and this represents one method of preventing diffusion. The rate of chemical change brought about by enzyme is affected by certain factors. For examples, some substances, like paralysers, partially or entirely, inhibit the action of the enzymes. The substances, called co-enzymes, are required for the action. The substances known as accelerators or activators greatly accelerate the rate of reaction. Temperature is an important factor in enzyme action. For each enzyme, there is a particular temperature, called the optimum temperature and lies between 35 and 45 °C, at which reaction proceeds most rapidly. Most of the enzymatic reactions are inhibited below 10 °C and destroyed by heating to 100 °C.

Important features that distinguish enzymes as drugs from all other drugs are (i) their great target binding affinity and specificity and (ii) catalytic properties, and these characteristics have resulted in the development of many enzyme drugs for a wide range of disorders (Vellard 2003). Proteolytic enzymes digest protein by aiding in the digestion process, breaking it down into amino acids. They can be taken as a supplement, but better yet, they can be found naturally in certain foods, e.g., papaya contains the proteolytic enzyme papain, a popular meat tenderizer. Papain found in papayas may be very helpful for the prevention of atherosclerosis and diabetic heart disease.

Proteolytic enzymes are known as proteases, e.g., pepsin, trypsin, and chymotrypsin are three main proteases. The protease enzyme breaks down protein found in meats, poultry, fish, nuts, eggs, and cheese, and may be helpful for people with food allergies or those who have difficulty digesting protein. Some manufacturers derive their enzymes from animal sources. For example, supplements that contain trypsin or chymotrypsin are extracted from livestock, while supplements that contain papain or bromelain come from plant sources.

Papain is the dried and purified latex of the fruit of *Carica papaya* and used as a digestant. Chymopapain is a nonpyrogenic proteolytic enzyme obtained from the latex of *Carica payaya* and employed in the treatment of herniated lumbar intervertebral disks. Bromelains is a mixture of protein-digesting and milk-clotting enzymes obtained from the juice of the pineapple, *Ananas comosus*. It is used as adjunctive therapy to reduce inflammation and oedema and to reduce tissue repair. Proteolytic enzymes modulate the inflammatory process by a variety of mechanisms, including reducing the swelling of mucous membranes, decreasing capillary permeability, and dissolving blood clot-forming fibrin deposits and micro-thrombi. By reducing the viscosity (thickness) of the blood, proteolytic enzymes improve circulation. This consequently increases the supply of oxygen and nutrients and the transport of harmful waste products away from traumatized tissue. Proteolytic enzymes also help break down plasma proteins and cellular debris at the site of an injury into smaller fragments. This greatly facilitates their passage through the lymphatic system, resulting in more rapid resolution of swelling, with the consequent relief of pain and discomfort in the bones and joints affected. These enzymes can help athletes recover faster from hard workouts and races.

Vellard (2003) mentioned 10 FDA-approved enzymes designated as orphan drugs in the USA. They are (i) adagen (pegademase bovine), for enzyme

replacement therapy (ERT) for adenosine deaminase (ADA) in patients with severe combined immunodeficiency disease (SCID); (ii) ceredase (alglucerase injection), for ERT in patients with Gaucher's disease type I; (iii) pulmozyme (dornase a), enzyme to reduce mucous viscosity and enable the clearance of airway secretions in patients with cystic fibrosis (CF); (iv) cerezyme (imiglucerase), for enzyme replacement therapy in patients with types I, II, and III Gaucher's disease; (v) oncaspar (pegaspargase), enzyme for treatment of acute lymphocytic leukemia; (vi) sucraid (sacrosidase), enzyme for treatment of congenital sucrase-isomaltase deficiency; (vii) elitek (rasburicase), enzyme for treatment of malignancy-associated or chemotherapy-induced hyperuricemia; (viii) fabrazyme (agalsidase beta), enzyme for treatment of Fabry's disease; (ix) aldurazyme (laronidase), enzyme for treatment of patients with mucopolysaccharidosis I (MPS I); and (x) replagal (α -Galactosidase A), enzyme for long-term enzyme replacement therapy for the treatment of Fabry's disease.

Enzymes are usually soluble in water. They usually accompany with glycosides. Some drugs like Wild Cherry, Almonds, Mustard and Wintergreen, owe their value not to the glycoside present, but to its decomposition products by the enzymes. Some important enzymes of medicinal importance are pancreatin of pancreas used to treat pancreatitis; trypsin of ox pancreas used to cure wounds, ulcers, abscesses, and fistulas and as anti-inflammatory agent; chymotrypsin of pancreas of ox used identically as trypsin; fibrinolysin utilized to treat venous thrombosis and pulmonary embolism; pepsin of the gastric juice employed to treat achylia gastrica; hyaluronidase found in microorganisms, leaches, snake venom, and mammalian testes, and used to facilitate the administration of fluids by hydronermolysis.

Peptide hormones

Hormones are secreted by endocrine glands of animals. Thyroxine, conjugated oestrogens, insulin, epinephrine, oxytocin, vasopressin, and gonadotropins are important mammalian hormones released directly into the blood. Thyroxin hormone of thyroid gland is used to treat thyroid insufficiency. Menopausal symptoms in females and dysmenorrhea are treated with conjugated oestrogens. Insulin, a polypeptide hormone secreted by the beta cells of the islets of Langerhans of pancreas gland, is employed to cure diabetes. Adrenal medulla of mammals secretes the hormone epinephrine (adrenaline) which is utilized as vasoconstrictor to cure acute asthma. Oxytocin, a polypeptide hormone secreted by posterior pituitary gland, causes contraction of uterine muscles, stimulates the ejection of milk in lactating mothers, induces labor in pregnant women, and stops hemorrhage after childbirth. Another peptide hormone of the posterior lobe of pituitary gland, vasopressin, is used in the treatment of intestinal paralysis and diabetes. Gonadotropins are secreted by the interior lobe of the pituitary gland which controls the production of sex hormones. They are employed to cure infertility and in cryptorchidism.

Microorganisms

Microorganisms (microbes) are the viruses, bacteria, and rickettsiae which are sources of many biological substances of immunization importance. These drugs

possess immunity against various infectious diseases. Immunity is acquired by administration of a vaccine, toxoid, or antitoxin like diphtheria. Vaccines are suspended microorganisms which may be obtained from viruses, bacteria, and rickettsiae. On introduction into body, a vaccine stimulates the production of antibodies against pathogenic microbes. Viral vaccines are prophylactic agents used against polio, smallpox, rabies, influenza, measles, and mumps. Rickettsial vaccine, prepared from gram-negative microorganisms, is the typhus vaccine which produces active immunity against typhus fever. Bacterial vaccine is the suspension of pathogenic bacteria in sodium chloride or other solvent. Bacteria vaccine includes Typhoid vaccine, Cholera vaccine, Plague vaccine, Pertussis vaccine (for whooping cough), and BCG vaccine (for tuberculosis).

The waste products of bacteria, called toxins, are dissolved in the surrounding culture medium after excretion. On treatment with formaldehyde, their toxic properties are reduced but their antigenic property is not affected. These products are called fluid toxoids which are precipitated with alum, aluminum hydroxide, or aluminum phosphate. The toxoids are used to induce artificial active immunity in susceptible individual. For example, tetanus toxoid and diphtheria toxoid are the microbial products used to produce immunity in young children against diphtheria, tetanus, and whooping cough.

Marine products

Marine products are used as thickening, emulsifying and suspending agents. Carrageenan from *Chondrus crispus* (Irish Moss) and alginates from species of *Laminaria*. Ascophyllum, *Ecklonia*, *Nereocystis*, and *Macrocystis* are used in adhesive formulations and as stabilizers, ingredients of ointment bases, suspending agent, and tablet disintegrating agents. Agar, obtained from species of *Gelidium* and *Gracilaria*, is used as laxative, emulsifier, suspending agent and in the preparation of vaginal capsules, suppositories, and nutrient media in bacteriological culture. Spermaceti, a solid waxy substance obtained from the oil of the sperm whale, *Physeter macrocephalus*, is used as a pharmaceutical aid for creams, ointments, cerates, soaps, cosmetics, etc. Shark liver oil, a fixed oil obtained from the liver of shark fish, *Hypoprion brevirostris*, is nutritive and used as a tonic and to treat xerophthalmia occurring due to deficiency of vitamin A. The marine fungus, *Cephalosporium acremonium*, produces the antibiotic cephalosporin C identical to penicillins. The strongly basic protein of low molecular weight, protamine, is obtained from the testes of the fish salmon. It is used as a heparin antagonist. Pralidoxime is produced from electric eel which acts as antidote for certain types of insecticide poisoning in humans. The Japanese red alga, *Digenea simplex*, contains the amino acid known as kainic acid from which an anthelmintic drug is prepared. Cod liver oil is the source of vitamins A and D.

An anticoagulant agent has been isolated from the sea anemone, *Rhodactis howesii*. Very potent anticancer agents, named dolastatin 1-9, are present in Indian Ocean sea hare. The marine annelid, *Lumbriconeris heteropoda*, is toxic to some insects. The richest natural source of prostaglandin is the soft coral *Plexaura homomalla*. Many toxins occur throughout the complete range of marine life; they

include irritants, CNS stimulants and depressants, haemolytic substances, and protoplasmic poisons. Extracts of various marine algae contain vitamin C, folic acid, foionic acid, niacin, and vitamin B.

Natural anesthetics

There are two main types of natural anesthetics such as (i) topical and (ii) internal. Topical anesthetics are used directly on skin or external surface to treat issues like toothaches, cuts, rashes, and burns while internal anesthetics are ingested and are used to treat conditions like general pain, headaches, and muscle aches. Anesthetics may also be grouped as local anesthetic, general anesthetic, antinociceptive, analgesic, sedative, etc., and may be derived from various phytochemicals including terpenoids (menthol, eugenol, thymol, carvacrol, linalool), alkaloids (cocaine, piperine, lappaconitine, bulleyaconitine A, 3-acetylaconitine), flavonoids (catechin, resveratrol, apigenin, kaempferol, baicalein, wogonin, chrysins, isoliquiritigenin), etc. For molecular mechanisms, anesthetics may interact with Na⁺ channel, aminobutyric acid type A (GABA_A) receptor, N-methyl-D-aspartate (NMDA) receptor, etc., and may also interact with cell membrane. Anesthetics are used for their ability to cause physical insensitivity. The feeling of pain may be blocked, numbed, or temporarily taken away. It is important to know that these herbal medications have potential interactions with other drugs; the patient might receive as part of treatments.

Natural anesthetics have been in use since antiquity has been continuing up to this day of modern medicine. Earlier, mandrake root (plants of the genus *Mandragora* which contain hallucinogenic and narcotic alkaloids) was used for its natural anesthetic properties, but it was replaced by other, less toxic alternatives. Cocaine, derived from the coca (*Erythroxylum coca*) leaf; methyl salicylate in oil of wintergreen (a group of aromatic plants; most species of the shrub genus *Gaultheria*, especially *Gaultheria procumbens*); and capsaicin, the active ingredient in cayenne and other hot peppers, etc., are a few examples natural topical anesthetics. In addition to capsaicin, cayenne also contains salicylates. Wintergreen is a mild anesthetic and good to treat toothaches or stomach aches. Wintergreen is the source of an essential oil that contains natural anesthetic components like salicylates; an effective pain reliever is present in wintergreen, and wintergreen cream can be used topically for arthritis, aching muscles, and gout. Wintergreen is also effective in treating the discomfort of backaches and the pain of tendinitis. A homemade anesthetic cream can be made by adding wintergreen leaves to white skin lotion and blending until a green color permeates the mixture. Opium (*Papaver somniferum*, its latex contains ~12% analgesic alkaloid morphine), curare (a South American vine that contains arrow poison alkaloid), etc., are commonly used plant-based anesthetics of the early days. The active ingredient of opium poppy is morphine, and other derivatives of the opium poppy have frequently been employed to anesthetize and eliminate pain. Large doses of opium were used to anesthetize surgical patients before ether was discovered. Laudanum, codeine, and heroin are some derivatives of the opium poppy that have been used extensively in western medicine. Opiate usage dropped after the addictive properties became known, but

some opium derivatives, such as codeine and hydrocodone, are still used to relieve pain. South American indigenous cultures used curare, the sap from various plants native to rainforests, as arrow poison on the tips of darts shot from blowguns to paralyze prey. The paralytic effect allows curare to be used as an intravenous anesthetic. Curare relaxes the muscles, keeping a patient still during surgery.

Black pepper (*Piper nigrum*, Piperaceae) contains piperine alkaloid, which helps to counteract pain in the body. Eugenol of clove oil (70–90% of the oil) extracted from flower buds, leaves and stems of clove trees (*Syzygium aromaticum* Myrtaceae), is a natural anesthetic and has been used topically for centuries to relieve toothaches, effective as benzocaine for topically numbing pain. Many hatcheries and research studies use clove oil to immobilize fish for handling, sorting, tagging, artificial reproduction procedures, and surgery and to suppress sensory systems during invasive procedures. Lavender (*Lavandula angustifolia*, Lamiaceae) pretty purple flower has been used for centuries as an antiseptic, topical anesthetic and sedative. Lavender oil contains linalool alcohol, ketone, sterzoaldehyde, etc., as active principles. Onions, garlic, ginger, tea tree oil, and Epsom salts may also function as anesthetics. Anesthetics thymol, a natural monoterpenic phenol derivative of cymene, is contained in thyme (*Thymus vulgaris*, Lamiaceae). Chewing betel leaf (*Piper betel*, Piperaceae) showed both infiltration and surface anesthetic effects that were almost comparable to those of lidocaine. Another natural anesthetic that contains salicylates is cayenne. All hot peppers have these pain-relieving constituents, in addition to the natural anesthetic capsaicin. Capsaicin is a popular ingredient in creams sold over the counter to rub on sore muscles and arthritic joints.

The plant kingdom is the most common source of natural anesthetics, but extreme cold can also be used to numb an area. Ice packs are often recommended to treat inflammation. The cold helps cools down blood vessels, reducing inflammation and anesthetizing the area to relieve pain. Running cold water over a sore muscle or aching joint can also relieve pain and inflammation. Figure 5.41 shows structure of cocaine, thymol, piperine, carvacrol, or cymophenol, ionlool isomers (coriandrol, licareol), resveratrol, and apigenin.

Anesthetic herbs create numbness and relieve or block pain. Anesthetic herbs other than mentioned above include arnica (*Arnica montana*, Asteraceae), black cohosh (*Actaea racemosa*, Ranunculaceae), butterbur and coltsfoots (*Petasites spp.*, Asteraceae), common butterbur (*Petasites hybridus*) California poppy (*Eschscholzia californica*, Papaveraceae), cannabis (*Cannabis sativa*, Cannabaceae), caraway (*Carum carvi*, Apiaceae), kava kava (*Piper methysticum*, Piperaceae), lobelia (*Lobelia inflata*, Campanulaceae), mullein (*Verbascum thapsus*, Scrophulariaceae), Pau D'Arco (*Tabebuia avellanedae*, Bignoniaceae), Skullcap (*Scutellaria baicalensis*, Lamiaceae), St. John's Wort (*Hypericum perforatum*, Hypericaceae), tea tree bark (*Melaleuca alternifolia*, Myrtaceae), turmeric (*Curcuma longa*, Zingiberaceae), valerian (*Valeriana officinalis*, Caprifoliaceae), Wild Lettuce (*Lactuca virosa*, Asteraceae), wild yam (*Dioscorea villosa*, Dioscoreaceae), willow bark (*Salix alba*, Salicaceae), wood betony (*Stachys officinalis*, Lamiaceae), yarrow (*Achillea millefolium*, Asteraceae), etc.

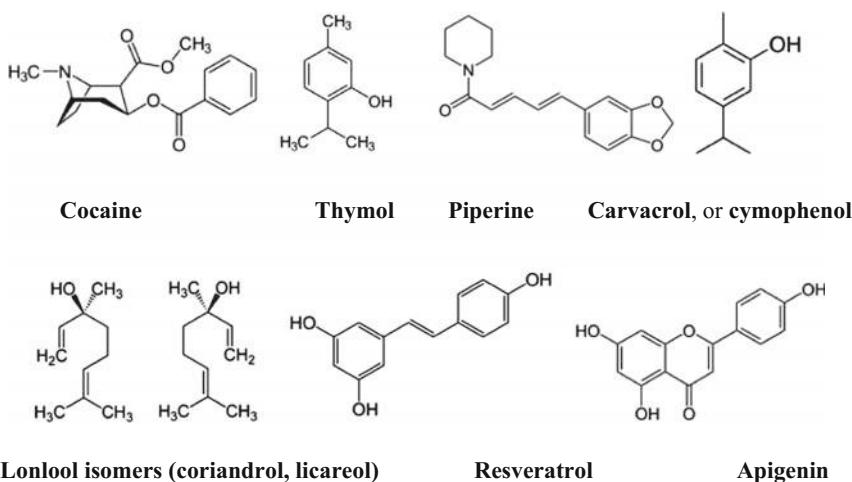


Fig. 5.41 Showing structure of cocaine, thymol, piperine, carvacrol, or cymophenol, lonlool isomers (coriandrol, licareol), resveratrol, and apigenin

5.4 Natural Sources, Classification, Chemistry, and Therapeutic Use of Cosmeceuticals

Natural sources, classification, and chemistry of cosmeceuticals

The term “cosmeceuticals,” derived from the combination of cosmetics and pharmaceuticals, is a hybrid combination. Cosmeceuticals are cosmetic-pharmaceutical hybrid products intended to improve the health and beauty of the skin by providing a specific result, ranging from acne-control and anti-wrinkle effects to sun protection. Cosmeceuticals have medicinal benefits depending on type of functional ingredients they contain. They are cosmetic products containing biologically active ingredients to add medical or drug-like, e.g., lotion, cream, ointment, etc., containing botanical, animal and marine extracts like antioxidants (vitamin C vitamin E—ascorbic acid, idebenone, lipoic acid, and tocopherol, tartaric acid); vitamins (vitamin A—retinoids, retinoic acid/tretinoin, adapalene and tazarotene; B vitamins); peptides (carnosine, copper tripeptide, palmitoyl oligopeptide, acetyl hexapeptide-3, soybean peptide, silk fibroin peptide, black rice oligopeptides, keratin peptide); essential oils (agarwood, lavender, chamomile, geranium, rosemary, sweet orange, eucalyptus oil and sandalwood oil), waxes (floral and plant essential waxes, Jasmine, and Rose waxes), oils (coconut oil, sunflower oil, olive oil, jojoba oil), natural color (turmeric—*Curcuma longa*, henna/mehendi leaf—*Lawsonia inermis*), saffron stigmas and styles—*Crocus sativus*, tea leaf—*Camellia sinensis*, coal tar dye), natural fragrances (essential oils, resins from lavender, lemon balm, rosemary and mint and thyme) parts of plants like leaves, buds, flowers, etc. Cosmetics containing botanicals are herbal cosmetics and for herbal cosmetics, permissible cosmetic ingredients are taken to form the base and one or

more herbal ingredients are added to it. Trees and herbs like *Azadirachta indica*, *Cocos nucifera*, *Aloe vera* spp., *Camellia sinensis*, *Calendula* spp., *Carica papaya*, *Curcuma longa*, *Cymbopogon* spp., *Daucus carota*, *Embelica officinalis*, *Eucalyptus* spp., *Ginkgo biloba*, *Helianthus annus*, *Lawsonia inermis*, *Rhodiola rosea*, *Rosea* spp., and similar many other herbs possess a vast and complex arsenal of bioactive phytochemicals (e.g., vitamins, antioxidants, oils and essential oils, hydrocolloids, proteins, terpenoids, etc.) that are able to calm or smooth, clean, restore, heal, and protect the skin and other parts of the body. Researchers support the use of antioxidants in topical applications (Burke 2007; Manosroi et al. 2011; Rohr et al. 2011). And Vitamin E, Vitamin C (ascorbic acid), idebenone, lipoic acid, and tocopherol are all effective antioxidants (McDaniel et al. 2005). Topical cosmeceutical peptides can be classified as signal peptides, carrier peptides, neurotransmitter inhibitor peptides, and enzyme inhibitor peptides (Schagen 2017). These peptides are used for collagen stimulation, wound healing, wrinkle smoothing, as well as antioxidant, antimicrobial (Rahnamaeian and Vilcinskas 2015). The composition of agarwood oil is exceedingly complex, consisting of >150 compounds, and ~70 of them are terpenoids, e.g., sesquiterpenes and chromones and other classes of compounds include agarofurans, cadinanes, eudesmanes, valencanes and eremophilanes, guaiaines, prezizanes, vetispiranes, simple volatile aromatic compounds as well as a range of miscellaneous compounds (Naef 2010). Most essential oils have cosmetic benefits in addition to their therapeutic properties. The essential oils have gained their importance in therapeutic, cosmetic, aromatic, fragrant, and spiritual uses (Svoboda and Deans 1995; Evans 2000). Aromatherapy, one of the complementary therapies, uses essential oils as the major therapeutic agents to treat several diseases or to get relief from numerous ailments like depression, indigestion, headache, insomnia, muscular pain, respiratory problems, skin ailments, swollen joints, urine associated complications, etc. (Ali et al. 2015). Turmeric has been used for centuries as an herbal medicine, a spice in cooking, in cosmetics, and dye. Curcumin is a yellow-orange dye obtained from turmeric and it has many valuable properties that can be exploited to treat dermatologic conditions (Chaudhari et al. 2015). Lawsone, 2-hydroxy-1,4-naphthoquinone or hennotannic acid, is a red-orange dye present in the leaves of the henna plant (*Lawsonia inermis*) as well as in the flower of water hyacinth (*Eichhornia crassipes*) (Dweck 2002). Saffron's golden yellow-orange color is primarily the result of α -crocin and crocin has been shown to be an antioxidant (Papandreou et al. 2006; Akhtari et al. 2013), neural protective (Ochiai et al. 2006; Zheng et al. 2006), and antiproliferative action (Escribano et al. 1996; Abdullaev Jafarova et al. 2002; Chryssanthi et al. 2007). Saffron contains more than 150 volatile and aroma-yielding compounds mainly terpenes, terpene alcohol, and their esters; and one important biological activity of saffron for cosmetics formulators. Figure 5.42 shows the structure of idebenone, lipoic acid (LA or α -LA), cadalene (in agar oil), tretinoin, adapalene, tazarotene (both third-generation topical retinoids), carnosine (*beta*-alanyl-L-histidine), copper tripeptide (glycyl-L-histidyl-L-lysine), palmitoyl pentapeptide-4, acetyl hexapeptide-3, curcumin (enol form), lawsone (hennotannic acid), and crocin carotenoid. Examples of some of the

zoochemicals used in cosmetics include (i) hyaluronic acid, produced from rooster combs is used in antiaging skincare products as it is an antioxidant, a humectant and boosts collagen synthesis; (ii) carmine, a red dye made of red pigment from the crushed female cochineal insect, is often used in lipsticks, rouge, eye shadow, etc., and also used in food and drinks; (iii) most collagen in skincare creams comes from chicken feet and animal horns, loss of collagen is one of the main signs of facial

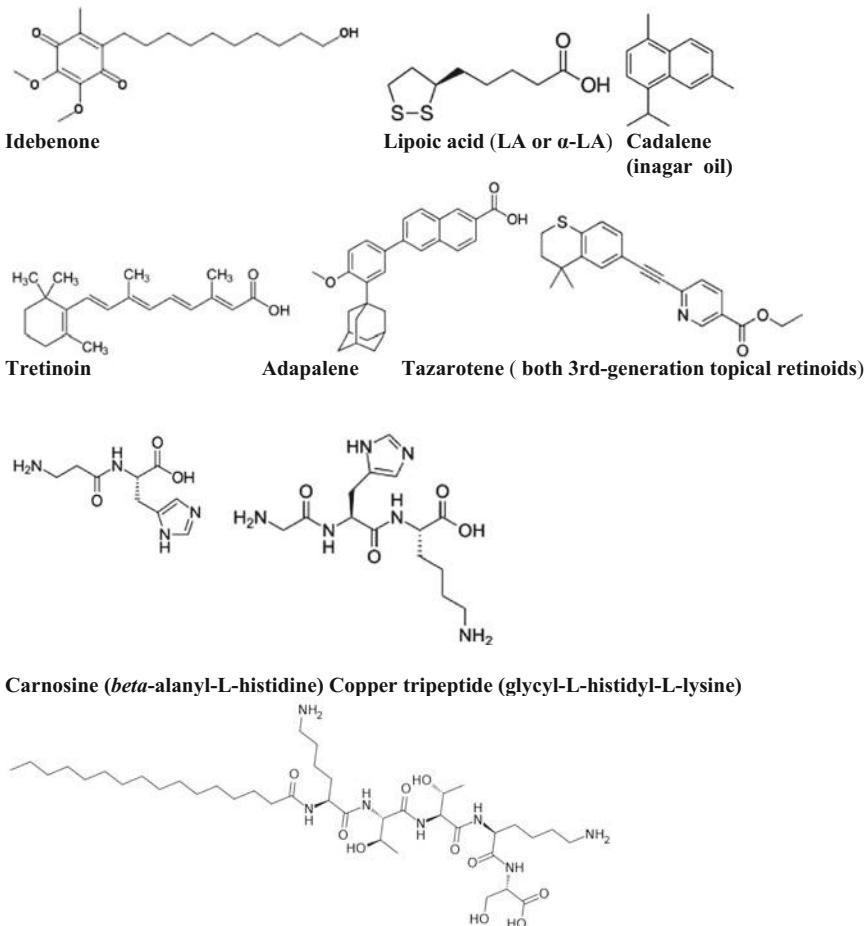


Fig. 5.42 Showing structure of idebenone, lipoic acid (LA or α -LA), cadalene (in agar oil), tretinoin, adapalene, tazarotene (both 3rd-generation topical retinoids), carnosine (*beta*-alanyl-L-histidine), copper tripeptide (glycyl-L-histidyl-L-lysine), palmitoyl pentapeptide-4, Acetyl hexapeptide-3, gurcumin (enol form), lawsone (hennotannic acid), and crocin carotenoid

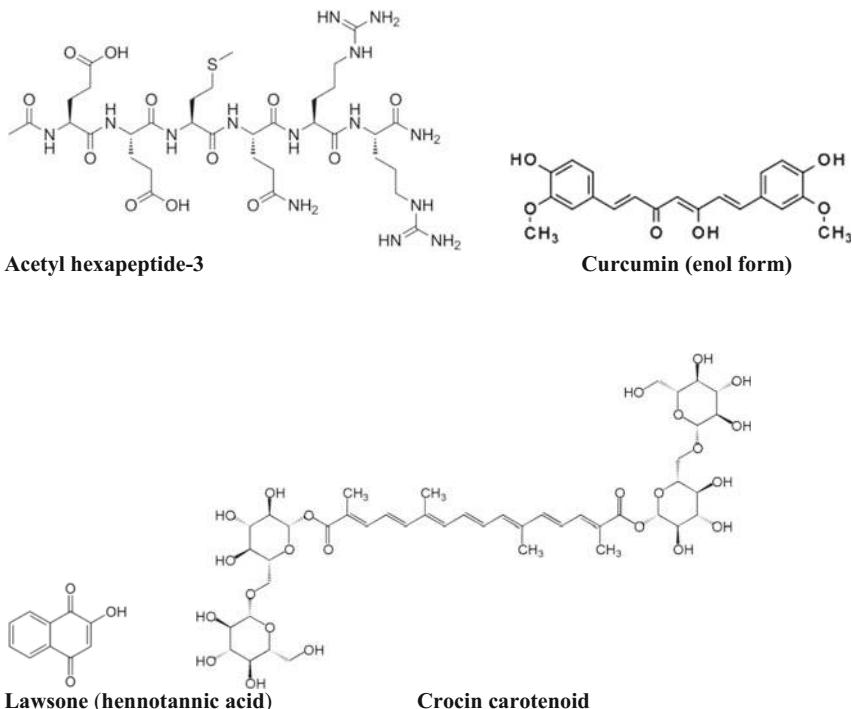


Fig. 5.42 (continued)

aging; (iv) glucosamine is derived from chicken bone marrow for the cosmetics industry; (v) ambergris from sperm whale used in cosmetics as a fixative; (vi) fake vanilla fragrance from cow dung; (vii) placental protein from animal placenta; (viii) animal-derived stearic acid derived from waste animal tissue of cow, pig, and sheep; (ix) crystalline guanine, extracted from fish scales, is used to produce shiny effect in shampoo, eye shadow, and nail polish; panthenol, comes from meat or honey, is used in shampoos and conditioners to moisturize and lubricate hair; (x) keratin, haircare product, is extracted from horns, hooves, feathers, quills, and hair of various animals; (xi) shellac, a resin secreted by the female lac insect, is used to create a shiny lacquer in products such as hairsprays, shampoos, mascara, lipstick, etc. Many of these chemicals are used as active ingredients of different cosmetic formulations for skin problems (like hyper pigmentation, skin wrinkling, skin aging, rough skin texture, etc.), hairspray, shampoo, etc. Figure 5.43 shows the structure of hyaluronic acid (an anionic, nonsulfated glycosaminoglycan), carmine (aluminum salt of carminic acid), glucosamine ambrein, ambroxan, ambrinol, and vanillin.

Hyaluronic acid is a common ingredient in skin care products, used as a lip filler in plastic surgery, applied to skin for healing wounds, burns, skin ulcers, and as a moisturizer. Glucosamine, an amino sugar, is primarily used to ease symptoms of

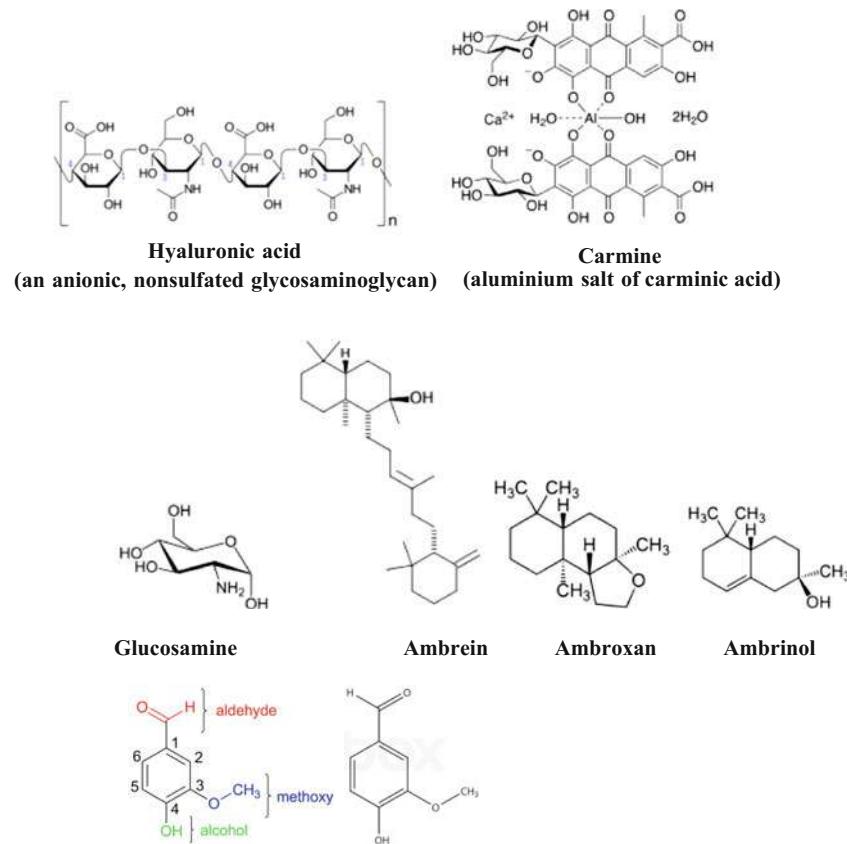


Fig. 5.43 Showing structure of hyaluronic acid (an anionic, nonsulfated glycosaminoglycan), carmine (aluminim salt of carminic acid), glucosamine ambrein, ambroxan, ambrinol, and vanillin

osteoarthritis and apart from this therapeutic use, glucosamine is also used in cosmetic products because it supports the formation of connective tissue and helps increasing moisture and promotes exfoliation and hydration. Raw honey is incredible for skin as it moisturizes skin and its antioxidants repair skin and protects it against oxidative and environmental damage. Ambrein terpene is present in ambergris (whale vomit), and amberin on oxidative breakdown yields ambroxan and ambrinol, the main odor components of ambergris used in perfume industry. The compound vanillin is responsible for the flavor and smell of vanilla, and natural vanillin comes from beans of a Mexican orchid following an extremely lengthy and expensive chemical process. So, scientists tried to make a synthetic version of vanillin in a lab. In 2006, Japanese researcher Mayu Yamamoto figured out how to extract vanillin from cow poop. She was awarded the Nobel Prize at Harvard University for this development. Most artificial vanillin now comes from two sources such as lignin and guaiacol.

Marine organisms including marine algae are rich sources of structurally diverse bioactive compounds like polyunsaturated fatty acids, pigments, and antioxidants for different biomedical products. A diverse group bioactive substances like terpenoids, carotenoids, tocopherol, phenolic compounds, polysaccharides (fucoidan, carrageenans, alginates, and agar), unsaturated fatty acids, mycosporin-like amino acids, and unsaturated fatty acids derived from marine algae are potential ingredients for cosmeceuticals (Agatonovic-Kustrin and Morton 2013). Many of these marine algae-derived compounds (vitamins, phytochemicals, enzymes, antioxidants, essential oils, etc.) are incorporated in skin care cosmeceuticals like creams, lotions, ointments, etc. (Kim et al. 2008). These bioactive ingredients used in topical cosmeceuticals protect function like antioxidant, provide UV radiation protection, inhibit melanogenesis, immunomodulator, control of cutaneous bacterial flora, antiaging, anti-wrinkle, anti-viral, anti-inflammatory, anticoagulant, antitumor, antihyperlipidemic agents, skin repair, skin hydration, gelling, stabilizer, etc. Functions of individual bioactive molecule or their group are given in Table 5.10.

Table 5.10 Cosmeceutical application of compounds derived from marine algae

Bioactive Component	Potential Function as Cosmeceutical	Other uses
1. Terpenoids	Photodamage, photoaging	
2. Carotenoids	UV filter, epidermal cells renewal, antioxidant, control of cutaneous bacterial flora	
3. Tocopherol	UV protection	
4. Phenolic compounds	UV protection	
5. Fucoidans	Antiaging, anti-wrinkle	Anti-viral, anti-inflammatory, anticoagulant, antitumor
6. Carrageenans	Gelling and thickening	Antitumor, anticoagulant, immunomodulatory, antihyperlipidemic, induction of experimental inflammation and inflammatory pain, anti-viral
7. Alginates	Face masks and body washes, skin repair, skin hydration, gelling, stabilizer	
8. Agars	Gelling, emulsifying	Bulking agent, laxative, anticoagulant, antioxidant
9. Unsaturated fatty acids	Antiaging, UV filters, anti-wrinkle, regeneration, skin hydration	Anti-inflammatory
10. Mycosporin-like amino acids	UV filters	

Source Agatonovic-Kustrin and Morton (2013)

Bioactive substances derived from marine resources have diverse functional roles as natural skin care agents, and these properties can be applied to the development of novel cosmetics as well as nutricosmetics from edible seaweeds and edible marine animals (Kim 2014).

The cosmeceuticals that are ingested orally are known as nutricosmetics. All these products are intended for the improvement of health and beauty of the skin and hair. They contain wide spectrum biological active ingredients of natural origin including moisturizer, vitamin, sun protector, skin whitener, free radical scavenger, etc., which are nutritional supplements and support the function and the structure of the skin (e.g., vitamin C, omega-3 fatty acids, carotenes, flavonoids, etc.). Vitamin C functions as an antioxidant and reduces the impact of free radicals in the skin, functions in the production of collagen in the dermis; omega-3 fatty acids, carotenes, etc. protect the skin from the damaging effects of ultraviolet light exposure, which may lead to accelerated skin aging and wrinkle formation (Katiyar 2002; Nichols and Katiyar 2010; Schagen 2012). Since 1990s, sales of nutricosmetics have increased dramatically to over 1 billion USD annually (Anonymous 2006). The various other terms by which cosmeceuticals can be substituted are active cosmetics, performance cosmetics, functional cosmetics, and dermataceuticals. Today's cosmeceuticals as well as nutricosmetics are serving as a bridge between personal care products and pharmaceuticals. The Cosmeceuticals are broad spectrum topical agents that lie somewhere between pure cosmetics (lipstick and rouge) and pure drug (antibiotics, and corticosteroids).

Classification of cosmeceuticals

Cosmeceuticals are classified on the basis of their use into three categories as (a) skin cosmeceutical product (e.g., antiaging creams, moisturizers, facial products, and lotions); (b) hair cosmeceutical product (e.g., gel and creams, hair colorants and dyes, shampoos, growth stimulators, and conditioners); and (c) others (e.g., lipstick, nail polish, toothpaste, and powders). On the basis of the active ingredient content and function, cosmeceuticals are classified into eight categories as (a) retinoids (e.g., vitamin A, niacinamide, panthenol, etc.); (b) sunscreens (e.g., ferulic acid, enzophenones, dihydroxybenzone, oxybenzone, sulisobenzene, etc.); (c) moisturizers (emollients, Jojoba esters, etc.); (d) hydroxyacids (e.g., citric acid, malic acid, lactic acid); (e) depigmenting agents (e.g., hydroquinone, ascorbic acid, kojic acid, glabridin, etc.); (f) exfoliants (e.g., salicylic acid, lactic acid, glycolic acid, etc.); (g) antioxidants (e.g., α -lipoic acid, vitamins A,C,E, niacinamide, N-acetylglucosamine, α -tocopherol, lactobenzoic acid, ubiquinone, polyphenols, etc.); and (h) proteins/peptides (e.g., pentapeptides-KTTKS or collagen pentapeptide—Lys-Thr-Thr-Lys-Ser, KTTKS); (i) growth factors. Figure 5.44 shows structure of niacinamide (nicotinamide), panthenol, ferulic acid, dihydroxybenzene, oxybenzone, sulisobenzene, 11-eicosenoic acid, hydroquinone, kojic acid, and glabridin.

Niacinamide, vitamin B₃, is a very effective skin-restoring ingredient that offers multiple benefits for aging skin. Panthenol (pantothenol), a provitamin of B₅, is used as a moisturizer to improve wound healing in pharmaceutical and cosmetic products. As a building block of lignocelluloses, such as pectin and lignin, ferulic

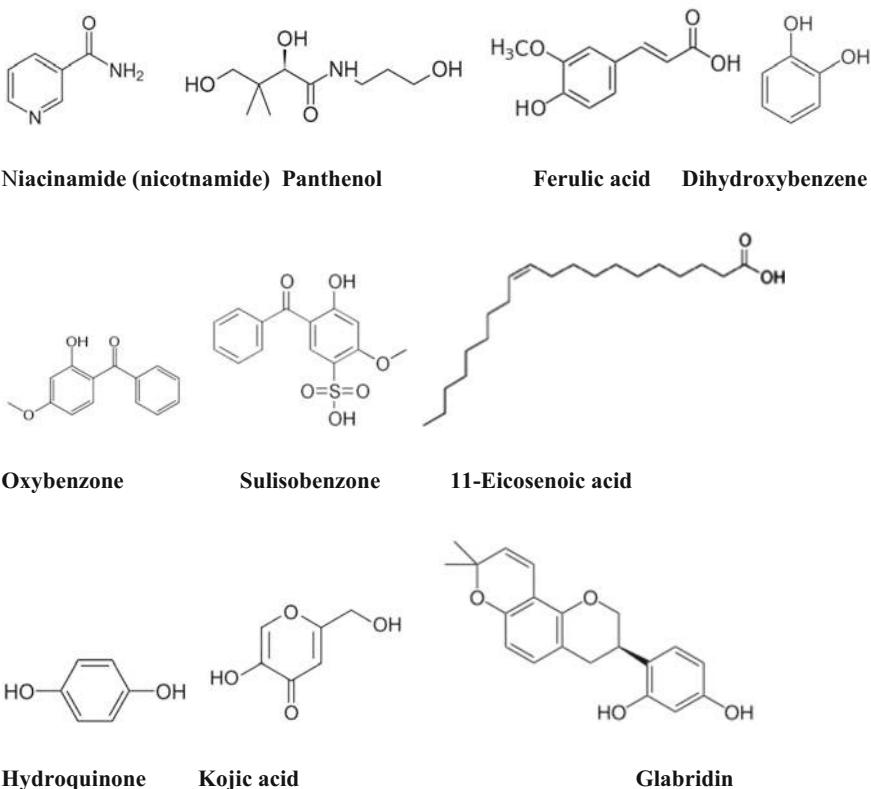


Fig. 5.44 Showing structure of Niacinamide (nicotinamide), Panthenol, Ferulic acid, Dihydroxybenzene, Oxybenzone, Sulisobenzene, 11-Eicosenoic acid, Hydroquinone, Kojic acid, and Glabridin

acid is ubiquitous in the plant kingdom. Oxybenzone belongs to the class of aromatic ketones (benzophenones) and it is a component of many sunscreen lotions. Sulisobenzene (benzophenone-4) is an ingredient in some sunscreens which protects the skin from damage by UVB and short-wave UVA ultraviolet light (Nohynek and Schaefer 2001). Jojoba oil, composed almost entirely (~97%) of monoesters of long chain fatty acids and alcohols and extracted from the seed of Jojoba plant (*Simmondsia chinensis*) is found as an additive in many cosmetic products (lotions and moisturizers, hair shampoos, and conditioners). 11-Eicosenoic acid (gondoic acid) is a monounsaturated omega-9 fatty acid found in jojoba oil as a major constituent (~76.70%) and in a variety of other plant oils and nuts (Miwa 1971). Hydroquinone (benzene-1,4-diol or quinol) is used as a topical application in skin whitening to reduce the color of skin. Kojic acid is a chelation agent produced by several species of fungi (e.g., *Aspergillus oryzae*), used in cosmetics to lighten skin and to treat skin diseases like melasma. Glabridin, an isoflavanone isoflavonoid of licorice (*Glycyrrhiza glabra*) roots, has been associated with a wide range of

biological properties such as antioxidant, anti-inflammatory, antiatherogenic, regulation of energy metabolism, estrogenic, neuroprotective, anti-osteoporotic, and skin whitening.

Different classes of cosmeceuticals are such as (i) Skin cosmeceutical products include antiaging creams, moisturizers, facial products, and lotions; (ii) Hair cosmeceutical products include gel and creams, hair colorants and dyes, shampoos, growth stimulators, and conditioners; and (iii) cosmeceutical products under other categories include lipstick, nail polish, toothpaste, powders, etc.

Skin cosmeceuticals are products that have medicinal or drug-like benefits are able to affect the biological functioning of skin owing to type of functional ingredients they contain. These are skin care products that go beyond coloring and adorning the skin and improve the functioning/texture of the skin by encouraging collagen growth by combating harmful effects of free radicals, thus maintaining keratin structure in good condition and making the skin healthier. OLAY is a well-known skin care line and contains vitamins A, C, D, E, selenium, and lycopene, pycnogenol plus zinc and copper. The treatment of aging skin with a cream containing a hormone such as estrogen results in a fresh appearance with a rejuvenating effect. Kuno and Matsumoto (2004) had patented an external agent for the skin comprising an extract prepared from olive plants as a skin beautifying component, in particular, as an antiaging component for the skin and/or a whitening component. Dry emollient preparation containing monounsaturated Jojoba esters was used for cosmeceutical purpose. Martin (2004) utilized plant extract of genus Chrysanthemum in a cosmetic composition for stimulating skin and/or hair pigmentation (Preetha and Karthika 2009).

Commonly used skin cosmeceuticals include (i) Hydroxy acid: Hydroxy acid also referred to as fruit acids; they are a common ingredient found in many cosmeceutical products. Examples include citric acid, malic acid, and lactic acid. AHAs improve skin texture and reduce the signs of aging by promoting cell seeding in the outer layers of the epidermis and by restoring hydration. AHAs also reduce the calcium ion concentration in the epidermis, and the resulting reduction of the calcium ion levels tends to promote cell growth and slow cell differentiation, thus giving rise to younger looking.

(ii) Botanicals: Botanicals comprise the largest category of cosmeceutical additives found into the market place today. Some botanicals that may benefit the skin include green tea extract, ferulic acid, and grape seed extract.

Ferulic acid: This compound, which is derived from plants, is considered to be a potent antioxidant, and has been shown to provide photo protection to skin. Furthermore, when ferulic acid is combined with vitamins C and E, the product has been shown to provide substantial UV protection for human skin. Moreover, Murray et al. (2008) reported that because its mechanism of action is different from sunscreens, and ferulic acid could be expected to supplement the sun protection provided by sunscreens.

Grape seed extract: This botanical has been established as a potent antioxidant and has been shown to speed wound contraction and closure. Topical application of

grape seed extract has also been shown to enhance the sun protection factor in humans.

(iii) Depigmenting agent: Skin-lightening agents added to product formulations have become increasingly popular and such products are in demand. Common depigmenting ingredients include hydroquinone, ascorbic acid (vitamin C), kojic acid, and licorice extract (glabridin).

Hydroquinone: Hydroquinone has been the popular agent of choice for skin lightening. The US FDA has proposed concentrations between 1.5 and 2% in skin lighteners. A recent study suggests that this concern has been based mainly on studies with animal models utilizing long-term exposure at high dosages that are carcinogenic. Routine topical application may pose no greater risk than that from levels present in common foods.

(iv) Exfoliants: Exfoliants promote skin turnover by removing adherent cells in the stratum corneum. Common exfoliants found in cosmeceutical preparations include salicylic acid (SA), lactic acid, and glycolic acid. There are concerns that repeated use of SA and AHAs could cause the dermis and epidermis to be more vulnerable to penetration by UV radiation.

(v) Moisturizers: Moisturizers restore water content to the epidermis and provide a soothing protective film. They improve the appearance and tactile properties of dry and aging skin, restore the normal barrier function of the skin, and reduce the release of inflammatory cytokines. Moisturizers comprise an important therapeutic component in the management of various skin conditions (e.g., eczema, psoriasis, pruritus, and aged skin).

(vi) Topical peptides: These are regarded as cellular messengers that are formed from amino acids and are designed to mimic peptide fragments with endogenous biologic activity. These pentapeptides (e.g., KTTKS) are comprised of a subfragment of type I collagen propeptide, and play a role in signaling fibroblasts to produce collagen in the skin, which can improve the appearance of wrinkles.

(vii) Retinoids: They are among the most common ingredients found in cosmeceuticals. In fact, they are the most studied and have the most data behind them. They consist of natural and synthetic derivatives of vitamin A that reduce hyperpigmentation and inhibit enzymes from breaking down collagen.

(viii) Sunscreen: Sunscreens are the single most important cosmeceutical, because they protect skin against solar radiation, which is the most important damaging environmental agent. As a result, they help to prevent the signs of aging. To be effective, sunscreens should provide broad spectrum coverage that includes both UVA and UVB blocking agents to inhibit photoaging and be part of a daily skin care regimen. Sunscreens contain active ingredients that act as ultraviolet filters.

(ix) Antioxidants: Antioxidants reduce free radical damage, thereby preventing impairment at the cellular level. They inhibit inflammation, which leads to collagen depletion, and they offer protection against photodamage and skin cancer. Common antioxidants include alpha-lipoic acid (ALA), L-ascorbic acid (vitamin C), niacinamide (vitamin B₃), N-acetyl-glucosamine (NAG), α -tocopherol, and ubiquinone.

Cosmetic Versus drug

The term cosmetic refers to a preparation designed to enhance the body superficially to hide a real comprehended deficiency or flaw, by direct application. This application is considered to be decorative, lacking in depth or significance, as opposed to a response to a medical requirement. The definition of a drug is more complex. Generally, a drug is a chemical substance which, when absorbed into a living organism, alters normal function. The pharmacology definition of a drug will apply—"a chemical substance used in the treatment, cure, prevention or diagnosis of disease or used to otherwise enhance physical or mental well-being, for a limited duration or indefinite period of time." Individual governments regulate the availability of drugs to the public as (i) Over-the-counter (OTC) medication is available from pharmacies; (ii) Behind-the-counter medication (BTC) medication must be dispensed by pharmacist, but does not require the authority of a doctor; and finally (iii) Prescription-only medicine (POM) can only be prescribed by a licensed medical professional.

There are also numerous bodies that regulate the drugs present in the market including (i) The Medicines and Healthcare products Regulatory Agency (MHRA)—is a government agency responsible for ensuring that medicines and medical devices work and are acceptably safe. They are responsible for public information as well the investigation and handling of complaints and patient feedback. (ii) The National Biological Standards Board (NBSB)—is a nondepartmental public body, established in 1975 by Act of Parliament. The board takes responsibility for safeguarding and advancing public health. In fact, FDA does not recognize the word as an official product type, but it regulates cosmetics under the Federal Food, Drug and Cosmetics Act (FDCA). The regulations of cosmeceuticals have not been harmonized between the USA, European, Asian, and other countries yet.

References

- Abdullaev Jafarova F, Caballero-Ortega H, Riverón-Negrete L, Pereda-Miranda R, Rivera-Luna R, Manuel Hernández J et al (2002) In vitro evaluation of the chemopreventive potential of saffron. *Rev Invest Clin* 54(5):430–436
- Aberg F, Appelkvist EL, Dallner G, Ernster L (1992) Distribution and redox state of ubiquinones in rat and human tissues. *Arch Biochem Biophys* 295(2):230–234
- Akhtari K, Hassanzadeh K, Fakhraei B, Fakhraei N, Hassanzadeh H, Zarei SA (2013) A density functional theory study of the reactivity descriptors and antioxidant behavior of crocin. *Comput Theor Chem* 1013:123–129
- Ali B, Ali N, Wabel A, Shams S, Ahamad A, Khan SA, Anwar F (2015) Essential oils used in aromatherapy: a systemic review. *Asian Pacific J Tropical Biomed* 5(8):601–611
- Allen MA, Burgess SG (1950) The losses of ascorbic acid during the large-scale cooking of green vegetables by different methods. *Br J Nutr* 4(2–3):95–100
- Allgeyer LC, Miller MJ, Lee SY (2010) Sensory and microbiological quality of yogurt drinks with prebiotics and probiotics. *J Dairy Sci* 93:4471–4479
- Aluru M, Xu Y, Guo R, Wang Z, Li S, White W et al (2008) Generation of transgenic maize with enhanced provitamin A content. *J Exp Bot* 59:3551–3562

- Anonymous (1973) Committee on Food Protection; Food and Nutrition Board; National Research Council (1973) Phytates. Toxicants occurring naturally in foods. National Academy of Sciences, pp 363–371
- Anonymous (1998) Dietary supplement fact sheet: Folate. Health Information. Office of Dietary Supplements, US National Institutes of Health
- Anonymous (2000) Dietary reference intakes: vitamin C, vitamin E, selenium, and carotenoids. Institute of Medicine, Food and Nutrition Board, National Academy Press, Washington. <https://doi.org/10.17226/9810>
- Anonymous (2004) Chambers Concise Dictionary. Allied Publishers. 2004. p. 451
- Anonymous (2005) Collins dictionary of biology, 3rd edn. © W. G. Hale, V. A. Saunders, J. P. Margham
- Anonymous (2017) USDA database for the flavonoid content of selected foods. https://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/Flav/Flav_R03.pdf
- Anthony JB, Harriet CB, Matti J, Dean JT (1993) Dietary betaine promotes generation of hepatic Sadenosylmethionine and protects the liver from ethanol-induced fatty infiltration. *Alcohol Clin Exp Res* 17(3):552–555
- Ayoub M, de Camargo AC, Shahidi F (2016) Antioxidants and bioactivities of free, esterified and insoluble-bound phenolics from berry seed meals. *Food Chemistry* 197(Part A):221–232
- Banerjee R (2007) Redox biochemistry. Wiley, New York, p. 35
- Becerra-Moreno A, Alanís-Garza P, Mora-Nieves JL, Mora-Mora JP, Jacobo-Velázquez DA (2014) Kale: an excellent source of vitamin C, pro-vitamin A, lutein and glucosinolates. *CyTA-J Food* 12:298–330
- Beck K, Conlon CA, Kruger R, Coad J, Stonehouse W (2011) Gold kiwifruit consumed with an iron-fortified breakfast cereal meal improves iron status in women with low iron stores: a 16-week randomized controlled trial. *Br J Nutr* 105:101–109
- Bender DA, Bender AE (2005) A dictionary of food and nutrition. Oxford University Press, New York
- Bieri JG, Evarts RP, Evarts (1974) γ -Tocopherol: metabolism, biological activity and significance in human vitamin E nutrition. *Am J Clin Nutrition* 27(9):980–986
- Biewenga GP, Haenen GR, Bast A (1997) The pharmacology of the antioxidant lipoic acid. *Gen Pharmacol* 29(3):315–331
- Birt DF, Hendrich S, Wang W (2001) Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacol Ther* 90(2–3):157–177
- Block G, Patterson B, Subar A (1992) Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr Cancer* 18:1–29
- Bourgis F (1999) S-methylmethionine plays a major role in phloem sulfur transport and is synthesized by a novel type of methyltransferase. *Plant Cell* 11(8):1485–1498
- Bracco P, Oral E (2011) Vitamin E-stabilized UHMWPE for total joint implants: A review. *Clin Orthop Relat Res* 469(8):2286–2293
- Bremer J (1983) Carnitine—metabolism and functions. *Physiol Rev* 63(4):1420–1480
- Brigelius-Flohé R, Traber MG (1999) Vitamin E: function and metabolism. *FASEB J.* 13(10): 1145–1155
- Brody T (1999) Nutritional biochemistry, 2nd edn. Academic Press, San Diego, 1999
- Bruckert E, Labreuche J, Amarenco P (2010) Meta-analysis of the effect of nicotinic acid alone or in combination on cardiovascular events and atherosclerosis. *Atherosclerosis* 210(2):353–361
- Burke KE (2007) Interaction of vitamins C and E as better cosmeceuticals. *Dermatol Ther* 20(5): 314–321
- Carmel R (2006) Cobalamin (Vitamin B-12). In: Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ (eds) Modern nutrition in health and disease. Lippincott Williams & Wilkins, Philadelphia, pp 482–497
- Cayen MN, Dvornik D (1979) Effect of diosgenin on lipid metabolism in rats. *J Lipid Res* 20(2): 162–174

- Chakraborty S, Chakraborty N, Agrawal L, Ghosh S, Narula K, Shekhar S et al (2010) Next-generation protein-rich potato expressing the seed protein gene AmA1 is a result of proteome rebalancing in transgenic tuber. *Proc Natl Acad Sci USA* 107:17533–17538
- Charalampopoulos D, Wang R, Pandiella SS, Webb C (2002) Application of cereals and cereal components in functional foods: a review. *Int J Food Microbiol* 79:131–141
- Chaudhari SP, Tam AY, Barr JA (2015) Curcumin: a contact allergen. *J Clin Aesthet Dermatol* 8(11):43–48
- Chauhan B, Kumar G, Kalam N, Ansari SH (2013) Current concepts and prospects of herbal nutraceutical: a review. *J Adv Pharm Technol Res* 4(1): 4–8. doi: [10.4103/2231-4040.107494](https://doi.org/10.4103/2231-4040.107494)
- Cheney G (1950) Anti-peptic ulcer dietary factor (vitamin ‘U’) in the treatment of peptic ulcer. *J Am Diet Assoc* 26(9):668–672
- Chow J, Klein EY, Laxminarayan R (2010) Cost-effectiveness of golden mustard for treating vitamin A deficiency in India. *PLoS ONE* 5(8):e12046
- Chryssanthi DG, Lamari FN, Iatrou G, Pylara A, Karamanos NK, Cordopatis P (2007) Inhibition of breast cancer cell proliferation by saponins constituents of different *Crocus* species. *Anticancer Res* 27(1A):357–362
- Combs GF (2001) The vitamins, fundamental aspects in nutrition and health, 2nd edn. Academic Press, San Diego, pp 245–272
- Combs GF Jr (2008) The vitamins: fundamental aspects in nutrition and health, 3rd edn. Elsevier, Ithaca. ISBN 978-0-12-183493-7
- Darby WJ, Jones E (1945) Treatment of sprue with synthetic *L. casei* factor (folic acid, vitamin M). *Experim Biol Med* 60(2):259–262
- De Nicola R, Bagatta M, Pagnotta E, Angelino D, Gennari L et al (2014) Comparison of bioactive phytochemical content and release of isothiocyanates in selected brassica sprouts. *Food Chem* 141:297–303
- Dietrich M, Brown CJ, Block G (2005) The effect of folate fortification of cereal-grain products on blood folate status, dietary folate intake, and dietary folate sources among adult non-supplement users in the United States. *J Am Coll Nutr* 24(4): 266–274. 17
- Duggal JK, Singh M, Attri N, Singh PP, Ahmed N, Pahwa S et al (2010) Effect of niacin therapy on cardiovascular outcomes in patients with coronary artery disease. *J Cardiovasc Pharmacol Therapeutics* 15(2):158–166
- Durrani AI, Schwartz H, Nagl M, Sontag G (2010) Determination of free [alpha]-lipoic acid in foodstuffs by HPLC coupled with CEAD and ESI-MS. *Food Chem* 120(4):38329–38336
- Dweck AC (2002) Natural ingredients for colouring and styling. *International J Cosmetic Sci* 24(5):287–302
- El-Sohaimy SA, Elsayed E, El-Saadani HMA (2010) Cloning and in vitro-transcription of chymosin gene in *E. coli*. *Open Nutraceuticals J* 3:63–68
- Escribano J, Alonso GL, Coca-Prados M, Fernandez JA (1996) Crocin, safranal and picrocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells in vitro. *Cancer Lett* 100(1–2):22–30
- Evans WC (2000) Trease and Evans pharmacognosy, 4th edn. WB Saunders Co, London
- Fenech M (2012) Folate (vitamin B9) and vitamin B12 and their function in the maintenance of nuclear and mitochondrial genome integrity. *Mutation Res Fundamental Mol Mech Mutagenesis* 733(1–2):21–33
- Flanagan JL, Simmons PA, Vehige J, Willcox MDP, Garrett Q (2010) Role of carnitine in disease. *Nutr Metab (Lond)* 7:30–44
- Galvano F, Fauci LL, Lazzarino G, Fogliano V, Ritieni A, Ciappellano S et al (2004) Cyanidins: metabolism and biological properties. *J Nutr Biochem* 15:2–11
- de la Garza DRI, Gregory JF, Hanson AD (2007) Folate biofortification of tomato fruit. *Proc Natl Acad Sci USA* 104:4218–4222
- Gerasimenko JV, Flowerdew SE, Voronina SG, Sukhomlin TK, Tepikin AV, Petersen OH et al (2006) Bile acids induce Ca²⁺ release from both the endoplasmic reticulum and acidic intracellular calcium stores through activation of inositol trisphosphate receptors and ryanodine receptors. *J Biol Chem* 281(52):40154–40163

- Ghorai S, Banik SP, Verma D, Chowdhury S, Mukherjee S, Khawala S (2009) Fungal biotechnology in food and feed processing. *Food Res Int* 42:577–587
- Ghosh D (2005) Anthocyanins and anthocyanin-rich extracts in biology and medicine: biochemical, cellular, and medicinal properties. *Curr Topics Nutraceutical Res* 3(2):113–124
- Glier MB, Green TJ, Devlin AM (2014) Methyl nutrients, DNA methylation, and cardiovascular disease. *Mol Nutr Food Res* 58:172–182
- Gossell-Williams M, Fletcher H, McFarlane-Anderson N, Jacob A, Patel J, Zeisel S (2005) Dietary intake of choline and plasma choline concentrations in pregnant women in Jamaica. *West Ind Med J* 54(6):355–359
- Gregory SKND (2011) Monogtaph: pantothenic acid. *Alternative Med Rev* 16(3):263–273
- Gropper SS, Smith JL, Groff JL (2009) Advanced nutrition and human metabolism. Wadsworth, Cengage Learning, Belmont
- Hajirahimkhan A, Dietz B, Bolton J (2013) Botanical modulation of menopausal symptoms: mechanisms of action? *Planta Med* 79(07):538–553
- Hamman JH, Tarirai C (2006) Functional excipients. *Chem Today* 24:57–62
- Hauschild K, Pickr-Freyer KM (2004) Evaluation of a new co-processed compound based on lactose and maize starch for tablet formulation. *AAPS Pharm Sci* 6(2):27–38
- Herbert V (1979) Pangamic acid (vitamin B15). *Am J Clin Nutr* 32(7):1534–1540
- Herbert V, Herbert R (1981) Pangamate (Vitamin B15), controversies in nutrition. Churchill Livingstone, New York, pp. 159–170
- Hosoe K, Kitano M, Kishida H, Kubo H, Fujii K, Kitahara M (2007) Study on safety and bioavailability of ubiquinol (Kaneka QHTM) after single and 4-week multiple oral administration to healthy volunteers. *Regul Toxicol Pharmacol* 47(1):19–28
- Hurrell RF (2003) Influence of vegetable protein sources on trace element and mineral bioavailability. *J Nutrit* 133(9):2973S–2977S
- Hyvonen P, Suojala L, Orro T, Haaranen J, Simola O, Rontved C, Pyorala SP (2006) Transgenic cows that produce recombinant human lactoferrin in milk are not protected from experimental *Escherichia coli* intramammary infection. *Infect Immun* 74:6206–6212
- Illum L, Fisher AN, Jabbal GJ, Davis SS (2001) Bioadhesive starch microspheres and absorption enhancing agent's acts synergistically to enhance the nasal absorption of polypeptides. *Int J Pharmacol* 222(1):109–119
- Iwasaki M, Inoue M, Otani T, Sasazuki S, Kurashi N, Miura T et al (2008) Plasma isoflavone level and subsequent risk of breast cancer among Japanese women: a nested case-control study from Japan Public Health Center-base prospective study group. *J Clin Oncol* 26(10):1677–1683
- Jack DB (1995) Keep taking the tomatoes—the exciting world of nutraceuticals. *Mol Med Today* 1(3):118–121
- Jeffrey CP (2009) Alcamo's fundamentals of microbiology: body systems edition. Jones & Bartlett Learning, Burlington, p 511
- Kähkönen MP, Heinonen M (2003) Antioxidant activity of anthocyanins and their aglycons. *J Agric Food Chem* 51(3):628–633
- Kamen B (1997) Folate and antifolate pharmacology. *Semin Oncol* 24(5 Suppl 18): S18-30–S18-39
- Karlic H, Lohninger A (2004) Supplementation of l-carnitine in athletes: does it make sense? *Nutrition* 20(7–8):709–715
- Kimura S, Furukawa Y, Wakasugi J, Ishihara Y, Nakayama A (1980) Antagonism of L(-) pantothenic acid on lipid metabolism in animals. *J Nutr Sci Vitaminol (Tokyo)* 26(2):113–117
- Klopfenstein TJ, Angel R, Cromwell G, Erickson GE, Fox DG, Parsons C et al (2002) Animal diet modification to decrease the potential for nitrogen and phosphorus pollution. *Council Agric Sci Technology* 21:175–188
- Kowdley KV, Mason JB, Meydani SN, Cornwall S, Grand RJ (1992) Vitamin E deficiency and impaired cellular immunity related to intestinal fat malabsorption. *Gastroenterology* 102(6): 2139–2142

- Kramer K, Packer L (2001) R-alpha-lipoic acid. In: Kramer K, Hoppe P, Packer L (eds) Nutraceuticals in health and disease prevention. Marcel Dekker, Inc., New York, pp 129–164
- Krutmann J, Humbert P (2010) Nutrition for healthy skin: Strategies for clinical and cosmetic practice. Springer, Berlin, p 153
- Kubo H, Fujii K, Kawabe T, Matsumoto S, Kishida H, Hosoe K (2008) Food content of ubiquinol-10 and ubiquinone-10 in the Japanese diet. *J Food Compos Anal* 21(3):199–210
- Kukuljan M, Vergara L, Stojilkovic SS (1997) Modulation of the kinetics of inositol 1,4,5-trisphosphate-induced [Ca²⁺]_i oscillations by calcium entry in pituitary gonadotrophs. *Biophys J* 72(2 Pt 1):698–707
- Larner J (2002) D-chiro-inositol—its functional role in insulin action and its deficit in insulin resistance. *Int J Experim Diabetes Res* 3(1):47–60
- LeBlanc JG, Milani C, De Giori GS, Sesma F, van Sinderen D, Ventura M (2013) Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr Opin Biotechnol* 24:160–168
- Leermakers ET, Moreira EM, Kieft-De Jong JC, Darweesh SK, Visser T, Voortman T et al (2015) Effects of choline on health across the life course: a systematic review. *Nutr Rev* 73(8):500–522
- Leonardi R, Zhang YM, Rock CO, Jackowski S (2005) Coenzyme A: back in action. *Prog Lipid Res* 44(2–3):125–153
- Liu MJ, Wang Z, Ju Y, Wong RN, Wu QY (2005) Diosgenin induces cell cycle arrest and apoptosis in human leukemia K562 cells with the disruption of Ca²⁺ homeostasis. *Cancer Chemother Pharmacol* 55(1):79–90
- Lodge JK, Youn HD, Handelman GJ et al (1997) Natural sources of lipoic acid: determination of lipoylysine released from protease-digested tissues by high performance liquid chromatography incorporating electrochemical detection. *J Appl Nutr* 49(1 & 2):3–11
- Magoulas PL, El-Hattab AW (2012) Systemic primary carnitine deficiency: an overview of clinical manifestations, diagnosis, and management. *Orphanet J Rare Diseases* 7:68
- Mahan L K, Escott-Stump S (eds) (2000) Krause's food, nutrition, & diet therapy, 10th edn. W.B. Saunders Company, Philadelphia. ISBN 0-7216-7904-8
- Malbasa RV, Loncar ES, Vitas JS, Canadianovic-Brunet JM (2011) Influence of starter cultures on the antioxidant activity of kombucha beverage. *Food Chem* 127:1727–1731
- Manosroi A, Chutoprapat R, Sato Y, Miyamoto K, Hsueh K, Abe M et al (2011) Antioxidant activities and skin hydration effects of rice bran bioactive compounds entrapped in niosomes. *J Nanosci Nanotechnol* 11(3):2269–2277
- Marino BS, Fine KS (2009) Blueprints pediatrics. Lippincott Williams & Wilkins, p 131
- Masalha R, Chudakov B, Muhamad M, Rudoy I, Volkov I, Wirguin I (2001) Cobalamin-responsive psychosis as the sole manifestation of vitamin B₁₂ deficiency. *Israeli Med Assoc J* 3(9):701–703
- Matthew D, Boris P, Michael F, Michael S, Athanasios L, de Valérie CL, Andrei O (2002) Complete reconstitution of the human coenzyme A biosynthetic pathway via comparative genomics. *J Biol Chem* 277(24):21431–21439
- McDaniel DH, Neudecker BA, DiNardo JC, Lewis JA II, Maibach HI (2005) Idebenone: a new antioxidant—Part I. Relative assessment of oxidative stress protection capacity compared to commonly known antioxidants. *J Cosmet Dermatol* 4(1):10–17
- McNeil SD (1999) Betaines and related osmoprotectants. Targets for metabolic engineering of stress resistance. *Plant Physiol* 120(4):945–949
- Mellors A, Tappel AL (1966a) The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol. *J Biol Chem* 241(19):4353–4356
- Mellors A, Tappel AL (1966b) Quinones and quinols as inhibitors of lipid peroxidation. *Lipids* 1(4):282–284
- Miadoková E (2009) Isoflavonoids – an overview of their biological activities and potential health benefits. *Interdiscip Toxicol* 2(4):211–218
- Miadokova E, Masterova I, Vlckova V, Duhova V, Toth J (2002) Antimutagenic potential of homoisoflavonoids from *Muscari* racemosum. *J Ethnopharmacol* 18:381–386

- Miller A, Korem M, Almog R, Galboiz Y (2005) Vitamin B12, demyelination, remyelination and repair in multiple sclerosis. *J Neurological Sciences* 233(1–2):93–97
- Miwa T (1971) Jojoba Oil wax esters and derived fatty acids and alcohols: gas chromatographic analyses. *J Am Oil Chem Soc* 48(6):259–266
- Monek RV, Builders PF, Kollerg WM, Eneje M, Kunle OO (2012) Physicochemical and binder properties of starch obtained from *Cyperus esculentus*. *Pharm Sci Technol* 3(2):379–388
- Moreno DA, Carvaja M, López-Berenguer C, García-Viguera C (2006) Review: chemical and biological characterisation of nutraceutical compounds of broccoli. *J Pharmaceutical Biomedical Analysis* 41(5):1508–1522
- Naef R (2010) The volatile and semi-volatile constituents of agarwood, the infected heartwood of *Aquilaria* species: a review. *Flavour Fragrance J* 26(2):73–87
- Naqvi S, Zhu C, Farre G, Ramessar K, Bassie L, Breitenbach JD et al (2009) Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. *Proc Natl Acad Sci USA* 106:7762–7767
- Nohynek GJ, Schaefer H (2001) Benefit and risk of organic ultraviolet filters. *Regul Toxicol Pharmacol* 33(3):285–299
- Ochiai T, Shimeno H, Mishima K, Iwasaki K, Fujiwara M, Tanaka H et al (2006) Protective effects of carotenoids from saffron on neuronal injury in vitro and in vivo. *Biochim Biophys Acta* 1770(4):578–584
- Okamoto T, Matsuya T, Fukunaga Y, Kishi T, Yamagami T (1989) Human serum ubiquinol-10 levels and relationship to serum lipids. *Int J Vitam Nutr Res* 59(3):288–292
- Oomah BD, Mazza G (2016) Flavonoids and antioxidative activities in buckwheat. *J Agric Food Chem* 64(25):5197–5206
- Otten JJ, Hellwig JP, Meyers LD (2008) Dietary reference intakes: the essential guide to nutrient requirements. The National Academies Press, Washington
- Papandreou MA, Kanakis CD, Polissiou MG, Efthimiopoulos S, Cordopatis P, Margariti M et al (2006) Inhibitory activity on amyloid-beta aggregation and antioxidant properties of *Crocus sativus* stigmas extract and its crocin constituents. *J Agric Food Chem* 54(23):8762–8768
- Patel AD, Prajapati NK (2012) Review on biochemical importance of Vitamin-U. *J Chem Pharm Res* 4(1):209–215
- Pietta PG (2000) Flavonoids as antioxidants. *J Nat Prod* 63:1035–1042
- Pifferi G, Santoro P, Pedrani M (1999) Quality and functionality of excipients. *IL Farmaco* 54: 1–14
- Prakash R, Gandotra S, Singh LK, Das B, Lakra A (2008) Rapid resolution of delusional parasitosis in pellagra with niacin augmentation therapy. *Gen Hosp Psychiatry* 30(6):581–584
- Pravst I, Žmitek K, Žmitek J (2010) Coenzyme Q₁₀ contents in foods and fortification strategies. *Crit Rev Food Sci Nutr* 50(4):269–280
- Raddatz G, Bisswanger H (1997) Receptor site and stereospecificity of dihydrolipoamide dehydrogenase for R- and S-lipoamide: a molecular modeling study. *J Biotechnol* 58(2): 89–100
- Rahnamaeian M, Vilcinskas A (2015) Short antimicrobial peptides as cosmetic ingredients to deter dermatological pathogens. *Appl Microbiol Biotechnol* 99:8847–8855
- Rajasekaran A, Sivagnanam G, Xavier R (2008) Nutraceuticals as therapeutic agents: a review. *Research J Pharm Tech* 1(4):171–174
- Rapiejko PJ, Northup JK, Evans T, Brown JE, Malbon CC (1986) G-proteins of fat-cells. Role in hormonal regulation of intracellular inositol 1,4,5-trisphosphate. *Biochem J* 240(1):35–40
- Reboul E, Richelle M, Perrot E, Desmoulins-Malezet C, Pirisi V, Borel P (2006) Bioaccessibility of carotenoids and vitamin E from their main dietary sources. *J Agric Food Chem* 54(23): 8749–8755
- Reljanovic M, Reichel G, Rett K, Lobisch M et al (1999) Treatment of diabetic polyneuropathy with the antioxidant thioctic acid (alpha-lipoic acid): a two year multicenter randomized double-blind placebo-controlled trial (ALADIN II). *Alpha Lipoic Acid in Diabetic Neuropathy. Free Radical Res* 31(3):171–179

- Rohr M, Rieger I, Jain A, Schrader A (2011) Influence of repetitive UVA stimulation on skin protection capacity and antioxidant efficacy. *Skin Pharmacol Physiol* 24(6):300–304
- Roig MG, Rivera ZS, Kennedy JF (1995) A model study on rate of degradation of L-ascorbic acid during processing using home-produced juice concentrates. *Int J Food Sci Nutr* 46(2):107–115
- Ryan-Borchers TA, Park JS, Chew BP, McGuire MK, Fournier LR, Beerman KA (2006) Soy isoflavones modulate immune function in healthy postmenopausal women. *Am J Clin Nutr* 83:118–125
- Scarpato R, Paganucci L, Bertoli A, Fiore L, Pistelli L, Federico G (2008) Licoflavone C attenuates the genotoxicity of cancer drugs in human peripheral lymphocytes. *Phytother Res* 22(12):1650–1654
- Schagen SK (2017) Review: topical peptide treatments with effective anti-aging results. *Cosmetics* 4:16–30
- Sebrell WH, Butler RE (1939) Riboflavin deficiency in man (Ariboflavinosis). *Public Health Rep* 54(48):2121–2131
- Sethi NK, Robilotti E, Sadan Y (2005) Neurological manifestations of vitamin B12 deficiency. *Int J Nutri Wellness* 2:1–12
- Shane B (2000) Folic acid, vitamin B-12, and vitamin B-6. In: Stipanuk M (ed) Biochemical and physiological aspects of human nutrition. W.B. Saunders Co., Philadelphia, pp 483–518
- Shay KP, Moreau RF, Smith EJ, Hagen TM (2008) Is alpha-lipoic acid a scavenger of reactive oxygen species in vivo? Evidence for its initiation of stress signaling pathways that promote endogenous antioxidant capacity. *IUBMB Life* 60(6):362–367
- Shen X, Xiao H, Ranallo R, Wu WH, Wu C (2003) Modulation of ATP-dependent chromatin-remodeling complexes by inositol polyphosphates. *Science* 299(5603):112–114
- Shindo Y, Witt E, Han D, Epstein W, Packer L (1994) Enzymic and non-enzymic antioxidants in epidermis and dermis of human skin. *J Invest Dermatol* 102(1):122–124
- Sibbesen O, Sorensen JF (2010) Polypeptides with xylanase activity. WIPO Patent Application
- Smith AR, Shenvi SV, Widlansky M, Suh JH, Hagen TM (2004) Lipoic acid as a potential therapy for chronic diseases associated with oxidative stress. *Curr Med Chem* 11(9):1135–1146
- Smitha RL, Cohenb SM, Doullc J, Ferond VJ, Goodman JI, Marnett LJ et al (2005) A procedure for the safety evaluation of natural flavor complexes used as ingredients in food: essential oils. *Food Chem Toxicol* 43(3):345–363
- Spencer JP (2008). Flavonoids: modulators of brain function? *British J Nutrition* 99:ES 60–77
- Steger DJ, Haswell ES, Miller AL, Wente SR, O'Shea EK (2003) Regulation of chromatin remodeling by inositol polyphosphates. *Science* 299(5603):114–116
- Svoboda KP, Deans SG (1995) Biological activities of essential oils from selected aromatic plants. *Acta Hort* 390:203–209
- Tanphaichitr V (1999) Thiamin. In: Shils ME, Olsen JA, Shike M et al (eds) Modern nutrition in health and disease, 9th edn. Lippincott Williams & Wilkins, Baltimore, p 1999
- Theresa LC, James PG (1996) Sexual pharmacology: drugs that affect sexual function. W. W. Norton and Co., New York, p 1996
- Traber MG, Atkinson J (2007) Vitamin E, antioxidant and nothing more. *Free Radic Biol Med* 43(1):4–15
- Traber MG, Stevens JF (2011) Free radical biology and medicine—vitamins C and E: beneficial effects from a mechanistic perspective. *Free Radic Biol Med* 51(5):1000–1013
- Trumbo PR (2006) Pantothenic Acid. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ (eds) Modern nutrition in health and disease, 10th edn. Lippincott Williams & Wilkins, Philadelphia, pp 462–467
- Truswell AS (2002) Cereal grains and coronary heart disease. *Eur J Clin Nutr* 56:1–14
- Tucci M, Benghuzzi H (2003) Structural changes in the kidney associated with ovariectomy and diosgenin replacement therapy in adult female rats. *Biomed Sci Instrum* 39:341–346
- Van der Put NMJ, Van Straaten HWM, Trijbels FJM, Blom HJ (2001) Folate, homocysteine and neural tube defects: an overview. *Experim Biol Med* 226(4):243–270
- Varinska L, Gal P, Mojzisova G, Mirossay L, Mojzis J (2015) Soy and breast cancer: focus on angiogenesis. *Int J Mol Sci* 16(5):11728–11749

- Ververidis F, Trantas E, Douglas C, Vollmer G, Kretzschmar G, Panopoulos N (2007) Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part I: chemical diversity, impacts on plant biology and human health. *Biotech J* 2(10):1214–1234
- Voet D, Voet JG, Pratt CW (2006) Fundamentals of biochemistry: life at the molecular level, 2nd edn. Wiley, Hoboken
- Wan P, Moat S, Anstey A (2011) Pellagra: A review with emphasis on photosensitivity. *The British Journal of Dermatology* 164(6):1188–1200
- Watanabe F, Yabuta Y, Tanioka Y, Bito T (2013) Biologically active vitamin B12 compounds in foods for preventing deficiency among vegetarians and elderly subjects. *J Agric Food Chem* 61:6769–6775
- Wei P, Liu M, Chen Y, Chen DC (2012) Systematic review of soy isoflavone supplements on osteoporosis in women. *Asian Pacific J Tropical Med* 5(3):243–248
- White EM (2009) Current status of metabolically engineered resveratrol. *MMG 445 Basic Biotechnol* 5:84–90
- Whitney E, Rolfes SR (2008) Understanding nutrition, 11th edn. Thomson Wadsworth, California, p 154
- Whitney E, Sharon RR (2011). Understanding nutrition, 12th edn. Wadsworth, Cengage Learning, California, p 100
- WHO (2015) WHO model list of essential medicines (19th List). World Health Organization, April 2015
- William DJ, Timothy B, Dirk M (2006) Andrews' diseases of the skin: clinical dermatology. Elsevier Health Sciences, Amsterdam (10th International edition)
- Wu CL, Huang AC, Yang JS, Liao CL, Lu HF, Chou ST et al (2011) Benzyl isothiocyanate (BITC) and phenethyl isothiocyanate (PEITC)-mediated generation of reactive oxygen species causes cell cycle arrest and induces apoptosis via activation of caspase-3, mitochondria dysfunction and nitric oxide (NO) in human osteogenic sarcoma U-2 OS cells. *J Orthop Res* 29(8):1199–1209
- Wu Q, Christensen LA, Legerski RJ, Vasquez KM (2005) Mismatch repair participates in error-free processing of DNA interstrand crosslinks in human cells. *EMBO Rep* 6(6):551–557
- Yamada K (2013) Cobalt: its role in health and disease. In: Sigel A, Sigel H, Sigel RKO (eds) Interrelations between essential metal ions and human diseases. Metal ions in life sciences. Springer, Berlin, pp 295–320
- Yang B, Wang J, Tang B, Liu Y, Guo C, Yang P et al (2011) Characterization of bioactive recombinant human lysozyme expressed in milk of cloned transgenic cattle. *PLoS ONE* 6(3): e17593
- Yildiz F (2005) Phytoestrogens in functional foods. Taylor & Francis Ltd., Boca Raton, pp 3–5, 210–211
- Zeisel SH, da Costa KA (2009) Choline: an essential nutrient for public health. *Nutr Rev* 67(11):615–623
- Zempleni J, Hassan YI, Wijeratne SS (2008) Biotin and biotinidase deficiency. *Expert Rev Endocrinol Metab* 3(6):715–724
- Zheng YQ, Liu JX, Wang JN, Xu L (2006) Effects of crocin on reperfusion-induced oxidative/nitrative injury to cerebral microvessels after global cerebral ischemia. *Brain Res* 1138:86–94

Chapter 6

Poisons, Hallucinogens, Teratogens, Pesticides, and Xenobiotics—Their Sources, Classification, Chemistry, and Metabolism



Abstract Poison causes irritation, injury, illness, or death if a person tastes it, smells, and gets it on skin or in eye and may be solid, liquid, sprays, vapor or gases, etc., or even a good thing when used in a wrong way. Botulinum toxin is the deadliest substance known to man. Poison, toxin, and venom may be differentiated: any substance that has a noxious effect on living organisms is poison, a toxin is a poison produced by a living organism in nature and toxicants are synthesized chemical substances while venom is a toxin injected by a bite or sting from a living organism into another (delivery method). The poison is often used to describe any harmful substance, particularly corrosive substances, carcinogens, mutagens, teratogens and harmful pollutants, and to exaggerate the dangers of chemicals. Physical sources such as particulate radiation (alpha and beta particles) or electromagnetic waves (X-rays, γ -rays, UV-rays) may cause harmful effects on living organisms. Paracelsus wrote: “Everything is poison; there is poison in everything; only the dose makes a thing not a poison”. Some poisons exert their effects on the part they come in contact with (local effect), some exert their effect on one or more organ systems after absorption (systemic effect), while some poisons have both local and systemic effects (combined effect). General symptoms of poisoning include (i) sick feeling, (ii) diarrhea, (iii) stomach pain, (iv) drowsiness, dizziness or weakness, (v) high temperature (38°C , 100.4°F or above), (vi) chills (shivering), (vii) loss of appetite, (viii) headache, etc. Poisonous compounds may be useful either for their toxicity as pesticides in agriculture or in industry as chemical reagents, solvents or complexing reagents, but less common in household use. Hallucination is distortion in perceptions of reality caused by hallucinogens such as psychoactive agents like mescaline, psilocybin, ibogaine, LSD, etc. Most hallucinogens are alkaloids or related substances and may be smoked or snuffed, swallowed fresh or dried, drunk in decoctions and infusions, absorbed directly through the skin, placed in wounds or administered as enemas. Teratogens affect the development of an embryo or fetus and include radiation, maternal infections, maternal metabolic factors, exposure to 2,4-D spraying, chemicals, drugs, etc. A pesticide and weedicides are chemicals that prevent, destroy, or repel pests including insects, termites, nematodes, molluscs, mice and other rodents, weeds, fungi and microorganisms bacteria and viruses. Medicinal drugs are divided with

respect to human organism are autobiogenous or natural, and (ii) xenobiotic or foreign. Biogenous drugs are involved in the conventional metabolic process while metabolic process of xenobiotics is subject to two major stages such as modification and conjugation. Metabolism of both biogenous substances and xenobiotics drugs is governed by the laws of enzyme kinetics. The metabolic conversion of xenobiotics is dependent on the occurrence of enzymes capable of catalyzing the conversion of these xenobiotics.

6.1 Poisons—Their Sources, Classification, Chemistry, Mode of Action, Symptoms of Poisoning Application and Application

A substance that causes injury, illness, or death, especially by chemical means is poison. A poison is something that can make a person sick if he tastes it, smells it, and gets it on skin or in eye. A product that may be safe in a normal dose may be unsafe at overdose levels. Poisons can be plants, liquids, powders, fumes, or sprays. Poison can also be a good thing used in a wrong way, like vitamins, medicines, perfumes, or hair spray. Poisons can be solids, liquids, or vapors (gases), e.g., (a) Solids—medicines, plants, botulin, botox, digoxin, strichine, granular drain cleaners; (b) Liquids—perfumes, toilet bowl cleaners, floor cleaners; (c) Sprays—window cleaners, perfumes, cleaners; and (d) Gases—natural gas, car exhaust, carbon monoxide. Botulinum toxin is believed to be the deadliest substance known to man.

Therefore, poison is any substance (solid, liquid or gas) which when introduced into the living body or brought into context with any part thereof will produce ill effects or death, by its local or systematic action or both. The fields of medicine (e.g., veterinary) and zoology often distinguish a poison from a toxin, and from a venom. A poison is a substance that has a noxious effect on living organisms. A toxin is a poison produced by a living organism in nature. A venom is a toxin injected by a bite or sting (this is exclusive to animals) from a living organism into another. The difference between venom and other poisons is the delivery method. Therefore, venom is a toxin and a toxin is a poison, not all poisons are toxins, not all toxins are venoms.

A same species may be venomous and poisonous, e.g., cobra is venomous (when toxin is injected or transferred by a bite) and poison (when toxin is ingested) while a bee is venomous only (transfer toxin by sting), a dart frog is poisonous only, etc. Poisons are any chemical substances that impact biological functions in other organisms, but depending on the method delivery, terminology may be different. For example, toxins are biologically produced chemical substances that impact biological functions in other organisms; toxicants are synthesized chemical substances that impact biological functions in other organisms; poisonous organisms secrete chemical substances that impact biological functions in other organisms;

and venomous creatures inject chemical substances that impact biological functions in other organisms.

Industry, agriculture, and other sectors use poisons for reasons other than their toxicity. Pesticides are one group of substances whose toxicity to various insects and other pests (e.g., rats, cockroaches, etc.) is their prime purpose. The term “poison” is often used colloquially to describe any harmful substance, particularly corrosive substances, carcinogens, mutagens, teratogens and harmful pollutants, and to exaggerate the dangers of chemicals. Paracelsus (1493–1541), the father of toxicology, wrote: “Everything is poison; there is poison in everything; only the dose makes a thing not a poison.”

Some poisons are also toxins, which is any poison produced by animals, vegetables or bacterium, such as the bacterial proteins that cause tetanus and botulism. A distinction between the two terms is not always observed, and their derivative forms “toxic” and “poisonous” are used as synonymous. Animal poisons delivered subcutaneously (e.g., by sting or bite) are also called *venom*. In normal usage, a poisonous organism is one that is harmful to consume, but a venomous organism uses venom to kill its prey or defend itself while still alive. Rarely, an organism can be both poisonous and venomous (Hutchinson et al. 2007). All living things produce substances to protect them from getting eaten, so the term “poison” is usually only used for substances which are poisonous to humans, while substances that mainly are poisonous to a common pathogen to the organism and humans are considered antibiotics, e.g., bacteria are a common adversary for *Penicillium chrysogenum* mold and humans, and since the mold’s poison only targets bacteria humans may use it for getting rid of bacteria in their bodies. Human antimicrobial peptides which are toxic to viruses, fungi, bacteria, and cancerous cells are considered a part of the immune system (Reddy et al. 2004).

Sources of poisons: Sources of poisons may be natural and synthetic such as:

(a) Natural sources

Natural sources include biological sources (e.g., microbial, plants, animals) and minerals sources. Biological sources of poisons or biotoxins fall into three major categories such as (i) microbial sources of poisons or toxins, poisons produced by bacteria, blue-green algae, dinoflagellates, golden brown algae, etc., (ii) plant sources of poisons or phytotoxins produced by higher plants, and (iii) animal sources of poisons or zootoxins produced by animals. The geographic distribution of poisonous organisms varies greatly; poison-producing microorganisms tend to be ubiquitous in their distribution. Poisonous plants and animals are found in greatest abundance and varieties in warm-temperate and tropical regions. Comparatively a few poisonous, venomous, or toxic organisms of any kind are found in polar latitudes.

Although the knowledge of the evolutionary significance and development of most poisons or biotoxins is largely speculative and poorly understood, they may have developed as part of the food procurement mechanism (e.g., in snakes; cnidarians, jellyfishes, and their relatives; mollusks, octopuses, and others; and spiders); as chemical defensive mechanism against the predators and herbivores

(e.g., in some snakes, fishes, arthropods, certain plant species); and others. The defense may be quite complex, e.g., for territorial rights, certain marine organisms and terrestrial plants may release into the water, air, or soil inhibitory substances that discourage the growth of other organisms (e.g., allelopathy); production of antibiotic substances by microorganisms (e.g., allelochemicals), etc. It is evident that certain substances, which may be toxic to one group of organisms, may serve a vital function in the life processes of the source organism. Thus, biotoxins play important roles in the regulation of natural populations.

(i) Microbial poisons

Microbial poisons are produced by the Monera (e.g., bacteria and cyanobacteria) and Protista (e.g., algae, protozoa, and others), and the Fungi (e.g., mold, mushroom and others).

Moneran toxins

Poisonous proteins when released outside from bacteria are referred to as bacterial exotoxins. The exotoxins are generally produced by Gram-positive organisms; but Gram-negative bacteria *Shigella dysenteriae* and *Vibrio cholerae* produce exotoxins. The exotoxins usually do not contain any nonprotein substances, and most are antigenic (stimulate the formation of antibodies). Endotoxins (inside the bacterial cell) are antigens composed of complexes of proteins, polysaccharides, and lipids. The protein part determines the antigenicity, and polysaccharide part determines the immunological specificity, and lipids possibly determine the toxicity. Cyanobacterial water blooms of blue-green algae have been responsible for the death of fishes, waterfowl, cattle, horses, swine, and other animals and they have also been implicated as causes of human intoxications.

Microbial poisons are toxins produced by microorganisms like bacteria, fungi, and others. Microbial toxins promote infection and disease by directly damaging host tissues and by disabling the immune system, e.g., bacterial toxins—*botulinum* neurotoxins are the most potent natural toxins known. Microbial toxins, however, have important uses in medical science and research, e.g., toxins are used to combat microbial virulence, in the development of novel anticancer drugs and other medicines.

Bacteria generate toxins which can be classified as either exotoxins (e.g., botulinum toxin produced by *Clostridium botulinum*; *Corynebacterium diphtheriae* toxin, produced during life-threatening symptoms of diphtheria; tetanospasmin produced by *Clostridium tetani*) or endotoxins (lipopolysaccharides LPS—large molecules consisting of lipid A, core polysaccharide and O-antigen). Exotoxins are generated and actively secreted; endotoxins remain part of the bacteria. Usually, an endotoxin is part of the bacterial outer membrane, and it is not released until the bacterium is killed by the immune system. Toxinosis is pathogenesis caused by the bacterial toxin alone, not necessarily involving bacterial infection (when the bacteria have died, but have already produced toxin, which are ingested), e.g., it can be caused by *Staphylococcus aureus* toxins. *Botulinum* neurotoxins (BoNTs) are the causative agents of the deadly food poisoning disease botulism; *Clostridium tetani*

produces tetanus toxin (TeNT protein), which leads to a fatal condition known as tetanus in many vertebrates (including humans) and invertebrates.

Alpha toxin or alpha-toxin (not to be confused with Aflatoxins) refers to several different protein toxins produced by bacteria, e.g., *Staphylococcus aureus*; alpha toxin, a membrane-disrupting toxin that creates pores causing hemolysis and tissue damage; *Clostridium perfringens* alpha toxin, a membrane-disrupting toxin with phospholipase C activity, which is directly responsible for gas gangrene and myonecrosis; *Pseudomonas aeruginosa* alpha toxin, etc.

Anthrax toxin is a three-protein exotoxin secreted by virulent strains of the bacterium, *Bacillus anthracis*—the causative agent of anthrax. Cyanotoxins are toxins produced by bacteria called cyanobacteria (also known as blue-green algae). Cyanotoxins usually target the nervous system (neurotoxins), the liver (hepatotoxins) or the skin (dermatotoxins). The chemical structure of cyanotoxins falls into three broad groups: cyclic peptides (microcystins, nodularins), alkaloids (anatoxin-a, anatoxin-a(s), Cylindrospermopsin, lyngbyatoxin-a) and lipopolysaccharides(LPS) (endotoxins). Diphtheria toxin is an exotoxin secreted by *Corynebacterium diphtheriae*, the pathogenic bacterium that causes diphtheria. Diphtheria toxin is a single polypeptide chain of 535 amino acids consisting of two subunits linked by disulfide bridges, known as an A-B toxin. The toxin causes the disease diphtheria in humans by gaining entry into the cell cytoplasm and inhibiting protein synthesis. Pertussis toxin (PT) is a protein-based AB₅-type exotoxin produced by the bacterium *Bordetella pertussis*, which causes whooping cough. Shiga toxins are named for Kiyoshi Shiga, who first described the bacterial origin of dysentery caused by *Shigella dysenteriae*. The toxin has two subunits—designated A (mol. wt. 32,000 D) and B (mol. wt. 7700 D)—and is one of the AB₅ toxins. Cholera toxin (CTX or CT) is protein complex secreted by the bacterium *Vibrio cholerae*. CTX is responsible for the massive, watery diarrhea characteristic of cholera infection.

Mycotoxins (fungal poisons)

A mycotoxin is a toxic secondary metabolite produced by fungi and is capable of causing disease and death in both humans and animals (Bennett and Klich 2003; Richard 2007). The term “mycotoxin” is usually reserved for the toxic chemical products produced by fungi that readily colonize crops (Turner et al. 2009). One mold species may produce many different mycotoxins, and several species may produce the same mycotoxin (Robbins et al. 2000). Toxic fungi can be roughly divided into two main categories on the basis of their size: the smaller microfungi (Ascomycetes and Deuteromycetes) and the larger mushrooms (Basidiomycetes). However, some Ascomycetes, e.g., poisonous false morel (*Gyromitra esculenta*), may attain a size as large as some of the mushrooms.

Different microfungi such as *Claviceps purpurea* (ergot) produces ergotoxine (a complex of toxic alkaloids—ergocryptine, ergocornine, ergocristine, and others); *Stachybotrys chartarum* produces stachybotryotoxin; *Aspergillus flavus* and other species, *Penicillium* species produce aflatoxin complex; *Fusarium sporotrichioides* and other *Fusarium* species produce fusariogenin, epichlorosporic acid,

fagicladosporic acid and these toxins like *Fusarium* species are also produced by *Cladosporium epiphyllum* and other species of *Cladosporium*; *Pithomyces chartarum* (*Sporidesmium bakeri*) produces sporidesmin; *Fusarium* species, *Rhizopus* species, *Aspergillus* species, *Penicillium islandicum*, and others produce luteoskyrin, islanditoxin, citrinin, citreoviridin, and others.

Different macrofungi such as poisonous mushrooms or toadstools are the widely distributed members of the class Basidiomycetes. Most deaths attributed to mushroom poisoning result from eating members of the genus *Amanita*. Representative poisonous mushrooms like *G. esculenta* produces gyromitrin; *Amanita muscaria*, *Inocybe patouillardii* and *Omphalotus olearius* produce muscarine; death cap—*Amanita phalloides* produces amanitine, phalloidine; *Psilocybe mexicana* produces psilocybin, psilocin.

Major groups of mycotoxin

(i) Aflatoxins (not to be confused with Alpha toxin produced by bacteria *Staphylococcus aureus*) are poisonous carcinogens that are produced by certain molds (*Aspergillus flavus* and *Aspergillus parasiticus*). They are regularly found in improperly stored staple commodities such as cassava, chili peppers, corn, cottonseed, millet, peanuts, rice, sesame seeds, sorghum, sunflower seeds, tree nuts, wheat, and a variety of spices. Aflatoxins form one of the major groupings of mycotoxins and at least 14 different aflatoxins are produced in nature (e.g., B₁, B₂, G₁, G₂, M₁, M₂, Q₁, etc.). (ii) Ochratoxin is a mycotoxin that comes in three secondary metabolite forms, A, B, and C. All are produced by *Penicillium* and *Aspergillus* species. The three forms differ in that Ochratoxin B (OTB) is a nonchlorinated form of Ochratoxin A (OTA) and that Ochratoxin C (OTC) is an ethyl ester form Ochratoxin A. (iii) Citrinin, a polyketide mycotoxin, is synthesized several species of *Penicillium* and *Aspergillus*. It causes different toxic effects, like nephrotoxic, hepatotoxic and cytotoxic effects. Citrinin is mainly found in stored grains, but sometimes also in fruits and other plant products. (iv) Ergot alkaloids are compounds produced as a toxic mixture of alkaloids in the sclerotia of species of *Claviceps*. (v) Patulin is a toxin produced by *Penicillium*, *Aspergillus*, and *Paecilomyces* fungal species. Although patulin has not been shown to be carcinogenic, it has been reported to damage the immune system in animals (Moss 2008). (v) *Fusarium* toxins are produced by over 50 species of *Fusarium* and they include a range of mycotoxins, such as fumonisins (affect the nervous systems of horses and may cause cancer in rodents), trichothecenes (causes chronic and fatal toxic effects in animals and humans), and zearalenone (not correlated to any fatal toxic effects in animals or humans). Fumonisins consist of Fumonisin B₁, Fumonisin B₂, and Fumonisin B₃ among others. Fumonisin B₁ is hepatotoxic and nephrotoxic in all animal species tested. Fumonisin B₂ is a structural analog of fumonisin B₁ and it is more cytotoxic than fumonisin B₁. Trichothecenes belong to sesquiterpene compounds. Zearalenone (ZEN) is a potent estrogenic metabolite. Zearalenone is the primary toxin, causing infertility, abortion or other breeding problems, especially in swine.

Mushroom poisoning (mycetism or mycetismus) refers to harmful effects from ingestion of poisonous substances present in a mushroom. At present approximately 100,000 known fungi species found worldwide and about 100 of them are poisonous to humans (Graeme 2014). Poisonous fungi including the most lethal mushrooms are (i) death cap (*Amanita phalloides*), (ii) destroying angels (*Amanita virosa* and *Amanita bisporigera*), and the (iii) fool's mushroom (*Amanita verna*), and two more (iv) the deadly webcap (*Cortinarius rubellus*), and (v) the fool's webcap (*Cortinarius orellanus*).

α -Amanitin or α -amanitin, a cyclic peptide of eight amino acids, is the most deadly of all the amatoxins, found in the death cap (*Amanita phalloides*), the destroying angel (*A. virosa* and *A. bisporigera*). It is also found in the mushrooms *Galerina marginata* and *Conocybe filaris*. The oral LD₅₀ of amanitin is approximately 0.1 mg/kg for rats. The phallotoxins consist of at least seven compounds (e.g., phalloidin prophalloin, phalloin, phallisin, phallacidin, phallacin, and phallisacin.), all of which are bicyclic heptapeptides (seven amino acids), isolated from the death cap mushroom (*Amanita phalloides*). Orellanine (orellanin), a pyridine N-oxide based compound, is a deadly poisonous mycotoxin found in Orellani group of mushrooms (*C. orellanus*, *C. rubellus*, *C. henrici*, *C. rainerensis* and *C. brunneofulvus*) of the family Cortinariaceae. Muscarine (L-(+)-muscarine, or muscarin), a deadly poisonous natural product, is found in *Inocybe* and *Clitocybe* mushrooms species (e.g., *C. dealbata*). Ergotamine is a secondary metabolite (natural product) and the principal alkaloid of ergot fungus (*C. purpurea*) and related fungi in the family Clavicipitaceae. Figure 6.1 shows structure of different microbial toxins such as tetrodotoxin (TTX), aflatoxin b₁, cylindrospermopsin, lipopolysaccharides (LPS), lyngbyatoxin-a, saxitoxin (STX), ochratoxin A, ochratoxin B, ochratoxin C, citrinin, patulin, fumonisin B₁, fumonisin B₂, trichothecenes, zearalenone, α -amanitin, phalloidin, orellanine, muscarine and ergotamine. Figure 6.1 shows structure of different microbial toxins—tetrodotoxin (TTX), aflatoxin b₁, cylindrospermopsin, lipopolysaccharides (LPS), lyngbyatoxin-a, saxitoxin (STX), ochratoxin A, ochratoxin B, ochratoxin C, citrinin, patulin, fumonisin B₁, fumonisin B₂, trichothecenes, zearalenone, α -amanitin, phalloidin, orellanine, muscarine, and ergotamine.

Dinoflagellate poisons

The dinoflagellates, microscopic one-celled autotrophic organisms and important producers of the primary food supply of the sea, during planktonic blooms (sometimes referred to as red tide because they discolor the water) multiply in large numbers and intoxicate shellfish as after ingestion, the poisons accumulate in their digestive glands. The majority of toxic blooms have been caused by *Alexandrium catenella*, *Alexandrium tamarensense*, and *Alexandrium fundyense*, which together comprise the *A. tamarensense* species complex (Balech 1985; Cembella 1998). They are most commonly involved in human intoxications (paralytic shellfish poisoning —PSP). PSP affects those who come into contact with the affected shellfish by ingestion (Clark et al. 1999). The paralytic shellfish poison saxitoxin (STX) is a tricyclic perhydropurine alkaloidal neurotoxin, the best-known paralytic shellfish

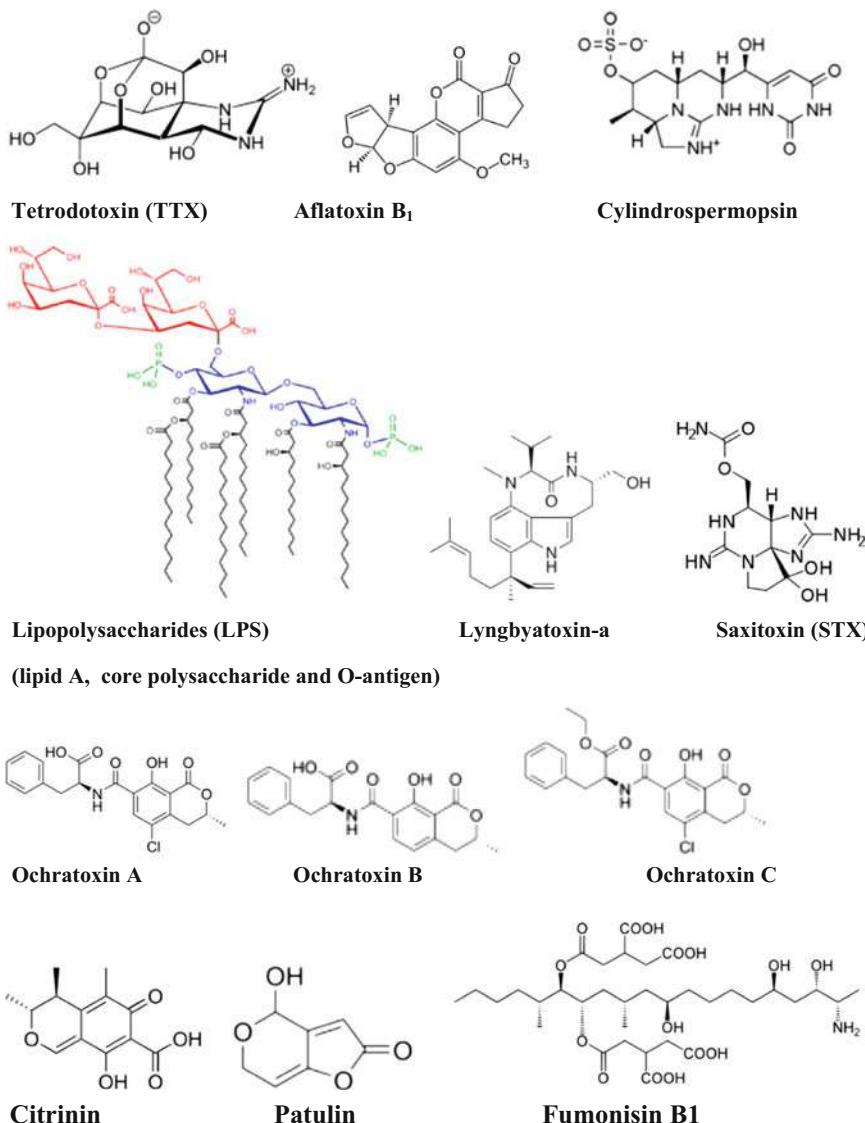
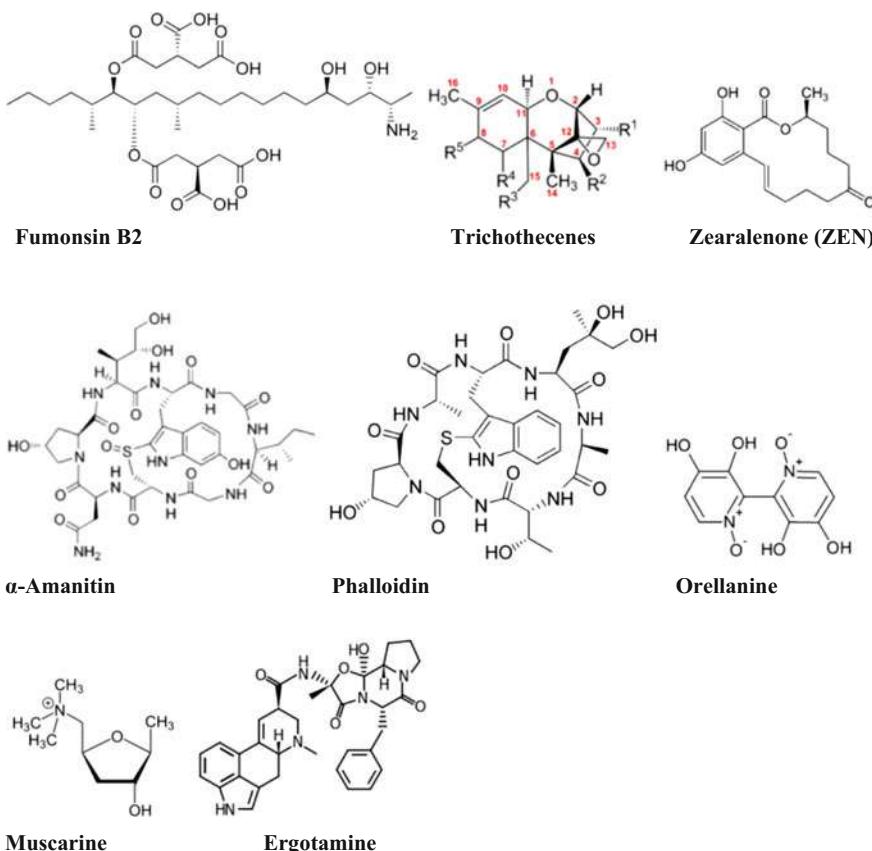


Fig. 6.1 Structure of different microbial toxins—tetrodotoxin (TTX), aflatoxin B₁, cylindrospermopsin, lipopolysaccharides (LPS), lyngbyatoxin-a, saxitoxin (STX), ochratoxin A, ochratoxin B, ochratoxin C, citrinin, patulin, fumonisins B₁, fumonisins B₂, trichothecenes, zearalenone, α -amanitin, phalloidin, orellanine, muscarine, and ergotamine

toxin (PST). The paralytic shellfish toxins (PSTs) also include neosaxitoxin (NSTX), gonyautoxins (GTX) and decarbamoylsaxitoxin (dcSTX). Prymnesin poison (phycotoxins) from *Prymnesium parvum* is known to be toxic to fish,

**Fig. 6.1** (continued)

causing mass fish deaths of brackish water around the world. Prymnesins ($C_{107}H_{154}Cl_3NO_{44}$) consist of a large polyether polycyclic core with several conjugate double and triple bonds, chlorine and nitrogen heteroatoms and sugar moieties including L-xylose; and three forms of prymnesin such as prymnesin 1 and 2, differing in their glycosylation, and prymnesin B₁ differing in backbone are known (Manning and La Claire 2010; Rasmussen et al. 2016). Figure 6.2 shows the structure of different Dinoflagellate poisons such as saxitoxin (STX), neosaxitoxin (NSTX), gonyautoxins 2 (GTX-2), decarbamoylsaxitoxin (dcSTX), and prymnesin 1.

(ii) Plant poisons

Plant that contains poisons is called poisonous plant and plant poisons are also called phytotoxins. There is significant overlap between plants considered poisonous and those with psychotropic properties. Many plants are commonly used as

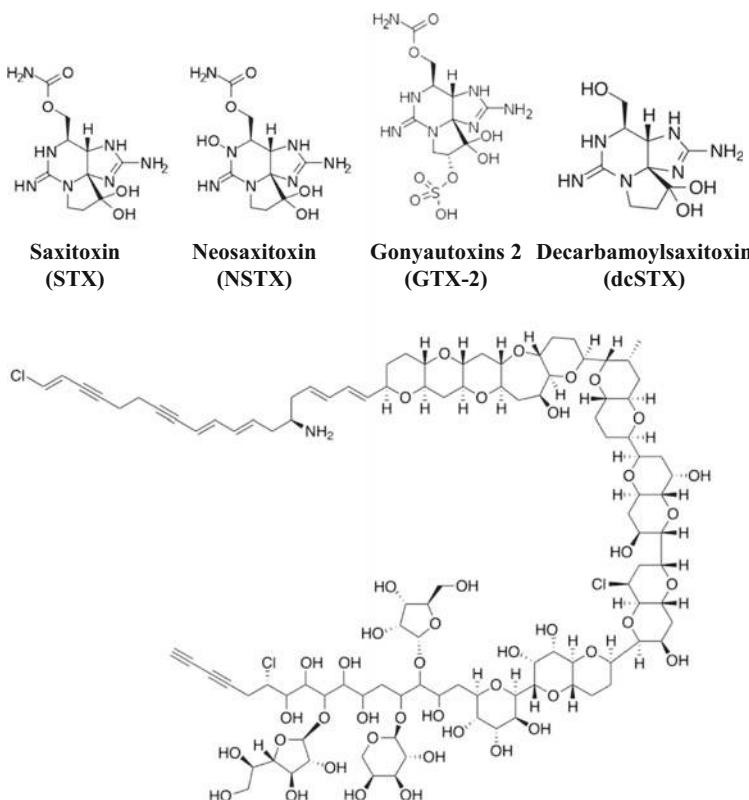


Fig. 6.2 Structure of different Dinoflagellate poisons—saxitoxin (STX), neosaxitoxin (NSTX), gonyautoxins 2 (GTx-2), decarbamoylsaxitoxin (dcSTX), and prymnesin 1

food possess toxic parts (e.g., apple seeds are mildly poisonous for containing a small amount of amygdalin, a cyanogenic glycoside; berries of Asparagus berries of the mature plant are poisonous as they contain furostanol and spirostanol saponins; Cassava roots and leaves contain cyanogenic glycosides—linamarin and lotaustralin). The entire plant may be poisonous, or a certain part of the plant such as root, stems, barks, seeds, leaves, berries, and flowers may be poisonous. Poisonous plants contain poisonous principles like alkaloids (pyridine–piperidine alkaloids, tropane alkaloids, isoquinoline alkaloids, indole alkaloids, imidazole alkaloids, steroid alkaloids, alkaloidal amines, purine bases, pyrrolidine group alkaloids, etc.), glycosides (steroidal glycosides, flavonoid glycosides, anthroquinone glycosides, cyanophoric glycosides, lactone glycosides, aldehyde glycosides, phenolic glycosides, etc.), toxic proteins (ricin, toxalbumins), oxalates, resins (cannabis resin), etc., and a large group of miscellaneous compounds whose chemical structure has not yet been determined. Poisonous plants may be classified in various

ways such as: (a) on the basis of their toxic effects, (b) on the basis of the chemical nature of their toxic constituents, (c) on the basis of their phylogenetic relationship, as well as (d) on the basis of their botanical characteristics. Poisonous plants may exhibit their poisonous effects after ingestion, upon contact, or they may produce photosensitization, and airborne allergies.

Plants that exhibit poisoning effects on ingestion by different secondary active principles include among others *Abrus precatorius* (abrin and abric acid); *Aconitum napellus* (aconite and a complex of other alkaloids); *Agrostemma githago* (githagin, agrostemmic acid (saponins); *Astragalus* sp. (locoine); *Atropa belladonna* (hyoscyamine, atropine, hyoscine, and a complex of other alkaloids); *Blighia sapida* (hypoglycin A, B); *Brassica napus* (glycosides isothiocyanates); *Cannabis sativa* (cannabinol, canabidiol, and related compounds); *Cicuta maculata* (cicutoxin); *Conium maculatum* (coniine, conhydrine, N-methyleleoniine, coniceine, and other alkaloids); *Croton tiglium* (croton, croton resin, ricinine); *Gloriosa superba* and *Colchicum autumnale* bulbs contain colchicine alkaloid poison; *Daphne mezereum* (glycoside involving aglycone dihydroxycoumarin); *Datura stramonium* (hyoscine, hyoscyamine, atropine); *Delphinium* sp. (delphinine, delphinoidine, delphisine, and other alkaloids); *Dieffenbachia seguine* (protoanemonine, calcium oxalate); *Digitalis purpurea* (glycosides, digitoxigenin, and others); *Dioscorea hispida* (diосcorine); *Erythroxylon coca* (cocaine and other alkaloids); *Hippomane mancinella* (physostigmine or a similar alkaloid plus a sapogenin); *Hyoscyamus niger* (hyoscyamine, hyoscine, atropine, and other alkaloids); *Jatropha curcas* (curcin); *Kalmia latifolia* (andromedotoxin); *Lathyrus sativus* (β -aminopropionitrile); sparteine, a class 1a antiarrhythmic agent and a sodium channel blocker, is predominant alkaloid in *Lupinus mutabilis*; *Manihot esculenta* (cyanophoric glycosides); *Melia azedarach* (bitter alkaloid azadirachtin); *Papaver somniferum* (morphine, codeine, thebaine, papavarine, narcotine); *Phytolacca americana* (phytolaccine); *Ricinus communis* (ricin, a toxalbumin); *Solanum nigrum* (solanine, a glycoalcaloid); rhizome of *Podophyllum peltatum* contains the non-alkaloid toxin podophyllotoxin; ouabain, a cardiac glycoside and traditional arrow poison, is extracted from the ripe seeds of *Strophantus gratus*; etc.

Plant poisons by contact include poison ivy, curare, poisonwood, spurge, spurge nettle, shiny-leaf stinging tree, tree nettle, e.g., *Euphorbia* sp. (a complex of substances including alkaloids, glycosides, and others); *Jatropha urens* (unknown toxin); *Dendrocnide photiniphylla* (5-hydroxytryptamine); *Strophantus* sp. (an alkaloid, trigonelline, and a large number of cardiac glycosides and aglycones used as an arrow poison); *Strychnos toxifera* (toxiferines, caracurines, and other alkaloids); etc. The toxic substance may be obtained directly from the plant, which thereupon acts on the skin (primary photosensitivity), or the toxicity may result from liver damage caused by the ingestion of a photosensitizing toxic plant (hepatic photosensitivity) resulting in deposition of a photosensitizing pigment in the skin; sunlight then causes redness of the skin, nervousness, swelling of the eyelids, convulsions, and prostration in farm animals. Plants that induce photosensitization include *Fagopyrum sagittatum* (fagopyrin, a naphthodianthrone derivative); *Hypericum perforatum* (hypericin, a naphthodianthrone derivative). Plants that

produce airborne allergies include *Acer negundo* (oleoresin and a water-soluble antigen) and others. Under certain ecological conditions, plants may become poisonous as a result of the accumulation of toxic inorganic minerals such as copper, lead, cadmium, fluorine, manganese, nitrates, or selenium.

Plant poisons of nightshade or deadly nightshade (*Atropa belladonna*, Solanaceae), a perennial herbaceous plant native to Europe, North Africa, and Western Asia, its roots, leaves and fruits contain poisonous alkaloids like atropine, hyoscyamine and scopolamine; all parts of all angel's trumpet plant genus (*Brugmansia*) contain the tropane alkaloids scopolamine and atropine; coniine alkaloid is the active principle of hemlock or poison hemlock (*Conium maculatum*, Apiaceae), a biennial herbaceous flowering plant, native to Europe and North Africa, bears fleshy, carrot like roots and can grow up to ten feet tall, hemlock toxicity primarily results from consumption, but poisoning can also result from inhalation, and from skin contact; poisonous alkaloids—strychnine and brucine present in the seeds of strychnine tree (*Strychnos nux vomica*, Loganiaceae), a medium sized tree found in South East Asia (including Bangladesh, India) and Australia; helenalin, a highly toxic sesquiterpene lactone, is found in *Arnica montana* and *Arnica chamissonis foliosa*; curare is a common name for arrow poisons alkaloids (toxic isoquinoline alkaloids like papaverine, sanguinarine, dihydrosanguinarine, protoverine, berberine, coptisine, protopine, and chelidonine; and indole alkaloids—strychnine, brucine, etc.) of the extract of various plants of families such as Menispermaceae (*Curarea toxicofera*, *C. tecunumina*, *Chondrodendron tomentosum*, *Sciadotenia toxifera*, etc.), Loganiaceae (*Strychnos toxifera*, *Strychnos guianensis*, *Strychnos castelnaei*, *Strychnos usambarensis*), Araceae (Aroideae), Piperaceae (*Artanthe* spp.), Aristolochiaceae native to Central and South America, i.e., there are dozens of plants from which active alkaloid principles with curarizing effects may be isolated; ricin is a highly toxic, naturally occurring lectin (a carbohydrate-binding protein) produced in the seeds of the castor oil plant (*Ricinus communis*, Euphorbiaceae), perennial shrub native to the south-eastern Mediterranean Basin, Eastern Africa, Bangladesh and India and today it is widespread throughout tropical regions. Datura is a genus of nine species of poisonous vespertine flowering plants (also known as devil's trumpets, and not to be confused with angel's trumpets of *Brugmansia* genus) belonging to the family Solanaceae. All parts of Datura plants contain dangerous levels of tropane alkaloids (highly poisonous hyoscyamine, atropine and scopolamine) and may be fatal if ingested by humans or other animals, including livestock and pets. It grows in the wild in all the warmer parts of the world. Abrin is a highly toxic toxalbumin found in the seeds of the rosary pea (or jequirity pea), *A. precatorius*. Abrin is a ribosome inhibiting protein like ricin, Amygdalin is a naturally occurring cyanogenic glycoside found notably in the seeds (kernels) of apricot, bitter almonds, apple, peach, and plum. Anisatin is an extremely toxic, insecticidally active component of the Japanese star anise, Shikimi plant (*Illicium anisatum*). Antiarins are cardiac glycoside poisons produced by the upas tree (*Antiaris toxicaria*). There are two forms, α -antiarin and β -antiarin. Aconitine is a toxin produced by the *Aconitum* plant, considered notorious for its toxic properties due to poison its alkaloid. *Argemone*

mexicana seeds contain 22–36% of a pale yellow nonedible oil (argemone oil or katkar oil), which contains the toxic alkaloids sanguinarine and dihydrosanguinarine; *Calla palustris* (water-arum) is very poisonous when fresh due to its high oxalic acid content. Cerberin, a steroid class cardiac glycoside, is a potent toxin related to digoxin and found in the seeds of *Cerbera* including the suicide tree (*Cerbera odollam*) and the sea mango (*Cerbera manghas*). Figure 6.3 shows the structure of different plant poisons such as cicutoxin (a neurotoxin), colchicine, sparteine, solanine, podophyllotoxin, ouabain, atropine, hyoscyamine, scopolamine (hyoscine), coniine, strychnine, brucine, helenalin, amygdalin, anisatin, α -antiariin, β -antiariin, aconitine, sanguinarine, dihydrosanguinarine, oxalic acid, and cerberin.

(iii) Animal poisons

Poisonous animals are widespread throughout the animal kingdom with the exception of Aves. Zootoxins can be divided into (i) oral poisons, i.e., poisonous when eaten; (ii) parenteral poisons, or venoms, i.e., produced by a specialized poison gland and administered or injected by bites or stings; and (iii) crinotoxins, i.e., produced by a specialized poison gland but are merely released usually by means of a pore or rarely released into the environment. Oral zootoxins are generally thought to be small molecules; majority venoms are large molecules, usually a protein; but little is known about the chemical properties of most crinotoxins. Animals in which poison glands are present and poison is released into the environment through a pore are crinotoxic animals. Poisonous Amphibians are poisonous as they possess only poison glands but not any true venom apparatus. The term poisonous may be used in the generic sense to refer to all three categories of zootoxins.

Animal poisons include the tetrodotoxin (TTX), derived from Tetraodontiformes, is a potent neurotoxin, Tetraodontiformes includes pufferfish, porcupinefish, ocean sunfish, and triggerfish as well as several other aquatic animals including blue-ringed octopuses, rough-skinned newts, and moon snails carry TTX; it is actually produced by certain infecting or symbiotic bacteria like *Pseudoalteromonas*, *Pseudomonas*, and *Vibrio* as well as other species found in the animals. Tetrodotoxin is a sodium channel blocker and is extremely toxic, the LD₅₀ for the mouse is 10 ng.

Box jellyfish (Cubozoa) are cnidarian invertebrates include several world's most venomous species such as *Chironex fleckeri*, *Carukia barnesi*, and *Malo kingi* and stings from these species are extremely painful and can be fatal to humans. The protein venom of box jelly fish inflict severe localized and systemic effects, including cutaneous pain, inflammation and necrosis, hypertension followed by hypotension, cardiovascular collapse, and cardiac arrest (Lumley et al. 1988; Currie and Jacups 2005; Brinkman et al. 2014).

There are several poisonous species of snakes in the world, and they belong to three families such as Elapidae (common cobra, king cobra, and krait); Viperidae (Russell's viper, pit viper, and saw-scaled viper) and Hydrophiinae (the sea snakes). Among these, the majority of bites and consequent mortality are attributable to only five species such as king cobra (*Ophiophagus hannah*), common cobra (*Naja naja*), Russell's viper (*Daboia russelii*), krait (*Bungarus coeruleus*), and saw-scaled viper

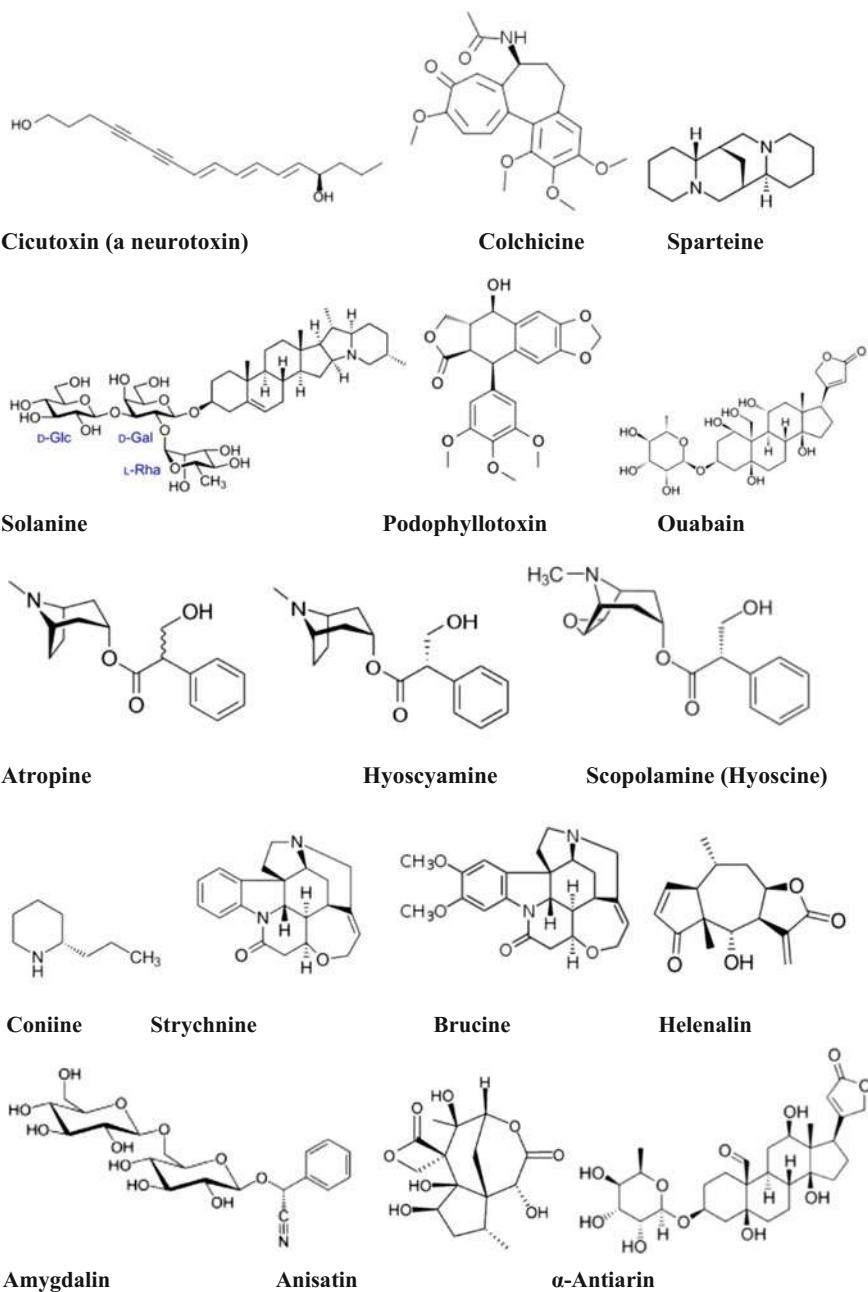
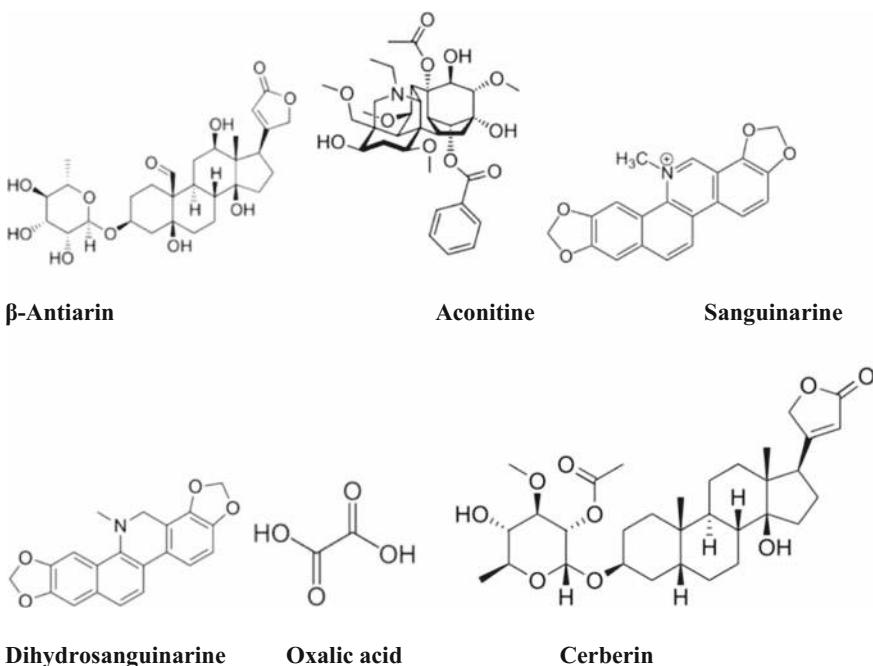


Fig. 6.3 Structure of different plant poisons—cicutoxin (a neurotoxin), colchicine, sparteine, solanine, podophyllotoxin, ouabain, atropine, hyoscyamine, scopolamine (hyoscine), coniine, strychnine, brucine, helenalin, amygdalin, anisatin, α -antiarin, β -antiarin, aconitine, sanguinarine, dihydrosanguinarine, oxalic acid and cerberin

**Fig. 6.3** (continued)

(*Echis carinatae*). Snake venoms are of three types: neurotoxic, hemotoxic, and myotoxic. The king cobra (*Ophiophagus hannah*, Elapidae) is the world's longest venomous snake endemic to forests of Southeast Asia including Bangladesh and India. The venom of this serpent consists primarily of neurotoxins—hadtotoxin (a short chain dimer), and several other compounds (Roy et al. 2010). The lethal neurotoxic protein affects the victim's central nervous system, resulting in severe pain, blurred vision, vertigo, drowsiness, and eventually paralysis and in serious envenomation, it progresses to cardiovascular collapse. Venomous snakes have certain common features and some other features that are unique to each of them.

Snake venoms are complex mixtures of proteins and peptides, enzymatic and nonenzymatic compounds, and they comprise >90% of the dry weight of the venom; also contain inorganic cations like sodium, calcium, potassium, and magnesium, and small amounts of zinc, iron, cobalt, manganese, and nickel. The metals in snake venoms are likely catalysts for metal-based enzymatic reactions. Snake venoms function as neurotoxins, coagulants, hemorrhagins, hemolitics, myotoxins, cytotoxins, and nephrotoxins. In medicine, cobra venom is a potential source of anticancer drugs and painkillers.

Marbled cone snail (*Conus marmoreus*, Conidae), predatory sea snail, is a species is venomous, like all cone snails. The toxins venoms are called conotoxins and these are various peptides consisting of 10–30 amino acid residues, typically have one or more disulfide bonds.

Blue-ringed octopuses (*Hapalochlaena lunulata*, *Hapalochlaena maculosa*, *Hapalochlaena fasciata*, *H. nierstraszi*, Octopodidae), four highly venomous species of octopus of tide pools and coral reefs in the Pacific and Indian Oceans from Japan to Australia, contain the powerful neurotoxin tetrodotoxin. The blue-ringed octopus, despite its small size, carries enough venom to kill 26 adult humans within minutes. Their bites are tiny and often painless, with many victims not realizing they have been envenomated until respiratory depression and paralysis start to set in.

The deathstalker scorpion (*Leiurus quinquestriatus*, Buthidae) is also known as Israeli or Palestine yellow scorpion, and can be found in desert and scrubland habitats ranging from North Africa through to the Middle East. It is one of the most dangerous species of scorpions. Its venom is a powerful mixture of neurotoxins (e.g., chlorotoxin, charybdotoxin, scyllatoxin, agitoxins), with a low lethal dose. Chlorotoxin is a 36-amino acid peptide which blocks small-conductance chloride channels (DeBin and Strichartz 1991). Charybdotoxin (CTX) is a 37 amino acid neurotoxin that blocks calcium-activated potassium channels (Laurent et al. 1993). Scyllatoxin consists of only 0.02% of the total protein in crude venom and it is a 31-residue peptide, with a helix and a short antiparallel β -sheet. It is a blocker of small-conductance Ca^{2+} -activated K^+ channels. Three types of agitoxin (e.g., agitoxin-1, -2, and -3) can be distinguished, each identified as comprising 38 amino acids. Agitoxin binds to the Shaker K^+ channel in *Drosophila* as well as to its mammalian homologue. It blocks this channel by binding with high affinity ($K_d < 1 \text{ nmol/L}$) to its external vestibule.

There are many other animals that are poisonous to humans (and other animals) belonging to different groups; and numerous animal species naturally produce chemical toxins which are used to kill or incapacitate prey or as a defense against predators. Venomous animals deliver these toxins as venom through a bite, sting, or other specially evolved mechanism. The relationships of representative poisonous animals and their position in the total framework of the animal kingdom can best be appreciated by categorizing them according to the group in which they belong. Poisonous animals include sponges—*Microciona prolifera* (toxin unknown); flatworms—*Leptoplana tremellaris* (toxin unknown); arthropods: blister beetles—*Cantharis vesicatoria* (cantharidin)—*Orthoporus*, *Rhinocrichus*, *Julus*, and *Spirobolus* spp. (toxin unknown); venomous ticks—*Ixodes* and *Ornithodoros* spp. (toxin unknown); fishes: sea lamprey—*Petromyzon marinus* (toxin unknown), Atlantic Ocean soapfish—*Rypticus saponaceus* (neurotoxic); amphibians: fire salamander—*Salamandra salamandra* (alkaloids samandarine, samandenone, samandine, samanine, samandarone, samandaridine, and others), toads—*Bufo* spp. (a poisonous secretion in the parotid glands and skin includes bufotoxin, bufo-genins, and 5-hydroxytryptamine; poison includes a complex of many substances), frogs—*Dendrobates*, *Physalaemus*, and *Rana* spp. (skin secretions are poisonous; histamine, bufotenine, physalaemin, serotonin, and other substances; composition varies with the species), tree frogs—*Hyla* and *Phyllobates* spp. (skin secretions are poisonous; batrachotoxin, steroid alkaloids, serotonin, histamine, and other

substances; bufotenine varies with the species); sea wasp—*Chironex fleckeri* (cardiotoxin), etc.

Representative venomous animals that inflict a sting include cnidarians: Portuguese man-of-war—*Physalia* species (tetramine, 5-hydroxytryptamine), sea anemone—*Actinia equina* (nature of venom unknown); mollusks: cone shell—*Conus* sp. (quaternary ammonium compounds and others), spotted octopus—*Octopus maculosus* (cephalotoxin, a neuromuscular poison); arthropods: kissing bug—*Triatoma* sp. (toxin unknown), puss caterpillar—*Megalopyge* species (toxin unknown), honeybee—*Apis* species (neurotoxin, hemolytic, melittin, hyaluronidase, phospholipase A, histamine, and others), bumblebee—*Bombus* sp. (similar to *Apis* venom), yellow jacket, hornet—*Vespula* sp. (similar to bee venom, also acetylcholine), wasp—*Polistes* and *Vespa* spp. (similar to bee venom, also acetylcholine), harvester ant—*Pogonomyrmex* sp. (bradykinin, formic acid, hyaluronidase, hemolytic, phospholipase A, and others), fire ant—*Solenopsis* sp. (similar to harvester ant venom), millipede—*Apheloria* sp. (hydrogen cyanide and benzaldehyde), centipede—*Scolopendra* sp. (hemolytic phospholipase and serotonin), brown spider—*Loxosceles* sp. (cytotoxic, hyaluronidase, hemolytic, and others), black widow—*Latrodectus* sp. (nurotoxin), tarantula—*Dugesiella* and *Lycosa* sp. (venom mild), scorpion—*Centruroides*, *Tityus*, and *Leiurus* spp. (neurotoxin, cardiotoxin, hemolytic, lecithinase, hyaluronidase, and others); echinoderms: crown-of-thorns starfish—*Acanthaster planci*, long-spined sea urchin—*Diadema setosum*, sea urchin—*Toxopneustes pileolus* (nature of poison unknown); sharks and rays: stingray—*Dasyatis* sp. (stingray venom, cardiotoxin); bony fish: weever fish—*Trachinus draco* (weever fish venom), scorpion fish—*Scorpaena* sp. (scorpion fish venom), stonefish—*Synanceja* sp. (stonefish venom); reptiles: gila monster—*Heloderma suspectum* (heloderma venom, primarily a neurotoxin); etc. Some animals are poisonous for ingestion and they include mollusks (e.g., octopus, squid, shellfish, and others); arthropods, e.g., crabs (*Demania toxica*, *Zozymus aeneus*, *Tachypleus tridentatus*), as well as some species of sharks (*Somniosus microcephalus*, *Thunnus thynnus*), eels (*Gymnothorax javanicus*), and other fish (*Ruvettus pretiosus*, *Euthynus pelamis*, *Arothron hispidus*), amphibian (*Taricha torosa*), reptile (*Eretmochelys imbricate*, *Dermochelys coriacea*), mammals (whale—*Balaenoptera borealis*, *Delphinapterus leucas*, polar bear—*Thalarctos maritimus*) may be poisonous for ingestion due to their poisonous contents (e.g., paralytic shellfish poison, saxitoxin, tetramine, ciguatoxin, choline, ciguatoxin, clupeotoxin, saurine, tetrodotoxin, tarichatoxin, chelonitoxin, etc.).

(iv) Mineral source

Mineral source includes various metal and nonmetal elements and their salts, compounds, etc. Metals and their compounds toxic to humans include heavy metals like mercury (Hg), manganese (Mn), lead (Pb), cadmium (Cd), nickel (Ni), and arsenic (As) and their compounds, beryllium oxide (BeO), and others. Chronic manganese exposure can damage the brain, resulting in a condition with symptoms similar to Parkinson's disease, such as slurred speech, masklike face, and rigidity. Mercury can also damage the brain, leading to behavioral changes. Methyl mercury

is especially toxic to the developing brain of a fetus. Mercury is also toxic to the peripheral nervous system, and to the kidney. Like mercury, lead is toxic to the nervous system and kidney. Lead tends to cause paralysis or weakness, indicative of peripheral nervous system damage and high level of lead intoxication results in severe brain damage and death. Irritation of the gastrointestinal tract is the major due to cadmium poisoning by ingestion causing nausea, vomiting, diarrhea, and abdominal cramps while chronic exposure causes kidney and lung damage. Arsenic compounds cause skin lesions, decrease in heart contractility, blood vessel damage, and injuries of the nervous system, kidney, and liver. Certain nickel and hexavalent chromium compounds, as well as beryllium oxide, are toxic to the lungs and can cause lung cancer. Mineral acid and alkali such as sulfuric (H_2SO_4) and hydrochloric acids (HCl), sodium hydroxide (NaOH), and potassium hydroxide (KOH) are corrosive to tissues on contact and can cause severe tissue injuries. Sulfuric acid, sodium hydroxide, and potassium hydroxide, etc., are active ingredients in drain cleaners, and their ingestion or spray can cause severe chemical burns of the mouth and esophagus or skin. Cyanide (CN) ions poison the oxidative metabolic machinery of cells so that insufficient energy is generated. Hydrogen sulfide (H_2S) and chlorine are highly toxic and irritating to the respiratory tract including pulmonary edema. Chronic fluoride (F) poisoning is called fluorosis, characterized by tooth mottling and increased bone density. (Si) and asbestos (a group of silicate minerals that share the same fibrous nature) remain in the lungs for long periods of time, and both produce lung fibrosis and asbestos, cause asbestosis, is a well-known human carcinogen. Sulfur dioxide (SO_2), an acidic gas, irritates the respiratory tract and causes violent coughing, shortness of breath, lung edema, and pneumonia. Ozone (O_3) and nitrogen oxides (NO_x), the oxidizing gases, cause respiratory irritation. Carbon monoxide (CO), an asphyxiating gas, binds to hemoglobin more strongly than oxygen, inhibits a hemoglobin molecule to carry its normal load of four oxygen molecules.

(b) Synthetic source

The majority agricultural and industrial chemicals are synthetic chemicals. The majority of agricultural chemicals include pesticides (e.g., insecticides, herbicides, fungicides, fumigants, and rodenticides) and plant growth regulators. Poisoning with agricultural and industrial chemicals occurs most often by either percutaneous, inhalation routes, or by ingestion.

The four main classes of insecticides are organophosphates (e.g., malathion, parathion); carbamates (e.g., carbaryl, carbofuran); chlorinated hydrocarbons (chlorophenothane—DDT, methoxychlor chlordecone—Kepone); and botanical insecticides derived from plants (natural) (e.g., pyrethrins, rotenone). Herbicides includes chlorophenoxyacetic acids (e.g., 2, 4-dichlorophenoxy-acetic acid—2, 4-D, 2,4,5-trichlorophenoxy-acetic—2,4,5-T); bipyridinium compounds (paraquat, diquat and others, e.g., e.g., diuron, monuron, atrazine, simazine, chlorpropham, alachlor). Fungicides are pentachlorophenol, creosote, ferbam, thiram; Fumigant nematocides include 1,2-dibromo-3-chloropropane—DBCP, ethylene dibromide and methyl

bromide; and Rodenticides include warfarin, strychnine and thallium). Daminozide —Alar is used as synthetic plant growth regulator.

Industrial chemicals refer to chemicals used neither in agriculture nor as drugs, it includes chemicals used in industry, as well as chemicals found in or near households. Industrial chemicals include Hydrocarbons (gasoline, toluene, xylene, hexanes, *n*-hexane, heptanes); Chlorinated hydrocarbons (chloroform, carbon tetrachloride, methylene chloride, and others); Alcohols (methanol, ethanol); Aldehydes (formaldehyde); various ketones, esters, aromatic amines and nitro compounds, anhydrides and isocyanates, and various inorganic compounds like polychlorinated biphenyls (PCB), polybrominated biphenyls (PBB), tetrachlorodibenzodioxin (TCDD), etc.

Organic compounds, e.g. hydrocarbons cause depression of the central nervous system and it is a common effect of most hydrocarbons. Hydrocarbons are lipid-soluble and dissolve in the membrane of nerve cells in the brain, perturbing their function and depression, such as drowsiness; x[occurs, as a result;] many of the hydrocarbons sensitize the heart to fibrillation by epinephrine. The hydrocarbon *n*-hexane also causes damage to peripheral nerves. Benzene is toxic to organs like the bone marrow that form blood cells and can lead to the production of leukemia.

Synthetic drugs and healthcare products may also cause poisoning and poisoning with drugs predominantly involves oral exposures and cause generally anorexia, nausea, vomiting, etc. resulting from gastrointestinal irritation, but irritation of the respiratory tract is rare.

Painkillers (analgesics) are the most commonly used drugs and account for many poisoning cases (e.g., aspirin, acetaminophen, morphine). Aspirin interferes with the oxidative burning of fuel by cells, causes dehydration and thirst, alters the pH in the body, and affects central nervous system and acetaminophen causes liver damage. Morphine causes nausea, vomiting, pinpoint pupil, depressed respiration, delusions, confusion, coma, and death.

Synthetic drugs include tranquilizers and sleeping pills (benzodiazepines such as diazepam, clonazepam, and chloridazepoxide, barbiturates, chloral hydrate, para-aldehyde, and meprobamate,); antipsychotic drugs (chlorpromazine, perphenazine, and haloperidol); nasal decongestants (ephedrine); antihistamines; cough medicine; antiseptics (hydrogen peroxide, benzoyl peroxide, resorcinol, benzalkonium chloride, parabens, and cetylpyridinium chloride); vitamins and iron pills (vitamin A, C, K, iron); antidepressants (tricyclic antidepressants—amitriptyline and imipramine, lithium salt); drugs of abuse (amphetamines, cocaine, phencyclidine, heroin, and methaqualone), cardiovascular drugs (digitalis e.g., digoxin and digitoxin, beta blockers (e.g., propanolol and metoprolol, verapamil, procainamide, quinidine); therapeutics for asthma (theophylline and aminophylline), etc.

The major toxicity from narcotic analgesics, like morphine, is depression of the central nervous system, especially the brain center controlling respiration. The cause of death in morphine overdoses is usually respiratory failure. Benzodiazepines have a wide margin of safety when used at prescribed doses, but barbiturates and others, the margin of safety is much narrower. Their major toxic effect is depression of the CNS leading to respiratory and cardiovascular failure.

Antipsychotic drugs rarely cause fatalities. They occasionally may block the action of the parasympathetic and sympathetic nervous systems and thus produce such undesired effects as dry mouth, blurred vision, drop in blood pressure, etc. Nasal decongestants, antihistamines, and cough medicine have a low potential to produce toxicity. Most antiseptics produce gastrointestinal irritation if ingested. Benzoyl peroxide and parabens applied to the skin may be toxic. Hexachlorophene, benzalkonium, and cetylpyridinium chloride, etc., are most toxic antiseptics and can cause injuries to internal organs. Hypervitaminosis A can result in skin lesions, edema, and liver damage. Chronic poisoning with vitamin A can cause neurological symptoms, including pain, anorexia, fatigue, and irritability. Hypervitaminosis C can lead to kidney stones and that of vitamin K increased destruction of red blood cells leading to anemia and the accumulation of bilirubin, and excess bilirubin can result in brain damage (kernicterus) in newborns. The toxicity of iron is a result of its corrosive action on the stomach and intestine leading to the development of shock. Some tricyclic antidepressants cause most of the fatal cases of poisoning.

Drugs of abuse (mind-altering drugs) are primarily toxic to the central nervous system; some (amphetamine and cocaine) cause hallucinations and delirium, and other (heroin) causes depressed respiration and coma; drowsiness, delirium and seizures (phencyclidine and methaqualone). Amphetamines also cause anorexia, nausea, vomiting, diarrhea and increased blood pressure and heart rate, palpitations, and abnormal heart rhythm. Cardiovascular drugs like digitalis (*e.g.*, digoxin) overdose usually cause anorexia, nausea, and vomiting, followed by pain and visual disturbances, and affect CNS, characterized by delirium and hallucinations. The major toxicities of beta blockers result from the blockage of sympathetic effects on the tracheobronchial tree (lung) and heart. Blockage produced by propranolol or metoprolol can cause bronchoconstriction and heart failure. Antiasthmatics drugs for treating asthma like caffeine stimulant also stimulate the central nervous system resulting excitement, delirium, rapid breathing, increased heart rate, and seizures with an overdose.

Classification of poisons (Fig. 6.4)

- (a) On the basis of origin poison may be (i) Natural poisons—they are of vegetable, animal or mineral origin and (ii) Synthetic poisons—they are derived from synthetic sources and include various types of chemicals.
- (b) In regard to poisoning, chemicals can be divided into three broad groups: (i) agricultural and industrial chemicals, (ii) drugs and healthcare products, and (iii) biological poisons—*i.e.*, plant and animal sources. There may be another group, the fourth group or category, (iv) ionizing radiation.
- (c) On the basis of the mode of action (clinical and forensic), poisons may be classified as (i) Corrosives—inorganic acids (hydrochloric acid, sulfuric acid), organic acids (carboxylic acid, oxalic acid, acetic acid, salicylic acid), vegetable acids (hydrocyanic) and alkalies (caustic soda, caustic potash, carbamates of sodium and potassium). (ii) Irritants—inorganic—metallic (arsenic, antimony, copper, lead, mercury, zinc, etc.) and nonmetallic (phosphorus, chloride, iodine, boron, etc.), organic—(plant origin—ergot, aloe, capsicum, and castor seed,

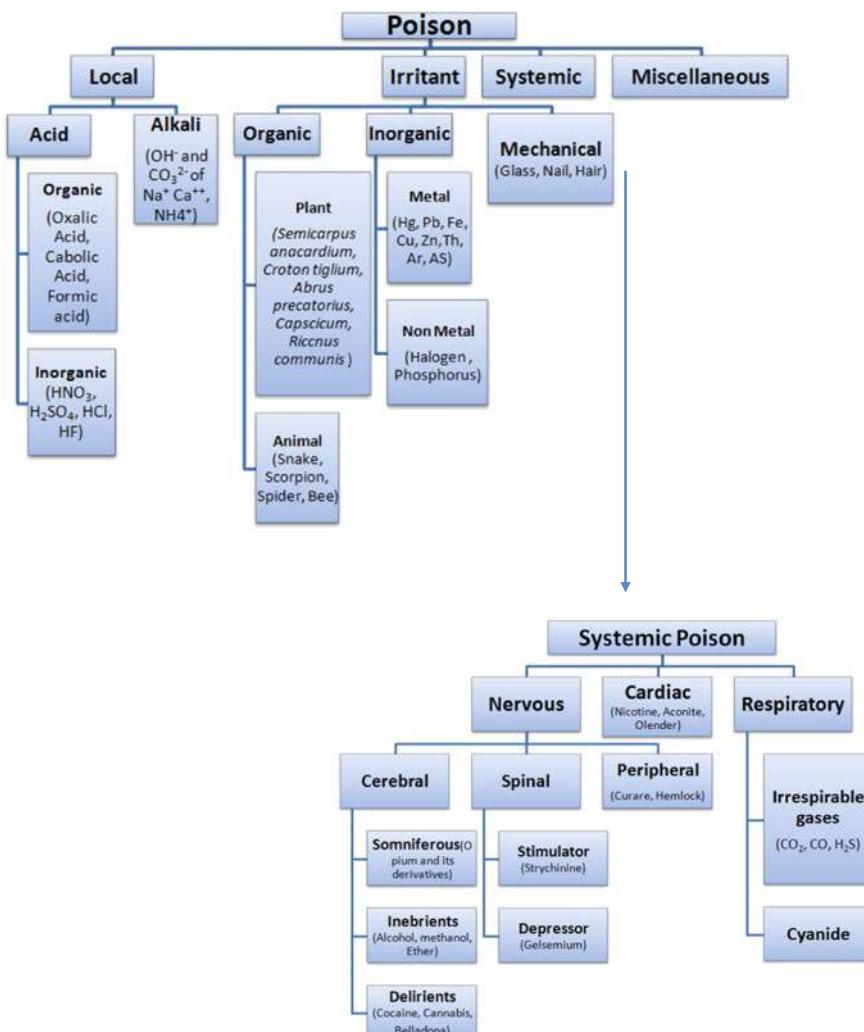


Fig. 6.4 Classification of poisons

castor oil, croton oil, etc., animal origin—venom of snake, scorpion, spiders and insects, etc.) and mechanical (coarsely powdered glass, chopped hair, dried sponge, diamond dust, etc.). (iii) Neurotoxin poisons—cerebral—somniferous—opium and its alkaloids, cinebriants, deleriants—datura, belladonna, hyoscyamus niger, cannabis indica, psychotropics inebriant—alcohol, anesthetics, sedatives, fuels, insecticides; spinal—excitatory, nuxvomica, depressant, and peripheral—sedative, hypnotics, cocaine, and curare. (iv) Cardiovascular poisons—antihypertensive, anticoagulants, cardiotoxic and asphyxiант poisons (digitalis, strophantus, aconite, oleander, nicotine, etc.).

(v) Respiratory depressants—poisonous gases like carbon mono oxide (CO), coal gas, etc. (vi) Miscellaneous poisons—antiphryretics poisons; nonsteroidal anti-inflammatory drugs; antihistamines; antiinfectives; hypoglycemic agents; food poisoning; and odds and ends (analgesics—aspirin, paracetamol, antipyretics, antihistamines, Stimulants—amphetamine and antidepressants—tricyclic compounds, hallucinogens may produce toxicity when consumed in higher doses and finally results in patient's death).

Radiation as poison: Radiation, radioactivity, and radioisotopes; their classification and effects

Radiation is a flow of energy through space or matter in the form of particles (e.g., alpha and beta particles) or electromagnetic waves (e.g., X-rays, gamma rays, ultraviolet-UV and visible light).

Radiation sources

Radiation is either natural or man-made. Natural radiation includes cosmic radiation, terrestrial radiation, radioisotopes inside human bodies, and radon gas. Cosmic radiation consists of charged particles from outer space, and terrestrial radiation of gamma rays from radionuclides in the Earth. Radioisotopes in human bodies come from the food, water, and air consumed. Cosmic and terrestrial radiation, together with radioisotopes inside human bodies, contribute to only one-third of the total natural radiation dose. The remaining two-thirds can be attributed to radon, a radioactive gas released from soil that may reach a high level inside buildings with poor ventilation. Man-made radiation consists of radiation from medical and dental diagnostic procedures, atmospheric tests of atomic bombs, emissions from nuclear plants, certain occupational activities, and some consumer products. The largest nonoccupational radiation sources are tobacco smoke for smokers and indoor radon gas for the nonsmoking population.

Classification and effects of radiation

Radiation can be classified as either ionizing or nonionizing depending on its ability to produce ions in the matter it interacts with. Ionizing radiation is more toxic than nonionizing radiation. Radioactivity is the emission of radiation caused by the disintegration of unstable nuclei of radioisotopes. After disintegration, a radioisotope may become a radioisotope of another element, which will further disintegrate. The disintegration series continues until a stable isotope is formed.

There are two classes of ionizing radiation: particulate (alpha particles, beta particles, neutrons, and positrons) and electromagnetic (Gamma rays and X-rays) ionizing radiation and electromagnetic (Gamma rays and X-rays) ionizing radiation. The toxic effect of ionizing radiation is related to the ionization, during ionization of tissues, mainly of water decomposes to generates H_2O^+ (by leaving one electron) and H_2O^- (by taking one electron) ions, which in turn dissociates almost immediately (1^{-16} seconds) into H^+ ions and OH radicals as well as OH^- ions and H radical, respectively. All these species with unpaired electrons are very reactive chemically to cause biological damage to DNA and proteins.

Alpha and beta particles are the most common in the environment and are biologically the most significant. Alpha particles, each consisting of 2 neutrons and 2 protons and thus a 2^+ charge, are the heaviest ionizing particles, do not penetrate tissue very well, turn many atoms in their short paths into ions, and thus produce intense tissue ionization. In contrast, beta particles are electrons of little mass, carrying 1 negative charge, penetrate well in soft tissues, but due to their low mass and low charge bring about only moderate ionization when they collide with atoms in their path. Gamma rays and X-rays are electromagnetic radiation of similar properties without any charge or mass, but gamma rays possess higher energy than X-rays. They can penetrate tissues easily creating moderate ionization along their paths. Biological damage is related to the degree of tissue ionization produced by radiation.

Ionizing radiation quickly kills rapidly dividing cells. In general, immature blood cells in bone marrow, cells lining the mucosa of the gastrointestinal tract, and cells in the lower layers of the epidermis and in hair follicles are the most rapidly dividing cells in the body. As a result, radiation leads to the decreased production of blood cells, nausea, vomiting, diarrhea, malabsorption by the intestine, skin burns, and hair loss. Because of its relatively selective lethal effect on rapidly dividing cells, however, ionizing radiation is used in the treatment of certain cancers. Some cells in the embryo and fetus also divide rapidly, and thus ionizing radiation can cause malformations and even fetal death. Ionizing radiation can also produce mutations by altering the DNA, and it can result in cancer.

Nonionizing radiation includes UV radiation, infrared radiation, microwaves, and radio frequencies, all of which are electromagnetic waves. The toxicity of radio frequencies is rather low. On the whole, nonionizing radiation is not as toxic as ionizing radiation, and the various forms of nonionization radiation share common target organs; particularly the skin and eyes.

The toxicity of ultraviolet light depends on its wavelength, e.g., UV-A (315–400 nm), UV-B (280–315 nm), UV-C (200–280 nm). UV-B is the major component of sunlight and accelerates the aging of skin by damaging the collagen fibers. UV-A affects primarily the skin and causes burns at high energy levels, toxicities of UV-B and UV-C are similar cause injuries to the eyes and skin, but UV-C is less toxic because it does not penetrate tissues as deeply. UV-B can cause skin cancer, which may be a result of the linking of thymidines, a base in DNA, produced by ultraviolet-B radiation. The major mechanism of toxicity of infrared radiation and microwaves is the production of heat in tissues. Lasers are high-energy light beams, visible and nonvisible, generated by atoms at an excited state and further amplified by optics. Like most other nonionizing radiation, lasers can produce skin burns.

Application of poisons

Poisonous compounds may be useful either for their toxicity as pesticides in agriculture or as reagents in chemical industry. Poisons are widely used in industry as chemical reagents, solvents or complexing reagents, e.g., carbon monoxide, methanol and sodium cyanide, respectively. They are less common in household use, with occasional exceptions such as ammonia and methanol.

Mode of action of poisons

- (i) **Local effect**—Some poisons exert their effects on the part they come in contact with, e.g., Corrosives (cause chemical destruction), atropine (produces mydriasis when applied into the eyes), aconite (cause of tingling and numbness of nerve ending), etc.
- (ii) **Systemic effect**—Some poisons exert their effect on one or more organ systems after absorption into the systemic circulation, e.g., opiates on the CNS, strychnine on the spinal cord, digitalis on heart, heavy metals on multiple systems, etc.
- (iii) **Combined effect**—Some poisons have both local and systemic effects, e.g., carbolic and oxalic acids, phosphorus, etc.

Symptoms of poisoning

General symptoms of poisoning include (i) sick feeling, (ii) diarrhea, (iii) stomach pain, (iv) drowsiness, dizziness or weakness, (v) high temperature (38°C , 100.4°F or above), (vi) chills (shivering), (vii) loss of appetite, (viii) headache, etc.

6.2 Hallucinogens and Teratogens—Their Sources, Classification, Chemistry, Mode of Action and Application

Hallucinogens

Hallucinogens are drugs that cause hallucinations, a visual terminology, when users see images, hear sounds, and feel sensations that seem very real but do not exist, i.e., distortions in perceptions of reality. They include a diverse group of drugs that cause an alteration in perception, thought, or mood and mescaline, psilocybin, ibogaine, LSD (lysergic acid diethylamide), etc., are some of the examples of common hallucinogen drugs. Hallucinogens cause their effects by disrupting the interaction of nerve cells and the neurotransmitter serotonin. A hallucinogen is a psychoactive agent and causes hallucinations, perception anomalies, and other substantial subjective changes in thoughts, emotion, and consciousness. Hallucinations are typically caused by psychedelics, dissociatives, or deliriants. Opioids and similar other psychoactive drugs are not explicitly hallucinogens because their psychoactivity is devoid of visual anomalies. Hallucinogenic drugs are characterized by (i) predominating changes in thought, perception, and mood over other effects; (ii) minimal intellectual or memory impairment; (iii) stupor, narcosis, excessive stimulation etc. are integral effects; (iv) minimal autonomic nervous system side effects; and (v) absence of addictive craving. Most hallucinogens are alkaloids and may be smoked or snuffed, swallowed fresh or dried, drunk in decoctions and infusions, absorbed directly through the skin, placed in wounds or administered as enemas.

Mescaline is derived from the peyote cactus (*Lophophora williamsii*), psilocin and psilocybin are found in about 100 varieties of mushrooms, and dimethyltryptamine (DMT) is found in a variety of seeds and plants. Solanaceous belladonna (*Atropa belladonna*), henbane (*Hyoscyamus niger*), mandrake (*Mandragora officinarum*), and datura (*Datura metel*) are examples of some other hallucinogens. They are topically active, i.e., they are absorbed directly through the skin. The hallucinogenic (psychoactive) species are widely distributed throughout the plant kingdom, but appear to be more prevalent in fungi and angiosperms and less frequent in bacteria, algae, lichens, bryophytes, ferns, and gymnosperms (Schultes 1969–1970). The seeds of many species of morning glory (e.g., *Ipomoea tricolor*) contain ergoline alkaloids such as the psychedelic ergonovine and ergine—D-lysergic acid amide (LSA), their effects are similar to that of LSD.

Classification of hallucinogens

The hallucinogenic properties can be ascribed to only a few kinds of organic constituents and they may be conveniently divided into two broad groups such as (a) nitrogenous and (b) non-nitrogenous compounds.

The nitrogenous compounds play greater role and comprise mostly of alkaloids or related substances such as (i) 3-carbolines, (ii) ergolines, (iii) indoles, (iv) isoquinolines, (v) isoxazoles, (vi) 3-phenylethylamines, (vii) quinolizidines, (viii) tropanes, and (ix) tryptamines. The non-nitrogenous hallucinogen compounds include (i) dibenzopyrans and (ii) phenylpropenes as well as other compounds such as catechols, alcohols, etc.

The hallucinogens may also be grouped into three major categories such as (i) psychedelics, (ii) dissociatives, and (iii) delirants. The psychedelics are further divided into three subgroups such as cannabinoids, empathogens, and serotonergics and they include cannabis (marijuana), methylenedioxymethamphetamine (MDMA), lysergic acid diethylamide (LSD), psilocybin mushrooms, etc.; the dissociative hallucinogens includes ibogaine, phencyclidine (PCP), ketamine, dextromethorphan (DXM), etc.; and the delirants include atropine, scopolamine (hyoscine), diphenhydramine (benadryl); etc.

Some principal components of cannabinoids from *Cannabis sativa* are cannabinol, tetrahydrocannabinol (THC), cannabidiol (CBD), cannabidiol-carboxylic acid, cannabigerol, and cannabichromene (Fig. 6.5).

Some of the poisonous fungi including toadstools of the genera *Amanita*, *Psilocybe*, and *Conocybe* produce extremely toxic chemicals that promote hallucinogenic effects, e.g., amatoxins. Three recognized classes of toxins are tryptamines (e.g., bufotenine), cyclic peptides (e.g., phallotoxins and amatoxins) and isoxazole alkaloids (e.g., ibotenic acid). Figure 6.6 shows the structure of toxic and hallucinogenic principles of *Amanitas* fungus.

A small spineless cactus—peyote cactus (e.g., *Lophophora williamsii*) that grows in the Southwestern part of the United States and Mexico has been used since long past by Mexican Indians for its pharmaceutical and pharmacological importance for its psychoactive alkaloids, particularly mescaline and others with marked hallucinogenic properties (Fig. 6.7).

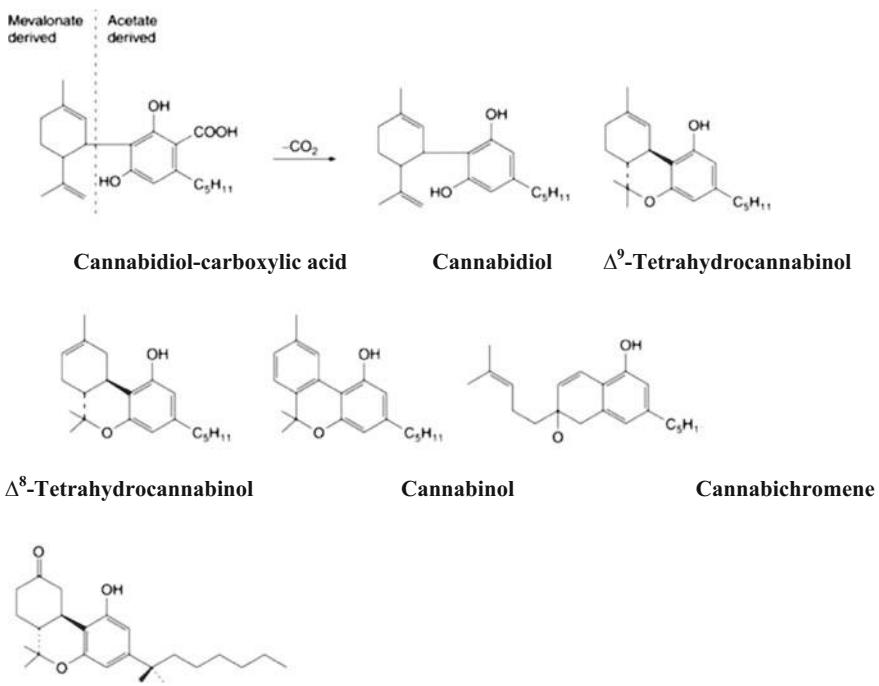


Fig. 6.5 Structure of principal cannabinoids of *Cannabis sativa* and synthetic analog-cannabidiol, Δ^9 -tetrahydrocannabinol, Δ^8 -tetrahydrocannabinol, cannabinol, cannabichromene, nabilone (a synthetic THC analog)

Teratogens

Teratogens are agents that can affect the development of an embryo or fetus. Teratogens may halt the pregnancy outright or may cause abnormalities (birth defect) in an exposed fetus. The effects depend on the nature of the teratogen, the timing at which the exposure occurs and, most likely, the genetic susceptibility of the mother and/or the fetus. Birth defect is a significant problem in livestock as well as in humans; causes are environmental rather than hereditary. The classes of teratogens include radiation (atomic weapons and radioiodine), maternal infections (cytomegalovirus, herpes virus, syphilis, *Toxoplasma*, and rubella virus), maternal metabolic factors (alcoholism, diabetes, folic acid deficiency, and endemic cretinism), exposure to 2,4-D spraying, chemicals and drugs (aminopterin, busulfan, cocaine, coumarin anticoagulants, cyclophosphamide, lithium, mercury, thalidomide, and retinoic acid). Teratogenic drugs like ACE inhibitors (benazepril, captopril, enalapril, fosinopril sodium, lisinopril, lisinopril, quinapril ramipril, etc.); the acne medication isotretinoin (accutane, retin-A); alcohol; androgens (male hormones); antibiotics (tetracycline, doxycycline, streptomycin, etc.); blood-thinners

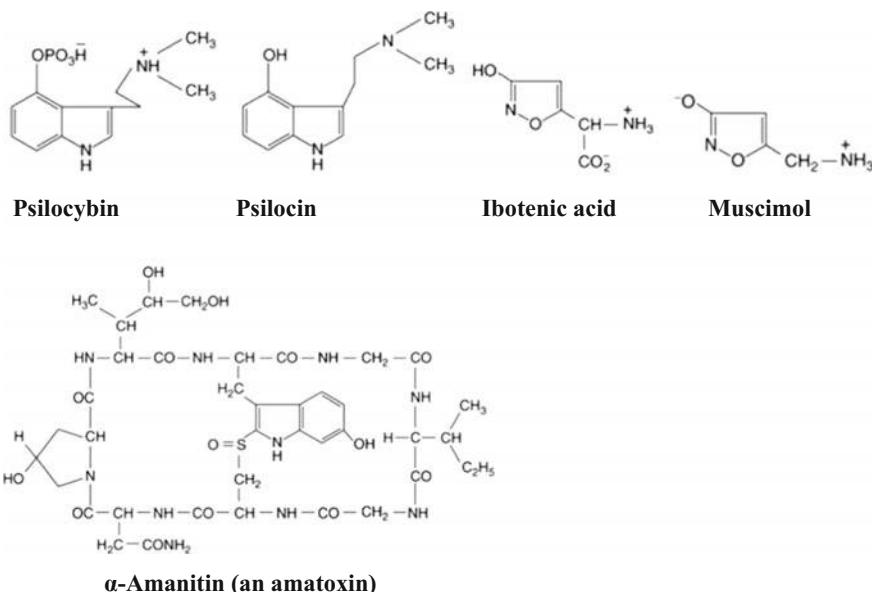


Fig. 6.6 The structure of toxic and hallucinogenic principles of *Amanitas* fungus—psilocybin, psilocin, ibotenic acid, muscimol, α -amanitin (an amatoxin)

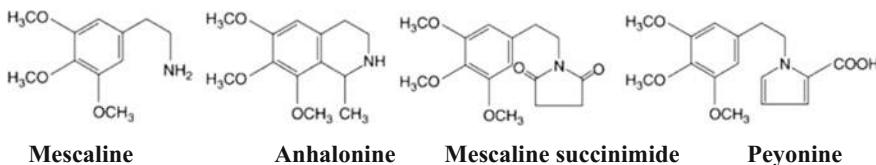


Fig. 6.7 Structure of the representative alkaloids of peyote—mescaline, anhalonine, mescaline succinimide, peyonine

(warfarin), etc., are just a few examples. Lack of nutrients, e.g., lack of folic acid in the nutrition in pregnancy for humans can result in spina bifida.

Many compounds synthesized by plants are known to be teratogenic in laboratory animals, but only a few have been shown by feeding trials to produce terata in livestock. Several teratogens are known definitely from teratogenic plants of *Lupinus*, *Veratrum*, *Conium* and *Leucaena* genera; several plants of *Astragalus*, *Nicotiana* and *Trachymene* genera are teratogenic with unidentified teratogens while several plants of *Datura*, *Prunus*, *Sorghum* and *Senecio* genera are suspected teratogenic plants (Keeler 1984).

Teratogenic agents can be environmental chemicals, maternal metabolic factors, drugs, or infections. A number of environmental chemicals including lead, methyl mercury, and polychlorinated biphenyls have been linked with birth defects in

exposed fetuses. Maternal metabolic factors associated with a significant risk of birth defects are maternal hyperglycemia and maternal phenylketonuria. Excessive alcohol intake in pregnancy has been linked with fetal growth retardation, microcephaly, and cardiac and other malformations. Many prescribed drugs can act as teratogens including some anticonvulsant agents, lithium, androgens, retinoids, and misoprostol.

Many other diverse groups of compounds, e.g., vitamin D, quinine, anagyrine and other alkaloids aspirin, marijuana cannabinoids, etc., have shown some evidence of teratogenicity in laboratory rodents and some of these compounds are synthesized by different plants of the genera *Lupinus*, *Veratrum*, *Conium*, *Astragalus*, *Nicotiana*, *Trachymene*, *Datura*, *Prunus*, *Sorghum*, *Senecio*, etc. Plants that cause congenital defects include *Lupinus*, *Lathyrus*, *Leucaena*, *Nicotiana*, *Conium*, *Astragalus*, *Oxytropis*, *Veratrum*, *Vicia*, *Salsola* spp., etc. Figure 6.8 shows the structure of teratogens—isotretinoin, piperidine and quinolizidine alkaloid teratogens found in lupines, poison-hemlock.

6.3 Pesticides—Their Sources, Classification, Chemistry Mode of Action and Application

Pesticides

A pesticide is a chemical used to prevent, destroy, or repel pests. A pesticide is usually toxic substance and kills animal or plant pests that cause economic damage to crop, ornamental plants and domestic. Pests include insects, termites, nematodes, molluscs, mice and other rodents, weeds, fungi and microorganisms including bacteria and viruses. All pesticides interfere with normal metabolic processes in the pest organisms and often are classified according to the type of organism they are intended to control, viz. insecticides, molluscacides, nematocides, rodenticides, fungicides, herbicides, and fumigants.

Weedicides

Any undesirable plant is known as weed. A weed may be a dandelion in a lawn, a thistle plant (Gokhru) in a vegetable garden, or mustard in a clove field. Undesirable plants in gardens interfere in the growth of cultivated plants by consuming most of the available water contents and minerals of the soil. If weeds are allowed to grow, they will soon acquire the possession of the garden and gradually destroy the more delicate, cultivated plants. Similarly, the quality of the field crops, especially grains, becomes poor due to presence of weed seeds. Weeds exert allelopathic interference on crops with the chemicals they secrete and, in addition, many of the weeds contain toxins, allergens, etc.

Corn cockle, *Agrostemma githago*, contains a cyanophore type of glycoside, and its seeds cause death when they are present in excessive quantities in wheat flour. A large number of plants give rise to allergic reactions in certain individuals; produce an antigen-antibody reaction which results in the liberation of histamine or

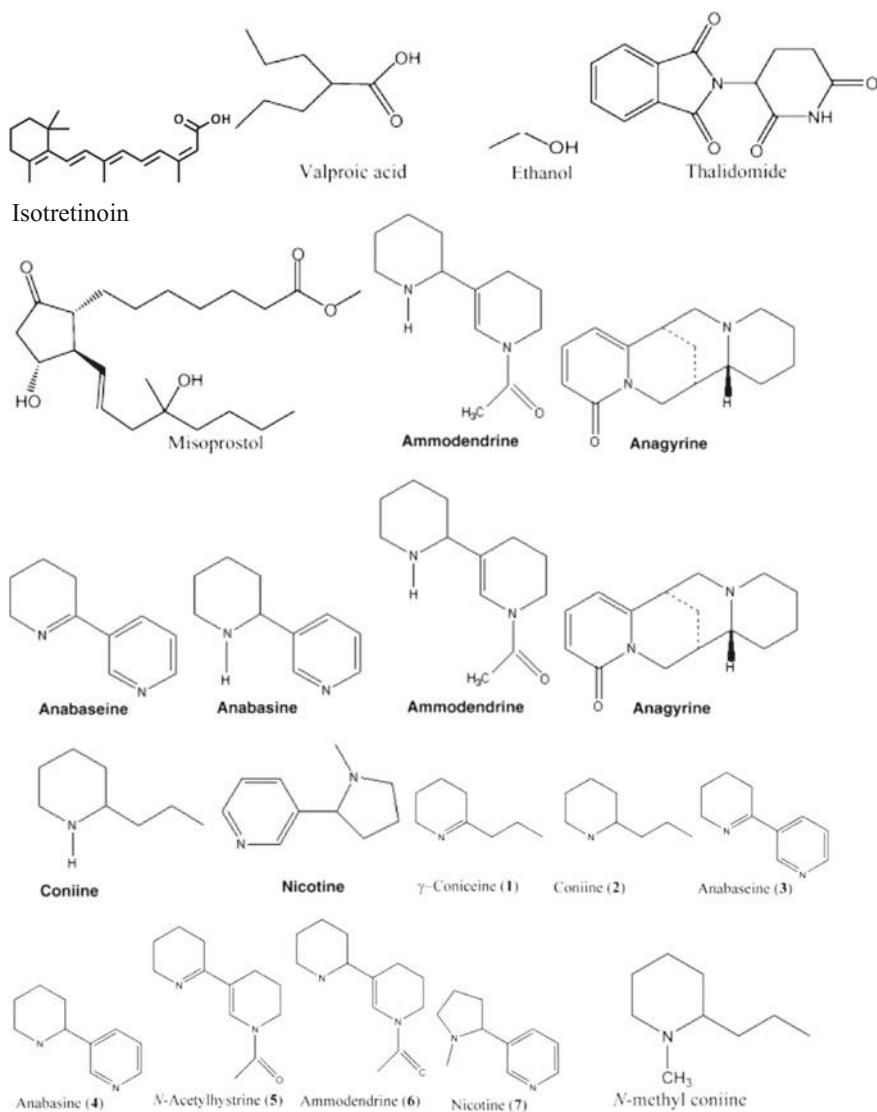


Fig. 6.8 Structure of teratogens—isotretinoin, piperidine and quinolizidine alkaloid teratogens

identical compounds causing allergic symptoms. Allergies are commonly asthma and dermatitis. Pollens of grasses like timothy (*Phleum pratense*), cocks foot (*Dactylis glomerata*) and perennial rye (*Lolium perenne*) as well as that of nettle (*Urtica dioica*), plantain (*Plantago* spp.) and ragweeds (*Ambrosia* spp.) is responsible for seasonal hay fever. A number of common moulds produce spores which cause rhinitis and asthma in sensitive individuals. *Rhus* spp. like *Rhus radicans* (poison ivy), *Rhus toxicodendron* (poison oak), *Rhus diversiloba* (Pacific poison

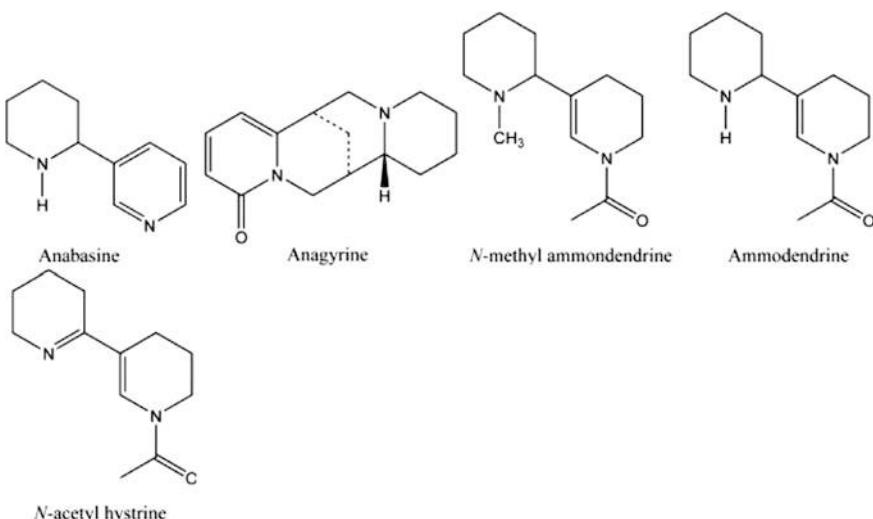


Fig. 6.8 (continued)

oak) and *R. venix* (poison elder) (fam. Anacardiaceae) contain allergens which produce severe dermatitis associated with watery blisters. Sesquiterpene lactones from the species of Asteraceae, Lauraceae and Magnoliaceae and from the Liverwort *Fruifoma* (Fam. Jubulaceae) are a major class of compounds causing allergic contact dermatitis in human. The fruits and seeds of *Menispermum canadense* and *Datura stramonium* are poisonous when swallowed. Some of the poisonous fungi when taken orally produce hallucinations. The examples are *Amanito*, *Psilocybe*, and *Conocybe*. Certain cacti contain protoalkaloids, some of which have marked hallucinogenic properties.

Natural contact insecticides

Leaf tobacco: It consists of the cured and dried leaves of the Virginia tobacco plant, *Nicotiana tabacum* (Fam. Solanaceae). The genus *Nicotiana* is comprised of about 100 species. *Nicotiana tabacum* is a tall annual herb indigenous to tropical America and widely cultivated. The stem is simple, bearing large, pubescent, ovate, entire, decurrent leaves, the veins of which are prominent and more or less hairy.

Nicotine (0.6–9%) is the characteristic alkaloid of the genus and is prepared commercially from waste material of the tobacco industry. A lesser amount of nornicotine and an aromatic compound, nicotianin, or tobacco camphor are also present in the herb. The characteristic flavor is due to the nicotianin which is formed during the curing of the leaves. The roots of *N. tabacum* contain about eight pyridine alkaloids, including nicotine, nornicotine, anabasine, and anatabine.

Nicotine is a pyridine-type alkaloid which is pale yellow, oily liquid, very hygroscopic; turns brown on exposure to air or light; acrid burning taste; develops odor of pyridine; volatile with steam. It forms salts with almost any acid and double

salt with many metals and acids. It is miscible with water below 60 °C, very soluble in alcohol, chloroform, ether, petroleum ether, etc. It is poisonous, being a local irritant and paralyzent.

Nicotine is used as insecticide and fumigant. As a contact poison, it is most effective as soap, i.e., as the laurate, I oleate, or naphthenate. As a stomach poison, a combination with bentonite has come into use. Nicotine sulfate in a 40% solution (Black leaf 40) is quite toxic to aphids; if the solution is alkalized, the toxicity is increased. Soap solution decomposes the sulfate to the free alkaloid which is considerably more poisonous to the insects. Nicotine is highly toxic. The symptoms include extreme, nausea, vomiting, evacuation of bowel and bladder, mental confusion, twitching and convulsions. The base is readily absorbed through mucous membranes and intact skin, but the salts are not.

Pyrethrum flowers (Synonyms-Pyrethrum Flower Heads, or Insect Flowers, Dalmatian insect powder; Persian insect powder). These are the dried flower heads of *Chrysanthemum cinerariaefolium* or of *C. marschallii* (Fam. Compositae). Pyrethrum contains about 0.5% of total pyrethrins (Pyrethrin I and Pyrethrin II).

Pyrethrum flowers are collected from 2 to 6 years old plants by hand. They are dried and stored. The plant is widely grown in Kenya, Ecuador, Japan, Yugoslavia, east central Africa, Brazil, and India.

The insecticidal activity of Pyrethrum arises from four esters, the pyrethrins I and II and the cinerins I and II. They are complex esters of chrysanthemum carboxylic acid and the monomethyl ester of chrysanthemum dicarboxylic acid with pyrethrolones and cinerolones. The pyrethroids (or rethroids) are synthetic compounds of a similar structure of the pyrethrins themselves. The most important pyrethroids are allethrin, furethrin, and cyclethrin.

The Pyrethrum flowers are a contact poison for insects. They are largely used in the form of powder, but sprays in which the active principles are dissolved in kerosene or other organic solvent. It can cause severe allergic dermatitis and systemic allergic reactions. Large amounts may cause nausea, vomiting, tinnitus, headaches, and other CNS disturbances.

Derris and Lonchocarpus: The roots of many species of Derris and Lonchocarpus (Fam. Leguminosae) show insecticidal properties. Derris consists of the dried rhizome and roots of *Derris elliptica*, *D. malaccensis* and possibly other species. Lonchocarpus are the dried roots of *Lonchocarpus utilis*, *L. itrucu* and some other species.

Derris is native of Malaya and cultivated there and in Burma, Thailand, Malaysia, Indonesia, and the Philippine Islands. The genus Lonchocarpus is grown mainly in Mexico, Central and South America, England, Africa, and Australia.

These roots contain rotenone (3–10%), deguelin, toxicarol, or tephrosin. Rotenone is a colorless crystalline substance which is insoluble in water but soluble in many organic solvents. All these compounds show insecticide properties. It is an insecticide which is widely used to control both chewing and sucking insects.

Derris and Lonchocarpus roots have been used as fish poisons. For dusting purposes, the powdered root is finally ground and diluted with a suitable carrier

(talc, clay) to a concentration of 1%. For spray purposes the powdered roots may be mixed with water or preferably with organic solvents such as ethylene dichloride, trichloroethylene, or chlorobenzene. Rotenone extracts with oil and emulsifying agents and extracts dissolved in paraffin oil are excellent household and cattle sprays. Rotenone decomposes upon exposure. Inhalation or ingestion of large doses may cause numbness of oral mucous membrane, nausea, vomiting, muscle tremors, and tachypnea.

Cevadilla seed (or Sabadilla): It consists of the seeds of *Schoenocaulon officinale* (Fam. Liliaceae), a plant found from Mexico to Venezuela. The seeds are dark brown to black, sharply pointed and about 6 mm long. The seeds contain veratrine alkaloids (2–4%) which is a mixture of cevadine (veratrine), veratridine, sabadilline (cevadilline), sabadine, sabadinine and sabatrine. The powdered seeds and preparations of “Veratrine” are used as dust or spray to control thrips and various true bugs which attack vegetables.

Ryania—the roots and stems of *Ryania speciosa* (Fam. Iacourtiaceae) contain 0.16–0.2% of alkaloids having insecticidal properties. Ryanodine, the principal alkaloid is a complex ester involving 1-fivrrolf-carboxylic acid. The plant is used in the control of various Lepidopterous larvae which attack fruits and particularly European corn borer, codling moth, and sugarcane borer. It may be used as a dust made from—a 40% I extract. Due to its low-toxicity, Ryania has no residue hazard.

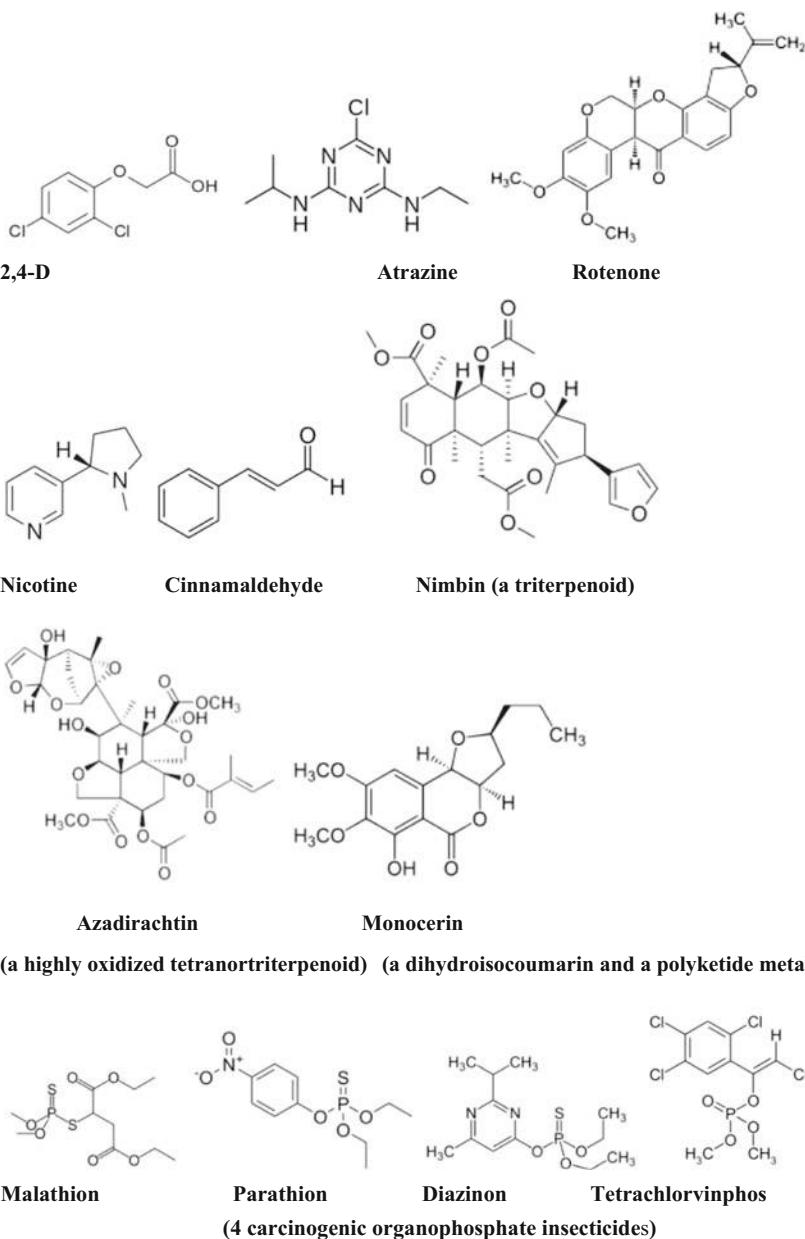
Repellents

The natural product Citronella oil has been used as a mean of preventing insect attack. The synthetic products include dimethyl phthalate, Ethohexadiol (Rutgers 612) and Butopyronoxyl. These compounds are mixed in Dimethyl phthalate in ratio of Dimethyl phthalate (6 parts), Ethohexadiol (2 parts) and Butopyronoxyl (2 parts), a synonym for this solution is 622 mixture. Diethyltoluamide is another effective insect repellent.

Herbicides

Plant growth regulators—the natural plant growth-promoting substance, gibberellin acid, is obtained from the fungus *Gibberella fujikurai* (Sawada). Six gibberellins, A₁, A₂, A₃, A₄, A₇, and A₉, have been isolated from filtrates of the fungus.

Chemically, gibberellins are the tetracyclic diterpenes. They are more highly functionalized than other groups of terpenoids. These compounds are produced in minute quantities within plants where they act as hormones of various developmental processes. Gibberellin-like compounds occur in higher plants. They are responsible for the development, maturation, budding, flower formation, fruit ripening, and various other growth processes. Substances like 2,4-D and 2,4,5-T also possess auxin-like activity, but they are more effective as herbicides. Figure 6.9 shows the structure of different pesticides.

**Fig. 6.9** Structure of different pesticides

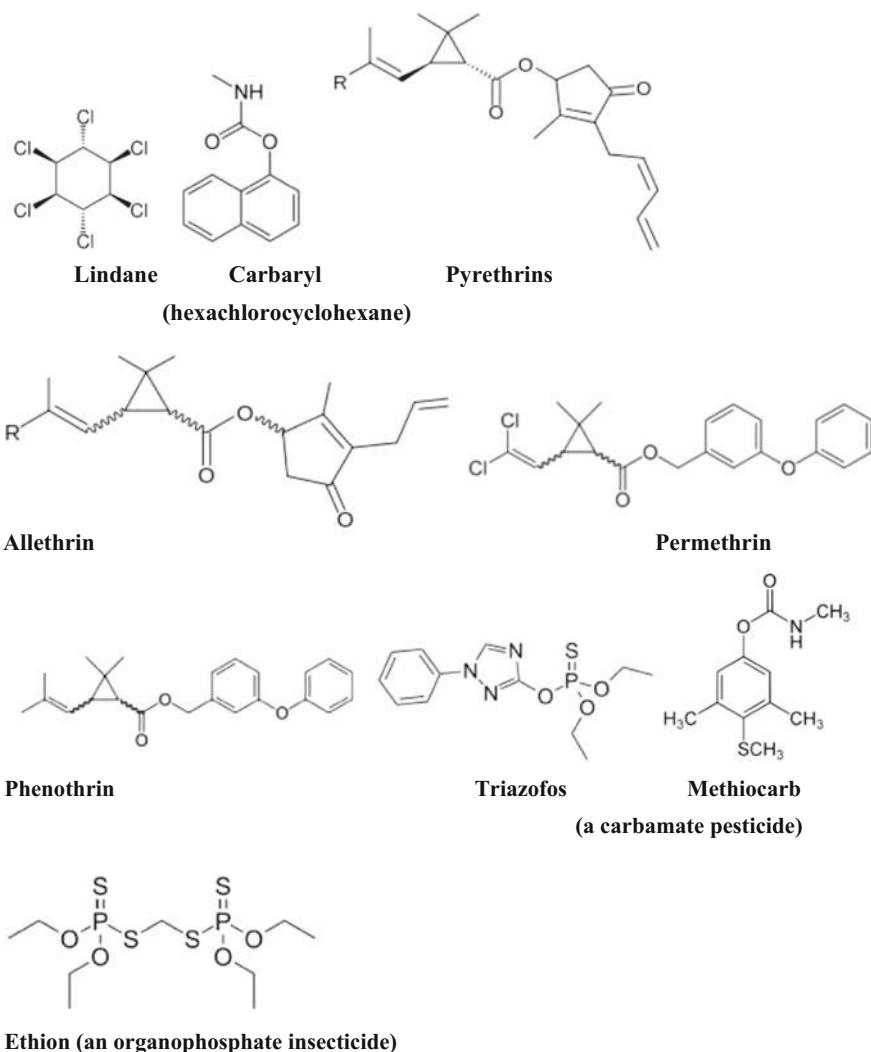


Fig. 6.9 (continued)

6.4 Xenobiotics—Their Sources, Classification, Chemistry and Metabolism

Biogenous and xenobiotic drugs and their metabolism

All medicinal drugs are divided with respect to human organism into following two groups: (i) Autobiogenous or natural, and (ii) Xenobiotic or foreign.

Autobiogenous or natural drugs are native products of the organism that are involved in biochemical processes occurring in the organism. Normally, xenobiotics do not occur in the human organism, or are found there in trace amounts. Xenobiotics are synthetic compounds or substances that are supplied from other organisms (chiefly microorganism and plants). Viewed formally, the substances that are vitamin for the human organism may be likened to foreign substances; none the less, while not being products of the human organism, they are essential for the accomplishment of its biochemical reactions. For this reason, such substances should be assigned to natural preparations.

Biogenous drugs or substances of medicinal value, as distinct from xenobiotics, are involved in the conventional metabolic process. Xenobiotics, in the course of their conversion, are subject to two major stages: (i) modification (nonsynthetic stage), and (ii) conjugation (synthetic stage).

In fact, the metabolism of biogenous substances and xenobiotics used as drugs is governed by the laws of enzyme kinetics. Biogenous substances, being natural substrates for enzymes, are converted at the rate characteristic of catalytic properties of the enzymes involved. The metabolic conversion of xenobiotics is dependent on the occurrence of enzymes capable of catalyzing the conversion of these xenobiotics. In the absence of enzymes, the xenobiotics behave as metabolically inert. Enzymes that catalyze the conversion of xenobiotic generally exhibit a less specificity. The drug metabolism in the organism may be represented within the frame work of a general scheme:



The drug metabolism is studied by determining the drugs and their metabolites in biological fluids, tissues and excretions and also by estimating the activity and kinetics of enzymes involved in the drug metabolism. The modification stage is an enzyme associated modification of the initial structure of a xenobiotic resulting either in a cleavage of bonds within the xenobiotic molecule, or in the insertion of additional functional groups (e.g., hydroxyl or amino groups) into its molecule or in a release of its functional groups blocked in the initial structure (e.g., by hydrolysis of ester or peptide bonds). The modification leads increased solubility of the xenobiotic (xenobiotic becomes more hydrophilic). Additional functional groups are needed to enable the xenobiotic to enter the conjugation stage. The conjugation stage is viewed as an enzyme-assisted process for building covalent bonds between the xenobiotic and biomolecules occurring in the organism's media (e.g., glucuronic acid, sulfates, etc.). Conjugation stage terminates in the synthesis of novel compound whose constituents are partly xenobiotic moiety and partly a conjugate (biomolecule).

Any novel drug proposed as a definite medicinal form, e.g., tablets, pills, powders, capsules, extracts, emulsion, solution, etc., requires a comprehensive investigation from the stand point of its effect on the organism.

Metabolism of xenobiotics and poisons

All the substances supplied to the organism in a variety of ways pass through several basically similar stages such as absorption, distribution (mechanical transport), and excretion. The transit rate of substance at these stages may either be increased, or lowered, depending on the structural features and physicochemical properties of a substance as well as on its affinity to biological molecules. The discipline, dealing with rate characteristics at the stages in which any substance entering the organism is involved, is referred to as chemobiokinetics which treats, in a broader sense, movements of substances in the living organism. Conceptually, chemobiokinetics is divided into three subdisciplines: pharmacokinetics, toxicokinetics, and biokinetics. Pharmacokinetics confines itself to the study of drugs; toxicokinetics, to the study of toxic substances; and biokinetics, to the study of substances not alien to the organism. In many respects, this classification is rather arbitrary, since the distinction between a drug and a poison in many instances may be evasive. Moreover, even autogenous compounds taken in improper doses may exhibit toxic properties.

The subsequent history of a substance after its uptake by the organism is dependent to a significant degree on the rates at which it is converted by various engines, i.e., on its metabolic transformations. In point of fact, the metabolism of biogenous substance and xenobiotics used as drugs is governed by the laws of enzymic kinetics. Biogenous substances, being natural substrates for enzymes, are converted at the rates characteristic of catalytic properties of the enzymes involved. The metabolic evolution of xenobiotics is dependent on the occurrence of enzymes capable of catalyzing the conversion of these xenobiotics. If no enzymes that are potentially capable of catalytic intervention of the xenobiotics are available, the xenobiotics behave as met a helically inert. Their history in the organism is therefore chronicled only in terms of absorption, transport, and excretion. Enzymes that catalyze the conversion of xenobiotics must, exhibit a low specificity toward an intrinsic substrate, since, viewed in this light, the latter is classified among foreign substances. Apparently, in the course of evolution, highly substrate specificity enzymes have laid a basis for the intrinsic metabolism in living organisms, while the enzymes with low specificity toward substrates have taken up defense functions aimed at the inactivation of extraneous invaders.

Biochemistry studies enzyme-assisted conversions of drugs in the organism by making use of appropriate methods and techniques. The drug metabolism is studied by determining the drugs and their metabolites in biological fluids, tissues, and excretions as well as by estimating the activity and kinetics of enzymes involved in the drug metabolism. Experimentally, the two approaches are used in the studies on metabolism of xenobiotics. In the clinic, the drug metabolism is assessed, as a rule, by measuring the concentration of administered drug and its metabolites in blood, urine, and other excretions.

Stages in the metabolism of xenobiotics

Biogenous substances, as distinct from xenobiotics, are involved in the conventional metabolic process. Xenobiotics, in the course of their conversion, are subject

to two major stages: modification (nonsynthetic stage) and conjugation (synthetic stage). The modification stage is an enzyme-assisted modification of the initial structure of a xenobiotic resulting either in a cleavage of bonds within the xenobiotic molecule, or in the insertion of additional functional groups (e.g., hydroxyl or amino group?) into its molecule, or in a release of its functional groups blocked in the initial structure (for example, by hydrolysis of ester or peptide bonds). The modification leads to an increased solubility of the xenobiotic (xenobiotic becomes more hydrophilic). Additional functional groups are needed to enable the xenobiotic to enter the conjugation stage. The conjugation stage is viewed as an enzyme-assisted process for building covalent bonds between the xenobiotic and biomolecules occurring in the organism's media (e.g., glucuronic acid, sulfates, and others). The conjugation stage terminates in the synthesis of a novel compound whose constituents are, on the one hand, a conjugate (biomolecule).

Relationship between metabolism of xenobiotics and their structure

Xenobiotics invaded into the organism are liable to a chain of modifications, or nonsynthetic conversions (oxidation-reduction, isomerization, cyclization, ring opening, and hydrolysis) carried out by the respective enzymes (oxidoreductases, isomerases, lyases, and hydrolases):

Depending on the number of functional groups in the molecule of modified xenobiotic, its conjugation can proceed by a variety of routes in which each of the xenobiotic functional groups becomes bound with a conjugating agent. If the xenobiotic is not functionalized (e.g., benzene), it cannot enter the conjugation stage. In contrast, if the introduced xenobiotic is in possession of an appropriate functional group (e.g., phenol), it may become immediately engaged in the conjugation stage. The knowledge of principles that govern I Sic enzyme-assisted conversions of xenobiotics provides an opportunity to prognosticate metabolic behavior of any xenobiotic taking into account its structural specificities.

Xenobiotic routes in the organism

Xenobiotics are either eliminated from the organism, or become accumulated in tissues. Xenobiotics are excreted; (a) as supplied (unmodified by enzymes); (b) as metabolites (modified by enzymes); (c) as conjugates (by action of conjugating enzymes); (d) as complexed with biomolecules (or example, metal-containing xenobiotics become bound to cysteine by glutathione and excreted as complexes). The xenobiotics that accumulate in the organism are those capable of interacting with macromolecules (proteins, nucleic acids, and lipid entities). For example, organochloric compounds, which are readily soluble in lipids, are quite resistant to catabolic conversion and are difficult to eliminate from the organism. They tend to accumulate in lipid-rich tissues. Heavy metals (mercury, cadmium, silver, arsenic, and lead) and preparations containing organometallic compounds become bound with proteins and likewise accumulate in the organism.

Metabolism and physiological action of drugs

Substances introduced into the organism may exhibit either medicinal or toxic properties. Commonly, any drug can exert both medicating and side (toxic) effects;

therefore, generally speaking, the more active the drug, the faster its toxic properties become manifest. During metabolism, the specific activity and toxicity of xenobiotics are susceptible to alterations.

Biological activity alterations show up in:

- (a) deactivation, i.e., a loss of medicinal or biological activity of drugs;
- (b) activation, i.e., induced activity of an inactive preparation;
- (c) modification of the major effect, i.e., when the administered drug, on having metabolized, exhibits properties different from those of the initial preparation.

The alterations of toxicity are manifested in:

- (i) deintoxication, i.e., a loss or reduced toxicity of drug;
- (ii) toxification, i.e., enhanced toxicity of drug.

The above instances may be exemplified as follows:

Deactivation is observed as the functional groups responsible for the biological activity of a drug are either eliminated from or blocked in the drug molecule. For example, the active sulphanilamide, after its conjugation with acetyl, is converted to an inactive acetylsulphamamide.

Activation is observed when the biologically active groups that have been blocked in the initial preparation become deblocked during metabolism. Modification of the major drug effect manifests itself as a variant of activation. For example, codeine (morphine 3-methyl ether) exhibits mainly antitussive and mildly analgesic action. When codeine undergoes demethylation in the organism, it converts to morphine, which is a strong analgetic. Deintoxication resembles deactivation and is a defense reaction to the toxic effect of a drug. For example, phenol is a toxic compound, while phenol sulfate, which is a product of phenol conjugation in the organism, is nontoxic.

Toxification shows up as an enhanced side effect due to a drug administered into the organism. By mechanism, toxification resembles activation. Occasionally, toxification is produced by "lethal" molecules synthesized from the introduced compounds during their metabolism in the organism. The lethal synthesis with the involvement of a xenobiotic leads to a metabolic block and to the death of organism. For example, the administered fluoroacetate enters the Krebs cycle in tissues to produce a toxic product, fluorocitrate, which blocks aconitate hydratase and interrupts conversion steps in the Krebs cycle. Toxification effects are taken into account in the development of chemicals against rodents and other vermin.

Localization of drug metabolism in the organism

Depending on the site of conversion of biogenous preparations and xenobiotics in the organism, the drug metabolism is classified into (i) cavitary (enteral), (ii) extracellular (humoral), and (iii) cellular, or tissue, types of metabolism.

- (i) The cavitary, or enteral, drug metabolism is effected by hydrolytic enzymes supplied to the cavity of gastrointestinal tract. Hydrolysis of biogenous preparations occurs with the involvement of pancreatic and intestinal

digestive enzymes. Xenobiotics whose molecules contain peptide, carboxyester, glycoside, amide, and phosphamide bonds are also liable to hydrolysis. This process involves proteolytic and lipolytic enzymes as well as enzymes capable of hydrolyzing glycoside bonds. In addition, a large group of esterases (e.g., carboxyesterases and phosphatases) and phosphamidases (involved in hydrolysis of phosphamide bonds in drugs) are found in the intestine. Trypsin while being a proteolytic, exhibits also an esterase activity and is capable of hydrolyzing the ester bonds in xenobiotics.

- (ii) Extracellular or humoral drug metabolism takes place in the extracellular fluids (after uptake and subsequent circulation of a drug in the organism), i.e., in the blood, lymph, cerebrospinal, and extracellular proper, fluids. Possibly, metabolic conversions therein are chiefly confined to hydrolysis of the preparations delivered (both biogenous and xenogenous). In the blood and other fluids, this function is performed by proteinases and esterases (e.g., pseudocholinesterase, phosphatases, and others). In the extracellular fluids, other enzymes, for example, alcohol dehydrogenase, amino oxidases, etc., are available in small amounts, but the activity of these enzymes is rather low. The contribution of the humoral metabolic link to the overall drug metabolism is insignificant. At the humoral level, drug hydrolysis is chiefly effected; however, if this hydrolysis plays a role in drug inactivation this metabolic link should be taken into account.
- (iii) Cellular (tissue) drug metabolism, the whole variety of metabolic transformations, including those of xenobiotics, are being accomplished in the cells. However, the substances, before being subjected to the action of enzymic systems, should be transported from the site of their introduction to the cells and allowed to penetrate the intracellular space through the cell membrane. Xenobiotics are transported by the same mechanisms as biogenous substances. In the blood plasma, they either become dissolved in the liquid medium, or adsorbed, mostly on albumin. In a dissolved or in a protein-bound state, xenobiotics are delivered to the cells (tissues). They gain access to the cells mostly by simple and facilitated diffusion; large molecules enter the cells by endocytosis. Xenobiotics synthetically derived from biogenous substances can be actively transported across the cell membranes using natural substance transport systems. Not all the tissues and organs are equally active when they convert xenobiotics. The most actively engaged organ is liver which is in possession of enzymes that perform modification and conjugation of drugs. The other organs and tissues are less active in the metabolism of xenobiotics.

The metabolic conversion of xenobiotics occurs in various organelles of the liver cells. The most powerful metabolic system is found in endoplasmic reticulum (in microsomes). The microsomes are fragments of endoplasmic reticulum that are formed, for example, on trituration of a tissue sample and spontaneously close into small bladder-like structures (vesicles). Thus, with reference on its localization, the metabolism of xenobiotics is differentiated into microsomal and extramicrosomal.

The extramicrosomal metabolism occurs in hyaloplasm, lysosomes, peroxisomes, and mitochondria.

The enzymic reactions conducive to conversion of xenobiotics may be divided into the following major groups: (i) Oxidation-reduction reactions; (ii) Hydrolytic reactions; (iii) Synthetic reactions, or conjugation reactions; (iv) Other reactions (isomerization, ring opening, etc., which are effected by isomerase and lyases).

Microsomal oxidation of substances

In the microsomes, there are found enzymic chains for oxidation of substances. These chains are represented by two short electron-proton transfer chains built into the membranes of endoplasmic reticulum or into microsomal membranes. Microsomal oxidation is connected with these chains. One of these chains is a monooxygenase oxidation chain (in which the source of electrons and protons is reduced NADP), and the other is a reductase oxidation chain, with reduced NAD as a supplier of electrons and protons $x[D-H-x]$. The source of NADP-H in the monooxygenase chain is the pentose phosphate cycle, and the source of NAD-H is glycolysis.

The reaction of substrate hydroxylation is a cyclic process which includes the following major steps.

1. Binding of the substrate to the oxidized form of cytochrome P_{450} to produce a ferrisubstrate complex. The cytochrome iron converts from a low-spin to a high-spin state (I).
2. The ferrisubstrate complex is subjected to a one-electron reduction by NADP-H-cytochrome P_{450} reductase (flavoprotein) to yield a reduced complex (II).
3. The molecular oxygen interacts with the reduced P_{450} complex to form a number of oxygenated intermediates of oxycytochrome 450 (III).
4. The latter is subjected to one-electron reduction with the participation of NADH-cytochrome B_5 -reductase (IV).
5. The next step involves an intramolecular rearrangement of the complex formed: one oxygen atom is reduced to water, while the other makes part of a hydroxyl group of the substrate molecule (V).
6. The hydroxylated reaction product dissociates to yield a low-spin oxidized form of cytochrome P_{450} (VI).

The microsomal NADPH-dependent monooxygenase chain is composed of flavoprotein (PP_2) with FAD for a coenzyme, and cytochrome P_{450} . Flavoprotein exhibits a NADPH-dehydrogenase activity, FAD acting as an acceptor for two protons and two electrons. From flavoproteins, electrons are transported onto cytochrome P_{450} , and protons are lost into the environment. Cytochrome P_{450} is the terminal self-oxidizable link of this chain. Like all the cytochromes, it belongs to hem o proteins. Its protein moiety is represented by a single polypeptide chain.

The molecular mass of cytochrome P150 is about 50,000. The cytochrome P₄₅₀ is capable of complexing with carbon monoxide, CO. The light absorption maximum for these complexes is at 450 nm; hence the name for the given cytochrome. Cytochrome P₄₅₀ performs a dual function: it activates molecular oxygen by transferring electrons onto it, and uses the activated oxygen to oxidize substances (R), with the concomitant formation of water. Consequently, one oxygen atom adds to the oxidizable substance (RO), and the other, by accepting two H⁺ ions from the medium, makes up water.

The NADH-dependent reductase oxidation chain occurs not only in the microsomal membranes; it is also available in the outer mitochondria membrane, in the nuclear membrane, and in the erythrocytic cell membranes. The reductase chain is thus included among the most rapid reactions of biological oxidation, but its function in the cell remains still unclear. The self-oxidizable component of this chain capable of activating the oxygen has never been identified either; quite probable that this function is exercised by cytochrome P15U itself. The NADPII-and NADH-dependent chains can exchange electrons; for example, the electrons from FP₂ and cytochromes may be transferred onto cytochrome P₄₅₀ to be used in the oxidation of substrates.

Occasionally, the microsomal monooxygenase chain is erroneously assigned the role of oxidizing only xenobiotics. Actually, this chain serves as a versatile biological system for oxidizing nonpolar compounds of any origin, since the cytochrome P₄₅₀, directly involved in oxidation, is located within the biomembrane lipid layer. A substrate to be oxidized by cytochrome P₄₅₀ must meet the only condition—to be a nonpolar species, i.e., here the specificity to the physicochemical properties, rather than to the structure of substrate should be emphasized.

The most widespread redox reactions as performed by NADPH- and NADH-dependent oxidation chains of microsomal membranes (endoplasmic reticulum) of the liver are summarized below (in accordance with A.I. Archakov's classification).

NADPH-dependent reactions. I. Oxidation of xenobiotics:

1. Oxidative N-, S-, and O-dealkylation. For example, the oxidative N-dealkylation of dimethylaniline proceeds according to the scheme:

NAD. H-dependent reactions:

I. Production of unsaturated fatty acids from saturated acids.

II. Reduction of semidehydroascorbic acid.

III. Hydroxylation reactions:

1. Hydroxylation of kynurenine.

2. Hydroxylation of phenols and aniline.

The activity of the monooxygenase chain for oxidation of substances in the liver is carried out on a large scale. Currently, the number of known chemical species to be oxidized by this chain exceeds 7000. Such a versatility of the monooxygenase chain

enables it to perform its main task—to render compounds supplied to it more polar. After this is accomplished, the compound treated by this chain becomes more soluble in aqueous medium and susceptible to other conversions facilitating its elimination from the organism. In most cases, the hydroxylation of xenobiotics leads to a reduction of their toxicity. But this is not always the case, e.g., the monooxygenase chain oxidizes the nontoxic benzopyrene (present in tobacco smoke and smoke products) to form the toxic carcinogen hydroxybenzopyrene. The production of toxic and biologically active substances in the monooxygenase oxidation chain is thus a kind of “retribution” for its versatility.

Extramicrosomal conversion of xenobiotics

Xenobiotics may as well be converted outside the microsomes of the cells of liver and other organ. For example, in the mitochondria, the oxidative deamination of aliphatic and aromatic amines to corresponding aldehydes takes place. In the soluble portion of cytoplasm, aliphatic alcohols (methanol, ethanol, butanol, and others) are oxidized by alcohol dehydrogenase to corresponding aldehydes which undergo further oxidation to organic acids by aldehyde dehydrogenase. In the peroxisomes, an alternative route to ethanol oxidation with the participation of catalase is possible according to the scheme:

Reactions of ring aromatization, or reduction of single bonds to double bonds in a ring, are also involved in redox conversions of xenobiotics.

Hydrolases, chiefly lysosomal hydrolases, participate in a large number of conversions. These enzymes encompass:

- (1) esterases for carboxylic acid esters (pseudocholine esterase, atropine esterase, cocaine esterase, tannin esterase, etc.);
- (2) esterases for phosphoric acid esters (phosphomonoesterases, phosphodiesterases, phosphoamidases, etc.);
- (3) esterases for sulfuric acid esters (various sulfatases);
- (4) esterases for glucuronides (β -glucuronidase).

Xenobiotic metabolites produced by the action of the enzymes of endoplasmic reticulum and other organelles are reactive intermediates. They can produce a side effect on the organism tissues, for example, mutagenic, carcinogenic, immunodepressive, and allergic.

Conjugation of xenobiotics: its mechanism and role

The conjugation stage, or synthetic stage, is essential for the formation of nontoxic and easily excretible drug metabolite? By their mechanism, the conjugation reactions are divided into two groups:

Reactions of I type. Initially, conjugating agents, i.e., biomolecules, are activated and then transferred onto xenobiotics to form conjugates. This type of conjugation reactions occurs in all tissues of the organism.

Reactions of II type. Initially, a xenobiotic is activated to be transferred onto a conjugating biomolecule to form a conjugate. This conjugation type is of rare occurrence and is only observed in liver and kidney.

Various groups for conjugation reactions of I and II types are distinguished, depending on the nature of a conjugating species involved. In I type reactions, glucuronide, sulfate, acetyl, methyl, thiosulfate conjugations are to be noted, and in the II type, glycine and glutamine conjugations.

Glucuronide conjugation

UUP-glucuronic acid is the source for glucuronic acid residues in this process. Endogenous substances and xenobiotics are subject to glucuronide conjugation (known are glucuronides of bilirubin, steroid hormones, vitamin D, etc.). Xenobiotics can enter glucuronide conjugation if they possess or have acquired, during modification, a hydroxyl, carboxyl, and amino group (commonly, in the aromatic ring), or, at least, a SH-group. The conjugation reaction proceeds with the participation of UDP-glucuronosyltransferase by the scheme. Among xenobiotics (drugs and poisons), susceptible to glucuronide conjugation are phenols, polyphenols, phenolic steroids, aromatic amino acids, and others.

Sulfate conjugation

Initially an active form of a conjugating agent, 3'-phosphoadenosine-5'-phosphosulfate (PAPS for short) is formed. PAPS, which may also be designated as $\text{PAP} \sim \text{SO}_3\text{H}$, is a source of labile sulfate groups used in the conjugation of natural compounds and xenobiotics. The natural substances subject to sulfate conjugation include endogenous toxic products of intestinal putrefaction of proteins, e.g., indole, scatol, phenols as well as steroids, iodothyronines, tocopherols, naphthoquinones, and others. To be able to enter the sulfate conjugation xenobiotics must, as a rule, possess a cyclic structure (carbocyclic or heterocyclic) and carry free OH and NH_2 groups.

The sulfate conjugation reaction proceeds with the involvement of a special enzyme, sulfotransferase, according to the scheme:

It should be emphasized that most substances whose structures are amenable to sulfate conjugation may, with an equal probability, be subjected to glucuronide conjugation. Obviously, the route of conjugation is dependent on the conditions at the site of localization of the enzymes responsible for a given conjugation, and on the relative specificity of these enzymes toward substrate.

Acetyl conjugation

The source of labile acetyl groups in this variety of conjugation reactions is acetyl-CoA, which is produced by degradation of carbohydrates, triacylglycerides, and amino acids. Endogenous substances and xenobiotics containing a free NH_2 group may be acetylated. *N*-Acetylation of is an essential biochemical reaction in the synthesis of monosaccharide derivatives (*N*-acetylglucosamine, *N*-acetylgalactosamine, and neuraminic acid) that are further used in the synthesis of heteropolysaccharides. *N*-Acetylation is also a route to neutralization of biogenous amines—serotonin, histamine, and others. *N*-Acetylation of histones and nonhistonic chromatin proteins is an important regulatory mechanism of DNA transcription. For endogenous substances, the only case of *O*-acetylation has been reported, which is a reaction of acetylcholine formation. Xenobiotics possessing a

free NH₂ group (commonly, on the aromatic ring) are subject to acetylation. This reaction is effected by means of a special acetyltransferase called arylamine-*N*-acetyltransferase (probably, several such enzymes are available). This enzyme exhibits a low specificity to xenobiotics to be acetylated. The reaction proceeds by the scheme.

Among the xenobiotics susceptible to acetylation, sulfanylamides, isonicotinic acid hydrazides, and aniline derivatives can be mentioned: these preparations are widely used in medical practice.

Methyl conjugation

In this reaction methyl groups derived from the active form of methionine, S-adenosylmethionine, serve as a conjugating agent. S-Adenosylmethionine is a participant in numerous reactions of methylation of endogenous compounds. It is also a methyl group donor for conjugation reactions of xenobiotics (RXH), which proceed with the involvement of methyltransferases according to the scheme. Xenobiotics containing an Ml; group or a heterocyclic nitrogen, as well as OH and SH groups, are subject to methylation by addition of methyl groups to X, O, and S atoms. Among the preparations used in therapy, liable to methylation are mono- and polyphenols, and heterocyclic compounds of pyridine, quinolint, isoquinoline, and thiouracil type.

Thiosulfate conjugation

This kind of conjugation is used in the enzymic detoxification of cyanides. The conjugating agent in this reaction is the thiosulfate sulfur (on rarer occasions, other sulfur-containing compounds). The transfer of sulfur from thiosulfate onto a cyanide ion is catalyzed by a specific enzyme, thiosulfate sulfurtransferase, according to the scheme.

The thiocyanate thus formed is much less toxic than cyanides. In the human tissues, the sources of thiosulfate are sulfur-containing amino acids. Thiosulfate conjugation is also feasible with cyanides of inorganic origin (cyanic acid, sodium and potassium cyanides) and organic origin (acetonitrile, acrylonitrile, benzonitrile, mandelonitrile, malononitrile, various cyanohydrin glucosides, and halocyanides), providing the latter release the cyanide ion by their hydrolysis in the organism.

Glycine conjugation

This reaction belongs to type II conjugations, which require a prior activation of the substrate rather than of the conjugating agent. In principle, any carboxylic acid can serve as a conjugation substrate. However, aliphatic acids are but rarely formed: therefore classical substrates are cyclic carboxylic acids.

The mechanism of glycine conjugation may be exemplified by the formation of hippuric acid. According to the mechanistic concept of type II conjugation reactions, the initial step of hippuric acid formation is activation of benzoic acid with the involvement of arylacyl-CoA synthetase by the scheme.

Then benzoyl (or, in a wider sense, any activated substrate in the reactions of this type) is transferred onto the glycine amino group. This process is catalyzed by acylglycine transferase, which is specific to acylation of only glycine, bypassing other amino acids:

Similarly, glycine conjugates of other compounds are formed: aromatic acids (nicotinic), phenyl-substituted acetic acids (phenylacetic and hydratropic), B-substituted propionic acid (B-0-tolylpropionic), substituted acrylic acids (cinnamic, furylacrylic, B-methylcinnamic, and phellandrenic), steroid acids (cholic and deoxycholic).

Glutamine conjugation

It is a rare variety of conjugation, distinctly observable in patients with phenylketonuria. In such patients, phenylacetic acid is formed in appreciable amounts; it is activated in kidney and liver to produce phenylacetyl-CoA and then it, transferred onto the glutamine. NH₂ group. Phenylacetyl-glutamine conjugate thus formed is then excreted in the urine. In normal humans, the glutamine conjugation of xenobiotics has never been reported.

Factors affecting drug metabolism

Drug metabolism is affected by a variety of factors including genetic, age, diet, hormone balance, organ-specific function, neuroendocrine, environmental factors, and the manner a drug has been administered.

Molecular-genetic mechanisms determinative of drug metabolism

The rate at which a drug supplied to the organism is metabolized is dependent on the number of enzymes involved in modification and conjugation of the drug. In enzymopathies associated with defective enzymes that are involved in drug metabolism, a decrease in the drug metabolism rate is observed. Since in the majority of case, the drug metabolism is conducive to a diminution in drug activity and toxicity, there may arise an unpredictable abnormal hypersensitivity of the organism tissues to the drug administered (if it has been applied in an active form), or high drug toxicity (side effect). The drug metabolism enzymopathy is often, the cause of negative effects produced by the drugs applied. Let us now consider certain typical enzymopathies.

Present in the blood are dialing esterase, which hydrolyzes acetylcholine, and nonspecific pseudocholinesterase capable of hydrolyzing, alongside acetylcholine, other carboxyesters. The two enzymes are produced in the liver. In enzymopathy of pseudocholinesterase, its activity in the blood plasma is lowered. In patients suffering from this enzymopathy, the administration of diacetylcholine as a muscle relaxant (an agent that specifically aids in reducing muscle tension), which can be hydrolyzed by pseudocholinesterase, leads to an abnormally prolonged action of this drug on the organism to a few hours, as compared to the normal action for several minutes.

Enzymopathies associated with conjugation reactions have been described in humans. Molecular diseases due to a defective UDP-glucuronosyltransferase are known. They manifest themselves as two forms of hereditary hyperbilirubinemia or jaundice: congenital hyperbilirubinemia, or congenital nonhemolytic jaundice (Crigler-Najjar syndrome), and familial juvenile idiopathic hyperbilirubinemia (Gilbert-Meulengracht syndrome). These molecular diseases are characterized by the disturbed glucuronide conjugation not only of bilirubin, but of other

endogenous substrates and drugs too. For this reason, the prescription of sulfanilamides, salicylates, and phenol-derived preparations, which are metabolized by glucuronide conjugation, leads to aggravated symptoms of the disease: even normal doses of these drugs produce a negative effect.

Enzymopathies associated with acetylation of xenobiotics have also been reported. In the diseased persons, the activity of arylalaine-N-acetyltransferase is low in persons suffering from these enzymopathies, which results in slow inactivation (by conjugation) of sulfanilamides and antituberculous drugs (p-aminosalicylic acid or isoniazid), with ensuing side effects on the organism tissues. In this connection, the inactivating response (fast or slow) of the patient's organism should be taken into consideration when the isoniazid therapy is prescribed to tuberculosis patients.

Age factor in drug metabolism is important. In neonates and infants (to the age of about eight weeks), the enzymic apparatus of xenobiotic metabolism is poorly developed. They exhibit, as distinct from the adult humans, the low activity of the mono-oxygenate chain toward oxidation of drugs and other enzymes; in particular, UUP-glucuronosyltransferase, as well as the low level of cytochrome 1 P4M. For this reason, as has previously been pointed out, the physiological jaundices in neonates are of more frequent occurrence. The childhood deficiency in these enzymes should not be confused with enzymopathies. As the young organism develops, the physiological enzymic deficiency disappears, while hereditary enzymopathies in adult humans persist. The drug detoxification insufficiency in children is conducive to that the side drug effects in them show up faster and with smaller drug doses.

Organ-specific—Factors in Drug Metabolism

Liver is the major organ responsible for drug metabolism. Therefore, in hepatic pathology, there occur disturbances in metabolic detoxification of drugs, which also leads to an increased toxicity of drugs and their uncommonly high activity.

Neuroendocrine factor determinative of drug metabolism

The state of neuroendocrine system influences the activity of drug metabolism enzymes. A state of tension or of stress leads to an increased secretion of corticotropin and, respectively, glucocorticoids which enhance the activity of drug metabolism enzymes. The drug inactivation is to a certain extent sex-determined, which is associated with a different action of the male and female sex hormones on the activity of drug metabolism enzymes. Androgens are inducers for enzymes of the monooxygenase oxidation chain and drug conjugation: therefore, in males, drugs are detoxified at a faster rate. The female organism, on the contrary, is slower in inactivating the drugs. Presumably, this is explained by the fact that estrogens and progesterone suppress the activity of UDP-glucuronosyltransferase and the microsomal hydroxylation of xenobiotics in the liver.

Drug metabolism in relation to administration mode determines the drug metabolic pathways. In enteral administration, the drugs undergo hydrolytic cleavage by gastrointestinal enzymes and on absorption, are directly supplied in the

portal vein blood to the liver. Therefore, the enteral mode provides for an intensive metabolic conversion of drugs and their faster inactivation. It ensues therefrom that the enteral mode of drug administration is safer as to an eventual toxic effect; on the other hand, larger drug doses are required to produce the desired specific effect. The specific features of enteral administration drug metabolism are being taken into account in developing the preparations for intestinal action. To this effect, the active groups should preferably be blocked in the chemical structure of a drug and liberated as the drug becomes hydrolyzed by intestinal enzymes.

In parenteral administration, the drug omits being involved in the cavitary metabolism step altogether and is supplied to the liver by a longer route. For this reason, the drug metabolism rate is significantly slower, and the probability of a deleterious side effect is higher. An advantage of the parenteral administration mode is a smaller drug dose required for attaining a maximal therapeutic effect.

Environmental factors in drug metabolism—a variety of environmental factors affect the activity of the organism tissue enzymes involved in the drug metabolism and thus influence the effectiveness and toxicity of drugs. Environmental factors such as light, ambient temperature, radiation, and others have been noted to influence the drug metabolism. The action of these factors is accomplished indirectly, via the neuroendocrine system. Simultaneously, the influence of endocrine factors on drug-detoxifying enzymes is varied in effect: some enzymes become activated, while others remain inert or inhibited in their activity. Therefore, the influence of environmental factors on the metabolism (and, consequently, activity and toxicity) of various drugs is dependent on the manner in which these factors affect the activity of enzymes that metabolize a definite chemical group of drugs.

The extended daytime duration reduces the activity of enzymic microsomal oxidation in the liver; on the contrary, the activity of enzymic systems in the night time is increased. For this reason, at night the drug metabolism rate by the enzymes of hepatic endoplasmic reticulum increases, while at day time, it decreases. Ionizing radiation on the organism decreases detoxifying ability of the hepatic monooxygenase chain of drug oxidation.

The dietary regimen can likewise substantially affect the drug metabolism. With most drugs, starvation leads to an inhibition of enzymic activity of microsomal oxidation and enhances the probability of drug intoxication. The alimentary deficiency in proteins produces roughly the same effect on drug metabolism. In B₁ and B₂, hypovitaminoses, the hydroxylation of xenobiotics in hepatic microsomes is reduced. The knowledge of these effects is used by practical physicians in rationalizing the drug prescription schemes and course of treatment.

In xenobiotic metabolism, the mechanism of enzymic process control by various drugs and compounds operable in the human organism merits a special mention. These drugs include agents acting as inducers or inhibitors for the synthesis of enzymic systems. Currently, over 200 preparations are known to be capable of exerting an inducing action on drug metabolism enzymes, primarily microsomal ones. They include butadiene (antiinflammatory), amidoprine (analgesic), novocain (local anesthetic), ethanol, and others. Phenobarbital (soporific) acts as the most

powerful inducer. It drastically enhances the synthesis of microsomal oxidation enzymes in the liver by affecting the genetic apparatus of the liver cells. The increased number of microsomal oxidation chains leads to an enhanced metabolism of endogenous compounds and xenobiotics oxidizable by enzymes of these chains. In addition, phenobarbital elicits the synthesis of UDP-glucuronosyltransferase and facilitates the conjugation stage in the metabolism of various materials. Other preparations exhibit a similar, but a markedly less pronounced effect.

The phenomenon of medicinal (in particular, phenobarbitalic) induction provides an explanation of the addiction to soporific agents of barbiturate family and to other drugs liable to detoxification by oxidation in the monooxygenase chain of endoplasmic reticulum of the liver and by glucuronide conjugation. Thus, phenobarbital may be envisioned as providing a ground for self-detoxification and for the detoxification of other compounds: for this reason, it is recommendable in practice, for example, in treating poisoning and in stimulating the metabolism of endogenous substances (for example, bilirubin in physiological jaundice of neonates and in congenital hyperbilirubinemias).

The prescription of inducer drugs together with other pharmaceuticals requires a judicious approach. Otherwise, the administered drug dose may lead to an unsatisfactory therapeutic effect.

Inducers for drug metabolism enzymes are biogenic preparations such as thiamine, riboflavin and their coenzymes, carnitine, pantothenic acid, androgens, and anabolic steroids; preparations of progesterone and estrogens inhibit these enzymes. Thus, the preparations of biogenic origin and xenobiotics may be profitably used to divert in the right direction the metabolism of endogenous materials and other compounds supplied to the patient's organism and control their toxicity and activity.

References

- Balech E (1985) The genus *Alexandrium* or *Gonyaulax* of the Tamarensis Group. In: Anderson DM, White AW, Baden DG (eds) Toxic dinoflagellates. Elsevier, New York, pp 33–38
- Bennett JW, Klich M (2003) Mycotoxins. Clin Microbiol Rev 16(3):497–516
- Brinkman DL, Konstantakopoulos N, McInerney BV, Mulvenna J, Seymour JE, Isbister GK et al (2014) *Chironex fleckeri* (Box Jellyfish) venom proteins: expansion of a cnidarian toxin family that elicits variable cytolytic and cardiovascular effects. J Biol Chem 289(8):4798–4812
- Cembella AD (1998) Ecophysiology and metabolism of paralytic shellfish toxins in marine microalgae. In: Cembella AD, Hallegraeff GM, Anderson DM (eds) Physiological ecology of harmful algal blooms. Springer, Berlin, pp 381–403
- Clark RF, Williams SR, Nordt SP, Manoguerra AS (1999) A review of selected seafood poisonings. Undersea Hyperb Med 26(3):175–184
- Currie BJ, Jacups SP (2005) Prospective study of *Chironex fleckeri* and other box jellyfish stings in the “Top End” of Australia’s Northern Territory. Med J Aust 183:631–636
- DeBin JA, Strichartz GR (1991) Chloride channel inhibition by the venom of the scorpion *Leiurus quinquestriatus*. Toxicon 29(11):1403–1408

- Graeme KA (2014) Mycetism: a review of the recent literature. *J Med Toxicol* 10(2):173–189
- Hutchinson DA, Mori A, Savitzky AH, Burghardt GM, Wu X, Meinwald J et al (2007) Dietary sequestration of defensive steroids in nuchal glands of the Asian snake *Rhabdophis tigrinus*. *Proc Natl Acad Sci USA* 104(7):2265–2270
- Keeler RF (1984) Teratogens in plants. *J Anim Sci* 58(4):1029–1039
- Laurent F, Michel A, Bonnet PA, Chapat JP, Boucard M (1993) Evaluation of the relaxant effects of SCA40, a novel charybdotoxin-sensitive potassium channel opener, in guinea-pig isolated trachealis. *Br J Pharmacol* 108(3):622–626
- Lumley J, Williamson JA, Fenner PJ, Burnett JW, Colquhoun DM (1988) Fatal envenomation by *Chironex fleckeri*, the north Australian box jellyfish. The continuing search for lethal mechanisms. *Med J Aust* 148:527–534
- Manning SR, La Claire JW (2010) Prymnesins: toxic metabolites of the golden alga, *Prymnesium parvum* Carter (Haptophyta). *Marine Drugs* 8(3):678–704
- Moss MO (2008) Fungi, quality and safety issues in fresh fruits and vegetables. *J Appl Microbiol* 104(5):1239–1243
- Rasmussen SA, Meier S, Andersen NG, Blossom HE, Duus JØ, Nielsen KF et al (2016) Chemodiversity of ladder-frame prymnesin polyethers in *Prymnesium parvum*. *J Nat Prod* 79(9):2250–2256
- Reddy KV, Yedery RD, Aranha C (2004) Antimicrobial peptides: premises and promises. *Int J Antimicrobial Agents* 24(6):536–547
- Richard JL (2007) Some major mycotoxins and their mycotoxicoses—an overview. *Int J Food Microbiol* 119(1–2):3–10
- Robbins CA, Swenson LJ, Nealley ML, Gots RE, Kelman BJ (2000) Health effects of mycotoxins in indoor air: a critical review. *Appl Occup Environ Hyg* 15(10):773–784
- Roy A, Zhou X, Chong MZ, d'Hoedt D, Foo CS, Rajagopalan N et al (2010) Structural and functional characterization of a novel homodimeric three-finger neurotoxin from the venom of *Ophiophagus hannah* (king cobra). *J Biol Chem* 285(11):8302–8315
- Schultes RE (1969–1970) Bull. Narcotics, 21, pt. 3, 3–16; pt. 4, 15–27; 22, pt. 1, 25–53 (1969–1970)
- Turner NW, Subrahmanyam S, Piletsky SA (2009) Analytical methods for determination of mycotoxins: a review. *Anal Chim Acta* 632(2):168–180

Chapter 7

Biotechnology, In Vitro Production of Natural Bioactive Compounds, Herbal Preparation, and Disease Management (Treatment and Prevention)



Abstract Biotechnology uses living systems to develop products and plant biotechnology generates useful products or services, e.g., different bioactive secondary metabolites including alkaloids, flavonoids and other phenolics, saponins, terpenoids, steroids, glycosides, tannins, volatile oils, etc., from plant cells, tissues or organs culture independent of geographical and climatic factors under aseptic conditions. These bioactive compounds are economically important as drugs (pharmaceuticals), flavors, perfumes (fragrances), pigments (dyes), agrochemicals as well as cosmetics, food additives, etc. Different strategies, e.g., genetic transformation of plants with *Agrobacterium rhizogenes*, hairy roots and others can be applied for the improvement of production of bioactive compounds of secondary metabolic origin. Recombinant DNA techniques can be used to manipulate metabolic pathways and produce protein pharmaceuticals such as antibodies, and protein hormones. Bioinformatics and genomics can find application in drug discovery from plant-based products and biotechnological procedures can enhance and advance the studies of medicinal plants. Molecular biotechnology uses laboratory techniques to study and modify nucleic acids and proteins for applications in areas such as human and animal health, agriculture, and the environment. Herbal extracts are now widely used in the management of chronic diseases like diabetes, hypertension, cancer, etc., as a part of CAM therapy. Plant-derived immune stimulators diverse small or large molecules (saponins, tomatine, inulin, polysaccharides), fungal β -glucans, complex molecules from marine sponge (α -galactosylceramide), shrimp chitin (chitosan), etc., have established adjuvant activity. Immunotherapy may be activation immunotherapy or suppression immunotherapy. Vaccines provide immune protection against diseases and plant-based edible vaccine production mainly involves the integration of transgene into the plant cells to produce the antigen protein for specific disease.

7.1 Biotechnology and Production of Bioactive Compounds and Techniques of Molecular Biotechnology

Biotechnology or biotech is the use of living systems and organisms to develop or make useful products or any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use, and plant biotechnology may be defined as generation of useful products or services from plant cells, tissues, and, often, organs (very small organ explants). Such cells, tissues, and organs are either continuously maintained *in vitro* or they pass through a variable phase to enable regeneration from them of complete plants which are ultimately transferred to the field.

Plants synthesize a large number of bioactive secondary metabolites including alkaloids, flavonoids and other phenolics, saponins, terpenoids, steroids, glycosides, tannins, volatile oils, etc. The application of secondary bioactive compounds falls into five main categories, viz. drugs (pharmaceuticals), flavors, perfumes (fragrances), pigments (dyes), agrochemicals as well as cosmetics, food additives, etc. Most of these metabolites are obtained from plants as *in vitro* synthesis of these compounds at industrial level is difficult due to complex chemical structures and complicated biosynthetic pathways. Biotechnology offers a valuable tool to produce these compounds of interest in a desired amount and an eco-friendly way.

In vitro plant cell culture technique, in which plant cells, tissues, and organs are cultivated under aseptic conditions (independent of geographical and climatic factors), offers alternatives for producing important metabolites. The process includes mainly four main approaches, viz. callus, suspension, immobilized cells, and differentiated cultures. Callus culture involves growing a disorganized aggregate of cells from plant explants by culturing on a semi-solid support, which contains nutrients and any hormones required to promote growth of the cells. Suspension cultures result when callus is suspended in liquid growth medium and growing cells as dispersed cell culture. With their relatively fast growth, suspension cultures are widely employed in the study of bioactive secondary metabolite production by plant cells. The advantages of this approach are obvious, as biomass production is rapid than that of whole plant, nutritional and environmental requirements can be easily controlled allowing the production of pharmaceutical throughout the year if necessary, nutrient uptake is enhanced by submerged culture conditions, which stimulate the multiplication rate and higher yield of bioactive compounds. In several studies, *in vitro* culture were found more efficient than whole plant for the production of different bioactive secondary metabolites, i.e., the level of production of ajmalicine, ajmaline, anthraquinones, benzylisoquinoline alkaloids, berberine, bisoclaurine, coniferin, diosgenin, ginseng, ginsenoside, glutathione, nicotine, rosmarinic acid, raucaffricine, shikonin, taxol, terpentine, tripdiolide, ubiquinone-10, etc., were high in *in vitro* culture compared to whole plant production following agronomic method

(Alamgir 2017). Plant cell culture systems represent a potential renewable source of valuable medicinal compounds, flavors, fragrances, and colorants, which cannot be produced by microbial cells or chemical synthesis.

Plant tissue culture technology could be a potential alternative approach for bioproduction of phytoconstituents of therapeutic value and might be attractive under certain conditions if, for example, the source plant is difficult to cultivate, has a long cultivation period or has a low metabolite yield; if chemical synthesis has not been achieved or if it is technically problematic. In vitro culture of plant cell, tissue, and organs forms an integral part of any plant biotechnology activity. By employing biotechnological techniques, it is possible to regulate the biosynthetic pathway of plant in order to enhance/decrease the synthesis of particular compound. Different strategies can also be applied for the improvement of production of bioactive compounds of secondary metabolic origin. The major obstacles faced in the study of medicinal plants include inaccurate identification and speciation, low yield of bioactive metabolites, variability of traditional protocols, etc.

Research in the area of plant tissue and organ culture technology has resulted in the production of many bioactive metabolites (alkaloids, terpenoids, steroids and sterols, saponins, phenolics, flavonoids, amino acids, C-phycocyanins, glycosides, etc.) for new therapeutics under defined conditions. Bioactive compounds may be derived from microbial, algal, and vegetable sources and are used as antioxidants and anti-inflammatory agents, anti-allergenic compounds, etc. A recent development to overcome the difficulties arising with cell suspension cultures is the genetic transformation of plants with *Agrobacterium rhizogenes*. Hairy roots have been found to be suitable for the production of secondary metabolites, because of their stable and high productivity in hormone-free culture conditions. Recombinant DNA techniques can be used to manipulate metabolic pathways and produce protein pharmaceuticals such as antibodies, and protein hormones. The new disciplines of Bioinformatics and Genomics can find application in drug discovery from plant-based products and biotechnological procedures can enhance and advance the studies of medicinal plants. Plant-transformed technology has now reached a platform of commercial reality. Despite the advances in biotechnology techniques, there are only a few successful examples of secondary compounds production at an industrial level.

The biotechnological importance of secondary metabolites lies in the fact that: (i) many of them are commercially valuable chemicals, (ii) a number of them are toxic and should be removed from food products, and (iii) they act as protective agents, used by the plant against insects, pathogens, or animal foragers. The various objectives achieved/achievable by plant biotechnology may be summarized as follows: (i) Useful biochemical production (large-scale cell cultures); (ii) Rapid clonal multiplication (adventitious shoot/bulb/protocorm); (iii) Virus elimination (thermo-, cryo-, or chemotherapy coupled with meristem culture); (iv) Rapid development of homozygous lines by producing haploids (anther culture, ovary culture, interspecific hybridization); (v) Production/recovery of difficult to produce hybrids (embryo rescue, in vitro pollination); (vi) Germplasm conservation of

vegetatively reproducing plants or those producing recalcitrant seeds (cryopreservation, slow growth cultures, DNA clones); (vii) Genetic modification of plants (somaclonal variation, somatic hybridization, cybridization, and gene transfer); and (viii) Creation of genome maps and use of molecular markers to assist conventional breeding efforts.

Plants are the tremendous source for the synthesis of bioactive secondary metabolites, which are economically important as drugs, flavor and fragrances, dye and pigments, pesticides, and food additives. Recent advances in the molecular biology, enzymology, and fermentation technology suggest that these plant products can be extracted from the aseptic culture of plant cell, tissue, and organ. Based on their biosynthetic origins, bioactive secondary metabolites of plant can be divided into three major groups such as (i) phenolic compounds; (ii) terpenoids; and (iii) nitrogen-containing alkaloids and sulfur-containing compounds. Compared to another group of secondary metabolites, phenolic compounds are largely responsible for beneficial effects on human health and these naturally occurring compounds are found largely in fruits, vegetables, cereals, and beverages. Phenolics that are not soluble are found in cell walls, while soluble phenolics are present within the plant cell vacuoles. Phenols are classified into different groups as a function of the number of phenol rings in the structure and their main classes include phenolic acids, flavonoids, stilbenes, and lignans and they include apigenin, diosmin, quercetin, kaempferol, eriodictyol, naringenin, hesperetin, baicalein, chrysins, catechin, morin, genistein, curcumin, colchicine, resveratrol, emodin, etc. Plant cell, tissue, and organ cultures have been established as routine work under sterile conditions from explants such as plant leaves, stems, roots, meristems, etc., for the production and extraction of hundreds of bioactive secondary metabolites (Karuppusamy 2009) (Table 7.1).

One of the most exciting aspects of cell culture technology is the potential for producing novel structures not observed in the parent plant, e.g., rutacultin by cultures of *Ruta graveolens* and sesquiterpene lactones by cultures of *Andrographis paniculata*. Table 7.2 shows some plant sources for different pharmaceutically valuable bioactive compounds.

The use of plant tissue cultures for the biotechnological production of bioactive phytoconstituents on commercial scale is attractive for several reasons. Tissue culture protocols have been developed for several plants but more are to be developed for many other species. Refined culture systems have improved the biochemical yields considerably, and over half a dozen cell cultures produce 2 g/l or more, of the biochemical.

7.1.1 Techniques of Molecular Biotechnology

Molecular biotechnology is the use of laboratory techniques to study and modify nucleic acids and proteins for applications in areas such as human and animal health, agriculture, and the environment. Molecular biotechnology results from the

Table 7.1 In vitro secondary metabolites from plant cell, tissue, and organs cultures

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
1. <i>Aconitum heterophyllum</i>	Aconites	MS + 2,4-D + Kinetin	Hairy root
2. <i>Adhatoda vasica</i>	Vasine	MS + BAP + IAA	Shoot culture
3. <i>Agastache rugosa</i>	Rosmarinic acid	MS + 2,4-D + Kinetin + 3% sucrose	Hairy root
4. <i>Agave amanuensis</i>	Saponins	MS + Kinetin	Callus
5. <i>Ailanthus altissima</i>	Alkaloids	MS + 2,4-D + Kinetin	Suspension
6. <i>Allium sativum</i>	Allin	MS + IAA + Kinetin	Callus
<i>Aloe saponaria</i>	Glucosides	MS + 2,4-D + Kinetin	Suspension
7. <i>Ambrosia tenuifolia</i>	Altamisine	MS + Kinetin	Callus
8. <i>Ammi majus</i>	Umbelliferone	MS + BAP	Shootlet
9. <i>Ammi visnaga</i>	Furanocoumarin	MS + IAA + GA3	Suspension
10. <i>Anchusa officinalis</i>	Rosmarinic acid	B5 + 2,4-D	Suspension
11. <i>Angelica gigas</i>	Deoursin	MS (Liq.) + 2,4-D + GA3	Hairy root
12. <i>Anisodus luridus</i>	Tropane alkaloids	MS + 2,4-D + BA	Hairy root
13. <i>Ammi majus</i>	Triterpenoid	MS + 2,4-D + BA	Suspension
14. <i>Arachys hypogaea</i>	Resveratrol	G5 + 2,4-D + Kin	Hairy root
15. <i>Armoracia lapathifolia</i>	Fusicoccin	MS + IAA	Hairy root
16. <i>Artemisia absinthium</i>	Essential oil	MS + NAA + BAP	Hairy root
17. <i>Artemisia annua</i>	Artemisinin	MS + IAA + Kinetin	Hairy root
18. <i>Artemisia annua</i>	Artemisinin	MS + NAA + Kinetin	Callus
19. <i>Aspidosperma ramiflorum</i>	Ramiflorin	MS + 2,4-D + BAP	Callus
20. <i>Aspidosperma ramiflorum</i>	Ramiflorin alkaloid	MS + 2,4-D + BAP + 30 g/l	Sucrose Callus
21. <i>Astragalus mongholicus</i>	Cycloartane saponin	MS + 2,4-D + Kin	Hairy root
22. <i>Astragalus mongholicus</i>	Cycloartane	MS + IAA + NAA	Hairy root
23. <i>Azadirachta indica</i>	Azadirachtin	MS + 2,4-D	Suspension
24. <i>Azadirachta indica</i>	Azadirachtin	MS + 2,4-D + Cyanobacterial elicitor	Suspension
25. <i>Beta vulgaris</i>	Betalain pigments	MS + IAA	Hairy root

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
26. <i>Brucea javanica</i>	Alkaloids	MS + 2,4-D + Kinetin	Suspension
27. <i>Brucea javanica</i>	Cathin	MS + IAA + GA3	Suspension
28. <i>Brugmansia candida</i>	Tropane	MS + 2,4-D + IAA	Hairy root
29. <i>Brugmansia candida</i>	Tropane alkaloid	MS + BA + NAA	Hairy root
30. <i>Bupleurum falcatum</i>	Saikosaponins	B5 + IBA	Root
31. <i>Bupleurum falcatum</i>	Saikosaponins	LS + 2,4-D	Callus
32. <i>Calystegia sepium</i>	Cuscohygrine	MS + 2,4-D + BA	Hairy root
33. <i>Camellia chinensis</i>	Flavones	MS + 2,4-D + NAA	Callus
34. <i>Camellia sinensis</i>	Theamine	MS + IBA + Kinetin	Suspension
35. <i>Campanula medium</i>	Polyacetylenes	MS + IAA + BA	Hairy root
36. <i>Canavalia ensiformis</i>	Canavanine	LS + NAA + Picloram	Callus
37. <i>Capsicum annum</i>	Capsiacin	MS + 2,4-D+ GA3	Callus
38. <i>Capsicum annum</i>	Capsiacin	MS + 2,4-D + Kin	Callus
39. <i>Capsicum annum</i>	Capsiacin	MS + 2,4-D + Kinetin	Suspension
40. <i>Cassia acutifolia</i>	Anthraquinones	MS + 2,4-D + kinetin	Suspension
41. <i>Cassia obtusifolia</i>	Anthraquinone	MS + TDZ + IAA	Hairy root
42. <i>Cassia senna</i>	Sennosides	MS + NAA + Kin	Callus
43. <i>Catharanthus roseus</i>	Indole alkaloids	MS + IAA	Suspension
44. <i>Catharanthus roseus</i>	Indole alkaloids	MS + NAA + Kinetin	Suspension
45. <i>Catharanthus roseus</i>	Vincristine	MS + 2,4-D + GA3	Suspension
46. <i>Catharanthus roseus</i>	Indole alkaloid	MS + 2,4-D + GA3 +Vanadium	Suspension
47. <i>Catharanthus roseus</i>	Catharathine	MS + 2,4-D + UV-B radiation	Suspension
48. <i>Catharanthus trichophyllum</i>	Indole alkaloids	MS + IAA + GA3	Hairy root

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
49. <i>Cayratia trifoliata</i>	Stilbenes	MS + IAA + GA3	Suspension
50. <i>Centella asiatica</i>	Asiaticoside	MS + 2,4-D	Hairy root
51. <i>Centella asiatica</i>	Asiaticoside	MS + 2,4-D + Kin	Callus
52. <i>Centella asiatica</i>	Asiaticoside	MS + BAP + IAA	Shoot
53. <i>Centella asiatica</i>	Asiaticoside	MS + 2,4-D	Hairy root
54. <i>Centranthus ruber</i>	Valepotriates	MS + IAA + Kin	Hairy root
55. <i>Cephaelis ipecacuanha</i>	Alkaloids	MS + IAA	Root
56. <i>Chaenatis douglasei</i>	Thiarbrins	MS + NAA	Hairy root
57. <i>Chrysanthemum cinerariaefolium</i>	Pyrethrins	MS + 2,4-D + Kinetin	Callus
58. <i>Cinchona ledgeriana</i>	Quinine	MS + 2,4-D	Hairy root
59. <i>Cinchona succirubra</i>	Anthraquinone	MS + IAA + GA3	Suspension
60. <i>Citrus</i> sp.	Limonin	MS + 2,4-D + Kinetin	Callus
61. <i>Coffea arabica</i>	Caffeine	MS + 2,4-D + Kinetin	Callus
62. <i>Coleus forskohlii</i>	Forskolin	MS + IAA + Kin	Hairy root
63. <i>Corydalis ambigua</i>	Corydaline	MS + IAA + 3% sucrose	Embryo
64. <i>Corydalis cava</i>	Corydaline	MS + IAA + GA3	Shoot
65. <i>Corydalis ophiocarpa</i>	Alkaloids	MS + 2,4-D + Kinetin	Callus
66. <i>Corydalis terminalis</i>	Corydaline	MS + 2,4-D + BAP	Callus
67. <i>Coscinium fenestratum</i>	Berberine	MS + 2,4-D + BAP	Callus
68. <i>Coscinium fenestratum</i>	Berberine	MS + IAA + BAP	Callus
69. <i>Coscinium fenestratum</i>	Berberine	MS + 2,4-D + GA3	Suspension
70. <i>Crataegus sinaica</i>	Flavonoid	MS + 2,4-D + NAA + BAP	Callus
71. <i>Croton sublyratus</i>	Plaunotol	MS + NAA + BA	Callus

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
72. <i>Cruciata glabra</i>	Anthraquinones	LS + NAA + Kinetin	Suspension
73. <i>Cryptolepis buchanani</i>	Cryptosin	B5 + 2,4-D + Kinetin	Callus
74. <i>Cymbopogon citratus</i>	Essential oil	MS + IAA + GA3	Shoot
75. <i>Datura stramonium</i>	Hyocyamine	MS + IAA	Hairy root
76. <i>Digitalis purpurea</i>	Cardenolides	MS + BA	Suspension
77. <i>Digitalis purpurea</i>	Cardioactive glycosides	MS + 2,4-D + BA	Hairy root
78. <i>Diocorea doryophora</i>	Diogenin	MS + 2,4-D + BA	Suspension
79. <i>Dioscorea deltoidea</i>	Diosgenin	MS + 2,4-D	Suspension
80. <i>Drosera rotundifolia</i>	7-Methyljuglone	MS + BAP + NAA	Shoot culture
81. <i>Duboisia leichhardtii</i>	Alkaloids	LS + NAA + BA	Callus
82. <i>Duboisia leichhardtii</i>	Scopolamine	MS + 2,4-D + BA	Hairy root
83. <i>Duboisia myoporoides</i>	Scopalamine	MS + IAA	Hairy root
84. <i>Echinacea purpurea</i>	Alkamides	MS + 2,4-D	Hairy root
85. <i>Eleutherococcus senticosus</i>	Eleuthrosides	MS + 2,4-D	Suspension
86. <i>Ephedra</i> sp.	L-Ephedrine	MS + Kinetin + 2,4-D	Suspension
87. <i>Eriobotrya japonica</i>	Triterpenes	LS + NAA + BA	Callus
88. <i>Eucalyptus tereticornis</i>	Sterols and phenolic compounds	MS + 2,4-D	Callus
89. <i>Fabiana imbricata</i>	Rutin	MS + NAA + 2,4-D	Callus and Suspension
90. <i>Fagopyrum esculentum</i>	Flavonol	MS + IAA + GA3	Hairy root
91. <i>Fagopyrum esculentum</i>	Rutin	MS + NAA	Hairy root
92. <i>Frangula alnus</i>	Anthraquinones	WPM + IAA + BAP	Callus
93. <i>Fritillaria unibracteata</i>	Alkaloids	MS + 2,4-D + Kin	Multiple shoot

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
94. <i>Fumaria capreolata</i>	Alkaloids	LS + IAA	Suspension
95. <i>Gentiana macrophylla</i>	Glucoside	MS + IAA + Kin	Hairy root
96. <i>Gentiana</i> sp.	Glucosides	B5 + Kinetin	Callus
97. <i>Gentianella austriaca</i>	Xanthone	MS + BAP	Multiple shoot
98. <i>Geranium thunbergii</i>	Tannin	MS + 2,4-D + BAP	Hairy root
99. <i>Ginkgo biloba</i>	Ginkoside-A	MS + NAA + Kinetin	Suspension
100. <i>Glehnia littoralis</i>	Furanocoumarin	LS + 2,4-D + Kinetin	Suspension
101. <i>Glycyrrhiza echinata</i>	Flavonoids	MS + IAA + Kinetin	Callus
102. <i>Glycyrrhiza glabra</i>	Triterpenes	MS + IAA + Kinetin + 2,4-D	Callus
103. <i>Glycyrrhiza glabra</i>	Glycyrrhizin	MS + 2,4-D + GA3	Hairy root
104. <i>Glycyrrhiza glabra</i>	Flavonoid	MS + IAA	Hairy root
105. <i>Gymnema sylvestre</i>	Gymnemic acid	MS + 2,4-D + IAA	Callus
106. <i>Gymnema sylvestre</i>	Gymnemic acid	MS + IAA + BA	Callus
107. <i>Gynostemma pentaphyllum</i>	Saponin	MS + 2,4-D + BAP	Hairy root
108. <i>Gypsophila paniculata</i>	Saponin	MS + IAA + TDZ	Root suspension
109. <i>Hemidesmus indicus</i>	Lupeol, Rutin	MS + BAP + NAA	Shoot culture
110. <i>Hyocyamus niger</i>	Tropane alkaloids	MS + 2,4-D + BA	Callus
111. <i>Hyocyamus niger</i>	Tropane alkaloids	MS + IAA + Kinetin	Hairy root
112. <i>Hyoscyamus albus</i>	Phytotoxins	MS + NAA + GA3	Hairy root
113. <i>Hyoscyamus muticus</i>	Hyoscyamine	MS + 2,4-D	Hairy root
114. <i>Hypericum perforatum</i>	Hypericin liquid	MS + NAA + GA3	Suspension
115. <i>Hypericum perforatum</i>	Hypericins	MS + BA + IAA	Multiple shoot

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
116. <i>Hypericum perforatum</i>	Hypericin	MS + BA + TDZ	Multiple shoot
117. <i>Hypericum perforatum</i>	Hyperforin	MS + 2,4-D + Leusine	Multiple shoot
118. <i>Hyssopus officinalis</i>	Triterpenes	G5 + 2,4-D + IAA	Suspension
119. <i>Hyssopus officinalis</i>	Sterols	MS + 2,4-D + NAA	Suspension
120. <i>Ipomoea cairica</i>	Lignan	MS + IAA + Kin	Callus
121. <i>Isoplexis isabelliana</i>	Anthraquinone	MS + 2,4-D + Kinetin	Suspension
122. <i>Lactuca virosa</i>	Sesquiterpene lactones	MS + 2,4-D	Hairy root
123. <i>Leontopodium alpinum</i>	Essential oil	MS + IAA + BA	Hairy root
124. <i>Linum flavum</i>	5-Methoxyphyllotaxin	MS salts + B5 Vitamins	Suspension
125. <i>Linum flavum</i>	Lignan	MS + IAA + GA3	Hairy root
126. <i>Lithospermum erythrorhizon</i>	Shikonin derivatives	LS + IAA + Kinetin	Suspension
127. <i>Lithospermum erythrorhizon</i>	Shikonin	MS + 2,4-D + Kinetin	Hairy root
128. <i>Lobelia cardinalis</i>	Polyacetylene glucosides	MS + 2,4-D	Hairy root
129. <i>Lycium chinense</i>	Cerebroside	MS + 2,4-D, Kinetin	Suspension
130. <i>Mentha arvensis</i>	Terpenoid	MS + BA + NAA	Shoot
131. <i>Momordica charantia</i>	Flavonoid	MS + BAP + NAA	Callus
132. <i>Morinda citrifolia</i>	Anthraquinones	B5 + NAA	Suspension
133. <i>Mucuna pruriens</i>	L-Dopa	MS + IAA	Suspension
134. <i>Myristica fragrans</i>	Myristin	MS + NAA + TDZ	Shoot
135. <i>Nandina domestica</i>	Alkaloids	MS + 2,4-D + Kinetin	Callus
136. <i>Nicotiana hesperis</i>	Anatabine	MS + IAA	Hairy root
137. <i>Nicotiana rustica</i>	Alkaloids	LS + 2,4-D + Kinetin	Callus

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
138. <i>Nicotianan tabacum</i>	Nicotine	MS + NAA + Kinetin	Suspension
139. <i>Ophiorrhiza rugosa</i> var. <i>decumbens</i>	Camptothecin	MS + BA + Kin	Shoot
140. <i>Panax ginseng</i>	Saponin and spogenins	MS + 2,4-D	Callus
141. <i>Panax ginseng</i>	Glycoside	MS + NAA + Kin	Hairy root
142. <i>Panax notoginseng</i>	Gensenosides	MS + 2,4-D + Kinetin	Suspension
143. <i>Papaver bracteatum</i>	Thebaine	MS + Kinetin + 2,4-D	Callus
144. <i>Papaver somniferum</i>	Alkaloids Morphine and codeine	MS + Kinein	Callus
145. <i>Papaver somniferum</i>	Codeine	LS + BA + NAA	Hairy root
146. <i>Peganum harmala</i>	Alkaloids	MS + 2,4-D	Suspension
147. <i>Perezia cuernavacana</i>	Sesquiterpene quinone	MS + IAA + BA	Hairy root
148. <i>Ophiorrhiza pumila</i>	Alkaloids	LS + 2,4-D + NAA	Callus
149. <i>Phytolacca americana</i>	Betacyanin	MS + 2,4-D	Suspension
150. <i>Picrasma quassoides</i>	Quassain	B5 + 2,4-D + Kinetin	Suspension
151. <i>Pimpinella anisum</i>	Essential oil	MS + IAA + BAP	Hairy root
152. <i>Piper solianum</i>	Piperine	MS + 2,4-D + BA	Suspension
153. <i>Plantago media</i>	Verbascoside	B5 + IAA + Kin	Callus
154. <i>Platycodon grandiflorum</i>	Polyacetylene	MS + 2,4-D	Hairy root
155. <i>Pluchea lanceolata</i>	Quercetin	MS + NAA + BAP	Callus
156. <i>Plumbago rosea</i>	Plumbagin	MS + CaCl ₂	Callus
157. <i>Plumbago zeylanica</i>	Plumbagin	MS + BAP + IBA	Hairy root
158. <i>Podophyllum hexandrum</i>	Podophyllotoxin	B5 + NAA	Suspension
159. <i>Podophyllum hexandrum</i>	Podophyllotaxin	MS + BAP + GA32	Shoot

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
160. <i>Polygala amarella</i>	Saponin	MS + IAA	Callus
161. <i>Polygonum hydropiper</i>	Flavonoids	MS + 2,4-D + Kinetin	Suspension
162. <i>Portulaca grandiflora</i>	Betacyanin	MS + 2,4-D + Kinetin	Callus
163. <i>Psoralea veris</i>	Saponins	MS + BAP + GA3	Shoot
164. <i>Psoralea corylifolia</i>	Isoflavones	MS + TDZ + BAP	Multiple shoot
165. <i>Ptelea trifoliata</i>	Alkaloids	MS + 2,4-D + Kinetin	Callus
166. <i>Rauvolfia sellowii</i>	Alkaloids	B5 + 2,4-D + Kinetin	Callus
167. <i>Rauvolfia serpentina</i>	Reserpine	LS + NAA +BA	Suspension
168. <i>Rauvolfia serpentina</i>	Serpentine	MS + BAP + IAA	Callus
169. <i>Rauvolfia serpentina</i>	Reserpine	MS + IAA + Cu ²⁺	Callus
170. <i>Rauvolfia tetraphylla</i>	Reserpine	MS + 2,4-D + Tryptophan	Callus
171. <i>Rhamnus catharticus</i>	Anthraquinones	WPM + Kin + 2,4-D	Callus
172. <i>Rheum ribes</i>	Catechin	MS + IBA + BA	Callus
173. <i>Rhodiola rosea</i>	Rosarin	MS + NAA + IAA	Callus culture
174. <i>Rhus javanica</i>	Gallotannins	LS + IAA + Kinetin	Root
175. <i>Rubia akane</i>	Anthraquinone	B5 + NAA + Kin	Hairy root
176. <i>Rubia akane</i>	Anthraquinone	MS + 2,4-D + Chitosan	Suspension
177. <i>Rubia tinctorum</i>	Anthraquinone	MS + 2,4-D	Hairy root
178. <i>Ruta</i> sp.	Alkaloids and coumarins	MS + 2,4-D + Kinetin	Callus
179. <i>Salvia miltiorrhiza</i>	Rosmarinic acid Cryptotanshinone	MS + 2,4-D + BA	Callus
180. <i>Salvia miltiorrhiza</i>	Rosmarinic acid Cryptotanshinone	MS + 2,4-D + Kinetin	Suspension
181. <i>Salvia officinalis</i>	Flavonoid	LMS + IAA + BAP	Multiple shoot
182. <i>Salvia officinalis</i>	Terpenoids	MS + 2,4-D + BA	Callus
183. <i>Saponaria officinalis</i>	Saponin	MS + IAA + TDZ	Suspension

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
184. <i>Saprosma fragrans</i>	Anthraquinone	MS + 2,4-D + NAA	Callus
185. <i>Scoparia dulcis</i>	Scopadulic acid	LMS + Kin + Phenyl urea	Callus
186. <i>Scopolia parviflora</i>	Alkaloids	LS + 2,4-D + IAA	Callus
187. <i>Scutellaria baicalensis</i>	Flavonoids	MS + IAA	Hairy root
188. <i>Scutellaria columnae</i>	Phenolics	MS + 2,4-D + Kinetin	Callus
189. <i>Serratula tinctoria</i>	Ecdysteroid	MS + 2,4-D + BA	Hairy root
190. <i>Sesamum indicum</i>	Napthaquinone	MS + NAA + Kinetin	Hairy root
191. <i>Silybum marianum</i>	Silymarin	MS + IAA + GA3	Hairy root
192. <i>Silybum marianum</i>	Flavonolignan	LS + TDZ	Root
193. <i>Silybum marianum</i>	Silymarin	MS + IAA + Kin	Hairy root
194. <i>Silybum marianum</i>	Silymarin	MS + IAA + BA	Callus
195. <i>Simmondsia chinensis</i>	Fixed oil	MS + TDZ + GA3	Callus
196. <i>Simmondsia chinensis</i>	Fixed oil	MS + IAA + 2iP	Callus
197. <i>Solanum aculeatissi</i>	Steroidal saponin	MS + 2,4-D	Hairy root
198. <i>Solanum chrysotrichum</i>	Saponin	MS + 2,4-D + Kinetin	Suspension
199. <i>Solanum laciniatum</i>	Solasodine	MS + 2,4-D + Kinetin	Suspension
200. <i>Solanum paludosum</i>	Solamargine	MS + BA + Kinetin	Suspension
201. <i>Stevia rebaudiana</i>	Stevioside	MS + BA + NAA	Callus
202. <i>Swertia japonica</i>	Amarogenetin	MS + IAA	Hairy root
203. <i>Tabernaemontana divaricata</i>	Alkaloids	MS + NAA + BA	Suspension
204. <i>Tagetes patula</i>	Thiophenes	MS + IAA + Kinetin	Hairy root
205. <i>Tanacetum parthenium</i>	Sesquiterpene	MS + 2,4-D + Kinetin	Hairy root

(continued)

Table 7.1 (continued)

Plant name	Active ingredient	Culture medium and growth regulator(s)	Culture type
206. <i>Taxus baccata</i>	Taxol baccatin III	B5 + 2,4-D + Kinetin + GA3	Suspension
207. <i>Taxus</i> spp.	Taxol	B5 + 2,4-D + BA	Suspension
208. <i>Thalictrum minus</i>	Berberin	LS + NAA + 2,4-D + BA	Suspension
209. <i>Tinospora cordifolia</i>	Berberin	MS + IAA + GA3	Suspension
210. <i>Torreya nucifera</i>	Diterpenoids	MS + 2,5-D	Suspension
211. <i>Trichosanthes kirilowii</i>	Protein	MS + IAA	Hairy root
212. <i>Trigonella foenum-graecum</i>	Saponins	MS + 2,4-D + Kinetin	Suspension
213. <i>Vaccinium myrtillus</i>	Flavonoids	MS + BAP + NAA	Callus culture
214. <i>Vinca major</i>	Vincamine	MS + BAP	Hairy root
215. <i>Vitis vinifera</i>	Anthocyanin	MS + BAP + NAA	Suspension
216. <i>Vitis vinifera</i>	Resveratrol	MS + IAA + GA3 + UV	Callus
217. <i>Withania somnifera</i>	Withaferin A	MS + BA	Shoot
218. <i>Withania somnifera</i>	Withaferin	MS + IAA + Kintin	Hairy root
219. <i>Withania somnifera</i>	Withanoloid A	MS + IAA + Kin	Hairy root
220. <i>Withania somnifera</i>	Steroidal lactone	MS + 2,4-D + BA	Callus
221. <i>Zataria multiflora</i>	Rosmarinic acid	MS + IAA + Kin	Callus

convergence of many areas of research, such as molecular biology, microbiology, biochemistry, immunology, genetics, and cell biology; and includes techniques such as molecular cloning, polymerase chain reaction, gel electrophoresis, macromolecule blotting and probing, microarrays, allele-specific oligonucleotide, high throughput screening (HTS), techniques of in vitro synthesis of bioactive molecules, etc. It is an exciting field fueled by the ability to transfer genetic information between organisms with the goal of understanding important biological processes or creating a useful product. Information from human genomics project has opened a myriad of opportunities to create new medicines and treatments, as well as approaches to improve existing medicines. Molecular biotechnology is a rapidly changing and dynamic field. The importance and impact of molecular biotechnology is being felt across the nation. Molecular biotechnology has applications in

Table 7.2 Pharmaceutically valuable bioactive compounds obtained from different plant sources

Compound	Plant species	Medicinal value
(i) Shikonin	<i>Lithospermum erythrorhizon</i>	Antiseptic (also used as dye for silk and cosmetics)
(ii) Berberine	<i>Coptis japonica</i>	Antibacterial, anti inflammatory
(iii) codeine	<i>Papaver somniferum</i>	Analgesic
(iv) Diosgenin	<i>Dioscorea deltoidea</i>	Antifertility agents
(v) Quinine	<i>Cinchona</i>	Antimalarial
(vi) Scopolamine	<i>Datura stramonium</i>	Antihypertensive
(vii) Vincristine	<i>Catharanthus roseus</i>	Antileukemic
(viii) Ajmalicine	<i>C. roseus</i>	
(ix) Taxol ^a	<i>Taxus species</i>	Breast and ovarian cancer treatment
(x) Artemisinin	<i>Artemisia</i> sp.	Antimalarial
(xi) Trichosanthin	<i>Trichosanthes</i> sp.	Cytotoxicity against HIV infected cells, immunosuppressant, induces abortion
(xii) Karasurin ^b		

^aActs on spindle-like colchicine; promotes dissolution of microtubules into tubulin molecules;

^bProteins isolated from rhizomes of the traditional Chinese medicinal plant

plant and animal agriculture, aquaculture, chemical and textile manufacturing, forestry, and food processing and the tools of molecular biotechnology can be applied to develop and improve drugs, vaccines, therapies, and diagnostic tests that will improve human and animal health.

7.2 Advantages of Tissue Cultures in Production of Useful Bioactive Compounds

In *in vitro* technique for plant bioactive compound production, plant cells, tissues and organs are cultivated under aseptic conditions independently of geographical and climatic factors. It offers an alternative approach for producing important bioactive metabolites in the face of different adverse circumstances such as circumstances loss of plant populations, genetic diversity, habitat degradation and, even, species extinction, etc. It has emerged as a viable biotechnological tool for the production of bioactive compounds that can be used in the most diversified areas and particularly with a view of an additional effort for sustainable conservation and rational utilization of biodiversity. Plant tissue culture technology could be a potential alternative approach for production of high-value bioactive phytoconstituents of therapeutic importance and might be attractive under certain conditions such as: when (i) the source plant is difficult to cultivate, (ii) has a long cultivation

period, (iii) has a low metabolite yield, (iv) chemical synthesis has not been achieved due to technical problem, etc. In addition, (v) novel compounds which are not generally found in the parent plants can be produced in the in vitro grown plants through plant tissue culture as well as (vi) stereo- and region-specific biotransformation of the plant cells can be performed for the production of bioactive compoundS from economical precursors.

With the increasing demand of the market for novel products derived from plants, in vitro culture has become a reliable technique for the mass production of plant material. These and a number of other advantages in using plant cell culture provide impetus for its use for large-scale production of important bioactive compounds at industrial level. These advantages are summarized as follows:

- (i) Plant cell cultures are independent from environmental factors;
- (ii) Production levels may be geared more accurately according to the market demand;
- (iii) By using characterized cell lines, a more consistent product quality and yield can be maintained;
- (iv) New routes of synthesis can be recovered from mutant cell lines which may lead to the development of novel products;
- (v) Culture of cells will reduce the pressure on already overexploited medicinal and other economically important plants.
- (vi) The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products;
- (vii) The advantage of the cell cultures include synthesis of bioactive secondary metabolites, running in controlled environment, independently from climate and soil conditions;
- (viii) The use of in vitro plant cell culture for the production of chemicals and pharmaceuticals has made great strides building on advances in plant science;
- (ix) The increased use of genetic tools and an emerging picture of the structure and regulation of pathways for secondary metabolism will provide the basis for the production of commercially acceptable levels of product;
- (x) The increased level of natural products for medicinal purposes coupled with the low product yields and supply concerns of plant harvest has renewed interest in large-scale plant cell culture technology;
- (xi) Knowledge of biosynthetic pathways of desired phytochemicals in plants as well as in cultures is often still in its infancy, and consequently strategies needed to develop an information based on a cellular and molecular level. These results show that in vitro plant cell cultures have potential for commercial production of secondary metabolites; and
- (xii) The introduction of newer techniques of molecular biology, so as to produce transgenic cultures and to effect the expression and regulation of biosynthetic pathways, is also likely to be a significant step toward making cell cultures more generally applicable to the commercial production of secondary metabolites.

7.3 Herbal Preparations and Disease Management (Prevention and Treatment)

Medicinal plants play an important role in preventing and treating of human diseases. The bioactive phytochemicals present in them play important role in health promotion and disease prevention. The curative efficacy of medicinal plants is due to these bioactive metabolites (pharmacologically active compounds), e.g., alkaloids have an antispasmodic, antimalarial, analgesic, diuretic activities; phenols and flavonoids have an antioxidant, anti-allergenic, antibacterial properties; terpenoids are known for their antiviral, anthelmintic, antibacterial, anticancer, antimalarial, anti-inflammatory properties; glycosides are reported for antifungal and antibacterial properties; and saponins are reported to have anti-inflammatory, antiviral, plant defense activities. The use of medicinal herbs and herbal preparation is an age-old tradition and the recent progress in modern therapeutics has stimulated the use of these natural therapeutics in the prevention and treatment of different diseases. Different bioactive compounds from different plant sources are found to be effective against a vast array of diseases, e.g., taxol from *Taxus brevifolia* and vinblastine and vincristine from *Catharanthus roseus*, topotecan and irinotecan from *Camptotheca acuminata*, etoposide and teniposide from *Podophyllum peltatum* show antitumor and anticancer activity; curcumin from *Cucumis longa* show anticancer, anti-inflammatory, hepatoprotective; flavonoid silymarin (silibinin) from *Silybum marianum* show anticancer, anti-inflammatory, liver tonic for hepatic disorders property; ricinine), lectin (ricin) from *Ricinus communis* show hepatoprotective, antioxidant, hypoglycemic, antitumor activity; tannins, shikimic acid compounds, triterpenoids, ellagic acid from *Terminalia chebula* show antioxidant, antidiabetic, renoprotective, hepatoprotective activity; steroid lactones, withanolides, notably withaferin A from *Withania somnifera* show chemopreventive, anticancerous, memory enhancer, and immunomodulatory properties and used in parkinson's and alzheimer's disorders; mono and sesquiterpenoids, zingerone and gingerols from *Zinziber officinalis* are anticancer, antioxidant, hepatoprotective, hypercholesterolaemic, anti-atherosclerotic; limonoids (nimbidinin), di- and tri- terpenoids from *Azadirachta indica* function as inhibitor of carcinoma, chemopreventive, inhibit colon cancer, antiallergic, blood purifier; piperidine, dehydropiperonaline of *Piper nigrum* are anticarcinogenic, anti-hyperlipidaemic, useful in epilepsy; diterpenoid furanolactones (tinosporin), isoquinoline alkaloids from *Tinospora cordifolia* function as immunomodulator, chemopreventive, cardioprotective, antidiabetic agents; aloin and emodin, campesterol, β -sisosterol from *Aloe vera* show healing properties, antiviral and antitumor activity antidiabetic, hepatoprotective, antiseptic effect; apigenin, taxol and ursolic acid, citral from *Ocimum sanctum* show antidiabetic, hepatoprotective antibacterial, antifungal, antipyretic, and anticancer properties; berberine from *Berberis vulgaris* works as antidiabetic, hepatoprotective, antimicrobial; digoxin, from *Digitalis lanata* is used in heart diseases; thymoquinone from *Nigella sativa* show antidiabetic, anticancer, antimicrobial, hepatorenal protective, and gastro-protective; quinquefuscin from *Cinchona robusta*

show antimalarial, antiparasitic effect; artemisinin from *Artemisia absinthium* is an antimalarial drug; ophelic acid, sawertiamarine, mangeferin and amarogenitine from *Swertia chirata* show antidiabetic antiviral, hepatorenal protective activities; allicin from *Allium sativum* is cardioprotective, anti-inflammatory; arjunic acid, tannic acid, tannins, saponins, gallic acid and phytosterols from *Terminalia arjuna* are cardioprotective, anticancer agents, hepatoprotective; emblicanin A, emblicanin B, punigluconin, and pedunculagin from *phyllanthus emblica* are antiviral, antimicrobial, anticancer, hepatoprotective and antidiabetic; ajmalicine and reserpine from *Rauvolfia serpentine* show hypotensive properties, phenol compounds from *Gynura procumbens* are antidiabetic, etc.

7.3.1 Herbal Extracts and Management of Chronic Diseases

Herbal medicines are the most preferred ways of complementary and alternative medicine (CAM) used all over the world for the management of different chronic diseases. Diabetes mellitus (DM), hypertension (HT), hyperlipidemia (HL), etc., among others, are the most prevalent chronic diseases in the world. Some other leading chronic diseases include osteoporosis, cardiovascular disease (heart attacks, stroke, ischemic cardiopathy), chronic obstructive pulmonary disease (COPD), asthma, epilepsy and seizures, obesity, oral health problems, hepatitis C and HIV/AIDS, dementia, Alzheimer's, autoimmune, and Parkinson's disease, schizophrenia, bipolar disorder, multiple sclerosis, glaucoma, etc. Patients with chronic diseases frequently or occasionally use herbal medicines including CAM all over the world for treatment because of diversity, low cost, and safety.

In a work with a total of 217 patients (55 male and 162 female and 56.6 ± 9.7 years mean age of the participants), Tulunay et al. (2015) noted about 29% patient used herbal medicine use and use among female gender was significantly higher ($P = 0.040$). Conventional medication use was found to be lower among herbal medicine consumers and the most frequently used herbs were lemon (39.6%) and garlic (11.1%) for HT, cinnamon (12.7%) for DM, and walnut (6.3%) for HL. A number of photochemicals (nutraceuticals), e.g., omega-3-fatty acids, dietary fibers, vitamins, antioxidants, plant sterols, flavonoids from the medicinal plants that have beneficial effects on the chronic diseases and nutraceutical photochemicals without any side effects, less cost and also abundant helps to prevent a number of chronic diseases and act as chronic fighters.

Diabetes management

Diabetes mellitus (DM) should not be confused with diabetes insipidus (DI). Diabetes insipidus occurs when a person's kidneys pass an abnormally large volume of urine that is insipid (dilute and odorless). In most people, the kidneys pass about 1–2 quarts of urine a day, but in person with DI, the kidneys can pass 3–20 quarts of urine a day. As a result, a person with DI may feel the need to drink large

amounts of liquids. DM results from insulin deficiency or resistance leading to high blood glucose, also called blood sugar. Diabetes insipidus and diabetes mellitus are unrelated, although both conditions cause frequent urination and constant thirst. Diabetes mellitus causes high blood glucose, or blood sugar, resulting from the body's inability to use blood glucose for energy. People with diabetes insipidus have normal blood glucose levels; however, their kidneys cannot balance fluid in the body. Excessive urination and extreme thirst as a result of inadequate output of the pituitary hormone ADH (antidiuretic hormone—vasopressin) (Fig. 7.1) or the lack of the normal response by the kidney to ADH. There are four types of diabetes insipidus; (i) central diabetes insipidus, (ii) nephrogenic diabetes insipidus, (iii) dipsogenic diabetes insipidus, and (iv) gestational diabetes insipidus. The most common symptom of DI is frequent urination and it is a rare disorder, i.e., a rare disease that causes frequent urination.

Diabetes mellitus (DM) is one of the most common and serious chronic diseases worldwide. DM is a chronic endocrine disorder involving most common metabolic disorders of carbohydrate, fat, and protein which are grouped under non-communicable disease (NCD). DM is characterized by elevated plasma glucose concentrations resulting from insufficient insulin. Type 1 diabetes is called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes and type 2 diabetes is called noninsulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes. The National Institute for Clinical Excellence (NICE) recommended target blood glucose level ranges for normal and diabetic blood sugar. For the majority of healthy individuals, normal blood sugar levels are as follows (NICE:Diabetes.co.uk: https://www.diabetes.co.uk/diabetes_care/blood-sugar-level-ranges).

(i) Between 4.0 and 6.0 mmol/L (72–108 mg/dL) when fasting; (ii) Up to 7.8 mmol/L (140 mg/dL) 2 h after eating. For people with diabetes, blood sugar level targets are as follows; (iii) Before meals: 4–7 mmol/L for people with type 1 or type 2 diabetes; (iv) After meals: under 9 mmol/L for people with type 1 diabetes and under 8.5 mmol/L for people with type 2 diabetes. Table 7.3 summarizes these data.

Fig. 7.1 Vasopressin, an antidiuretic hormone (ADH) also arginine vasopressin (AVP) or argipressin. It is synthesized as a peptide prohormone in neurons in the hypothalamus, and then converted to AVP

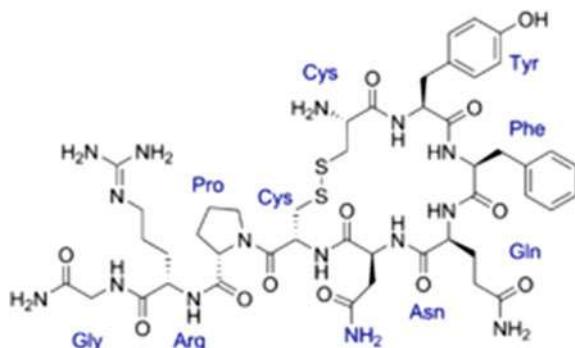


Table 7.3 NICE recommended target blood glucose level ranges

Target levels by type	Upon waking	Before meals (pre-prandial)	At least 90 min after meals (postprandial)
Nondiabetic ^a		4.0–5.9 mmol/L	under 7.8 mmol/L
Type 2 diabetes		4–7 mmol/L	under 8.5 mmol/L
Type 1 diabetes	5–7 mmol/L	4–7 mmol/L	5–9 mmol/L
Children w/type 1 diabetes	4–7 mmol/L	4–7 mmol/L	5–9 mmol/L

^aThe non-diabetic figures are provided for information but are not part of NICE guidelines

Table 7.4 Blood sugar levels in diagnosing diabetes

Blood sugar levels in diagnosing diabetes			
Plasma glucose test	Normal	Predabetes	Diabetes
Random	Below 11.1 mmol/L below 200 mg/dL	N/A	11.1 mmol/L or more 200 mg/dL or more
Fasting	Below 6.1 mmol/L below 108 mg/dL	6.1–6.9 mmol/L to 108–125 mg/dL	7.0 mmol/L or more 126 mg/dL or more
2 h postprandial	Below 7.8 mmol/L below 140 mg/dL	7.8–11.0 mmol/L to 140–199 mg/dL	11.1 mmol/L or more 200 mg/dL or more

The data on the range of blood sugar levels in diagnosing diabetes and prediabetes and prediabetes are shown in the Table 7.4.

Random plasma glucose test: A blood sample for a random plasma glucose test can be taken at any time. This does not require much planning and is, therefore, used in the diagnosis of type 1 diabetes when time is of the essence.

Fasting plasma glucose test: A fasting plasma glucose test is taken after at least 8 h of fasting and is, therefore, usually taken in the morning. The NICE guidelines regard a fasting plasma glucose result of 6.1–6.9 mmol/L as putting someone at higher risk of developing type 2 diabetes, particularly when accompanied by other risk factors for type 2 diabetes.

Oral glucose tolerance test (OGTT): An oral glucose tolerance test involves first taking a fasting sample of blood and then taking a very sweet drink containing 75 g of glucose. After having this drink, you need to stay at rest until a further blood sample is taken after 2 h.

HbA1c test for diabetes diagnosis: An HbA1c test does not directly measure the level of blood glucose, however, the result of the test is influenced by how high or low your blood glucose levels have tended to be over a period of 2–3 months.

Indications of diabetes or prediabetes are given under the following conditions:

- (i) **Normal:** Below 42 mmol/mol (6.0%)
- (ii) **Prediabetes:** 42–47 mmol/mol (6.0–6.4%)
- (iii) **Diabetes:** 48 mmol/mol (6.5% or over)

A comprehensive herbal drug therapeutic regimen offers time-tested safe and effective support to conventional therapy in the management of diabetes. This is combination with adequate dietary management and physical activity would provide an integrated approach to the management of this deadly disease, particularly Type 2 diabetes. Development of type 2 diabetes has been linked to β -cell failure coupled with *insulin* resistance and obesity. Adipose tissue, known as the fat store, secretes a number of hormones and proteins collectively termed adipokines some of which regulate *insulin* sensitivity.

Diabetes is a metabolic, endocrine disorder which is characterized by hyperglycemia and glucose intolerance due to insulin resistance and researchers have confirmed that inflammation is closely involved in the pathogenesis of diabetes and its complications. Herbal medications have long been used in the treatment and prevention of T2DM in both traditional Chinese medicine (TCM) and traditional Indian medicine (Ayurveda, Unani). Existing therapeutic drugs used for diabetes increase various secondary complications including cardiovascular disease, kidney failure, liver injury, dizziness, mental disorders, weight gain, and skin diseases. Nowadays, the key therapeutic agents to treat T2DM and its complications, sulfonylureas, metformin, and insulin-sensitizing glitazones all improve metabolic control and lead to control of various circulating inflammation mediators through innate immunity-related signaling pathways. Sulfonylureas and metformin are main drugs to prevent the T2DM, and sulfonylureas increase insulin production from pancreatic β -cells, while metformin suppresses glucose production in the liver and meanwhile increases insulin sensitivity in peripheral tissues. Glitazones, another antidiabetic drug, binds to peroxisome proliferator-activated receptors (PPARs), beginning a transcriptional activity that leads to improved insulin action through reducing the secretion of inflammatory markers. Consequently, glitazones reduced levels of CRP, PAI-1, TNF- α , and other inflammatory markers. These drugs showed better antidiabetic nature and also have the comparable anti-inflammatory potential.

Other therapeutic approaches for T2DM that would act as principals in the inflammatory system have been proposed in the form of salicylates, an anti-inflammatory therapeutic that inhibits I κ B kinase (IKK), and also lowering the glucose level through improvement of beta cell function. Various well-established nonsteroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase inhibitors (e.g., ibuprofen, naproxen) are able to improve glucose-mediated macrosomia. Researchers are searching for efficient natural therapeutic targets with less or no side effects from natural products'-derived bioactive molecules to improve insulin resistance and associated complications through suppression of inflammatory signaling pathways.

Table 7.5 Some selected botanical therapeutics and their proposed mode of carbohydrate metabolism

Botanical therapeutics	
Scientific name	Proposed actions
<i>Hoodia gordonii</i>	WL, decreases appetite
<i>Opuntia</i> spp.	Decreases LDL; decreases TG; decreases PPG; decreases IS
<i>Cinnamomum cassia</i> , <i>Cinnamomum verum</i> , and others	Increases IS; decreases FPG; decreases PPG; decreases BP; decreases LDL; decreases TG
<i>Artemisia dracunculus</i> L.	Increases IS; decreases PPG
<i>Momordica charantia</i>	Increases IS; decreases FPG; decreases PPG; decreases LDL; decreases TG
<i>Trigonella foenum-graecum</i>	Increases IS; decreases FPG; decreases LDL; decreases TG
<i>Gymnema sylvestre</i>	Increases IS; decreases FPG; decreases PPG; decreases LDL; decreases TG; increases Ins sec
<i>Allium sativum</i>	Decreases BP; decreases LDL
<i>Ginkgo biloba</i>	Decreases BP
<i>Panax</i> spp.	Decreases BP
<i>Aloe vera</i>	Increases IS; decreases FPG
<i>Coccinia indica</i>	Increases IS; decreases FPG

Source Cefalu et al. (2011)

BP = blood pressure; LDL = LDL-cholesterol; TG = triglycerides; FPG = fasting blood glucose; PPG = postprandial blood glucose; IS = insulin sensitivity; WL = weight loss; and Ins sec = insulin secretion.

There are a huge number of active medicinal plants and its natural bioactive molecules that have already reported the therapeutic nature against diabetes. Several medicinal plants have been used since ancient times to manage and prevent diabetes and associated conditions (Table 7.5).

All parts of antidiabetic plants are not equally important for the preparation of hypoglycemic extract, so different parts (including the whole plant to leaf, root, rhizome, flower, fruit, seed, etc.) of antidiabetic plants are used for their desired hypoglycemic activity as shown in Table 7.6.

Mode of action of different bioactive phytoconstituents in hyperglycemia

Different bioactive phytoconstituents follow similar or different ways to alleviate hyperglycemic activity as

- (i) Alkaloids—Inhibit alpha glucosidase and decrease glucose transport through the intestinal epithelium, e.g., berberine (roots, stem bark of *Berberis* spp., *T. cordifolia*); casuarine 6-O- α -glucoside (bark of *Syzygium malaccense*); catharanthine, vindoline, and vindoline (leaf and stem of

Table 7.6 Some selected antidiabetic plants with hypoglycemic activity distributed in their whole body or in different parts

Sl. no.	Plant/part	Name of plants
(i)	Whole plant	<i>Abies pindrow, Achyranthes aspera, Ajuga iva, Aloe vera, Anacardium occidentale, Andrographis paniculata, Capsicum frutescens, Cryptolepis sanguinolenta, Enicostemma littorale, Ficus religiosa</i>
(ii)	Roots	<i>Clausena anisata, Glycrrhiza glabra, Helicteres isora, Pandanus odoratus</i>
(iii)	Rhizome	<i>Nelumbo nucifera</i>
(iv)	Bulb	<i>Allium cepa, Allium sativum</i>
(v)	Modified root	<i>Ipomoea batatas</i>
(vi)	Aerial parts	<i>Artemisia pallens, Bidens pilosa, Bixa orellana, Teramnus labialis</i>
(vii)	Leaves	<i>Aloe barbadensis, Annona squamosa, Averrhoa bilimbi, Azadirachta indica, Beta vulgaris, Camellia sinensis, Cassia alata, Eclipta alba, Eucalyptus globulus, Euphrasia officinale, Ficus carica, Gymnema sylvestre, Gynura procumbens, Ipomoea aquatica, Mangifera indica, Myrtus communis, Memecylon umbellatum, Morus indica, Ocimum sanctum, etc.</i>
(viii)	Flower	<i>Cassia auriculata, Gentiana olivieri, Musa sapientum</i>
(ix)	Fruit	<i>Carum carvi, Coriandrum sativum, Embellica officinalis, Juniperus communis, Momordica charantia, Xanthium strumarium</i>
(x)	Seed	<i>Acacia arabica, Agrimony eupatoria, Lupinus albus, Luffa aegyptiaca, Lepidium sativum, Mucuna pruriens, Punica granatum</i>
(xi)	Stem	<i>Amaranthus spinosus, Coscinium fenestratum</i>
(xii)	Bark	<i>Cinnamomum zeylanicum, Croton cajucara</i>

C. roseus); calystegine B2 (fruits of *Nicandra physalodes*); cryptolepine (*Cryptolepis sanguinolenta*); harmane, norharmane, (*Tribulus terrestris*); jambosine (bark, seeds, fruits, *Syzygium cumini*); jatrorrhizine, magnoflorine, palmatine (*T. cordifolia*); javaberine A, javaberine A hexaacetate, javaberine B hexaacetate (roots of *Talinum paniculatum*); lepidine and semilepidine (seeds of *Lepidium sativum*); lupanine (*Lupinus perennis*); mahanimbine (leaf of *Murraya koenigii*); piperumbellactam A (branches of *Piper umbellatum*); radicamines A and B (*Lobelia chinensis*); swerchirin (*S. chirayita*); tecomine (*Tecoma stans*); trigonelline (seeds of *Trigonella foenum-graecum*; 1-deoxynojirimycin (leaf and bark of *Morus alba*);

- (ii) Imidazoline compounds—Stimulates insulin secretion in glucose-dependent manner; they are insulinotropic, e.g., the imidazoline RX871024 was found to increased basal- and glucose-stimulated insulin release in vitro and in vivo;
- (iii) Polysaccharides—Increased the levels of serum insulin, reduce the blood glucose levels and improve tolerance of glucose. polysaccharides like

- aconitans A-D (roots of *Aconitum carmichaeli*); atractans A (rhizome of *Atractylodes japonica*); ganoderans A and B (fruit bodies of *Ganoderma lucidum*); galactomannan gum (seeds and tubers of *Cyamopsis tetragonolobus* and *Amorphophallus konjac*);
- (iv) Flavonoids—Suppressed the glucose level, reduced plasma cholesterol and triglycerides significantly, and increased their hepatic glucokinase activity probably. Bengalenoside flavonoids (stem bark of *Ficus benghalensis*); cyanidin-3-galactoside; epigallocatechin gallate (leaf of *Camellia sinensis*); (-)-3-O-galloylepicatechin, (-)-3-O-galloylcatechin (*Bergenia ciliata*); genistein (*Glycine* spp. and soya beans); hesperidin, naringin (*Citrus* spp.); prunin (stem of *Amygdalus davidiana* var. *davidiana*); kaempferitrin (leaf of *Bauhinia forficata*); kaempferol (leaf of *Jindai*, Soybean); kolaviron (*Garcinia kola*); leucodelphinidin (bark of *Ficus benghalensis*); mangiferin (rhizome of *Anemarrhena asphodeloides*); marsupsin, pterostilbene (heartwood of *Pterocarpus marsupium*); quercetin (*Chamaecostus cuspidatus*); Rutin; shamimin (leaf of *Bombax ceiba*); leaves
- (v) Dietary fibers—Effectively adsorbed glucose, retard glucose diffusion, and inhibit the activity of alpha-Amylase and may be responsible for decreasing the rate of glucose absorption and concentration of postprandial serum glucose; and
- (vi) Terpenoids and Steroids- α -amyrin acetate (fruits of *Ficus racemosa*); andrographolide (leaf of *A. paniculata*); 3 ω -acetoxy-16 β -hydroxybetulinic acid (stem bark of *Zanthoxylum gilletii*); basic acid (root bark of *Bumelia santorum*); charantin (fruit and seed of *Momordica charantia*); christinin-A (leaf of *Zizyphus spina-christi*); colosolic acid, maslinic acid (leaf of *Lagerstroemia speciosa*); corosolic acid (leaf of *Vitex* spp.); elatosides E (root cortex of *Aralia elata*); escins-IIA and IIB (seeds of *Aesculus hippocastanum*); forskolin (*Coleus forskohlii*); ginsenosides (rhizome of *Panax* sp.); gymnemic acid IV (leaf of *Gymnema sylvestre*); momordin ic (fruit of *Kochia scoparia*); β -sitosterol (*A. indica*); senegin derivatives (*Polygala senega*);
- (vii) Saponin, (Triterpenoid + steroid)—Stimulates the release of insulin and related compounds;
- (viii) Glycosides—Kalopanax (stem bark of *Kalopanax pictus*); jamboline/antimellin (seeds of *S. cumini*); myrciacitriins I and II and myrciaphenones A and B (leaf of *Myrcia multiflora*); neomyrtillin (leaf of *Vaccinium myrtillus*); pelargonidin 3- O - α -L rhamnoside (bark of *F. bengalensis*); pseudoprototinosaponin AIII and prototinosaponin AIII (rhizome of *A. asphodeloides*); vitexin, isovitexin and isorhamnetin 3- O - β -D-rutinoside (leaf of *Microcos paniculata*);
- (ix) Miscellaneous compounds—allicin (bulb of *A. sativum* and *Allium cepa*); bellidifolin (*Swertia japonica*); bakuchiol (*Otholobium pubescens*); curcumoids (rhizome of *C. longa*); ellagitannins (fruit of *T. chebula*);

Some of the antidiabetic plants have insulin mimetic or insulin secretory activity (Table 7.7).

Table 7.7 List of plants having insulin mimetic or insulin secretory activity and their mechanism of action

S. no.	Plant botanical name	Family	Mechanism of action
1	<i>Abies pindrow</i>	Pinaceae	Insulin secretagogue activity
2	<i>Acacia arabica</i>	Leguminosae	Release of insulin from pancreas
3	<i>Agrimony eupatoria</i>	Leaves	Insulin releasing and insulin like activity
4	<i>Aloe barbadensis</i>	Liliaceae	Stimulating synthesis and release of insulin
5	<i>Annona squamosa</i>	Annonaceae	Increased plasma insulin level
6	<i>Averrhoa bilimbi</i>	Oxalidaceae	Increase serum insulin level
7	<i>Bixa orellana</i>	Bixaceae	Increase plasma insulin concentration and increase insulin binding on insulin receptor
8	<i>Boerhavia diffusa</i>	Nyctaginaceae	Increase plasma insulin concentration
9	<i>Camellia sinensis</i>	Theaceae	Increase insulin secretion
10	<i>Capsicum frutescens</i>	Solanaceae	Increase insulin secretion and reduction of insulin binding on the insulin receptor
11	<i>Cinnamomum zeylanicum</i>	Lauraceae	Elevation in plasma insulin level
12	<i>Clausena anisata</i>	Rutaceae	Stimulate secretion of insulin
13	<i>Eucalyptus globulus</i>	Myrtaceae	Increase insulin secretion from clonal pancreatic beta line (BRIN-BD 11)
14	<i>Ficus religiosa</i>	Moraceae	Initiating release of insulin
15	<i>Hibiscus rosasinensis</i>	Malvaceae	Stimulate insulin secretion from beta cells
16	<i>Helicteres isora</i>	Sterculiaceae	Decrease plasma triglyceride level and insulin sensitizing activity
17	<i>Ipomoea batata</i>	Convolvulaceae	Reduce insulin resistance and blood glucose level
18	<i>Juniperus communis</i>	Pinaceae	Increase peripheral glucose consumption and induce insulin secretion
19	<i>Olea europaea</i>	Oleaceae	Increase insulin release and increase peripheral uptake of glucose
20	<i>Swertia chirata</i>	Gentianaceae	Stimulates insulin release from islets

(continued)

Table 7.7 (continued)

S. no.	Plant botanical name	Family	Mechanism of action
21	<i>Scoparia dulcis</i>	Scrophulariaceae	Insulin-secretagogue activity
22	<i>Tinospora crispa</i>	Menispermaceae	Anti-hyperglycemic, stimulates insulin release from islets
23	<i>Urtica dioica</i>	Urticaceae	Increase insulin secretion
24	<i>Vinca rosea</i>	Apocynaceae	Beta cell rejuvenation, regeneration and stimulation
25	<i>Zingiber officinale</i>	Zingiberaceae	Increase insulin level and decrease fasting glucose level

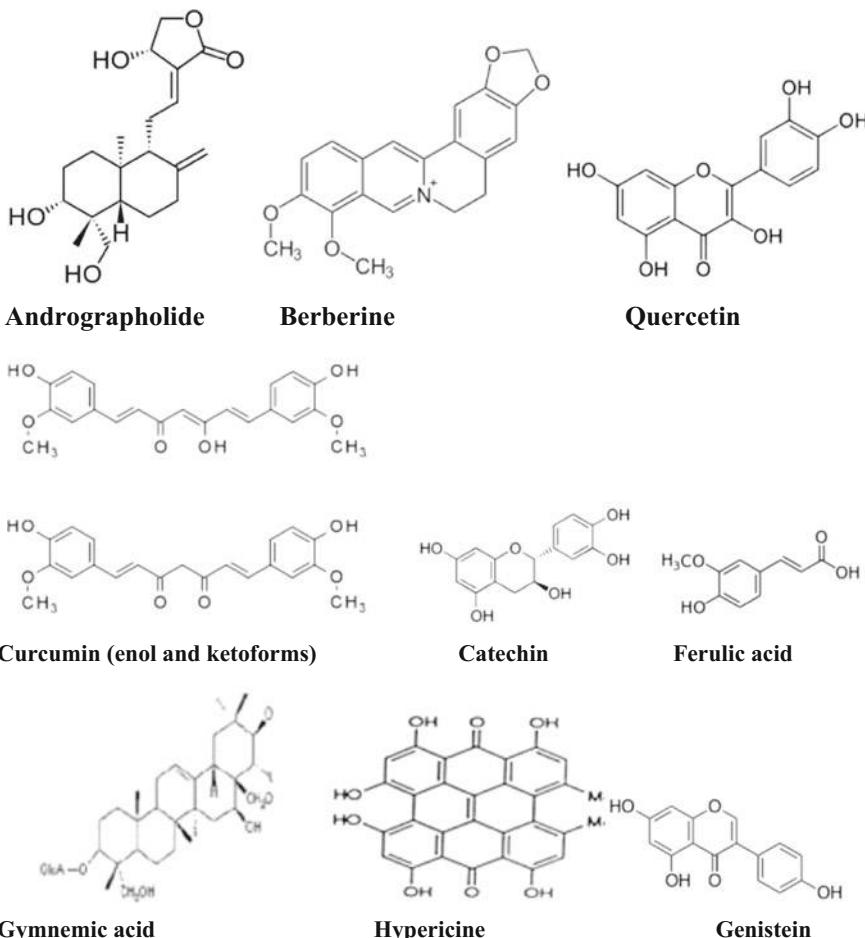
Bioactive compounds having insulin secretagogues or insulin mimetic activity, however, may be different in different plants (Table 7.8).

Structures of some phytoconstituents with hypoglycemic activity are given in Fig. 7.2.

Medicinal plants or the bioactive compounds in their extracts show glucose-lowering either through insulin-mimicking activity, enhanced β -cells regeneration, or glucose uptake. Phytoconstituents like alkaloids (as major role of alkaloids) inhibits alpha-glucosidase and decrease glucose transport through the intestinal epithelium; imidazoline compounds stimulate insulin secretion in a glucose-dependent manner; polysaccharides increase the level of serum insulin, reduced the blood glucose level of serum insulin, reduce the blood glucose level, and enhance tolerance to glucose; flavonoids suppress the glucose level, saponin stimulates the release of insulin, and blocks the formation of glucose in the bloodstream (Bhushan et al. 2010). The antidiabetic principles of bitter melon are a mixture of steroidal saponins, insulin-like peptides, alkaloids, and triterpenoids. Alkaloids such as aconitine, anisodamine, charantane, and leurosine, showed antidiabetic effects. Dietary flavonoids and acarbose synergistically inhibit α -glucosidase and lower postprandial blood glucose.

Hypertension (HTN) management

Hypertension (HTN) or high blood pressure (BP) is a chronic medical condition in which the BP in the arteries is elevated. BP is a combination of systolic and diastolic pressure. According to WHO criteria, hypertension means elevated blood pressure levels above 140/90 mmHg (Lifton 1996). Systolic pressure represents blood force, or pressure, while the heart is beating and diastolic pressure stands for blood pressure when the heart is at rest. Systolic pressure is always the first or top measurement in a blood pressure reading. In a reading of 130/80, 130 represents systolic pressure and 80 represents diastolic pressure. In prehypertension, systolic numbers range from 120 to 129 and diastolic numbers are less than 80. HTN has been named the “silent killer” as it is asymptomatic and the major contributor or risk factor for cardiovascular morbidity and mortality (Gavras 2009). In 2000,



R₁ R₂

Gymnemic acid 1: Tigloyl Ac

Gymnemic acid 2: 2-Methylbutyloyl Ac

Gymnemic acid 3: 2-Methylbutyloyl H

Gymnemic acid 4: Tigloyl H

Fig. 7.2 Showing structure of phytoconstituents having insulin mimetic activity

26.4% of the world's population suffered hypertension and it is predicted that this rate would increase by 60% in 2025 (Kearney et al. 2005).

Ranges of BP are (i) normal: less than 120/80 mmHg, (ii) prehypertension: systolic between 120–129 and diastolic <80, (iii) stage 1 high blood pressure:

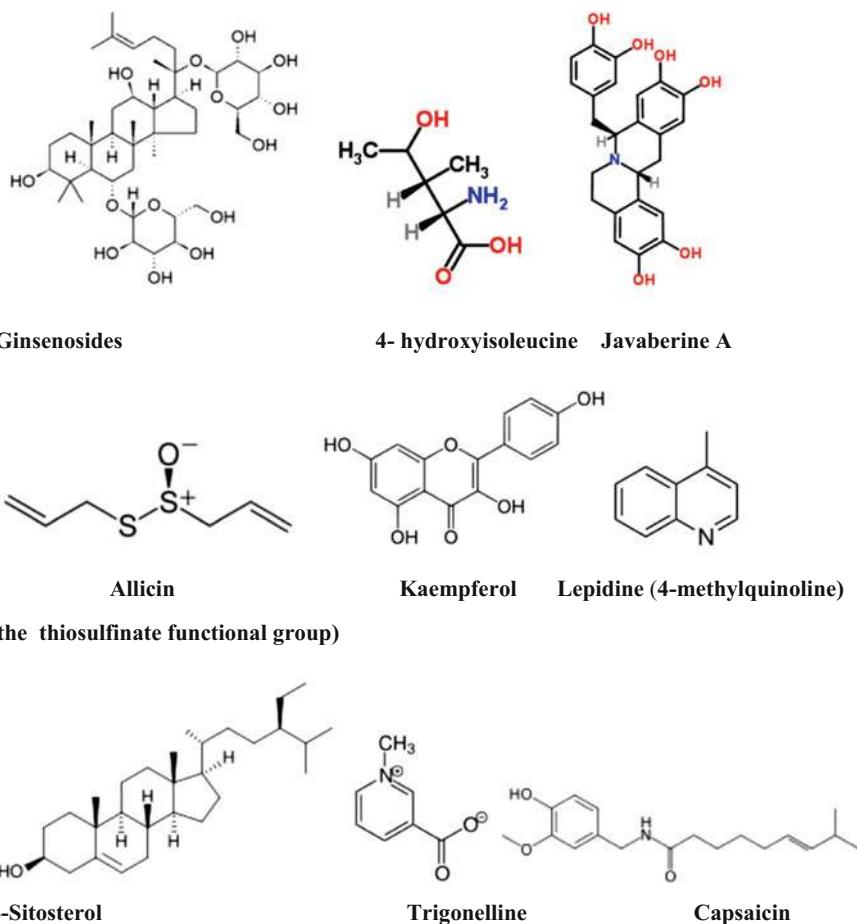


Fig. 7.2 (continued)

systolic between 130 and 139 or diastolic between 80 and 89, (iv) stage 2 high blood pressure: systolic at least 140 or diastolic at least 90 mmHg. According to Barker et al. (1995), the categories of hypertension in adults may be different (Table 7.9).

Although both numbers (systolic and diastolic readings) are significant, after age ~ 50 , the systolic number is most important and only 10% of high blood pressure cases are due to secondary or identifiable causes such as medications, or conditions and diseases of other organs. High blood pressure happens when the pressure on the arteries and blood vessels becomes too high and the arterial wall becomes distorted causing extra stress on the heart. Long-term high blood pressure increases the risk of stroke, heart attack, and diabetes. Results of high blood pressure include arterial damage, aneurysm, heart failure, blocked or ruptured blood

Table 7.8 List of plants and their constituents having insulin secretagogues or insulin mimetic activity

S. no.	Plant botanical name	Family	Active constituents
1	<i>Aloe vera</i>	Liliaceae	Pseudoprototinosaponin AIII and prototinosaponins AIII
2	<i>Anemarrhena asphodeloides</i>	Liliaceae	Mangiferin and mangiferin-7-O- β -D' glucoside
3	<i>Bauhinia variegata</i>	Caesalpiniaceae	Roseoside
4	<i>Camellia sinensis</i>	Theaceae	Epigallocatechin gallate
5	<i>Citrullus colocynthis</i>	Cucurbitaceae	Beta-pyrazol-1-ylalanine
6	<i>Ephedra distachya</i>	Ephedraceae	L-ephedrine
7	<i>Eriobotrya japonica</i>	Rosaceae	Cinchonain ib
8	<i>Eugenia jambolana</i>	Myrtaceae	Pandanus odoratus (Toei-hom) a 4-hydroxybenzoic acid
9	<i>Ficus benghalensis</i>	Moraceae	Leucocyanidin 3-O-beta-D-galactosyl cellobioside, leucopelargonidin-3-O-alpha-L rhamnoside
10	<i>Glycyrrhiza radix</i>	Fabaceae	Glycyrrhetic acid, dihydroxy gymnemic triacetate
11	<i>Momordica charantia</i>	Cucurbitaceae	Momordicin, charantin, and galactose-binding lectin
12	<i>Panax ginseng</i>	Araliaceae	Polypeptides
13	<i>Prunella vulgaris</i>	Labiatae	Jiangtang su
14	<i>Psidium guajava</i>	Myrtaceae	Strictinin, isostrictinin and pedunculagin
15	<i>Pterocarpus marsupium</i>	Fabaceae	Epicatechin
16	<i>Semen coicis</i>	Gramineae	Coixans
17	<i>Stevia rebaudiana</i>	Asteraceae	Stevioside, steviol
18	<i>Swertia chirayita</i>	Gentianaceae	Swerchirin
19	<i>Teucrium polium</i>	Lamiaceae	Apigenin
20	<i>Trigonella foenum-graecum</i>	Leguminosae	4-hydroxyleucine and hydroxyisoleucine
21	<i>Zizyphus spina-christi</i>	Rhamnaceae	Christinin-A

Table 7.9 Categories of hypertension in adults (measured in mmHg)^a

Stages	Systolic BP range	Diastolic BP range
Stage 1 (mild)	Systolic BP 140–159	Diastolic BP 90–99
Stage 2 (moderate)	Systolic BP 160–179	Diastolic BP 100–109
Stage 3 (severe)	Systolic BP 180–209	Diastolic BP 110–119
Stage 4 (very severe)	Systolic BP >210	Diastolic BP >120

^aBarker et al. (1995)

vessels, reduced kidney function, vision loss, loss of cognitive function (concentration, memory, and ability to learn), metabolic syndrome (high cholesterol and insulin, atherosclerosis and increased waist size), etc. Frequently, there are no symptoms as blood pressure increases, but warning signs for very high BP include usually chest pains, confusion, headaches, ear noise or buzzing, irregular heartbeat, nosebleeds, tiredness or vision changes, etc. High-salt diet, emotional stress, alcohol, caffeine, smoking, obesity, inactivity, birth control pills, heavy-metal poisoning, etc., are the probable causes of high blood pressure. Patients with BP higher than 130/80 mmHg with concomitant presence of diabetes or kidney disease require further treatment. Exercise HTN is an excessively high elevation (systolic values between 200 and 230 mmHg) in BP during exercise. Exercise HTN may indicate that an individual is at risk for developing HTN at rest. Persistent HTN is one of the risk factors for strokes, heart attacks, heart failure, and arterial aneurysm, and is a leading cause of chronic kidney failure. Moderate elevation of arterial BP leads to shortened life expectancy (Table 7.10). Different causes of hypertension are described in Table 7.8.

Recent evidence indicate that hypertension and raised blood pressure are increasing partly because of the increase in risk factors including smoking, obesity, and high use of alcohol, social insecurity and anxiety, and lack of exercise. Both dietary and lifestyle changes as well as medicines can improve BP control and decrease the risk of associated health complications.

Conventional antihypertensives are usually associated with many side effects. Conventional medicines used in the treatment of hypertension are classified as follows:

- (i) Diuretics—diuretics help the kidneys eliminate excess salt and water from the body's tissues and blood and include loop diuretics (bumetanide, ethacrynic acid, furosemide, torsemide); thiazide diuretics (epitizide, hydrochlorothiazide and chlorothiazide, bendroflumethiazide, methyclothiazide, polythiazide); thiazide-like diuretics (indapamide, chlorthalidone, metolazone); potassium-sparing diuretics (amiloride, triamterene, spironolactone, eplerenone);
- (ii) Adrenergic receptor antagonists—beta blockers (atenolol, bisoprolol, betaxolol, carteolol, carvedilol, labetalol, metoprolol, nadolol, nebivolol, oxprenolol, penbutolol, pindolol, propranolol, timolol, etc.); alpha blockers (doxazosin, phentolamine, indoramin, phenoxybenzamine, prazosin,

Table 7.10 Various causes of hypertension

Hypertension type	Cause
Primary hypertension (essential hypertension)	Increased sympathetic nervous system activity Increased production of sodium-retaining hormones and vasoconstrictors Deficiencies of vasodilators such as prostacyclin and nitric oxide Inappropriate or increased renin secretion, resulting in increased production of angiotensin II and aldosterone. Genetic predisposition
Secondary hypertension	Renal: acute glomerulonephritis, chronic nephritis, polycystic disease, diabetic nephropathy and hydronephrosis Endocrine: Acromegaly, Hypothyroidism, Hyperthyroidism, Hypercalcaemia (hyperparathyroidism) Adrenal: Cortical: Cushing syndrome, primary aldosteronism, congenital adrenal hyperplasia, apparent mineralocorticoid excess (licorice) Medullary: Pheochromocytoma, extra-adrenal chromaffin tumors, Carcinoid Exogenous hormones: estrogen, glucocorticoids, mineralocorticoids, sympathomimetics, tyramine-containing food, monoamine oxidase inhibitors Systolic hypertension: Increased cardiac output Aortic valvular insufficiency, Arteriovenous fistula, patent ductus arteriosus Thyrotoxicosis, Rigidity of aorta Iatrogenic hypertension Pregnancy-induced hypertension Neurological disorders: Increased intracranial pressure -brain tumors -encephalitis -respiratory acidosis

terazosin, tolazoline); and mixed alpha and beta blockers (bucindolol, carvedilol, labetalol);

- (iii) Adrenergic receptor agonists—centrally acting adrenergic drugs or alpha-2 adrenergic receptor agonist—clonidine, guanabenz, guanfacine, methyl-dopa, moxonidine;
- (iv) Calcium channel blockers—calcium channel blockers block the entry of calcium into muscle cells in artery walls and include dihydropyridines (amlodipine, lacidipine, cilnidipine, clevidipine, felodipine, isradipine, lercanidipine, levamldipine, nicardipine, nifedipine, nimodipine, nisoldipine, nitrendipine) and non-dihydropyridines (diltiazem, verapamil);
- (v) ACE inhibitors—ACE inhibitors inhibit the activity of angiotensin-converting enzyme (ACE), an enzyme responsible for the conversion of angiotensin I into angiotensin II, a potent vasoconstrictor. Examples include captopril, enalapril, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, trandolapril, benazepril, etc.;

- (vi) Angiotensin II receptor antagonists—Angiotensin II receptor antagonists work by antagonizing the activation of angiotensin receptors (e.g., azilsartan, candesartan, eprosartan, irbesartan, losartan, olmesartan, olmesartan medoxomil, telmisartan, valsartan, fimasartan, etc.);
- (vii) Aldosterone antagonists—eplerenone, spironolactone; and
- (viii) Vasodilators—sodium nitroprusside, hydralazine.

Figure 7.3 showing structure of some conventional antihypertensives drugs that are used to treat hypertension.

Many of these antihypertensive agents have some side effects, e.g., diuretics may cause muscle cramps, dizziness, extreme tiredness, dehydration, blurred vision, abnormal heart rate, skin rash, and others; ACE inhibitors may cause cough, skin rash, vomiting, kidney failure, fever, sore throat, diarrhea, and others; and side effects come with the use of calcium channel blockers are fatigue, headache, diarrhea, constipation, skin rash, edema, etc. Herbs do not cause side effects like weakness, tiredness, drowsiness, impotence, cold hands and feet, depression, insomnia, abnormal heartbeats, skin rash, dry mouth, dry cough, stuffy nose, headache, dizziness, swelling around eyes, constipation or diarrhea, fever, etc. Herbal medicines have been gaining more attention in the treatment of hypertension both in the developed and developing countries because of their wide biological and medicinal activities, diversity, ease of availability, higher safety margins and low cost than novel pharmaceuticals. In the past few decades, a lot of concerted efforts have been channeled into researching the local plants with antihypertensive therapeutic values and a lot of concerted efforts have been channeled into researching into local plants with antihypertensive therapeutic values, in which some of these medicinal plants have been validated and others disproved. Many of the following medicinal plants have so far been scientifically studied and reported to have hypotensive or antihypertensive effects:

Ajwain (*Carum copticum*), American Ginseng (*Panax quinquefolius*), Amur Cork Tree (*Phellodendron amurense*), Arjuna (*T. arjuna*), Asafetida (*Ferula assafoetida*), Ashwagandha (*W. somnifera*), Avocado (*Persea americana*), Banana (*Musa sapientum*), Barberry (*B. vulgaris*), Basil (*Ocimum basilicum*), Black bean (*Castanospermum australe*), Black cumin (*N. sativa*), Black Mangrove (*Lumnitzera racemosa*), Black plum (*Vitex doniana*), Black Walnut (*Juglans nigra*), Bilberry (*V. myrtillus*), Biting Stonecrop (*Sedum acre*) Borage (*Borago officinalis*), Breadfruit (*Artocarpus altilis*), Broccoli (*Brassica oleracea*) Buchu (*Agathosma betulina*), Cantaloupe or musk melon (*Cucumis melo*), Cardamon (*Elettaria cardamomum*), Carrot (*Daucus carota*), Cat's Claw herb (*Uncaria tomentosa*), Celery seed (*Apium graveolens* var. *dulce*), Chaksu (*Cassia absus*), Chicory (*Cichorium intybus*), Chinese Knotweed or Fo-Ti (*Polygonum multiflorum*), Chocolate or cocoa bean (*Theobroma cacao*), Cicely (*Myrrhis odorata*), Cinnamon (*Cinnamomum verum* or *C. tamala*), Coconut root (*Cocos nucifera*), Coffee weed (*Cassia occidentalis*), Custard apple (*Annona reticulata*), Dandelion (*Taraxacum officinale*), Dodder (*Cuscuta reflexa*), Dong Quai (*Angelica sinensis*), Flaxseed (*Linum usitatissimum*), French Lavender (*Lavandula stoechas*), Hyssop (*Hyssopus officinalis*), Juniper—

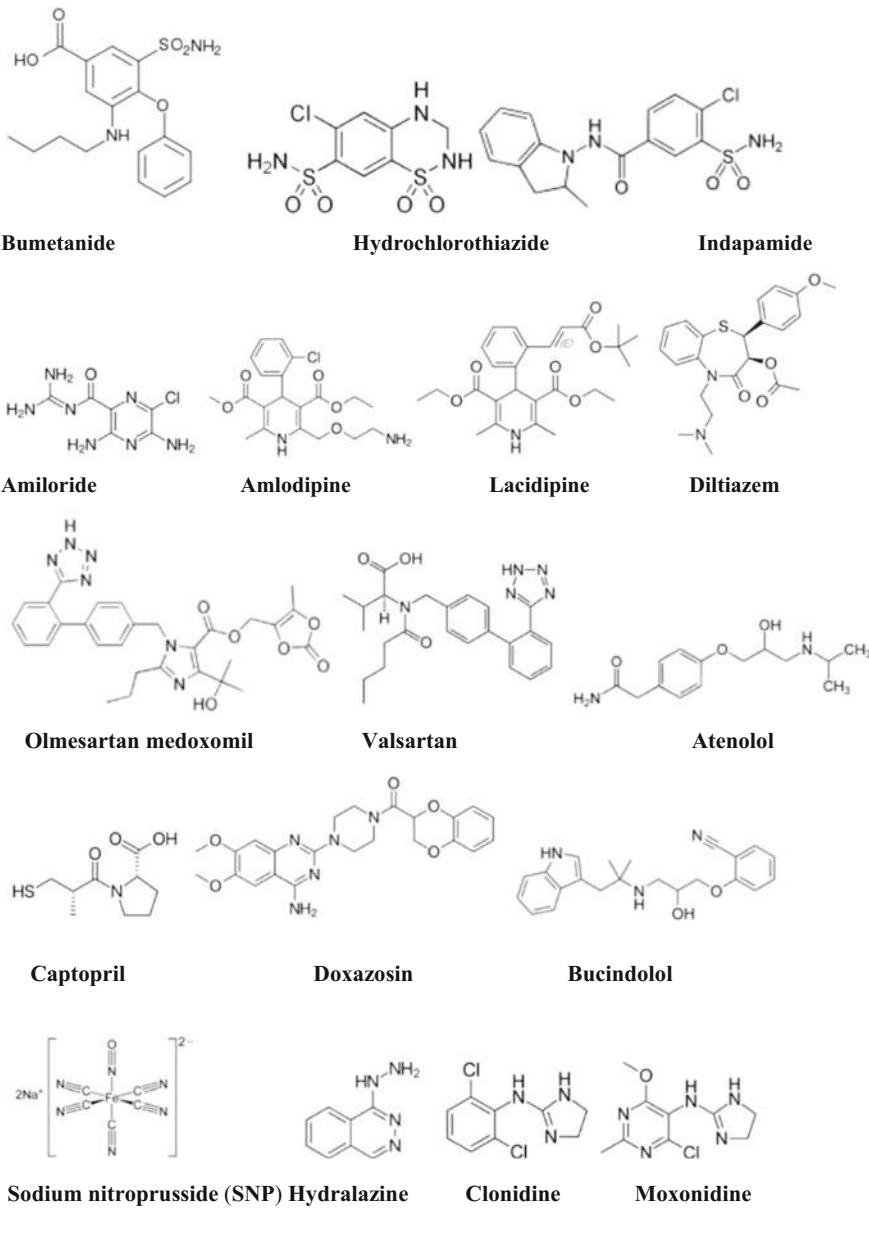


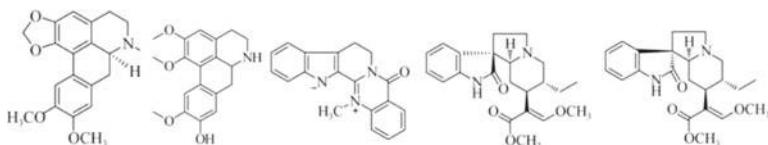
Fig. 7.3 Structure of some conventional antihypertensives drugs that are used to treat hypertension

(*Juniperus communis*), Kudzu (*Pueraria lobata*), Lemon Grass—(*Cymbopogon citratus*), Garlic (*A. sativum*), Guan Mu Tong (*Aristolochia manshuriensis*), Ginger (*Zingiber officinale*), Ginseng (*Panax* sp.), Hardy Fuchsia (*Fuchsia magellanica*),

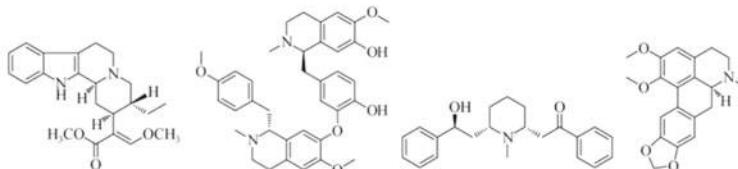
Fig. 7.4 Showing structure of some hypotensive bioactive natural products of secondary metabolic origin. Alkaloids: (+)-Dicentrine, Laurotetanine, Dehydroevodiamine, Rhynchophylline, Isorhynchophylline, Dihydrocorynantheine, Isoliensinine, (-)-Lobelaine, (+)-Nantenine, Puqienine A, Puqienine B, Puqienine E, Reserpine, Deserpidine, Tetrandrine; Diterpenes: Forskolin, Stevioside, a diterpenoid glycoside, 14-Deoxy-11, 12-didehydroandrographolide (DDA), a diterpenoid, *ent*-Kaur-16-en-19-oic acid and *ent*-kaur-16-en-15-one-19-oic acid are two kaurane-type diterpenes; Essential oil constituents: α -Pinene, α -Terpinene, γ -Terpinene, p-Cymene, Carvacrol, Thymol, and Linalool; Coumarins: (+)-Praeruptorin A, a coumarin, Imperatorin, a dietary furanocoumarin, Ostruthol, a furanocoumarin; Flavonoids: Quercetin, Isorhamnetin, Astragalin, Orientin, Cardamonin, Alpinetin; flavonoids vicenin-2 (6,8-di- β -D-glucopyranosyl-5,7,4'-trihydroxyflavone), butin (7,3',4'-trihydroxyflavanone), and 3'-hydroxydaidzein (7,3',4'- trihydroxyisoflavone); Others:33-Daleformis

Harmal (*Peganum harmala*), Hawthorn (*Crataegus* spp.), Chinese Hawthorn (*Crataegus pinnatifida*), Indian plantago (*Plantago* sp.), Karpurvali (*C. forskohlii*), Kudzu (*P. lobata*), Lasaf (*Capparis cartilaginea*), Maritime pine (*Pinus pinaster*), Mistletoe (*Viscum album*), Motherwort (*Leonurus cardiaca*), Murungai leaf (*Moringa oleifera*), Nela nelli (*Phyllanthus amarus*), Oat (*Avena sativa*), Oliver (*Rhaptoperatum coriaceum*), Osbeck (*Desmodium styracifolium*), Parsley (*Petroselinum crispum*), Pau d'Arco (*Tabebuia avellanedae*), Periwinkle (*Vinca minor*), Pima cotton leaf (*Gossypium barbadense*), Pomegranate (*Punica granatum*), Punarnava (*Boerhavia diffusa*), Purslane (*Portulaca oleracea*), Radish (*Raphanus sativus*), Rauvolfia (*Rauvolfia serpentina*), Red Sage (*Salvia miltiorrhiza*), Roselle (*Hibiscus sabdariffa*), Self heal (*Prunella vulgaris*), Sesame (*Sesamum indicum*), Spinach (*Spinacia oleracea*), Soybean (*Glycine max*), Sticky nightshade (*Solanum sisymbriifolium*), Stinging nettle (*Urtica dioica*), Stone breaker (*Lepidium latifolium*), Sunflower (*Helianthus annuus*), Swamp lily (*Crinum gallacum*), Sweet orange (*Citrus sinensis*), Tea (*Camelia sinensis*), Tomato (*Lycopersicon esculentum*), Umbrella tree or cork wood (*Musanga cecropioides*), Virginia dayflower (*Commelina virginica*), Watermelon (*Citrullus lanatus*), Wheat bran (*Triticum aestivum*), Wild tomato (*Solanum sisymbriifolium*), Wild Yam (*Dioscorea villosa*), Yarrow (*Achillea millefolium*). Many of these plants contain bioactive compounds of secondary metabolic origin, fibers, and high-level potassium, or all components. Ren-Ren et al. (2015) while examining the antihypertensive activity of different plant sources such as *Stevia rebaudiana*, *Hippophae rhamnoides*, *Fritillaria pugiensis*, *Evodia rutaecarpa*, *C. forskohlii*, *Peucedanum ostruthium*, *Salvia miltiorrhizae*, *Nandina domestica*, *Uncaria rhynchophylla*, and *Musa sapientum*, put emphasis on the research and development of natural lead compounds with antihypertensive activity, including alkaloids, diterpenes, coumarins, flavonoids, and peptides.

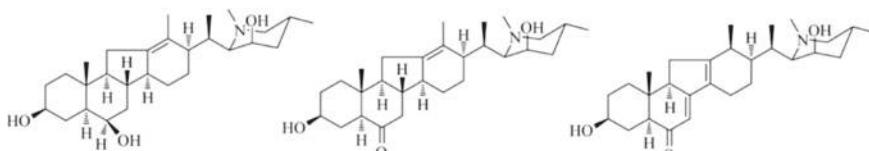
Phytochemical screening confirms the presence of (i) alkaloids-(+)-dicentrine, laurotetanine, dehydroevodiamine, rhynchophylline, isorhynchophylline, dihydrocorynantheine, isoliensinine, (-)-lobelaine, (+)-nantenine, puqienine A, puqienine B and puqienine E, reserpine, deserpidine, tetrandrine; (ii) saponins; (iii) polyphenols-



(+)-Dicentrine **Laurotetanine** **Dehydroevodiamine** **Rhynchophylline** **Isorhynchophylline**



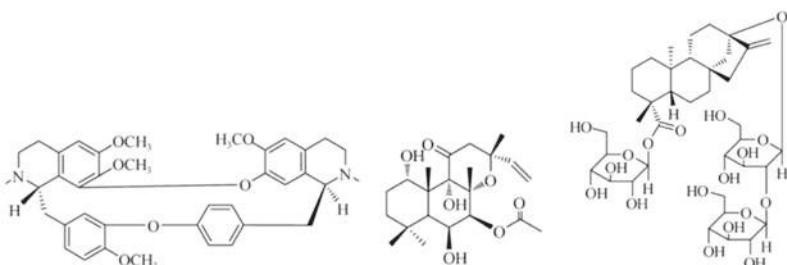
Dihydrocorynantheine **Isoliensinine** **(-)-Lobeline** **(+)-Nanteni**



Puqienine A, **Puqienine B** **Puqienine E**

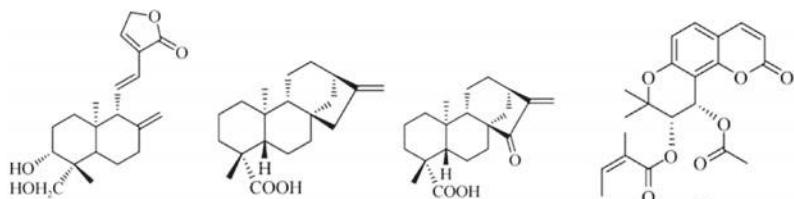


Reserpine (R1=OCH₃; R2=3,4,5-trimethoxybenzoyl) **Deserpidine (R1=H; R2=3,4,5-trimethoxybenzoyl)**



Tetrandrine **Forskolin** **Stevioside**

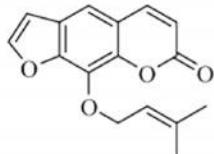
isochroman-4-one XJP; (iv) phenolic glycosides; (v) flavonoids—quercetin, isorhamnetin, stragalin, orientin, cardamonin, alpinetin; vicenin-2, butin, and 3'-hydroxydaidzein; (vi) coumarins (+)-praeruptorin A, imperatorin, ostruthol; (vii) diterpenes and terpenoids—forskolin, stevioside, 14-deoxy-11,



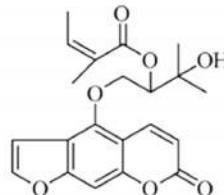
DDA

Two kaurane-type diterpenes

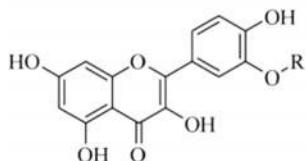
24-(+)-Praeruptorin A, a coumarin



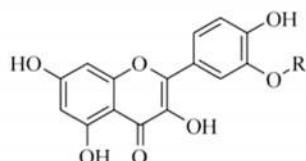
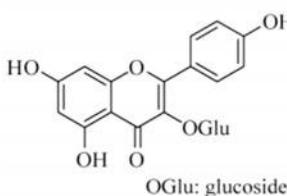
Imperatorin



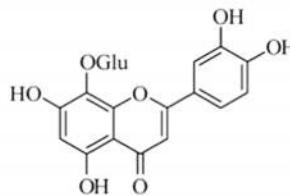
Ostruthol -two furanocoumarins



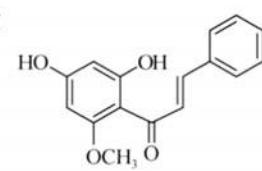
Quercetin (R=H)

Isorhamnetin (R=CH₃)

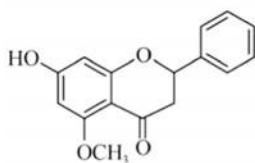
Astragalin



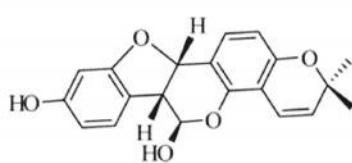
Orientin



Cardamonin

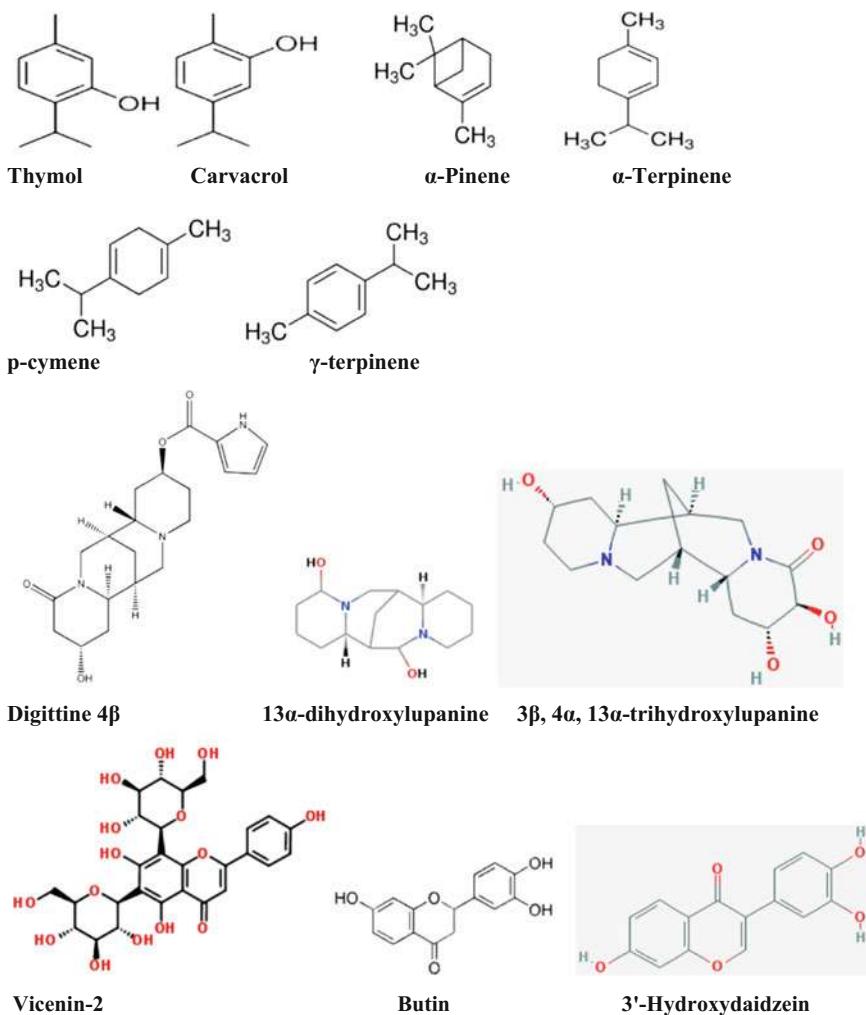


Alpinetin



Daleformis

Fig. 7.4 (continued)

**Fig. 7.4** (continued)

12-didehydroandrographolide-DDA; major constituents of essential oil— α -pinene, α -terpinene, γ -terpinene, p-cymene, carvacrol, thymol, and linalool; (viii) anthraquinones; (ix) tannins; (x) phytosterols; (xi) cardiac glycosides; (xii) peptides (Heshiko), and (xiii) others—daleformis, ursolic, moronic acids, Magnesium lithospermate B; are important groups of bioactive compounds, which played a dominant role in the treatment of hypertension. Structure of some of these bioactive antihypertensive compounds are shown below (Fig. 7.4).

Cancer management

Cancer is a class of diseases characterized by out-of-control cell growth. *Cancer* is the uncontrolled growth of abnormal cells in the body. *Cancer* develops when the body's normal control mechanism stops working. Old cells do not die and instead grow out of control, forming new, abnormal cells. These extra cells may form a mass of tissue, called a tumor. Some cancers, such as leukemia, do not form tumors. There are >200 different types of cancer and each is classified by the type of cell that is initially affected. Cancers respond to treatment in different ways, some types of cancer are best treated with surgery, others respond better to drugs (chemotherapy), radiation, etc., and often 2 or more treatments are used to get the best results.

Cancer is divided into five stages using the international TNM system—0, I, II, III, IV (T stands for primary tumor, N for lymph nodes, and M for distant metastases). Stage 0 means the presence of a small carcinoma *in situ* that has not spread. Stage IV means cancer that has spread widely or cancer that has metastasized. In some cases, the stages have subclasses. When it spreads from the primary site via the bloodstream or lymphatic system to other organs, cancer starts to produce metastases and become attached to other organs. From there they begin to divide and invade space. Different cancers typically metastasize in certain organs, e.g., in the liver, lungs, adrenal glands, brain, and bones. The symptoms caused by metastases vary according to their location.

Medicinal plants have been used to prevent and to treat various diseases and they comprise a source of bioactive pharmaceuticals with beneficial health effects. Certain bioactive components from the plants have been confirmed for their anticancer activities. These include curcumin from turmeric, genistein from soybean, EGCG from green tea polyphenols from green tea, resveratrol from grapes, sulforaphane from broccoli, isothiocyanates from cruciferous vegetables, silymarin from milk thistle, diallyl sulfide from garlic, lycopene from tomato, rosmarinic acid from rosemary, apigenin from parsley, gingerol from gingers, vitamin E from plant oil, boron-rich natural compound, hydroxytyrosol from virgin olive oil, phytoestrogens, etc. Up to now, cancer remains to be one of the leading causes of death in the world and modern drug-targeted therapies has undeniably improved cancer patients' cares, but the advanced metastasized cancer remains untreatable. Cancer chemoprevention with natural bioactive phytochemicals is an emerging strategy to prevent, impede, delay, or cure cancer.

Phytochemicals used as cancer chemopreventive

- (i) Apigenin is a flavone present in vegetables such as parsley, celery, chamomile, and *Moringa peregrina*. It demonstrates cytotoxic activities against breast cancer cell lines (MCF 7), colon cell line (HCT 116), and its cytotoxic activity is comparable to that of doxorubicin;
- (ii) Curcumin (diferuloylmethane) is the major components of popular spice turmeric, *C. longa* L., a member of the ginger family. Its anticancer effects have been studied for colon cancer, breast cancer, lung metastases, and brain tumor;

- (iii) Crocetin from, *Crocus sativus* L. Saffron is a food colorant present in the dry stigmas of the plant and it is a potential agent for a novel anticancer drug against hepatocellular carcinoma;
- (iv) Cyanidin is an extract of pigment from red berries such as grapes, blackberry, cranberry, raspberry, or apples and plums, red cabbage, and red onion. It possesses antioxidant and radical-scavenging effects which may reduce the risk of cancer;
- (v) Indole-3-carbinol (I3C) is found in brassica vegetables, such as broccoli, cauliflower, collard greens. Diindolylmethane (DIM) is a digestion derivative of indole-3-carbinol via condensation formed in the acidic environment of the stomach. Both are studied for their anticarcinogenic effects;
- (vi) Epigallocatechin gallate (EGCG) is the most abundant catechin compounds in green tea. EGCG can be beneficial in treating brain, prostate, cervical, and bladder cancers.
- (vii) Fisetin is a flavone found in various plants such as *Acacia greggii*, *Acacia berlandieri*, *Eurasian smoketree*, parrot tree, strawberries, apple, persimmon, grape, onion, and cucumber Fisetin alleviates aging effects in the yeast or fruit fly, exerts anti-inflammatory effect in LPS-induced acute pulmonary inflammation and anticarcinogenic effects in HCT-116 human colon cancer cells;
- (viii) Genistein is an isoflavone originates from a number of plants such as lupine, fava beans, soybeans, kudzu, and psoralea, and coffee. Functioning as antioxidant and anthelmintic, genistein has been found to have antangiogenic effects (blocking formation of new blood vessels), and may block the uncontrolled cell growth associated with cancer, most likely by inhibiting the enzymes that regulate cell division and cell survival (growth factors).
- (ix) Gingerol is the active component of fresh ginger with distinctive spiciness. Gingerol has been studied for its anticancerous effects for the tumors in colon, breast and ovarian and pancreas;
- (x) Kaempferol is a natural flavonol isolated from tea, broccoli, witch-hazel, grapefruit, brussels sprouts, apples, etc., and has been studied for pancreatic cancer and lung cancer;
- (xi) Lycopene is a bright red pigment and phytochemical from tomatoes, red carrots, watermelons, and red papayas. It demonstrates antioxidant activity and chemopreventive effects in many studies, especially for prostate cancer;
- (xii) Phenyl isothiocyanate (PEITC), along with sulforaphane from cruciferous vegetables, such as watercress, broccoli, cabbage, etc., have been studied for induction of apoptosis in cell lines. PEITC has shown very strong potency against melanoma. It has been intensively studied for chemoprevention against breast cancer cells, non-small-cell lung cancer, cervical cancer, osteogenic sarcoma U-2 OS, prostate cancer, and myeloma cell lines;

- (xiii) Resveratrol is a natural phenol and can be found in the red grapes skin, peanuts, and in other fruits. It is cancer chemopreventive;
- (xiv) Rosmarinic acid (RA) is a natural antioxidant found in culinary spice and medicinal herbs such as lemon balm, peppermint, sage, thyme, oregano, and rosemary to treat numerous ailments. Rosemary extracts play important roles in anti-inflammation, antitumor, and antiproliferation in various in vitro and in vivo studies;
- (xv) Sulforaphane is an organosulfur compound obtained from cruciferous vegetables such as broccoli, Brussels sprouts, and cabbages. The enzyme myrosinase in GI tract transforms glucoraphanin into sulforaphane upon damage to the plant such as from chewing.
- (xvi) Triterpenoids are biosynthesized in plants by cyclization of squalene, a triterpene hydrocarbon and precursor of all steroids. This group of phytochemicals are subclassified into cucurbitanes, dammaranes, ergostanes, friedelanes, lanostanes, limonoids, lupanes, oleananes, tirucallanes, ursanes, and the list is still growing. Various in vitro and in vivo studies have been conducted for chemoprevention and therapy of breast cancer, and pancreatic cancer using triterpenoids.
- (xvii) Light-exposed mushroom could be an excellent source of Vitamin D. Vitamin D has been involved in breast cancer, colon cancer, ovarian cancer, and pancreatic cancer;
- (xviii) Vitamin E includes both tocopherols and tocotrienols, a fat-soluble antioxidant, and exists in many foods including wheat germ oil, sunflower oil, and safflower oils. Alphatocopherol is the most bioactive form of vitamin E that stops the production of reactive oxygen species (ROS) when fat undergoes oxidation. There are reports that both tocopherols and tocotrienols have antitumor effects due to their antioxidant properties, and tocotrienols show stronger bioactivity and both show antiproliferative, proapoptotic, and COX-2 inhibiting effects in in vitro studies.

Plant-derived compounds have been an important source of several clinically useful anticancer agents (Cragg and Newman 2005). These include vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide, derived from epipodophyllotoxin, and paclitaxel (Taxol). A number of promising new agents are in clinical development based on selective activity against cancer-related molecular targets, including flavopiridol and combretastatin A4 phosphate, while some bioactive compounds which failed in earlier clinical studies are stimulating renewed interest. Different bioactive compounds respond differently to different types of cancers (Table 7.11).

The mechanisms of action of plant-derived anticancer drugs are numerous and most of them induce apoptotic cell death. Chemotherapy is the treatment of cancer cells with one or more antineoplastic cytotoxic agents, inhibitor, antimitotic, and anti-microtubule, which mainly targets the rapidly proliferating cancer cells leading to the induction of cell death. Figure 7.5 showing structure of some bioactive herbal anticancer agents.

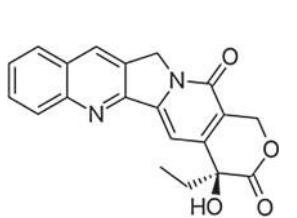
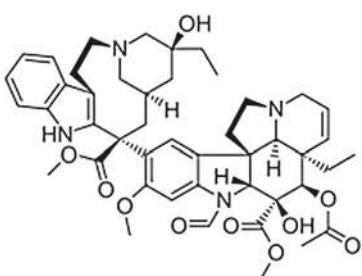
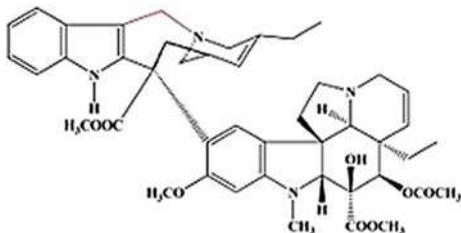
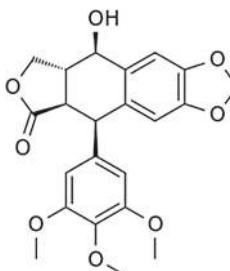
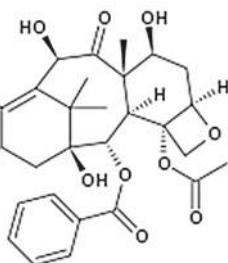
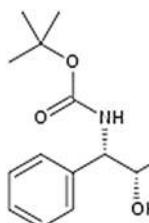
Table 7.11 Influence of different bioactive compounds in different cancers

Sl. no.	Metabolites	Groups	Plant species	Type of cancer
1	Cucumin	Phenolic	<i>Curcuma longa</i>	Colorectal
2	Phenol	Phenolic	<i>Zingiber officinale</i>	Cancer
3	Resveratrol	Phytoalexin	GrapesVitis	Breast
4	Genistein	Flavonoids		Leukemia
5	Biocalein	Flavonoids	Shosiko	Hepatocellular
6	Hydroxystaurosporin	Alkaloid	<i>Viscom album</i>	Ovarian cancer
7	Lectin	Lectins	Banana	Cancer
8	Xanthorrhizol	Terpenoids	<i>Curcuma longa</i>	Cancer

Camptothecin (CPT) is a topoisomerase inhibitor, isolated from the bark and stem of *Camptotheca acuminata*; Vinca alkaloids are a set of antimitotic and anti-microtubule alkaloid agents originally derived from the periwinkle plant *C. roseus*, include vinblastine, vincristine, vindesine, and vinorelbine. Additional researched vinca alkaloids include vinca minor, vincristine, and vinburnine; podophyllotoxin (PPT) is a non-alkaloid toxin lignan extracted from the roots and rhizomes found in *P. peltatum*; *T. brevifolia* (Pacific yew) have been used as the basis for two chemotherapy drugs, docetaxel and paclitaxel; ingenol mebutate (ingenol-3-angelate) is a substance found in the sap of the plant *Euphorbia peplus* and an inducer of cell death. Trastuzumab emtansine (Kadcyla) is an antibody conjugated to a synthetic derivative of the cytotoxic principle of the Ethiopian plant *Maytenus ovatus*. It used to treat breast cancer.

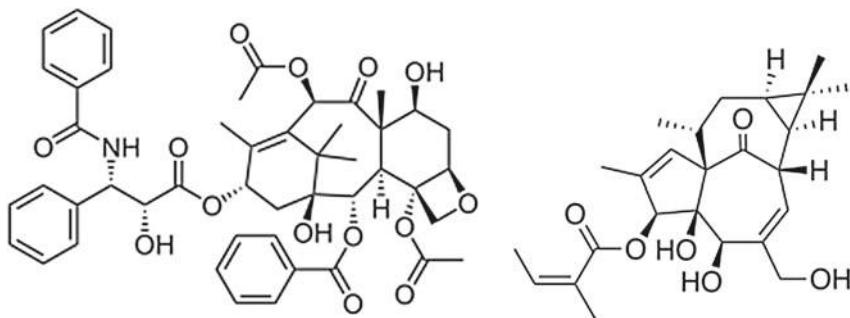
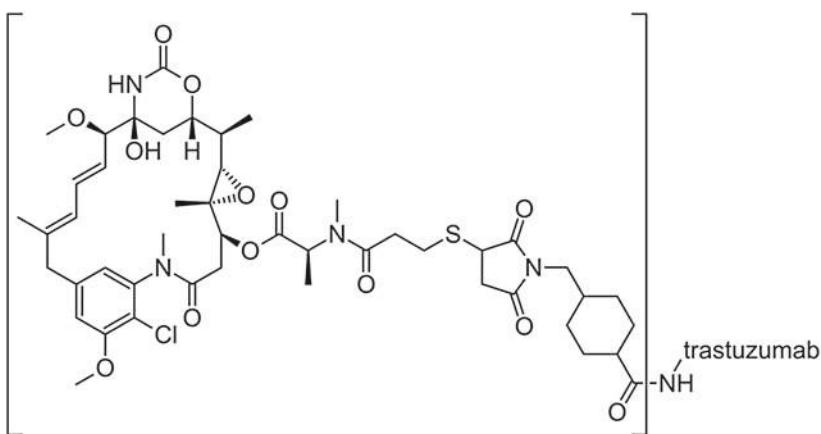
7.3.2 Viral Disease Management with the Use of Antiviral Bioactive Phytoconstituents

Viruses are acellular particulate matter made up of nucleic acids and proteins. They cause many infectious viral diseases including HIV, Influenza, Herpes simplex virus (HSV), dengue, chikungunya, Zika, hepatitis A (HSV), hepatitis B (HSB), hepatitis C (HCV), etc., and recent outbreaks in the advent of globalization and ease of travel have underscored their prevention as a critical issue in safeguarding public health. Despite the progress made in immunization and drug development, many viruses lack preventive vaccines and efficient antiviral therapies, which are often beset by the generation of viral escape mutants. Viral diseases pose a great risk to human health as viral infections are tough to control due to mutative nature of the viral genomes. Medicinal plants provide diverse bioactive phytochemicals which play synergetic role in maintaining human health and there is the constant emergence of new resistant viral strains which demands novel antiviral agents with fewer side effects and cell toxicity (Kapoor et al. 2017). So, identifying novel antiviral drugs is of critical importance and natural products are an excellent source for such

**Camptothecin (CPT)****Vinblastine****Vincristine****Vinorelbine****Podophyllotoxin (PPT)****Docetaxel****Fig. 7.5** Showing structure of some anticancer bioactive herbal agents

discoveries. Polyphenols, alkaloids, flavonoids, saponins, quinones, terpenes, proanthocyanidins, lignins, tannins, polysaccharides, steroids, thiosulfonates, and coumarins are prominent bioactive phytochemicals, which have been observed to combat viral infections.

Lin et al. (2014) summarize the antiviral activities from several natural products and herbal medicines against some notable viral pathogens including coronavirus (CoV), coxsackievirus (CV), dengue virus (DENV), enterovirus 71 (EV71),

**Paclitaxel (PTX)****Ingenol mebutate****Trastuzumab emtansine (Kadcyla)****Fig. 7.5** (continued)

hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus, human immunodeficiency virus (HIV), influenza virus, measles virus (MV), and respiratory syncytial virus (RSV) and found that natural products and herbal ingredients possessed high antiviral activity.

Many antiviral bioactive phytoconstituents such as polysaccharides, lectins, proteins, alkaloids, terpenes, flavonoids, polyphenols, etc., are useful as antiviral agents. Their working mechanisms are different, e.g., polysaccharides inhibit viral replication and viral binding to cell; lectins from banana inhibit virus penetration (HIV), reverse transcriptase and N-glycohydrolases; proteins (GAP31) inhibit viral DNA integration and viral replication, panaxagin reverse transcriptase and inhibit

viral protein synthesis; alkaloids block virus binding, inhibit virus growth, reduce viral titers in lungs (HIV, HSV); terpenes (saponins) inhibit the virus replication; flavonoids are inhibitory on reverse transcriptase, blocking RNA synthesis (HIV, HSV, influenza); polyphenols inhibit the viral cell entry by modulating the viral surface structure and affect the expression of virus proteins on cell surface, etc.

Many medicinal plants possess significant antiviral properties owing to the presence of a large array of different bioactive molecules in them. Researchers are in favor of the use of less toxic antiviral bioactive molecules from natural sources instead of using nucleic acid analogs, protease inhibitors or other toxic synthetic molecules as antiviral therapeutics. Polyphenols, alkaloids, flavonoids, saponins, quinones, terpenes, proanthocyanidins, lignins, tannins, polysaccharides, steroids, thiosulfonates, and coumarins are prominent bioactive phytochemicals, which have been observed to combat viral infections. Examples of some other bioactive antiviral phytochemical agents from plant sources include chalcones (ketone), spiroketal-enol (ether derivative), honokiol, limonoids (lignin), Swerilactones (lactones), xanthohumol (chalcone), decanoylphorbol-13 acetate (diterpene), oleanine, dammarenolic acid and saikosaponins (triterpene and triterpenoid), excoecarianin, loliolide (tannins), jubanines (alkaloids), quercetin (flavonoid), sennoside a (glycoside), silvestrol (benzofuran), sjp-l-5 (ligningomisin), etc. Figure 7.6 showing molecular mechanism of action of antiviral phytochemicals.

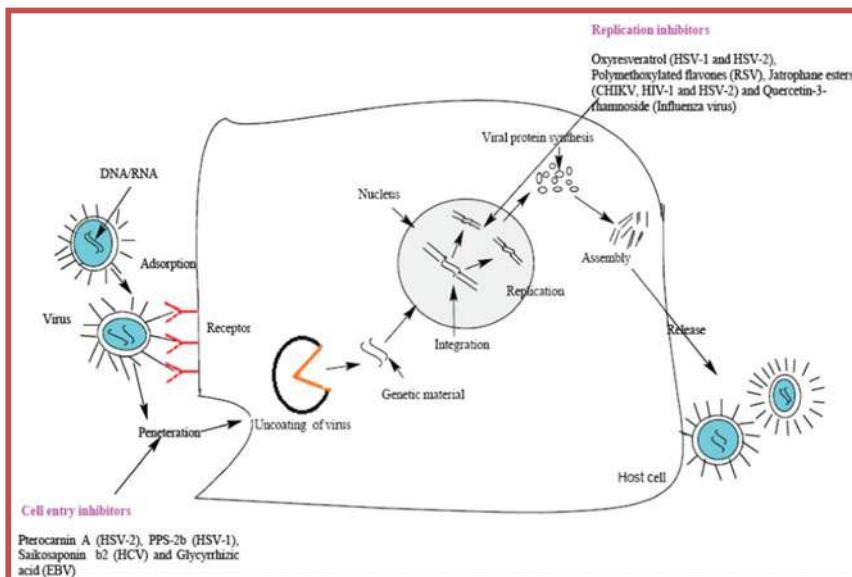
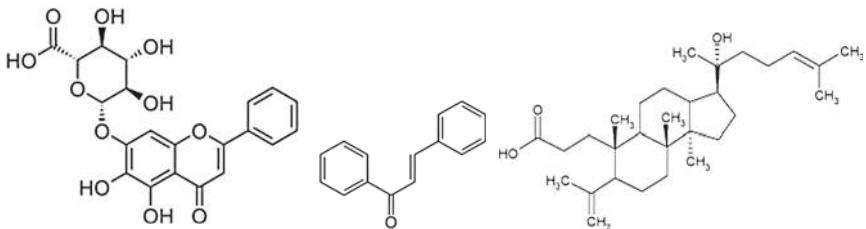
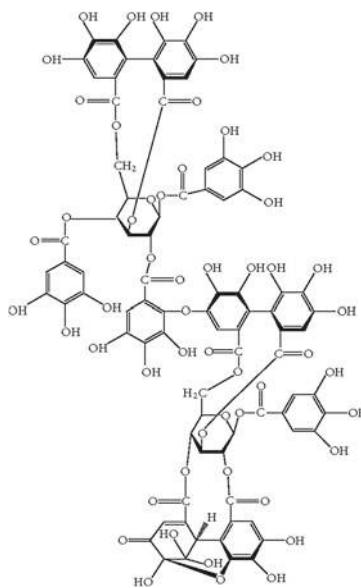
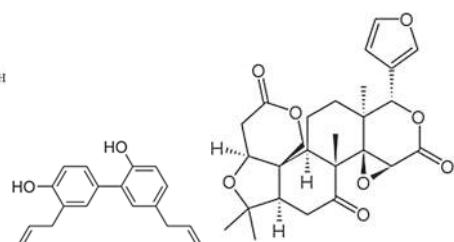
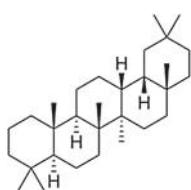
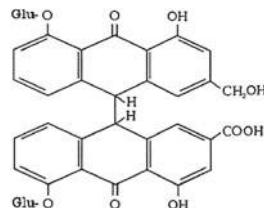


Fig. 7.6 Molecular mechanism of action of antiviral phytochemicals. Adsorption of the virus on to the surface of the target cell—entry of the uncoated virus particle into host cell—replication—assembly—release. Adsorption of the virus on to the surface of the target cell may be inhibited by phytomolecules such as Pterocarin A, PPS-2b, Saikosaponin glycyrrhetic acid; replication process may be inhibited by some of the phytomolecules such as Oxyresveratrol, Polymethoxylated Flavonoids, Jatrophane esters, and Quercetin or its derivatives

Table 7.12 Some antiviral bioactive compounds from medicinal plants with name of the chemicals, class and their activity

Phytochemicals	Class	Active against virus	Plant/plant parts
Baicalin	Flavone glycoside	DENV	<i>Scutellaria baicalensis</i> (roots)
Chalcones	Aromatic ketone	Influenza A (H1N1)	<i>Glycyrrhiza inflate</i> (roots)
Dammarenolic acid	Triterpenoid	Retroviruses	<i>Aglaia</i> sp. (bark)
Decanoylphorbol-13 acetate	Diterpene	CHIKV	<i>Croton mauritianus</i> (leaves)
Excoecarianin,	Tannins	HSV-2, HCV	<i>Phyllanthus urinaria</i> (whole plant)
Honokiol	Lignan	DENV-2	Magnolia tree (roots, bark)
Jubanines	Cyclopeptide alkaloids	PEDV	<i>Ziziphus jujuba</i> (roots)
Limonoids	Lignin	HCV	<i>Swietenia macrophylla</i> (stem)
Oleanane	Triterpenes	PDEV	<i>Camellia japonica</i> (flowers)
Quercetin	Flavonoid	HCV	<i>Embelia ribes</i> (seeds)
Saikosaponins	Terpenoid	HCV	<i>Bupleurum kaoi</i> (roots)
Sennoside A	Glycoside	HIV-1	<i>Rheum palmatum</i> (roots)
Silvestrol	Benzofuran	Ebola virus	<i>Aglaia foveolata</i> (leaves, bark)
SJP-L-5	Ligningomisin	HIV-1	<i>Schisandra micrantha</i> (roots)
Spiroketal-enol	Ether	HSV-1 HSV-2	<i>Tanacetum vulgare</i> (rhizome)
Swerilactones	Lactones	HBV	<i>Swertia mileensis</i> (whole plant)
Xanthohumol	Chalcone	BVDV	<i>Humulus lupulus</i> (whole plant)

Some antiviral bioactive compounds from medicinal plants with the name of the chemicals, class, their activity, and structure have been given above (Table 7.12: Fig. 7.7). Baicalin is a flavone glycoside. It is the glucuronide of baicalein found in several species in the genus *Scutellaria*, including *Scutellaria baicalensis* and *Scutellaria lateriflora*. Chalcone is an aromatic ketone and an enone that forms the central core for a variety of important biological compounds, which are known collectively as chalcones or chalconoids. Honokiol is a lignan isolated from the bark, seed cones, and leaves of trees belonging to the genus *Magnolia*.

**Baicalin****Chalcone****Dammarenolic acid****Excoecarianin****Honokiol****Limonoids****Oleanane (natural triterpene)****Sennoside-A (senna glycoside or senna)****Fig. 7.7** Structure of some antiviral bioactive compounds derived from medicinal plants

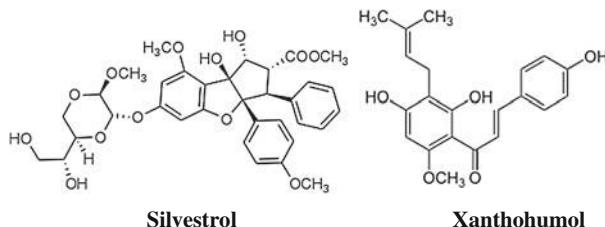


Fig. 7.7 (continued)

7.4 Natural Immunopotentiators, Vaccine and Biotechnology in Health Care

Medicinal plants are now widely used worldwide to address a variety of diseases for their prevention and treatment. Disease prevention is categorized into three levels such as (i) primary prevention (intending to decrease the number of new cases of a disorder or illness by health promotion/education, immunization); (ii) secondary prevention (intending to lower the rate of established cases or prevalence of illness by screening and prompt management); and (iii) tertiary prevention (intending to decrease the amount of disability associated with an existing disorder by rehabilitation and other means).

Disease prevention focuses on strategies that reduce the risk of disease, identify risk factors, or detect disease in its early, most treatable stages and examples of disease prevention activities include regular health examination, immunizations, calcium and Vitamin D supplements to reduce the risk of osteoporosis, blood pressure and assessment of cholesterol and screening for illnesses such as breast, cervical, colorectal and prostate cancer. Medicinal plants play vital roles in disease prevention and their promotion and use fit into all existing prevention strategies and Sofowora et al. (2013) provided a list of >50 plants with potentials in preventive medicine practice. Prevention is better than cure is no doubt the golden rule in health management today and there is a clear need for prevention of a disease developing in the first place. Herbal immunotherapy may be helpful in this regard to reach the desired goal.

7.4.1 Natural Immunopotentiators and Vaccine Adjuvants from Plants and Other Sources

Immunopotentiators may be derived from different natural sources such as plants, fungi, marine organisms, and others. Plant-derived immune stimulators consist of a diverse range of small molecules or large polysaccharides, e.g., saponins, tomatine,

inulin, etc., and fungi produce a range of potential candidate molecules, e.g., β -glucans. Other complex molecules that have established adjuvant activity include α -galactosylceramide (from marine sponge), chitosan (from shrimp chitin), and peptides (in bee venom). Some organisms like endophytic fungi and bees, produce immunostimulants using compounds obtained from plants (Woods et al. 2017).

Immunotherapy and herbal immune boosters

Prevention is better than cure and the proverb implies that it is unwise to become diseased and go under treatment to get rid of the curse of the imposed disease rather the disease should be prevented before the attack. It is always safe to prevent the enemy before the attack. Boosting the immune system and prohibiting the entry of microorganisms is better than acting upon them later. Immunotherapy is a medical term defined as the treatment of disease by inducing, enhancing, or suppressing an immune response. Immunotherapy designed to elicit or amplify an immune response are classified as activation immunotherapy, while immunotherapy that reduce or suppress are classified as suppression immunotherapy.

The active agents of immunotherapy are collectively called immunomodulators and they comprise a diverse array of recombinant, synthetic, and natural preparations, often cytokines (Table 7.13). Some of these substances, such as granulocyte colony-stimulating factor (G-CSF), interferons, imiquimod, and cellular membrane fractions from bacteria are already licensed for use in patients. Others including IL-2, IL-7, IL-12, various chemokines, synthetic cytosine phosphate-guanosine (CpG), oligodeoxynucleotides, and glucans are currently being investigated extensively in clinical and preclinical studies. Immunomodulatory regimens offer an attractive approach as they often have fewer side effects than existing drugs, including less potential for creating resistance in microbial diseases.

Cell-based immunotherapies have been proven to be effective for some cancers. Immune effector cells such as lymphocytes, macrophages, dendritic cells, natural killer cells (NK Cell), cytotoxic T-lymphocytes (CTL), etc., work together to defend the body against cancer by targeting abnormal antigens expressed on the surface of the tumor due to mutation.

Immune booster is a stimulation of the immune system through intake of the vaccine, toxoid (e.g., detoxified bacterial products, which keeps its antigenic properties) or ready to use antibodies (immunoglobulins). The natural active body's immune booster occurs as a result of its infection, and the natural passive immune booster occurs as a result of the maternal antibodies' transport through the placenta

Table 7.13 Some immunomodulators—the active agents of immunotherapy

Agent	Example
Interleukins	IL-2, IL-7, IL-12
Cytokines	Interferons, G-CSF, Imiquimod
Chemokines	CCL3, CCL26, CXCL7
Other	Cytosine phosphate-guanosine, oligodeoxynucleotides, glucans

to a fetus or to a newborn's body with witch's milk. In the case of the man-made passive immunization, antibodies are inserted into a body. Vaccines and detoxified bacterial products protect body for a long time, sometimes till one's dying day. The ready to use antibodies provide a temporary protection only; they have to be administering again in case of re-infection.

There are two ways of the man-made active immune booster, viz., (i) intake of the live but attenuated (weakened) microorganisms and (ii) intake of the killed microorganisms, their toxins or antigens. In both cases, a person gets vaccine or toxins, which do not cause a disease by themselves, but stimulate the immune system, making it able to recognize and to attack the particular microorganism. Generally, vaccines are administered parenterally (by injection) except live polio vaccine which is administered orally (by mouth). A possibility of using of the aerosols (when the vaccine gets into a body through the mucous membranes of a nose) is exploring for certain types of vaccines in addition to these two methods of vaccination.

The immune system is a complex and fantastic body system that protects people from bacteria, viruses, toxins, and other dangerous pathogens (germs). However, people often take their immune system for granted until something goes wrong with it. In the past, medical science primarily worked on ways of curing already-existing illness and disease, but today there is a growing emphasis on preventing illness by boosting the immune system's strength. Boosting body's immune system can help keep the body in peak condition to fight, repelling and destroying infections, viruses, and bacteria. There are many ways to help keep immune system functioning at its highest potential. If someone takes care of the immune system, immune system will, in turn, take care of him. Physical exercise keeps the body fit and active as well as helps the body to circulate lymph throughout the body. Simply, 30 min of moderate exercise a day can help the immune system to optimally carry out its defense. In addition, healthy and balanced diet, sufficient amounts of quality sleep, good hygiene habit, emotional health, healthy weight, practice prevention, etc., help to maintain sound health and immune system.

Natural immune system boosters and herbal antibiotics are known to prevent infections and illnesses. Synthetic and chemical agents may have adverse effects on the body. In this condition, herbal agents act fantabulously. Herbal agents are found to have zero side effects and show their action. Herbal immunity boosting agents are safe and effective. This phenomenon is present since ages, e.g., the concept of chyawanprash of Ayurvedic traditions developed >3000 years ago. Chyawanprash is a mixture of a large number of herbs (25–80) including amla and other ingredients and is claimed to posses antioxidants and immunomodulatory properties. There are many herbal immune boosters available in nature.

Natural remedies as immune system boosters

Natural and herbal remedies can provide much needed assistance in strengthening the immune system and getting the body's natural defense mechanisms in top shape. Holistic medicine recognizes that illness is caused primarily by weakened immune systems and not by pathogens. It is important to remember that

microorganisms like the flu virus, TB, etc., are around people all the time, but mostly they manage to resist becoming ill. Boosting the immune system naturally can allow the human body to fight off infectious agents without the drawbacks of conventional medication. There is a wide selection of medicinal herbs well known for their immune strengthening properties that are even safe for children too. Allowing the body to resolve infections without antibiotics will also help to strengthen the immune system against future attacks.

Black Elderberries (*Sambucus nigra*), a natural immune booster, are native to Europe and have a long history of use in herbal medicine. *Echinacea purpurea*, an American herb, has become famous for its antiviral, antifungal, and antibacterial properties. It is an excellent immune system tonic that boosts the body's immunity by stimulating the production of immune cells. *Astragalus membranaceous* has been used for centuries in traditional Chinese medicine to tone the immune system. *Astragalus* is an ideal remedy for anyone who is prone to recurrent infections such as the common cold, as it is able to increase the body's resistance and immune response to illness. *V. album* is commonly known to enhance the immune-stimulating properties of other ingredients, and it encourages repair of damaged cells. There are many herbs proven to improve immune function. Natural ingredients with immune boosting properties are *A. paniculata*, *Chondrus crispus* (Irish moss), *Crataegus monogyna* (Hawthorn). *C. oxycanthoides*, *Eleutherococcus senticosus* (Siberian ginseng), *Ficus carica*, *Glycyrrhiza glabra*, *H. officinalis*, *Hypoxis rooperi* (African star grass/wild potato), *Inula helenium*, *Mentha piperita*, *Olea europaea* (olive), *Panax ginseng* and other *Panax* spp., *P. emblica*, *Rosmarinus officinalis* (rosemary), *Schisandra chinensis*, *Solidago virgaurea*, *Thymus vulgaris* (thyme), *Verbascum thapsus* (mullein), *W. somnifera*, etc., act as excellent immune boosters. Over 30 species of medicinal mushrooms including *Auricularia auricula*, *A. polytricha*, *Agaricus bisporus* (Common mushroom), *A. blazei* (God's mushroom), *A. subrufescens*, *Agrocybe aegerita* (Chestnut mushroom), *Boletus edulis*, *Coprinus comatus*, *Cordyceps sinensis* (Caterpillar fungus), *Flammulina velutipes* (Enokitake), *Fomes fomentarius* (Tinder Conk mushroom), *G. lucidum* (Reishi), *Grifola frondosa* (Maitake), *Hericium erinaceus* (Lion's mane mushroom), *Hypsizygus tessellates*, *Inonotus obliquus* (Chaga Mushroom), *Lentinula edodes* (Shiitake), *Phallus indusiatus*, *Phellinus linteus* (Mesima), *Pleurotus citrinopileatus*, *P. djamor*, *P. ostreatus* (Oyster mushroom), *P. eryngii* (King Oyster mushroom), *Piptoporus betulinus*, *P. betulinus* (Birch bracket mushroom), *Schizophyllum commune* (Split-gill), *Sparassis crispa* (Cauliflower mushroom), *Tinder polypore*, *Trametes versicolor* (Turkey tail), *Tricholoma matsutake*, *Ustilago maydis*, and *Volvariella volvacea* are important for this purpose.

Medicinal mushrooms are used to treat and prevent a wide array of illnesses through their use as immune stimulants, immune modulators, adaptogens, and antioxidants. All of these natural medicines will go a long way in helping strengthen immune system against illness, disease, and infection. As immune stimulants, these natural products can be used to help treat cancer and fight infections by initiating an immune response which results in higher levels of white blood cells, cytokines, and antibodies and complement proteins. While not one of

the top immune-enhancing herbs, ginger (*Z. officinale*) does benefit the immune system. It has antibacterial, antiviral and antiparasitic activity. It has activity against certain types of cancer. It can stimulate phagocytosis and activate T-cells and modulate TH2.

Herbal immune boosting preparation

Herbal immune boosting preparation at home can be made with the following ingredients like 4 parts Echinacea root, 2 parts Thyme, 1 part Licorice and 1 part Elderberries. A cup of hot tea be prepared by adding 1 or 2 teaspoons of the premixed herbs to 1 cup of boiling water. Let it steep for 5–10 min, strain, and add honey (raw is best) to taste. To make a pitcher of tea for storing in the refrigerator, use 10 teaspoons or so of the premixed herbs for 8 cups of boiling water. Let it cool for a while and strain. Discard the used herbs, and put the tea in the refrigerator to drink over ice or reheated.

Biotechnology and high-tech herbal medicine

Biotechnology has applications in four major industrial areas, including health care (medical), crop production and agriculture, nonfood (industrial) uses of crops and other products (e.g. biodegradable plastics, vegetable oil, biofuels), and environmental uses. Biotechnology in healthcare sector along with the development of infrastructure, manpower, research, etc., includes development and manufacturing of biological reagents, biodiagnostics, biotherapeutics, preventive, therapeutic and, prophylactic vaccines, etc., and promoted biomedical innovation. The production of peptide hormones, new interferons and other lymphokines by the microbial and cell cultures, and new enzyme inhibitors of microbial origin are the most important for health care and pharmacy. Biotechnology is about to change health care and its delivery in profound ways; pharmacogenomics and the new genomic tools emerging from the biotechnology have revolutionized medicine and transformed the understanding of health and the provision of healthcare. Biotechnology has contributed to the discovery and manufacturing of traditional small molecule pharmaceutical drugs as well as drugs that are the product of biotechnology—biopharmaceutics. Examples of high-tech herbal medicine are plant-based vaccines, use of potato tubers as a biofactory for recombinant antibodies, etc.

Herbal vaccines

Vaccines help in stimulating the antibodies produced in human and animals and provide immune protection against several diseases. Plant-based vaccine production mainly involves the integration of transgene into the plant cells. Herbal or plant-based vaccine technologies involve the integration of the desired genes encoding the antigen protein for specific disease into the genome of plant tissues. The plants then start producing the exact protein that will be used for vaccinations. Agrobacterium-mediated gene transfer and transformation via genetically modified plant virus are the common methods that have been used to produce effective vaccines. New methods such as biolistic, electroporation, agroinfiltration, sonication, polyethylene glycol treatment, etc., have been developed to increase the efficiency of former methods. The flexibility of the plant expressed vaccine system,

combined with its low cost and ability to massively scale may provide vaccine protection not only to citizens of the developed as well as developing countries of the world that cannot currently afford vaccines. Other uses of plant-expressed vaccines including the successful creation of edible bananas that protect against the Norwalk virus. Table 7.14 showing examples of some plant-based vaccines for human and animal diseases.

Prevention is better than cure as the proverb implies that it is unwise to become diseased and go under treatment to get rid of the curse of the imposed disease rather the disease should be prevented before the attack. It is always safe to prevent the enemy before the attack. Boosting the immune system and prohibiting the entry of microorganisms is better than acting upon them later. Immunotherapy is a medical term defined as the treatment of disease by inducing, enhancing, or suppressing an immune response. Immunotherapy designed to elicit or amplify an immune response are classified as activation immunotherapy, while immunotherapy that reduce or suppress are classified as suppression immunotherapy.

Activation immunotherapies—cancer immunotherapy

Cancer immunotherapy attempts to stimulate the immune system to reject and destroy tumors. Dr. William Coley used Coley's Toxins in the late 1800s as crude immunotherapy with some success. Immuno-cell therapy for cancer was first introduced by Rosenberg and his colleagues of National Institute of Health, USA. In the late 80s, they published an article in which they reported a low tumor regression rate (2.6–3.3%) in 1205 patients with metastatic cancer who underwent different types of active specific immunotherapy (ASI), and suggested that immuno-cell therapy along with specific chemotherapy is the future of cancer immunotherapy. Initially, immunotherapy treatments involved administration of cytokines such as interleukin. Thereafter, the adverse effects of such intravenously administered cytokines lead to the extraction of the lymphocytes from the blood and expanding in vitro against tumor antigen before injecting the cells with appropriate stimulatory cytokines. The cells will then specifically target and destroy the tumor expressing antigen against which they have been raised.

The concept of this treatment started in the US in the 80s and fully fledged clinical treatments on a routine basis have been in practice in Japan since 1990. Randomized controlled studies in different cancers resulting in significant increase in survival and disease-free period have been reported and its efficacy is enhanced by 20–30% when cell-based immunotherapy is combined with other conventional treatment methods. BCG immunotherapy for early stage (noninvasive) bladder cancer utilizes *instillation* of attenuated live bacteria into the bladder, and is effective in preventing recurrence in up to two-thirds of cases. Topical immunotherapy utilizes an immune enhancement cream (imiquimod) which is an interferon producer causing the patients own killer T-cells to destroy warts, actinic keratoses, basal cell cancer, vaginal intraepithelial neoplasia, squamous cell cancer, cutaneous lymphoma, and superficial malignant melanoma. Injection immunotherapy uses mumps, candida the HPV vaccine or trichophytin antigen

Table 7.14 The plant-based vaccines production for human and animal diseases

Disease	Pathogens	Plants	Transformation method	References
(i) Avian H5N1 influenza	Hemagglutinin protein of H5N1	<i>Nicotiana benthamiana</i>	Agrobacterium	Greer (2015)
(ii) Bluetongue	Bluetongue virus	<i>Nicotiana benthamiana</i>	Agroinfiltration	Thuenemann et al. (2013)
(iii) Dengue	Dengue virus type 2 E glycoprotein (EII)	<i>Nicotiana tabacum</i> cv. MD69	Agrobacterium tumefaciens	Kim et al. (2009)
(iv) Diabetics	Insulin	Safflower	Agrobacterium tumefaciens	Penney et al. (2011)
(v) Diarrheal	Norwalk virus	<i>Nicotiana benthamiana</i>	Agrobacterium tumefaciens	Lai and Chen (2012)
(vi) Diarrheal	Enterotoxigenic <i>Escherichia coli</i>	Corn	—	Tacket et al. (2004)
(vii) Ebola	Ebola virus	<i>Nicotiana benthamiana</i>	Agroinfiltration	Phoolcharoen et al. (2011)
(viii) Foot-and-mouth disease	Foot-and-mouth disease virus	<i>Stylosanthes guianensis</i> cv. Reynva II	—	Wang et al. (2008)
(ix) Gaucher disease	Taliglucerase alfa	Carrot	Stable transformation	Malabadi et al. (2012)
(x) Hepatitis B	Hepatitis B surface antigen	Tomato	Agrobacterium tumefaciens	Li et al. (2011)
(xi) Human immunodeficiency	HIV	Tobacco	Agroinfiltration	Strasser et al. (2009)
(xii) Nerve agents attack	Acetylcholinesterase	Tobacco	PEGylated	Atsmon et al. (2015)
(xiii) Rabies	Rabies virus	<i>Nicotiana benthamiana</i> , tomato	Agroinfiltration	Perea Arango et al. (2008)
Tuberculosis	<i>Mycobacterium tuberculosis</i>	<i>Arabidopsis thaliana</i>	Agrobacterium	Rigano et al. (2004)

injections to treat warts (HPV induced tumors). Lung cancer has been demonstrated to potentially respond to immunotherapy.

Dendritic cell-based immunotherapy

Dendritic cells (DCs) are professional antigen-presenting cells (APC). They are found in most tissues of the body and are particularly abundant in those that are interfaces between the external and internal environments (e.g., skin, lungs, mucosa, and lymphoid tissues and in the lining of the gastrointestinal tract). DCs get their name from their surface projections that resemble the dendrites of neurons. Their main function is to process antigens and present them to T cells to promote immunity to foreign antigens and tolerance to self-antigens. They also secrete cytokines to regulate immune responses. Once activated, they migrate to the lymph nodes where they interact with T-cells and B-cells to initiate and shape the adaptive immune response. They act as messengers between the innate and the adaptive immune systems.

Dendritic cells can be stimulated to activate a cytotoxic response towards an antigen. Dendritic cells, a type of antigen-presenting cell, are harvested from a patient. These cells are then either pulsed with an antigen or transfected with a viral vector. Upon transfusion back into the patient, these activated cells present tumor antigen to effector lymphocytes (CD4 + T cells, CD8 + T cells, and B cells). This initiates a cytotoxic response to occur against cells expressing tumor antigens (against which the adaptive response has now been primed). The cancer vaccine Sipuleucel-T is one example of this approach. DC-based vaccinations represent a promising approach for the immunotherapy of cancer and infectious diseases as DCs play an essential role in initiating cellular immune responses.

T-cell adoptive transfer

Adoptive cell transfer uses T-cell-based cytotoxic responses to attack cancer cells. T-cells that have a natural or genetically engineered reactivity to a patient's cancer are generated *in vitro* and then transferred back into the cancer patient. One study using autologous tumor-infiltrating lymphocytes was an effective treatment for patients with metastatic melanoma. This can be achieved by taking T-cells that are found in the tumor of the patient, which are trained to attack the cancerous cells. These T-cells are referred to as tumor-infiltrating lymphocytes (TIL) and are then encouraged to multiply *in vitro* using high concentrations of IL-2, anti-CD3, and allo-reactive feeder cells. These T-cells are then transferred back into the patient along with exogenous administration of IL-2 to further boost their anticancer activity.

Thus far, a 51% objective response rate has been observed; and in some patients, tumors shrank to undetectable size.

The initial studies of adoptive cell transfer using TIL, however, revealed that persistence of the transferred cells *in vivo* was too short. Before reinfusion, lymphodepletion of the recipient is required to eliminate regulatory T-cells as well as normal endogenous lymphocytes that compete with the transferred cells for homeostatic cytokines. Lymphodepletion was made by total body irradiation prior to transfer of the expanded TIL. The trend for increasing survival as a function of

increasing lymphodepletion was highly significant ($P = 0.007$). Transferred cells expanded in vivo and persisted in the peripheral blood in many patients, sometimes achieving levels of 75% of all CD8 $^{+}$ T-cells at 6–12 months after infusion. Clinical trials based on adoptive cell transfer of TILs for patients with metastatic melanoma are currently ongoing at the National Cancer Institute (Bethesda, MD, USA), Moffitt Cancer Center (Tampa, FL, USA), MD Anderson Cancer Center (Houston, TX, USA), Sheba Medical Center (Tel Hashomer, Israel), Herlev University Hospital (Herlev, Denmark) and NKI Antonie van Leeuwenhoek (Amsterdam, Netherlands).

Autologous immune enhancement therapy

The autologous immune enhancement therapy (AIET) is an autologous immune cell-based therapy, wherein the patient's own peripheral blood-derived NK cells Cytotoxic T-lymphocytes and other relevant immune cells are expanded *in vitro* and then re-infused to tackle cancer. There are also studies proving their efficacy against hepatitis C viral infection, chronic fatigue syndrome, and HHV6 infection.

Genetically engineered T-cells

Genetically engineered T-cells are created by infecting patient's cells with a virus that contains a copy of a T cell receptor (TCR) gene that is specialized to recognize tumor antigens. The virus is not able to reproduce within the cell, however, integrates into the human genome. This is beneficial as new TCR gene remains stable in the T-cell. A patient's own T-cells are exposed to these viruses and then expanded nonspecifically or stimulated using the genetically engineered TCR. The cells are then transferred back into the patient and ready to have an immune response against tumor. Morgan et al. (2006) demonstrated that the adoptive cell transfer of lymphocytes transduced with retrovirus encoding TCRs that recognize a cancer antigen are able to mediate antitumor responses in patients with metastatic melanomas. This therapy has been demonstrated to result in objective clinical responses in patients with refractory stage IV cancer. The Surgery Branch of the National Cancer Institute (Bethesda, Maryland) is actively investigating this form of cancer treatment for patients suffering aggressive melanomas. The use of adoptive cell transfer with genetically engineered T-cells is a promising new approach to the treatment of a variety of cancers.

In one case study, United States doctors from the Clinical Research Division, led by Dr. Cassian Yee at Fred Hutchinson Cancer Research Center in Seattle had successfully treated a patient with advanced skin cancer by injecting the patient with immune cells cloned from his own immune system. The patient was free from tumours within 8 weeks of treatment. Dr. Cassian Yee described the research findings at The Cancer Research Institute International 2008 Symposia Series. Responses, however, were not seen in other patients in this clinical trial. Larger trials are now under way.

Immune recovery

The potential use of immunotherapy is known to restore the immune system of patients with immune deficiencies as result of infection or chemotherapy. For

example, cytokines have been tested in clinical trials interleukin-7 has been in clinical trials for HIV and cancer patients. In addition, interleukin-2 has also been tested in HIV patients.

Vaccination

Antimicrobial immunotherapy, which includes vaccination, involves activating the immune system to respond to an infectious agent.

Suppression immunotherapies

Immune suppression dampens an abnormal immune response in autoimmune diseases or reduces a normal immune response to prevent rejection of transplanted organs or cells.

Immunosuppressive drugs

Immunosuppressive drugs are important tools in the management of organ transplantation and autoimmune disease. Immune responses depend on lymphocyte proliferation, and cytostatic drugs are immunosuppressive. Glucocorticoids are somewhat more specific inhibitors of lymphocyte activation, whereas inhibitors of immunophilins more specifically target T lymphocyte activation. Immunosuppressive antibodies target an increasingly broad array of steps in the immune response, and there are still other drugs that modulate immune responses.

Immune tolerance

Immune tolerance is the process by which the body naturally does not launch an immune system attack on its own tissues. An immune tolerance therapy seeks to reset the immune system so that the body stops mistakenly attacking its own organs or cells in autoimmune disease or accepts foreign tissue in organ transplantation. A brief treatment should then reduce or eliminate the need for lifelong immunosuppression and the chances of attendant side effects, in the case of transplantation, or preserve the body's own function, at least in part, in cases of type 1 diabetes or other autoimmune disorders.

Allergies

Immunotherapy is also used to treat allergies. While other allergy treatments (such as antihistamines or corticosteroids) treat only the symptoms of allergic disease, immunotherapy is the only available treatment that can modify the natural course of the allergic disease, by reducing sensitivity to allergens.

A 1-to-5-year individually tailored regimen of injections may result in long-term benefits. Recent research suggests that patients who complete immunotherapy may continue to see benefits for years to come. Immunotherapy does not work for everyone and is only partly effective in some people, but it offers allergy sufferers the chance to eventually reduce or stop symptomatic/rescue medication.

The therapy is indicated for people who are extremely allergic or who cannot avoid specific allergens. For example, they may not be able to live a normal life and completely avoid pollen, dust, mites, mold spores, pet dander, insect venom, and certain other common triggers of allergic reactions. Immunotherapy is generally not indicated for food or medicinal allergies. Immunotherapy is typically individually

tailored and administered by an allergist (allergologist) or through specialized physician offices. Injection schedules are available in some healthcare systems and can be prescribed by family physicians. This therapy is particularly useful for people with allergic rhinitis or asthma.

The therapy is particularly likely to be successful if it begins early in life or soon after the allergy develops for the first time. In the past, this was called a serum, but this is an incorrect name. Most allergists now call this mixture an allergy extract. The first shots contain very tiny amounts of the allergen or antigen to which one is allergic. With progressively increasing dosages over time, one's body adjusts to the allergen and becomes less sensitive to it, in a process known as desensitization. A recently approved sublingual tablet (Grazax), containing a grass pollen extract, is similarly effective with few side effects, and can be self-administered at home, including by those patients who also suffer from allergic asthma, a condition which precludes the use of injection-based desensitization. To read more about this topic, see allergy and hyposensitization.

Helminthic therapies

Recent research into the clinical effectiveness of Whipworm ova (*Trichuris suis*) and Hookworm (*Necator americanus*) for the treatment of certain immunological diseases and allergies means that these organisms must be classified as immunotherapeutic agents. Helminthic therapy is being investigated as a potentially highly effective treatment for the symptoms and or disease process in disorders such as relapsing-remitting multiple sclerosis Crohn's, allergies and asthma. The precise mechanism of how the helminths modulate the immune response, ensuring their survival in the host and incidentally effectively modulating autoimmune disease processes, is currently unknown. However, several broad mechanisms have been postulated, such as a re-polarization of the Th1/Th2 response, and modulation of dendritic cell function. The helminths downregulate the pro-inflammatory Th1 cytokines, Interleukin-12 (IL-12), Interferon Gamma (IFN- γ) and Tumour Necrosis Factor-Alpha (TNF- α), while promoting the production of regulatory Th2 cytokines such as IL-10, IL-4, IL-5, and IL-13.

That helminths modulate host immune response is proven, as the core assertion of the hygiene hypothesis appears to have been, with the recent publication of a study demonstrating that co-evolution with helminths has shaped at least some of the genes associated with Interleukin expression and immunological disorders, like Crohn's, ulcerative colitis and Celiac Disease. Much of the research that has been published now indicates a key role, for what has been traditionally regarded as disease-causing organisms, so that their relationship to humans as hosts should not be classified as parasitic, rather as mutualistic, symbionts.

7.5 Biotechnology of Disease Prevention

Most developments in biotechnology originated for their potential applications in health care of both human and animal. And it is in this sector that the contributions of biotechnology are more frequent, more notable and more rewarding (both financially and psychologically). It is difficult to summarize the whole gamut of contributions in a text of limited space; these could be grouped under the following broad heads: (i) disease prevention, (ii) disease detection, (iii) therapeutic agents, (iv) correction of genetic diseases, (v) fertility control, and (vi) forensic medicine. It is aimed to highlight the major developments under each of these categories by citing appropriate examples.

Disease prevention (Vaccines)

Prevention of diseases is the most desirable, most convenient and highly effective approach to health, this is achieved by vaccination or immunization using biological preparations called vaccines. Vaccines represent an invaluable contribution of biotechnology as they provide protection against even such diseases for which effective cures are not yet available. The effectiveness of vaccines may be appreciated from the fact that smallpox, once a dreaded disease the world over, has been completely eradicated from the world; the last case of smallpox was reported in 1977. The various vaccines can be grouped under the following types: (i) conventional vaccines (live vaccines, inactivated pathogens), (ii) purified antigen vaccines, and (iii) recombinant vaccines (recombinant proteins/polypeptides, DNA vaccines) (Fig. 7.8).

An ideal vaccine

An ideal vaccine or vaccination protocol should have the following features:

- (i) It should not be tumorigenic or toxic or pathogenic, i.e., it should be safe.
- (ii) It should have very low levels of side effects in normal individuals.
- (iii) It should not cause problems in individuals with the impaired immune system.
- (iv) It should not spread either within the vaccinated individual or to other individuals (live vaccines).
- (v) It should not contaminate the environment.
- (vi) It should be effective in producing long-lasting humoral and cellular immunities.

Conventional vaccines

Conventional vaccines consist of whole pathogenic organisms which may either be killed (most bacterial vaccines and some viral vaccines), or live vaccines where the virulence of pathogens is greatly reduced or attenuated (most viral vaccines). Conventional vaccines, although highly effective and relatively easy to produce at low cost, suffer from the following limitations:

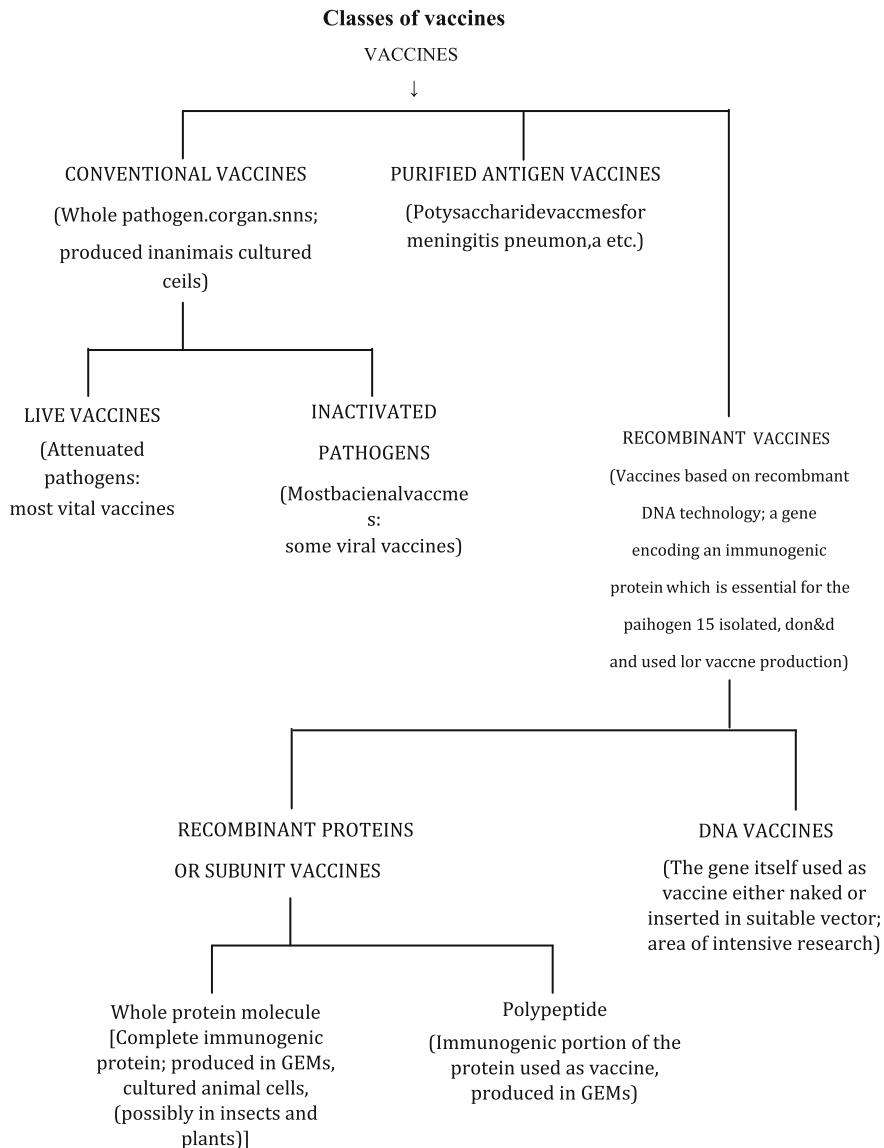


Fig. 7.8 Classes of different vaccines either in commercial use or in various stages of development (DNA vaccines)

- in many cases, live vaccines have to be used since killed pathogen vaccines are ineffective;
- live vaccines are generally based on cultured animal cells, hence expensive tissue culture setup is essential;
- live vaccines are heat-labile due to the pathogen inactivation by heat;

- (iv) conventional vaccines carry a variable risk of disease development due to the occasional presence of active virus particles (in case of inactivated vaccines; e.g., outbreak of foot-and-mouth disease in Europe), or reversion to virulence after replication in the vaccinated individuals (in case of attenuated live vaccines; 1 or 2×10^{-6} cases in case of live polio vaccine); and
- (v) in many cases, they are difficult to produce, e.g., hepatitis b virus does not grow in high titer in cultured cells;

These limitations have prompted the successful development of vaccines based on purified antigens and of recombinant vaccines, which are rather costly, at least for the present.

Purified antigen vaccines

These vaccines are based on purified antigens isolated from the concerned pathogens, i.e., these are nonrecombinant). Since they do not contain the organism, the risk of pathogenicity is avoided. However, (their cost is higher due to the steps involved in purification and vaccine preparation, and many of the isolated antigens are poorly immunogenic. Successful examples of such vaccines are mostly from bacteria, e.g., vaccines based on polysaccharide antigens from the bacterial cell wall capsules of *Neisseria meningitis* (causing meningitis) and *Streptococcus pneumoniae* (causing pneumonia).

Many bacteria produce exotoxins which are highly immunogenic. But these toxins produce toxic effects, the intensity of which decreases with storage and this decline is accelerated by heat, formaldehyde, and other chemicals. Fortunately, many exotoxins that have lost their toxicity retain their immunogenicity. They are called toxoids and are used as effective vaccines, e.g., toxoids of the pathogens causing tetanus, diphtheria, gangrene, etc. Precipitation of toxoids with alum enhances their immunogenicity. The toxoid vaccines are quite effective and cheap.

Some toxoids are very good adjuvants, i.e., increase the immunogenicity of other antigens, e.g., diphtheria toxoid. For example, the B polysaccharide of *Haemophilus influenzae* is poorly immunogenic. But when the B polysaccharide is combined with diphtheria toxoid. Its immunogenicity is greatly increased. In many cases, such adjuvant activities can be used to great advantage since most of the isolated antigens from pathogens are poorly immunogenic.

Recombinant vaccines

A recombinant vaccine contains either a protein or a gene encoding a protein of a pathogen origin that is immunogenic and critical to the pathogen function; the vaccine is produced using recombinant DNA technology. The vaccines based on recombinant proteins (=proteins produced by recombinant DNA technology) are also called subunit vaccines. The logic of such vaccines, in simple terms, is as follows. Proteins are generally immunogenic, and many of them are critical for the pathogenic organism. The genes encoding such proteins can be identified and isolated from a pathogen and expressed in *E. coli* or some other suitable host for a mass production of the proteins.

The concerned proteins are then purified and mixed with suitable stabilizers and adjuvants, if required, and used for immunization.

The different steps involved in the development of a recombinant protein-based vaccine may be simply summarized as follows: (i) The first step is to identify a protein that is both immunogenic and critical for the pathogen. (ii) The gene encoding this protein is then identified and isolated. (iii) The gene is integrated into a suitable expression vector and introduced into a suitable host where it expresses the protein in large quantities. (iv) The protein is then isolated and purified from the host cells, and (v) It is used for the preparation of vaccine. The host organisms used for expression of immunogenic proteins to be used as vaccines may be any one of the following:

- (i) A genetically engineered microorganism, e.g., yeast for the expression of hepatitis 8 surface antigen (HBsAg) used as vaccine against hepatitis B virus (this vaccine is approved for marketing in India) (Table 7.15).

Table 7.15 Products generated from genetically engineered microbes (GEMS). The products are either in current therapeutic use or in advanced stages of development

Product	GEM	Application
Currently in therapeutic use insulin ^a	<i>E. coli</i> and yeast	Diabetes
Human growth hormone	<i>E. coli</i>	Dwarfism
Interferons ^a	<i>E. coli</i>	Viral diseases, cancer, AIDS
Interleukins	<i>E. coli</i>	Various cancers
Hepatitis B surface antigen ^a	Yeast	Vaccine against hepatitis B
Streptokinase	<i>E. coli</i>	Thrombolysis
Epidermal growth factor	<i>E. coli</i> Yeast	Wound and burn healing
Granulocyte macrophage colony-stimulating factor ^a	Yeast	Cancer, AIDS
Granulocyte colony-stimulating factor ^a Bovine growth hormone	<i>E. coli</i>	Cancer, AIDS, bone marrow transplantation
Bovine growth hormone	<i>E. coli</i>	Increased milkyield
Tumour necrosis factor		Sepsis, cancer

In advanced stages of development

Atrial natriuretic factor (ANF)	Yeast	Hypertension and kidney diseases Thrombolysis
Plasminogen activator (including chimacrics and modifications)	<i>E. coli</i>	Cardiac treatment and organ transplants
Superoxide dismutase	<i>E. coli</i>	Antitumour and antiviral therapy Thrombolysis
Urokinase	<i>E. coli</i>	Neuropathic ulcers
Fibroblast growth factor	<i>E. coli</i> and Yeast	Osteoporosis, metabolism
Insulin like growth factor	<i>E. coli</i> and Yeast	Peripheral neuropathies Diabetic ulcers

(continued)

Table 7.15 (continued)

Product	GEM	Application
Nerve growth factor	<i>E. coli</i> and Yeast	Blood substitute
Platelet derived growth factor	<i>E. coli</i> Yeast	Cystic fibrosis
Haemoglobin (adult)	Yeast	Viral infection
Thymosin α 1	<i>E. coli</i>	

^aApproved for marketing in India. *Source* Ghosh (1995)

- (ii) Cultured animal cells, e.g., HBsAg expressed in CHO (Chinese hamster ovary Chinese hamster ovary) cell line and C-127 cell line; the vaccine is in advanced stages of development.
- (iii) Transgenic plants, e.g., HBsAg, HIV-1 (human immunodeficiency virus I) epitope. Rhinovirus 14 epitope (the last two for use as vaccines against HIV-1; in experimental stages).
- (iv) Insect larvae; the gene is integrated into a baculovirus (DNA viruses) which is used to infect insect larvae. Often, a very high quantity of the recombinant protein is produced. For example, up to 68% of the total protein of *Spodoptera exigua* larvae infected with a recombinant baculovirus is the recombinant protein. This protein can be purified for use as vaccine or may be used in diagnostic tests without purification. This approach has also been used to produce monoclonal antibodies to uncommon antigens. e.g., Alzheimer's protein.

So far a large number of recombinant immunogenic proteins of pathogens have been produced and evaluated. In general, a majority of such proteins are ineffective or only poorly effective in immunization. In some cases, at least, this problem may be due to the essential requirement for the immunogenic protein to be present in a specific aggregate form, e.g., HBsAg can be used for effective immunization only when it forms virus-like particles (of about 22 nm). Thus far hepatitis B vaccine is the only good example of a recombinant protein vaccine. An antimalarial vaccine based on the recombinant circumsporozoite protein of the sporozoite stage of the parasite (*Plasmodium falciparum*) is in advanced stages of development.

Recombinant polypeptide vaccines

Generally, the whole protein molecule is not necessary for immunogenicity; the immunogenic property is usually confined to a small portion of the protein molecule. For example, the immunogenicity of foot-and-mouth disease virus coat protein is due to its amino acids 114–160, and also 201–213. Segments of proteins containing either of these two amino acid sequences are effective in immunization; they induce antibodies which neutralize the virus and thereby provide protection against the foot-and-mouth disease. Similarly, the immunogenicity of the coat protein of feline leukemia virus (FLV) is due to a 14-amino acid long segment; this segment produced a partial immunogenic response in guinea pigs. Table 7.15 shows

products generated from genetically engineered microbes (GEMS). The products are either in current therapeutic use or in advanced stages of development.

In some cases, the immunogenic protein may be composed of two or more distinct polypeptides. In such cases, it may be desirable to use only one of the polypeptides as a vaccine for various reasons. For example, the cholera enterotoxin (produced by *Vibrio cholerae*) consists of 3 polypeptides, viz., A₁, A₂, and B polypeptides. The A polypeptides are toxic, while the B polypeptide is nontoxic but immunogenic. The gene encoding B polypeptide has been cloned, and the recombinant B polypeptide thus produced is being used as a vaccine against cholera; the recombinant B polypeptide is used, in combination with inactivated cholera cells, as an oral vaccine in place of the conventional injectable vaccine.

Recombinant protein or polypeptide vaccines are very safe since whole organisms are not involved. They are also of high efficacy. But (i) their cost is very high and often prohibitive, since they are produced by either bacterial, fermentation, or in animal cell cultures. (ii) They have to be stored at low temperatures since heat destabilizes the proteins, and (iii) This makes their storage and transportation, especially in developing countries, problematic and often limiting.

DNA vaccines

Recently, vaccines based on DNA are being developed, and the results obtained with influenza virus (advanced stages of testing) are quite exciting: these are regarded as the third revolution in vaccines. The strategy of DNA vaccines is as follows. The gene encoding the relevant immunogenic protein is isolated, cloned and then integrated into a suitable expression vector. This preparation is introduced into the individual to be immunized. The gene is ultimately expressed in the vaccinated individual and the immunogenic protein is expressed in sufficient quantities to invoke both humoral and cell-mediated immunities. It may be pointed out that cell-mediated immune response is essential for recovery from infectious diseases. The various approaches for DNA vaccines are as follows: (i) injection of pure DNA (or RNA) preparation into muscle; (ii) use of vectors (e.g., vaccinia viruses, adenoviruses, retroviruses, *E. coli*, *Salmonella typhimurium*, herpes viruses, etc.) for delivery of the gene, (iii) reimplantation of autologous cells (cells of the individual to be vaccinated) into which the gene has been transferred, and (iv) particle gun delivery of plasmid DNA which contains the gene in an expression cassette.

Injection of pure DNA or RNA into the skeletal muscle leads to the uptake and expression of the DNA in the muscle cells. When a gene encoding an immunogenic protein is so introduced, its expression also results in immunization of the individuals. This approach has potential for delivery of DNA vaccines. The DNA most likely enters the skeletal muscle cells through transient discontinuities in their plasma lemma produced by stretching of the muscle cells during exercise.

Another approach is to remove cells from the body of an individual into which the concerned immunogen encoding gene is introduced and expressed. These cells are then reintroduced into the body of the individual in a variety of ways, e.g., simple infusion, implantation, encapsulation, etc. This approach, although more cumbersome, has the advantage of enabling control of the modified cells within containment.

The immunogen encoding gene may be integrated into an expression plasmid, which is purified, coated on gold or tungsten particles and introduced into skin cells by a particle gun. Antigen-encoding genes introduced into the skin of mice and guinea pigs elicited humoral immune response; it is not known if cellular immunity is also induced. Plasmid DNA is noninfectious, heat stable and offers other advantages over viral/bacterial vectors. The skin cells are usually shed off in a few days after the inoculation so that there is no long-term persistence of the modified cells.

The approach holding considerable promise employs a live vector for the delivery of immunogen-encoding gene into the vaccinated individuals. The most advanced and promising vectors are: vaccinia viruses, adenoviruses, *E. coli*, *S. typhimurium*, other poxviruses, herpesviruses etc. The concerned gene is introduced into the genome of selected viral/bacterial vector which is suitably attenuated, and the live microorganisms are used for vaccination. Of the various vectors studied, vaccinia virus appears to be the most promising.

Vaccinia virus (W) is a close relative of the variola virus causing smallpox and was used as the vaccine to generate protection against smallpox. This virus has many useful features including stability in freeze-dried preparations, low production cost, and simple administration through ruptured skin cells. VV offers 19 possible sites for integration and expression of foreign genes. Generally, antigen encoding genes are inserted within its thymidine kinase (TK) locus which makes the virus TK and attenuates its pathogenicity. Further attenuation of VV can be achieved by integration, in its genome, of lymphokine genes like interferon gamma (IFN- γ) or interleukin-2 (IL-2). IFN- γ and IL-2 have, in addition, adjuvant activity and promote the immunogenicity of the introduced antigen to a level comparable to complete Freund's adjuvant.

A large number of genes encoding antigenic proteins have been integrated into the W genome which was then used for vaccination. The antigens included viral proteins like rabies virus glycoprotein, herpes simplex virus glycoprotein D, hepatitis B surface antigen, vesicular stomatitis glycoprotein etc. These recombinant vaccinia viruses induced both humoral and cellular immunity, and protected the immunized animals from the concerned viruses. Recently, a highly effective vaccine against rinderpest virus has been developed by inserting the viral genes H and F in the VV genes TK and HA. Cattle immunized with the recombinant VV vaccine were completely protected even when they were challenged by a more than 1000 times the normally lethal inoculum. The recombinant VV vaccine against rabies is also highly effective. Some antigens of HIV have been produced by recombinant VV in experimental animals and human volunteers; these antigens induced detectable cellular immunity to the expressed HIV protein. A recombinant VV containing the chimeric gene for IFN- γ and the structural proteins of HIV-1 is a quite promising candidate for a safe vaccine against HIV-1.

Several antigen encoding genes, each from a different pathogen, may be incorporated into a single VV genome. Such a recombinant VV vaccine will produce immunity to several diseases from a single inoculation; such vaccines are

called polyvalent vaccines. There is little data on the efficiency of such vaccines. The use of DNA for immunization is often called genetic immunization.

Recombinant VV (i) is not transmitted from vaccinated to contact animals. (ii) and induces both humoral and cellular immune responses. But (i) individuals previously immunized or exposed to infection by VV may respond poorly to recombinant VV vaccines. In addition, (ii) children and adults with congenital or acquired immunodeficiency may run the risk of severe infections; this could be resolved by incorporating the VV genome the gene IL-2 or IFN- γ .

DNA vaccines offer the following advantages: (i) purification and preparation of DNA for vaccines is easier, cheaper and more rapid, (ii) they are safer and more specific because of high purity, and (iii) they elicit a more potent immune response than purified protein vaccines.

Disease diagnosis

An accurate diagnosis of the disease and its causal organism is critical to its effective management and cure. Conventionally, disease diagnosis is based on the following.

- (i) Microscopy. The specimen (tissue, body fluid, excreta, pus, exudates, etc.) are subjected to microscopic examination for detection of the causal organisms, e.g., stool examination for ova and cyst.
- (ii) Culture of the specimen on specific and selective media to allow specific pathogens to grow, which are then tested for their susceptibility to various therapeutic agents, e.g., antibiotics.
- (iii) Detection and measurement of the pathogen-specific antibodies produced by the patient in response to the invasion by pathogen, e.g., in case of viral infections.

These tests are often tedious, take a long time (e.g., culture methods), may yield ambiguous results (e.g., immunological assays since they are based on polyclonal antibodies), and some of them cannot be applied in certain cases (e.g., antibody titer estimation in case of latent viral infections). Novel diagnostic approaches have been developed by biotechnology which are precise and very rapid, viz., (i) probes and (ii) monoclonal antibodies.

Probes

Probes are small (15–30 bases long) nucleotide (DNA/RNA) sequences used to detect the presence of complementary sequences in nucleic acid samples. Both DNA and RNA are used as probes. The probes can be prepared in many ways, and are either radioactively or non-radioactively labeled. Probes are being used in clinical diagnosis for the detection of microorganisms in various samples, e.g., tissues, excreta, body fluids, etc. Use of probes for disease diagnosis offers several advantages over the conventional diagnostic tools which are briefly listed below.

- (i) They are highly specific, relatively rapid, and much simpler.
- (ii) They are extremely powerful especially when combined with PCR; even a single molecule in the test sample can be detected.
- (iii) Since the culture of microbes is not required, the risk of accidental infection to laboratory personnel is eliminated and considerable time is saved.
- (iv) It is applicable to even such organisms which cannot be cultured.
- (v) Probes detect even latent viral infections which do not lead to an increase in antibody titer in the blood.
- (vi) A single species-specific probe can identify all the serotypes of a pathogen (Each of them may require a separate antibody).
- (vii) Pure probe preparations are relatively easily obtained.

However, since probes are usually radioactively labeled, they present a health hazard in handling and disposal. Therefore, the emphasis is shifting to non-radioactively labeled probes.

Probes are available for the detection of a variety of pathogenic microorganisms (Table 7.16). Probes can be used as follows: (i) hybridization (dot blot, Southern, in situ) and (ii) ligase chain reaction (LCR).

Hybridization—DNA may be isolated from the test samples and subjected to Southern blot or dot blot hybridization with the probe. For dot blot analyses, test samples like blood are generally lysed directly on the nitrocellulose filter. A probe can hybridize with a test.

DNA sample only when the latter contains the complementary sequence. The probes used in diagnostic assays are highly specific to the concerned pathogenic microorganisms. Therefore, a positive hybridization signal of a test DNA sample with a given probe reveals the presence of concerned microorganism.

Probes are used for hybridization assays using microscopic preparations of tissues. Generally, the tissues are fixed in formalin, embedded in paraffin, sectioned and stained with conventional stains like eosin and hematoxylin for routine examination. Subsequently, probes are used for in situ hybridization to detect the presence of concerned pathogens. This approach has proved quite useful for the detection of viral pathogens. The cytopathological data obtained by routine microscopic observations can then be correlated with the presence of specific pathogens to obtain a greater insight.

Table 7.16 Some selected microorganisms against which probes are available

Protozoa	Helminths	Bacteria	Viruses
Leishmania (Kala-azar)	Schistosomes (human blood flukes)	<i>Legionella</i> <i>Mycobacterium tuberculosis</i> complex	Herpes virus type 2
Trypanosoma (Sleeping sickness)	Wuchereria and Brugia (Filaria)	<i>Mycobacterium</i> spp. <i>Mycobacterium avium - sutracellulare</i>	Herpes virus type 1 and 2
Plasmodium (Malaria)	Onchocerca (River blindness) Trichinella Taenia solium	<i>Mycoplasma pneumoniae</i> <i>Chlamydia campylobacter</i> spp.	

Ligase chain reaction

In ligase chain reaction, (i) the clinical sample is prepared according to a protocol which liberates the DNA present in the sample. (ii) The prepared clinical sample is added to a reaction mixture containing thermostable DNA ligase, a vast excess of two double-strand oligonucleotide probes specific to the pathogen to be detected, and NAD (nicotinamide adenine dinucleotide). The two probes are blunt-ended, represent contiguous segments of the pathogen genome, and each of them is 15–30 bases long, therefore the target sequence is 35–60 bases long. The target sequence must be specific to the pathogen to be detected. (iii) The reaction mixture is heated in a thermocycler (also used for PCR) to 94 °C to ensure strand separation of both the target DNA and the probes; (iv) The temperature is then lowered to 55 °C to allow the probes to pair with the target DNA. Now, ligase joins the adjacent 3'-OH of one strand of probe 1 to the 5' phosphate of probe 2 strand; the complementary strands of the two probes are also similarly joined. The product of ligation of the two probes is called amplicon.

The second cycle of the ligase chain reaction (LCR) is initiated by heating the reaction mixture to 94 °C. In this and subsequent cycles of LCR, both the target DNA and the amplicons serve as targets for probes and, as a result, for amplification; this leads to an exponential amplification of the amplicons. The amplicons are detected by gel electrophoresis of the reaction mixture, ethidium bromide staining, and viewing under UV light. There will always be one band corresponding to the probes used for amplification. A second band, equal in size of the sum of the sizes of the two probes. It will appear only when the target sequence, i.e., the concerned pathogen is present in the test sample.

LCR (i) is highly efficient; it detects as few as 200–300 target molecules in a sample. (ii) It is highly specific and rarely produces false positive signals, which is in contrast to PCR. (iii) The LCR procedure allows automated detection by employing fluorescence or hapten labeled probes. LCR has been used to detect a wide variety of infectious agents, e.g., *Chlamydia trachomatis*, *Mycobacterium tuberculosis*, *Neisseria gonorrhoeas*, herpes simplex virus (HSV), HIV, hepatitis B virus, Hepatitis C virus, etc.

During LCR, the blunt-ended probe duplexes, not paired to the target sequence, also become ligated at a low frequency; this is called target-independent ligation.

This limits the sensitivity of LCR to between 200 and 300 target molecules per sample. This problem can be overcome by using probe molecules having staggered ends; this is called gap ligase chain reaction (G-LCR). G-LCR is able to detect as few as 5 target molecules/sample. G-LCR also uses a thermostable DNA polymerase. Another modification of LCR, called asymmetric gap LCR (AG-LCR), also utilizes reverse transcriptase, and is specific for RNA detection.

Monoclonal antibodies

A monoclonal antibody (Mab) preparation is specific to a single antigenic determinant (epitope) of a single antigen. Mabs are usually produced from hybridoma clones; each such clone is derived by the fusion of a single myeloma cell with a single antibody producing lymphocyte. Mabs are being produced against a variety

of antigens and being employed for many purposes including diseases diagnosis. Mabs are employed for (i) classification of blood groups (ABO, Rh, etc.), (ii) clear and specific detection of pathogens, (iii) and a very early and accurate detection of cancers.

The immunological assays generally employed for diagnostic purposes are varied. Of these, ELISA is the most rapid convenient and highly efficient. More recently a technique called immuno-PCR has been developed; this can be used to detect rare antigens at the single cell level, and even single antigen molecules/sample can be detected.

Immuno-PCR technique uses PCR amplification of a marker DNA segment attached to an antibody for detection of the antigen for which this antibody is specific. The protocol of immuno-PCR may be stated in simple terms as follows:

- (i) Test samples suspected of containing the antigen are added to microtitre plate wells and the antigen is immobilized on the surface of wells.
- (ii) The free binding sites on the microtitre well surfaces are suitably blocked.
- (iii) The antibody specific to the antigen to be detected is added to the wells. It forms antigen-antibody complex; this occurs only in those wells where the antigen is present. Free antibodies are removed by washing.
- (iv) Now, the molecular linker is added; it binds to the fragment crystallizable region (Fc region) of the antibody. A streptavidin-protein A chimera is the most versatile molecular linker. This chimeric linker binds to the Fc domain of antibodies due to the protein A sequences. It also binds to biotinylated DNA molecules due to its streptavidin moiety. The linker molecule is already complexed with the marker DNA molecule when it is added to the microtitre wells.
- (v) A segment of the marker DNA is amplified by PCR.
- (vi) The PCR products are analyzed by gel electrophoresis. For a large-scale application, the PCR products can be labeled using fluorochromes and haptens which permit their rapid, even automated detection as in the case of LCR.

The antigen-antibody complex will be formed only in those microtitre wells which contain the target antigen (the antigen for which antibody employed in the test is specific). Therefore, PCR amplification will occur only in such wells; in other wells there will be no PCR products. Therefore, all test samples which yield PCR products will contain the target antigen.

It is critical that after each step, up to step (iv) a thorough washing is done to remove unbound and nonspecifically bound antibody and marker DNA molecules; this increases the precision of the assay.

Immuno-PCR is (i) highly precise and extremely sensitive; it is several orders of magnitude more sensitive than ELISA. Therefore, (ii) it can detect rare antigens and (iii) diagnose pathological conditions much earlier. It is (iv) extremely versatile so that it can be applied to even single cells, can yield quantitative estimates of the antigen, and is amenable to automation. In addition, (v) it is relatively simple.

Table 7.17 Few selected examples of autoantibodies used as disease-specific marker for diagnosis

Antibody	Antigen specific	Associated clinical condition
Anti-ds DNA	B form of DNA	Systemic lupus erythematosus
Anti-Jo- 1	Histidyl-tRNA synthetase	Polymyositis
Anti-RNA polymerase I	Subunits of RNA polymerase I complex	Scleroderma
Anti-Centromere	Centromeric proteins	CREST (a subset of scleroderma)
Anti-acetylcholine receptor	Acetylcholine receptor	Myasthenia gravis
Anti-mitochondrial	Pyruvate dehydrogenase complex	Primary biliary cirrhosis

^ads = double stranded

Autoantibodies are those antibodies that are specific to those antigens which are normally tolerated by the immune system, and are typical constituents of cells and tissues of the animal in question; such antigens are called autoantigens. Autoantibodies are produced in conditions of autoimmunity which may be simply described as ‘an attack by the immune system on the host itself’. These antibodies are either cell-specific or non-organ specific. They recognize a variety of cellular and subcellular components, including the components of replication, transcription, RNA processing, RNA translation, and protein processing. The antigenic species of autoantibodies can be useful in clinical diagnosis of the associated autoimmune diseases (Table 7.17).

Detection of genetic diseases

Human beings suffer from several hundred genetic diseases almost all of which are produced by single recessive mutations. Many of these ailments can be managed but there—is no cure for any of them, except for the fast emerging option of gene therapy (Sect. 7.5). Their incidence can be minimized by an early detection of the afflicted fetuses which are then aborted. Therefore, when a woman gets pregnant the probability of her having a child suffering from a genetic disorder is estimated based on the histories of her and her husband’s families, and from the knowledge of previous births, if any. In case of risk, further investigation is carried out for a clear-cut and specific diagnosis.

Obtaining fetal cells: Earlier fetal cells were obtained by amniocentesis, i.e., withdrawal of amniotic fluid (which has free cells of fetal or developing human embryo origin) with the help of a hypodermic syringe. But amniocentesis is applicable only 18 weeks or later after the pregnancy, which is rather late for an abortion. Therefore, fetal cells are now obtained from biopsies of trophoblastic villi which are an external part of the human embryo and later form a part of the placenta. The biopsy is performed during 6th or 8th week of pregnancy (using an endoscope passed through the cervix of uterus), usually provides 100 µg of pure fetal DNA.

Fetal cells present in the amniotic fluid obtained by amniocentesis are recovered by centrifugation and cultured to obtain sufficient cells for various analyses. But the

tissue obtained from a biopsy of trophoblastic villi is usually enough for assays and in Rico culture may not be necessary.

Disease detection: The fetal cells are used for detection of the genetic disorders in one of the following ways.

- (i) Determination of karyo type of cells provides information on various syndromes produced by gross chromosomal aberrations.
- (ii) Most of the genetic diseases produce defective proteins/enzymes or no enzymes; many of these proteins have been identified and some of these can be assayed. The fetal cells are used to assay the concerned enzyme activities to detect such genetic diseases. At least 35 genetic diseases can be detected by assaying activities of specific enzymes.
- (iii) In case of some genetic diseases, the concerned gene mutation may alter (either abolish or produce) the recognition site for a restriction enzyme. The RFLP so produced can be detected by Southern hybridization; a sequence of the concerned gene is used as a probe. For example, in case of sickle cell anemia, the mutation from GAG to GTG eliminates a recognition site for the restriction enzyme Mst II (CCTNAGG) in the β globin gene (β gene) of hemoglobin. DNAs from a normal (β^A) and the test individuals (including fetal samples) are digested with Mst II, subjected to gel electrophoresis and probed with a sequence of β globin gene. If the test individuals have normal β globin (β^A) gene, the bands detected in their Southern blots will be comparable to those of normal DNA. A sickle cell mutant of the β globin (β) gene will change this pattern in a detectable manner (Fig. 9.4). Heterozygotes will show the bands present in both normal and sickle cell DNAs.
This approach is applicable to only those disorders in which the gene mutation changes the restriction pattern. As a result, this approach is not of general application.
- (iv) A more general approach utilizes oligonucleotide probes representing the sequence altered by the gene mutation causing the genetic disease. Typically, a set of two separate probes are used for each disease: one probe is complementary to the normal sequence, while the other is complementary to the mutant sequence. The probes are radiolabelled and used to probe Southern blots; under appropriate conditions, the probes can distinguish the normal and mutant DNA samples.

A set of two 19-mer (19 base long) oligonucleotide probe has been successfully used to detect sickle cell anemia. One of the two probes (β^S probe) contains the sequence complementary to that changed by the sickle cell mutation, while the other (β^A probe) is complementary to the same segment of the normal allele. The Southern blots of normal individuals ($\beta^A \beta^A$) hybridize only with the β^A probe, those of sickle cell homozygotes ($\beta^S \beta^S$) only with the β^S probe, while those of the heterozygotes hybridize with both β^A and β^S probes. Similarly, other mutant genes (e.g., α -antitrypsin gene implicated in pulmonary emphysema) differing from the

normal allele for a single base could be detected using this approach. This approach is of more general application than detection of RFLPs. But, this assay can be used only in such cases where the base sequence of the gene segment containing the mutation (for both normal and mutant alleles) is known to allow the synthesis of the two oligonucleotide probes. Probes can be prepared for mutations due to base substitution, insertion or deletion in the concerned genes.

Disease treatment

Treatment of diseases utilizes a wide variety of preparations of both biological and abiological origins. The preparations of biological origin may either be crude (e.g., Ayurvedic medicines, some allopathic drugs, etc.) or purified to various degrees. Many of such compounds are obtained from plants, but a large number of them originate from microorganisms, cultured cells, and recombinant organisms.

Products from nonrecombinant organisms

Therapeutic agents from non-recombinants organisms may originate from the following systems.

Microorganisms. A large number of pharmaceuticals originate from microorganisms; they range from whole microorganisms, e.g., spores of *Lactobacillus* sporogenes, through biomass used as food/feed supplements, e.g., single cell proteins, to a variety for highly valuable compounds like antibiotics, vitamins, enzymes, organic acids, etc.

Plant cell cultures. Some biochemicals of pharmaceutical value are produced by cultured plant cells, e.g., shikonin, berberine, ginseng biomass, and Taxol. Taxol is produced from cell cultures of *Taxus* spp. grown in 75,000 l bioreactors and is used for the treatment of breast and ovarian cancers.

Animal cell cultures. Cultured animal cells are the source of several compounds used in the treatment of diseases, e.g., angiogenic factor, interleukin-2, β -interferon, etc.

Products from recombinant organisms

The products obtained from non-recombinant organisms are limited to their natural capabilities. Genetic engineering has, however, removed this limitation and genes from any organism can be transferred and expressed into any other organism. This has enabled the production of a large number of recombinant proteins, i.e., proteins produced by genetic engineering or recombinant DNA technology in microorganisms, cultured animal cells and, more recently, in plants (grown in the field).

Genetically engineered microorganisms. A large number of human genes encoding pharmaceutically valuable proteins have been cloned and expressed in microorganisms. Initially, *E. coli* was used as the host for obvious reasons of the ease in cloning. But yeast is fast becoming the host of choice for production of recombinant proteins. Several of the recombinant proteins, used for treatment of diabetes mellitus (protein, insulin), dwarfism [protein, human growth hormone (hGH)], cancer (proteins, interferons, interleukins, granulocyte macrophage colony-stimulating factor), thrombosis (streptokinase) and AIDS (e.g., interferons.

granulocyte macrophage colon\stimulating factor). Many other useful recombinant proteins are in advanced stages of development.

Animal cell cultures. More recently, cultured animal cells have been preferred for the expression of human genes encoding pharmaceutically valuable proteins. Some of these proteins have already been approved for therapeutic use, e.g., hGH (for dwarfism), tissue plasminogen activator (tPA; thrombolysis) erythropoietin (anemia), and blood clotting factor VIII (hemophilia); the last two of the proteins have been approved for marketing in India. In addition, more than a dozen recombinant proteins are in advanced stages of development.

Transgenic plants. Plants are highly desirable in many ways for commercial production of recombinant proteins of value. A large number of transgenes encoding pharmaceutically valuable proteins have been expressed in plants. There is at least one example of commercial production of a recombinant protein, hirudin, encoded by a synthetic gene expressed in *Brassica napus*. More examples are likely to follow in the near future.

Advantages. Production of recombinant proteins of pharmaceutical value in microorganisms, cultured animal cells or plants offers several important advantages over their conventional routes of production.

- (i) Production costs are reduced, in some cases drastically. For example, in case of interferon, the cost was reduced to about 150 times the cost of the conventional product derived from human blood.
- (ii) There is no risk of contamination by AIDS or any other virus as is the case with conventionally produced proteins, usually from human sources.
- (iii) They can be produced in far greater quantities than it is possible for conventional products.
- (iv) In some cases, proteins of human origin have become available, e.g., insulin, while conventionally only that animal origin could be obtained.

Interferons

Interferons (IFNs) are members of a large group of proteins called cytokines which affect a wide range of target cells and tissues by binding to specific receptors present on the surface of their target cells. In these and the following respect, cytokines resemble hormones: they are released into the bloodstream and other body fluids. But they differ from hormones in the following features: they are produced by a variety of cell types (and not by specific endocrine organs).

Interferons were originally identified due to their antiviral activity. The protection from viral infections by interferons is independent of the immune system. The interferons are of three major types: IFN- α IFN- β . IFN- γ and IFX- α group consists of a family of closely related proteins and is usually divided into two subfamilies. IFN- α I and IFN- α II, and is mainly produced by leucocytes. IFN- β is a single protein species, and is produced by both leucocytes and fibroblasts.

IFN- α and IFN- β share common receptors on the surface of target cells (Table 7.18), and are mainly induced by viral infections. In contrast, IFN- γ is

Table 7.18 Interferons, number of amino acids, and others

Interferon (IFN)	Number of amino acids	Produced by	Induced by	Receptors
IFN- α I subfamily	166–172	Leucocytes	Viral infection	IFN- α and IFN- β share common receptors
IFN- α II subfamily (= IFN- ω)	166–172	Leucocytes	Viral infection	
IFN- β	166	Fibroblasts and leucocytes	Viral infection	
IFN- γ	143	T-lymphocytes	Antigenic stimulation of T-lymphocytes	Distinct receptors

produced only by T-lymphocytes in response to antigen-activation, and has distinct receptors from those for IFN- α and IFN- β .

Mode of IFN action is not clearly understood, and more than one pathway may be involved. When IFN- α or IFN- γ bind to their receptors one or more tyrosine kinases become activated. This leads to the phosphorylation and consequent activation of transcription factors like 1SGF 3 (interferon-stimulated gene factor 3) which are then translocated from the cytoplasm into the nucleus. These factors then stimulate the transcription of a number of genes, including those encoding 2',5'-oligoadenylate synthetases, double-stranded RNA activated protein kinases (PKR) and Mx proteins. These and some other gene products protect the cells against a range of viruses. In addition, IFNs induce the following effects, the basis of which is not well understood.

1. Inhibition of viral replication by IFN- α , IFN- β , IFN- γ .
2. Protection of cells against other intracellular parasites (all IFNs).
3. Inhibition of cell division in some normal and transformed cells (all IFNs).
4. Regulation of cell differentiation (all IFNs).
5. Induction of cytokines, e.g., IL-1, tumor necrosis factor, or colony-stimulating factors (by IFN- γ only).
6. Activation of macrophages by IFN- γ only.
7. Activation of natural killer cells (all IFNs) etc.

IFN- α is being used on a significant scale for the treatment of hepatitis B. In addition, interferons have been approved for use in the treatment of cancer and other viral diseases, including AIDS.

Growth factors

Growth factors belong to the group of proteins called cytokines—they can alter cell production, organogenesis, and disease susceptibility in animals. The effect produced by a growth factor will mainly depend on the presence of other growth factors, the target cells and their receptors on target cells. The nomenclature of

cytokines is confusing and terms like interleukins, growth factors, colony-stimulating factors, etc., are used often for a single protein. The term interleukin is the preferred one and new leucocyte products are designated by this name followed by a number, e.g., interleukin-13. The various growth factors can be grouped into the following families: (i) insulin like growth factors (IGF, e.g., IGF-I and IGF-II), (ii) nerve growth factors (NGF), (iii) epidermal growth factors (EGF), (iv) transforming growth factor β (TGF- β), (v) platelet-derived growth factors (PDGF), (vi) fibroblast growth factors (FGFs), (vii) hepatocyte growth factors (HGF), and (viii) hemopoietic growth factors (at least 16 cytokines; affect production and function of blood cells).

Many of the growth factors have been approved for treatment of human diseases. Erythropoietin (EPO) is used on a considerable scale for the treatment of anemia. Interleukin-2 in conjunction with LAK cells are being used for cancer therapy. Similarly, granulocyte macrophage colony-stimulating factor and granulocyte colony-stimulating factor are used to accelerate neutrophil recovery after chemotherapy or bone marrow transplantation. EPO is also used to stimulate red blood cell production in kidney dialysis or cancer patients. Several other growth factors have been/are likely to be approved for similar and other applications.

Antisense nucleotides as therapeutic agents

A very effective and specific approach for the treatment of a variety of diseases is to design and use oligonucleotides (say 25–35 bases long) complementary to the 5' end of the parasite mRNAs; such oligonucleotides are called antisense oligonucleotides. The antisense oligonucleotide may be linked to an acridine for increased effectiveness. When such oligonucleotides were used on cultured blood parasite *Trypanosoma brucei*, the parasite was killed. This approach has been quite successful in the treatment of cancer, and antisense oligonucleotides are in various stages of evaluation.

Monoclonal antibodies

Monoclonal antibodies (Mabs) have several therapeutic applications, e.g., (i) to provide passive immunity against diseases, (ii) in treatment of diseases like leprosy (Mab preparations specific against the pathogen are administered at regular intervals), (iii) to deliver toxin molecules (as immunotoxins) specifically to cancer cells, and (iv) to deliver radioactivity to cancer cells.

Drug designing

This approach aims at designing drugs which specifically and selectively fit into the critical sites of the target molecules, thereby inactivating the latter. The target molecule may be an enzyme (concerned with either metabolism or DNA replication), a hormone receptor or some other important molecule involved in a disease. The aim of drug designing is to develop highly efficient drugs which have little or no side effects.

Drug delivery and targeting

Drugs are normally delivered either orally or parenterally (by injection). They become distributed in the whole body tissues and fluids, and only a small portion

reaches the diseased tissue/organ. This necessitates a much larger dose of expensive drugs, and may often produce severe undesirable effects in other organs/tissues. Further, oral route of drug administration is much more desirable than that by injection for obvious reasons. But this route is unsuitable for the new class of protein/peptide drugs due to poor uptake; this is because of proteolytic degradation in the gastrointestinal tract and poor permeability of the intestinal mucosa to these high molecular weight therapeutic agents. The following approaches are being developed for a more efficient and/or targeted delivery of drugs:

- (i) Peptide/protein drugs may be delivered by oilier routes, e.g., nasal, buccal, rectal, ocular, pulmonary, and vaginal routes; of these the nasal route appears to be the most promising. The epithelial membranes present barriers to drug uptake which can be overcome by using compounds that enhance permeability of these membranes. Some commonly used permeability enhancers are: sodium glycocholate, sodium deoxycholate, dimethyl- β cyclodextrin, etc. But prolonged use of these enhancers causes changes in the mucosal surface. Therefore, new and better enhancers are being developed.
- (ii) A variety of drugs can be encapsulated in liposomes, which are small lipid vesicles produced artificially. But liposomes have the disadvantages of larger size and poor tissue or cell type selectivity (liposomes become concentrated in liver and spleen). Tissue selectivity of liposomes can be greatly increased by attaching to their surface specific ligands, e.g., monoclonal antibodies (Mabs). When polyethylene glycol is also attached to the surface of such liposomes (having Mabs on their surface), the circulation time as well as the site-specific delivery of liposomes is greatly increased; such liposomes are called "stealth liposomes". Liposomes hold great promise as a DNA delivery system in gene therapy, but they have to be injected into the subjects.
- (iii) Polymers have been used as drug delivery systems; the drug is generally released by cleavage of the drug from the polymer, swelling of the polymer (for drugs trapped within the polymeric chains), through osmotic pressure generated pores, or simple diffusion. Polyesters are the widely studied biodegradable products; their hydrolysis yields nontoxic alcohols and organic acids. Polyesters of lactic acid and glycolic acid are the most widely used polyesters for slow release of drugs having large molecules, e.g., proteins, polysaccharides, and oligonucleotides. The drugs being delivered by the polymer systems include insulin growth factors, steroids, anticancer drugs, etc.
- (iv) The most effective system for site-directed delivery of drugs and other moieties (called drug targeting) is based on monoclonal antibodies. Immunotoxins serve as a good example of this approach. The application of this technology is limited by the low availability of Mabs specific to a given tumor cell type and also by the changing surface decorations (antigens displayed on the cell surface) of tumor cells. Intensive research efforts are directed in this very important area of disease treatment, and many important developments may be expected in the near future.

Artificial tissues/organs

Effective treatment of many ailments like burns, injuries, etc., requires tissue/organ transplants. Production of implantable tissues in vitro is called tissue engineering. Artificial skin produced in vitro is already in therapeutic use. It is hoped that artificial cartilage will also become available for therapeutic use in the near future. Studies are also focussing on the development in vitro of other organs like bone, liver, etc.

Gene therapy

Human beings suffer from more than 5000 different diseases caused by single gene mutations, e.g., cystic fibrosis, acatalasia, Huntington's chorea, Tay-Sachs disease, Lesch-Nyhan syndrome, sickle cell anemia, mitral stenosis, Hunter's syndrome, hemophilia, several forms of muscular dystrophy, etc. In addition, many common disorders like cancer, hypertension, atherosclerosis and mental illness seem to have genetic components. Malignant cells may arise due to mutations in two types of genes, viz., oncogenes and tumor suppressor genes; both the types of mutant alleles are involved in malignant transformation of cells.

Gene therapy may be defined in broad general terms as follows: Introduction of a normal functional gene into cells which contain the defective allele of the concerned gene with the objective of correcting a genetic disorder or an acquired disorder. Application of gene therapy involves the following basic developments in genetics, molecular biology and biotechnology: (i) identification of the gene that plays the key role in the development of a genetic disorder, (ii) determination of the role of its product in health and disease, (iii) isolation and cloning of the gene, and (iv) development of an approach for gene therapy.

The candidate disorders for gene therapy are selected on the basis of the following criteria: (i) the disease should be life threatening, (ii) the gene responsible for the disease has been cloned, (iii) a precise regulation of the gene should not be required, and (iv) a suitable delivery system should be available.

Types of Gene Therapy

Gene therapy may be classified into two types: (i) germline gene therapy and (ii) somatic cell gene therapy. In case of germline gene therapy, germ cells, i.e., sperms or eggs (even zygotes), are modified by the introduction of functional genes which are ordinarily integrated into their genomes. Therefore, the change due to therapy is heritable and passed on to later generations. This approach, theoretically, is highly effective in counteracting the genetic disorders. However, this option is not considered, at least for the present, for application in human beings for a variety of technical and ethical reasons.

In somatic cell gene therapy, the gene is introduced only in somatic cells, especially of those tissues in which expression of the concerned gene is critical for health. Expression of the introduced gene relieves/eliminates symptoms of the disorder, but this effect is not heritable as it does not involve the germline. Somatic cell therapy is the only feasible option, and clinical trials have already started mostly for the treatment of cancer and blood disorders. This approach is divided

into two groups on the basis of the end result of the process: (i) addition or augmentation gene therapy and (ii) targetted gene transfer.

Augmentation therapy

In this type of somatic cell gene therapy, the functional gene is introduced in addition to the defective gene endogenous to die cell (s), i.e., the modified cells contain both the defective (endogenous) as well as the normal (introduced) copies of the gene. There are two general approaches to augmentation therapy. The first approach was used in the first two patients on whom gene therapy was attempted to correct the genetic disorder called Severe Combined Immune Deficiency (SCID) syndrome produced by adenosine deaminase (ADA) deficiency. (i) Normal/ADA gene copies were produced by cloning and then (ii) packed into a defective retrovirus; most of the viral genes were replaced by the ADA gene, (iii) Lymphocytes were isolated from the patients, and (iv) the recombinant retroviruses were used to infect the lymphocytes. Finally, (v) the infected cells expressing the A/14 gene were injected back into the patients. The normal ADA gene was expressed in the patients, and ADA deficiency was partially corrected; this resulted in an improvement in the patient's immune system.

A variety of viral vectors have been used to deliver genes into target/stem cells. e.g., lymphocytes, bone marrow cells, cultured in vitro. The stem cells themselves are obtained either from the concerned patient (more desirable) or from a matched donor. The reservations about the safety of retroviral vectors are sought to be solved by developing suicide vectors which cannot replicate alter the delivery of the gene. The other main problems of this approach are: (i) low frequency of transfection of stem cells, (ii) stability of the integrated gene, (iii) duration of the gene expression, (iv) lack of proper regulation of gene expression, etc. More recently, interest has focussed on physical methods of gene delivery like Ca^{2+} phosphate coprecipitation, particle gun, electroporation, etc.

The second approach is the direct injection of DNA into the tissues either as protein complexes (in order to bring about the receptor-mediated transfer of DNA into a specific tissue, e.g., liver) or even as naked DNA into muscle or skin. Interestingly, these cells take up the DNA and express the gene product. Exciting results have been obtained with experimental familial hypercholesterolemia, where LDL receptor levels have been augmented by injection of the gene as a sialoglycoprotein complex. The problems in this approach as well, relate to the frequency of cells taking up and expressing the gene and. more particularly, the duration of expression.

The gene delivery methods used for gene therapy can also be used for the treatment of cancer or AIDS. In case of cancer, a toxin encoding gene can be delivered into the cancer cells. Similarly, appropriate interleukin genes can be delivered to boost the body's defense mechanisms (in case of AIDS).

Targetted Gene Transfer

Targetted gene transfer or gene targeting uses homologous recombination to replace the endogenous gene with the functional introduced gene. The first case of such gene transfer was used to disrupt the human (5-globin gene in cultured cells.

Subsequently, over 100 mammalian genes have been modified by this approach. Gene targeting can be used either to inactivate (by disruption) a functional endogenous gene or to correct a defective one. The initial gene targeting to disrupt the human β -globin gene used a double selection strategy called positive-negative selection.

The vectors employed for gene targeting are of two types: (i) insertion vectors and (ii) replacement vectors. The insertion vector is linearized by restriction cleavage within the sequence to be targeted; the targeted sequence provides the site for recombination and is different from the gene to be introduced. Hence, the sequence to be introduced is located in the inner region of the vector and is flanked by the sequences involved in recombination. A recombination of such a vector with its homologous cellular sequences produces a duplication of the targeted sequence; this is called insertional recombination. In contrast, a linearized replacement vector has the two halves of the target gene at its two ends. Recombination occurs within the two halves of the target gene, replacing a portion of the endogenous gene sequence by that of the introduced gene: this is called replacement recombination. There is no duplication of sequences, and the target gene becomes disrupted. Therefore, this approach can not be used for gene therapy.

A strategy has been devised to modify only a small sequence of the target gene without the attendant gene duplication/disruption produced by insertional/replacement recombination. This approach, called in-out method of gene targeting, consists of the following two steps:

- (i) The first step called "in" step, is targeted gene transfer using an insertion vector; the appropriately targeted cell will have a gene duplication.
- (ii) The second step, termed as "out" step, depends on either intrachromosomal recombination (between the introduced and the endogenous genes) or unequal sister chromatid exchange between homologous chromosomes. The recombination product of interest is a chromosome which has only a single and functional copy of the introduced gene.

The in-out strategy has been tested using the HGPRT (hypoxanthine-guanine phosphoribosyltransferase) gene. The gene was targeted into a mouse embryonic stem cell line; subsequently, it has been successfully used with some other genes. This procedure is ideal for gene therapy.

Gene targeting is the strategy of choice for gene therapy for the following reasons. (i) The targeted gene is changed in a precise and specific manner. (ii) The introduced functional gene is placed in the same context, i.e., it is flanked by the same DNA sequences, as the replaced endogenous gene. And, (iii) no other gene of the genome is affected. The major limitation of the approach is the low frequency of homologous recombination; this problem, however, is being removed by refinements of the technique. The feasibility of gene targeting has been demonstrated in a number of different cell types for several different genes. It is expected that targeted therapy would become feasibility for many genetic diseases in the near future.

References

- Alamgir ANM (2017) Therapeutic use of medicinal plants and their extracts: volume 1, Pharmacognosy. In: Rainsford KD (ed) Progress in drug research, vol 73. pp 403–426
- Atsmon J, Brill-Almon E, Nadri-Shay C et al (2015) Preclinical and first-in-human evaluation of PRX-105, a PEGylated, plant-derived, recombinant human acetylcholinesterase-R. *Toxicol Appl Pharmacol* 287(3):202–209
- Barker LR, Burton JR et al (eds) (1995) Principles of Ambulatory Medicine, 4th edn. Williams & Wilkins, Baltimore, Md, pp 803–843
- Bhushan MS, Rao CHV, Ojha SK, Vijayakumar M, Verma A (2010) An analytical review of plants for anti diabetic activity with their phytoconstituent and mechanism of action. *IJPSR*. 1(1):29–46
- Cefalu WT, Stephens JM, Ribnicky DM (2011) Diabetes and herbal (botanical) medicine, Chapter 19. In: Benzie IFF, Wachtel-Galor S (eds) Herbal medicine: biomolecular and clinical aspects, 2nd edn. CRC Press/Taylor & Francis, Boca Raton (FL)
- Cragg GM, Newman DJ (2005) Plants as a source of anti-cancer agents. *J Ethnopharmacol* 100:72–79
- Gavras H (2009) Pathogenesis of hypertension: a review. *J Med Sci* 2(1):25–28
- Ghosh PK (1995) Role of biotechnology in health care in India: present and future. *Biotech Dev Rev*, pp 1–22. (June–Dec 1995)
- Greer AL (2015) Early vaccine availability represents an important public health advance for the control of pandemic influenza. *BMC Res Notes* 8(1):191
- Kapoor R, Sharma B, Kanwar SS (2017) Antiviral phytochemicals: an overview. *Biochem Physiol* 6:220. <https://doi.org/10.4172/2168-9652.1000220>
- Karuppusamy S (2009) A review on trends in production of secondary metabolites from higher plants by in vitro tissue, organ and cell cultures. *J Med Plants Res* 3(13):1222–1239
- Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J (2005) Global burden of hypertension: analysis of worldwide data. *Lancet* 365:217–223
- Kim MY, Yang MS, Kim TG (2009) Expression of dengue virus e glycoprotein domain III in non-nicotinic transgenic tobacco plants. *Biotechnol Bioprocess Eng* 14(6):725–730
- Lai H, Chen Q (2012) Bioprocessing of plant-derived virus-like particles of Norwalk virus capsid protein under current Good Manufacture Practice regulations. *Plant Cell Rep* 31(3):573–584
- Li T, Sun JK, Lu ZH, Liu Q (2011) Transformation of HBsAg (hepatitis B surface antigen) gene into tomato mediated by Agrobacterium tumefaciens. *Czech J Genet Plant Breed* 47(2):69–77
- Lifton R (1996) Molecular genetics of human blood pressure variation. *Science* 272(5262): 676–680
- Lin LT, Hsu WC, Lin CC (2014) Antiviral natural products and herbal medicines. *J Tradit Complement Med* 4(1):24–35
- Malabadi RB, Meti NT, Mulgund GS, Nataraja K, Kumar SV (2012) Recent advances in plant derived vaccine antigens against human infectious diseases. *Res Pharm* 2(2):8–19
- Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM et al (2006) Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science* 314(5796):126–129
- Penney CA, Thomas DR, Deen SS, Walmsley AM (2011) Plant-made vaccines in support of the millennium development goals. *Plant Cell Rep* 30(5):789–798
- Perea Arango I, Loza Rubio E, Rojas Anaya E, Olivera Flores T, de la Vara LG, Lim GMA (2008) Expression of the rabies virus nucleoprotein in plants at high-levels and evaluation of immune responses in mice. *Plant Cell Rep* 27(4):677–685
- Phoolcharoen W, Bhoo SH, Lai H et al (2011) Expression of an immunogenic Ebola immune complex in *Nicotiana benthamiana*. *Plant Biotechnol J* 9(7):807–816
- Ren-Ren BAI, Xiao-Ming WU, Jin-Yi XU (2015) Reviews: current natural products with antihypertensive activity. *Chinese J Natural Medicines* 13(10):0721–0729

- Rigano MM, Alvarez ML, Pinkhasov J et al (2004) Production of a fusion protein consisting of the enterotoxigenic *Escherichia coli* heat-labile toxin B subunit and a tuberculosis antigen in *Arabidopsis thaliana*. *Plant Cell Rep* 22(7):502–508
- Sofowora A, Ogunbodede E, Onayade A (2013) The role and place of medicinal plants in the strategies for disease prevention. *Afr J Tradit Complement Altern Med* 10(5):210–229
- Strasser R, Castilho A, Stadlmann J et al (2009) Improved virus neutralization by plant-produced anti-HIV antibodies with a homogeneous β 1, 4-galactosylated N-glycan profile. *J Biol Chem* 284(31):20479–20485
- Tacket CO, Pasetti MF, Edelman R, Howard JA, Streitfield S (2004) Immunogenicity of recombinant LT-B delivered orally to humans in transgenic corn. *Vaccine* 22(31–32): 4385–4389
- Thuenemann EC, Meyers AE, Verweij J, Rybicki EP, Lomonosoff GP (2013) A method for rapid production of heteromultimeric protein complexes in plants: assembly of protective bluetongue virus-like particles. *Plant Biotechnol J* 11(7):839–846
- Tulunay M, Aypak C, Yikirkhan H, Gorpelioglu S (2015) Herbal medicine use among patients with chronic diseases. *J Intercult Ethnopharmacol* 4(3):217–220
- Wang DM, Zhu JB, Peng M, Zhou P (2008) Induction of a protective antibody response to FMDV in mice following oral immunization with transgenic *Stylosanthes* spp. as a feedstuff additive. *Transgenic Res* 17(6):1163–1170
- Woods N, Niwasabutra K, Acevedo R, Igoli J, Altwaijry NA, Tusiimire J et al (2017) Natural vaccine adjuvants and immunopotentiators derived from plants, fungi, marine organisms, and insects, Chapter 11. In: Schijns VEJC, O'Hagan DT (eds) Immunopotentiators in modern vaccines, 2nd edn. Academic Press, Copyright © 2017 Elsevier Ltd., pp 211–229

Chapter 8

Molecular Pharmacognosy—A New Borderline Discipline Between Molecular Biology and Pharmacognosy



Abstract Molecular pharmacognosy is based on molecular identification technologies and has provided an identification basis of crude drugs at the gene level. Molecular pharmacognosy includes the systematic assortment of varieties of traditional herbal medicine and Chinese herbs, and studies quality, standardization, conservation, and diversity of medicinal plants. The molecular pharmacognosy in twenty-first century embraces many items from the study of molecular taxonomy, phylogenetic evolution of medicinal plants and animals, conservation of endangered species, molecular identification of medicinal raw materials, controlling metabolic pathways and biosynthetic regulation of secondary metabolites in plants, conservation of biodiversity, genetic engineering, tissue culture technology, production of pollution-free medicinal plants, structure-activity relationships with a drug potential, etc. to genetic engineering technology based on molecular cloning and the related tissue culture technology, especially molecular marker technology based on PCR. Molecular pharmacognosy is a rising discipline combining molecular biology and pharmacognosy, and its development depends on systems biology with reference to genomics, proteomics, and metabolomics, and also involved methods of modern biotechnology. Molecular biology has become vital to drug discovery from medicinal plants, through the determination and accomplishment of appropriate screening assays directed toward physiologically relevant molecular targets. DNA markers based tools for molecular identification of traditional medicinal materials are considered more reliable for authentication of herbal materials.

Molecular pharmacognosy is a science dealing with crude drugs, which refers to either a kind of new product that originates in plants, animals, and minerals or natural medicinal materials that are directly used for medical care or as raw materials for medicine after a simple processing. As a subject, pharmacognosy has gone through four stages of development: pharmacognosy in ancient times, pharmacognosy in early modern times, pharmacognosy in modern times, and period of natural pharmacognosy. Molecular pharmacognosy is based on molecular identification technologies and has provided an identification basis of traditional

medicine at the gene level. Molecular pharmacognosy has been given the following main tasks: systematic assortment of varieties of traditional herbal medicine including Chinese herbs and study of quality standardization, conservation of medicinal plant and animal biodiversity, and research of sustainable utilization of crude drug resources, medicinal plant marker breeding and new variety cultivation, gene regulation of metabolic pathway and directional control of the quality of Chinese herbal medicines, the use of genetic engineering and tissue culture technique to achieve high-level expression and production of natural active ingredients or genetically modified ingredients, genetic engineering, and green pollution-free medicinal plant.

8.1 Concept of Molecular Pharmacognosy and Its Development

Pharmacognosy, originally coined from the Greek words “pharmakon” meaning drug and “gnosis” meaning knowledge in 1811 by the Austrian physician Schmidt and used later in 1815 by Seydler in his work *Analecta Pharmacognostica*, was involved in the study of crude drugs of plant and animal origin and developed as an important area of pharmacy education in the twentieth century. Pharmacognosy, in earlier days, was primarily concerned with the study of morphology and taxonomy of crude drugs, then isolation, structure elucidation of active constituents, biological activity of crude drugs, and recently involved in high throughput screening for discovering high value drugs or novel lead compounds for certain newly emerged diseases or effective against drug-resistant pathogens. To cope up with the increasing development and specialization in other branches of science including pharmacy, at present, pharmacognosy appears to be a multidisciplinary science that developed over the years, adapted, and accommodated within itself all the progressive scientific challenges including science of biogenic drugs, modern analytical techniques, quality control of herbal products, pharmaceuticals, poisons, medicinal foods, purified active extracts, fractions, essential oil isolation and characterization, and even application of molecular docking techniques.

The discovery of DNA structure in 1953 has changed the scenario of all branches of life science with special impact on molecular biology. This led to the generation of many interdisciplinary sciences including molecular pharmacognosy. Lu-qi Huang, professor of Pharmacognosy at the Institute of Chinese *materia medica*, China, first introduced the term “Molecular Pharmacognosy” in 1995. He proposed three theoretical bases for molecular pharmacognosy such as (i) it brought biologically related branches as pharmacognosy into a molecular level; (ii) pharmacognosy combined both crude drugs that contain DNA in their cells and molecular biology that is based on DNA as its material base; and (iii) the study level of crude drugs in pharmacognosy developed from organism, tissue, organ, and cell into genetics. Thus, the development of pharmacognosy was closely related to

molecular biology, promoting the study of pharmacognosy into a molecular level. Molecular pharmacognosy deals with the study of classification, identification, cultivation, quality control and protection of crude drugs, and production of effective element at molecular level. Based on theories and methods of pharmacognosy and molecular biology, molecular pharmacognosy is a promising and prospective branch in pharmacognosy.

Many techniques of molecular biology such as molecular markers, recombinant DNA, gene chip technique that is used for gene expression profiles and construction of genomic library, and also elicitors which are compounds stimulating plant defense, and thus can be used to increase secondary metabolites production, are applied to pharmacognosy. Molecular pharmacognosy is concerned with the assessment of drug purity (genuine or false) and quality, and thus excellent varieties can be researched and cultured for high yield, maximum quality, and fast growth. Molecular markers with important traits can be searched and undergo breeding. Molecular pharmacognosy is also concerned with gene regulation of metabolic pathway as an attempt to improve the content of active constituents and the quality of herbal drugs. The molecular pharmacognosy in twenty-first century embraces many items from the study of molecular taxonomy, phylogenetic evolution of medicinal plants and animals, conservation of endangered species, molecular identification of medicinal raw materials, controlling metabolic pathways and biosynthetic regulation of secondary metabolites in plants, conservation of biodiversity, genetic engineering, tissue culture technology, production of pollution free medicinal plants, structure-activity relationships with a drug potential, etc., to genetic engineering technology based on molecular cloning and the related tissue culture technology, especially molecular marker technology based on PCR. Molecular pharmacognosy deals with crude drugs at the genetic level with a theoretical and methodological basis on molecular biology. At the edge between pharmacognosy and molecular biology, molecular pharmacognosy has developed as a new intermediate branch.

8.2 Pharmacognosy at the Molecular Level

Molecular pharmacognosy is a science dealing with the study of classification, identification, cultivation, and protection of crude drugs and production of effective element at the molecular level, e.g., it discusses the application of molecular biology in resource science and authentication of traditional herbal medicine including TCM. The techniques and methods of molecular biology have been widely applied in pharmacognosy fields as indicated by international development trends of pharmacognosy studies on molecular level analyzed by bibliometric methods using the SCIE database on Web of Science. The number of international pharmacognosy literature on a molecular level is increasing year by year with a focus on molecular identification and genetic diversity. Chinese scientists issued high-impact factor journals papers and high citations amount in the international

forefront. The international pharmacognosy research on molecular level has developed rapidly and the USA, China, and Japan have close cooperation having a significant influence of Chinese research—the molecular mechanism of the formation of Daodi Herbs may become the next hotspot. At the boundary between pharmacognosy and molecular biology, molecular pharmacognosy has developed as a new borderline discipline and using the method and technology of molecular biology, molecular pharmacognosy focuses on resolving a wide range of challenging problems, such as distinguishing herbal and animal drug populations by molecular marker assay, conserving and utilizing wild resources on the basis of knowledge of genetic diversity, investigating the mechanism of active compound accumulation, and obtaining new resources with higher quality through genetic engineering.

Molecular pharmacognosy is a rising discipline combining molecular biology and pharmacognosy, and its development depends on systems biology with reference to genomics, proteomics, and metabolomics and also involved methods of modern biotechnology. Molecular biology has become vital to drug discovery from medicinal plant, through the determination and accomplishment of appropriate screening assays directed toward physiologically relevant molecular targets.

8.3 Development of Species Biology and Molecular Systematics

The evolutionary process by which biological populations evolve to become distinct species is called speciation and speciation depends on a measure of reproductive isolation, a reduced gene flow. Modern species concept has been widely accepted by taxonomists, and pharmacognosists can never ignore theory and fruits in species biology. Systematics—a system for imposing order (classify) to explain the biological diversity of nature. This attempt to examine and classify the biological diversity of nature is called systematics. Molecular systematics is the use of molecular genetics to study the evolution of relationships among individuals and species. The goal of systematic studies is to provide insight into the history of groups of organisms and the evolutionary processes that create diversity among species. Development of species biology and molecular systematics provides an effective weapon and basis for the study of system and evolution, classification, and identification. Evolutionists rapidly incorporated the evolutionary aspects of development of species in the Linnaean system and developed it into a phylogenetic classification. Evolution is not only an incident that just happened in the past but it can also be observed in the present and can be used to predict the future by employing molecular systematics to compare data across genes, individuals, populations, and species.

The importance of phylogenetic trees (estimates of evolutionary history) are that they allow biology to be predictive; as much as a chemist can use the periodic table

of elements to predict chemical reactions, biologists can use phylogenetic trees to analyze biological variation and make predictions about behavior, morphology, and physiology, as well as biomolecular structure and other biological attributes. Phylogeny is also an integral part of interpreting any co-evolutionary relationships such as host and parasite, e.g., the coevolution of insects and their host plants, the plants evolve chemical defenses against the insects, which then evolve resistance to the chemicals. Interest in phylogeny waned over much of the nineteenth century, replaced by an emphasis on genetics, physiology, and geographic variances.

DNA diversity is the essence of biodiversity. Molecular makers based on DNA polymorphism analysis and molecular systematics based on genome sequence analysis can directly test DNA variation patterns and determine the key units to protect, so the study of molecular systematics of medicinal plants and animals may presume developing status and endangered degree of the population, thus rendering new operative methods for measurement of biodiversity and countermeasures taken to protect rare medicinal plant and animal resources. Moreover, the application of research findings in molecular systematics based on DNA polymorphism makes the work of hunting for and enlarging the scope of medicinal plant and animal resources more effective and efficient. Expounding the relevance among genetic relationship of DNA molecules, active ingredients, and efficacy in combination of chemical taxonomy, obtaining molecular genetic background of important chemical elements so as to identify whether unknown plants have the genes to produce specific chemical composition, are shortcuts to hunt for and enlarge the scope of crude drug materials by use of molecular systematics.

A revolution in molecular biology took place in the 1960s. Methods for determining the molecular structure of proteins and amino acids allowed biologists to begin to estimate phylogenetic relationships. The exponential growth of molecular systematics in the late twentieth century is due to a combination of increased sophistication in molecular biology techniques, and computer advances in hardware and software that allowed scientists to model large and complex data sets.

Molecular systematists use a variety of techniques to derive phylogenetic trees. Polymerase chain reaction (PCR) is used to investigate variations of DNA on a large scale. Gene amplification is also fundamental to new approaches to DNA fingerprinting. Scientists can use “molecular clocks” to predict both past and future molecular divergences in genes. This theory claims that molecular change is sufficiently constant to determine how current genetic lineages branch off from a common ancestor and to determine when the branching occurred. Genetic markers are used to make inferences about relationships between environment and morphology, as well as physiology and behavior.

The applications of molecular systematics in medicine are particularly important. The ability to predict the course of evolution allows scientists to track epidemic pathogens, research zoonotic viruses (animal viruses that are transmissible to humans), understand the evolution of pharmaceuticals and drug resistance, and make predictions about emerging diseases. For example, phylogenetic studies of a form of influenza called influenza A have revealed reliable evolutionary behavior that can be used to predict how the viruses that cause influenza will evolve. This

allows scientists to prepare vaccines for future strains in advance. Research into when simian immunodeficiency virus began to be transmitted to humans is vital to understanding how the transmission occurred and perhaps to prevent future zoonotic transmissions.

8.4 Molecular Identification of Traditional Medicinal Materials

Traditional medicines are widely used by a large number of people (~80%) all over the world for health care purposes. In recent years, the use and demand of herbal preparations have been growing across the world including the developed countries and medicinal plants have gained popularity and attention among physicians and patients. TCM, as alternative medicines, is becoming one of the most widely used therapies throughout the world (Normile 2003). Adulteration, substitution, use of inherent toxic herbs, fraudulent action contamination, by misidentification, confusion of species, and inappropriate labeling of the herbal source materials have been the life-threatening problems of herbal preparations. There are several reports of incidence that adulterants or substitutes caused serious intoxications and even deaths (But 1994; But et al. 1996; Zhao et al. 2007; Mazzanti et al. 2008; Ng et al. 2009) and, as a result, a reliable identification method is important for safety, efficacy, and quality assurance of such preparations.

In practice, the identification of medicinal plants relies mainly on morphological, anatomical, and phytochemical characters. Many pharmacopeias refer to macroscopic and microscopic evaluation (morphology and histology) and chemical profiling (TLC-, HPLC-, and GC-fingerprinting) for quality control and standardization of raw and processed herbs (Chan 2003; Siow et al. 2005; Wagner et al. 2011). Microscopic examination of drugs requires botanical expertise for the unequivocal authentication (because related species often possess similar features) and chemical variability within the plant material often hinders the confirmation of its botanical identity as the chemical composition is affected by growth and storage conditions as well as by the harvesting process. With the development of molecular biology, improvement of molecular biotechnology, plant genetics, and related analytical methods in the recent decades, DNA markers-based tools for molecular identification of traditional medicinal materials are considered more reliable for authentication of herbal materials (Kumar et al. 2009). Meanwhile, various DNA-based molecular marker techniques are applied in many fields and their application is remarkably increasing for species characterization in medicinal plants (Shaw et al. 2002; Zhang et al. 2007; Sucher and Carles 2008). DNA markers use nucleotide sequences to identify species; it takes preference over the other two markers being not age-dependent, tissue-specific, and having a higher discriminating power. Various types of DNA-based molecular techniques are utilized to evaluate DNA polymorphism. These are hybridization-based methods; polymerase chain reaction

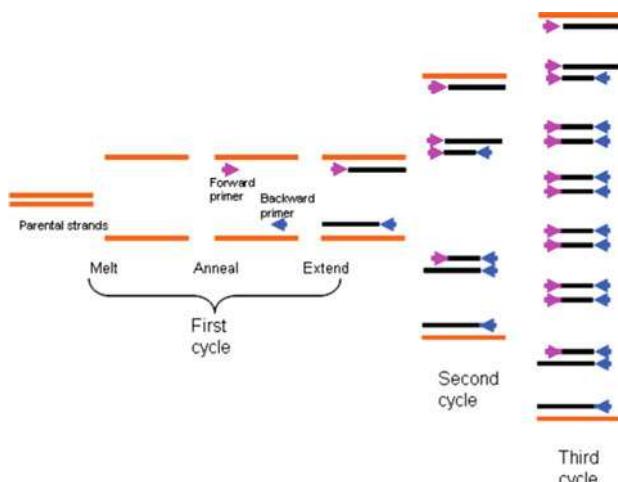
(PCR)-based methods, and sequencing-based methods. Based on the development of these techniques in the last three decades, they are classified into three classes such as (i) The first-generation molecular markers, including RFLPs, RAPDs, and their modifications; (ii) The second-generation molecular markers, including SSRs, AFLPs, and their modified forms, (iii) The third-generation molecular markers including ESTs and SNPs, followed by the development of DNA sequencing or DNA barcoding, next-generation sequencing (NGS), etc.

PCR (polymerase chain reaction), developed in 1983 by Kary Mullis, is a technique used in molecular biology to amplify a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. PCR is now an almost indispensable technique used in biological works including agricultural, medical, etc., for a variety of purposes such as molecular identification, DNA fingerprinting including DNA sequencing and DNA-based phylogeny, genetic engineering, molecular archeology, molecular epidemiology, diagnosis, molecular ecology, classification of organisms, genotyping, genomic cloning, genome projects, gene mutation screening, gene expression, drug discovery, bioinformatics, and in similar other studies.

DNA template (the sample DNA that contains the target sequence to amplify), Deoxyribonucleoside triphosphates (dNTPs), PCR buffer, primers (forward and reverse), Taq polymerase, etc. are the components of PCR. PCR contains 20–30 cycles following each other. Every cycle contains three consecutive steps such as (i) Denaturation (strand separation) step—the double-stranded DNA is heated to 94–96 °C for separating the strands when the hydrogen bonds which hold the two strands together are broken; (ii) annealing or connecting (primer binding) step—after separating the two DNA strands, the temperature is lowered (45–60 °C) so the primers can connect to the DNA strands. This temperature depends on the primers used in the PCR reaction, usually 5 °C lower than the melting point of the primers; (iii) Elongation (synthesis of new DNA) step—the DNA polymerase (Taq polymerase) enzyme synthesises the complementary strand of DNA beginning from the primer (72 °C). This temperature depends on the polymerase enzyme used in the reaction.

Polymerase chain reaction (PCR) is an efficient and cost-effective molecular tool to copy or amplify small segments of DNA or RNA. PCR combines the principles of complementary nucleic acid hybridization with those of nucleic acid replication that are applied repeatedly through numerous cycles. It results in the exponential production of the specific target DNA/RNA sequences by a factor of 10^7 within a relatively short period. PCR *in vitro* amplification technique can amplify a single copy of nucleic acid target using two synthetic oligonucleotides “primers” that bind to the target genomic sequence, which are extended by a Taq polymerase (a thermostable DNA polymerase). An automated process of repeated cycles (usually 25–40) of denaturation of the template DNA (at 94 °C), annealing of primers to their complementary sequences (50 °C), and primer extension (70 °C) is employed for the amplification of target sequence. Primer is a short segment of nucleotides, which is complementary to a section of the DNA or RNA, which is to be amplified

in the PCR. Two short DNA sequences designed to bind to the start (forward primer) and end (reverse primer) of the target sequence is used in PCR. Taq polymerase is a thermally stable DNA polymerase originally isolated from the thermophilic bacterium *Thermus aquaticus*, which resist inactivation during denaturation temperatures and allows primer extension at high temperature. Once the first round is completed, the process is repeated by cycling back to the first reaction temperature and next round of denaturation, annealing and extension are started (*an automatic process in thermocycler*). This three-step temperature cycle is repeated approximately 30 times, which results in exponential amplification of target gene sequence (Fig. 8.1).



Three cycles of polymerase chain reaction (PCR)

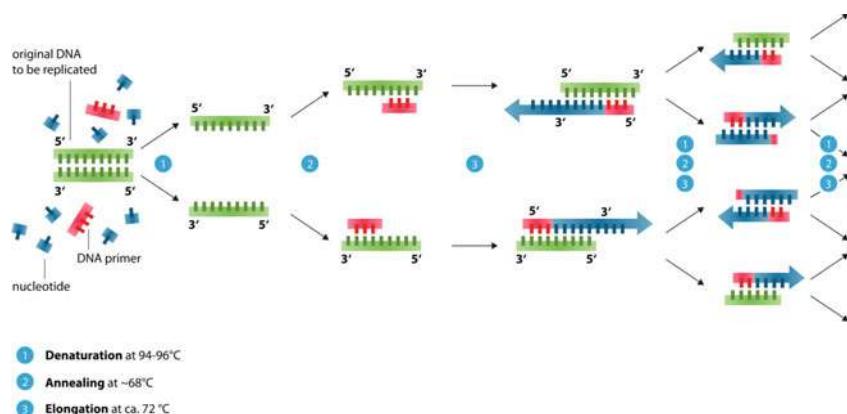


Fig. 8.1 PCR—polymerase chain reaction. Source <http://www.obgynacademy.com/basicsciences/fetology/genetics/>

There are several types of PCR such as (i) Real-Time PCR; (ii) Nested PCR; (iii) Multiplex PCR; (iv) Quantitative PCR; (v) Arbitrary Primed PCR, etc. These are different purpose-oriented modified forms of PCR. Benefiting in the first place from PCR techniques, DNA markers have become a powerful tool for identification and authentication of plant, animal, fungal, and bacterial species (Kaplan et al. 2004; Yip et al. 2007; Pereira et al. 2008; Hao et al. 2010). Contrary to chemical fingerprinting, which is strongly influenced by the age of the sample, physiological conditions, environmental factors, cultivation area, harvesting period, drying, and storage conditions, DNA is an extremely stable macromolecule that is not affected by external factors and, therefore, can be recovered from fresh, dried, and even processed biological material. Additionally, the marker molecules are not tissue-specific, and thus can be detected at all stages of organism development. Moreover, only a small amount of a sample is sufficient for analysis.

Types of DNA markers used in plant genome analysis

There are various types of DNA-based molecular techniques that are used to evaluate DNA polymorphism in order to authenticate plant taxa (Kaplan et al. 2004; Yip et al. 2007; Sucher and Carles 2008; Shaw et al. 2009; Heubl 2010). These are hybridization-based methods, polymerase chain reaction (PCR)-based methods (e.g., random PCR amplification, species-specific PCR primers), and sequencing-based methods. In recent times, the use of multilocus sequence analysis (MLSA), commonly used in phylogenetic studies, has proven its discriminatory power. Additionally, DNA microarrays are a promising new development for sensitive and high-throughput taxon identification (Schena et al. 1998; Trau et al. 2002). Application of one or several of the molecular identification techniques based on DNA fingerprinting such as (i) restriction fragment length polymorphism (RFLP), (ii) arbitrarily primed PCR (AP-PCR), (iii) random amplified polymorphic DNA (RAPD), (iv) amplified fragment length polymorphism (AFLP), (v) direct amplification of length polymorphisms (DALP), (vi) polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), (vii) inter-simple sequence repeats (ISSRs), (viii) sequence characterized regions (SCARs), (ix) sequence tag sites (STSs), (x) cleaved amplified polymorphic sequences (CAPS), (xi) microsatellites or simple sequence repeats (SSRs), (xii) expressed sequence tags (ESTs), (xiii) single nucleotide polymorphisms (SNPs), (xiv) diversity arrays technology (DArT), (xv) polymerase spiral reaction (PSR) and isothermal amplification, (xvi) DNA microarray (DNA chip or biochip), (xvii) DNA sequencing (DNA barcoding), next generation sequencing (NGS), etc., may provide the alternative means for authentication and quality assessment, alternative to conventional organoleptic and chemical authentication methods, and such molecular techniques are often more superior in accuracy, sensitivity, resolution, and reproducibility (Wang et al. 2001; Zhang et al. 2003; Gong et al. 2006; Li et al. 2007; He et al. 2010; Heubl 2013). These major molecular identification techniques have been developed and applied since early 1990s to identify traditional medicinal materials and these techniques are capable of differentiating traditional medicinal materials and their adulterants and substitutes, and in some cases, distinguishing closely

related species, subspecies, varieties, cultivars, and species of animal and botanical medicinal materials from different localities. Analysis of DNA markers is more reliable than the chemical markers. They provide an efficient and accurate means of testing the authenticity of several hundreds of samples simultaneously, whereas the conventional chemical-based methods usually take several days for verification. In order for any compound to act as a chemical marker, it should be unique to that particular species. Not all plants have a unique chemical compound and also the same chemical marker is used for the identification of two or more plants. Moreover, the concentration of secondary metabolites and other biochemical markers may change due to environmental factors, and hence correct identification of the botanicals is difficult, whereas genetic markers are unique and are not affected by age, physiological conditions, and environmental factors.

DNA barcoding, microarrays-based markers, and Next Generation Sequencing (NGS)-based markers are of relatively recent development. The use of a particular marker is dependent on objectives of the researcher. Table 8.1 gives information on different types of markers used in the study of medicinal plants.

RFLP (Restriction fragment length polymorphism)

RFLP analysis was one of the first techniques to be widely used for detecting variations at the DNA level (Fig. 8.2). The principle of this method is based on the comparison of banding patterns from DNA sequences digested with specific restriction enzymes (e.g., HaeIII, EcoRI, BamHI). Restriction enzymes are endonucleases produced by bacteria with the function to cut specific DNA sequence motifs of invading DNA molecules. Each enzyme has a specific, typically palindromic recognition sequence. Consequently, it recognizes and cuts DNA in a predictable way, resulting in a reproducible set of DNA fragments of different lengths. If two organisms (strains, individuals, or species) differ in the distance between sites of cleavage of a particular restriction endonuclease, then also the length of the fragments produced differs. These differences in fragment lengths can be detected by gel electrophoresis, hybridization, and visualization. The technique is time-consuming, costly, labor intensive, and requires a large quantity of good quality or undegraded DNA (Weising et al. 2005). RFLP combined with DNA hybridization has mainly been used for phylogenetic studies in the past, e.g., in *Lupinus* (Yamazaki et al. 1993), *Hedysarum* (Trifi-Farah and Marrakchi 2001), *Triticum* (Mori et al. 1997), *Musa* (Gawel et al. 1992), and for detection of *Dendrobium* (Li et al. 2005) and *Fritillaria* (Tsoi et al. 2003).

Microsatellites or SSR (Simple sequence repeats)

Microsatellites also known as simple sequence repeats (SSRs), short tandem repeats (STRs), or simple sequence length polymorphisms (SSLPs) are the smallest class of simple repetitive DNA sequences (Litt and Luty 1989; Gupta et al. 1996). Based on tandem repeats of short (2–6 bp) DNA sequences, these markers are highly polymorphic due to variation in the number of repeat units and dispersed throughout most eukaryotic genomes (Fig. 8.3).

Table 8.1 Different types of markers used in the study of medicinal plants

Plant	Marker type	No. of markers	Total no. of bands	Polymorphic fragments	Polymorphic percentage	Average bands/primer
<i>Jatropha curcas</i>	AFLP	7 pairs	770	680	88	110
<i>Achillea</i> sp.	AFLP	9 pairs	313	301	96.1	33.4
<i>Incarvillea young husbandii</i>	AFLP	7 pairs	332	185	55.7	20
<i>Rosa damascena</i>	AFLP	23 pairs	966	—	—	42
<i>Huperzia serrata</i>	AFLP	7 pairs	615	532	86.5	38
<i>Gardenia jasminoides</i>	AFLP	11 pairs	244	165	67.6	15
<i>Ocimum</i> spp.	AFLP	8 pairs	253	150	59.5	31
<i>Calathea</i> spp.	AFLP	6 pairs	733	497	67.8	41.1
<i>Oroxylum indicum</i>	RAPD	40	387	188	49.6	9.6
<i>Pongamia pinnata</i>	RAPD	18	210	22	10.4	1.2
<i>Zeyheria montana</i>	RAPD	9	105	65	61.9	11.6
<i>Ocimum basilicum</i>	RFLP	9	163	83	50.9	9.22
<i>Rhodiola rosea</i>	SSR	5 pairs	12	12	100	1.8
<i>Lycium chinense</i>	SSR	18 pairs	108	91.4	84.7	6
<i>Punica granatum</i>	SSR	12 pairs	34	12	35	3
<i>Chrysanthemum</i> spp.	ISSR	22	182	148	81.8	6.7
<i>Magnolia officinalis</i>	ISSR	12	137	114	83.2	9
<i>Heritiera littoralis</i>	ISSR	11	117	89	76	8

Source Tharachand et al. (2012)

Randomly Amplified Polymorphic DNA (RAPD)

The RAPD technology utilizes short synthetic oligonucleotides (10 bp long) of random sequences as primers to generate a high number of anonymous DNA fragments via PCR reaction (Fig. 8.4). A large number of amplification products is generally separated on agarose gels and stained with ethidium bromide or SyBRgreen. Because of the simplicity (no prior sequence information is necessary), low costs, efficiency in developing a large number of DNA markers in a short time, and requirement for less sophisticated equipment, RAPDs have found a wide range of applications. Although the RAPD method is easy to perform, the issue of reproducibility has been an important concern. In fact, the RAPD reaction is far more sensitive than conventional PCR because of the length of a single and

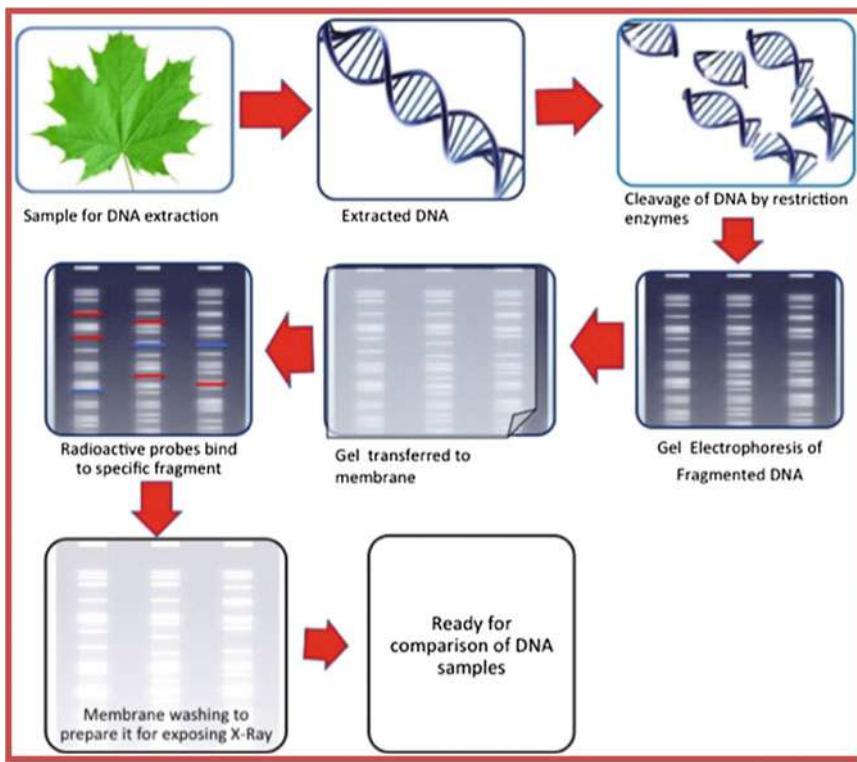


Fig. 8.2 Pictorial view showing methodology of restriction fragment length polymorphism (RFLP)

arbitrary primer, which is used to amplify anonymous regions of a given genome. Special care is needed for keeping out contaminant DNA (from infections and parasites) in the material to avoid misleading patterns. RAPDs are inherited as dominant recessive characters which mean that homozygotes and heterozygotes cannot be distinguished.

RAPD markers have found a wide range of applications in the authentication of medicinal plants. The technique has been applied in many plant groups like *Glycyrrhiza* (Yamasaki et al. 1994), *Atractylodes* (Kohjyouma et al. 1997; Chen et al. 2001), *Astragalus* (Cheng et al. 2000), *Scutellaria* (Hosokawa et al. 2000), *Panax* (Lim et al. 2007), *Aconitum* (Cole and Kuchenreuther 2001), *Ginkgo* (Fan et al. 2004), *Phyllanthus* (Dnyaneshwar et al. 2006), *Rehmannia* (Cheng et al. 2002), and others.

Arbitrary polymerase chain reaction (AP-PCR)

Arbitrarily chosen primers (ACP-PCR) is a special variation of RAPD, which uses single primers approximately 10–50 bp in length (Welsh and McClelland 1990). In AP-PCR, the amplification follows three steps. In the first two cycles, annealing is

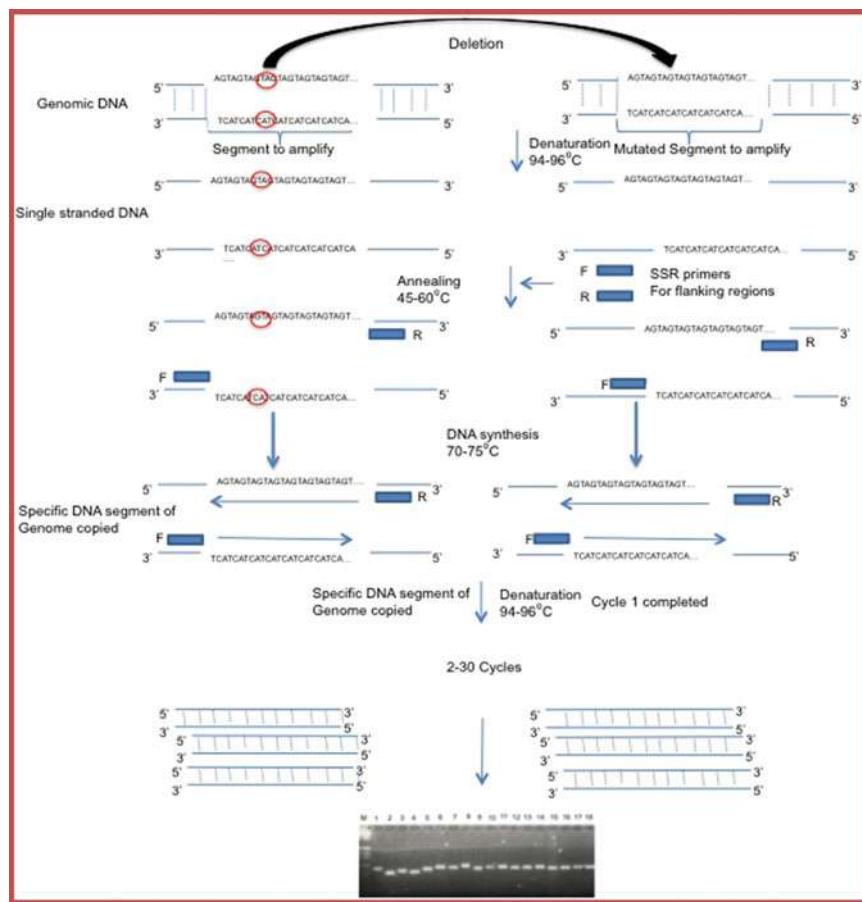


Fig. 8.3 Pictorial view showing methodology of microsatellites or simple sequence repeats (SSR)

under nonstringent conditions. Higher primers are used in the first cycle. Often primers of variable length are used and products are mostly analyzed on polyacrylamide gels. AP-PCR has been applied to various groups for identification of species and analysis of genetic variation (Munthali et al. 1992; Kersten et al. 2007). Similar to RAPDs, reproducibility can be a problem for fingerprints generated by a single primer because small changes in annealing conditions can affect banding pattern.

DNA amplification fingerprinting (DAF)

DNA amplification fingerprinting (DAF) is a variant of the RAPD technique and was developed by Caetano-Anolle's et al. (1991).

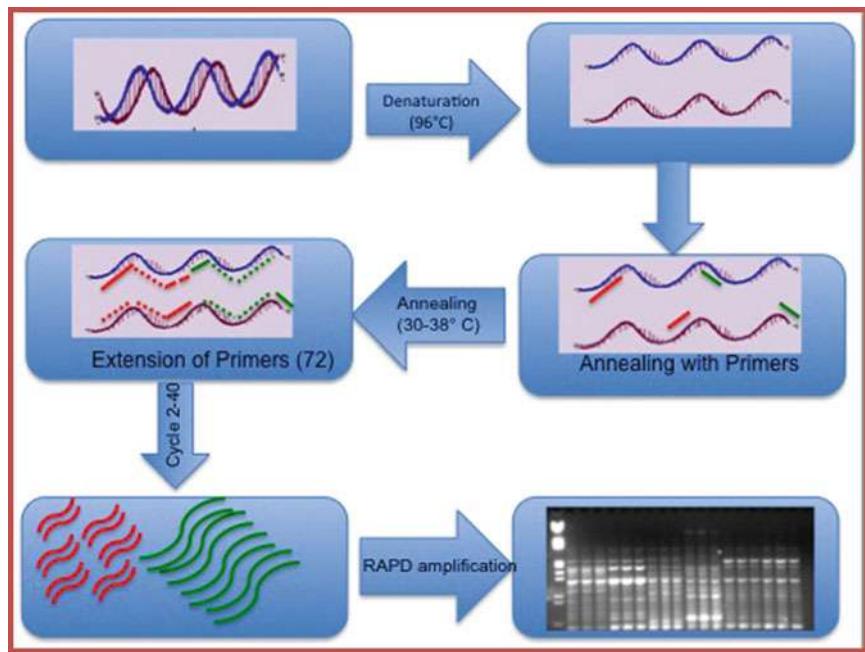


Fig. 8.4 Pictorial view showing methodology of randomly amplified polymorphic DNA (RAPD)

Amplified fragment length polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) originally developed by Zabeau and Vos (1993) is a powerful tool for DNA fingerprinting of organismal genomes and it combines the use of RFLP and PCR techniques (Fig. 8.5). AFLP is a whole-genome fingerprinting method based on selective amplification of restriction fragments. This multilocus approach needs no prior sequence information; it is highly reproducible with the ability to screen a large number of loci (ca. 50–100 fragments per reaction) for polymorphisms. It is a very useful technique for DNA fingerprinting, especially when very little information on the genome of the plant under study is available (Blears et al. 1998; Mueller and Wolfenbarger 1999). Compared to the widely used RFLP, AFLP is faster, less labor intensive, and provides more information. Because of the highly informative fingerprinting profiles, AFLPs can be applied in studies involving genetic identity, parentage, identification of clones and cultivars, and in phylogenetic studies of closely related species. AFLP analyses have been used in *Panax* (Ha et al. 2002; Choi et al. 2008), *Actaea* (Zerega et al. 2002), *Plectranthus* (Passinho-Soares et al. 2006), *Caladium* (Loh et al. 1999), *Cannabis* (Datwyler and Weible 2006), and *Rehmannia* (Qi et al. 2008).

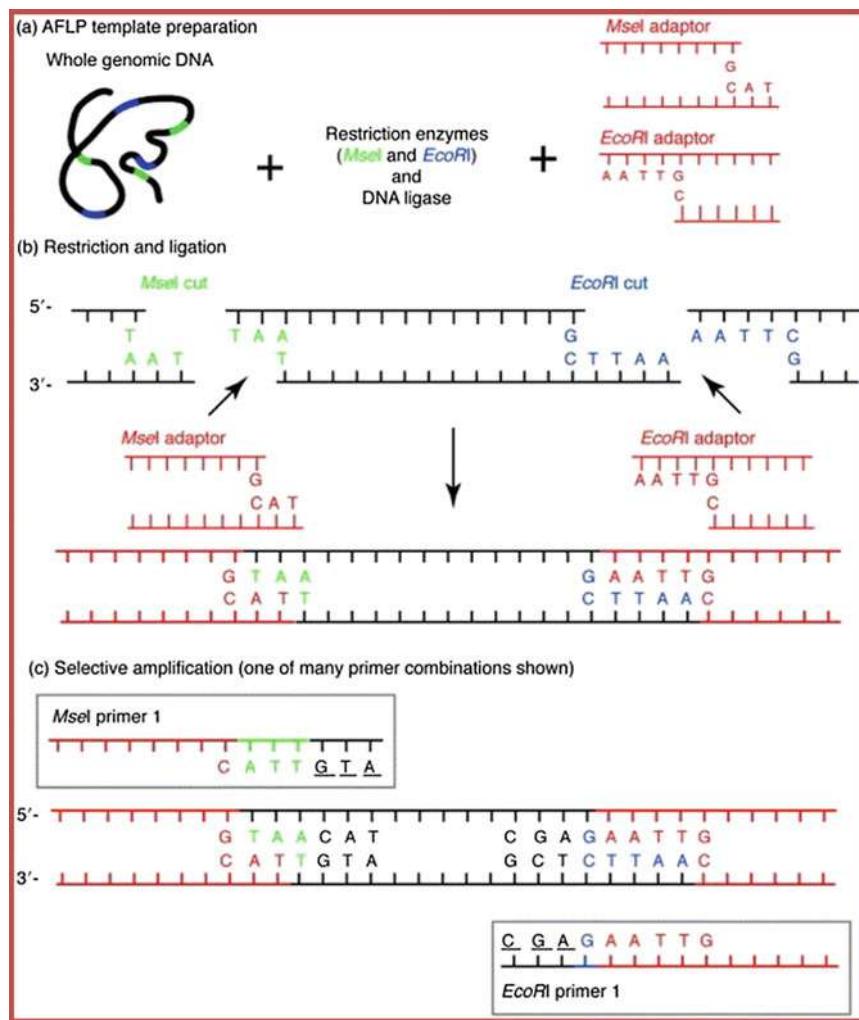


Fig. 8.5 Pictorial view showing methodology of amplified fragment length polymorphism (AFLP). DNA digestion with restriction enzymes (a) adaptor ligation; (b) Selective amplification; (c) Prior to selective amplification, preamplification is carried out with a single nucleotide extension, followed by selective amplification using three 3-bp extension (Mueller and Wolfenbarger 1999)

Inter-simple sequence repeat (ISSR)

In higher plants, ISSR markers are frequently applied because they are known to be abundant, very reproducible, highly polymorphic and easy to use (Zietkiewicz et al. 1994; Borne et al. 2002; Borne and Branchard 2004). ISSR (Fig. 8.6), also known as anchored simple sequence repeat (ASSR), has been used in genetic fingerprinting, gene tagging, phylogenetic analysis, species and cultivar identification,

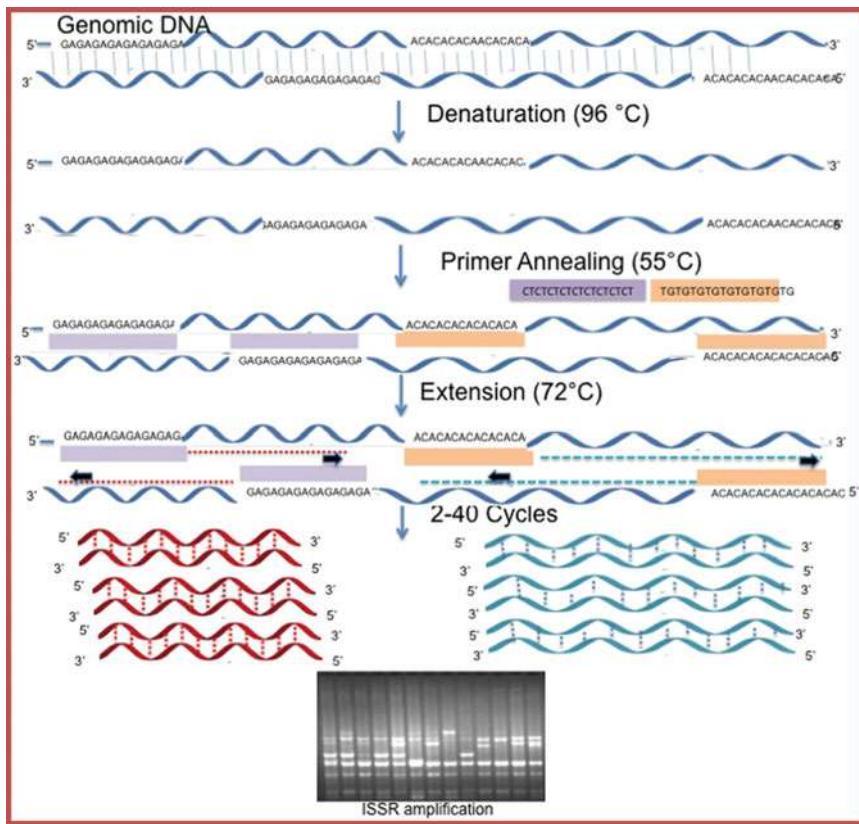


Fig. 8.6 Pictorial view showing methodology of inter-simple sequence repeat (ISSR)

and assessment of hybridization (Kurane et al. 2009). The ISSR technique is nearly identical to RAPD except that ISSR primers are designed from microsatellite regions and are longer (approximately 14 bp or more) than RAPD primers. Microsatellites are very short stretches of DNA that are “hypervariable,” expressed as different variants within populations and among different species. ISSRs have been used for screening genetic diversity and authentication of *Dendrobium* (Shen et al. 2006), *Cistanche* (Shi et al. 2009), *Fritillaria* (Li et al. 2009), *Salvia* (Song et al. 2010), *Rehmannia* (Wang et al. 2005a, b), *Vitex* (Hu et al. 2007), *Cannabis* (Kojoma et al. 2002), *Rhodiola* (Xia et al. 2007), *Cymbidium* (Wang et al. 2009), *Ammopiptanthus* (Ge et al. 2005), *Swertia* (Joshi and Dhawan 2007), *Glycyrrhiza* (Yao et al. 2008), and *Houttuynia* (Wu et al. 2005).

Randomly amplified microsatellite polymorphism(RAMPO)

The RAMPO method is also termed random amplified hybridization microsatellite (RAHM) or randomly amplified microsatellites (RAMS) and combines arbitrarily primed PCR (RAPD) with microsatellite hybridization to produce polymorphic

genetic fingerprints (Weising and Kahl 1998; Weising et al. 2005). The method is mainly used for identification and discrimination of genotypes within and among populations, cultivars, and germplasm, e.g., in *Ficus* (Chatti et al. 2007) and *Phoenix dactylifera* (Soumaya et al. 2008). RAMPOs share their fate with other marker technologies that are partly or totally based on blot hybridization. These methods are barely used anymore because more convenient marker systems are available for most purposes.

Selective amplification of microsatellite polymorphic loci (SAMPL)

The SAMPL technique was introduced by Morgante and Vogel (1994). It combines the high and controllable multiplexing rate of the AFLP technique with the high levels of microsatellite polymorphism using AFLP-type primers together with compound microsatellite primers (Weising et al. 2005). SAMPLs differ from AFLPs using primers with compound microsatellite motifs in combination with oligonucleotides complementary to the end-ligated adapters for the selective amplification step (Paglia et al. 1998). This multiplexing genome profiling technique has not been used adequately in plant genomics, although a few reports have already documented its potential for detecting polymorphisms (Molina and Kahl 2002). This method was used for analysis of genetic diversity in *Cicer* (Winter et al. 1999), *Lactuca* (Witsenboer et al. 1997), and *Tribulus* (Sarwat et al. 2008).

Directed amplification of minisatellite-region DNA (DAMD)

DAMD is a DNA fingerprinting method based on amplification of the regions rich in minisatellites at relatively high stringencies using previously found variable number of tandem repeats (VNTR) core sequences as primers (Heath et al. 1993; Somers and Demmon 2002). Minisatellites also known as VNTR or hypervariable repeats (HVR) are similar to microsatellites (SSR) except that the tandem repeat DNA sequences are longer and generally consist of 10–60-bp motifs. Recently, DAMD-PCR has been applied successfully for genotyping of *wheat* cultivars, and rice species (Zhou et al. 1997). The method has been used for authentication of *Panax* (Ha et al. 2002), *Capsicum* (Ince et al. 2009), *Salvia* (Karaca et al. 2008), and *Morus* (Bhattacharya et al. 2005).

Single nucleotide polymorphism (SNP)

Single nucleotide polymorphisms (SNPs) are widely observed between individuals, ecotypes, and species serving as efficient molecular markers particularly in genetic analysis and breeding programs, also including ecological and evolutionary studies. SNPs are single-base pair positions in the genomes of two (or more) individuals, at which different sequence alternatives (alleles) exist. Polymorphisms result from point mutations (either transition or transversion events) causing single base pair differences between DNA sequences. According to most recent estimates, one SNP occurs every 100–300 bp (or every 1000 bp) in any genome (Kwok 2001). SNPs are co-dominant, single-locus, biallelic markers, and they are the most abundant molecular markers known so far. SNPs have been applied in authentication of *Perilla* varieties (Luo et al. 2006), *Dendrobium officinale* (Ding et al. 2008), *Panax* cultivars (Wang et al. 2009), *Boehmeria* varieties (Li et al. 2010).

Amplification refractory mutation system (ARMS)

ARMS, also known as allele-specific polymerase chain reaction (AS-PCR), is a simple, timesaving, and effective method for detecting any mutations involving single base changes (SNPs) or small deletions. It has become a standard technique that allows the discrimination of alleles (Newton et al. 1989). The ARMS technique has been applied in authentication of *Alisma* (Li et al. 2007), *Panax* (Zhu et al. 2004; Diao et al. 2009), *Rheum* (Yang et al. 2004), *Dendrobium* (Ding et al. 2008; Qian et al. 2008), and *Curcuma* (Sasaki et al. 2002).

Cleaved amplified polymorphic sequence (CAPS or PCR-RFLP)

CAPS, originally named PCR-RFLP, is a combination of PCR of target DNA and subsequent digestion with a restriction enzyme (Maeda et al. 1990; Lum et al. 2005). PCR-RFLP has been used for authentication of *Alisma* (Li et al. 2007), *Angelica* (Watanabe et al. 1998), *Sinopodophyllum* and *Dysosma* (Gong et al. 2006), *Ephedra* (Guo et al. 2006), *Fritillaria* (Wang et al. 2005a, b, 2007), *Artemisia* (Lee et al. 2009), *Panax* (Do et al. 2001; Um et al. 2001; Diao et al. 2009; Lu et al. 2010), *Actinidia* (Zhao et al. 2007), *Atractylodes* (Mizukami et al. 2000), *Glehnia* (Mizukami et al. 1993a, b), *Astragalus* (Lu et al. 2009), *Dendrobium* (Zhang et al. 2005), *Duboisia* (Mizukami et al. 1993a, b), and *Codonopsis* (Fu et al. 1999).

Sequence characterized amplified region (SCAR)

SCAR, a new type of RAPD-derived molecular marker, was introduced by Paran and Michelmore in 1993 and it circumvented several of the drawbacks inherent to RAPDs (Fig. 8.7). A SCAR marker can be used to rapidly amplify a diagnostic nucleic acid from herbal materials using a pair of specific oligonucleotide primers designed from polymorphic RAPD (McDermott et al. 1994; Semagn et al. 2006) or ISSR (Albani et al. 2004) fragments. The SCAR technique has been used for authentication of *Panax* (Wang et al. 2001; Choi et al. 2008) and for discrimination of species of *Artemisia* (Lee et al. 2006), *Phyllanthus* (Dnyaneshwar et al. 2006; Theerakulpisut et al. 2008), *Pueraria* (Devaiah and Venkatasubramanian 2008a), *Sinocalycanthus* (Ye et al. 2006), *Embelia* (Devaiah and Venkatasubramanian 2008b), and *Lycium* (Sze et al. 2008).

SSCP (Single-strand confirmation polymorphism)

SSCP is a powerful mutation detection system. The principle of this technique is that under a neutral condition, the single-stranded DNA (ssDNA) folds into a tertiary structure. Differences in DNA sequences (often a single base pair) alter the single-stranded DNA in the tertiary conformation (by differential folding), which in turn affect the mobility of the ssDNA in a gel. Based on their mobility differences, SNPs can be detected (Orita et al. 1989). The method is not frequently applied for authentication, e.g., in *Boesenbergia* (Techaprasan et al. 2008).

MSAP (Methylation-sensitive amplified polymorphism)

This tool is a modification of the AFLP technique and was developed for monitoring the state of genomic DNA methylation. Genomic DNA is double-digested with one of the methylation-sensitive enzymes *Hpa*II or *Msp*I, and then with the methylation-insensitive *Eco*RI. The resulting fragments are ligated with the

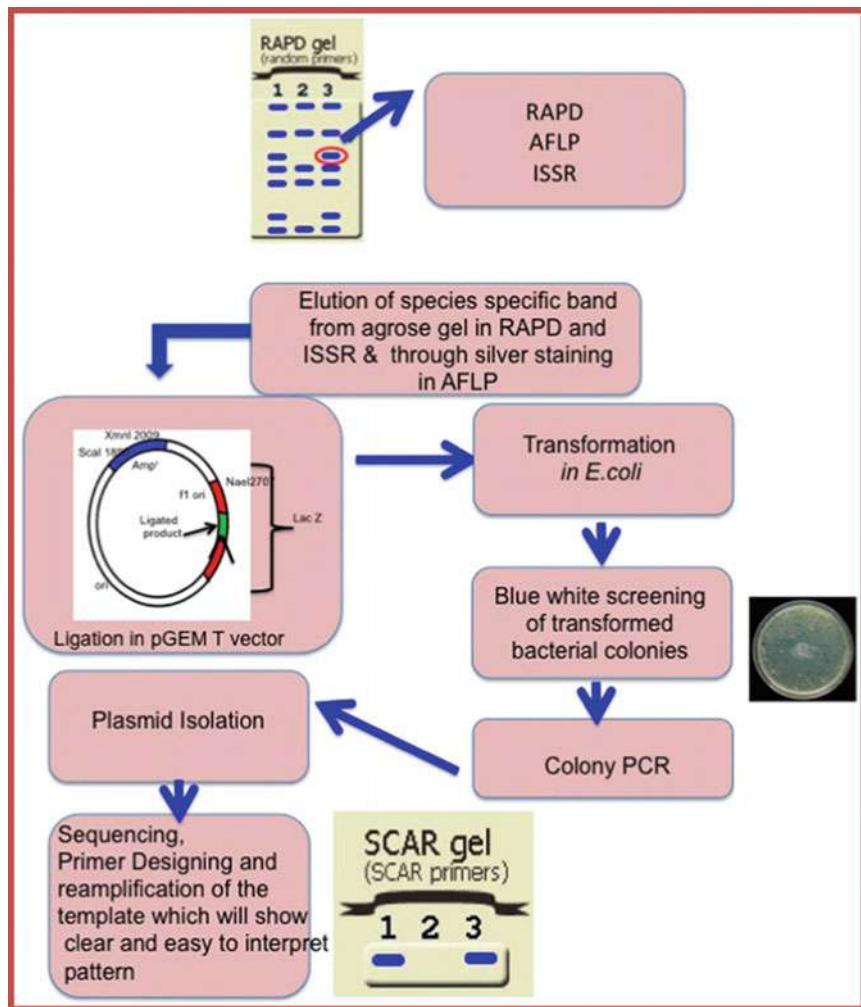


Fig. 8.7 Pictorial view showing methodology of sequence characterized amplified region (SCAR)

corresponding double-stranded adapters; a first preselective amplification is carried out followed by a selective amplification step. MSAP was first developed to determine DNA methylation events in dimorphic fungi (Reyna-Lopez et al. 1997) and later adapted for the detection of cytosine methylation in the rice genome (Xiong et al. 1999), pepper (Portis et al. 2004), apple (Xu et al. 2000), and *Siberian ginseng* (Chakrabarty et al. 2003).

LAMP (Loop-mediated isothermal amplification)

Notomi et al. (2000) developed the loop-mediated isothermal amplification or LAMP that amplifies target nucleic acids with high specificity, efficiency, and

rapidity under isothermal conditions. This method relies on auto-cycling strand displacement DNA synthesis performed by a DNA polymerase with high strand displacement activity. Because LAMP recognizes the target by six distinct sequences initially and by four distinct sequences afterward, it is expected to amplify the target sequence with high selectivity (Nagamine et al. 2001, 2002). This technique was applied to detect *Panax ginseng* (Sasaki et al. 2008), the botanical source of Ginseng (Ginseng Radix), and to distinguish this species from *Panax japonicus*. It was also used for the detection of *Lophophora williamsii* (Sasaki et al. 2009) and *Curcuma longa* (Sasaki and Nagumo 2007).

SDA (Subtracted diversity array)

PCR-based plant identification techniques are often limited by their low throughput, whereas hybridization-based microarray technology represents a rapid and high-throughput tool for genotype identification. Using an innovative technique, a “Subtracted Diversity Array” (SDA) was constructed from a pooled genomic DNA library of 49 angiosperm species, from which pooled non-angiosperm genomic DNA was subtracted (Jayasinghe et al. 2007). The subtraction was carried out using the Clontech PCR-Select cDNA Subtraction Kit. This new SDA method was shown to be superior to conventional molecular identification methods in terms of accuracy, sensitivity, and efficiency, as well as capacity for high-throughput and broad application. The SDA technique was validated for potential genotyping use. Niu et al. (2011) showed that SDAs technique is suitable to differentiate two ginseng species, *P. ginseng* and *Panax quinquefolius*, that are frequently mixed for adulteration.

MLPA (Multiplexed ligase-dependent probe amplification)

The multiplexed ligase-dependent probe amplification (MLPA) assay is well suited to medicinal plant species identification (Barthelson 2009; Shen and Wu 2009). MLPA is a semi-quantitative PCR-based technique initially developed by Schouten et al. (2002). It uses the sensitivity of the polymerase chain reaction but increases the specificity by including a key ligation step for those MLPA probes that hybridize to a DNA sequence. Several key features distinguish this technique from other PCR-based techniques, due to its low costs, excellent sensitivity, reliability, and ease of development and implementation, the MLPA technique has become a very popular research and diagnostic tool.

DNA-based molecular markers have acted as very useful tools in various fields like taxonomy, physiology, embryology, plant breeding, ecology, genetic engineering, etc. DNA-based markers have their applications in fingerprinting genotypes, determining the seed purity, and in phylogenetic analysis by which the conservation of the plant can be made easy. The innovation of polymerase chain reaction (PCR) made the development of DNA-based markers easier. Recently, various molecular techniques like random amplified polymorphic DNA (RAPD) (Xu et al. 2002), simple sequence repeats (SSRs) (Kim et al. 2007; Kim and Chung 2007), restriction fragment length polymorphism (RFLP) (Ngan et al. 1999; Del Serrone et al. 2006), and subtracted diversity array (SDA) (Niu et al. 2011) have been reported to authenticate medicinal herbs. Polymerase chain reaction (PCR) has

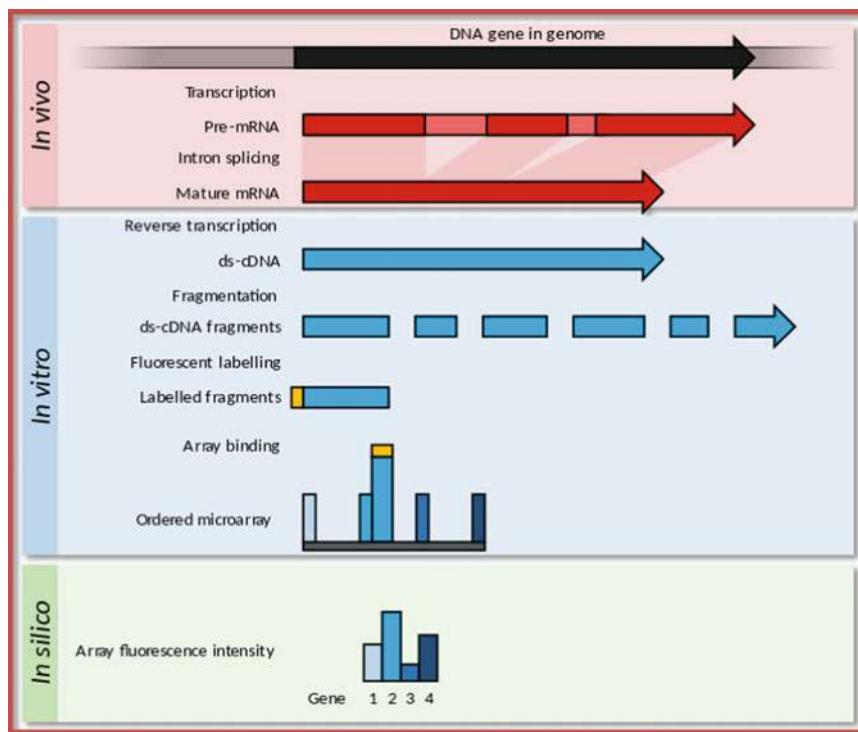


Fig. 8.8 Summary of DNA Microarrays: Within the organisms, genes are transcribed and spliced (in eukaryotes) to produce mature mRNA transcripts (red). The mRNA is extracted from the organism and reverse transcriptase is used to copy the mRNA into stable ds-cDNA (blue). In microarrays, the ds-cDNA is fragmented and fluorescently labeled (orange). The labeled fragments bind to an ordered array of complementary oligonucleotides, and measurement of fluorescent intensity across the array indicates the abundance of a predetermined set of sequences. These sequences are typically specifically chosen to report on genes of interest within the organism's genome

given scientists a powerful arsenal of molecular tools as it can be used to exponentially amplify trace amounts of DNA. With the help of PCR, it becomes possible to detect DNA of specific natural products in processed drugs and audit the quality of CPM.

DNA microarray (DNA chip or biochip technology)

The DNA chip technology developed by Fodor et al. (1991) enables the production of a “biochip” designed to identify fluorescent-labeled DNA or RNA fragments through their hybridization to oligonucleotide probes (Figs. 8.8 and 8.9). DNA microarrays are a high-throughput technology for simultaneous analysis of multiple genes in many taxa or samples (Fodor et al. 1993; Gershon 2002).

A DNA microarray (also commonly known as DNA chip or biochip) is a collection of microscopic DNA spots attached to a solid surface. Scientists use DNA

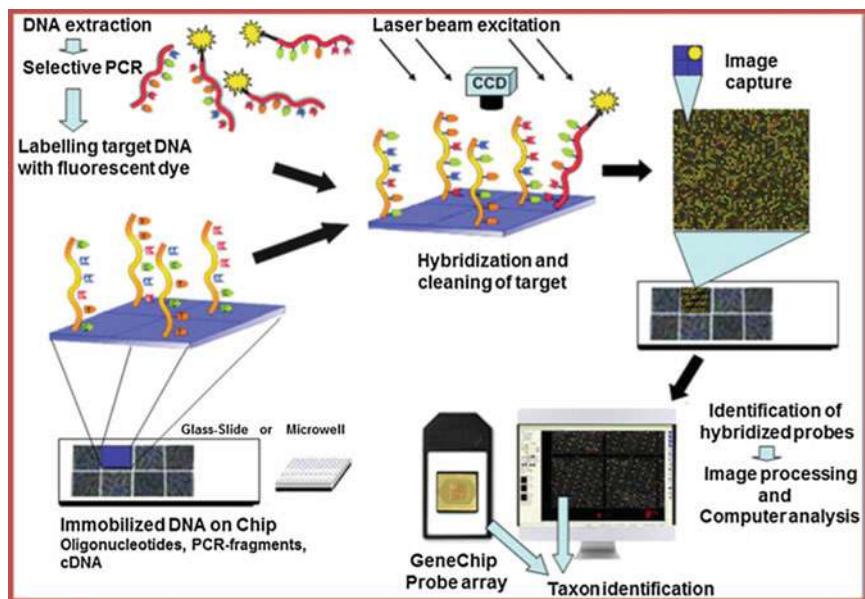


Fig. 8.9 Workflow overview with major steps of an automated and high-throughput DNA microarray platform for species identification

microarrays to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome. Each DNA spot contains picomoles (10^{-12} mol) of a specific DNA sequence, known as probes (or reporters or oligos). These can be a short section of a gene or other DNA element that are used to hybridize a cDNA or cRNA (also called antisense RNA) sample (called target) under high-stringency conditions. Probe-target hybridization is usually detected and quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the target.

This technique has been applied for the identification of various species of *Fritillaria* (Tsoi et al. 2003), *Dendrobium* (Li et al. 2005; Zhang et al. 2003), and *Bupleurum* (Lin et al. 2008). The nucleotide sequences of the nuclear 18S rRNA gene of 13 *Panax* taxa were determined and on the basis of the nucleotide differences, a DNA microarray (PNX array) was developed for the identification of various *Panax* drugs (Zhu et al. 2008). A silicon-based DNA microarray was designed and fabricated for the identification of toxic traditional Chinese medicinal plants. Species-specific oligonucleotide probes were derived from the 5S ribosomal RNA gene of *Aconitum carmichaelii*, *A. kusnezoffii*, *Alocasia macrorrhiza*, *Croton tiglium*, *Datura inoxia*, *D. metel*, *D. tatula*, *Dysosma pleiantha*, *Dy. versipellis*, *Euphorbia kansui*, *Hyoscyamus niger*, *Pinellia cordata*, *P. pedatisecta*, *P. ternata*, *Rhododendron molle*, *Strychnos nux-vomica*, *Typhonium divaricatum*, and *T. giganteum* (Carles et al. 2005). The analyses demonstrated that DNA

microarray-based technology can provide a rapid, high throughput tool for correct botanical identification, for authentication of crude plant materials, standardization, and for quality control being used for hundreds of samples simultaneously (Debouck and Goodfellow 1999).

DNA Barcoding

DNA barcoding, a term first created by Hebert et al. (2003) is a novel molecular and bioinformatical tool designed to provide rapid, accurate, automatable, and cost-effective identification of species. Contrary to other molecular methods, it can be used on a large scale and with high reliability. For DNA barcoding, the unique nucleotide sequence patterns of small DNA fragments (400–800 bp) are used as specific reference collections to identify specimens and to discover cryptic taxa (Vijayan and Tsou 2010). DNA barcoding uses a short genetic marker from a standard locus (alternatively from nuclear, mitochondrial, or plastidial DNA) of an organism (Fig. 8.10). An ideal and successful DNA barcode marker should be suitable for a wide range of taxa (breadth of taxonomic application), routinely retrievable with a universal primer pair, be short enough to be accessible to

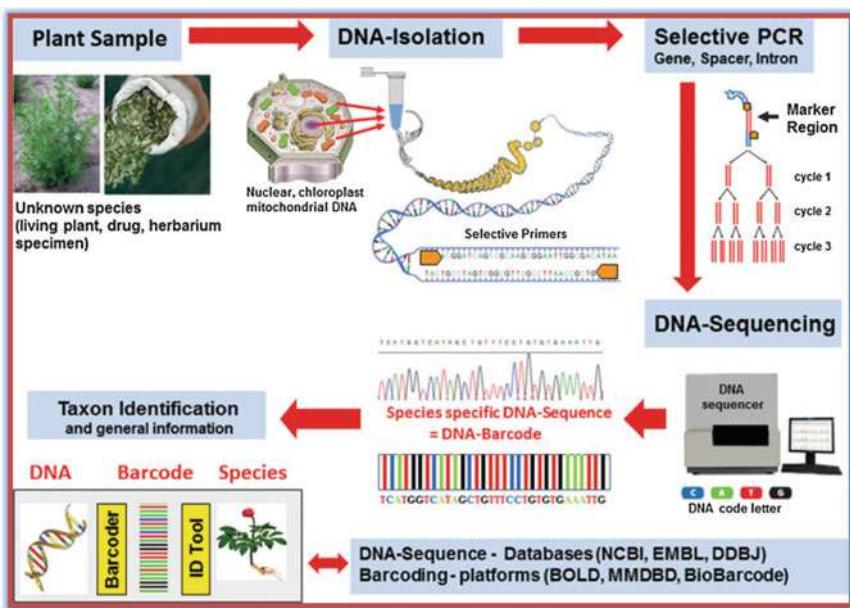


Fig. 8.10 Major steps in the DNA barcoding process. These procedures include tissue sampling, DNA extraction, polymerase chain reaction and marker amplification, PCR product check, and sequencing. The end product of this procedure will be a unique, species-specific “barcode” sequence of the marker (gene, spacer, and intron) that can be used for species identification and submitted to a DNA database (e.g., GenBank-NCBI, Bold). The original specimen is kept as a voucher. All collateral information (identification, collection data, etc.) are stored along with the DNA barcode sequence. *Source* Heubl (2013)

bidirectional sequencing, and provide a unique sequence for maximal discrimination among species which means high variation between species but conserved within the species, so that the intra-specific variation will be insignificant (CBOL 2009).

DNA barcoding, an approach to identify species based on sequences from a short, standardized DNA region, opens up a unique avenue for the identification of organisms (Hebert et al. 2003; Hebert and Gregory 2005). Among the several candidate DNA barcodes, the internal transcribed spacer (ITS) of the nuclear ribosomal DNA (nrDNA) has been recently proposed for incorporation into the core barcode, and has the potential to be used as a standard DNA barcode to identify medicinal plants and their close relatives (Chen et al. 2010; Li et al. 2011). *Peucedanum praeruptorum* L., a traditional Chinese herb (Qian-hu), is commonly used for dispelling wind-heat and expectorant and loss of energy, but due to high market demand and similar morphological characters and, there are many substitutes and adulterants of *P. praeruptorum*. Under the circumstances, DNA barcoding would be an approach to identify species based on sequences from a short, standardized DNA region, and results showed that *P. praeruptorum*, its substitutes and 23 adulterants could be easily distinguished at the DNA level, and the internal transcribed spacer (ITS) sequence could be used for the identification of *P. praeruptorum* and to distinguish it from common substitutes and adulterants (Zhou et al. 2014).

NGS (Next generation sequencing)

The advent of reversible chain-termination reaction in sequencing coupled with high-resolution detection, popularly termed as Next Generation or NGS allows concurrent sequencing of a large number of molecules, therefore, generating vast amount of sequence data simultaneously. The technique (Fig. 8.11) is based on the principle that DNA templates are first fragmented, and thereafter immobilized on a solid support. These fragments are to be amplified and sequenced. Three main technologies/strategies that are in practice for NGS are commercialized by Roche/ 454 Life Sciences (Indianapolis, IN), Illumina/Solexa Genome Analyzer (San Diego, CA), and Applied Iosystems/SOLiD System (orange county, CA). All of them retain their distinctive enzyme systems, sequencing chemistry, hardware, and software engineering (Mardis 2008; Shendure and Ji 2008; Metzker 2010); also, the sequencing reads obtained with these technologies varies in total sequencing output.

Assortment of varieties of herbs, based on classical taxonomy, can be applied to systematic assortment, classification, and identification, but too many human factors are involved, especially for planted groups of herbal medicines, such as identification of authentic raw materials, which still remains a problem. Development of species biology and molecular systematics provides an effective weapon and basis for the study of system and evolution, classification and identification. Modern species concept has been widely accepted by taxonomists, and pharmacognosists can never ignore theory and fruits in species biology and molecular systematics. Penetration of molecular systematics and application of

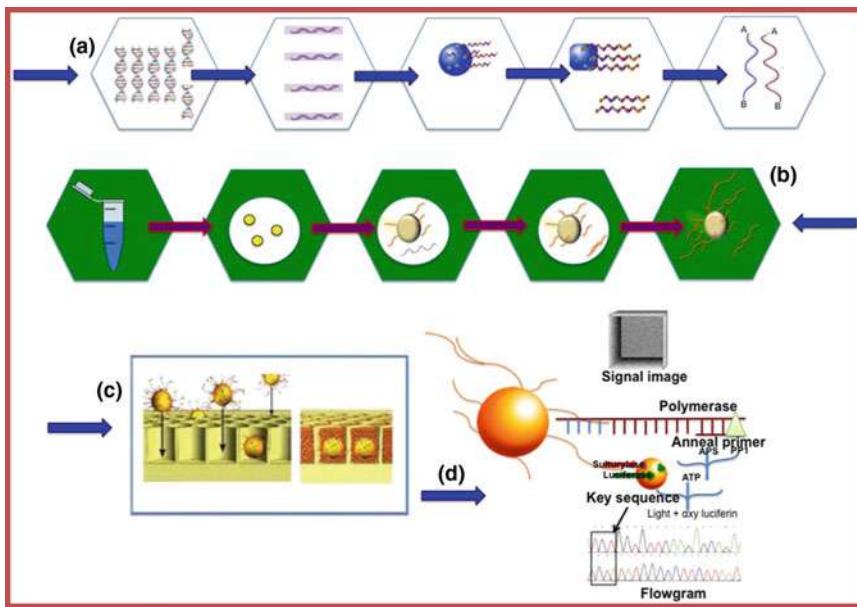


Fig. 8.11 Outline of the Roche/454 sequencer workflow. **a** Single-strand template DNA library preparation; **b** Emulsion-based clonal amplification; **c** Depositing DNA beads into the PicoTiter Plate device; **d** Sequencing by synthesis. (Next Generation Sequencing and Whole Genome Selection in Aquaculture, Ed. Zhanjiang (John) Liu (2011) Blackwell Publishing Ltd.)

biological engineering technology provide crude drugs classification and identification with an effective weapon and basis to test molecules. To establish a method and system to identify crude drugs based on species biology, molecular systematics, Chinese medicinal resources, and herbalism and to further the development of systematic assortment and standardization of quality of Chinese herbal medicines at the population, individual even genetic level, fall into one of the major concerns of molecular pharmacognosy.

8.5 Basic Methods of Systems Biology

Systems biology is the computational and mathematical modeling of complex biological systems; a biology-based interdisciplinary field of study that focuses on complex interactions within biological systems, using a holistic approach to biological research (Fig. 8.12). Systems biology is aimed at analyzing the behavior and interrelationships of biological systems (Kitano 2002) and is characterized by the synergistic combination of experimentation, theory, and computation. System biology is a new field of integrated science that aims at system level understanding

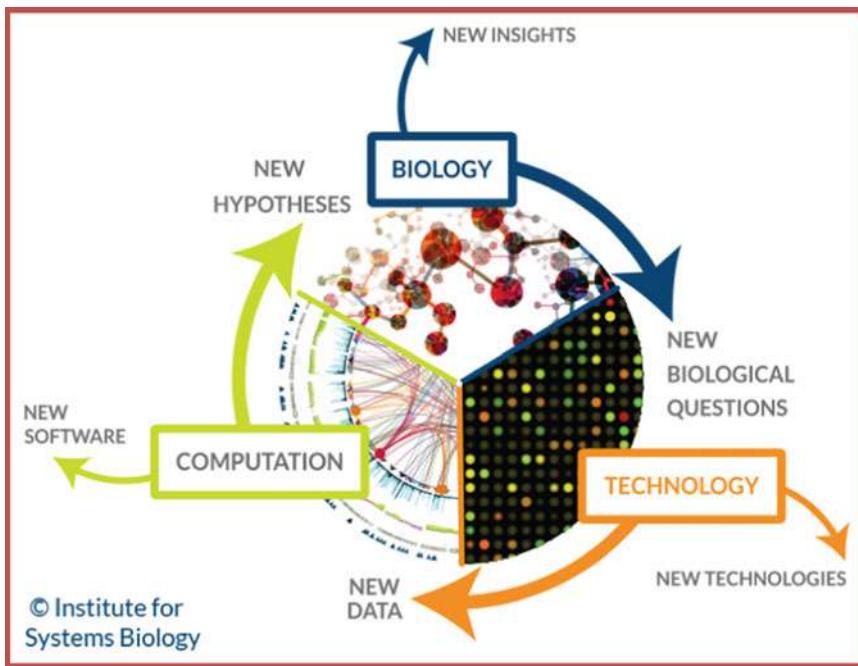


Fig. 8.12 Systems biology-computational and mathematical modeling of complex biological systems, a biology-based interdisciplinary field of study that focusing on complex interactions within biological systems and a holistic approach to biological research

of biological systems, and it is the first time that one may be able to understand biological systems grounded in molecular level as a consistent framework of knowledge after genomics and proteomics were put forward. Different molecular biology focusing on the individual ingredient, system biology concentrates on the constitution of all the compositions such as gene, RNA, protein, etc., in a biological system and correlations of these compositions under specific condition. It is a powerful tool to explore biology fully.

The developments in the molecular biosciences have made possible a shift to combined molecular and system-level approaches to biological research under the name of Systems Biology. It integrates many types of molecular knowledge, which can best be achieved by the synergistic use of models and experimental data. Many different types of modeling approaches are useful depending on the amount and quality of the molecular data available and the purpose of the model. Analysis of such models and the structure of molecular networks have led to the discovery of principles of cell functioning overarching single species. Two main approaches of systems biology can be distinguished as (i) Top-down systems biology is a method to characterize cells using system-wide data originating from the Omics in combination with modeling. Those models are often phenomenological but serve to

discover new insights into the molecular network under study; (ii) Bottom-up systems biology does not start with data but with a detailed model of a molecular network on the basis of its molecular properties. In this approach, molecular networks can be quantitatively studied leading to predictive models that can be applied in drug design and optimization of product formation in bioengineering.

Systems biology has been responsible for some of the most important developments in the science of human health and environmental sustainability. It is a holistic approach to deciphering the complexity of biological systems that start from the understanding that the networks that form the whole of living organisms are more than the sum of their parts. It is collaborative, integrating many scientific disciplines—biology, computer science, engineering, bioinformatics, physics, and others—to predict how these systems change over time and under varying conditions and to develop solutions to the world's most pressing health and environmental issues. Systems biology, ultimately, creates the potential for entirely new kinds of exploration and drives constant innovation in biology-based technology and computation. A fundamental tenet of systems biology is that solving challenging biological problems always requires the development of new technologies in order to explore new dimension of data space. New data types require novel analytical tools. This virtuous cycle of biology driving technology driving computation can exist only in a cross-disciplinary environment where biologists, chemists, computer scientists, engineers, mathematicians, physicists, physicians, and others can come together in teams to tackle grand challenges. Institute for systems biology (ISB) concentrates on the study of relationships and interactions between various parts of biological systems, and advocates an interdisciplinary approach to biological research and describes the “innovation engine” (depicted below) that drives human ability to develop intellectual property.

From 2000 onwards, the concept has been used widely in biology in a variety of contexts. The genome projects including humans are examples of applied systems thinking in biology which has led to new, collaborative ways of working on problems in the biological field of genetics. Systems biology finds its roots in the quantitative modeling of enzyme kinetics, mathematical modeling of population dynamics, simulations developed to study neurophysiology, control theory and cybernetics, synergistics, etc.

One of the aims of systems biology is to model and discover emergent properties, properties of cells, tissues, and organisms functioning as a system whose theoretical description is only possible using techniques of systems biology (Figs. 8.13, 8.14, and 8.15). These typically involve metabolic networks or cell signaling networks (Bu and Callaway 2011).

Systems biology can be considered from a number of different aspects like (i) as a field of study of the interactions between the components of biological systems, and how these interactions give rise to the function and behavior of that system (e.g., the enzymes and metabolites in a metabolic pathway or the heart beats) (Snoep and Westerhoff 2005; Noble 2006); (ii) as a series of operational protocols used for performing research, viz., a cycle composed of theory, analytic or computational modeling to propose specific testable hypotheses about a biological

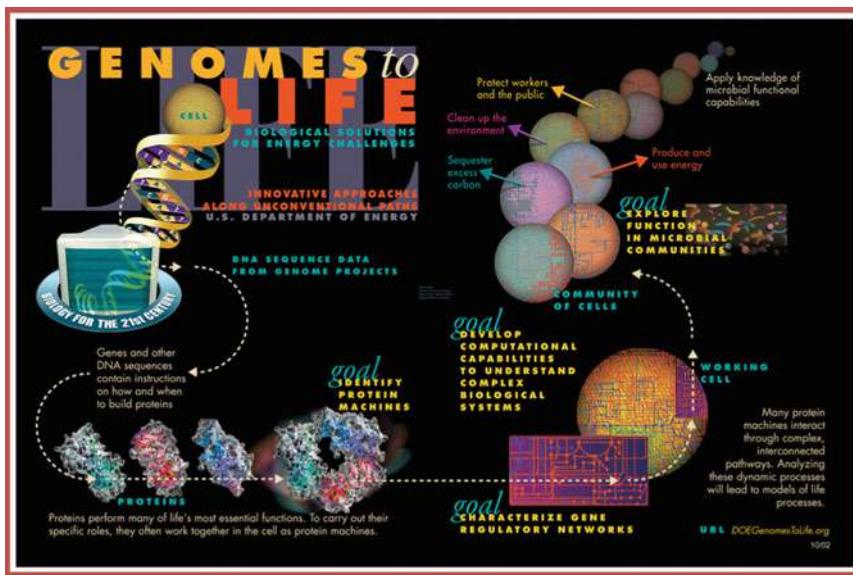


Fig. 8.13 An illustration of the systems approach to biology

system, experimental validation, and then using the newly acquired quantitative description of cells or cell processes to refine the computational model or theory (Kholodenko and Sauro 2005); transcriptomics, metabolomics, proteomics, and high-throughput techniques are used to collect quantitative data for the construction and validation of models (Chiara and Gerolamo 2009); (iii) as the application of dynamical systems theory to molecular biology; (iv) as a socioscientific phenomenon defined by the strategy of pursuing integration of complex data about the interactions in biological systems from diverse experimental sources using interdisciplinary tools and personnel (Baitaluk 2009). Systems biology refers to a cluster of peripherally overlapping concepts rather than a single well-delineated field.

Systems biology has the ability to obtain, integrate, and analyze complex data sets from multiple experimental sources using interdisciplinary tools including the typical technology platforms like (i) phenomics (organismal variation in phenotype as it changes during its life span); (ii) genomics (organismal deoxyribonucleic acid dna sequence, including intra-organisamal cell specific variation, i.e., telomere length variation); (iii) epigenomics or epigenetics (organismal and corresponding cell specific transcriptomic regulating factors not empirically coded in the genomic sequence, i.e., DNA methylation, histone acetylation and deacetylation, etc.); (iv) transcriptomics (organismal, tissue or whole cell gene expression measurements by DNA microarrays or serial analysis of gene expression); (v) interferomics (organismal, tissue, or cell-level transcript correcting factors, i.e., RNA interference); (vi) proteomics (organismal, tissue, or cell-level measurements of proteins and peptides via two-dimensional gel electrophoresis, mass spectrometry or

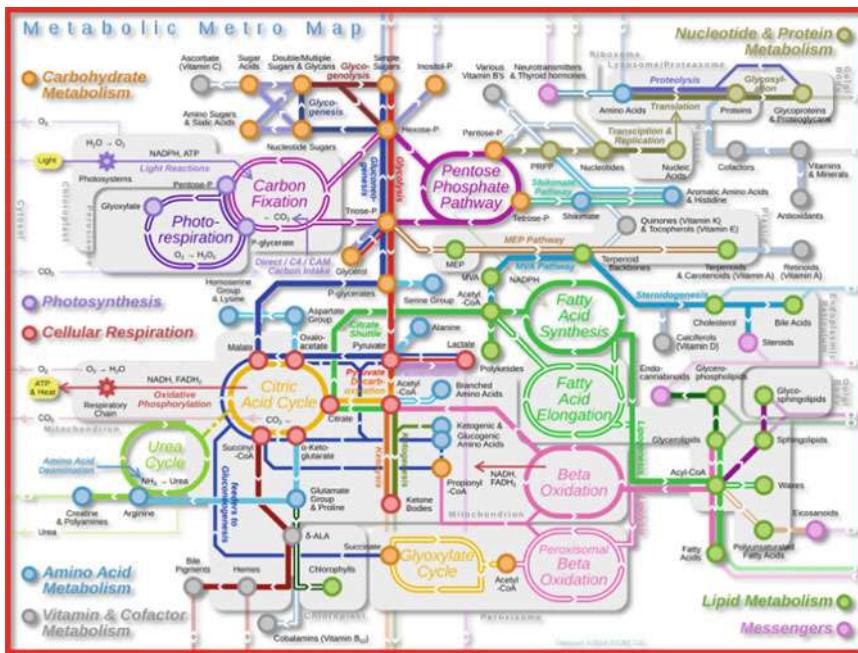


Fig. 8.14 Major metabolic pathways in metro-style map. Click any text (name of pathway or metabolites) to link to the corresponding article. Single lines: pathways common to most lifeforms. Double lines: pathways not in humans (occurs in, e.g., plants, fungi, and prokaryotes). Orange nodes: carbohydrate metabolism. Violet nodes: photosynthesis. Red nodes: cellular respiration. Pink nodes: cell signaling. Blue nodes: amino acid metabolism. Gray nodes: vitamin and cofactor metabolism. Brown nodes: nucleotide and protein metabolism. Green nodes: lipid metabolism

multi-dimensional protein identification techniques, i.e., advanced HPLC systems coupled with mass spectrometry, sub disciplines include phosphoproteomics, glycoproteomics, and other methods to detect chemically modified proteins); (vii) metabolomics (organismal, tissue, or cell-level measurements of small molecules known as metabolites); (viii) glycomics (organismal, tissue, or cell-level measurements of carbohydrates); (ix) lipidomics (organismal, tissue, or cell-level measurements of lipids); etc. In addition to the identification and quantification of the cellular molecules, further techniques that analyze the dynamics and interactions within a cell include (x) interactomics (organismal, tissue, or cell-level study of interactions between molecules, i.e., protein-protein interactions-PPI); (xi) neuro-electrodynamics (organismal, brain computing function as a dynamic system, underlying biophysical mechanisms and emerging computation by electrical interactions); (xii) fluxomics (organismal, tissue, or cell-level measurements of molecular dynamic changes over time); (xiii) biomics (systems analysis of the biome); (xiv) molecular biokinematics (the study of “biology in motion” focused on how cells transit between steady states; various technologies utilized to capture

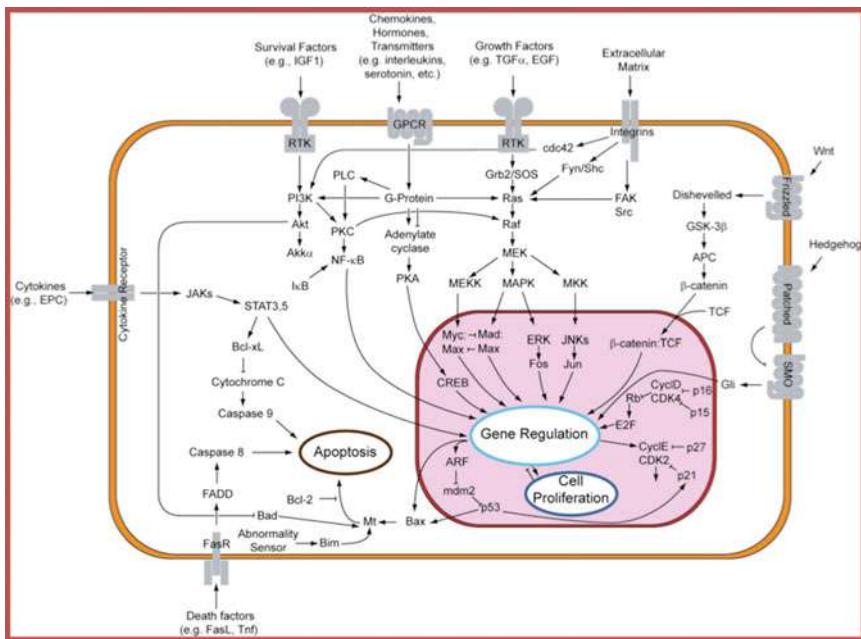


Fig. 8.15 Signal transduction pathways

dynamic changes in mRNA, proteins, and post-translational modifications; (xv) semiomics (analysis of the system of sign relations of an organism or other biosystems); (xvi) physiomics (a systematic study of physiome in biology); etc.

Cancer systems biology is an example of the systems biology approach, which can be distinguished by the specific object of study (tumorigenesis and treatment of cancer). It works with the specific data (patient samples, high-throughput data with particular attention to characterizing cancer genome in patient tumor samples) and tools (immortalized cancer cell lines, mouse models of tumorigenesis, xenograft models, Next Generation Sequencing methods, siRNA-based gene knocking down screenings, computational modeling of the consequences of somatic mutations and genome instability) (Barillo et al. 2012). The long-term objective of the systems biology of cancer is ability to better diagnose cancer, classify it and better predict the outcome of a suggested treatment, which is a basis for personalized cancer medicine and virtual cancer patient in more distant prospective. Significant efforts in computational systems biology of cancer have been made in creating realistic multi-scale in silico models of various tumors (Byrne 2010).

The investigations are frequently combined with large-scale perturbation methods, including gene-based (RNAi, mis-expression of wild-type, and mutant genes) and chemical approaches using small molecule libraries. Robots and automated sensors enable such large-scale experimentation and data acquisition. These technologies are still emerging and many face problems that the larger the quantity

of data produced, the lower the quality. A wide variety of quantitative scientists (computational biologists, statisticians, mathematicians, computer scientists, and physicists) are working to improve the quality of these approaches and to create, refine, and retest the models to accurately reflect observations. The systems biology approach often involves the development of mechanistic models, such as the reconstruction of dynamic systems from the quantitative properties of their elementary building blocks (Gardner et al. 2003; di Bernardo et al. 2005). For instance, a cellular network can be modeled mathematically using methods coming from chemical kinetics and control theory. Due to the large number of parameters, variables, and constraints in cellular networks, numerical and computational techniques are often used (e.g., flux balance analysis) (Tavassoly 2015).

8.6 Conservation of Medicinal Plant and Animal Biodiversity and Sustainable Utilization of Crude Drugs Resources

Biodiversity is the variability among living organisms (e.g., plants, animals, and microbes) from all sources such as terrestrial, marine, and other aquatic ecosystems and the ecological complexes of which they are part. Biodiversity provides support for drug discovery and the availability of medicinal resources (Mendelsohn and Balick 1995) and health issues such as dietary health and nutrition security, infectious disease, medical science, and medicinal resources, social and psychological, etc. are influenced by biodiversity (Gaston et al. 2007). A significant proportion of drugs are derived, directly or indirectly, from different biological sources, e.g., plants, animals and micro-organisms of terrestrial and marine ecosystems, and ~80% of the world population depends on medicines from nature for primary health care; natural products have a long history of supporting significant economic and health innovation (Hunter 1996; Bowen 1999; Dyke 2008). Conservation and sustainable management of biodiversity can sustain human and environmental health, generate employment, and enhance export earnings.

Biodiversity reflects the number, variety, and variability of plants, animals, and other living organisms and includes diversity within species (genetic diversity), between species (species diversity), and between ecosystems (ecosystem diversity). Natural substances have long served as sources of therapeutic drugs, e.g., digitalis (from foxglove), ergotamine (from contaminated rye), quinine (from cinchona), and salicylates (willow bark) are some classical examples. Wild and domestic animals and their by-products (e.g., hooves, skins, bones, feathers, and tusks) form important ingredients in the preparation of curative, protective, and preventive medicine (Adeola 1992), in addition, a significant portion of the currently available non-synthetic and/or semi-synthetic pharmaceuticals in clinical use is comprised of drugs derived from biological sources like plants, animals, microbes, and mineral products (Farnsworth and Morris 1976; Farnsworth and Soejarto 1985;

Soejarto 1996). Over 50% of commercially available drugs are based on bioactive compounds extracted (or patterned) from non-human species (Grifo et al. 1997), including some life-saving medicines such as cytarabine, derived from a Caribbean sponge, which is reputed as the single most effective agent for inducing remission in acute myelocytic (Chivian 2001). Some other examples of drugs from biological sources include quinidine to treat cardiac arrhythmias, D-tubocurarine to help induce deep muscle relaxation without general anesthetics, vinblastine to fight Hodgkin's disease, vincristine for acute childhood leukemias, combadigitalis to treat heart failure, ranitidine to fight ulcers, levothyroxine for thyroid hormone replacement therapy, enalapril maleate to reduce high blood pressure, etc. (Anonymous 1997; Chivian 1997).

Drug discovery from natural sources involve a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques; and medicinal-plant-based drug discovery still remains an important area for the discovery of important leads against various pharmacological targets. The decline in biodiversity is largely the result of the rise in the global population, rapid industrialization, indiscriminate deforestation, overexploitation of natural resources, pollution, and global climate change. Biodiversity loss diminishes the supplies of raw materials for drug discovery and biotechnology, causes a loss of medical models, affects the spread of human diseases, and threatens food production and water quality. Its reduction has direct effects on the discovery of potential medicines. It is of utmost importance that plant biodiversity be preserved, to provide future structural diversity and lead compounds for the sustainable development of human civilization at large. As medicinal plants are globally valuable sources of herbal products are disappearing at a high speed, both conservation strategies, e.g., in situ and ex situ conservation and cultivation practices and resource management, e.g., good agricultural practices and sustainable use solutions, should be adequately taken into account for the sustainable use of medicinal plant resources. Biotechnical approaches (e.g., tissue culture, micropropagation, synthetic seed technology, and molecular marker-based approaches) should be applied to improve yield and modify the potency of medicinal plants.

8.7 Molecular Breeding Marker in Herbal Drug Technology and New Variety Cultivation

Molecular breeding (MB) may be defined as the use of genetic manipulation performed at DNA molecular levels to improve characters of interest in plants and animals and it includes genetic engineering or gene manipulation, molecular marker-assisted breeding, genomic selection, etc. In genetics, a molecular marker (identified as genetic marker) is a fragment of DNA that is associated with a certain location within the genome. Molecular markers are used in molecular biology and biotechnology to identify a particular sequence of DNA in a pool of unknown

DNA. Molecular marker-assisted breeding (MAB) implies the application of molecular biotechnologies, specifically molecular markers, in combination with linkage maps and genomics, to alter and improve plant or animal traits on the basis of genotypic assays. This term is used to describe several modern breeding strategies, including marker-assisted selection (MAS), marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), and genome-wide selection (GWS) or genomic selection (GS) (Ribaut et al. 2010).

Genetic markers are the biological features that are determined by allelic forms of genes or genetic loci and can be transmitted from one generation to another, and thus they can be used as experimental probes or tags to keep track of an individual, a tissue, a cell, a nucleus, a chromosome or a gene. Genetic markers used in genetics and plant breeding can be classified into two categories: (i) classical markers and (ii) DNA markers (Xu 2010). Classical markers include morphological markers, cytological markers, and biochemical markers. DNA markers have developed into many systems based on different polymorphism-detecting techniques or methods (Collard et al. 2005). There are many types of genetic or DNA markers including (i) Restriction Fragment Length Polymorphism (RFLP), (ii) Random Amplified Polymorphic DNA (RAPD), (iii) Amplified Fragment Length Polymorphism (AFLP), (iv) Variable Number Tandem Repeat (VNTR), (v) Oligonucleotide Polymorphism (OP), (vi) Random Amplified Polymorphic DNA (RAPD), (vii) Single Nucleotide Polymorphism (SNP), (viii) Allele-Specific Associated Primers (ASAP), (ix) Inverse Sequence-tagged Repeats (ISTR), (x) Inter-retrotransposon Amplified Polymorphism (IRAP), etc. They can be further categorized as dominant or co-dominant. Dominant markers allow for analyzing many loci at one time, e.g., RAPD. A primer amplifying a dominant marker could amplify at many loci in one sample of DNA with one PCR reaction. Co-dominant markers analyze one locus at a time. A primer amplifying a co-dominant marker would yield one targeted product.

There are many types of genetic markers, each with particular limitations and strengths. Within genetic markers, there are three different categories: (i) First-Generation Markers, (ii) Second-Generation Markers, and (iii) New-Generation Markers (Meuwissen et al. 2001). These types of markers may also identify dominance and co-dominance within the genome (Jannink et al. 2010). Identifying dominance and co-dominance with a marker may help identify heterozygotes from homozygotes within the organism. Co-dominant markers are more beneficial because they identify more than one allele, thus enabling someone to follow a particular trait through mapping techniques. These markers allow for the amplification of particular sequence within the genome for comparison and analysis. Molecular markers are effective because they identify an abundance of genetic linkage between identifiable locations within a chromosome and are able to be repeated for verification. They can identify small changes within the mapping population enabling distinction between a mapping species, allowing for segregation of traits and identity. They identify particular locations on a chromosome, allowing for physical maps to be created. Last, they can identify how many alleles an organism has for a particular trait (biallelic or poly allelic) (Heffner et al. 2009).

Among the techniques that have been extensively used and are particularly promising for application to plant breeding are the restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), microsatellites or simple sequence repeat (SSR), and single nucleotide polymorphism (SNP). According to a causal similarity of SNPs with some of these marker systems and fundamental difference with several other marker systems, the molecular markers can also be classified into SNPs (due to sequence variation, e.g., RFLP) and non-SNPs (due to length variation, e.g., SSR) (Gupta et al. 2001). The marker techniques help in the selection of multiple desired characters simultaneously using F_2 and backcross populations, near isogenic lines, doubled haploids and recombinant inbred lines.

Molecular breeding is the application of molecular biology tools in plant (Stephen and Rita 2008) and animal breeding (Dekkers and Hospital 2002; Jack 2012). The areas of molecular breeding include (i) Quantitative trait loci (QTL) mapping or gene discovery; (ii) Marker-assisted selection and genomic selection (Meuwissen et al. 2001; Heffner et al. 2009; Jannink et al. 2010); (iii) Genetic engineering; (iv) Genetic transformation, etc.

Genomic markers as mentioned have particular strengths and weakness, so, consideration and knowledge of the markers are necessary before use. For instance, a RAPD marker is dominant (identifying only one band of distinction) and it may be sensitive to reproducible results. This is typically due to the conditions in which it was produced. RAPD's are used also under the assumption that two samples share a same locus when a sample is produced. Different markers may also require different amounts of DNA. RAPD's may only need 0.02 g of DNA while an RFLP marker may require 10 g of DNA extracted from it to produce identifiable results. SNP markers have turned out to be a potential tool in breeding programs in several crops.

Genetic markers in herbal drug technology

Genetic markers have proved their utility in fields like taxonomy, physiology, embryology, genetics, etc. As the science of plant genetics progressed, researchers have tried to explore the molecular markers techniques for their applications in commercially important plants such as food crops, horticultural plants, etc. and recently in pharmacognostic characterization of herbal medicine. Genetic markers in herbal drug technology serve the following purposes:

Genetic variation or genotyping

Results on the investigation on geographical variation at the genetic level clearly indicated that geographical conditions affected the active constituents of the medicinal plant and their activity profiles (Oleszek et al. 2002). RAPD-based molecular markers have been found to be useful in differentiating different accessions of *Taxus wallichiana* (Shasany et al. 1999), *Azadirachta indica* (Farooqui et al. 1998), *Juniperus communis* (Adams et al. 2002), *Codonopsis pilosula* (Fu et al. 1999), *Allium schoenoprasum* (Friesen and Blattner 1999), *Andrographis paniculata* (Padmesh et al. 1999), etc., collected from different geographical regions.

Authentication of medicinal plants

DNA-based techniques have been widely used for authentication of plant species of medicinal importance. This is especially useful in case of those that are frequently substituted or adulterated with other species or varieties that are morphologically and/or phytochemically indistinguishable. Dried fruit samples of *Lycium barbarum* were differentiated from its related species using RAPD markers (Zhang et al. 2001).

Marker-assisted selection of desirable chemotypes

Along with authentication of species identity, prediction of the concentration of active phytochemicals may be required for quality control in the use of plant materials for pharmaceutical purposes. Identification of DNA markers that can correlate DNA fingerprinting data with quantity of selected phytochemical markers associated with that particular plant would have extensive applications in quality control of raw materials. AFLP analysis has been found to be useful in predicting phytochemical markers in cultivated *Echinacea purpurea* (Baum et al. 2001) germplasm and some related wild species. RAPD fingerprint has been developed to support the chemotypic differences in oil quality of three different genotypes of *Pelargonium graveolens* (Shasany et al. 2002) and flavonoid composition of *Aconitum* species (Fico et al. 2003).

Marker-assisted medicinal plant breeding

Marker-assisted breeding involves the use of DNA markers linked with DNA sequences of interest. Inheritance pattern of sequences/traits can be confirmed even prior to expression using RFLP, RAPD, AFLP, and minisatellites markers (Yu et al. 1991; Ma et al. 1994). A high content artemisinin producing plant variety “CIM-Arogya” was developed at CIMAP, Lucknow through marker-assisted breeding. Selection of genotype with increased biomass led to selection of higher artemisinin yielding variety (Khanuja et al. 2007). An increase in artemisinin from 0.15 to 1.16% on dry weight basis was recorded in this variety. Thus, marker-assisted breeding could also be used in developing high secondary metabolite yielding plants. Thus, DNA-based molecular markers have acted as versatile tools in plant genome analysis and are specifically important in differentiating different plant species and their varieties. Various techniques like RFLP, RAPD have been successfully applied for characterization of semi-processed and processed herbal drug materials. Being environmentally stable and specific, DNA markers could gain wide popularity in quality control and standardization of medicinal plant materials. Although DNA analysis is currently considered to be cutting-edge technology, it has certain limitations due to which its use has been limited to academia.

ISSR-PCR has been found to be an efficient and reliable technique for the identification of zygotic plantlets in citrus interloploid crosses (Tusa et al. 2002). Molecular markers have been used as a tool to verify sexual and apomictic offspring of intraspecific crosses in *Hypericum perforatum*, a well-known antihelminthic and diuretic (Steck et al. 2001). An attempt has been made toward marker-assisted selection of fertile clones of garlic with the help of RAPD markers (Etoh and Hong

2001). RAPD markers have been successively used for selection of micropropagated plants of *Piper longum* for conservation (Ratnaparkhe et al. 1995; Shaw and But 1995; Parani et al. 1997).

8.8 Gene Regulation of Metabolic Pathway and Directional Control of the Quality of Herbal Medicines

A metabolic pathway is a linked series of chemical reactions that occur within a cell; and the reactants, products, and intermediates of an enzymatic reaction are known as metabolites. In a metabolic pathway, the product of one enzyme acts as the substrate for the next. These enzymes often require minerals, vitamins, and other cofactors to function. Different metabolic pathways function in a given compartment of the eukaryotic cell, e.g., the citric acid cycle, electron transport chain, and oxidative phosphorylation take place in the mitochondrial membrane while glycolysis, pentose phosphate pathway, and fatty acid biosynthesis occur in the cytosol of a cell (Nicholson 1971; Pratt et al. 2013).

Metabolic pathways are classified as (i) anabolic pathways, characterized by their ability to synthesize molecules with the utilization of energy, and (ii) catabolic pathways, characterized by their ability to break down complex molecules by releasing energy in the process. The two pathways complement each other in that the energy released from one (catabolic pathway) is used up by the other (anabolic pathway). The amphibolic pathway may be either catabolic or anabolic based on the need for or the availability of energy (Berg et al. 2012). Metabolic pathways may also be (i) primary and (ii) secondary metabolic pathways. Products of the primary metabolic pathways are primary metabolites—compounds that are directly involved in the growth and development of a plant—whereas products of the secondary metabolic pathways are secondary metabolites—compounds, although important, are not essential to the functioning of the plant. Pathways are required for the maintenance of homeostasis within an organism and the flux of metabolites through a pathway is regulated depending on the needs of the cell and the availability of the substrate.

Plants produce a wide spectrum of secondary metabolites that play critical roles in plant–environment interactions and against biotic and abiotic stresses. Many of these secondary metabolites (e.g., vinblastine, nicotine, artemisinin, taxol, ginsenosides, etc.) have high pharmaceutical efficacy for a wide range of diseases (cancer, asthma, malaria, etc.) valued and have been used for the treatment of wide range of diseases, e.g., vinblastine for tumor, nicotine for asthma, artemisinin for malaria, taxol and ginsenosides for different cancer treatments. Because of these beneficial health effects of secondary metabolites, many scientists have been focusing on the comprehensive study of regulation and production of secondary

metabolites. Controlled transcription of biosynthetic genes is one of the major mechanisms regulating secondary metabolism in plants.

As the major source of active ingredients in medicinal herbs including the Chinese herbs is secondary metabolites and the presence or absence of secondary metabolites and their amount decide the quality of herbal medicine, emphasis should be given on the basic research of secondary metabolite biosynthesis, especially on the research of gene regulation of key enzymes. Genetic engineering will be an invaluable tool in the twenty-first century to overcome the rate-limiting steps in biosynthesis of secondary metabolites and could provide targets for genetically engineering biochemical pathways to produce augmented amounts of active ingredients (compounds) and new compounds for future improvement of the resources of herbal medicines. Targeted expression genes may be used to channel metabolic flow into metabolic pathways in one hand, and in other, gene silencing tools may be used to reduce or eliminate undesirable toxic compounds with the help of genetic engineering.

A gene or genetic regulatory network (GRN) is a collection of molecular regulators that interact with each other and with other substances in the cell to govern the gene expression levels of mRNA and proteins. The regulator can be DNA, RNA, protein, and complexes of these. The interaction can be direct (through transcribed RNA) or indirect (through translated protein). In general, each mRNA molecule goes on to make a specific protein or set of proteins such as (i) structural protein that will accumulate at the cell membrane or within the cell to give it particular structural properties; (ii) biologically active protein or enzyme (a micro-machine) that catalyses a certain reaction such as the breakdown of a food source or toxin; and (iii) regulatory proteins, and these are the transcription factors (TFs) that are the main players in regulatory networks or cascades.

TFs include a wide number of proteins that initiate and regulate the transcription of several genes. TFs are sequence-specific DNA-binding proteins that interact with the regulatory regions of the target genes and modulate the rate of transcriptional initiation by RNA polymerase (Yang et al. 2012). Many TFs have been characterized for their roles in regulating biosynthetic pathways at the transcriptional level. By binding to the promoter region at the start of other genes they (TFs) turn them on, initiating the production of another protein, and so on. Some TFs are inhibitory. Several transcription factor families (e.g., MYC, MYB, WRKY, and AP2/ERF (Apetala2/ethylene responsive factor) have been found to be involved in the regulation of secondary metabolism in different medicinal plants and, in addition, the biosynthesis and proper accumulation of secondary metabolites are also induced by signaling molecule jasmonic acid (JA) (Afrin et al. 2015) (Fig. 8.16). List of JA-mediated TFs involved in secondary metabolites production in several medicinal plants including *Catharanthus roseus* (TIAAs-terpenoid indole alkaloids, such as vinblastine and vincristine), *Artemisia annua* (artemisinin), *Nicotiana* spp. (phenylpropanoids, nicotine, volatile terpenes), *Taxus* spp. (taxol), and *Panax quinquefolius* (ginsenoside) have also been given by Afrin et al. (2015).

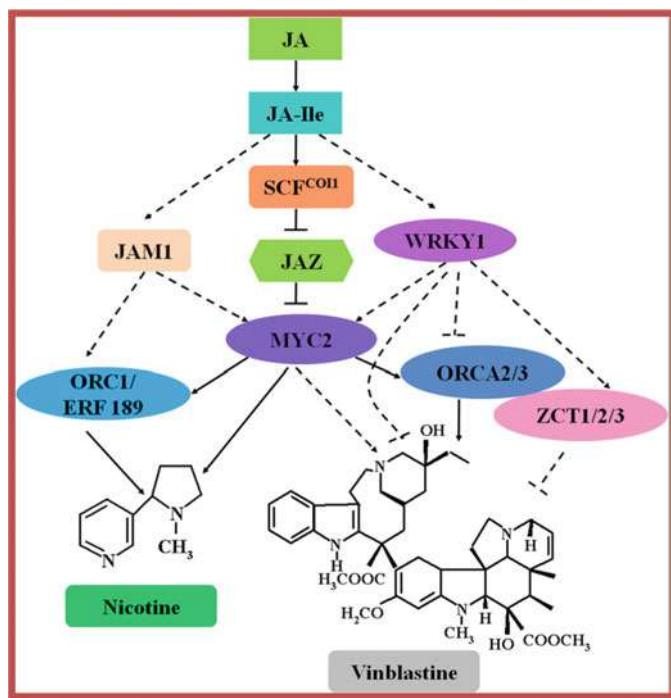


Fig. 8.16 JA-mediated transcriptional regulation of vinblastine synthesis in *Catharanthus roseus* and nicotine synthesis in *Nicotiana tabacum*. Solid and dashed arrows indicate proven and hypothetical (yet to be experimentally established) links, respectively. Arrows indicate positive interactions and T-bars indicate negative interactions. COII CORONATINE INSENSITIVE 1, ERF ethylene response factor, JA jasmonic acid, JA-Ile JA-isoleucine; JAZ jasmonic acid ZIM domain, JAM1 JA factor-stimulating MAPKK 1, ORCA 2/3 octadecanoid-responsive *Catharanthus* AP2-domain proteins 2 and 3, SCF Skp–Cullin–F-box-type E3 ubiquitin ligase, ZCT zinc-finger *Catharanthus* transcription factor. Source Afrin et al. (2015)

8.9 Biological Process of the Formation of Secondary Metabolites in Medicinal Plants

Secondary metabolites play an important role in plant defense against herbivory and other interspecies defenses, herbal medicines, food flavoring, and recreational drugs. Secondary metabolites clearly provide most of the therapeutic activity of medicinal plants. Several distinct secondary metabolites derived from medicinal plants are extensively used in modern medicines in the world since they carry out a number of protective functions like boosting the immune system, protect the body from free radicals, cardiovascular illnesses, killing of pathogenic germs, etc.

During long period evolution, plants struggling to survive gradually gain the ability to synthesize various kinds of secondary metabolites with bioactivities. These compounds played important role in defending insects, herbivores, microbial

pathogens, competing with other plants, and facilitating pollination and reproduction. Based on the structures, the secondary metabolites can be classified into alkaloids, flavonoids, phenylpropanoids, quinones, terpenoids, steroids, tannins, and proteins. These compounds are biosynthesized through series enzyme-catalyzed reactions using simple building blocks in different ways. There are several main biosynthetic pathways in plants, including shikimic acid pathway (phenylpropanoids), mavalonic acid pathway (quinones), 2-C-methyl-D-erythritol-4-phosphate pathway (quinones), amino acid pathway (alkaloids), acetate-malonate pathway (fatty acid, phenols and quinones), and combined pathways (flavonoids). Secondary metabolites are not directly involved in the normal growth, development, and reproduction of the organism; they are primarily involved in the overall maintenance (homeostasis) of the organism. Secondary metabolites specifically modulate health-maintaining processes, including excretion of waste and toxic products from the body, i.e., sustaining the overall health and functional status of the cells within organ systems of the body, the principal function of secondary metabolites, e.g., the biotransformation of tryptophan, a primary metabolite, into Actinomycin, which is a secondary metabolite.

8.10 Application of Systems Biology in Secondary Metabolites Study

Secondary metabolites found in nature have long been a source of drugs, including antibiotics, antifungal agents, and chemotherapeutics, and it is likely that many more valuable compounds remain to be discovered. All metabolites occur as a result of activity within biological systems. Secondary metabolites are used as flavors, fragrances, colorants, or pharmaceuticals and they (pharmaceuticals) are the main active ingredients in herbal medicine to ensure the quality of crude drugs. Secondary metabolites are ultimately derived from primary products of photosynthesis through multiple enzymatic steps encoded by the genome of each plant. A better understanding of metabolite synthesis and the regulation thereof will be increasingly important for improving the sustainability and efficiency of useful plant production, but unfortunately, knowledge about the genetic mechanism both primary and secondary metabolites synthesis is far from complete. Availability of genome sequences data of certain plants and the development of functional genomics tools have allowed the elucidation of metabolite syntheses by a systems biology approach (Goff et al. 2002; Yu et al. 2002). The mining and exploitation of the data obtained from genomics and the related research areas of genome-wide transcriptomics, proteomics, and metabolomics will bring researchers into a new era of understanding of biological systems (Fig. 8.17).

The thought and approach adopted in systems biology are a powerful tool to explore biology fully, along with the development of modern molecular and information biology, omics integration like genomics, transcriptomics, proteomics,

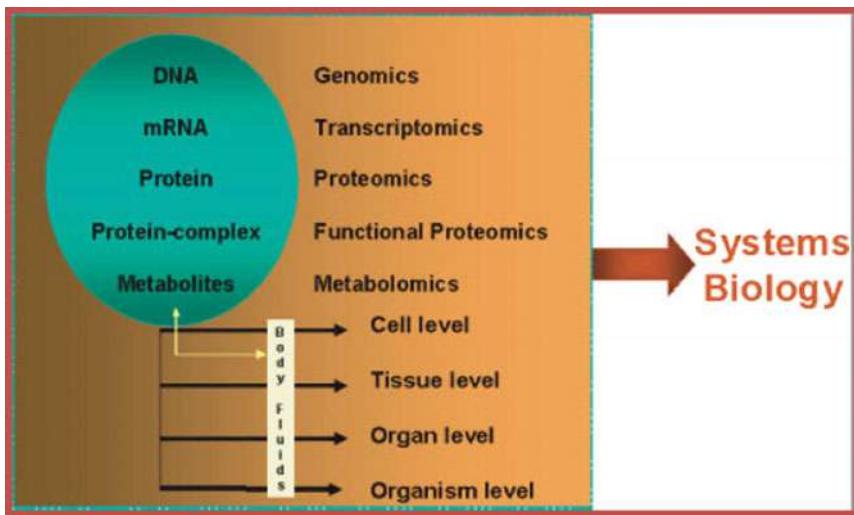


Fig. 8.17 The different levels of measurement in a systems approach. *Source* Wang et al. (2005a, b)

and metabolomics will bring new opportunities for the study of secondary metabolites of medicinal plant. It has great significance to apply this holistic and systematic method in researches on biosynthetic pathway, signal transduction, ecological environment, and metabolic engineering of the formation of the secondary metabolites of medicinal plants, and in building secondary metabolite biosynthesis gene expression and regulation system model, in order to explain the origin of the active ingredients of medicinal plants, formation mechanism of the Chinese herbs, metabolic engineering effecting active ingredients of medicinal plants, and the rational exploitation and utilization of resources of medicinal plants systematically. Metabolic engineering using systems biology tools is increasingly applied to overproduce secondary metabolites for their potential industrial production.

In most described cases, biosynthetic pathways for secondary metabolites are organized as biosynthetic gene clusters (BGCs), i.e., all required genes for the production of a secondary metabolite are encoded within such BGCs. Biosynthetic gene clusters include genes encoding core biosynthetic enzymes (e.g., polyketide synthase and non-ribosomal peptide synthetase), genes encoding tailoring enzymes, genes encoding for specific precursor biosynthesis pathways, cluster-situated regulators, and often genes encoding transporters or resistance factors are physically clustered on the chromosome. Advances in metabolomics and its integration into systems biology research are being made possible by combining expertise from biology, chemistry, instrumentation, computer science, physics, and mathematics. Given that the era of such true interdisciplinary cooperation is only starting, many

exciting discoveries are to be expected in the coming years. System biology, a holistic approach to understand biological complexity, involves the study of biological systems viewed as integrated and interrelated networks of genes, proteins, and biochemical reactions. Systems biology is the ultimate phenotyping and it opens up the possibility of studying the effect of complex mixtures, such as those used in herbal medicine including TCM, in complex biological systems; abridging it with molecular pharmacology. This approach is considered to have the potential to revolutionize natural product research and to advance the development of scientific-based herbal medicine. Systems biology will prove to be a valuable tool in TCM and herbal research.

8.11 Use of Genetic Engineering and Tissue Culture Technique for the Production of Active Ingredients

Plant tissue culture has emerged as an alternative to whole plant cultivation in the production of valuable secondary metabolites. At present, cells, adventitious roots, hairy roots, shoots, and embryos have been successfully cultivated for the large-scale production of secondary metabolites. Recent advances in plant cell, tissue, and organ culture research mainly focus on optimization of culture conditions, composition comparison, elicitors, transgenic technology, and genetic stability. Adventitious roots culture of *P. ginseng* and *E. purpurea* has reached the scale of 1–10 kL. Some molecular biological techniques, such as transgenic technology and genetic stability are increasingly used in the studies on plant tissue cultures. The studies on elicitors have deepened into the induction mechanism, including signal molecules, functional genes, and so on. More and more biological elicitors, such as *A. niger* and yeast are used to increase the active compounds in plant tissue cultures. Table 8.2 lists the production of active compounds through plant tissue culture or synthetic biology method.

Transgenic technology

Plant transgenic technology is a method of inducing variation of organisms heritable traits, by which exogenous genes transformed into the host plants are expressed after consolidation. The plantlets transformed with some genes can significantly improve the corresponding features of plants and produce high levels of active compounds or high biomass of plants. Agrobacterium-mediated method is not only the first transgenic method applied to plants but also the most widely used method for plant transformation. In the plant tissue culture, plant transgenic technology is mainly applied in the establishment of hairy root culture and suspension cells.

Hairy roots transformed by Ri plasmid grow fast and contain the expression of complete metabolic pathways, which provide a broad prospect for the industrial production of secondary metabolites of medicinal plants. Furthermore, hairy roots

Table 8.2 Production of secondary metabolites in medicinal plants through bioengineering

Components	Secondary metabolites	Plants	Sources	References
Terpenoids	Taxuyunnanine c, taxol	<i>Taxus chinensis</i> (Pilger) Rehd.	Cell, biosynthesis	Gao et al. (2010) and Zhou et al. (2015)
	Saponin	<i>Panax quinquefolius</i> L.; <i>A. Senticosus</i> ; <i>P. ginseng</i> ; <i>Bupleurum falcatum</i>	Cell, hairy root, biosynthesis	Hao et al. (2010), Zhao et al. (2015), Tao et al. (2011), Balusamy et al. (2013) and Moses et al. (2014)
	Glycyrrhizic acid	<i>Glycyrrhiza uralensis</i> Fisch.	Hairy root	Zhang et al. (2011) and Yang et al. (2014)
	Artemisinin	<i>Artemisia annua</i> Linn	Biosynthesis	Paddon et al. (2013)
Flavonoids	Flavonoids	<i>Ginkgo biloba</i> Linn; <i>Saussurea involucrate</i> Kar. et Kir. ex Maxim.	Cell; hairy root	Hao et al. (2010), Hu et al. (2011), Zhang et al. (2013) and Qiao et al. (2011)
	Licochalcone a, total flavonoid	<i>G. uralensis</i>	Hairy root	Zhang et al. (2011)
Alkaloids	Scopolamine, hyoscyamine	<i>Datura stramonium</i> Linn	Hairy root	Sun et al. (2013)
	Vincamine	<i>Catharanthus roseus</i>	Hairy root	Verma et al. (2014)
Penyl propanoids	Coumarins	<i>Angelica archangelica</i> L.	Cell	Tomas et al. (2012)
Phenolic acids	Phenolic acids, chlorogenic acid	<i>Eryngium planum</i> L.	Cell	Kikowska et al. (2012)
	Caffeic acid	<i>E. purpure</i>	Adventitious root	Cui et al. (2013)
Quinones	Acetylshikonin	<i>Arnebia euchroma</i> (Royle) Johnst; <i>Radix Arnebiae</i> Seu. <i>Lithospermi</i>	Cell, hairy root	Baranek et al. (2012), Li et al. (2010) and He et al. (2010)
	Anthroquinones	<i>Morinda officinalis</i> How	Hairy root	Zheng et al. (2014)
Steroids	Phytoecdysteroids	<i>Achyranthes bidentata</i> Blume	Cell	Wang et al. (2013)

Source Wang et al. (2017)

provide a lot of plant materials for new drug screening because the biotransformation can produce many new compounds. At present, ginsenosides and berberine have achieved commercial production by hairy root culture (Kim et al. 2011).

8.12 Genetic Engineering and Green Pollution-Free Medicinal Plant

One of the tasks for molecular pharmacognosy is to improve the ability of medicinal plants against pests via genetic engineering. Genetic engineering enables scientists to create plants, animals, and microorganisms by manipulating genes in a way that does not occur naturally. However, in the development of medicinal plant genetic engineering, the safety of transgenic medicinal plants should be taken into consideration. Genetic engineering can be used to improve the disease resistance, insect resistance, herbicides resistant ability of medicinal plant; such technology can improve the medicinal plant yield and increase the content of active substances in medicinal plants. Thus, the potent biotechnology can play an important role in protection and large area planting of medicinal plants and the problem of pesticide pollution in medicinal plants that has aroused public concern, for its harmful effects on environment, health, and export of medicinal herbs including the Chinese herbal medicines. Good agricultural practices (GAP) for medicinal plants may be helpful for regulation of quality production and the standardization of herbal drugs (Chan et al. 2012). A GAP approach by advocating green pollution-free medicinal plant cultivation ensures high quality, safe and pollution-free herbal drugs. Chinese medicine refers to the pollution-free medicinal plants in compliance with Green standards of medicinal plants and preparations for import and export. China released strict management on the marks of traditional Chinese medicine.

8.13 Metabolomics of Medicinal Plants: Genomics, Proteomics, and Metabolomics

Plant secondary metabolites play an important role in the fields of food, medicine, agriculture, and biofuels. Secondary metabolites are an important focus of crop breeding and metabolic engineering research. The study of the structure and function of the genome comes under genomics. Further, elaborated in sub-class like structural, functional, and comparative genomics. Functional genomics, which includes transcriptomics, proteomics, and metabolomics, opens a new avenue for deciphering secondary metabolism. The study of the proteome includes full complement of proteins made by the cell. The use of genome sequence analysis to determine the capability of the cell, tissue, or organism to synthesize small molecules comes under metabolomics (Primrose and Twyman 2003). It is also defined as systematic study of the distinctive chemical fingerprints that specific cellular processes leave behind. Metabolomics with reference to natural product is one of the important bases for studying the relation between the composition of complex and variable mixtures of plant-derived remedies as well as their biological effects. Plant metabolomics starts with the analysis of as many as probable detectable individual bioactive moiety that is present in the material. It also helps to study the secretion,

percentage, and composition of individual herbal marker compound, e. g., study of metabolites in *Ginkgo biloba* leaves and effect of harvesting time (sunrise and sunset) on their flavonoids contents (Wang et al. 2005a, b).

Metabolomics, the comprehensive and global analysis of diverse metabolites produced in cells and organisms, has greatly expanded metabolite fingerprinting and profiling as well as the selection and identification of marker metabolites. The methodology typically employs multivariate analysis to statistically process the massive amount of analytical chemistry data resulting from high-throughput and simultaneous metabolite analysis. Although the technology of plant metabolomics has mainly developed with other post-genomics in systems biology and functional genomics, it is independently applied to the evaluation of the qualities of medicinal plants based on the diversity of metabolite fingerprints resulting from multivariate analysis of non-targeted or widely targeted metabolite analysis. One advantage of applying metabolomics is that medicinal plants are evaluated based not only on the limited number of metabolites that are pharmacologically important chemicals but also on the fingerprints of minor metabolites and bioactive chemicals. In particular, score plot and loading plot analyses, e.g., principal component analysis (PCA), partial-least-squares discriminant analysis (PLS-DA), and discrimination map analysis such as batch-learning self-organizing map (BL-SOM) analysis are often employed for the reduction of a metabolite fingerprint and the classification of analyzed samples. Based on recent studies, we now understand that metabolomics can be an effective approach for comprehensive evaluation of the qualities of medicinal plants. In this review, we describe practical cases in which metabolomic study was performed on medicinal plants, and discuss the utility of metabolomics for this research field, with focus on multivariate analysis.

8.14 The Goal of Molecular Pharmacognosy

With a theoretical basis on pharmacognosy, molecular pharmacognosy presents problems to the field of study in pharmacognosy whose major contents can be summarized by authenticity and excellence as presented as follows: (a) Discerning the false from the genuine so as to settle the problem of variety confusion. Due to rise in scope of use and dosage of medicine, plants, animals, and parts with similar appearance or homonym are taken as the same drugs to be used in different regions, thus leading to a lot of confusion; therefore, it is necessary to discern the false from the genuine in terms of their origins and distribution areas. Only in this way can quality be guaranteed. (b) Quality assessment: A systematic study is conducted on crude drugs with multi-origin and genuine quality, including place of origin, harvesting, processing, storage, and the influence of transportation upon active ingredients to confirm high-quality variety and factors that may have an effect on it. More than that, excellent varieties should be researched and cultured to achieve fast growth, high quality, and high yield in order to meet the needs of medication.

Scientific connotation of all specific information of authenticity and excellence of herbal medicine is related to the difference in their DNA. The fake and the genuine may have different DNA composition due to the difference in their origin of varieties; thus, DNA, the genetic material, differentiates the genuine from the fake. Therefore, the scientific connotation of studying molecular pharmacognosy is to research DNA and its relation to authenticity and excellence of crude drugs.

References

- Adams RP, Pandey RN, Leverenz JW, Diggard N, Hoeghe K, Thorfinnsson T (2002) Molecular Markers in herbal drug technology. *Sci Hortic* 96:303–312
- Adeola MO (1992) Importance of wild animals and their parts in the culture, religious festivals, and traditional medicine, of Nigeria. *Environ Conserv* 19(2):125–134
- Afrin S, Huang JJ, Zhi-Yong Luo ZY (2015) JA-mediated transcriptional regulation of secondary metabolism in medicinal plants. *Sci Bull* 60(12):1062–1072
- Albani MC, Battey NH, Wilkinson MJ (2004) The development of ISSR-derived SCAR markers around the seasonal flowering locus (SFL) in *Fragaria vesca*. *Theor Appl Genet* 109:571–579
- Anonymous (1997) Biodiversity and human health: a guide for policymakers. Center for Biodiversity and Conservation (CBC), American Museum of Natural History, New York, NY. <http://research.amnh.org/biodiversity/acrobat/policy.pdf>
- Baitaluk M (2009) System biology of gene regulation. *Biomed Inf Methods Mol Biol* 569:55–87
- Balusamy SRD, Kim YJ, Rahimi S, Lee OR, Lee S, Yang DC (2013) Transcript pattern of cytochrome P450, antioxidant and ginsenoside biosynthetic pathway genes under heavy metal stress in *Panax ginseng* Meyer. *B Environ Contam Tox* 90(2):194–202
- Baranek KS, Pietrosiuk A, Naliwajski MR, Kawiak A, Jeziorek M, Wyderska S, Łojkowska E, Chinou I (2012) Effect of l-phenylalanine on PAL activity and production of naphthoquinone pigments in suspension cultures of *Arnebia euchroma* (Royle) Johnst. *In Vitro Cell Dev Biol –Plant* 48(5):555–564
- Barillo E, Calzone L, Hupe P, Vert JP, Zinovyev A (2012) Computational systems biology of cancer. Chapman & Hall, CRC Mathematical & Computational Biology, p 461
- Barthelson RA (2009) Protocols for in vitro cultures and secondary metabolite analysis of aromatic and medicinal plants. Identification of medicinal plants and plant sequences: multiplexed MLPA assay. *Methods Mol Biol* 547(3):277–288
- Baum BR, Mechanda S, Livesey JF, Binns SE, Arnason JT (2001) Predicting quantitative phytochemical markers in single *Echinacea* plants or clones from their DNA fingerprints. *Phytochemistry* 56:543–549
- Berg JM, Tymoczko JL, Stryer L, Gatto GJ (2012) Biochemistry, 7th edn. W.H. Freeman, New York, p 429
- Bhattacharya E, Dandin SB, Ranade SA (2005) Single primer amplification reaction methods reveal exotic and indigenous mulberry varieties are similarly diverse. *J Biosci* 30:669–677
- Blears M, De Grandis S, Lee H, Trevors J (1998) Amplified fragment length polymorphism (AFLP): a review of the procedure and its applications. *J Ind Microbiol Biotechnol* 21:99–114
- Borne B, Goraguer F, Joly G, Branchard M (2002) Genetic diversity in European and Argentinian cultivated potatoes (*Solanum tuberosum* subsp. *tuberosum*) detected by inter-simple sequencerepeats (ISSRs). *Genome* 45:481–484
- Bornet B, Branchard M (2004) Use of ISSR fingerprints to detect microsatellites and genetic diversity in several related *Brassica* taxa and *Arabidopsis thaliana*. *Hereditas* 140:245–248
- Bowen BW (1999) Preserving genes, species, or ecosystems? Healing the fractured foundations of conservation policy. *Mol Ecol* 8:S5–S10

- Bu Z, Callaway DJ (2011) Proteins MOVE! Protein dynamics and long-range allostery in cell signaling. *Adv Protein Chem Struct Biol* 83:163–221
- But PPH (1994) Herbal poisoning caused by adulterants or erroneous substitutes. *J Trop Med Hyg* 97:371–374
- But PPH, Tomlinson B, Cheung KO, Yong SP, Szeto ML, Lee CK (1996) Adulterants of herbal products can cause poisoning. *Br Med J* 313:117
- Byrne HM (2010) Dissecting cancer through mathematics: from the cell to the animal model. *Nat Rev Cancer* 10(3):221–230
- Caetano-Anolle's G, Bassam BJ, Gresshoff PM (1991) DNA amplification fingerprinting: a strategy for genome analysis. *Plant Mol Biol Rep* 9:294–307
- Carles M, Cheung MK, Moganti S, Dong TT, Tsim KW, Ip NY et al (2005) A DNA microarray for the authentication of toxic traditional Chinese medicinal plants. *Planta Med* 71:580–584
- CBOL Plant Working Group (2009) A DNA barcode for land plants. *Proc Natl Acad Sci USA* 106:12794–12797
- Chakrabarty D, Yu KW, Paek KY (2003) Detection of DNA methylation changes during somatic embryogenesis of *Siberian ginseng* (*Eleuterococcus senticosus*). *Plant Sci* 165:61–68
- Chan K (2003) Some aspects of toxic contaminants in herbal medicines. *Chemosphere* 52:1361–1371
- Chan K, Shaw D, Simmonds MS, Leon CJ, Xu Q, Lu A et al (2012) Good practice in reviewing and publishing studies on herbal medicine, with special emphasis on traditional Chinese medicine and Chinese materia medica. *J Ethnopharmacol* 140:469–475
- Chatti K, Saddoud O, Salhi Hannachi A, Mars M, Marrakchi M, Trifi M (2007) Analysis of genetic diversity and relationships in a Tunisian Fig (*Ficus carica*) germplasm collection by random amplified microsatellite polymorphisms. *J Integr Plant Biol* 49:386–391
- Chen KT, Su YC, Lin JG, Hsin LH, Su YP, Su CH et al (2001) Identification of *Atractylodes* plants in Chinese herbs and formulations by random amplified polymorphic DNA. *Acta Pharm Sin* 22:493–497
- Chen SL, Yao H, Han JP, Liu C, Song JY, Shi LC et al (2010) Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS ONE* 5:e8613
- Cheng KT, Su B, Chen CT, Lin CC (2000) RAPD analysis of *Astragalus* medicines marketed in Taiwan. *Am J Chin Med* 28:273–278
- Cheng JL, Huang LQ, Shao AJ, Lin SF (2002) RAPD analysis on different varieties of *Rehmannia glutinosa*. *Zhongguo Zhongyao Zazhi* 27:505–508
- Chiara R, Gerolamo L (2009) Statistical tools for gene expression analysis and systems biology and related web resources. In: Krawetz S (ed) Bioinformatics for systems biology, 2nd edn. Humana Press, pp 181–205
- Chivian E (1997) Global environmental degradation and species loss: implications for human health. In: Grifo F, Rosenthal J (eds) Biodiversity and human health. Island Press, Washington, pp 7–38
- Chivian E (2001) Environment and health: 7. Species loss and ecosystem disruption—the implications for human health. *CMAJ* 164(1):66–69
- Choi YE, Ahn CH, Kim BB, Yoon ES (2008) Development of species specific AFLP-derived SCAR marker for authentication of *Panax japonicus* C. A. MEYER. *Biol Pharm Bull* 31:135–138
- Cole CT, Kuchenreuther MA (2001) Molecular markers reveal little genetic differentiation among *Aconitum noveboracense* and *A. columbianum* (Ranunculaceae) populations. *Am J Bot* 88:337–347
- Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica* 142:169–196
- Cui HY, Baque MdA, Lee EJ, Paek KY (2013) Scale-up of adventitious root cultures of *Echinacea angustifolia* in a pilot-scale bioreactor for the production of biomass and caffeic acid derivatives. *Plant Biotech Rep* 7(3):297–308
- Datwyler SL, Weible GD (2006) Genetic variation in hemp and marijuana (*Cannabis sativa* L.) according to amplified fragment length polymorphisms. *J Forensic Sci* 51:371–375

- Debouck C, Goodfellow PN (1999) DNA microarrays in drug discovery and development. *Nat Genet* 21:48–50
- Dekkers JCM, Hospital F (2002) The use of molecular genetics in the improvement of agricultural populations. *Nat Rev Genet* 3(1):22–32
- Del Serrone P, Attorri L, Gallinella B, Gallo FR, Federici E, Palazzino G (2006) Molecular identification of *Panax ginseng* C. A. Meyer in ginseng commercial products. *Nat Prod Commun* 1:1137–1140
- Devaiah KM, Venkatasubramanian P (2008a) Development of SCAR marker for authentication of *Pueraria tuberosa* (Roxb. ex. Willd.) DC. *Curr Sci* 94:1306–1308
- Devaiah KM, Venkatasubramanian P (2008b) Genetic characterization and authentication of *Embelia ribes* using RAPD-PCR and SCAR marker. *Planta Med* 74:194–196
- di Bernardo D, Thompson MJ, Gardner TS, Chobot SE, Eastwood EL, Wojtovich AP et al (2005) Chemogenomic profiling on a genome-wide scale using reverse-engineered gene networks. *Nat Biotechnol* 23(3):377–383
- Diao Y, Lin XM, Liao CL, Tang CZ, Chen ZJ, Hu ZL (2009) Authentication of *Panax ginseng* from its adulterants by PCR-RFLP and ARMS. *Planta Med* 75:557–560
- Ding G, Zhang DZ et al (2008) SNP, ARMS and SSH authentication of medicinal *Dendrobium officinale* Kimura et Migo and application for identification of Fengdou drugs. *Biol Pharm Bull* 31:553–557
- Dnyaneshwar W, Preeti C, Kalpana J, Bhushan P (2006) Development and application of RAPDSCAR marker for identification of *Phyllanthus emblica* L. *Biol Pharm Bull* 29:2313–2316
- Do KR, Hwang WJ, Lyu YS, An NH, Kim HM (2001) Molecular authentication of *Panax ginseng* species by RAPD analysis and PCR-RFLP. *Biol Pharm Bull* 24:872–875
- Dyke FV (2008) Conservation biology: foundations, concepts, applications. Springer Science & Business Media, Berlin. ISBN 978-1-4020-6890-4
- Etoh T, Hong CJ (2001) RAPD markers for fertile garlic. *Acta Hort (ISHS)* 555:209–212
- Fan XX, Shen L, Zhang X, Chen XY, Fu CX (2004) Assessing genetic diversity of *Ginkgo biloba* L. (Ginkgoaceae) populations from China by RAPD markers. *Biochem Genet* 42:269–278
- Farnsworth NR, Morris RW (1976) Higher plants: the sleeping giant for drug development. *Am J Pharm* 148:46–52
- Farnsworth NR, Soejarto DD (1985) Potential consequence of plant extinction in the United States on the current and future availability of prescription drugs. *Econ Bot* 39(3):231–240
- Farooqui N, Ranade SA, Sane PV (1998) RAPD profile variation amongst provenances of neem. *Biochem Mol Biol Int* 45:931–939
- Fico G, Spada A, Bracab A, Agradic E, Morellib I, Tomea F (2003) RAPD analysis and flavonoid composition of *Aconitum* as an aid for taxonomic discrimination. *Biochem Syst Ecol* 31:293–301
- Fodor SP, Read JL, Pirrung MC, Stryer L, Lu AT, Solas D (1991) Light-directed, spatially addressable parallel chemical synthesis. *Science* 251:767–773
- Fodor SP, Rava RP, Huang XC, Pease AC, Holmes CP, Adams CL (1993) Multiplexed biochemical assays with biological chips. *Nature* 364(6437):555–556
- Friesen N, Blattner FR (1999) RAPD analysis reveals geographic differentiations within *Allium schoenoprasum* L. *Planta Med* 65:157–160
- Fu RZ, Wang J, Zhang YB, Wang ZT, But PP, Li N, Shaw PC (1999) Differentiation of medicinal *Codonopsis* species from adulterants by polymerase chain reaction-restriction fragment length polymorphism. *Planta Med* 65:648–650
- Gao MB, Zhang W, Li XT, Ruan CJ, Fan SD (2010) Expression profiling of genes involved in *Taxuyunnanine C* biosynthesis in cell suspension cultures of *Taxus chinensis* by repeated elicitation with a newly synthesized jasmonate, *in situ* absorption and sucrose feeding. *China Biotech* 30(8):31–36
- Gardner TS, di Bernardo D, Lorenz D, Collins JJ (2003) Inferring genetic networks and identifying compound mode of action via expression profiling. *Science* 301(5629):102–105

- Gaston KJ, Warren PH, Devine-Wright P, Irvine KN, Fuller RA (2007) Psychological benefits of greenspace increase with biodiversity. *Biol Lett* 3(4):390–394
- Gawel NJ, Jarret RL, Whittemore AP (1992) Restriction fragment length polymorphism (RFLP)-based phylogenetic analysis of *Musa*. *Theor Appl Genet* 84:286–290
- Ge XJ, Yu Y, Yuan YM, Huang HW, Cheng YAN (2005) Genetic diversity and geographic differentiation in endangered *Ammopiptanthus* (Leguminosae) populations in desert regions of Northwest China as revealed by ISSR analysis. *Ann Bot* 95:843–851
- Gershon D (2002) Microarray technology: an array of opportunities. *Nature* 416:885–891
- Goff SA, Ricke D, Lan TH, Presting G, Wang R, Dunn M et al (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). *Science* 296(5565):92–100
- Gong W, Fu CX, Luo YP, Qiu YX (2006) Molecular identification of *Sinopodophyllum hexandrum* and *Dysosma* species using cpDNA sequences and PCR-RFLP markers. *Planta Med* 72:650–652
- Grifo F, Newman D, Fairfield A, Bhattacharya B, Grupenhoff JT (1997) The origins of prescription drugs. In: Grifo F, Rosenthal J (eds) *Biodiversity and human health*. Island Press, Washington, DC, pp 131–163
- Guo Y, Tsuruga A, Yamaguchi S, Oba K, Iwai K, Sekita S et al (2006) Sequence analysis of chloroplast chlB gene of medicinal *Ephedra* species and its application to authentication of *Ephedra* herb. *Biol Pharm Bull* 29:1207–1211
- Gupta PK, Balyan HS, Sharma PC, Ramesh B (1996) Microsatellites in plants: a new class of molecular markers. *Curr Sci* 70:45–54
- Gupta PK, Roy JK, Prasad M (2001) Single nucleotide polymorphisms: a new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. *Curr Sci* 80:524–535
- Ha WY, Shaw PC, Liu J, Yau FC, Wang J (2002) Authentication of *Panax ginseng* and *Panax quinquefolius* using amplified fragment length polymorphism (AFLP) and directed amplification of minisatellite region DNA (DAMD). *J Agric Food Chem* 50:1871–1875
- Hao DC, Chen SL, Xia PG, Peng Y (2010) Authentication of medicinal plants by DNA-based markers and genomics. *Chin Herb Med* 2(4):250–261
- He J, Wong KL, Shaw PC, Wang H, Li DZ (2010) Identification of the medicinal plants in *Aconitum* L. by DNA barcoding technique. *Planta Med* 76:1622–1628
- Heath DD, Iwama GK, Devlin RH (1993) PCR primed with VNTR core sequences yields species specific patterns and hypervariable probes. *Nucleic Acids Res* 21:5782–5785
- Hebert PD, Gregory TR (2005) The promise of DNA barcoding for taxonomy. *Syst Biol* 54: 852–859
- Hebert PD, Cywinski A, Ball SL, de Waard JR (2003) Biological identifications through DNA barcodes. *Proc R Soc Biol Sci Ser B* 270:313–321
- Heffner EL, Sorrells ME, Jannink JL (2009) Genomic selection for crop improvement. *Crop Sci* 49:1–12
- Heubl G (2010) New aspects of DNA-based authentication of Chinese medicinal plants by molecular biological techniques. *Planta Med* 76(17):1963–1975
- Heubl G (2013) Chapter 2: DNA-based authentication of TCM-plants: current progress and future perspectives. In: Wagner H, Ulrich-Merzenich G (eds) *Evidence and rational based research on Chinese drugs*, Springer, Wien, pp 27–85
- Hosokawa K, Minami M, Kawahara K, Nakamura I, Shibata T (2000) Discrimination among three species of medicinal *Scutellaria* plants using RAPD markers. *Planta Med* 66:270–272
- Hu YM, Han XH, Zhou Q (2011) A study on the flavonoids production by *Ginkgo biloba* suspension cell culture. *Acta Agric Univ Jiangxiensis* 33(2):360–363
- Hu Y, Zhang Q, Xin H, Qin LP, Lu BR, Rahman K et al (2007) Association between chemical and genetic variation of *Vitex rotundifolia* populations from different locations in China: its implication for quality control of medicinal plants. *Biomed Chromatogr* 21:967–975
- Hunter ML (1996) *Fundamentals of conservation biology*. Blackwell Science. ISBN 978-0-86542-371-8

- Ince AG, Karaca AGM et al (2009) Development and utilization of diagnostic DAMD-PCR markers for Capsicum accessions. *Genet Res Crop Evol* 56:211–221
- Jack CMD (2012) Application of genomics tools to animal breeding. *Curr Genomics* 13(3): 207–212
- Jannink JL, Lorenz Aaron J, Iwata H (2010) Genomic selection in plant breeding: from theory to practice. *Brief Funct Genomics* 9(2):166–177
- Jayasinghe R, Kong S, Coram TE, Kaganovich J, Xue CC, Li CG et al (2007) Construction and validation of a prototype microarray for efficient and high-throughput genotyping of angiosperms. *Plant Biotechnol J* 5(2):282–289
- Joshi P, Dhawan V (2007) Assessment of genetic fidelity of micropropagated *Swertia chirayita* plantlets by ISSR marker assay. *Biol Plantarum* 51(1):22–26
- Kaplan J, Chavan P, Warude D, Patwardhan B (2004) Molecular markers in herbal drug technology. *Curr Sci* 87:159–165
- Karaca M, Ince AG, Ay ST, Turgut K, Onus AN (2008) PCR-RFLP and DAMD-PCR genotyping for *Salvia* species. *J Sci Food Agric* 88:2508–2516
- Kersten T, Daniel C, König GM, Knöß W (2007) The potential of PCR-related methods to identify medicinal plants in herbal medicinal products. *Planta Med* 73:256
- Khanuja SPS, Pau SI, Shasany AK, Gupta AK, Darokar MP et al (2007) High Artemisinin yielding plant genotype 'CIM-Arogya' US 20070089211 P1
- Kholodenko BN, Sauro HM (2005) Systems biology: definitions and perspectives. In: Alberghina L, Westerhoff HV (eds) *Topics in current genetics*, vol 13. Springer, Berlin, pp 357–451
- Kikowska M, Budzianowski J, Krawczyk A, Thiem B (2012) Accumulation of rosmarinic, chlorogenic and caffeic acids in *in vitro* cultures of *Eryngium planum* L. *Acta Physiol Plant* 34(6):2425–2433
- Kim J, Chung KW (2007) Isolation of new microsatellite-containing sequences in *Acanthopanax senticosus*. *J Plant Biol* 50:557–561
- Kim J, Jo BH, Lee KL, Yoon ES, Ryu GH, Chung KW (2007) Identification of new microsatellite markers in *Panax ginseng*. *Mol Cells* 24:60–68
- Kim JA, Kim YS, Choi YE (2011) Triterpenoid production and phenotypic changes in hairy roots of *Codonopsis lanceolata* and the plants regenerated from them. *Plant Biotech Rep* 5(3): 255–263
- Kitano H (2002) Systems biology: a brief overview. *Science* 295(5560):1662–1664
- Kohjyouma M, Nakajima S, Namura A, Shimizu R, Mizukami H, Kohda H (1997) Random amplified polymorphic DNA analysis and variation of essential oil components of *Atractylodes* plants. *Biol Pharm Bull* 20:502–506
- Kojoma M, Lida O, Makino Y, Sekita S, Satake M (2002) DNA fingerprinting of *Cannabis sativa* using inter-simple sequence repeat (ISSR) amplification. *Planta Med* 68:60–63
- Kumar P, Gupta VK, Misra AKL, Modi DR, Pandey BK (2009) Potential of molecular markers in plant biotechnology. *Plant Omics J* 2(4):141–162
- Kurane J, Shinde V, Harsulkar A (2009) Application of ISSR marker in pharmacognosy: current update. *Phcog Rev* 3(6):216–228
- Kwok PY (2001) Methods for genotyping single nucleotide polymorphisms. *Annu Rev Genom Human Genet* 2:235–258
- Lee MY, Doh EJ, Park CH, Kim YH, Kim ES, Ko BS et al (2006) Development of SCAR marker for discrimination of *Artemisia princeps* and *A. argyi* from other *Artemisia* herbs. *Biol Pharm Bull* 29:629–633
- Lee JH, Lee JW, Sung JS, Bang KH, Moon SG (2009) Molecular authentication of 21 Korean *Artemisia* species (Compositae) by polymerase chain reaction-restriction fragment length polymorphism based on *trnL-F* region of chloroplast DNA. *Biol Pharm Bull* 32(11): 1912–1916
- Li CF, Li XR, Wang F (2010) Arnebia hairy roots total sugar and polysaccharide content analysis. *Northwest Plant* 30(1):180–183

- Li T, Wang J, Lu Z (2005) Accurate identification of closely related *Dendrobium* species with multiple species-specific gDNA probes. *J Biochem Biophys Methods* 62:111–123
- Li X, Ding X, Chu B, Ding G, Gu S, Qian L et al (2007) Molecular authentication of *Alisma orientale* by PCR-RFLP and ARMS. *Planta Med* 73:67–70
- Li K, Wu W, Zheng Y, Dai Y, Xiang L, Liao K (2009) Genetic diversity of *Fritillaria* from Sichuan province based on ISSR. *Zhongguo Zhongyao Zazhi* 34:2149–2154
- Li DZ, Gao LM, Li HT, Wang H, Ge XJ et al (2011) Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *Proc Natl Acad Sci USA* 108:19641–19646
- Lim W, Mudge KW et al (2007) Utilization of RAPD markers to assess genetic diversity of wild populations of North American ginseng (*Panax quinquefolium*). *Planta Med* 73:71–76
- Lin WY, Chen LR, Lin TY (2008) Rapid authentication of *Bupleurum* species using an array of immobilized sequence-specific oligonucleotide probes. *Planta Med* 74:464–469
- Litt M, Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am J Hum Genet* 44:397–401
- Liu ZJ (2011) Next generation sequencing and whole genome selection in aquaculture. Wiley-Blackwell
- Loh JP, Kiew R, Kee A, Gan LH, Gan YY (1999) Amplified fragment length polymorphism (AFLP) provides molecular markers for the identification of *Caladium bicolor* cultivars. *Ann Bot (Lond)* 84:155–161
- Lu KT, Lee HC, Liu FS et al (2009) Discriminating astragalus radix from hedysarum radix in Chinese medicine preparations using nested PCR and DNA sequencing methods. *J Food Drug Anal* 17:380–385
- Lu KT, Lee HC, Liu FS et al (2010) Identification of Ginseng Radix in Chinese medicine preparations by nested PCR-DNA sequencing method and nested PCR-restriction fragment length polymorphism. *J Food Drug Anal* 18:58–63
- Lum MR, Potter E, Dang T, Heber D, Hardy M, Hirsch AM (2005) Identification of botanicals and potential contaminants through RFLP and sequencing. *Planta Med* 71:841–846
- Luo YM, Zhang WM, Ding XY, Shen J, Bao SL, Chu BH et al (2006) SNP marker and allele-specific diagnostic PCR for authenticating herbs of *Perilla*. *Acta Pharm Sin* 41:840–845
- Ma ZQ, Sorrells ME, Tanksley SD (1994) RFLP markers linked to powdery mildew resistance genes Pm1, Pm2, Pm3 and Pm4 in wheat. *Genome* 37:871–875
- Mardis ER (2008) Next-generation DNA sequencing methods. *Annu Rev Genom Human Genet* 9:387–402
- Maeda M, Uryu N, Murayama N, Ishii H, Ota M, Tsuji K, Inoko H (1990) A simple and rapid method for HLA-DP genotyping by digestion of PCR-amplified DNA with allele specific endonucleases. *Hum Immunol* 27:111–121
- Mazzanti G, Battinelli L, Daniele C, Costantini S, Ciaralli L, Evandri MG (2008) Purity control of some Chinese crude herbal drugs marketed in Italy. *Food Chem Toxicol* 46:3043–3047
- McDermott JM, Brandle U, Dutly F, Haemmerli UA, Keller S, Muller KE, Wolf MS (1994) Genetic variation in powdery mildew of barley: development of RAPD, SCAR and VNTR markers. *Phytopathology* 4:1316–1321
- Mendelsohn R, Balick MJ (1995) The value of undiscovered pharmaceuticals in tropical forests. *Econ Bot* 49(2):223–228
- Metzker ML (2010) Sequencing technologies—the next generation. *Nat Rev Gen* 11(1):31–46
- Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157(4):1819–1829
- Mizukami H, Ohbayashi K, Kitamura Y, Ikenaga T (1993a) Restriction fragment length polymorphisms (RFLPs) of medicinal plants and crude drugs. I. RFLP probes allow clear identification of *Duboisia* interspecific hybrid genotypes in both fresh and dried tissues. *Biol Pharm Bull* 16:388–390
- Mizukami H, Ohbayashi K, Umetsu K, Hiraoka N (1993b) Restriction fragment length polymorphism of medicinal plants and crude drugs. II. Analysis of *Glehnia littoralis* of different geographical origin. *Biol Pharm Bull* 16:611–661

- Mizukami H, Okabe Y, Kohda H, Hiraoka N (2000) Identification of the crude drug Atractylodes rhizome (Byaku-jutsu) and Atractylodes lancea rhizome (So-jutsu) using chloroplast *trnK* sequence as a molecular marker. *Biol Pharm Bull* 23:589–594
- Molina C, Kahl G (2002) Genomics of two banana pathogens, genetic diversity, diagnostics, and phylogeny of *Mycosphaerella fijiensis* and *M. musicola*. In: Jain SM (ed) Banana improvement, cellular and molecular biology, and induced mutations. FAO/IAEA, Vienna
- Morgante M, Vogel J (1994) Compound microsatellite primers for the detection of genetic polymorphisms. US Patent Application 08/326456
- Mori N, Moriguchi T, Nakamura C (1997) RFLP analysis of nuclear DNA for study of phylogeny and domestication of tetraploid wheat. *Genes Genet Syst* 72:153–161
- Moses T, Pollier J, Almagro L (2014) Combinatorial biosynthesis of saponins and saponins in *Saccharomyces cerevisiae* using a C-16 α-hydroxylase from *Bupleurum falcatum*. *PNAS* 111(4):1634–1639
- Mueller UG, Wolfenbarger L (1999) AFLP genotyping and fingerprinting. *Trends Ecol Evol* 14:389–394
- Munthali M, Ford-Lloyd BV, Newbury HJ (1992) The random amplification of polymorphic DNA for fingerprinting plants. *PCR Methods Appl* 1:274–276
- Nagamine K, Watanabe K, Ohtsuka K, Hase T, Notomi T (2001) Loop-mediated isothermal amplification reaction using a nondenatured template. *Clin Chem* 47:1742–1743
- Nagamine K, Hase T, Notomi T (2002) Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Mol Cell Probes* 16:223–229
- Newton CR, Graham A, Heptinstall LE, Powell SJ, Summers C, Kalsheker N et al (1989) Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Res* 17:2503–2516
- Ng KY, Cheng CI, Xu HX (2009) Safety issues of Chinese medicine: a review of intoxication cases in Hong Kong. *Chin Herb Med* 1(1):29–39
- Ngan F, Shaw P, But P, Wang J (1999) Molecular authentication of Panax species. *Phytochemistry* 50:787–791
- Nicholson DE (1971) An introduction to metabolic pathways by S. Dagley, vol. 59, 2nd edn. Sigma Xi, The Scientific Research Society. p 266
- Niu L, Mantri N, Li CG, Xue C, Wohlmuth H, Pang EC (2011) Detection of *Panax quinquefolius* in *Panax ginseng* using subtracted diversity array. *J Sci Food Agric* 91:1310–1315
- Noble D (2006) The music of life: biology beyond the genome. Oxford University Press, Oxford, p 176
- Normile D (2003) The new face of traditional Chinese medicine. *Science* 299:188–190
- Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T (2000) Loopmediated isothermal amplification of DNA. *Nucleic Acids Res* 28(12):E63
- Oleszek W, Stochmal A, Karolewski P, Simonet AM, Macias FA, Tava A (2002) Flavonoids from *Pinus sylvestris* needles and their variation in trees of different origin grown for nearly a century at the same area. *Biochem Syst Ecol* 30:1011–1022
- Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T (1989) Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphism. *Proc Natl Acad Sci USA* 86:2766–2770
- Paddon CJ, Westfall PJ, Pitera DJ (2013) High-level semi-synthetic production of the potent antimalarial artemisinin. *Nat* 496:528–532
- Padmesh P, Sabu KK, Seenii S, Pushpangadan P (1999) The use of RAPD in assessing genetic variability in *Andrographis paniculata* Nees, a hepatoprotective drug. *Curr Sci* 76:833–835
- Paglia GE, Olivieri AM, Morgante M (1998) Towards second-generation STS (sequence-tagged sites) linkage maps in conifers, a genetic map of Norway spruce (*Picea abies* K.). *Mol Gen Genet* 258:466–478
- Parani M, Anand A, Parida A (1997) Application of RAPD fingerprinting in selection of microp propagated plants of *Piper longum* for conservation. *Curr Sci* 73(1):81–83

- Passinho-Soares H, Felix D, Kaplan MA, Margis-Pinheiro M, Margis R (2006) Authentication of medicinal plant botanical identity by amplified fragmented length polymorphism dominant DNA marker: inferences from the *Plectranthus* genus. *Planta Med* 72:929–931
- Pereira F, Carneiro J, Amorim A (2008) Identification of species with DNA-based technology: current progress and challenges. *Recent Pat DNA Gene Seq* 2:187–200
- Portis E, Acquadro A, Comino C, Lanteri S (2004) Analysis of DNA methylation during germination of pepper (*Capsicum annuum* L.) seeds using methylation-sensitive amplification polymorphism (MSAP). *Plant Sci* 166:169–178
- Pratt DV, Judith GV, Charlotte W (2013) Fundamentals of biochemistry: life at the molecular level, 4th edn. Wiley, Hoboken, NJ, pp 441–442
- Primrose SB, Twyman RM (2003) Principles of genome analysis and genomics. Blackwell publishing, USA, p 7
- Qi J, Li X, Song J, Eneji AE, Ma X (2008) Genetic relationships among *Rehmannia glutinosa* cultivars and varieties. *Planta Med* 74:1846–1852
- Qian L, Ding G et al (2008) Molecular authentication of *Dendrobium loddigesii* Rolfe by amplification refractory mutation system (ARMS). *Planta Med* 74:470–473
- Qiao XL, Jiang SG, Lv XG, Li FX, Zhao DX (2011) Effects of phytohormones on plant regeneration and production of flavonoids in transgenic *Saussurea involucrata* hairy roots. *Biotechnology* 27(1):69–75
- Ratnaparkhe MB, Gupta VS, Ven Murthy MR, Ranjekar PK (1995) Genetic fingerprinting of pigeonpea (*Cajanus cajan* (L.) Millsp) and its wild relatives using RAPD markers. *Theor Appl Genet* 91:893–898
- Reyna-Lopez GE, Simpson J, Ruiz-Herrera J (1997) Differences in DNA methylation patterns are detectable during the dimorphic transition of fungi by amplification of restriction polymorphism. *Mol Gen Genet* 253:703–710
- Ribaut JM, de Vicente MC, Delannay X (2010) Molecular breeding in developing countries: challenges and perspectives. *Curr Opin Plant Biol* 13:1–6
- Sarwat M, Das S, Srivastava PS (2008) Analysis of genetic diversity through AFLP, SAMPL, ISSR and RAPD markers in *Tribulus terrestris*, a medicinal herb. *Plant Cell Rep* 27(3):519–528
- Sasaki Y, Nagumo S (2007) Rapid identification of *Curcuma longa* and *C. aromatica* by LAMP. *Biol Pharm Bull* 30:2229–2230
- Sasaki Y, Fushimi H et al (2002) Sequence analysis of Chinese and Japanese Curcuma drugs on the 18S rRNA gene and *trnK* gene and the application of amplification-refractory mutation system analysis for their authentication. *Biol Pharm Bull* 25:1593–1599
- Sasaki Y, Komatsu K, Nagumo S (2008) Rapid detection of *Panax ginseng* by loop-mediated isothermal amplification and its application to authentication of Ginseng. *Biol Pharm Bull* 31(9):1806–1808
- Sasaki Y, Fujimoto T, Aragane M, Yasuda I, Nagumo S (2009) Rapid and sensitive detection of *Lophophora williamsii* by loop-mediated isothermal amplification. *Biol Pharm Bull* 32(5): 887–891
- Schena M, Heller RA, Theriault TP, Konrad K, Lachenmeier E, Davis RW (1998) Microarrays: biotechnology's discovery platform for functional genomics. *Trends Biotechnol* 16(7):301–306
- Schouten JP, McElgunn CJ, Waaijer R, Zwijsenborg D, Diepvens F, Pals G (2002) Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res* 30(12):e57
- Seamagn K, Bjornstad A, Ndjiondjop MN (2006) An overview of molecular marker methods for plants. *Afr J Biotechnol* 5:2540–2568
- Shasany AK, Kukreja AK, Saikia D, Darokar MP, Khanuja SPS, Kumar S (1999) Drug discovery and development: traditional medicine and ethnopharmacology. *PGR Newslet* 121:27–31
- Shasany AK, Aruna V, Darokar MP, Kalra A, Bahl JR (2002) Bansal RP Khanuja SPS (2002) RAPD marking of three *Pelargonium graveolens* genotypes with chemotypic differences in oil quality. *J. Med. Aromat. Plant Sci.* 24:729–732
- Shaw PC, But PP (1995) Authentication of *Panax* species and their adulterants by random-primed polymerase chain reaction. *Planta Med* 61(5):466–469

- Shaw PC, Ngan FN, But PPH, Wang J (2002) Molecular markers in Chinese medicinal materials. In: Shaw PC, But PPH (eds) Authentication of Chinese medicinal material by DNA technology. World Scientific Publishing, Singapore
- Shaw PC, Wong KKL, Chan AWK, Wong WC, But PPH (2009) Patent applications for using DNA technologies to authenticate medicinal herbal material. *J Chin Med* 4:1–11
- Shen Y, Wu BL (2009) Designing a simple multiplex ligation-dependent probe amplification (MLPA) assay for rapid detection of copy number variants in the genome. *J Genet Genomics* 36(4):257–265
- Shen J, Ding XY, Ding G, Liu DY, Tang F, He J (2006) Studies on population difference of *Dendrobium officinale* II establishment and optimization of the method of ISSR fingerprinting marker. *Zhongguo Zhongyao Zazhi* 31:291–294
- Shendure J, Ji H (2008) Next-generation DNA sequencing. *Nat biotech* 26(10):1135–1144
- Shi HM, Wang J, Wang MY, Tu PF, Li XB (2009) Identification of *Cistanche* species by chemical and inter-simple sequence repeat fingerprinting. *Biol Pharm Bull* 32:142–146
- Siow YL, Gong Y, Au-Yeung KK, Woo CW, Choy PC (2005) Emerging issues in traditional Chinese medicine. *Can J Physiol Pharmacol* 83:321–334
- Snoep JL, Westerhoff HV (2005) Systems biology: definitions and perspectives. In: Alberghina L, Westerhoff HV (eds) Topics in current genetics, vol 13. Springer, Berlin, pp 13–30
- Soejarto DD (1996) Biodiversity prospecting and benefit-sharing: perspectives from the field. *J Ethnopharmacol* 51:1–15
- Somers DJ, Demmon G (2002) Identification of repetitive, genome-specific probes in crucifer oilseed species. *Genome* 45:485–492
- Song Z, Li X, Wang H, Wang J (2010) Genetic diversity and population structure of *Salvia miltiorrhiza* Bge in China revealed by ISSR and SRAP. *Genetica* 138:241–249
- Soumaya R, Dakhlaoui-Dkhil S, Salem AOM, Zehdi-Azouzi S, Rhouma A, Marrakchi M et al (2008) Genetic diversity and phylogenetic relationships in date-palms (*Phoenix dactylifera* L.) as assessed by random amplified microsatellite polymorphism markers (RAMPOs). *Sci Hortic* 117:53–57
- Steck N, Messmer M, Schaffner W, Bueter KB (2001) Molecular marker as a tool to verify sexual and apomictic off-spring of intraspecific crosses in *Hypericum perforatum*. *Plant Biol* 622–628
- Stephen PM, Rita HM (2008) Molecular plant breeding as the foundation for 21st century crop improvement. *Plant Physiol* 147:969–977
- Sucher JN, Carles MC (2008) Genome-based approaches to the authentication of medicinal plants. *Planta Med* 74:603–623
- Sun JW, Zhang H, Wang FY, Sun YM, Sun M (2013) Affect of Methyl jasmonate on datura main tropane alkaloids accumulation and release of hairy roots. *China J Chin Mater Med* 38(11): 1712–1718
- Sze SCW, Song JX, Wong RNS, Feng YB, Ng TB, Tong Y et al (2008) Application of SCAR (sequence characterized amplified region) analysis to authenticate *Lycium barbarum* (wolfberry) and its adulterants. *Biotech Appl Biochem* 51:15–21
- Tao JH, Pu XL, Jiang S (2011) Effect of endophytic fungal elicitors on growth and atracylodin accumulation of cell suspension cultures of *Atractylodes lancea*. *China J Chin Mater Med* 36(1):27–33
- Tomas S, Marie K, Jirina S (2012) Effects of zinc and cadmium ions on cell growth and production of coumarins in cell suspension cultures of *Angelica archangelica*L. *Ceska a Slovenska Farmacie* 61(6):261–266
- Tavassoly I (2015) Dynamics of cell fate decision mediated by the interplay of autophagy and apoptosis in cancer cells. Springer International Publishing. ISBN 978-3-319-14961-5
- Techaprasan J, Klinbunga S, Jenjittiku T (2008) Genetic relationships and species authentication of Boesenbergia (Zingiberaceae) in Thailand based on AFLP and SSCP analyses. *Biochem Syst Ecol* 36(5–6):408–441
- Tharachand C, Immanuel SC, Mythili MN (2012) Molecular markers in characterization of medicinal plants: An overview. *Res Plant Biol* 2(2):01–12

- Theerakulpisut P, Kanawapee N, Maensiri D, Bunnag S, Chantaranothai P (2008) Development of species-specific SCAR markers for identification of three medicinal species of phyllanthus. *J Syst Evol* 46:614–621
- Trau D, Lee TM, Lao AI, Lenigk R, Hsing IM, Ip NY et al (2002) Genotyping on a complementary metal oxide semiconductor silicon polymerase chain reaction chip with integrated DNA microarray. *Anal Chem* 74:3168–3173
- Trifi-Farah N, Marrakchi M (2001) Hedysarum phylogeny mediated by RFLP analysis of nuclear ribosomal DNA. *Gen Res Crop Evol* 48:339–345
- Tsoi PY, Wu HS, Wong MS, Chen SL, Fong WF, Xiao PG et al (2003) Genotyping and species identification of Fritillaria by DNA chip technology. *Acta Pharm Sin* 4:185–190
- Tusa N, Abbet L, Ferrante S, Lucreti S, Scarano MT (2002) Identification of zygotic and nucellar seedlings in citrus interploid crosses by means of isozymes, flow cytometry and ISSR-PCR. *Cell Mol Biol Lett* 7(2B):703–708
- Um JY, Chung HS, Kim MS, Na HJ, Kwon HJ, Kim JJ et al (2001) Molecular authentication of *Panax ginseng* species by RAPD analysis and PCR-RFLP. *Biol Pharm Bull* 24:872–875
- Verma P, Khan SA, Mathur AK, Shanker K, Kalra A (2014) Fungal endophytes enhanced the growth and production kinetics of Vinca minor hairy roots and cell suspensions grown in bioreactor. *Plant Cell Tiss Org* 118(2):257–268
- Vijayan K, Tsou CH (2010) DNA barcoding in plants: taxonomy in a new perspective. *Curr Sci* 99(11):1513–1541
- Wagner H, Bauer R, Melchart D, Xiao PG, Staudinger A (2011) Chromatographic fingerprint analysis of herbal medicines: thin-layer and high performance liquid chromatography of Chinese drugs, vols I and II, 2nd edn. Springer, Wien
- Wang J, Ha WY, Ngan FN, But PP, Shaw PC (2001) Application of sequence characterized amplified region (SCAR) analysis to authenticate Panax species and their adulterants. *Planta Med* 67:781–783
- Wang CZ, Li P, Ding JY, Jin GQ, Yuan CS (2005a) Identification of *Fritillaria pallidiflora* using diagnostic PCR and PCR-RFLP based on nuclear ribosomal DNA internal transcribed spacer sequences. *Planta Med* 71:384–386
- Wang J, Li JL, Jing Li J, Li JX, Liu SJ, Huang LQ et al (2017) Production of active compounds in medicinal plants: from plant tissue culture to biosynthesis. *Chinese Herbal Medicines* 9(2):115–125
- Wang M, Robert JA, Henrie JK, Joop HJ, Van N, Renger FW et al (2005b) Metabolomics in the context of systems biology: bridging traditional Chinese medicine and molecular pharmacology. *Phytother Res* 19:173–182
- Wang MW, Hao XJ, Chen KX (2007) Biological screening of natural products and drug innovation in China. *Philos Trans R Soc Lond B Biol Sci* 362(1482):1093–1105
- Wang HZ, Wu ZX, Lu JJ et al (2009) Molecular diversity and relationships among *Cymbidium goeringii* cultivars based on inter-simple sequence repeat (ISSR) markers. *Genetica* 136(3): 391–399
- Wang QJ, Zheng LP, Sima YH, Yuan HY, Wang JW (2013) Methyl jasmonate stimulates 20-hydroxyecdysone production in cell suspension cultures of *Achyranthes bidentata*. *Plant Omics* 6(2):116–120
- Watanabe A, Araki S, Kobari S, Sudo H, Tsuchida T, Uno T et al (1998) In vitro propagation, restriction fragment length polymorphism, and random amplified polymorphic DNA analyses of Angelica plants. *Plant Cell Rep* 18:187–192
- Weising K, Kahl G (1998) Hybridization-based microsatellite fingerprinting of plants. In: Caetano-Anolles G, Gresshoff PM (eds) *DNA markers: protocols, applications, and overviews*. Wiley, New York, pp 238–243
- Weising K, Bybom H, Wolff K, Kahl G (2005) DNA fingerprinting in plants. Principles, methods, and application, 2nd edn. Taylor and Francis, Boca Raton, London, NY, Singapore
- Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res* 18:7213–7218

- Winter E, Pfaff T, Udupa SM, Hiittel B, Sharma PC, Sahi S et al (1999) Characterization and mapping of sequence-tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. Mol Gen Genet 262:90–101
- Witsenboer H, Vogel J, Michelmore RW (1997) Identification, genetic localization, and allelic diversity of selectively amplified polymorphic loci in lettuce and wild relatives (*Lactuca* spp.). Genome 40:923–936
- Wu W, Zheng YL, Chen L, Wei YM, Yang RW, Yan ZH (2005) Evaluation of genetic relationships in the genus *Houttuynia* Thunb. In China based on RAPD and ISSR markers. Biochem Syst Ecol 33:1141–1157
- Xia T, Chen S, Chen S, Zhang D, Zhang D, Gao Q et al (2007) ISSR analysis of genetic diversity of the Qinghai-Tibet plateau endemic *Rhodiola chrysanthemifolia* (Crassulaceae). Biochem Syst Ecol 35:209–214
- Xiong LZ, Xu CG, Saghai-Marof MA, Zhang QF (1999) Pattern of cytosine methylation in an elite rice hybrid and its potential lines, detected by a methylation-sensitive amplification polymorphism technique. Mol Gen Genet 261:439–446
- Xu Y (2010) Molecular plant breeding. CAB International, Wallingford, UK, Cambridge, MA
- Xu M, Li X, Korban SS (2000) AFLP-based detection of DNA methylation. Plant Mol Biol Rep 18:361–368
- Xu H, Fabricant DS, Piersen CE, Bolton JL, Pezzuto JM, Fong HH et al (2002) A preliminary RAPD-PCR analysis of *Cimicifuga* species and other botanicals used for women's health. Phytomedicine 9:757–762
- Yamasaki M, Sato A, Shimomura K, Saito K, Murakoshi I (1994) Genetic relationships among *Glycyrrhiza* plants determined by RAPD and RFLP analyses. Biol Pharm Bull 17(11):1529–1531
- Yamasaki M, Sato A, Saito K, Murakoshi I (1993) Molecular phylogeny based on RFLP and its relation with alkaloid patterns in *Lupinus* plants. Biol Pharm Bull 16:1182–1184
- Yang DY, Hirotoshi F, Shao-Qing C, Katsuko K (2004) Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and amplification refractory mutation system (ARMS) analyses of medicinally used rheum species and their application for identification of *Rhei* rhizoma. Biol Pharm Bull 27:661–669
- Yang CQ, Fang X, Wu XM et al (2012) Transcriptional regulation of plant secondary metabolism. J Integr Plant Biol 54:703–712
- Yang R, Wang LQ, Liu Y (2014) Research progress on tissue culture of *Glycyrrhizae Radix et Rhizoma*. Chin Tradit Herb Drugs 45(12):1796–1802
- Yao H, Zhao Z, Chen DF, Chen JK, Zhou TS (2008) ISSR primer screening and preliminary evaluation of genetic diversity in wild populations of *Glycyrrhiza uralensis*. Biol Plantarum 52(1):117–120
- Ye Q, Qiu YX, Quo YQ, Chen JX, Yang SZ, Zhao MS et al (2006) Species-specific SCAR markers for authentication of *Sinocalycanthus chinensis*. J Zhejiang Univ Sci B 7:868–872
- Yip PY, Chau CF, Mak CY, Kwan HS (2007) DNA methods for identification of Chinese medicinal materials. J Chin Med 2:1–19
- Yu ZH, Mackill DJ, Bonman JM, Tanksley SD (1991) Tagging genes for blast resistance in rice via linkage to RFLP markers. Theor Appl Genet 81:471–476
- Yu J, Hu S, Wang J, Wong GK, Li S, Liu B et al (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). Science 296:79–92
- Zabeau M, Vos P (1993) Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application number 92402629.7. Publication number 0534858A1
- Zerega NJC, Mori S, Lindqvist C, Zheng Q, Motley TJ (2002) Using amplified fragment length polymorphisms (AFLP) to identify black cohosh (*Actaea racemosa*). Econ Bot 56:154–164
- Zhang KY, Leung HW, Yeung HW, Wong RN (2001) Differentiation of *Lycium barbarum* from its related *Lycium* species using random amplified polymorphic DNA. Planta Med 67(4): 379–381

- Zhang HC, Liu JM, Chen HM, Gao CC, Lu HY, Zhou H, Li Y et al (2011) Up-regulation of licochalcone A biosynthesis and secretion by tween 80 in hairy root cultures of *Glycyrrhizana uralensis* Fisch. Mol Biol 47(1):50–56
- Zhang N, Sun GL, Dai JG, Yang YF, Liu HW, Qiu DY (2013) Sequencing and analysis of the transcriptome of *Ginkgo biloba* L. cells. China Biotech 33(5):112–119
- Zhang YB, Wang J, Wang ZT, But PP, Shaw PC (2003) DNA microarray for identification of the herb of *Dendrobium* species from Chinese medicinal formulations. Planta Med 69:1172–1174
- Zhang M, Zhang DZ, Xu XH, Zhang T, Wang ZT (2005) 5S rRNA gene spacer sequences from *Ligularia* medicinal plants and the identification of HPAs-containing species. Chin J Nat Med 3:38–40
- Zhang YB, Shaw PC, Sze CW, Wang ZT, Tong Y (2007) Molecular authentication of Chinese herbal materials. J Food Drug Anal 15:1–9
- Zhao S, Chen X, Song J, Pang, X, Chen S (2015) Internal transcribed spacer 2 barcode: a good tool for identifying *Acanthopanax* cortex. Front Plant Sci 6:840
- Zhao ZL, Leng CH et al (2007) Identification of *Dryopteris crassirhizoma* and the adulterant species based on cpDNA rbcL and translated amino acid sequences. Planta Med 73:1230–1233
- Zhou Z, Bebeli PJ, Somers DJ, Gustafson JP (1997) Direct amplification of minisatellite-region DNA with VNTR core sequences in the genus *Oryza*. Theor Appl Genet 95:942–949
- Zhou J, Wang W, Liu M, Liu Z (2014) Molecular authentication of the traditional medicinal plant *Peucedanum praeruptorum* and its substitutes and adulterants by DNA—barcoding technique. Pharmacogn Mag. 10(40):385–390
- Zhu S, Fushimi H, Cai S, Komatsu K (2004) Species identification from Ginseng drugs by multiplex amplification refractory mutation system (MARMS). Planta Med 70:189–192
- Zhu S, Hirotoshi Fushimi H, Komatsu K (2008) Development of a DNA microarray for authentication of ginseng drugs based on 18S rRNA gene sequence. J Agric Food Chem 56(11):3953–3959
- Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20(2):176–183

Chapter 9

Methods of Qualitative and Quantitative Analysis of Plant Constituents



Abstract Plant kingdom harbors an inexhaustible source of active drug ingredients. Phytochemical techniques play a significant role in searching raw materials and resources for pharmaceutical industry. Drug discovery is a lengthy procedure and it involves a number of successive processes including (i) extraction, (ii) separation, (iii) isolation of the constituents of interest, (iv) purification, (v) characterization, and (vi) identification of the isolated compounds and also their quantitative estimation. Both dried and fresh plant materials may be used for extraction following different procedures with (water, ether, acetone, methanol, ethanol, chloroform, etc.) or without (expression, sublimation, and distillation) the use of solvents. Extraction with water may be without (infusion) or with boiling (decocction) and extraction with organic solvents involves maceration, percolation, soxhlet extraction, etc. Phytochemical screening of crude extract can be performed with the appropriate tests for different active ingredients, e.g., alkaloids (Dragendorff, Mayer, Hager, and Wagner's spot test), tannins (Ferric chloride test), Anthraquinone (Bornträger's test), flavonoids (Shinoda test-HCL test and Lead acetate test), glycosides (Fehling's test and Glacial acetic acid test), Cardiac glycosides (Kellar–Kiliani test), terpenoids and steroids (H_2SO_4 test), saponins (Foam test), fixed oil (Spot test), Amino acids and proteins (Ninhydrin test and copper sulfate test) and terpenes (Lieberman–Burchard), steroid (Liebermann–Burchard test), phenol (phenol test), and tannins (Braemer's test), etc. High-Throughput Screening (HTS) is a recent approach to accelerated drug discovery (e.g., screening a few thousand compounds per day or per week) and consists of several steps such as target identification, reagent preparation, compound management, assay development and high-throughput library screening including combinatorial chemistry, genomics, protein, and peptide libraries. The HTS method is more frequently utilized in conjunction with analytical techniques such as NMR or coupled methods, e.g., LC-MS/MS. The extracted chemical constituents are separated by various separation techniques such as fractional distillation, fractional liberation, fractional crystallization, chromatography, HPLC, etc. Isolation is a crucial step in the analysis of medicinal plants and the basic operation included steps, such as prewashing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of analytic extraction and also increasing the

contact of sample surface with the solvent system. Phytochemical characterization primarily may be initiated with the help of qualitative tests for the screening of phytochemical compounds. Characterization and identification of the separated and isolated constituents are the final steps in the photochemical analysis of plants. A pure compound is characterized and identified by determining its various physical and chemical properties like R_f value, melting point, optical values, nature and type of crystals, types and number of elements and functional groups present in the molecule, etc., by the use of different chemical tests and reactions, chromatographic techniques, crystallographic and spectroscopic methods, etc. The pure compounds are further used for the determination of structure and biological activity. In addition, various non-chromatographic techniques (immunoassay—MAbs, phytochemical screening assay, and FTIR) can also be used to facilitate the identification of the bioactive compounds. Bioassay (brine shrimp toxicity assay, crown gall tumor inhibition assay, potato disc antitumor assay-PDA, animal toxicity assay, antiviral, antimicrobial and antifungal assays, antimitotic assay, etc.) is a life-based activity-directed isolation process and its goal is to isolate bioactive compounds with certain definite degree of LD/LC/IC₅₀ value as a proof of cytotoxicity. Polymerase chain reaction (PCR)-based DNA technology of molecular biology now appears to be the basic analytical procedure in molecular pharmacognosy.

Modern medicine has evolved from folk and traditional systems of medicine only after thorough chemical and pharmaceutical screening. Although the use of synthetic compounds led to a decline in the use of plants in modern medicine, the side effects associated with synthetic medicines made their use unfavorable, and thus plants remain the favorable choice a major source of medicinal compounds. Plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Seventy-four percent of 119 plant-derived drugs were discovered as a result of chemical studies to isolate the active substances responsible for their traditional use (Farnsworth and Soejarto 1991). Phytochemical techniques played a significant role in searching raw materials and resources for pharmaceutical industry. Preliminary phytochemical tests are helpful in finding and locating chemical constituents which are source of pharmacologically active principles. Harborne (1984), in a treatise on phytochemical methods, described several techniques of plant analysis.

A wide range of technologies are available for the extraction of active components and essential oils from medicinal and aromatic plants. The choice depends on the economic feasibility and suitability of the process to the particular situation. Phytochemical analysis of drug principles is of primary importance in order to extract them in the pure form for use in pharmaceutical and medicinal preparations and it involves a number of successive processes such as (a) extraction, (b) separation, (c) isolation of the constituents of interest, (d) purification, (e) characterization, and (f) identification of the isolated compounds and also their quantitative estimation.

9.1 Extraction of Plant Constituents

Extraction is the first step in the analysis of plant constituents. It is a process of removing or taking out or separating the chemical constituents from the plant tissues with or without the use of solvents. Various methods of extraction are available for this process, which are described in the following pages.

Methods

A number of extraction methods are available for extracting plant constituents. The particular method to be used depends on the nature of the constituents, type of the plant material and the purpose of the extraction. Both dried and fresh plant materials may be used for extraction. The dried material must be powdered before extraction while the fresh samples may be directly extracted or homogenized before extraction. Extraction of plant constituents may be done with or without the use of a solvent.

(a) Methods of extraction without solvents

(i) Expression: In this method, the constituents are extracted or squeezed out from the plant material due to crushing and expressing effects of the heavy pressure applied. Plant constituents like fixed oils and fats are extracted by this method. Here, the fresh or dry material is subjected to hydraulic pressure with the application of heat (hot expression) or without it (cold expression). Hot expression method is applied to thermostable fatty constituents. On the completion of this extraction procedure, the residual plant material left is called cake, e.g., mustard, sesame, sunflower, and coconut oils and cakes are produced from dry seeds of mustard (*Brassica nigra*), sesame (*Sesamum indicum*), sunflower (*Helianthus annuus*), and dry kernel of coconut (*Cocos nucifera*), respectively. Most vegetable oils are extracted by this method.

(ii) Sublimation (phase transition from solid to gas form without liquid phase): This method is applicable to the extraction of sublimable constituents from plant materials, such as the extraction of caffeine from tea leaves. In this process, the powdered plant material is heated in a wide-mouthed container covered with a glass sheet. The sublimable constituent is first vaporized, and then condensed on the lower surface of the cover.

(iii) Distillation: Distillation is also a method of hot extraction, specifically used for the extraction of volatile oils and other volatile plant constituents. In extracting volatile oils, the plant material is placed in a distillation flask (with sufficient water in case of dried materials), which is connected to a receiver through a condenser. The material is heated directly or steam, generated in a connecting flask, is passed through it. The volatile oil co-distils with the steam, condenses in the condenser and is collected in the receiver along with water. The immiscible oil is then separated from the water and dried by anhydrous sodium sulfate.

(b) Methods of extraction with aqueous or organic solvents

With solvents, successful determination of bioactive compounds from herbal sources is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent extractant includes low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, etc. In addition, the choice of solvent depends on the targeted compounds to be extracted and is influenced by what is intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be nontoxic and should not interfere with the bioassay. The various solvents that are used in the extraction procedures are water, ether, acetone, methanol, ethanol, chloroform, etc., and these are used to extract different bioactive compounds (Table 9.1).

Extraction with aqueous solvents: In this method, fresh (intact, chopped or homogenized) or dried (intact, crushed or powdered) materials are extracted in a solvent such as water by allowing the material to remain suspended in the solvent over time without (infusion) or with boiling (decoction). The resultant extract, infusion or decoction is then filtered through a coarse cotton cloth or cotton or glass wool or filter paper. The process of infusion is distinct from decoction, which involves boiling the plant material, or percolation, in which the water passes through the material (as in a coffeemaker).

Extraction with organic solvents (alcohol, chloroform, petroleum ether, etc.)

(i) Maceration: In this method, the plant material is soaked, usually in a less volatile organic solvent, over a period of time with constant or occasional stirring or agitation. The supernatant liquid (extract) is then either decanted or filtered through a plug of cotton or glass wool. The process is repeated for complete extraction.

Table 9.1 Solvents used for different bioactive component extraction

Water	Ethanol	Methanol	Chloroform	Pet. ether	Acetone
Anthocyanins	Tannins	Anthocyanins,	Terpenoids	Alkaloids	Phenol
Starches	Polyphenols	Terpenoids,	Flavonoids	Terpenoids	Flavonols
Tannins	Polyacetylenes	Saponins,		Coumarins	
Saponins	Flavonol	Tannins,		Fatty acids	
Terpenoids	Sterols	Polyphenols			
Lectins	Alkaloids	Xathoxylenes, Totarol (diterpene), Quassinoids, Lactones, flavones, Phenones (aromatic ketone with a phenyl group)			

(ii) Percolation: Extraction by this method involves the use of a percolator, a glass tube with a fitted tap, in which the powdered plant material is packed, sometimes moistened with water to facilitate extraction. The extracting solvent, usually an organic solvent, is then poured on the material. The solvent slowly percolates through the plant material and passes out through the opened tap and is collected in a receiver placed below it. The solvent is allowed to percolate continuously through the plant material until the extraction is complete. This method is applicable to the extraction of most plant constituents.

(iii) Soxhlet extraction: This is a continuous process of extraction with a hot organic solvent. Soxhlet extractors are particularly used for this method of extraction. The powdered plant material is taken in a thimble, which is placed in the Soxhlet extractor. The extractor, which has a siphoning system, is fitted on top of a round bottom flask. A condenser is fitted at the top of the extractor. Enough quantity of the extracting solvent is poured into the flask placed on a heating mantle. On heating, the solvent evaporates, rises to the condenser, where it condenses and drains back to the extractor holding the thimble with the plant material. When the extractor becomes full with the hot solvent, the solvent siphons down to the flask along with the extracted constituents. The recycling of the evaporated solvent is allowed to continue until the extraction is complete.

9.2 Phytochemical Screening of Secondary Metabolites

After obtaining the crude extract or active fraction from plant material, phytochemical screening can be performed with the appropriate tests as shown in Table 9.2. Phytochemicals include basically a large number of secondary metabolites and phytochemical screening is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of chemical compounds in a mixture and an important tool in bioactive compound analyses.

Phytochemicals such as alkaloids (Dragendorff, Mayer, Hager, and Wagner's spot test), tannins (Ferric chloride test), Anthraquinone (Borntrager's test), flavonoids (Shinoda test–HCL test and Lead acetate test), glycosides (Fehling's test and Glacial acetic acid test), Cardiac glycosides (Kellar–Kiliani test), terpenoids and steroids (H_2SO_4 test), saponins (Foam test), fixed oil (Spot test), Amino acids and proteins (Ninhydrin test and copper sulfate test) and terpenes (Liberman–Burchard), steroid (Liebermann–Burchard–test), phenol (phenol test), and tannins (Braemer's test), etc., are generally assessed by different qualitative screening tests.

High-throughput screening

High-Throughput Screening (HTS) is an approach to drug discovery that has gained widespread popularity over the last two decades and has become a standard method for drug discovery in the pharmaceutical industry. It is basically a process of screening and assaying a large number of biological modulators and effectors against selected and specific targets. It is used not only among industrial scientists

Table 9.2 A brief summary of phytochemical screening of secondary metabolite

Secondary metabolite	Name of test	Methodology/composition of the reagent	Result(s)
(1) Alkaloid	Dragendorff's test	Solution of potassium bismuth iodide prepared from basic bismuth nitrate, tartaric acid and potassium iodide; spot a drop of extract on a small piece of precoated TLC plate; spray the plate with Dragendorff's reagent	Orange spot or reddish-brown precipitate (except with caffeine and a few other alkaloids)
	Meyer's reagent	Potassiomercuric iodide solution	Cream precipitate
	Hager's reagent	A saturated solution of picric acid	Yellow precipitate
	Tannic acid	Tannic acid	Precipitation
	Murexide test for caffeine	residue is exposed to ammonia vapor	Purine alkaloids produce pink color
	Wagner's test	Iodine in potassium iodide add 2 ml filtrate with 1% HCl + steam. Then add 1 ml of the solution with 6 drops of Wagner's reagent	Reddish-brown precipitate
	TLC method 1	Solvent system: Chloroform: methanol: 25% ammonia (8:2:0.5). Spots can be detected after spraying with Dragendorff reagent	Orange spot
	TLC method 2	Wet the powdered test samples with a half diluted NH ₄ OH and lixiviated with EtOAc for 24 h at room temperature. Separate the organic phase from the acidified filtrate and basify with NH ₄ OH (pH 11–12). Then extract it with chloroform (3X), condense by evaporation and use for chromatography. Separate the alkaloid spots using the solvent mixture chloroform and methanol (15:1). Spray the spots with Dragendorff's reagent	Orange spot

(continued)

Table 9.2 (continued)

Secondary metabolite	Name of test	Methodology/composition of the reagent	Result(s)
(2) Anthraquinone	Borntrager's test	Heat about 50 mg of extract with 1 ml 10% ferric chloride solution and 1 ml of concentrated hydrochloric acid. Cool the extract and filter. Shake the filtrate with equal amount of diethyl ether. Further, extract the ether extract with strong ammonia	Pink or deep red coloration of aqueous layer
	Borntrager's test	Add 1 ml of dilute (10%) ammonia to 2 ml of chloroform extract	A pink-red color in the ammoniacal (lower) layer
(3) Cardiac glycosides	Kellar-Kiliani test	Add 2 ml filtrate with 1 ml of glacial acetic acid, 1 ml ferric chloride and 1 ml concentrated sulphuric acid	Green-blue coloration of solution
	Kellar-Kiliani test	Dissolve 50 mg of methanolic extract in 2 ml of chloroform. Add H ₂ SO ₄ to form a layer	Brown ring at interphase
	TLC method	Extract the powdered test samples with 70% EtOH on rotary shaker (180 thaws/min) for 10 h. Add 70% lead acetate to the filtrate and centrifuge at 5000 rpm/10 min. Further centrifuge the supernatant by adding 6.3% Na ₂ CO ₃ at 10,000 rpm/10 min. Dry the retained supernatant and redissolved in chloroform and use for chromatography. Separate the glycosides using EtOAc-MeOH-H ₂ O (80:10:10) solvent mixture	The color and hR _f values of these spots can be recorded under ultraviolet (UV254 nm) light
(4) Flavonoid	Shinoda test	To 2–3 ml of methanolic extract, add a piece of magnesium ribbon and 1 ml of concentrated hydrochloric acid	Pink red or red coloration of the solution
	TLC method	Extract 1 g powdered test samples with 10 ml	The color and hR _f values of these spots

(continued)

Table 9.2 (continued)

Secondary metabolite	Name of test	Methodology/composition of the reagent	Result(s)
		methanol on water bath (60 °C/5 min). Condense the filtrate by evaporation, and add a mixture of water and EtOAc (10:1 mL), and mix thoroughly. Retain the EtOAc phase and use for chromatography. Separate the flavonoid spots using chloroform and methanol (19:1) solvent mixture	can be recorded under ultraviolet (UV254 nm) light
	NaOH test	Treat the extract with dilute NaOH, followed by addition of dilute HCl	A yellow solution with NaOH, turns colorless with dilute HCl
(5) Phenol	Phenol test	Spot the extract on a filter paper. Add a drop of phosphomolybdic acid reagent and expose to ammonia vapors	Blue coloration of the spot
(6) Phlobatannin	–	2 ml extract was boiled with 2 ml of 1% hydrochloric acid HCl	Formation of red precipitates
(7) Pyrrolizidine alkaloid	–	Prepare 1 ml of oxidizing agent, consisting of 0.01 ml hydrogen peroxide (30% w/v) stabilized with tetrasodium pyrophosphate (20 mg/ml) and made up to 20 ml with isoamylacetate, and add to 1 ml of plant extract. Vortex the sample and add 0.25 ml acetic anhydride before heating the sample at 60°C for 50–70 s. Cool the samples to room temperature. Add 1 ml of Ehrlich reagent and place the test tubes in water bath (60 °C) for 5 min. Measure the absorbance at 562 nm. The method of Holstege et al. (1995) should be used to confirm results of the screening method	Peaks were compared with the GC-MS library

(continued)

Table 9.2 (continued)

Secondary metabolite	Name of test	Methodology/composition of the reagent	Result(s)
(8) Reducing sugar	Fehling test	Add 25 ml of diluted sulphuric acid (H_2SO_4) to 5 ml of water extract in a test tube and boil for 15 min. Then cool it and neutralize with 10% sodium hydroxide to pH 7 and 5 ml of Fehling solution	Brick red precipitate
(9) Saponin	Frothing test/foam test	Add 0.5 ml of filtrate with 5 ml of distilled water and shake well	Persistence of frothing
	TLC method	Extract two grams of powdered test samples with 10 ml 70% EtOH by refluxing for 10 min. Condense the filtrate, enrich with saturated N-BuOH, and mix thoroughly. Retain the butanol, condense and use for chromatography. Separate the saponins using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. Expose the chromatogram to the iodine vapors	The color (yellow) and hR_f values of these spots were recorded by exposing chromatogram to the iodine vapors
(10) Steroid	Liebermann–Burchardt test	To 1 ml of methanolic extract, add 1 ml of chloroform, 2–3 ml of acetic anhydride, 1–2 drops of concentrated sulphuric acid	Dark green coloration
	–	To 1 ml of extract, add 2 ml acetic anhydride and 2 ml concentrated sulphuric acid H_2SO_4	Color change to blue or green
	TLC method	Extract two grams of powdered test samples with 10 ml methanol in water bath (80 °C/15 min). Use the condensed filtrate for chromatography. The sterols can be separated using chloroform, glacial	The color (Greenish black to Pinkish black) and hR_f values of these spots can be recorded under visible light

(continued)

Table 9.2 (continued)

Secondary metabolite	Name of test	Methodology/composition of the reagent	Result(s)
		acetic acid, methanol and water (64:34:12:8) solvent mixture. The color and hRf values of these spots can be recorded under visible light after spraying the plates with anisaldehyde sulphuric acid reagent and heating (100 °C/6 min)	
(11) Tannin	Braemer's test	10% alcoholic ferric chloride will be added to 2–3 ml of methanolic extract (1:1)	Dark blue or greenish gray coloration of the solution
(12) Terpenoid	Liebermann–Burchard test	To 1 ml of methanolic extract, add 1 ml of chloroform, 2–3 ml of acetic anhydride, 1–2 drops of concentrated sulphuric acid	Pink or red coloration
	Salkowski test	5 ml extract was added with 2 ml of chloroform and 3 ml of concentrated sulphuric acid H_2SO_4	Reddish-brown color of interface
(13) Volatile oil	–	Add 2 ml extract with 0.1 ml dilute NaOH and small quantity of dilute HCl. Shake the solution	Formation of white precipitates

but also among academic researchers. HTS assays are used for screening of different types of libraries, including combinatorial chemistry, genomics, protein, and peptide libraries. The main goal of the HTS technique is to accelerate drug discovery by screening large compound libraries at a rate that may exceed a few thousand compounds per day or per week. It is of vital importance because parallel and combinatorial chemical synthesis generate a vast number of novel compounds. High-throughput screening methods are also used to characterize metabolic, pharmacokinetic, and toxicological data about new drugs. HTS technology can reduce the costs of drug development. HTS consist of several steps such as target identification, reagent preparation, compound management, assay development and high-throughput library screening.

HTS is one of the newest techniques used in drug design and also applied in biological and chemical sciences. This method, due to utilization of robots, detectors, and software that regulate the whole process, enables a series of analyses of chemical compounds precisely in a short time, even >100,000 compounds per

day. The HTS method is more frequently utilized in conjunction with analytical techniques such as NMR or coupled methods, e.g., LC-MS/MS.

9.3 Separation of Plant Constituents

After extraction from the plant material the mixtures of the extracted chemical constituents are separated by various separation techniques and methods for further analysis, isolation, purification, and identification of the individual compounds present in the mixture.

9.3.1 *Separation Techniques*

A large number of separation techniques are used to separate, isolate, and characterize various mixtures of extracted plant constituents for further analysis. Some of these techniques are briefly described below:

(i) Fractional distillation: Volatile constituents having different boiling points when occurring in mixtures are conveniently separated from each other by distilling the mixture at different temperatures. Complete and discrete separation cannot always be obtained by this method,

(ii) Fractional liberation: Mixtures of basic plant constituents, like alkaloids are often separated by this method. In separating a mixture of alkaloids with different degree of basicity, the mixture is first slightly acidified and shaken with a strong organic solvent (e.g., chloroform). The weakly basic alkaloids will thus be extracted from the mixture. The mixture is then gradually basified in increasing order with the addition of an aliquot of ammonia solution at a time and after each addition, the mixture is shaken with the organic solvent and fractions is collected separately. In this way, the alkaloids are fractionally liberated depending on their degree of basicity.

(iii) Fractional crystallization: This separation technique exploits the differential solubility of the components of a mixture in a particular solvent. The least soluble one crystallizes out first from the mixture on cooling when it is dissolved in a minimum quantity of the hot solvent. On separation of these crystals, the others crystallize out in descending order depending on their degree of solubility, the most soluble one being the last.

Chromatography: Out of all the various separation techniques, the chromatographic methods are the most useful and successful techniques of general application. These methods brought a revolutionary advancement in the methods of analysis of plant constituents. Chromatography is the most widely applicable separation method currently used in practically all experiments that must rely on resolution of mixtures.

Original concept of chromatography

In its classical form (invented by M. Tswett, a Russian botanist in 1906), chromatography is a method of separating substances by filtering their solutions through a column of a finely powdered adsorbent, filled into a glass tube, and then washing (developing) the column with a solvent. This results in the separation of the various components of the mixture due to their selective adsorption. The separated substances form distinct zones in the column. They are then separated by cutting the column or are washed down fractionally. This original concept of chromatography was suitable for only colored substances, like pigments. Over the years, the chromatographic techniques have undergone tremendous modification and improvement making chromatography a highly efficient method, which is capable of separating not only colored substances but also closely related colorless substances of all descriptions.

Importance of Chromatography

The importance of chromatography may be summarized as follows:

(a) It is one of the most important analytical tools; (b) it serves as a means for the resolution of mixtures and for the isolation and partial description of the separated substances; (c) it permits the separation and partial description of unsuspected and unknown substances; (d) it is an indispensable laboratory method in all sciences dealing with chemical substances and their reactions; and (e) it is among the most selective and most widely applicable separatory techniques yet devised.

Classification and nomenclature of chromatographic process

Chromatography is a separation technique where separation is affected by a number of ways and by the application of various separating agents as separation by (a) adsorption, (b) partition, (c) ion exchange, (d) electric charge, and (e) particle size difference.

In these methods of separation, two different phases are involved. One of them, which may be a solid or liquid, serves as the carrier or holder of the mixture to be separated. This is called the stationary phase. The other, which may be a liquid or a gas, moves through the stationary phase pushing or carrying the components of the mixture over it at different speeds depending on the affinity of the individual components for the two phases. This moving phase is called the mobile phase. The whole chromatographic process depends on the activities of these two phases. Chromatographic process may be broadly divided into the following major groups.

- (a) **Adsorption chromatography:** In this technique, a pure solid in powdered form is used as the stationary phase and a liquid or gas as the mobile phase.
- (b) **Partition chromatography:** In this case, a liquid coated on an inert solid support serves as the stationary phase and a liquid or a gas as the mobile phase.
- (c) **Ion-exchange chromatography:** In this method, separation is carried out on some solid ion-exchangers by the use of an aqueous solution as the mobile phase.
- (d) **Electrochromatography or Electrophoresis:** In Electrophoresis, electric charge is used to effect separation of the components of a mixture. This can be

used either as partition or as adsorption chromatography. In this technique, an electrolyte serves as the mobile phase or the medium.

- (e) **Gel filtration:** In this method of separation, porous solids with a defined narrow distribution of pores are used as the stationary phase and a liquid as the mobile phase. This is also called a molecular sieve process or exclusion chromatography as the separation of the different components of a mixture depends on the size of their molecules.

Most of these chromatographic processes may be carried out in a number of different ways. For example, they can be carried out by using columns of adsorbents or on thin layers of adsorbents spread on glass slabs, or on thin layers of adsorbents spread on glass slabs, or on paper, or by the use of gases. Depending on the specific medium used or specific technique employed chromatographic methods are also variously named. For example,

- (i) **Column chromatography**, when the adsorption or partition chromatographic separations are carried out on a column of adsorbents packed in a glass or metallic tube.
- (ii) **Thin-layer chromatography**, when the adsorption or partition chromatography is performed on a thin layer of adsorbents spread on glass plates, metal or aluminum sheets.
- (iii) **Paper chromatography**, when the partition chromatographic process is carried on a filter paper.
- (iv) **Gas chromatography**, when gas is used as the mobile phase to carry out the adsorption (Gas–solid chromatography or GSC) or partition chromatography (Gas–Liquid Chromatography or GLC).
- (v) **High-performance liquid chromatography (HPLC)**, It is a liquid column chromatographic technique, which is highly mechanized, sophisticated, efficient, and speedy in operation. It employs relatively narrow columns and operates at ambient temperatures of up to about 200 °C and pressures of up to 2000 atm. This can be used both as an adsorption and partition chromatographic technique.

Column chromatography

Principle and process

The principle involved in column chromatography is that of the original concept of chromatography put forward by M. Tswett in 1906. The solution of the mixture to be separated is first allowed to be absorbed at the topmost part of a column of adsorbent moderately tightly packed in a glass or metallic tube. The compound mixture is then pushed through, that is, the column is developed with a suitable organic solvent called eluant. The components of the mixture are resolved into separate bands as they move down the column. The separated bands move down the column at different speeds due to their variable affinity for the adsorbent (the stationary phase) and the developing solvent (the mobile phase). As more eluent is added and allowed to percolate through the column the separated bands of the mixture are successively eluted from the column. Each of such eluted solution is

called elute, which normally represents the solution of one component of the mixture.

Methods of column chromatography

The column chromatographic process may be used both as an adsorption and a partition chromatography.

Adsorption column chromatography: In this case, the powdered adsorbent is packed in a glass or metallic tube to make a column and an organic liquid is used to develop it. Here, the adsorbent, which is a solid, acts as the stationary phase and the organic liquid as the mobile phase.

Partition column chromatography: When column chromatography is used as a partition chromatographic process, a liquid is used as the stationary phase and another liquid, usually an organic solvent, is used as the mobile phase. The liquid stationary phase is carried on an inert support, which is usually a normal adsorbent. In order to do that the liquid stationary phase is thoroughly mixed with the support and then packed in the glass or metal tube. Development of the column with the liquid mobile phase is then carried out in the usual manner.

Adsorbents used in column chromatography

The adsorbents that are used as stationary phase and carrier in column chromatography include the following:

Silica gel (silicic acid): Silica gel is the most popular adsorbent used in both column and thin-layer chromatography. It is slightly acidic in nature and occurs in powders of various particle sizes. Commercially different grades of Silica gel are now available which differ in their adsorbent capacity and other properties.

Alumina (aluminum oxide): Alumina is also widely used as an adsorbent for both column and thin-layer chromatography. Chemically it is basic in nature and is more reactive than silica gel. Like silica gel, alumina is also available in various commercial grades.

Kieselguhr (diatomaceous earth): This is chemically a neutral adsorbent, more commonly used in column chromatography. It is mainly used as a support for the liquid stationary phase in partition column chromatography.

Cellulose powder: Cellulose is less commonly used as an adsorbent or carrier in column chromatography. But it is used in many instances in thin-layer chromatography when combination of advantages of both paper and thin-layer chromatographic processes are desired in one.

Polyamides: Polyamides have been used for the chromatography of some organic compounds like phenols, flavones, and quinines. Starch, Rubber, and Polyvinylchloride have also been employed as adsorbents in both column and thin-layer chromatography.

Preparation of the Column

Adsorbents and carriers used to prepare the column can be packed in the tubes by the following two methods. The tubes used for preparing the column must have at their lower ends a sintered glass disc or cotton wool plug or rubber stopper with the hole covered with a filter paper or cotton wool. They must be fitted at this end with a stoppered tap.

Dry-packing method: This is the simplest and fastest method. The powder is poured into the tube, which is tapped lightly during the filling. The powder forms a lightly packed column which is washed first with the liquid stationary phase and then with the mobile phase (in case of a partition column) and only with the mobile phase (in case of an adsorption column). If a lightly packed column is required, the adsorbent must be tamped down by gently hitting the tube on a hard surface.

Wet packing method: This is a slightly laborious method. This can be accomplished in two ways: (1) by preparing a slurry of the adsorbent or carrier in the mobile phase, which is quickly poured into the tube and the adsorbent allowed settling down. The column is then washed with more of the mobile phase; (2) by first pouring the developing solvent in the tube and then gradually adding the adsorbent or carrier in small quantities. After each addition, the adsorbent is agitated and slowly pressed down with a glass or metallic plunger (made from a rod, which has at one end a perforated disc slightly smaller in diameter than the column). The wet-packed column should also be washed down with excess of the mobile phase, but it should never be allowed to go dry. The column is packed up to three-fourth length of the tube and a plug of cotton wool or glass wool or disc of filter paper is placed on top of the column to prevent it from further disturbance.

Uses of column chromatography

The column chromatographic methods have been extensively used in the resolution of mixtures and isolation of all types of organic compounds. It has also been successfully utilized in the separation of a number of inorganic substances. Repeated column chromatography is a popular method of initial purification of many natural substances.

Thin-layer chromatography

Although thin-layer chromatography has been known in principle for many years, its rapid growth began only in 1958 when E. Stahl demonstrated its wide applicability. Thin-layer chromatography (TLC) is now one of the most popular and widely used separation techniques. The reasons for this are many which include the following: (a) ease of operation, (b) wide application to a great number of different samples, (c) high sensitivity, (d) higher speed of separation, and (e) relatively low cost.

The Technique

Basically, TLC is an adsorption chromatographic technique in which the adsorbent, acting as the stationary phase, is coated on a glass slab or plastic sheet or aluminum foil in the form of a uniform thin layer. In order to prepare a stable layer of the adsorbent on the plate, a suitable binding agent or binder (usually 4% CaSO_4) is

used along with the adsorbent. Thin-layer chromatography can be equally used as a partition or ion-exchange separation technique.

Preparation of the TLC plates

In preparing the plates, the adsorbent, with or without binder, is made into slurry with a proportionate quantity of distilled water. The slurry is then quickly poured into a spreader. This consists of a rectangular box open at the top and a hollow cylinder that can be rotated through 180° by a handle. When it is rotated through 180°, the slurry empties and passes out through an adjustable slit at the bottom of the spreader to give a layer of desired thickness. The plates to be coated are placed edge to edge on a stable support. The spreader is held over the first plate and when the slurry starts coining out on rotating the cylinder, the spreader is drawn across the plates. The coated plates are left for a while on the support for settling and air drying of the adsorbent. They are then carried on some suitable racks to an oven where they are activated by heating at a temperature of 110–135 °C for half an hour to one hour depending on the adsorbent used. After activation, the plates are stored in a desiccator to stop reabsorption of moisture. The usual thickness for a thin layer of adsorbents used for analytical work is 250 micron.

Adsorbents of thin-layer chromatography

Most of the adsorbents used in TLC, either as stationary phases or carriers, are the same as those of the column chromatography. But they differ from the latter in their fine-grained structures.

Preparation and development of thin-layer chromatograms

Samples of mixtures are taken into solution usually in an organic solvent. They are applied on the thin layer as spots at one end on the plate in a straight line about 3 cm above the edge and 1.5 cm away from the margins. Capillary tubes are used for applying the spots. Care must be taken not to disturb or break the layer of the adsorbent in the process. When the spots are dry, the plates are placed inside a chromatographic tank containing the mobile phase taking care not to submerge the applied spots under the mobile phase. In that case, the compounds will be washed away by the mobile phase and be lost. The mobile phase should be poured into the tank at least 30 min before dipping the spotted plate, that is, the inner atmosphere of the tank must be allowed to saturate with the vapors of the mobile phase before starting the exercise. This is better achieved by lining the inner side of the tank with a filter paper or cloth soaked with the mobile phase. After dipping the plate, the tank should be closed firmly and left undisturbed until the operation is complete. The solvent or mobile phase runs along the thin layer in an ascending manner due to capillary actions. As the mobile phase moves up, it carries the components of the mixture along with it. They move at different speeds depending on their affinities for the mobile and stationary phases, and are thus resolved into separate spots. When the solvent reaches a reasonable height (about 15 cm) the operation is stopped, the solvent front marked and the plate is dried using suitable techniques.

Visualization and identification of the separated compounds

The separated compounds can be located in a number of ways such as (a) by examining the chromatogram (the developed plate) in daylight for any colored spot, (b) by exposing the chromatogram under ultraviolet light for any fluorescent spot, (c) by spraying the chromatogram with a suitable spray reagent for converting the separated compounds into colored spots.

Any spot detected by the above methods must be marked by an encircling line with a sharp pencil or needle. The relative positions of the separated compounds are expressed in terms of their rate of flow value, the retention factor (R_f), which is usually a physical constant for a given compound (X). The retention factor (R_f) may be defined as the ratio (X/Y) of the distance traveled by the substance (X) from the original point of application to the distance traveled by the solvent (Y), usually expressed as a fraction of two decimal places. If R_f value of a solution is zero, the solute remains in the stationary phase, and thus it is immobile; if 1, then the solute has no affinity for the stationary phase and travels with the solvent front. For example, if a compound travels 2.1 cm and the solvent front travels 2.8 cm, $(2.1/2.8)$ the R_f value = 0.75. R_f value depends on temperature and the solvent used in the experiment, so several solvents offer several R_f values for the same mixture of compound.

Adsorbents of thin-layer chromatography

Most of the adsorbents used in TLC, either as stationary phases or carriers, are the same as those of the column Chromatography. But they differ from the latter in their fine-grained structures.

Preparation and development of thin-layer chromatograms

Samples of mixtures are taken into solution usually in an organic solvent. They are applied on the thin layer as spots at one end on the plate in a straight line about 3 cm above the edge and 1.5 cm away from the margins. Capillary tubes are used for applying the spots. Care must be taken not to disturb or break the layer of the adsorbent in the process. When the spots are dry, the plates are placed inside a chromatographic tank containing the mobile phase taking care not to submerge the applied spots under the mobile phase. In that case, the compounds will be washed away by the mobile phase and be lost. The mobile phase should be poured into the tank at least 30 min before dipping the spotted plate, that is, the inner atmosphere of the tank must be allowed to saturate with the vapors of the mobile phase before starting the exercise. This is better achieved by lining the inner side of the tank with a filter paper or cloth soaked with the mobile phase. After dipping the plate, the tank should be closed firmly and left undisturbed until the operation is complete. The solvent or mobile phase runs along the thin layer in an ascending manner due to capillary actions. As the mobile phase moves up it carries the components of the mixture along with it. They move at different speeds depending on their affinities for the mobile and stationary phases, and are thus resolved into separate spots. When the solvent reaches a reasonable height (about 15 cm) the operation is stopped, the solvent front marked and the plate is dried using suitable techniques.

Visualization and Identification of the Separated Compounds

The separated compounds can be located in a number of ways as follows:

- By examining the chromatogram (the developed plate) in daylight for any colored spot;
- By exposing the chromatogram under ultraviolet light for any fluorescent spot;
- By spraying the chromatogram with a suitable spray reagent for converting the separated compounds into colored spots.

Any spot detected by the above methods must be marked by an encircling line with a sharp pencil or needle. The relative positions of the separated compounds are expressed in terms of their rate of flow value (R_f), which is usually a physical constant for a given compound. R_f value is denoted as the distance the compound has moved from the original point of application (say X) divided by the distance the mobile phase, that is the solvent front, has traveled from that point (say Y). Thus,

$$\frac{X}{Y} \text{ XRF Value} = \text{--- and } \frac{Y}{Y} \text{ HRF Value} = \text{---} \times 100$$

Application of thin-layer chromatography

As mentioned before, thin-layer chromatography has a very wide applicability. It is applied both as a qualitative and quantitative method for the separation and isolation of almost all kinds of natural and synthetic organic or inorganic chemical substances. In the medical and pharmaceutical fields, TLC is applied for the detection and separation of many substances like amino acids in food protein, hallucinogenic alkaloids in plants, steroids in the urine of newborn infant, morphine in the blood of addicts, pesticides in soil, etc.

Paper chromatography

Principle and technique

Paper Chromatography is a simple and efficient preparative technique and has a wide applicability like thin layer Chromatography. In this technique, separation of mixtures is accomplished on a filter paper strip which acts as the support medium on which the solution of the mixture is applied. Filter paper contains about 15–20% of its weight of water. When chromatographed on filter paper, separation of mixtures of substances is affected by continuous partition between the liquid mobile phase flowing along the paper and the water held in the paper, which acts as the stationary phase. Paper chromatography is thus a partition chromatographic technique.

Preparation of a paper chromatogram

The solution of the mixture to be separated is applied as a spot near one end of a prepared filter paper strip. The paper is then supported in an airtight tank which has an atmosphere saturated with the mobile phase and water vapors. Development of the chromatogram in this case can be achieved by an ascending technique as in TLC or by a descending technique. In the ascending technique, the mobile phase is either poured

into the tank directly or taken in trough placed at its bottom. The end of the paper strip holding the spot of the component mixture is dipped into the mobile phase up to just below the spot as in TLC. In the descending technique, trough containing the mobile phase is positioned near the top of the tank and the paper is hanged from the trough by the end of the strip which holds the spot, but the spot remaining outside the trough. The mobile phase runs downward along the paper and the chromatogram is developed. Naturally, the speed of development is higher in this case.

As the mobile phase runs up or down, as the case may be the components also move along the paper at varying rates, depending on the differences in their partition coefficients between the stationary phase (water in the filter paper) and the mobile phase. When the solvent front travels to the desired distance, the paper is withdrawn and rapidly dried.

Visualization and identification of the separated components

After the chromatogram has been dried, the separated components are located by the same methods as those described under thin layer chromatography. In addition to spraying, the paper chromatography can also be dipped into the relevant spray reagent to locate the spots. The relative position and identification of the separated components are determined by their R_f values as in thin layer chromatography.

Ion-exchange chromatography

Principle and technique

Ion-exchange chromatography is based on the ability of many solid materials to exchange ions with a solution. When mixtures of ions of opposite charge are passed through the appropriate type of ion-exchangers they are easily separated. The higher the charge on an ion the greater its affinity for an ion-exchanger, thus ions of different charge strength in a mixture are easily separated by elution from a column of the exchanger. If a solution containing a mixture of large and small ions is passed through a column of coarse-grained ion-exchanger, the smaller ions are retained and the eluate contains the larger ions. These various properties of ion-exchangers are exploited in separating mixtures of compounds by ion-exchange chromatography.

Ion-exchange chromatographic technique is mainly carried out on both adsorption and partition columns. Sometimes thin layers of suitable ion-exchangers and ion-exchange papers are also used in this chromatographic technique.

Preparation of columns and method of operation

Columns of ion-exchangers are made by the wet packing method. The tube is three-fourth filled with the mobile phase (usually an aqueous solution containing various ionic components) and the slurry of the ion-exchanger is poured down the wall of the tube using a funnel with a bent stem. The exchanger is allowed to settle down and then back washed in order to remove any entrapped air. The level of the mobile phase in the tube is lowered to the top of the column. The sample in solution is then added slowly by a bent pipette down the wall of the tube just on the top of the column and allowed to soak in. When the sample has soaked in, the walls of the tube are rinsed at least three times with the mobile phase—each portion being allowed to soak in before the next one is added. The column is then developed with

the mobile phase at a controlled flow rate and suitable fractions are collected by fraction collectors and analyzed.

Types of ion-exchangers

Ion-exchangers are ionic in nature and highly permeable. They are able to exchange ions with aqueous solutions. Ion-exchangers are generally solids (but some are used as solutions) and the aluminosilicates of the alkali metals, the zeolites, and some minerals such as sodalite, apatite, and kaollnite are examples of natural ion-exchangers.

Synthetic ion-exchangers are cross-linked polyelectrolytes. They may be inorganic or organic in chemical composition. Aluminosilicates are the common synthetic inorganic ion-exchangers used in chromatography. They are prepared by mixing solutions of aluminum sulfate and sodium silicate. They possess a high concentration of replaceable cations. Synthetic organic ion-exchangers include ion-exchange resins, sephadex, and cellulose ion-exchangers. Ion-exchange resins are made by co-polymerising styrene and di-vinylbenzene, and various functional groups are introduced in them to incorporate ionic properties. Sephadex is a cross-linked dextran polymer.

Application of Ion-exchange chromatography

Ion-exchange chromatography is widely used in the separation of amino acids, proteins, protein hydrolysates, and other ionized compounds.

Electrochromatography

Principle and technique

Electrochromatographic or electrophoretic methods are based on the differential migration of components of mixtures in an electric field. In the electrophoretic separation of mixtures, a filter paper strip or a thin layer of hydrophilic gels is used as the carrier or stationary phase. The filter paper strip or the thin layer is impregnated with a solution of an electrolyte (usually a buffer solution) and supported in the center. Its two ends are then dipped into solutions in which electrodes are immersed. A spot of the mixture is placed on the paper or thin layer, the whole apparatus is sealed and a potential difference of about 2–10 V/cm is applied along the paper or thin layer. The components in the mixture move toward either the anode or the cathode according to the type of the charge of the ions of the components. The speed of movement of a given substance depends on the magnitude of the ionic charge and the size and shape of the particular molecule.

Application of electrochromatography

Electrochromatography or electrophoresis has been successfully employed in the separation of many alkaloids, plant acids, the component sugars of cardiac glycosides, and anthraquinone derivatives.

Gel filtration

Principle and technique

Separation of substances by gel filtration or molecular sieving is accomplished by the use of columns of hydrophilic gels that are prepared from starch, agar, polyacrylarnide, polyvinylcaritol, and cross-linked dextrans. The particles of such gels

possess pores of different sizes, and when packed in a column they leave inter-granular spaces. The process of separation of the components of a mixture in this method depends on the relative sizes of their molecules. When a liquid mobile phase is percolated through the column, on the top part of which the solution of the mixture of the components to be separated has been placed, it permits the components with larger molecules, which do not enter the pores of the particles, to pass rapidly down the column with the mobile phase through the inter-granular spaces. But the components with smaller molecules, which are able to enter the gel particle pores, become evenly distributed across the column and pass slowly down its length at varying speeds. This differential movement of the components through the column causes their separation. Thin layers of such gels can also be used in thin layer chromatography.

Application of gel filtration

Gel filtration has been used in the separation of proteins, peptides, amino acids, and polysaccharides.

Gas chromatography

Gas chromatography includes all those chromatographic processes in which a solid or a liquid coated on a solid support is used as the stationary phase and a gas is used as the mobile phase. In the gas chromatographic technique, the components of the mixture to be separated are dissolved in a suitable organic solvent and placed on top of the column of the stationary phase by an injection device. They are then carried or transported through the stationary phase in the gaseous or vapor form. Thus, this separation technique can be applied to separating only those substances, which can be vaporized at the operating temperatures.

Classification

Based on the principle of separation and the type of the stationary phase, gas chromatographic techniques can be broadly divided into two as (a) gas-solid chromatography (GSC) or adsorption gas chromatography where a solid (usually an adsorbent) is used as the stationary phase and a neutral gas as the mobile phase, and (b) gas-liquid chromatography (GLC) or partition gas chromatography, where a liquid coated on an inert support is used as the stationary phase and an inert gas as the mobile phase.

Principle and technique

The stationary phase in gas Chromatography is used as a column, which is packed in a glass or metallic tube. Its internal diameter ranges from 2 to 10 mm and the length from 1 to 20 mm. The liquid, solid, or gaseous substance to be separated is placed on one end of the column by means of a suitable injection device and then driven through the column by an inert carrier gas (the mobile phase). The rate of flow of the mobile phase is adjusted to a constant speed that may range from 0.3 to 10 L/h. The components of the mixture are separated as they pass through the column, and the separated fractions gradually emerge at the other end of the column along with the mobile phase. The substances in the emerging mobile phase are then detected or characterized by measuring a physical or chemical property in a detector device, usually a cathetometer. This measurement is continuously recorded on a moving chart by a recording device.

Thus, results in a diagram in which the observed figures are plotted against time. This plotted chart or diagram is the gas chromatogram.

The separated components are identified in terms of their Retention time (R_t). Retention time of a compound is the interval of time between the introduction of the mixture into the column and the elution of that compound from the column.

Gas chromatography assembly

The basic components of a gas chromatographic assembly include a gas cylinder or a gas generator with a control valve, an injection device, a thermostat oven in which is accommodated the column, a detector device directly connected to the column, and an amplifier and recorder connected to the detector.

Application of gas chromatography

Gas chromatography, particularly the GLC, has been very successfully used both as a qualitative and quantitative method for analysis of a large number of natural substances of pharmaceutical importance, such as volatile oils, camphor, plant acids, some alkaloids, resins, saponins, and cardioactive glycosides and aglycones.

High-performance liquid chromatography

High-performance liquid chromatography or high-pressure liquid chromatography or simply HPLC is a kind of sophisticated and mechanized column chromatography that can also be used both as an adsorption and partition chromatography. But the tubes used in HPLC for preparing the columns of the adsorbents or carriers are narrow with internal diameters of 2–8 mm and the particles of the adsorbents have an average diameter of less than 50 microns.

HPLC apparatus

The essential components of a HPLC instrument include (i) a reservoir for the mobile phase; (ii) a pump to regulate the flow of the mobile phase; (iii) a damping device or pressure gauge for smoothing out the flow rate; (iv) a sampling device through which the sample is introduced on top of the column; (v) a column to hold the stationary phase; (vi) a detector to detect the sample components in the column effluent; and (vii) a recorder which records the separated components on a moving chart in the form of peaks. A fraction collector is also provided for the isolation of sample components.

Technique of operation

From the solvent reservoir, the pump delivers a constant flow of the mobile phase which is smoothed out by means of the damping device. The mobile phase flows via the sampling device to the column and percolates through the carrying the separated components of the sample mixture. On being eluted from the column, the separated sample components are sensed by the detector and monitored or recorded by the potentiometric recorder.

Application of HPLC

Since HPLC gives much improved and more rapid separation than classical column chromatography, it is finding increasing use in numerous areas. It has been successfully used in the separation and isolation of most secondary metabolites of

plants which include carboxylic acids and derivatives, flavonoids, terpenes, plant pigments, steroids, various glycosides, amines, alkaloids, antibiotics, and toxins.

9.4 Isolation and Characterization of Drug Principles from Plant and Other Natural Sources

Natural products from medicinal plants provide wide opportunities for new drug leads because of their unique chemical diversity. Literally, there are thousands of phytochemicals with many effective beneficial biological activity such as anti-cancer, antimicrobial, antioxidant, antidiarrheal, analgesic, and wound healing activity with less adverse effects. The unlimited chemical diversity is useful in screening programs aimed to discover new therapeutic drugs from botanicals, which contain various types of bioactive compounds. For this, the analytical methodologies, particularly isolation and characterization of active ingredients in botanicals and herbal preparations along with extraction process, play a significant role. The analysis of bioactive compounds present in the plant extracts involves the applications of common phytochemical screening assays, chromatographic techniques such as HPLC and TLC as well as non-chromatographic techniques such as immunoassay, Fourier Transform Infrared (FTIR), etc. (Fig. 9.1).

Isolation

Isolation is the crucial first step in the analysis of medicinal plants because it is necessary to isolate the desired chemical components from the plant materials for further separation and characterization. The basic operation included steps such as prewashing, drying of plant materials or freeze drying, grinding to obtain a

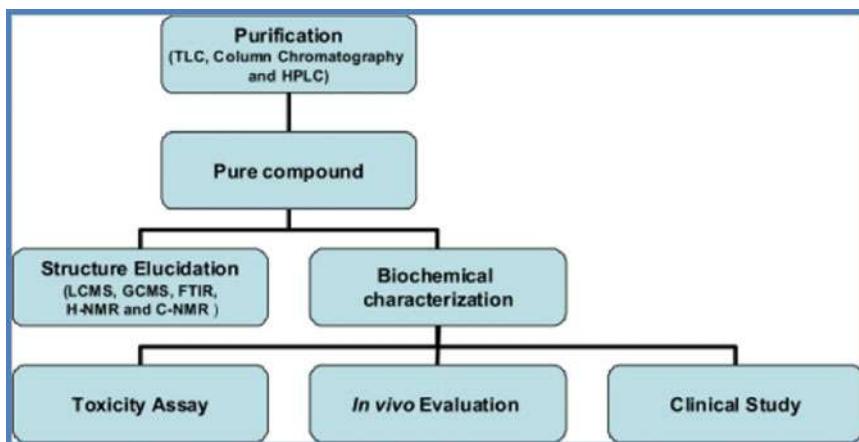


Fig. 9.1 General approaches used in extraction, isolation, and characterization of bioactive compound from plants extract

homogenous sample and often improving the kinetics of analytic extraction, and also increasing the contact of sample surface with the solvent system. Proper actions must be taken to assure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples. As the target compounds may be nonpolar to polar and thermally labile, the suitability of the methods of isolation must be considered.

Characterization of plant drug principles

Phytochemical characterization primarily may be initiated with the help of qualitative tests for the screening of phytochemical compounds. However, plant extracts usually occur as a combination of various types of bioactive compounds or phytochemicals with different polarities and, therefore, their separation is a big challenge for the process of identification and characterization of bioactive compounds. Characterization and identification of the separated and isolated constituents are the final steps in the photochemical analysis of plants. In order to accomplish these, plant constituents must be obtained in a very pure form. For this purpose, they are subjected to various purification processes after being separated and isolated by the various separation techniques. These purification processes include repeated column chromatography and preparative thin layer and paper chromatography. The chromatographically pure compounds are then further purified by repeated crystallization from suitable solvents or solvent mixtures. A pure compound thus obtained is characterized and identified by determining its various physical and chemical properties like R_f value, melting point, optical values, nature and type of crystals, types and number of elements and functional groups present in the molecule, etc. Determination of these various physicochemical properties of a chemical compound is done by the use of different chemical tests and reactions, chromatographic techniques, crystallographic and spectroscopic methods, etc. It is out of the scope of this book to discuss the topic in any further detail.

It is a common practice in isolation of these bioactive compounds that a number of different separation techniques such as TLC, column chromatography, flash chromatography, Sephadex chromatography, and HPLC should be used to obtain pure compounds. The pure compounds are then used for the determination of structure and biological activity. Besides, non-chromatographic techniques such as immunoassay, which use monoclonal antibodies (MAbs), phytochemical screening assay, Fourier-transform infrared spectroscopy (FTIR), can also be used to obtain and facilitate the identification of the bioactive compounds.

Non-chromatographic techniques

Immunoassay

Immunoassays use monoclonal antibodies against drugs and low molecular weight natural bioactive compounds and are becoming important tools in bioactive compound analyses. They show high specificity and sensitivity for receptor binding analyses, enzyme assays, and qualitative as well as quantitative analytical techniques. Enzyme-linked immunosorbent assay (ELISA) based on MAbs are in many cases more sensitive than conventional HPLC methods. Monoclonal antibodies can be produced in specialized cells through a technique known as hybridoma

technology (Shoyama et al. 2003). The following steps are involved in the production of monoclonal antibodies via hybridoma technology against plant drugs:

- (i) A rabbit is immunized through repeated injection of specific plant drugs for the production of specific antibody, facilitated due to the proliferation of the desired B cells.
- (ii) Tumors are produced in a mouse or a rabbit.
- (iii) From the above two types of animals, spleen cell (these cells are rich in B cells and T cells) are cultured separately. The separately cultured spleen cells produce specific antibodies against the plant drug, and against myeloma cells that produce tumors.
- (iv) The production of hybridoma by fusion of spleen cells to myeloma cells is induced using polyethylene glycol (PEG). The hybrid cells are grown in selective hypoxanthine aminopterin thymidine (HAT) medium.
- (v) The desired hybridoma is selected for cloning and antibody production against a plant drug. This process is facilitated by preparing single cell colonies that will grow and can be used for screening of antibody producing hybridomas.
- (vi) The selected hybridoma cells are cultured for the production of monoclonal antibodies in large quantities against the specific plant drugs.
- (vii) The monoclonal antibodies are used to determine similar drugs in the plant extract mixture through enzyme-linked immunosorbent assay (ELISA).

Fourier-transform infrared spectroscopy (FTIR)

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract (Eberhardt et al. 2007; Hazra et al. 2007). In addition, FTIR spectra of pure compounds are usually so unique that they are like a molecular “fingerprint”. For most common plant compounds, the spectrum of an unknown compound can be identified by comparison to a library of known compounds. Samples for FTIR can be prepared in a number of ways. For liquid samples, the easiest is to place one drop of the sample between two plates of sodium chloride. The drop forms a thin film between the plates. Solid samples can be milled with potassium bromide (KBr) and then compressed into a thin pellet which can be analyzed. Otherwise, solid samples can be dissolved in a solvent such as methylene chloride, and the solution is then placed onto a single salt plate. The solvent is then evaporated off, leaving a thin film of the original material on the plate.

9.5 Bioassay Techniques

Bioassay is a life-based technique. A simple and rapid bioassay can serve as starting point of drug discovery, which is, of course, a long, tedious, and expensive process that requires multidisciplinary collaborative efforts involving botanists, pharmacognostics, chemists, pharmacologists, toxicologists, clinicians, and many others.

Bioassay is an activity-directed isolation process and its goal is to isolate bioactive compounds with certain definite degree of LD/LC/IC₅₀ (the half maximal lethal dose/lethal concentration/inhibitory concentration) value as a proof of cytotoxicity. For bioassay results interpretation, know-how about the basic of probit analysis is necessary. Probit analysis is a specialized type of regression model used to analyze binomial response variables. Regression is a method of fitting a line to experimental data to compare the relationship of the response variable or dependent variable (Y) to the independent variable (X), while $Y = a + bX + e$ (where a = y-intercept, b = the slope of the line, e = error term). Probit transforms the sigmoid dose/stimulus-response curve to a straight line that can then be analyzed by regression either through least squares or maximum likelihood. Probit analysis can be done by (i) using tables to estimate the probits and fitting the relationship by eye; (ii) hand calculating the probits, regression coefficient, and confidence intervals (with 95% CI); or (iii) with the help of statistical package such as SPSS. Probit analysis is used to analyze many kinds of dose-response or binomial response experiments in a variety of fields including toxicology. There are many endpoints used to compare the differing toxicities of chemicals, but the LC50 (liquids) or LD50 (solids) are the most widely used outcomes of the modern dose-response experiments. ED50 (median effective dose), LD50 (median lethal dose), or LC50 (median lethal concentration) are the values corresponding to a probability of 0.50.

Benchtop bioassays can be integrated into the research programs for phytochemical (natural product) analysis targeted to new drug discovery or lead compounds. Rahman et al. (2001) described in the “Bioassay techniques for drug development” several benchtop and primary bioassay screening techniques. Bioassays can be divided into various broad groups based on the target life forms on which they are carried out.

Based on the target life forms on which the assays are carried out, the following groups of bioassays are recognized:

- (i) Whole animals;
- (ii) Isolated organs of vertebrates;
- (iii) Lower organisms, e.g., fungi, bacteria, insects, molluscs, lower plants, etc.;
- (iv) Cultured cells, e.g., cancer cells and tissues of human or animal origin;
- (v) Isolated subcellular systems, e.g., enzymes, receptors, etc.

The goal of an activity-directed isolation process is to isolate bioactive compounds which are capable of curing or alleviating a human or animal ailment and which can either be ultimately developed as established drugs directly or which can provide interesting structural leads. The process of drug development is long, tedious, and expensive, requiring a multidisciplinary collaboration between botanists, pharmacognosists, chemists, pharmacologists and toxicologists, and clinicians. Simple and rapid bioassays can serve as starting points for such multidisciplinary efforts directed at drug discovery. The purpose of these bioassays is to rapidly screen for interesting biological activities, which can then be followed by more detailed mechanism-based studies of a multidisciplinary nature.

Benchtop and primary bioassay screening**(i) Toxicity assays****i.i Brine shrimp toxicity (BST) assay**

Bioactive compounds are often toxic to shrimp larvae. Hence, in vivo lethality to shrimp larvae can be used as a rapid and simple preliminary monitor for bioactive compounds. The eggs of the brine shrimp *Artemia salina* (Leach) are readily available as fish food in pet shops. When placed in artificial seawater (0.38% saline water), the eggs hatch within 48 h and provide large numbers of larvae. These tiny shrimp larvae have been extensively used as a tool to monitor the cytotoxicity of samples under study. This is a rapid, inexpensive, in-house, general bioassay, which has been developed for screening, fractionation, and monitoring of physiologically active natural products (Meyer et al. 1982; McLaughlin 1991).

Materials

- (i) *Artemia salina* Leach (brine shrimp eggs);
- (ii) Sea salt;
- (iii) Small tank with perforated dividing dam and cover to grow shrimps; lamp to attract shrimps;
- (iv) Syringes: 5.0 ml, 0.5 ml, 100 µl and 10 µl;
- (v) 2 dram vials (9 per sample + 1 control);
- (vi) Magnifying glass;
- (vii) Organic solvents (methanol, dichloromethane, chloroform, DMSO, etc.);
- (viii) Distilled Water;
- (ix) Pasteur pipettes;
- (x) Aluminum foil;
- (xi) Test sample (crude extract, pure natural product, synthetic compound, etc.).

Methods

The following steps are involved in the brine shrimp lethality assay:

- (i) Artificial seawater is prepared by dissolving 3.8 g sea salt per liter of water and filtered;
- (ii) Seawater is placed in a small unequally divided tank and shrimp eggs added to the larger compartment of the tank which is darkened by covering it with aluminum foil. The illuminated compartment attracts shrimp larvae (nauplii) through perforations in the dam;
- (iii) Kept 2 days at room temperature (22–29 °C) for the shrimps to hatch and mature;
- (iv) Prepared vials for testing containing initially 1000, 100, 10, and 5 µg extract/ml with three replicates for each concentration making a total of nine vials; weighed 20 mg of sample and added 2 ml of organic solvent (20 mg/2 ml); from this solution transferred 500, 50, 5 or 2.5 µl to vials

corresponding to 1000, 100, 10 or 5 µg/ml, respectively. Evaporated the volatile organic solvents overnight under nitrogen, and then place under high vacuum for about 30 min;

Alternatively, polar insoluble materials may be dissolved in DMSO, and up to 50 µl may be added per 5 ml of seawater before DMSO toxicity affects the results.

- (v) After 2 days (when the brine shrimp larvae have matured), added 5 ml seawater to each vial and added 10 shrimps per vial with the help of pasteur pipette (30 shrimps per dilution). The vials are maintained under illumination;
- (vi) After 24 h have elapsed, count and record the number of surviving shrimps, with the aid of a 3× magnifying glass;
- (vii) Analyze data with the help of a suitable software program for Probit analysis to determine LD/LC₅₀ values and 95% confidence intervals;
- (viii) Additional dilutions of less than 5 µg/ml may be needed for potent materials. Intermediate concentrations can be prepared and tested to narrow the confidence intervals.

i.ii Brine shrimp microwell cytotoxicity assay

A new microplate assay for cytotoxicity or lethality determination using brine shrimp (*Artemia salina*) has been developed which gives results comparable to the vial method described above under the heading of brine shrimp lethality assay. The assay reliably correlates with kenacid blue (KB) cell toxicity assays, and thus provides a convenient means by which the presence of cytotoxic natural products may be detected during the fractionation and isolation of natural products.

Materials

- (i) Brine shrimp eggs (*Artemia salina*);
- (ii) Sea salt;
- (iii) Dried yeast;
- (iv) 96-Well microplates (flat plates with multiple “wells” used as small test tubes);
- (v) Dimethylsulfoxide (DMSO);
- (vi) Pasteur pipette;
- (vii) Binocular microscope (10.30×);
- (viii) Methanol;
- (ix) Incubator;
- (x) Beaker; and
- (xi) Test sample (plant extract, pure natural product or synthetic compound).

Methods

Brine shrimp microwell cytotoxicity assay typically consists of the following assay steps:

- (i) Artificial seawater is prepared by dissolving sea salt in distilled water (40 g/L) supplemented with 6 mg/L dried yeast.
- (ii) Brine shrimp eggs (*Artemia salina*) are hatched in artificial seawater during 48 h incubation in a warm room (22–29 °C).
- (iii) Brine shrimp larvae (nauplii) are collected with a Pasteur pipette after attracting the organisms to one side of the vessel with a light source. Nauplii are separated from the eggs by pipetting them 2–3 times in small beakers containing seawater.
- (iv) The test sample (20 mg of crude extracts or 4 mg for pure compound) is made up to 1 mg/ml in artificial seawater (water-insoluble compounds or extracts can be dissolved in 5 ml dimethyl sulfoxide (DMSO) prior to adding sea water).
- (v) Serial dilutions are made in wells of 96-well microplates in triplicate in 100 µl seawater.
- (vi) A suspension of nauplii containing 10–15 brine shrimp larvae (100 ml) are added to each well with the help of a Pasteur pipette and the covered microwell plate is incubated at 22–29 °C for 24 h.
- (vii) The plates are then examined under a binocular microscope (12.5×) and the number of dead (nonmobile) nauplii in each well counted.
- (viii) 100 µl methanol is then added to each well, and after 15 min the total number of shrimps in each well is counted.
- (ix) LC50 values are then calculated using Probit analysis following Goldstein (1964) or with the help of SPSS.

i.iii Crown gall tumor inhibition assay (potato disc antitumor assay-PDA)

Crown gall is a neoplastic disease of plants which is induced by the gram-negative bacteria *Agrobacterium tumefaciens*. The bacteria possess large Ti (tumor-inducing) plasmids which carry genetic information (T DNA) that transform normal, wounded, plant cells into autonomous tumor cells. Since the mechanism of tumor induction is similar to that in animals, this test system has been used to evaluate and prescreen the antitumor/cytotoxic properties of plant extracts or natural products. The results suggest that the potato disc assay is a safe, simple, rapid, and inexpensive in-house screen for 3PS (plastic-antibodies, plasmonics, and photovoltaic-cells) antitumor activity. It is statistically more predictive of 3PS (P 388 leukemia) activity than either the 9KB (human nasopharyngeal carcinoma) or 9PS (murine leukemia) cytotoxicity assays. The assay also gives an indication of tumor-promoting or carcinogenic properties of the test samples (Ferrigni et al. 1982).

Materials

- (i) Laminar flow hood or clean air chamber;
- (ii) Fresh, disease-free potato tubers (preferably red);
- (iii) Organic solvents (DMSO and ethanol);
- (iv) Broth culture of *Agrobacterium tumefaciens* strain B6 (ATCC)*;
- (v) 1.5 Sterile cork borer;
- (vi) Millipore filters (0.22 mm);
- (vii) Disposable or autoclavable gloves;
- (viii) Difco agar;
- (ix) Autoclave;
- (x) Sterile Petri dishes;
- (xi) Pipettes;
- (xii) Test tubes;
- (xiii) Large glass tray;
- (xiv) Aluminum foil;
- (xv) Parafilm;
- (xvi) Liquid hypochlorite bleach;
- (xvii) Sterile special cutter (3 cm long small knives fixed parallel with each other on a wooden frame with holder) or scalpel;
- (xviii) Incubator;
- (xix) Dissecting compound microscope;
- (xx) Lugol's solution (5% I₂ + 10% KI in H₂O); and
- (xxi) Test sample (crude extract, pure natural product or synthetic compounds).

Methods

The potato disc antitumor assay involves the following steps:

- (i) Fresh potato tubers (disease-free) of moderate size are sterilized by soaking in liquid bleach for 10 min.
- (ii) A core cylinder of tissue is removed from the potato by means of a sterilized cork borer. Two cm long ends of each potato cylinder should be discarded and the remainder of the cylinder is cut into discs of uniform thickness (0.5 cm) by a special cutter or scalpel under aseptic conditions.
- (iii) The potato discs are then transferred to 1.5% agar plates (1.5 g of agar/100 ml distilled water, autoclaved and 20 ml of agar solution is poured in each sterile Petri dish). Five potato discs should be placed in each Petri dish and 3–5 dishes are used for each test sample along with the same number of dishes for the control.
- (iv) 8 mg of sample is dissolved in 2 ml of DMSO in a test tube and filtered through a Millipore filter into another sterile tube. 0.5 ml of this solution is then added to 1.5 ml of sterile autoclaved distilled water, and then 2 ml of a broth culture of *A. tumefaciens* (a 48 h culture containing 5×10^9 cell/ml) is added.

- (v) Controls are prepared by filtering 0.5 ml DMSO through a Millipore filter into 1.5 ml of sterile distilled water and adding to tubes containing 2 ml of a broth culture of *A. tumefaciens*.
- (vi) One drop (0.05 ml) is drawn from these test tubes using a sterile pipette, and it is used to inoculate each potato disc, spreading it over the disc surface. The process starting from the cutting of the potatoes to the inoculations should be completed within 30 min in order to avoid contamination.
- (vii) The Petri dishes are incubated at room temperature (27 °C), the lids being taped down by using parafilm to minimize moisture loss.
- (viii) After 12 days of inoculation, the tumors are counted with the aid of a dissecting microscope after staining with Lugol's solution (the tumors can also be counted without using Lugol's solution). The tumor cells lack starch. The number of tumors in the control is used as a reference for activity.
- (ix) The results are derived from the number of tumors on test discs versus those on the control discs. Inhibition is expressed as a negative percentage and stimulation is expressed as a positive percentage. 20% inhibition in two or more independent assays is considered as a significant activity of a test sample.

i.iv Animal toxicity assay

Materials

- (i) Albino, laboratory-bred strain of the house mouse BALB/c mice (30 mice per test sample and 6 mice for control);
- (ii) Syringes;
- (iii) Saline solution (0.85% sterile NaCl);
- (iv) Autoclave; and
- (v) Test sample (plant extract, pure natural product or synthetic compound).

Methods

The following steps are involved in animal toxicity assay:

- (i) Six groups of five mice each are injected intraperitoneally with different dilutions of test samples (50, 100, 150, 200, and 250 mg dissolved in saline).
- (ii) The control group of the animals is only administered sterile saline.
- (iii) The animals are kept in observation for one week and deaths of animals are recorded.
- (iv) LD₅₀ is calculated by the standard method (Goldstein 1964; Kazmi et al. 1990).

i.v Antimicrobial assays

For this type of work, complete and in detail, biosafety precautions should be observed because many test microbes (e.g., bacteria, fungi, etc.) as well as viruses (e.g., HIV retrovirus) are highly pathogenic to human and other organisms. All

manipulations of viral material should be performed in strictly controlled (contained) environment (laminar flow chamber or glove box) using specially designed disposable gloves for virus handling. All contaminated materials and disposable items should be collected in autoclave bags and autoclaved at 120 °C before disposal; or burned in a controlled incinerator, away from urban areas.

Agar diffusion assay

Materials

- (i) Test organisms, e.g., *Escherichia coli* (NCTC 10418), *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* (NCTC 6571), *Pseudomonas aeruginosa* (ATCC 10145);
- (ii) Nutrient broth;
- (iii) Sterile cork borers;
- (iv) Petri dishes (14 cm dia);
- (v) Pipettes (0.1 and 1 ml);
- (vi) Organic solvent;
- (vii) Incubator;
- (viii) Standard antibiotics (streptomycin, ampicillin, etc.); and
- (ix) Test sample (crude extract, pure natural product, or synthetic compound).

Methods

The agar diffusion assay consists of the following sequential steps:

- (i) 10 ml aliquots of nutrient broth is inoculated with the test organisms and incubated at 37 °C for 24 h.
- (ii) Using a sterile pipette, 0.6 ml of the broth culture of the test organism is added to 60 ml of molten agar which has been cooled to 45 °C, mixed well and poured into a sterile Petri dish (for the 9 cm Petri dish, 0.2 ml of the culture is added to 20 ml of agar). Duplicate plates of each organism are prepared.
- (iii) The agar is allowed to set and harden and the required numbers of holes are cut using a sterile cork borer ensuring proper distribution of holes (cups) in the periphery and one in the center. Agar plugs are removed. Different cork borers should be used for different test organisms.
- (iv) Using a 0.1 ml pipette, 100 µl of the test sample dissolved in an appropriate solvent is poured into appropriately labeled cups (these are marked at the back of the cup before filling). The same concentrations of the standard antimicrobial agents (streptomycin 1 mg/ml and ampicillin 10 µg/ml) and the solvent (as control) are used.
- (v) The plates are left at room temperature for 2 h to allow diffusion of the sample and incubated face upwards at 37 °C for 24 h.
- (vi) The diameter of the zones of inhibition is measured to the nearest mm (the cup size also being noted) (Kavanagh 1963; Leven et al. 1979).

Agar dilution assay

Materials

- (i) Test bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Agrobacterium tumefaciens*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, etc.;
- (ii) Nutrient agar (composition g/L, peptone 5 g/L, NaCl 5 g/L, beef extract 15 g/L, yeast extract 15 gm/L, pH 7.2, and agar 20 gm/L);
- (iii) Organic solvents (ethanol or acetone);
- (iv) Test tubes;
- (v) Incubator;
- (vi) Pipettes (0.5 ml, 1 ml, 10 ml); and
- (vii) Test sample (crude extract, pure natural product or synthetic compound).

Methods

The following sequential steps are involved in agar dilution assay:

- (i) A loopful of the bacterial culture from the slant is inoculated in the nutrient broth and incubated at $37^\circ \pm 1$ °C for 24 h.
- (ii) The fresh broth (20 ml) is seeded with 0.25 ml of the 24 h broth cultures and a twofold serial dilution method is followed as described below. The test sample is dissolved in water (in case of water-soluble samples) or in an organic solvent (ethanol or acetone) to obtain a 10 mg/ml solution. A 0.2 ml solution of the test material is added to 1.8 ml of the seeded broth and this forms the first dilution.
- (iii) 1 ml of this dilution is diluted further with 1 ml of the seeded broth to produce the second dilution, and the process is repeated until six such dilutions are obtained.
- (iv) A set of tubes containing only seeded broth is kept as control and suitable solvent controls are also maintained.
- (v) After incubation for 24 h at $37^\circ \pm 1$ °C, the last tube with no visible growth of the microorganism is taken to represent the minimum inhibitory concentration (MIC) of the test sample which is expressed in mg/ml.

Microtitre plate method

If a 96-well microtitre plate is available then three dilutions of a test sample can be tested against 24 microorganisms (or two extracts against 12 microorganisms) simultaneously. In that case, the following procedure as described sequentially may be followed:

- (i) The test sample is dissolved/suspended in a physiological Tris buffer (pH 7.2) or in a mixture of polyethylene glycol 400 (PEG 400) and physiological Tris buffer (4:6) (15 ml).
- (ii) The solubilized test sample (2.0 ml) (or its four-fold dilutions, 1/4, 1/16, etc.) is warmed and mixed with an equal amount of liquid agar medium at 50 °C, thereby affording the dilutions 1/2, 1/8, 1/32, etc.

- (iii) The microtitre plate is warmed with an infrared lamp and the holes of lanes B-D or FH are filled with these dilutions (0.3 ml per hole). The holes in lanes A and E are filled with the control consisting of a mixture of the solubilizing buffer (2.0 ml) and culture medium (0.2–0.3 ml per hole).
- (iv) The infrared lamp is removed to allow the materials to solidify at room temperature and all the holes are then inoculated with 1:100 dilution (5 µl) of overnight cultures of test bacteria ($\pm 10^3$ bacteria).
- (v) Inoculation is carried out for 24 h at 36 °C, and a light microscope is then used to compare the growth of test organisms against the control. Test samples showing inhibitory effects at all three dilutions (1/2, 1/8, and 1/32) are subjected to further investigations (Srivastava 1984).

Direct bioautography method

Bioautography can be employed as a method for localizing antibacterial activity on a chromatogram. The “agar diffusion” technique involves transfer of the antibacterial compound from the chromatographic plate to an inoculated agar plate by diffusion, and visualization of the zones of inhibition. An improved simpler version of this method, which avoids extensive microbiological equipment and problems associated with differential diffusion of compounds from the chromatogram to the agar plate, may be prepared (Hamburger and Cordell 1987).

In this method, a suspension of a microorganism in a suitable broth is applied to TLC plate, which is then incubated in a humid atmosphere to allow growth of the bacteria. Zones of inhibition are then visualized by a dehydrogenase activity detecting reagent, a tetrazolium salt, which is converted by the bacteria into the intensely colored product. The antibacterial compounds appear as colorless spots against a colored background.

Preparation of bacterial suspension

Materials

- (i) Culture flasks (1 L);
- (ii) Inoculation loops;
- (iii) Shaker bath;
- (iv) Laminar flow hood;
- (v) Disposable centrifugation tubes (50 ml);
- (vi) Centrifuge;
- (vii) Colorimeter;
- (viii) Disposable pipettes (5, 10 ml);
- (ix) Nutrient broth and nutrient agar (BB1);
- (x) Culture tubes;
- (xi) ATCC cultures of *B. subtilis* (ATCC # 6633) and *E. coli* (ATCC # 25922); and
- (xii) Test sample (crude extract, pure natural product; or synthetic compound).

Methods

The direct bioautography method consists of following sequential steps:

- (i) Lyophilized ATCC bacterial culture is resuspended in the recommended broth. Agar slants are then inoculated. The bacteria can be kept on agar slants at 4 °C and should be checked for viability and contamination every month. For safety reasons, it is recommended to store a part of the initial inoculum in liquid nitrogen as a backup in case the slants are accidentally contaminated.
- (ii) Nutrient broth (NB) (300 ml in 1 L flasks) is inoculated with *B. subtilis* or *E. coli* (maintained on agar slants) and kept at 37 °C on a shaker at 80 rpm for 36–48 h.
- (iii) The suspension is centrifuged for 10 min at 1500 rpm (in 50 ml centrifugation tubes) and the supernatant is discarded.
- (iv) The bacteria is resuspended in 3–4 ml of fresh NB (SI). 2 ml of suspension is diluted to 20 ml by adding NB; the turbidity is determined by measuring the absorbance at 560 nm.
- (v) The solution SI is diluted such that turbidity of a 1:10 dilution has approx. 0.84A (approx. 109 bacteria/ml).
- (vi) The material is dispensed in cryovials (2 ml) and stored in liquid N₂.
- (vii) Aliquots of 18 ml NB are dispensed in sterile culture tubes. These tubes are then stored at 4 °C for some weeks.

Bioautography

Materials

- (i) Aluminum backed silica gel TLC sheets, GF 254 (Merck);
- (ii) Disposable surgical gloves;
- (iii) Autoclavable polyethylene boxes (larger than 20 × 20 cm);
- (iv) Chromatography paper (Whatman);
- (v) Pyrex glass dishes (rectangular, 10 × 15 cm);
- (vi) Autoclave bags (Fisher Scientific);
- (vii) TLC sprayer, glass;
- (viii) TLC tank;
- (ix) Ethanol;
- (x) Lysol disinfectant
- (xi) Roller device, 10 cm (should be autoclavable);
- (xii) Double-sided adhesive tape;
- (xiii) Safety hood, glove box or glove bags (Aldrich Chemicals);
- (xiv) Polyethylene stoppers (autoclavable);
- (xv) Syringe with Luer lock (20 ml);
- (xvi) Disposable membrane filters with Luer lock;
- (xvii) Disposable graduated TLC micropipettes (glass, 5 µl);
- (xviii) 19. *p*-Iodonitrotetrazolium violet (INT) (Sigma);
- (xix) HPLC grade water (fresh from Millipore unit);

- (xx) Cryovial (2 ml) of conc., bacterial suspension;
- (xxi) Nutrient broth (18 ml); and
- (xxii) Test sample (crude extract, pure natural product or synthetic compound).

Methods

The sequential steps involved in bioautography assay are as follows:

- (i) TLC chromatograms* (20×20 cm) are developed in a suitable solvent system (the mobile phase has to be sufficiently volatile so that it can be removed completely. Traces of organic solvents will otherwise inhibit bacterial growth).
[*Substantial quantities of crude extract (or fractions) should be spotted on the TLC plates if the test sample is too little, the chances of missing the active constituents will increase. On the other hand, a very high concentration of the test sample may lead to imperfect separation on TLC. The recommended quantity of crude extract is 10–100 mg/spot. Suitable organic solvents include CHCl_3 , CH_2Cl_2 , MeOH , H_2O , CCl_4 , i-PrOH, acetone, ether (avoid: toluene, benzene, *n*-BuOH, acids, and bases).]
- (ii) TLC is run in duplicate (one for the bioautogram, one for comparison).
- (iii) Both plates are dried carefully with a hair-drier and UV absorbing spots are marked on both plates (UV 254 + 366 nm). To avoid contamination of the plate, the silica layer of the plate should not be touched, especially the one which will be used for bioautography. A control TLC plate is also kept. The silica layer of the control TLC plate should be covered in order to minimize the oxidation of compounds. The control TLC can be sprayed with a suitable chromogenic reagent such as phosphomolybdic acid, ceric sulfate– $\text{Ce}(\text{SO}_4)_2 \cdot 4\text{H}_2\text{O}$, vanilline, Dragendorff's reagent or simply placed in a tank with iodine crystals the following day. The stained control TLC should be covered with parafilm to avoid discoloration.
- (iv) A roller device is wrapped with a layer of chromatography paper of suitable size and fixed with double-sided adhesive tape.
- (v) Polyethylene box (autoclaved) is lined with the chromatography paper, and soaked with fresh HPLC grade water (from Millipore unit) (approx. 20–30 ml). Polyethylene stoppers (6–8) and placed in the box (the stoppers will support the TLC plate and keep it separate from the wet paper).
- (vi) The cryovial (2 ml) is thawed quickly at 37°C ; 18 ml NB is added and poured into Pyrex glass dish.
- (vii) The roller device is carefully soaked with the bacterial suspension and gently applied onto the silica layer. The process is repeated until the silica is soaked with the liquid (approx. 5–6 ml for TLC plate 20×20 cm). (Another approach: dip the plate into the Pyrex dish and let the excess of liquid pour off. The zones of inhibition may, however, be less sharply visible by this method).
- (viii) The bioautogram is placed into the polyethylene box and the lid is closed. The exterior is rinsed with EtOH 70%.

- (ix) Incubate overnight at 37 °C.
- (x) Aqueous solution of INT (20 mg/ml) is prepared and filtered through the membrane filter into the glass TLC sprayer.
- (xi) The bioautogram is sprayed with approx. 5 ml of TTC solution. The plate can be sprayed directly in the polyethylene box.
- (xii) Incubate for 4 h at 37 °C.
- (xiii) Open lid, spray bioautogram with approx. 5–10 ml EtOH, 70%.
- (xiv) Stain control TLC with suitable chromogenic reagent.
- (xv) The bioautogram is evaluated by comparison with the control TLC. Zones of inhibition (indicating the presence of antibacterial compound) on the bioautogram appear as white spots on a pink background. Documentation by Polaroid photography is recommended (optional).

Antifungal assays

Agar tube dilution assay

There is a considerable need to discover new fungitoxic compounds in view of the many plant and human fungal diseases. Some of the important fungal diseases of crop plants are potato late blight, tobacco blue mold, hop downy mildew, Dutch elm disease, ergot of rye, cereal rusts, corn blight, grape downy mildew, etc., and human fungal diseases are athletes foot, aspergillosis, actinomycosis, histoplasmosis, coccidiomycosis, etc.

Materials

- (i) Test fungi (mostly dermatophytes) such as *Epidermophyton floccosum*, *Trichophyton mentogrophytes*, *T. rubrum*, *T. simii*, *T. schoenleinii*, *Microsporum canis*, *Pseudallescheria boydii*, *Candida albicans*, etc.;
- (ii) Sabouraud dextrose agar (composition in gm/l, pepto complex 10, and glucose 40, agar15);
- (iii) Dimethyl sulfoxide (DMSO);
- (iv) Screw test tubes;
- (v) Incubator;
- (vi) Micropipettes;
- (vii) Magnetic stirrer;
- (viii) Autoclave;
- (ix) Standard antifungal drugs such as amphotericin-B, miconazole, ketoconazole, flueytopsine, etc.; and
- (x) Test sample (crude extract, pure natural product or synthetic compound).

Methods

The following sequential steps are involved in agar tube dilution assay:

- (i) Test sample is dissolved in sterile DMSO to serve as stock solution.
- (ii) Sabouraud dextrose agar is prepared by mixing Sabouraud 4% glucose agar and agar-agar in distilled water.

- (iii) It is then stirred with a magnetic stirrer to dissolve it and a known amount is dispensed into screw-capped test tubes.
- (iv) Test tubes containing media are autoclaved at 121 °C for 15 min.
- (v) Tubes are allowed to cool to 50 °C and the test sample of desired concentrations pipetted from the stock solution into the nonsolidified Sabouraud agar media.
- (vi) Tubes are then allowed to solidify in a slanting position at room temperature.
- (vii) Each tube is inoculated with a 4 mm diameter piece of inoculum removed from a seven-day old culture of fungi.
- (viii) All culture containing tubes are inoculated at optimum temperature of 28–30 °C for growth for 7–10 days. Humidity (40–50%) is controlled by placing an open pan of water in the incubator.
- (ix) Cultures are examined at least twice weekly during the incubation.
- (x) After the incubation for 7–10 days, the test tubes with no visible growth of the microorganism are taken to represent the minimum inhibitory concentration (MIC) of the test sample which is expressed in µg/ml.

Antiviral assays

Anti-HIV assay

AIDS (Acquired Immune Deficiency Syndrome) is an immunosuppressive disease which has caused widespread deaths through opportunistic infections and malignancies. A retrovirus, the human immunodeficiency virus (HIV), has been identified as the aetiologic agent causing this disease and approaches to development of drugs against AIDS are, therefore, based on finding substances which can inhibit HIV replication. There are several points at which the intervention of the replicative cycle can be carried out. The inhibition of a multifunctional enzyme, reverse transcriptase (which is a virus-specific RNA-dependent DNA polymerase) offers a possible mode of intervention of the virus life cycle since it transcribes the viral RNA genome to DNA which is ultimately incorporated as pro-viral DNA into the cellular genome. Recently, a colorimetric test has been reported which is simple, sensitive, and rapid involving the transformation of a tetrazolium salt to a colored formazan derivative by living cells but not by dead cells or culture medium.

Materials

- (i) CO₂ incubator with temperature control;
- (ii) Tetrazolium salt, XTT;
- (iii) Dimethyl sulfoxide (DMSO);
- (iv) T4 lymphocytes (CEM cell lines);
- (v) HIV-1 virus (extreme caution)†;
- (vi) Spectrophotometer;
- (vii) Compound microscope;
- (viii) 96-well plates;

- (ix) AZT;
- (x) Multichannel pipettes; and
- (xi) Test sample (crude extract, pure natural product, or synthetic compound).

Methods

The anti-HIV assay consists of following sequential steps:

- (i) The test sample is dissolved in dimethyl sulfoxide, then diluted 1:100 in cell culture medium before preparing serial half-log 10 dilutions. T4 lymphocytes (CEM cell line) are added and after a brief interval HIV-1 is added resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound (test sample) serve as a toxicity control, and infected and uninfected cells without the compound serve as basic controls.
- (ii) Cultures are incubated at 37 °C in a 5% carbon dioxide atmosphere for 6 days.
- (iii) The tetrazolium salt, XTT, is added to all the wells, and cultures are incubated to allow formazan color development by viable cells.
- (iv) Individual wells are analyzed spectrophotometrically for quantitative formazan production and, in addition, are viewed microscopically for detection of viable cells and confirmation of protective activity.
- (v) Drug-treated virus-infected cells are compared with drug-treated noninfected cells and with other appropriate controls (untreated infected and untreated noninfected cells, drug-containing well without cells, etc.) on the same plate.
- (vi) The data is reviewed in comparison with other tests done at the same time and the activity is determined.

Antimitotic assay

Antimitotic assay using sea urchin eggs

Inhibition of cell division is a measure of the antimitotic activity of chemical compounds.

Antimitotic chemical compounds such as vinblastine and podophyllotoxin have been shown to inhibit cell division of fertilized sea urchin eggs and starfish oocytes. The following bioassay provides an easy method for detecting the antimitotic activity of chemical compounds (Jacobs et al. 1981; White and Jacobs 1981).

Materials

- (i) Male and female sea urchins (*Strongylocentrotus purpuratus*)*;
- (ii) KCl, 0.5–0.6 M;
- (iii) Light microscope;
- (iv) Seawater (made by adding 3.8 g of sea salt per liter of distilled water)+;
- (v) Small vials;
- (vi) Incubator;
- (vii) Test tubes;
- (viii) Syringe; and
- (ix) Test sample (crude extract, pure natural product, or synthetic compounds).

The sequential steps involved in antimitotic assay on sea urchin eggs are as follows:

- (i) Sexually mature male and female sea urchins are induced to spawn by the injection of a small amount of KCl solution.
- (ii) White sperms are collected from the male and kept in a small test tube at ice temperature.
- (iii) The eggs collected from the female urchins are washed with cold seawater, and resuspended in seawater (400 ml) to produce a slurry.
- (iv) Sperm (1–2 drops) is added to 50 ml seawater and 1 ml of this solution is added to the slurry of eggs for fertilization to occur.
- (v) Aliquots of the mixture are treated with different concentrations (16–50 mg/ml) of the test sample within five minutes after fertilization. If the test sample is not soluble in water, some organic solvent such as propylene glycol in microliter quantities can be used. An equal quantity of solvent should be added to the control vial.
- (vi) The embryos are allowed to proceed to the first cleavage by placing them on ice after 2–3 h incubation.
- (vii) The incubations are carried out at 14° or 15 °C with frequent agitation of the cells to minimize settling, promote contact inhibition and to ensure efficient sample distribution.
- (viii) Inhibition of cleavage can be observed from random populations totaling 500–600 eggs under a light microscope after an incubation time of 2–3 h. If 80–100% inhibition of cleavage occurred at ~16 mg/ml, the compound is considered to be active.
- (ix) The results are expressed as a percentage (the number of cells cleaved divided by the number of cells not cleaved) relative to a solvent-treated control.

Antimalarial assays

Four species of the parasite *Plasmodium* such as *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* are responsible for causing malaria disease. The female anopheline mosquitoes infected with *Plasmodium* sporozoites (parasites in the salivary gland of female anopheline mosquitoes are called sporozoites) transfer the *Plasmodium* sporozoites with the saliva to the blood of humans or other vertebrate hosts by bites during blood meal. Sporozoites enter the skin and travel through the bloodstream to the liver, where they multiply into merozoites, which return to the bloodstream. Merozoites infect red blood cells, where they develop through several stages to produce either more merozoites or gametocytes. Gametocytes are taken up by a mosquito and infect the insect, the gametocytes develop into male and female gametes which fertilize each other, forming a zygote, zygotes then develop into a motile ookinete, which develops into an oocyst, oocysts divide many times to produce large numbers of small elongated sporozoites. These sporozoites migrate to the salivary glands of the mosquito where they can be injected into the blood of the next host the mosquito bites, repeating the life cycle.

In the initial stage (the pre-erythrocytic phase), the sporozoites disappear from the blood and invade the liver. The malaria symptoms are not apparent at this stage. Infected blood cells containing many more merozoites when burst to release many more merozoites to infect again more erythrocytes, thereby multiplying the number of infected erythrocytes. When the erythrocytes burst, an intense feeling of cold is produced in the patient, causing shivering.

Well plate *in vitro* schizonticidal assay

Materials

- (i) Malarial parasite (*P. falciparum*) culture;
- (ii) 96 well microtiter plates;
- (iii) Medium RPMI-1640 buffered with 40 mM TES and supplemented with 2.0 g l-1 glucose and 10% human O + serum; pH 7.4;
- (iv) Fresh human RBCs;
- (v) Double distilled deionized water;
- (vi) Dimethyl sulfoxide (DMSO);
- (vii) Absolute methanol;
- (viii) Giemsa stain;
- (ix) CO₂ incubator;
- (x) Light microscope;
- (xi) Chloroquine; and
- (xii) Test sample (plant extract, pure natural product or synthetic compounds).

Methods

The procedure adopted for antimalarial assay consists of following sequential steps:

- (i) A drop of the malarial parasite culture is taken and at least 500 erythrocytes are counted, making a note of the number that contains parasites (excluding gametocytes). To estimate the percentage parasitemia, the number of infected erythrocytes is divided by 5.
- (ii) The culture is diluted so that final parasitemia is 2.0%.
- (iii) The culture is grown at 37 °C in the presence of 5% CO₂ for 32 h. The erythrocytes are centrifuged and resuspended in fresh culture medium RPMI-1640, supplemented with 10% human O + serum.
- (iv) 100 µl aliquots are distributed into sterile 96-well microtiter plate and 10 µl containing various concentrations of the test compound (solubilized in 0.5% DMSO/MeOH) is added and culture is placed in humidified CO₂ (5%) incubator at 37 °C.
- (v) Negative (−ve) control is administered 10 µl PB S in place of the drug while positive (+ve) control contains standard drug (s).
- (vi) Thin blood films of the culture are prepared after 24, 36, and 72 h. The slides are stained with Giemsa stain and the number of parasites per 1000 erythrocytes are determined by microscopic examination.

- (vii) Total parasitemia is calculated and plotted as percentage control against concentration and the half maximal inhibitory concentration (IC_{50}) is determined.

Larvicidal assay

Malaria, yellow fever, and dengue fever are some of the most widespread parasitic diseases prevalent in the tropics and subtropics. Mosquitoes of the genera *Anopheles* and *Aedes* are vectors of these diseases. One way of controlling these tropical diseases is by eliminating the vector of the parasite. Plant-derived and other natural larvicides can be used for the elimination of these vectors (Zarroug et al. 1988).

Materials

- (i) Common mosquitoes such as *Anopheles arabiensis* (vector of malaria), *Aedes aegypti* (vector of yellow fever) and *Culex quinquefasciatus* (urban nuisance mosquito);
- (ii) Temperature controlled insectary;
- (iii) Turf (a piece of soil with grass and plant roots);
- (iv) Baby food such as Farex;
- (v) Rabbits;
- (vi) Small quantities of fresh human blood;
- (vii) Glucose;
- (viii) Petri dishes;
- (ix) Filter paper;
- (x) Distilled water; and
- (xi) Small bowls.

Methods

The larvicidal assay involves the following sequential steps:

- (i) Mosquitoes are maintained in the insectary with a temperature range of 28–30 °C and 70–80% relative humidity. A piece of turf is added as a source of food for young larvae.
- (ii) After molting to the second instar stage, the larvae are fed with a small amount of baby food. Adults are fed on rabbits. Female *An. arabiensis* are occasionally fed with human blood. Male *An. arabiensis* are fed on a 10% glucose solution.
- (iii) Mosquito eggs are collected on moist filter paper placed on a petri dish. These eggs can be kept in a regulated atmosphere (26–28 °C, 70–80% rel. humidity) for up to six months.
- (iv) The larvae hatch readily when the eggs are placed in a bowl of tap water.
- (v) Different concentrations of test sample (1000, 500, 250, 100, 50, and 10 ppm) are dissolved in distilled water (small bowls) for the test. A minimum of eight replicates are required for each concentration.

- (vi) One-day-old larvae (25 per bowl) are then transferred into bowls containing the test sample with caution. The exposure period is 24 h during which no food is offered to the larvae.
- (vii) The killing effect of the test sample is assessed after 30 min and 24 h of exposure.
- (viii) Percent mortality is calculated to represent the larvicidal activity of each sample.

Molluscicidal activity

The snail *Biomphalaria glabrata* acts as the vector for rapidly spreading the tropical endemic schistosomiasis disease and over 200 million people are infected with this disease. Vector control is always considered to be an important step in the irradiation of tropical diseases. The molluscicidal assay provides a simple tool to screen different natural products for the control of vector snails (Hostettmann et al. 1982).

Materials

- (i) Snails of the species *Biomphalaria glabrata* kept in aquaria with a continuous circulation of water through a filtering system (water temperature at 24 °C);
- (ii) Distilled water;
- (iii) Petri dishes;
- (iv) Microscope;
- (v) Ultrasonic bath; and
- (vi) Test sample (crude extract, pure natural product or synthetic compound).

Methods

The following steps are involved in the molluscicidal activity assay:

- (i) Solutions of the test sample in distilled water (32, 16, 8, 4, and 2 ppm) are prepared with two replicates for each treatment. If the compounds or extracts are weakly soluble in water, solutions should be placed in an ultrasonic bath for one hour prior to the bioassay.
- (ii) Two snails of uniform size (average diameter of the shell, 9 mm) are placed in each concentration of the test samples.
- (iii) After 24 h these snails are transferred to Petri dishes, light is shone from the bottom of the Petri dish and the heartbeat is checked by a microscope.
- (iv) Mortality is counted at each concentration and the result of the bioassay is recorded as +ve (active) or -ve (inactive).

Piscicidal assay

There is a close relationship between molluscicidal and piscicidal activities, since the molluscicidal compounds are also usually very toxic to fish. Piscicidal compounds often possess other bioactivities such as insecticidal, plant growth inhibitory, insect antifeedant, antitumor, cocarcinogenic and irritant activities (Kawazu 1981).

Materials

- (i) Killie-fish (*Oryzias latipes*)—Commercially available as an ornamental fish;
- (ii) Organic solvents (methanol, acetone, DMSO, etc.);
- (iii) Air pump;
- (iv) Pipettes;
- (v) Beakers (200 ml);
- (vi) Incubator;
- (vii) Water tank;
- (viii) Fish food; and
- (ix) Test sample (crude extract, pure natural product or synthetic compound).

Methods

The piscicidal activity assay involves the following sequential steps:

- (i) Killie-fish averaging 350 mg in weight and 3–3.5 cm in length are reared in a water tank and not fed for two days, prior to the test.
- (ii) Various concentrations of test samples in organic solvents such as methanol (5000, 2500, 1000, and 500 ppm) are prepared.
- (iii) These solutions are then added to beakers containing 150 ml water and aerated for two days with an air pump before the test.
- (iv) Organic solvent alone serves as a control.
- (v) Five killie-fish are introduced into beakers containing various concentrations of test samples. These beakers are then kept in an incubator to maintain the temperature between 18 and 19 °C.
- (vi) Fish that die during the test are immediately removed from the solutions to avoid toxic effects.
- (vii) The number of surviving fish in each beaker is recorded after 24 h after the initial introduction.
- (viii) The results are represented as minimum lethal concentrations (MLC).
- (ix) If more than one fish dies in the control beaker, the test should be repeated.
- (x) If test sample is poorly soluble in methanol, the minimum possible quantity of acetone or DMSO can be used. The same quantity of this solvent should be mixed into the control.

Assays for agrochemicals

***Lemna minor* for phytotoxicity and growth stimulating assay**

Weeds are one of the major factors of poor agricultural productivity in the developing world. Synthetic weedicides (herbicides) are often expensive, toxic, and nonspecific. Weedicides from natural sources having improved characteristics could, therefore, have a promising future. The *Lemna minor* phytotoxicity assay is a useful primary screen for weedicide search. This bioassay has the added advantage of being able to predict the growth stimulating effect of the test sample.

The *Lemna* assay is a quick measure of phytotoxicity of the materials under investigation.

Lemna minor L. (duckweed) (Lemnaceae) is a miniature (1.5 × 1.5 mm) aquatic thalloid monocot. *Lemna* plants consist of a central oral frond or mother frond with two attached daughter fronds and a filamentous root. These plants are generally found in water ponds and other freshwater bodies (Einhelling et al. 1985).

Materials

- (i) *Lemna minor* L*;
- (ii) E-Medium **(about 80 ml per compound);
- (iii) Syringes: 10 ml, 100 µl, 500 µl, and 2 µl;
- (iv) 2 dram vials (40 per compound);
- (v) Large glass container with glass lid to hold vials; (stopcock grease should be applied on the top edges of the tank in order to form a seal with the lid to avoid moisture loss);
- (vi) Growth chamber with temperature range of 27–29 °C and continuous fluorescent and incandescent light; and
- (vii) Test sample (crude extract, pure natural product or synthetic compound).

Methods

The commonly used procedure for *Lemna* assay consists of the following sequential steps:

- (i) Prepare inorganic medium (E-Medium)*: add KOH pellets to attain pH 5.5–6.0.
- (ii) Prepare vials for testing: 10 vials per dose (500, 50, 5 ppm, control) #.
 - a. Weigh 15 mg of compound or extract and dissolve in 15 ml solvent.
 - b. Add 1000, 100, and 10 µl solutions to vials for 500, 40, and 5 ppm; allow solvent to evaporate overnight.
 - c. Add 2 ml of E-medium, and then a single plant containing a rosette of three fronds to each vial. Only healthy and green rosettes should be used.
- (iii) Place vials in a glass dish filled with about 2 cm water, seal container with stopcock grease and glass plate.
- (iv) Place dish with vials in growth chamber for seven days at 26 °C under fluorescent and incandescent light.
- (v) Count and record number of fronds per vial on day 3 and day 7.
- (vi) Analyze data as percent of control with ED50 computer program † to determine FI50 values and 65% confidence intervals.

*E-MEDIUM

Constituent	mg/l
KH ₂ PO ₄	680
KNO ₃	1515
Ca(NO ₃) ₂ .4 H ₂ O	1180
MgSO ₄ .7 H ₂ O	492
H ₃ BO ₃	2.86

MnCl ₂	3.62
FeCl _{3.6} H ₂ O	5.40
ZnSO _{4.7H₂O}	0.22
CuSO _{4.5H₂O}	0.08
Na ₂ MoO _{4.2H₂O}	0.12
EDTA	11.2

Hypoglycemic/antidiabetic activity assays

The evaluation of medicinal plants and their active natural principles may be helpful for searching new drugs to treat diabetes. A number of bioassays have been established for this purpose (Akhtar et al. 1981).

Antidiabetic activity assay on normal and alloxan-diabetic rabbits/male Wistar albino rats

Materials

- (i) Alloxan monohydrate (BDH);
- (ii) Carboxymethylcellulose (CMC);
- (iii) D-Glucose;
- (iv) Xylene;
- (v) Male, adult, healthy albino rabbits (750–1100 g)/albino rats;
- (vi) *o*-Toluidine reagent;
- (vii) Wooden animal (rabbit/rats) holder;
- (viii) Stainless steel feeding needles;
- (ix) Distilled water;
- (x) Plastic syringe;
- (xi) Syringe;
- (xii) Ethyl alcohol;
- (xiii) Cotton; and
- (xiv) Test sample (crude extract, pure natural product or synthetic compound).

Methods

The antidiabetic activity assay involves the following sequential steps:

Preparation of diabetic rabbits/rats

- (i) A group of rabbits is made diabetic by injecting intravenously 150 mg/kg body weight of alloxan monohydrate. Extreme caution is required to avoid accidental injection in the human body.
- (ii) Eight days after injection, the blood glucose levels of all the surviving rabbits are determined by the *o*-toluidine method (as described below).
- (iii) Rabbits/rats with blood glucose levels of 200–500 mg/100 ml are considered as diabetic and employed for the bioassay.

Grouping of rabbits/rats

- (i) Normal and alloxan-diabetic rabbits are randomly divided into five groups of six animals each.
- (ii) Group 1 serves as a control and receives orally 10 ml of 1% carboxymethyl cellulose (CMC) in water.
- (iii) A 0.2 ml sample of blood is immediately collected from group I animals for the blood glucose determination.
- (iv) Blood samples are also drawn at 5, 10, and 24 h intervals after the administration of 1% CMC.
- (v) The animals of groups II, III, IV, and V are treated orally with 0.25, 0.5, 1.00, and 1.5 g/kg body weight of test sample suspended in 1% CMC in water, respectively.

Preparation and administration of drug suspension

- (i) The amount of test sample required for each rabbit is calculated on body weight basis.
- (ii) The required quantity of extract is suspended in 6 ml of 1% CMC (in water) solution and the final volume made up to 10 ml.
- (iii) The test sample is then administrated orally to each animal by using a stainless feeding needle on a plastic syringe containing 10 ml of the suspension.
- (iv) The feeding needle is inserted into the stomach through the esophagus and the plunger pressed slowly and steadily (immediate sneezing and coughing indicate penetration of the needle into the lung; in this case, the animal should be rejected and another animal should be taken instead).

Collection of blood

- (i) After test sample administration, the animal is held in a wooden rabbit holder and immediately 0.2 ml of blood is collected from an ear vein.
- (ii) Similar samples of 0.2 ml of blood are also collected at 5, 10, and 24 h time intervals. (To prevent coagulation of blood, it is sometimes necessary to dampen the test animal's ear with xylene to promote flow of blood. Xylene causes an inflammatory response, resulting in the blood vessel enlargement and dilation.)
- (iii) After collecting the blood, the pricked side of the ear is rubbed with cotton wool soaked with ethyl alcohol to protect the rabbit against infection.

Determination of blood glucose

- (i) Blood glucose is determined by the method of Fings et al. (1970) using the *o*-toluidine reagent. The *o*-toluidine method is one of the most widely used manual methods.

Statistical analysis

- (i) The blood glucose levels in the various groups are expressed in mg/100 ml (Means \pm SEM) and the data is statistically analyzed by using the variance technique with factorial arrangement.
- (ii) The decrease in blood glucose levels of normal and diabetic rabbits produced by different doses of test sample, found at different time intervals, are compared by using Duncan's Multiple New Range Test (Snedecor 1965).
- (iii) The standard curve for glucose estimation can be drawn by plotting blood glucose levels of normal rabbits (mg/100 ml) at various time intervals (hours) after oral administration of 1% CMC solution and test sample (0.25, 0.5, 1.0, and 1.5 g/kg body weight) orally, suspended in 1% CMC.

Diuretic activity assay

Plant extracts and pure natural products often have a diuretic action which can be screened by a simple *in vivo* bioassay (Kawashima et al. 1985; Schales and Schales 1941).

Materials

- (i) Male Wistar rats (196 ± 1 g);
- (ii) Bicarbonate saline;
- (iii) Flame photometer;
- (iv) Osmometer;
- (v) Measuring cylinder;
- (vi) Metabolism cage;
- (vii) Gavage; and
- (viii) Test sample (crude extract, pure natural product or synthetic compound).

Methods

The diuretic activity assay involves the following sequential steps:

- (i) The rats are randomly divided into five groups of 12 each.
- (ii) The animals are fasted overnight and allowed free access to drinking water.
- (iii) Different concentrations of test sample dissolved in a vehicle (sodium bicarbonate) are administered at a volume of 50 ml/kg by gavage to different groups of rats.
- (iv) A control group of rats is administered with a pure vehicle (bicarbonate saline) at a volume of 50 ml/kg by gavage.
- (v) Individual rats are placed in a metabolism cage. Urine is collected into a graduated cylinder and its volume is recorded at 30 min intervals for 4 h.
- (vi) Urinary concentrations of sodium and potassium are determined by a flame photometer. Chloride concentration in the urine is measured by the method of Schales and Schales (1941).
- (vii) Urinary osmolality is determined with an osmometer. Eight significant and dose-related increases in urinary excretion of water (UV) are compared to the vehicle-treated control.

- (viii) Means \pm SEM can be presented as figures. Statistical significance can be calculated according to the WSD method for comparison of the data among the means of the experimental group.

Anti-inflammatory assay

Rat paw edema assay

Materials

- (i) Male Wistar rats (Nossan, 120–140 g);
- (ii) Indomethacin (Sigma);
- (iii) Carboxymethylcellulose (CMC) (Sigma);
- (iv) Diethyl ether;
- (v) Syringes (0.1 ml, 0.5 ml);
- (vi) Carrageenan (Sigma);
- (vii) Plethysmometer; and
- (viii) Test sample (crude extract, pure natural product or synthetic compound).

Methods

The sequential steps involved in the anti-inflammatory assay are the following:

- (i) Male Wistar rats are fasted for 12 h before the experiment.
- (ii) Groups of at least five rats are given 0.5 ml of test sample suspended in 0.5% carboxymethylcellulose.
- (iii) One group of five rats is given the standard drug indomethacin (5 mg/kg) in 0.5% CMC.
- (iv) The control group of five rats is given only the vehicle (0.5 ml of 0.5% CMC).
- (v) After one hour of drug administration, rats are lightly anesthetized with diethyl ether and paw edema is induced by single subplanar injection of 0.1 ml of 1% carrageenan.
- (vi) Paw volumes are measured using a water plethysmometer immediately before the injection of carrageenan and at hourly intervals for 5 h thereafter.
- (vii) The volume of edema is expressed for each rat as the difference before and after the injection of carrageenan.
- (viii) The percent inhibition of edema is calculated for each group (test sample-treated group and standard drug-treated group) versus its vehicle-treated control group.
- (ix) Data are analyzed using unpaired student's t-test and a $p < 0.05$ (probability) is taken as significant (Aquino et al. 1991).

In vitro screening active principles for anti-inflammatory activity

The anti-inflammatory activity of plant extract may also be studied by using inhibition of albumin denaturation technique following standard techniques (Mizushima and Kobayashi 1968; Sakat et al. 2010; Leelaprakash and Dass 2011). The reaction mixture consisted of test extracts and 1% aqueous solution of bovine albumin

fraction, pH of the reaction mixture was adjusted using small amount of 1 N HCl. The sample extracts were incubated at 37 °C for 20 min, and then heated to 51 °C for 20 min, after cooling the samples the turbidity was measured at 660 nm. (UV Visible Spectrophotometer Model 371, Elico India Ltd) The experiment was performed in triplicate. Aspirin, Diclofenac sodium, and Indomethacin were used as standard drugs. The percentage inhibition of protein denaturation was calculated as follows: Percentage inhibition = (Abs Control – Abs Sample) × 100/Abs control.

Albumin denaturation inhibitory activity

The assay was carried out by adopting the methods described by Kumari et al. (2015) with some modifications in which the volume of each component in the reaction mixtures was reduced by half. The plant extracts and positive standards (ibuprofen and diclofenac) were prepared at a concentration of 0.1% each (1.0 mg/ml). A reaction vessel for each mixture was prepared consisting of 200 µl of egg albumin, 1400 µl of phosphate buffered saline, and 1000 µl of the test extract. Distilled water instead of extracts were used as a negative control. Afterward, the mixtures were incubated at 37 °C for 15 min, and then heated at 70 °C for 5 min. After cooling, their absorbances were measured at 660 nm (Jasco V-630 Spectrophotometer, Japan) and the data were processed by Spectra Manager system. The inhibition percentage of protein denaturation was calculated using the following formula:

$$\% \text{ Denaturation inhibition} = (1 - D/C) \times 100$$

where D is the absorbance reading of the test sample, and C is the absorbance reading without test sample (negative control).

In vitro anticancer screening

In the cancer field, in vitro assays are primarily of two types, namely, molecular assays or cellular assays. Molecular assays are directed at a single subcellular target and they are, therefore, highly particular. They are of particular importance when a specific mechanism is of interest in a drug discovery program, and binding assays or inhibition assays can be used to discover new compounds having a specific type of activity. Because of their specificity, such assays result in a low hit rate from a large number of diverse samples screened. A battery of such screens is often used in conjunction in order to detect compounds working by more than one mechanism. A disadvantage is that interesting and important bioactive compounds not acting by the particular mechanism for which the screen is setup will be missed.

Cellular assays may be divided into two types, namely, (a) cytotoxicity assays, and (b) other assays types (including morphological assays). A simple example of a cytotoxicity assay may be to measure the 50% growth inhibitory concentration against a single cell line, but this could lead to a large number of “active” materials, many of which could be uninteresting substances such as detergents, heavy metals, protein denaturants, nonselective DNA alkylating agents, mitochondrial poisons; etc. Selecting out the really interesting compounds from a large number of “hits” can be difficult. The final choice of the type of assay to be employed must depend

on the precise interest of the researcher. It is possible to use both types of assays in conjunction with one another, the initial cytotoxicity assays giving a large number of positive leads, which are then further screened by biochemical assays to select compounds acting through mechanisms of interest. One must, however, take care of employing antagonistic screens, i.e., the positive leads of one assay should be further tested in another assay which is working by an unrelated mechanism.

Cell growth and cytotoxicity assays

The following are the four main types of nonradioactive cell growth and cytotoxicity assays:

- (i) cell or colony counts, and assays;
- (ii) macromolecular dye binding;
- (iii) metabolic impairment; and
- (iv) membrane integrity.

No single method is universally appropriate for all situations. Each has limitations, and all are subjected to potentially serious artifacts under certain circumstances. Cell and colony counts are time-consuming, tedious, and sensitive to minor variations in methodology. Dye binding assays come closest to fulfilling the ideal requirements for growth and cytotoxicity assays. They are simple, rapid, reliable, sensitive, and quantitative but do require access to ELISA reader. Metabolic impairment assays measure the decay of enzyme activity or metabolite concentration following toxic insult. They are generally more complex and artifact prone than dye binding assays. Membrane integrity assays measure the ability of cells to exclude impermeant extracellular molecules, either be colorimetric or fluorescent or require an ELISA reader and/or a fluorescent plate reader. They tend to be fewer artifacts prone than metabolic impairment assays.

Experimental design

Seeding density

Seeding density basically depends on cell size, growth rate, and assay duration and must be determined individually for each cell type. However, in a 48–72 h assay, seeding density is usually kept between 5×10^3 – 10^4 cells/well in 96-well microtiter plate.

Drug solubilization

Stock solutions (1.0 mg/0.05 ml) of polar compounds are made in water while of nonpolar compounds are made in 1:1 EtOH DMSO and then diluted with complete medium to the final test concentration. DMSO is toxic to most cells at concentrations above 0.5%. Preliminary experiments should be carried out to determine its toxicity threshold for each individual cell line.

Assay duration

Assay duration depends on growth rate and seeding density and should be determined individually for each cell type. However, for most transformed cell lines 48–72 h period is usually adequate to detect the effect of the drug.

Control wells

Every test plate should include following five different types of samples:

- (i) Medium blanks (MB) growth medium with no cells or drugs;
- (ii) Drug blanks (DB) growth medium with drug but no cells;
- (iii) -ve control cells plus medium;
- (iv) +ve control cells plus standard drug(s); and
- (v) Test medium plus cells plus test compound.

A. Dye binding assay

Sulforhodamine B (SRB) assay

Sulforhodamine B (SRB) is a bright pink aminoxanthene dye. Under mildly acidic conditions, SRB binds to basic amino acid residues of TCA fixed proteins. It provides a stable end point that does not have to be measured within any fixed period of time. Once stained and air dried, plates can be kept for months before solubilization and reading. This assay has proven particularly useful in large-scale drug screening.

Materials

1. Human tumor cell lines H157 and H1299 lung carcinoma, HT-144 malignant melanoma, Zr-75-1 and MCF7 breast carcinoma, SK-CO-1 and SW403 colon carcinoma, SK-OV-3 ovarian carcinoma, and HT 1376 bladder carcinoma;
2. Tissue culture flasks 25 cm²;
3. 96-well microtiter plates;
4. Medium RPMI-1640 buffered with 2.2 g 1–1 NaHCO₃ supplemented with 10% heat inactivated fetal bovine serum (HIFBS); pH 7.4;
5. DMSO-etOH 1:1;
6. Double distilled deionized water;
7. 10% Trichloroacetic acid (TCA);
8. 0.1% SRB in 1.0% glacial acetic acid
9. 10 mM unbuffered Tris base;
10. CO₂ incubator;
11. ELISA reader;
12. Adriamycin, *cis*-Platin, 5-fluorouracil, mitomycin C, and vinblastine; and
13. Test sample (plant extract, pure natural product or synthetic compounds)

The general strategic procedure for SRB assay is given below:

1. Cells are seeded onto 96-well microtiter plates at a concentration of 5 × 10⁴–10⁵ cells ml⁻¹, volume 200 µl/well.
2. Plates are incubated at 36.5 °C in humidified CO₂ (10%) incubator for 24 h.
3. Old medium is removed and fresh medium is added.
4. 10 µl containing various concentrations of test compound is added; whereas +ve and -ve control has standard drug (s) and no drug, respectively.

5. Plates are incubated for next 48–72 h at 36.5 °C in humidified CO₂ (10%) incubator.
6. After incubation, medium is removed from the wells and 200 µl of 10% TCA is added. Plates are kept at 4 °C for 30 min.
7. TCA is removed, plates are washed gently under tap water and air dried at room temperature.
8. 100 µl SRB reagent is added to each well and left for 15 min.
9. SRB is removed, wells are washed four times with 1.0% acetic acid and air dried.
10. Stain is solubilized with 0.2 ml 10 mM unbuffered Tris base and absorbance is measured at 540 nm.
11. ED₅₀ value of compounds possessed cytotoxic activity is calculated.

B. Cellular biomass assays

1. Propidium iodide (PI) assay

Thionin, Azure A, and Toluidine Blue O are biomass stains that approach the protein stains in sensitivity. Propidium iodide is a general biomass stain that binds to RNA, DNA, proteins, and glycosaminoglycans.

Materials

1. Human tumor cell lines H157 and H1299 lung carcinoma, HT-144 malignant melanoma, Zr-75-1 and MCF7 breast carcinoma, SK-CO-1 and SW403 colon carcinoma, SK-OV-3 ovarian carcinoma, and HT 1376 bladder carcinoma;
2. Tissue culture flasks 25 cm²;
3. 96-well microtiter plates;
4. Medium RPMI-1640 buffered with 2.2 g l – 1 NaHCO₃ supplemented with 10% heat-inactivated fetal bovine serum (HIFBS); pH 7.4;
5. DMSO-EtOH 1:1;
6. Double distilled deionized water;
7. 20% propidium iodide in distilled water (light sensitive);
8. CO₂ incubator;
9. Fluorescent plate reader;
10. Adriamycin, *cis*-Platin, 5-fluorouracil, mitomycin C, and vinblastine; and
11. Test sample (plant extract, pure natural product or synthetic compounds).

The following procedure is used for the PI assay:

1. Cells are seeded onto 96-well microtiter plates at a concentration of 5 × 10⁴–10⁵ cells ml⁻¹, volume 200 µl/well.
2. Plates are incubated at 36.5 °C in humidified CO₂ (10%) incubator for 24 h.
3. Old medium is removed and fresh medium is added.

4. 10 μ l containing various concentrations of test compound is added whereas +ve and -ve control has standard drug(s) and no drug, respectively.
 5. Plates are incubated for next 48–72 h at 36.5 °C in humidified CO₂ (10%) incubator.
 6. Plates are kept at –30 °C for 2–6 h and thawed at 50 °C for 15 min.
 7. To each well, 50 μ l of 20% PI stock solution is added so that final PI concentration is 400 μ g/ml.
 8. Plates are incubated in dark for 60 min at room temperature.
 9. Fluorescence is read at 530/590–620 nm in a fluorescent plate reader and ED is calculated.
2. Hoechst 33258 fluorescence assay

Hoechst 33,258 is a UV-excited blue bisbenzimidazole dye which selectively intercalates into the A-T rich regions of DNA.

Materials

1. Human tumor cell lines H157 and H1299 lung carcinoma, HT-144 malignant melanoma, Zr-75-1 and MCF7 breast carcinoma, SK-CO-1 and SW403 colon carcinoma, SK-OV-3 ovarian carcinoma, and HT 1376 bladder carcinoma.
2. Tissue culture flask 25 cm²;
3. 96-well microtiter plates
4. Medium RPMI-1640 buffered with 2.2 g l⁻¹ NaHCO₃ supplemented with 10% heat-inactivated fetal bovine serum (HIFBS); pH 7.4;
5. DMSO-EtOH 1:1;
6. Double distilled deionized water;
7. TNE Buffer (10 mM Tris, 1 mM EDTA, 2 M NaCl, pH 7.4);
8. 2% Hoechst 33,258 in TNE buffer (light sensitive);
9. CO₂ incubator;
10. Fluorescent plate reader;
11. Adriamycin, *cis*-platin, 5-fluorouracil, mitomycin C, and vinblastine; and
12. Test sample (plant extract, pure natural product or synthetic compounds).

The procedure used for the Hoechst 33,258 fluorescence assay is given below:

1. Cells are seeded onto 96-well microtiter plates at a concentration of 5×10^4 – 10^5 ml⁻¹, volume 200 μ l/well.
2. Plates are incubated at 36.5 °C in humidified CO₂ (10%) incubator for 24 h.
3. Old medium is removed and fresh medium is added.
4. 10 μ l containing various concentrations of test compound is added whereas +ve and -ve control has standard drug(s) and no drug, respectively.
5. Plates are incubated for next 48–72 h at 36.5 °C in humidified CO₂ (10%) incubator.
6. Medium is removed from the wells.
7. Plates are kept at –80 °C for 1–2 h and thawed at 50 °C for 15 min.
8. To each well, 100 μ l distilled water is added and plates are incubated at room temperature for 1 h.

9. Plates are refreezed at -80°C for 90 min and thawed at room temperature.
10. 0.1 ml of TNE containing $20 \mu\text{l ml}^{-1}$ of Hoechst 3325 dye is added and mixed well on a plate shaker.
11. Plates are incubated in dark for 90 min at room temperature.
12. Fluorescence is read at 350/460 nm in a fluorescent plate reader and ED₅₀ is calculated.

Some common *in vitro* enzyme-based bioassays

Protease inhibition assays

Proteases or proteinases are the proteolytic enzymes which play a vital role in the normal physiological functions of cells, e.g., protein maturation, digestion, blood coagulation, control of blood pressure, immune response, etc. (Fig. 9.2). A variety of diseases such as cancer, pulmonary emphysema, muscular dystrophy, arthritis, pancreatitis, etc., are associated with the excessive activity of proteases. The role of proteases in diseases, therefore, provides targets for the possible treatment of a wide range of diseases by protease inhibitors as therapeutic agents from natural sources.

1. Proteases and their specific chromogenic substrates

Protease Substrate

- (i) Chymotrypsin n-succinyl-l-phenylalanine p-nitroanilide
- (ii) Trypsin Bz-DL-Arg-p-nitroanilide;

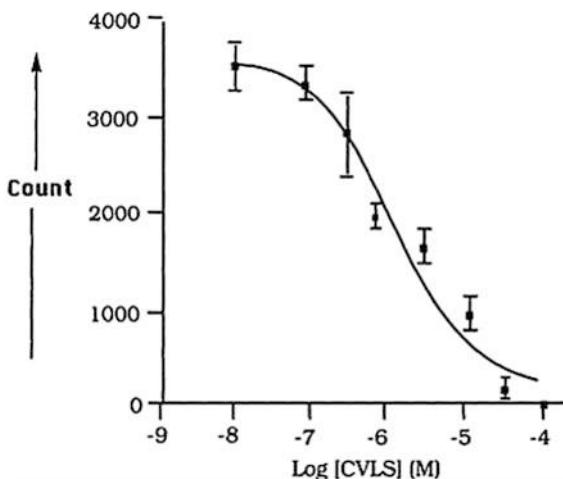


Fig. 9.2 CVLS competition for farnesylation of biotinyl-lamin. The assay contained final concentrations of 1 mg enzyme, 10 mM [³H]-FPP, 70 nM biotinyl-lamin, and indicated concentrations of CVLS; incubations were performed for 60 min at room temperature. Data points, representing the mean \pm SEM of triplicate determinations, were made at indicated CVLS concentrations. The curve represents the fit of data to a one-site competition equation using the nonlinear least squares curve fitting program, Prism

- (iii) Elastase Suc-(Ala)3-p-nitroanilide;
- (iv) Carboxypeptidase A Hippuryl-L-phenylalanine
- (v) Papain Bz-DL-Arg-*p*-nitroanilide.

Materials

1. Tris(hydroxymethyl) aminomethane;
2. Buffer A(pH 7.5):
 - Tris-HCl (1 M)
 - NaCl (0.5 M);
3. Buffer B (pH 8.6):
 - Tris-HCl (0.4 M)
 - MgCl₂ (5 mM);
4. Buffer C (pH 7.5):
 - Tris-HCl (50 mM)
 - L-Cysteinum chloride (1mM)
 - EDTA.Na₂ (2 mM);
5. Lithium chloride (10% w/v);
6. DMSO;
7. HCl(5 M);
8. Volumetric flasks;
9. Measuring cylinders;
10. Beakers;
11. Micropipettes;
12. pH meter;
13. UV/Vis spectrophotometer;
14. Deionized water;
15. Timer;
16. Eppendorf tubes (siliconized);
17. 96-well microplates (flat bottom);
18. Microplate reader; and
19. Test sample (crude extract, pure natural or synthetic compounds).

Principle

Chromogenic substrates of proteases have a specific amino acid sequence linked to a chromophore such as *p*-nitroaniline. The action of a specific protease on its substrate causes the release of the chromophore which is measured as increase in absorbance in a recording spectrophotometer. Therefore, the amount of chromophore liberated is proportional to the activity of the enzyme.

(a) Preparation of solutions:

1. Tris-HCl Buffer
 - 121.14 gm of Tris (hydroxymethyl)-aminomethane are dissolved in deionized water.
 - The pH is adjusted to the required value with HCl (5 M), and the volume is made up to 1 L with water.

2. Assay buffers for proteases.
3. Proteases can be dissolved in the following buffers.
4. Substrates solutions can be dissolved in the following buffers.

(b) Assay procedure

Assay buffer 400 μ l
Enzyme solution 100 μ l
Test sample 1 ml
Substrate solution 1.5 ml

1. 500 μ l of assay buffer is dispensed in a clean and dry test tube.
2. 500 μ l of protease enzyme solution is added.

Protease buffer Molar concentration (M) buffer pH

1. Chymotrypsin Tris-HCl 0.4 7.5;
2. Trypsin Tris-HCl 0.4 7.5;
3. Elastase Tris-HCl 0.4 8.6;
4. Carboxypeptidase A A—7.5;
5. Leucine B—8.6; and
6. Papain C—7.5.

Proteases buffer concentration (mM) buffer pH enzyme (Units/ml)

1. -ChyipotrypsinTris-HCl 50 7.5 9.0;
2. Trypsin Tris-HCl 50 7.5 150;
3. Elastase Tris-HCl 50 8.6 0.6;
4. Carboxypeptidase A LiCl (10% w/v)—0.4;
5. Leucine amino-peptidase B—8.6 4.0; and
6. Papain C—7.5 6.0.

Substrates (mM) buffer Molar concentration (mM) buffer pH substrate concentration

1. N-Suc-Phe-*p*-nitroanilide Tris-HCl 50 7.5 2.6;
 2. Bz-DL-Arg-*p*-nitroanilide Tris-HCl 50 7.5 1.0;
 3. Suc (Ala)3-*p*-nitroanilide Tris-HCl 50 8.6 1.55;
 4. Hippuryl-L-Phenylalanine A—7.5 1.70; and
 5. L-leucine-*p*-nitroanilide B—8.6 17.40.
3. 1.0 ml of test sample is added and the contents are mixed.
 4. The assay mixture is incubated at 37 °C for 30 min.
 5. Finally, 1.0 ml of substrate solution is added and the absorbance is monitored continuously in a recording spectrophotometer for 15 min at appropriate wavelength (410 nm for all substrates except for hippuryl-L-phenylalanine, 254 nm).

96-well microplate assay procedure is as follows:

1. 50 µl portions of assay buffer are dispensed in each well of a flat bottom 96-well microplate.
2. 50 µl of protease enzyme solution is added.
3. 100 µl of test sample is added and the contents are mixed.
4. The assay mixture is incubated at 37 °C for 30 min in a microplate reader.
5. 100 µl of substrate solution are dispensed and the absorbance is monitored continuously in the microplate reader for 15 min with appropriate wavelengths.

[Note: The organic solvent used to dissolve the test sample may affect the enzyme activity.]

Controls should be run to measure the change in enzyme activity. Positive controls should also be run with appropriate standard inhibitors. Conditions of the assays should be chosen such that the maximum amount of test sample should be added to each assay with a minimum amount of organic solvent. Although many organic solvents can be used but DMSO is one of the most convenient as it is water miscible and directly compatible with these assay systems.

Calculation of percentage inhibition:

Percentage inhibition in enzyme activity after incubation can be calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

Tyrosinase inhibition assay

Tyrosinase is an enzyme responsible for the synthesis of dermal melanin pigment from L tyrosine and L-DOPA (dihydroxyphenylalanine) within the melanocytes on the melanosomes. It has several functions including hydroxylation of L-tyrosine and oxidation of L-DOPA to dopaquinone and subsequent autopolymerization to melanin. The overproduction of melanin has been associated with the condition of hyperpigmentation of skin such as melasma and ephelides. Therefore, the inhibition of tyrosinase enzyme is reflective of controlling hyperpigmentation and associated conditions.

Materials

1. Phosphate buffer (0.1 M, pH 6.8);
2. L-3,4,-Dihydroxyphenylalanine (L-DOPA);
3. L-Tyrosine;
4. Mushroom tyrosinase enzyme;
5. DMSO;
6. Volumetric flasks;
7. Measuring cylinders;
8. Beakers;

9. Micropipettes;
10. pH meter;
11. Timer;
12. Water bath;
13. Deionized water;
14. UV/VIS spectrophotometer;
15. Eppendorf tubes (siliconized);
16. 96-well microplates (flat bottom);
17. Microplate reader; and
18. Test sample (crude extract, pure natural product, or synthetic compound).

Tyrosinase first hydroxylates L-tyrosine to L-DOPA and then oxidizes L-DOPA to dopaquinone which is subsequently converted to dopachrome. The activity of tyrosinase enzyme is proportional to the amount of dopachrome liberated that is measured at 475 nm in a spectrophotometer.

(1) Preparation of solutions

(a) Phosphate buffer (0.1 M, pH 6.8).

(I) Solution-A (0.2 M Na₂ HPO₄)

35.6 gm of Na₂HPO₄•2H₂O is dissolved in water and the volume is made up to 1 L with water.

(II) Solution-B (0.2 M, NaH₂ 4•2HPO₂ O)

31.2 gm of NaH₂PO₄•2H₂O is dissolved in water and the volume is made up to 1 L with water. 51 ml of solution-B are mixed with 49 ml of solution-A and diluted to a total of 200 ml of water.

(b) Preparation of enzyme and substrate solutions

Enzyme buffer concentration (M) buffer pH enzyme concentration (Units/ml);

Mushroom Phosphate 0.1 6.8 60;

Tyrosinase; and

Substrates: Substrate concentration (mM)

- (i) L-DOPA Phosphate 0.1 6.8 2.55
- (ii) L-Tyrosine Phosphate 0.1 6.8 1.70

(2) Assay Procedure

Two types of substrates can be used for this assay, which are given as follows:

- (i) L-DOPA and (ii) L-Tyrosine

- (1) 1.0 ml of phosphate buffer is dispensed in a test tube.
- (2) 500 µl of mushroom tyrosinase enzyme solution is added.
- (3) 500 µl of test sample is added, mixed, and incubated at 25 °C for 10 min.
- (4) 1.0 ml substrate solution is finally added.
- (5) The absorbance is monitored continuously in a recording spectrophotometer at 475 nm for 20 min.

(3) 96-Well Microplate Assay Format:

- (1) 100 µl of phosphate buffer is dispensed in each well of a 96-well microplate.
- (2) 50 µl of mushroom tyrosinase enzyme solution is added.
- (3) 50 µl of test sample is added, mixed and incubated at 25 °C for 10 min.
- (4) 100 µl substrate solution is finally added.
- (5) The absorbance is monitored continuously in a microplate reader at 475 nm for 20 min.

(4) Calculation of Percentage Inhibition:

The percentage inhibition in enzyme activity can be calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

Hyaluronidase Inhibition Assay

Hyaluronidase, a mucopolysaccharide hydrolyzing enzyme, has been shown to involve in various diseases. The enzyme is functionally related to vascular permeability and inflammatory reactions. Since the enzyme exists in an inactive form, its *in vivo* activation by metal ions including calcium ions is related to the degranulation of mast cells, causing release of mediators that cause allergy including inflammation.

Hyaluronate, a mucopolysaccharide, consisting of repeating subunits of D-glucuronic acid and N-acetyl-D-glucosamine, is a viscous lubricating agent present in synovial fluid in joints. In rheumatoid arthritis, its excessive degradation by hyaluronidase may lead to decrease in amount and molecular weight of hyaluronate, thus producing arthritic symptoms. The search for inhibitors of hyaluronidase enzyme may lead to the isolation of new potent anti-allergic and anti-inflammatory drugs.

Materials

1. Acetate Buffer (0.1 M, pH 3.5)
Sodium acetate (0.1 M)
Glacial acetic acid (0.1 M);
2. Hyaluronidase enzyme (from bovine testes);
3. Potassium hyaluronate;
4. Calcium chloride (2.5 mM);
5. Sodium hydroxide (0.4 N);
6. Potassium tetraborate (0.8 N, pH 9.1 adjusted with 5 M KOH);
7. *p*-Dimethylaminobenzaldehyde;
8. Screw cap test tubes (Pyrex);

9. Micropipettes;
10. Water bath;
11. HCl (10 N);
12. Timer;
13. UV/VIS spectrophotometer;
14. pH meter;
15. Volumetric flasks;
16. Beakers;
17. Measuring cylinders;
18. Deionized water; and
19. Test sample (crude extract, pure natural product or synthetic compound).

Principle

Hyaluronidase enzyme hydrolytically cleaves the (1–4) bond in hyaluronic acid liberating the product with a terminal N-acetyl-D-glucosamine moiety that reacts with alkali to form a glucoxazoline intermediate compound. This intermediate reacts with *p*-dimethylaminobenzaldehyde to produce a colored product which is measured at 585 nm.

(1) Preparation of buffers and solutions:

(a) Acetate Buffer (0.1 M , $\text{pH } 3.5$)

(I) Section A:

16.4 gm of sodium acetate is dissolved in water and the volume is made up to 1 L with water.

(II) Section B:

11.55 ml of glacial acetic acid is mixed with water and the volume is made up to 1 L with water.

46.3 ml of solution-B are mixed with 3.7 ml of solution-A and diluted to a total of 100 ml of water.

(b) Solvent for *p*-dimethylaminobenzaldehyde:

12.5 ml of HCl (10 N) is mixed with glacial acetic acid and the volume is made upto100 ml with the same acid.

(c) *p*-Dimethylaminobenzaldehyde (67 mM)

Stock solution:

p-DMAB (10 gm) is dissolved in *p*-DMAB-solvent and the volume is made up to 100 ml with the same solvent.

Shortly before the use, 1 ml of the stock solution is diluted to 10 ml with glacial acetic acid.

(d) Preparation of Enzyme and Substrate Solutions:

(2) Assay Procedure

(a) Activation of Hyaluronidase Enzyme

- (I) 400 µl of hyaluronidase enzyme solution (350 N.F. units/ml of acetate buffer) is taken in a screw cap test tube.
- (II) 100 µl of CaCl₂ solution (2.5 mM in acetate buffer) is added to this test tube and incubated at 37 °C for 20 min to activate the enzyme.
- (b) Inhibition of Activated Hyaluronidase Enzyme
 - (I) 100 µl of test sample or vehicle is added to the activated enzyme.
 - (II) This mixture is incubated at 37 °C for 20 min in a water bath.
 - (III) 500 µl of potassium hyaluronate (1.2 mg/ml of acetate buffer) is added and incubation is carried out again at 37 °C for 20 min.
 - (IV) The enzyme reaction is stopped by adding 100 µl of NaOH (0.4 N) and 100 µl of potassium tetraborate (0.8 N, pH 9.1).
 - (V) The mixture is heated in a boiling water bath for exactly 3 min and the tubes are cooled water tap water.
 - (VI) 3 ml of *p*-DMAB solution (67 mM) is added, mixed, and incubated at 37 °C for 20 min for color development.
 - (VII) The absorbance of the mixture is measured at 585 nm in a spectrophotometer against blank.
- (c) Inhibition of the activation of inactive hyaluronidase enzyme
 Hyaluronidase Acetate 0.1 3.5 350
 Potassium hyaluronate Acetate 0.1 3.5 1.2
 - (I) 400 µl of hyaluronidase enzyme solution (350 N.F. Units/ml of acetate buffer) is taken in a screw cap test tube.
 - (II) 100 µl of test sample or vehicle is added, mixed and incubated at 37 °C for 20 min.
 - (III) 100 µl of CaCl₂ (2.5 mM in acetate buffer) is added and incubated at 37 °C for 20 min.
 - (IV) The procedure described from step 3 onward in protocol B is repeated.

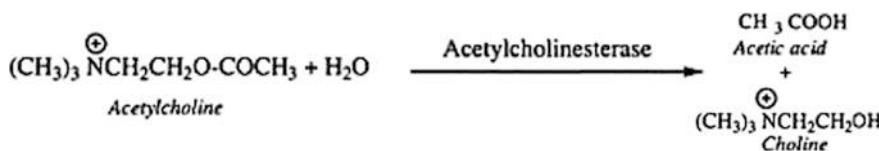
Calculation of Percentage Inhibition

The percentage inhibition in enzyme activity can be calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

Acetylcholinesterase Inhibition Assay

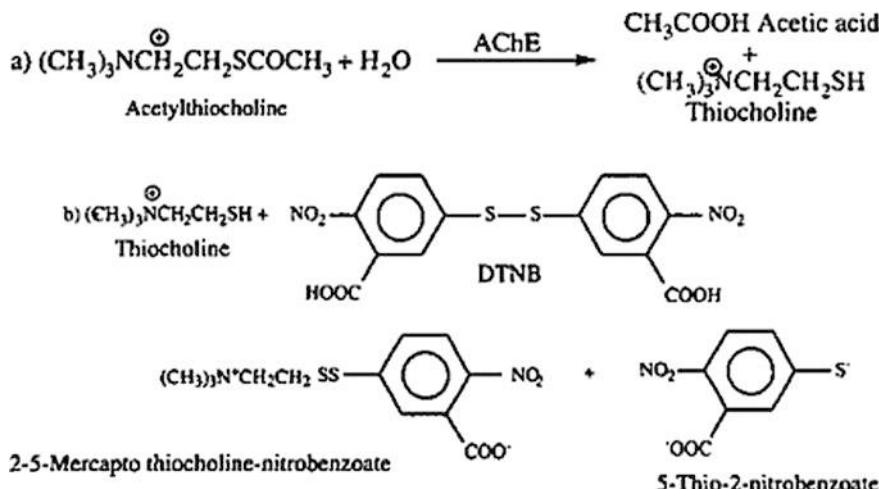
Acetylcholinesterase (acetylcholine acylhydrolase, EC 3.1.1.7) plays an important role in the central and peripheral nervous systems, along with the acetylcholine receptor, in the transmission of action potential across nerve–nerve and neuromuscular synapses. The enzyme's physiological task is the hydrolytic destruction of the cationic neurotransmitter, acetylcholine.



Because of the pivotal role that acetylcholinesterase (AChE) plays in the nervous system, it has long been an attractive target for the rational design and discovery of mechanism-based inhibitors. Some inhibitors of acetylcholinesterase are known to be useful for the treatment of Alzheimer's disease, senile dementia, ataxia, and for improving the long-term memory processes by enhancing cholinergic activity.

Spectrophotometric Assay

The principle involves the measurements of the rate of production of thiocholine, as acetylthiocholine is hydrolyzed by acetylcholinesterase. Hydrolysis of acetylthiocholine is accompanied by a continuous reaction between the thiocholine liberated and DTNB (dithiobisnitrobenzoic acid) which produces the yellow anion of 5-thio-2-nitrobenzoic acid.



The rate of anion production is measured from the absorbance at 412 nm.

Materials

1. Phosphate buffer-1 (0.1 M, pH 8.0);
 2. Phosphate buffer-2 (0.1 M, pH 7.0);
 3. Buffered Ellman's reagent, DTNB;
 4. Acetylthiocholine iodide, 75 mmol/l;
 5. Acetylcholinesterase (AChE);

6. Micropipettes;
7. Glass pipettes;
8. Stopwatch;
9. Water bath;
10. UV/VIS spectrophotometer; and
11. Test sample (crude extract, pure natural product, or synthetic compound)

(1) Preparation of Reagents

(a) Phosphate buffer-1(0.1 M) (For enzyme and test)

- (I) Dissolve 15.6 g of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in 750 ml water.
- (II) Check the pH at 25 °C.
- (III) Adjust to 8.0 by adding NaOH solution (100 mmol/l).
- (IV) Make volume up to 1 liter by adding distilled water.
- (V) Store the buffer solution-1 at 4 °C.

This buffer solution is stable as long as no microbial contamination occurs.

(b) Phosphate buffer-2(0.1 M) (For Ellman's reagent, DTNB)

- (I) Dissolve 15.6 g of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in 750 ml water.
- (II) Check the pH at 25 °C.
- (III) Adjust to pH 7.0 by adding NaOH solution (100 mmol/l).
- (IV) Make volume up to 1 liter by adding distilled water.
- (V) Store the buffer solution-2 at 4 °C.

This buffer solution is stable as long as no microbial contamination occurs.

(c) Buffered Ellman's Reagent, (DTNB, 0.1 M/1; NaHCO_3 , 17.85 mmol/l)

- (I) Dissolve 39.6 mg DTNB in 10 ml phosphate buffer-2 solution.
- (II) Add 15 mg NaHCO_3 .
- (III) Store in dark bottle at 4 °C.

This solution is stable for 4 weeks if stored in dark bottles.

(d) Acetylthiocholine iodide(75 mmol/l) (Substrate)

- (I) Dissolve 108.35 mg acetylthiocholine iodide in 5 ml of water.
- (II) Store at 4 °C.

This solution should not be kept for more than 7 days.

(e) Acetylcholinesterase(Enzyme)

- (I) The enzyme solution is prepared by dissolving the enzyme in phosphate buffer-1 so that the concentration of the enzyme in the reaction mixture is about 0.0025 U/ml.
- (II) Keep in an iced water bath at 5 °C.

(f) Test sample solution

The test sample should be dissolved in the proper solvent (preferably water) with desired concentration, but for water-insoluble compounds the effects of other solvents on the enzyme activity should be checked prior to the experiment; the controls should receive the same volume of the solvent.

The following steps are involved in the acetylcholinesterase inhibition assay:

1. Take 2.81 ml of phosphate buffer-1.
2. Add 30 µl of test sample solution.
3. Add 30 µl of enzyme stock solution.
4. Add 100 µl of DTNB stock solution to this.
5. Incubate for 5–10 min at 25 °C.
6. Add 30 µl of substrate stock solution.

(2) Calculation of Percentage Inhibition

The percentage inhibition in enzyme activity can be calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

9.6 Qualitative and Quantitative Analysis of Secondary Metabolites

9.6.1 Qualitative Analysis of Secondary Metabolites

1. Test for alkaloid by different alkaloid detecting reagents

The qualitative chemical tests used for detection of alkaloids depend on the character of alkaloids to give precipitate as salts of organic acids or with compound of heavy metals like Hg, Au, Pt, etc. Generally, alkaloids can be detected by two groups of reagents, e.g., (i) alkaloidal precipitants, and (ii) alkaloidal color reagents. Neither of these reagents, however, is sufficient enough for the identification of alkaloids. Both of them together with the confirmatory specific tests (e.g., rubreserine test, Warren's test, Vitali-Morin's test, etc.) are needed for complete identification of the alkaloid. The alkaloidal precipitating reagents may give precipitate, i.e., false positive reaction with other plant constituents like proteins, amino acid, tannins, volatile oils, some flavonoids and coumarins, and a variety of oxygenated natural products, and therefore, it is necessary to remove these substances from the plant extract prepared for alkaloid test.

Extraction for analysis

Collected plant samples were washed carefully with tap water, rinsed with distilled water, air dried for 1 h, and shade dried. They were ground into powder (coarsely) and stored at room temperature. The extract of the samples were prepared by soaking 100gm of dried powder in 200 ml of methanol for 32 h. The extracts were filtered using Whatman filter paper No. 42, the filtrate was evaporated to dry under vacuum, and then dissolved in water for analysis.

For spot test of alkaloids following standard method (Webb 1949; Amarasingham et al. 1964; Aplin and Cannon 1971), the plant extract for qualitative analysis may also be prepared by taking 5 g fresh material, chopped and pasted with 10 ml 2% HCl (1:2), transferred to a screw cap test tube (20 ml), and then heated in a water bath at 60 °C for 1 h. The extract was filtered after cooling using Whatman filter paper No. 1. Two drops of plant extract were put on clean dry microscopic groove slide and then added one drop of alkaloid detecting reagent (2:1) to it. The relative abundance of precipitate formed (if any) indicates the presence of alkaloid in the extract or plant material and graded by signs as + (slight), 2+ (moderate), 3+ (substantial), and 4+ (heavy amount), while absence of precipitate is indicated by - sign.

(i) Alkaloidal precipitants

(a) Dragendorff's reagent (potassium bismuth iodide)

To 2 ml of the ethanolic extract taken in a test tube, 5 ml of distilled water was added, 2 M HCl was added until an acid reaction occurs. To this 1 ml of Dragendorff's reagent (potassium-bismuth-iodide solution-bismuth nitrate, nitric acid, potassium iodide and water) was added along the sides of the test tube. Formation of orange or orange-red precipitate indicates the presence of alkaloids.

(b) Hager's reagent (saturated picric acid solution)

To 2 ml of the ethanolic extract taken in a test tube, a few drops of Hager's reagent (saturated solution of picric acid—most acidic phenol) were added along the sides of test tube. Formation of yellow precipitate confirms the presence of alkaloids.

(c) Wagner's reagent (iodine-potassium Iodide solution)

2 ml of ethanolic extract taken in a test tube was acidified with 1.5% v/v of HCl and a few drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide was dissolved in 5 ml of water and the volume was made 100 ml with distilled water) were added along the sides of test tube. Formation of reddish-brown precipitate indicates the presence of alkaloids.

(d) Mayer's reagent (potassium mercuric iodide)

To 2 ml of ethanolic extract taken in a test tube, a few drops of the Mayer's reagent (1.36 g of mercuric chloride is dissolved in 60 ml of distilled water; 5 g of potassium iodide is dissolved in 20 ml of distilled water; two are then mixed and the volume was adjusted to 100 ml with distilled water) was added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids. It is the most generally used alkaloidal reagents. The solution should be

added to distinctly acidic solution of the alkaloid, only few drops of the reagent should be used and the solution should not contain acetic acid or alcohol.

(e) Marme's reagent (potassium cadmium iodide)

To 2 ml of the ethanolic extract taken in a test tube, a few drops of Marme's reagent (potassium cadmium iodide) were added along the sides of test tube. Formation of yellow precipitate confirms the presence of alkaloids.

(f) Tannic acid reagent

To 2 ml of ethanolic extract taken in a test tube, a few drops of tannic acid were added along the sides of the test tube. Alkaloids give a buff color precipitate with tannic acid.

(g) Picrolonic acid reagent

To 2 ml of ethanolic extract taken in a test tube, a few drops of picrolonic acid were added along the sides of the test tube. Alkaloids give a yellow color precipitate with this acid.

(ii) Alkaloidal color reagents

Most of the color reagents are very sensitive (1 µg of alkaloids can be detected), but not sufficiently specific for the identification purposes. The test is applied on a white color porcelain slab or in a porcelain dish. The results should be recorded directly on the addition of the reagent and the sequence of color changes.

Common alkaloidal color reagents

- (a) **Froehd's reagent** (sulfomolybdic acid) 5 mg of molybdic acid or sodium molybdate dissolved in 1 ml of pure conc. H_2SO_4 .
- (b) **Mandalin's reagent** (sulphovanadic acid) 1 g of ammonium vanadate dissolved by gentle heating with 200 g of pure conc. H_2SO_4 .
- (c) **Marquis' reagent** (formaldehyde-sulfuric acid) 2 to 3 drops of 40% formaldehyde solution mixed with 3 ml of well-cooled pure conc. H_2SO_4 .
- (d) **Erdmann's reagent** (conc. H_2SO_4 containing HNO_3) 10 drops of a mixture of 10 drops of conc. HNO_3 and 100 ml of water is added to 20 ml % pure conc. H_2SO_4 .
- (e) **Modified Dragendorff's reagent** (spray reagent for chromatography) It is a solution of 5 ml (1.6% solution of Bismuth subnitrate in 20% acetic acid) + 5 ml (40% aqueous solution of KI) + glacial acetic acid and water to make 100 ml. It gives orange color with alkaloids.

2. Test for amino acids

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatman No. 1 filter paper and the filtrate is subjected to test for Amino acids.

(a) Ninhydrin test

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) are added to 2 ml of aqueous filtrate. Appearance of purple color indicates the presence of amino acids.

3. Test for carbohydrates**(a) Molish' s test**

To 2 ml of plant sample extract, two drops of alcoholic solution of α -naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of the test tube. A violet ring indicates the presence of carbohydrates.

(b) Benedict' s test

To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 min. A characteristic colored precipitate indicates the presence of sugar.

(c) Fehling's solution

It is used for the detection of reducing sugars. 34.66 g of copper sulfate was dissolved in distilled water and the volume was made to 500 ml (Solution-A). 173 g of potassium sodium tartrate and 50 g of sodium hydroxide in D/W was dissolved and volume was made up to 500 ml (solution-B). The two solutions were mixed in equal volume for prior use.

To 2.5 ml of filtrate, 2.5 ml of Fehling's solution was added to a 10 ml test tube. The mixture is heated on a boiling water bath for 2 min. A characteristic brick red colored precipitate indicates the presence of reducing sugar.

4. Test for Fixed oils and Fats**(a) Spot test**

A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

(b) Saponification test

A few drops of 0.5 N alcoholic potassium hydroxide solution is added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated on a water bath for 2 h. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

5. Test for Glycosides

For 50 mg of the extract, it is hydrolyzed with concentrated hydrochloric acid for 2 h on a water bath, filtered and the hydrolysate is subjected to the following tests.

(a) Borntrager's test

To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink color indicates the presence of glycosides.

(b) Legal's test

50 mg of extract is dissolved in pyridine, sodium nitroprusside solution is added and made alkaline using 10% NaOH. Presence of glycoside is indicated by pink color.

6. Test for Phenolic compounds and Tannins**(a) Ferric Chloride test**

Ferric Chloride (alcoholic): A 5% w/v solution of ferric chloride in 90% alcohol is used for the detection of phenols.

The extract (50 mg) is dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution are added. A dark green color indicates the presence of phenolic compound.

(b) Gelatin test

The extract (50 mg) is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

(c) Lead acetate test

A 25% basic lead acetate solution is used for the detection of flavonoid.

The extract (50 mg) is dissolved in of distilled water and to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

(d) Alkaline reagent test

An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

(e) Magnesium and Hydrochloric acid reduction

The extract (50 mg) is dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid (dropwise) are added. If any pink to crimson color develops, presence of flavonol glucosides is inferred.

Test for flavonoids when ammonium test and aluminum chloride test did not confirm the presence of flavonoids in the methanolic plant extract

A small quantity of the extract is heated with 10 ml of ethyl acetate in boiling water for 3 min. The mixture is filtered and the filtrates are used for the following test.

- a. Ammonium Test: The filtrate was shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration was not observed at ammonia layer which indicates the absence of the flavonoid from the plant extract.
- b. Aluminum Chloride Test: The filtrates were shaken with 1 ml of 1% aluminum chloride solution and observed for light yellow color, which did not appear indicating the absence of flavonoids. The light yellow color indicates the

presence of flavonoid and when dilute NaOH and HCl is added the yellow solution turns colorless.

(f) Test for tannins:

Ferric Chloride test and Lead Subacetate test confirmed the presence of tannins in the plant extract.

A small quantity of the extract is boiled with 5 ml of 45% solution of ethanol for 5 min. Each of the mixture is cooled and filtered. The different filtrates were used for the following test:

a. Ferric Chloride Test: 1 ml each of filtrate is diluted with distilled water and two drops of ferric chloride is added. A transient greenish to black color indicated the presence of Tannins.

b. Lead Subacetate Test: 1 ml of the different filtrate was added with three drops of lead subacetate solution. A creamy gelatinous precipitation, indicates positive test for Tannins.

7. Test for phytosterols

a. Salkowski test: In 2 ml of plant extract, 2 ml of chloroform and 2 ml of concentrated H_2SO_4 was added and shaken well. Chloroform layer appeared red and acid layer greenish yellow fluorescent. This confirms the presence of sterols.

b. Liebermann–Burchard test: 2 ml of methanolic plant extract was mixed with chloroform. 1-2 ml acetic anhydride and two drops of concentrated H_2SO_4 from the side of the test tube was added in the mixture. First red, then blue, and finally green color indicates the presence of sterols.

8. Test for terpenoids:

Salkowski test The extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show a positive result of the presence of terpenoids.

9. Test for Proteins

The extract (100 mg) was dissolved in 10 ml of distilled water and filtered through Whatman No. 1 filter paper and the filtrate was subjected to test for proteins.

(a) Millon's test:

To 2 ml of filtrate, few drops of Millon's reagent was added. Appearance of a white precipitate indicated the presence of proteins.

(b) Biuret test:

2 ml of filtrate was treated with 1 drop of 2% copper sulfate solution. To this, 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink color ethanolic layer indicated the presence of protein.

10. Test for Saponins:

The extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. A 2 cm layer of foam indicates the presence of saponins.

11. Test for Gum and Mucilages:

The extract (100 mg) was dissolved in 10 ml of distilled water and to this 2 ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of Gums and Mucilages.

12. Test for Volatile Oil

For volatile oil estimation, 50 mg of powdered material (crude drug) was taken and subjected to hydro-distillation. The distillate was collected in graduate tube of the assembly, wherein the aqueous portion automatically separated out from the volatile oil.

13. Test for Anthraquinones:

10 ml of benzene was added in 6 g of the *Ephedra* powder sample in a conical flask and soaked for 10 min, and then filtered. Further 10 ml of 10% ammonia solution was added to the filtrate and shaken vigorously for 30 s and pink, violet, or red color indicated the presence of anthraquinones in the ammonia phase.

14. Test for Tannins:

10 ml of bromine water was added to the 0.5 g aqueous extract. Decoloration of bromine water showed the presence of tannins.

15. Test for Saponins:

5.0 ml of distilled water was mixed with aqueous crude plant extract in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins.

Tests for Flavonoids

Shinoda Test: Pieces of magnesium ribbon and concentrated HCl were mixed with aqueous crude plant extract; appearance of pink color after a few minutes indicated the presence of flavonoid.

Alkaline Reagent Test: 2 ml of 2.0% NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow color was produced, which became colorless when we added two drops of diluted acid to the mixture. This result showed the presence of flavonoids.

16. Tests for Glycosides:

Liebermann's Test: Added 2.0 ml of acetic acid and 2 ml of chloroform with whole aqueous plant crude extract. The mixture was then cooled and we added H_2SO_4 concentrated. Green color showed the entity of aglycone, steroidal part of glycosides.

Keller-Kiliani Test: A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% FeCl_3 mixture was mixed with the 10 ml aqueous plant extract and 1 ml H_2SO_4 concentrated. A brown ring formed between the layers, which showed the entity of cardiac steroid glycosides.

Salkowski's Test: We added 2 ml H_2SO_4 concentrated to the whole aqueous plant crude extract. A reddish-brown color formed which indicated the presence of steroid aglycone part of the glycoside.

17. Test for Terpenoids:

2.0 ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water path and then boiled with 3 ml of H_2SO_4 concentrated. A gray color formed which showed the entity of terpenoids.

18. Test for Steroids:

2 ml of chloroform and concentrated H_2SO_4 were added with the 5 ml aqueous plant crude extract. In the lower chloroform layer, red color appeared that indicated the presence of steroids.

9.6.2 *Quantification of Phytochemicals in Crude Extract of Medicinal Plants*

9.6.2.1 Quantitative Estimation of Alkaloids (Alkaloidal Assays)

Methods of assay of drugs and galenical preparations containing alkaloids may differ from that of pure alkaloid, e.g., assay of ipecac or its liquid extract is an aqueous volumetric titration, while the assay of emetine is spectrophotometric (U.S.P) or nonaqueous titration in (B.P). Assay of colchicum corm powder is gravimetric, while assay for colchicine alkaloid is colorimetric or spectrophotometric. So, there may be different methods for use in the analysis of alkaloids.

Different methods of assaying alkaloids

- (i) Gravimetric assays, e.g., caffeine in tea;
- (ii) Volumetric aqueous titration, e.g., atropine in *Hyoscyamus muticus*;
- (iii) Nonaqueous titration, e.g., ephedrine and most pure alkaloids;
- (iv) Colorimetric assays, e.g., ergot alkaloids;
- (v) Spectrophotometric assays, e.g., colchicines;
- (vi) Fluorimetric assay, e.g., quinine; and
- (vii) Chromatographic assays.

Gravimetric method of determination of caffeine in tea

Caffeine is a weak base, which does not form stable salts with acids, and even if salts are formed it is easily dissociated in aqueous media, consequently, volumetric aqueous titration assay is unsuitable for determination of caffeine contents. Three different methods can be applied such as (i) Gravimetric; (ii) Colorimetric; and (iii) Nonaqueous titration methods can be used for assay of caffeine.

Isolation of Caffeine from Tea

The powdered tea leaves are extracted with boiling water and filtered; The filtrate is purified with Pb acetate solution (to precipitate tannins and other impurities); Excess lead is removed by addition Na_2HPO_4 followed by filtration; Caffeine is extracted from the filtrate with chloroform and is purified by recrystallization from water.

Gravimetric method of analysis

This method essentially consists of the following steps:

- (i) Weigh accurately 2 g of powdered tea and boil in a conical flask under reflux condenser with 100 ml of water for 30 min;
- (ii) Filter the solution while hot on a piece of cotton and reflux again with 50 ml water for 15 min;
- (iii) Filter the aqueous extract through the same filter and wash with 10 ml of H_2O ;
- (iv) Concentrate the combined aqueous extracts to about 50 ml in a porcelain dish, (with continuous stirring with a glass rod to prevent sublimation of caffeine), then cool;
- (v) Transfer the concentrated solution to a separating funnel, wash the dish with 10 ml of water then add the washings to the separator;
- (vi) Extract with three successive quantities of 40, 40, and 20 ml of chloroform, wash the mixed chloroformic extracts with 10 ml of N/1 NaOH (to remove any resinous matter), then wash with 10 ml water. Test for complete extraction with Wagner's reagent;
- (vii) Filter the chloroformic extract on anhydrous Na_2SO_4 ; and
- (viii) Distil off the chloroformic extract in a pre-weighed flask on a water bath. Transfer the flask to a desiccator for 2 h and weigh till constant weight.

Calculation

$$\% \text{ of caffeine (w/w)} = \frac{\text{wt. of the residue}}{\text{wt. of tea}} \times 100$$

Spectrophotometric method of determination of Alkaloids

The total alkaloid content may be determined according to UV/Visible spectrophotometer method and this method is based on the reaction between alkaloid and bromocresol green. One gram (1 g) each of SMCM root, leaf, seed, or any

desired plant part or whole herbaceous plant (or when used plant extract, 1 mg plant extract) was added into dimethyl sulphoxide (DMSO) and dissolved it. To this mixture, 1 ml of 2 N HCl added and filtered, 1 ml of this solution was transferred to separatory funnel and washed with 10 ml chloroform. One ml of this solution was transferred to a separating funnel, and then 5 ml of bromocresol solution and 5 ml of phosphate buffer (pH adjusted to neutral with 0.1 N NaOH) were added. The mixture was shaken with 1, 2, 3, and 4 ml chloroform and the complex formed was fractioned with chloroform by vigorous shaking. The fractions were collected in a 10 ml volumetric flask and diluted to volume with chloroform. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of standard solutions of atropine (20, 40, 60, 80, and 100 µg/ml) were prepared in the same manner as described above. Absorbance for test and standard solutions were recorded against blank at 470 nm with a UV/Visible spectrophotometer. The total alkaloid content was calculated from standard curve against absorbance and expressed as mg of AE/g of extract.

9.6.3 Spectrophotometric Method of Determination of Total Phenolic Content

Extraction

Thirty grams of powdered samples were extracted by 100 ml of aqueous ethanol (ethanol: water, 70:30 v/v). The solution was subject to agitation during 1 h at ambient temperature in darkness and then filtered. The extraction procedure was repeated twice in the same conditions. All filtrates were combined, evaporated at 40 °C under vacuum using a Büchi 461 rotary evaporator. The hydro-alcohol extraction value was determined as follows: % yield = [(M₁ – M₀)/M₂] × 100, where M₀ is the weight of the empty flask (g), M₁ is the weight of the flask after evaporation (g) and M₂ is the weight of the seeds powder (g). The obtained extract was kept away from light at low temperature.

Method of analysis

This is a colorimetric oxidation/reduction method where the extracts reacted with Folin–Ciocalteu reagent and then neutralized with sodium carbonate solution. The Folin–Ciocalteu Spectrophotometric method may be used for determination of total phenolic content in plant extracts of big-leaf mahogany. To a 25 ml volumetric flask, 1 ml of extract and 9 ml of distilled water was taken. One ml of Folin–Ciocalteu phenol reagent was added to this mixture and shaken well. After 5 min, 10 ml of 7% sodium carbonate (Na₂CO₃) solution was added to the mixture and the volume was adjusted to 25 ml with distilled water. A standard curve was developed using different concentrations of gallic acid (20, 40, 40, 60, 80, and 100 µg/ml).

Incubated for 90 min at room temperature and the absorbance values for test and standard solutions were noted against blank at 550 nm with a UV/Visible Spectrophotometer. Total phenol content was expressed as mg of GAE/gm of extract or the total phenolic expressed as g gallic acid equivalents (GAE) per 100 g of dry weight (dwt).

9.6.4 Spectrophotometric Method of Determination of Tannins

Total tannin content may be determined by using Folin–Ciocalteu Spectrophotometric method. This method essentially consists of the following few steps:

- (i) 0.1 ml plant extract was added to a 10 ml volumetric flask containing 7.5 ml of distilled water, 0.5 ml of Folin–Ciocalteu phenol reagent and 1 ml of 35% Na_2CO_3 solution and the content was diluted up to the mark with distilled water;
- (ii) The mixture was shaken well and kept at room temperature for 30 min;
- (iii) Using a set standard solutions of gallic acid (20, 40, 60, 80, and 100 $\mu\text{g}/\text{ml}$) were prepared in the same manner as described earlier;
- (iv) Absorbance for test and standard solutions was measured against blank at 725 nm with a UV/Visible spectrophotometer;
- (v) The tannin content was expressed in terms of mg of GAE/g of extract.

9.6.5 Spectrophotometric method Determination of total Flavonoids

The aluminum chloride colorimetric method may be employed for quantitative estimation of flavonoids in the crude extracts. For this, 1 ml of extract and 4 ml of distilled water were taken into a 10 ml volumetric flask. To this flask, 0.3 ml of 5% sodium nitrite (NaNO_2) was added and after 5 min 0.3 ml of 10% aluminum chloride (AlCl_3) was mixed, and then after another 5 min 2 ml of 1 M sodium hydroxide (NaOH) was added and the content was diluted to 10 ml with distilled water. A standard curve was prepared with quercetin or catechin (20, 40, 60, 80, and 100 $\mu\text{g}/\text{ml}$) solution. The absorbance was measured after 15 min for test and standard solutions against blank at 510 nm in UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE or CE/g of extract or per 100 g of dry weight (dwt.).

9.7 Molecular Biology: PCR-Based DNA Technology

Polymerase chain reaction (PCR) is a technique used in molecular biology to amplify a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Principles

PCR amplifies a specific region of a DNA strand (the DNA target). Most PCR methods amplify DNA fragments of between 0.1 and 10 kilo base pairs (kbp), although some techniques allow for amplification of fragments up to 40 kbp in size. The amount of amplified product is determined by the available substrates in the reaction, which become limiting as the reaction progresses.

A basic PCR setup requires several components and reagents, including:

- (i) a DNA template that contains the DNA target region to amplify;
- (ii) a DNA polymerase, an enzyme that polymerizes new DNA strands; heat-resistant Taq polymerase is especially common, as it is more likely to remain intact during the high-temperature DNA denaturation process;
- (iii) two DNA primers that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strands of the DNA target (DNA polymerase can only bind to and elongate from a double-stranded region of DNA; without primers there is no double-stranded initiation site at which the polymerase can bind); specific primers that are complementary to the DNA target region are selected beforehand, and are often custom-made in a laboratory or purchased from commercial biochemical suppliers;
- (iv) deoxynucleoside triphosphates, or dNTPs (sometimes called “deoxynucleotide triphosphates”; nucleotides containing triphosphate groups), the building blocks from which the DNA polymerase synthesizes a new DNA strand;
- (v) a buffer solution providing a suitable chemical environment for optimum activity and stability of the DNA polymerase;
- (vi) bivalent cations, typically magnesium (Mg) or manganese (Mn) ions; Mg^{2+} is the most common, but Mn^{2+} can be used for PCR-mediated DNA mutagenesis, as a higher Mn^{2+} concentration increases the error rate during DNA synthesis;
- (vii) monovalent cations, typically potassium (K) ions.

The reaction is commonly carried out in a volume of 10–200 μ l in small reaction tubes (0.2–0.5 ml volumes) in a thermal cycler. The thermal cycler heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction (see below). Many modern thermal cyclers make use of the Peltier effect, which permits both heating and cooling of the block holding the PCR tubes simply by reversing the electric current. Thin-walled reaction tubes permit favorable thermal conductivity to allow for rapid thermal equilibration. Most thermal cyclers have

heated lids to prevent condensation at the top of the reaction tube. Older thermal cyclers lacking a heated lid require a layer of oil on top of the reaction mixture or a ball of wax inside the tube.

Procedure

Typically, PCR consists of a series of 20–40 repeated temperature changes, called cycles, with each cycle commonly consisting of two or three discrete temperature steps (see figure below). The cycling is often preceded by a single temperature step at a very high temperature [$>90\text{ }^{\circ}\text{C}$ ($194\text{ }^{\circ}\text{F}$)], and followed by one hold at the end for final product extension or brief storage. The temperatures used and the length of time they are applied in each cycle depend on a variety of parameters, including the enzyme used for DNA synthesis, the concentration of bivalent ions and dNTPs in the reaction, and the melting temperature (T_m) of the primers. The individual steps common to most PCR methods are as follows:

(i) Initialization: This step is only required for DNA polymerases that require heat activation by hot start PCR. It consists of heating the reaction chamber to a temperature of $94\text{--}96\text{ }^{\circ}\text{C}$ ($201\text{--}205\text{ }^{\circ}\text{F}$), or $98\text{ }^{\circ}\text{C}$ ($208\text{ }^{\circ}\text{F}$) if extremely thermostable polymerases are used, which is then held for 1–10 min.

(ii) Denaturation: This step is the first regular cycling event and consists of heating the reaction chamber to $94\text{--}98\text{ }^{\circ}\text{C}$ ($201\text{--}208\text{ }^{\circ}\text{F}$) for 20–30 s. This causes DNA melting, or denaturation, of the double-stranded DNA template by breaking the hydrogen bonds between complementary bases, yielding two single-stranded DNA molecules.

(iii) Annealing: In the next step, the reaction temperature is lowered to $50\text{--}65\text{ }^{\circ}\text{C}$ ($122\text{--}149\text{ }^{\circ}\text{F}$) for 20–40 s, allowing annealing of the primers to each of the single-stranded DNA templates. Two different primers are typically included in the reaction mixture: one for each of the two single-stranded complements containing the target region. The primers are single-stranded sequences themselves, but are much shorter than the length of the target region, complementing only very short sequences at the 3' end of each strand.

It is critical to determine a proper temperature for the annealing step because efficiency and specificity are strongly affected by the annealing temperature. This temperature must be low enough to allow for hybridization of the primer to the strand, but high enough for the hybridization to be specific, i.e., the primer should bind *only* to a perfectly complementary part of the strand, and nowhere else. If the temperature is too low, the primer may bind imperfectly. If it is too high, the primer may not bind at all. A typical annealing temperature is about 3–5 °C below the T_m of the primers used. Stable hydrogen bonds between complementary bases are formed only when the primer sequence very closely matches the template sequence. During this step, the polymerase binds to the primer-template hybrid and begins DNA formation.

(iv) Extension/elongation: The temperature at this step depends on the DNA polymerase used; the optimum activity temperature for the thermostable DNA

polymerase of Taq (*Thermus aquaticus*) polymerase is approximately 75–80 °C (167–176 °F), though a temperature of 72 °C (162 °F) is commonly used with this enzyme. In this step, the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding free dNTPs from the reaction mixture that are complementary to the template in the 5'-to-3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent (elongating) DNA strand. The precise time required for elongation depends both on the DNA polymerase used and on the length of the DNA target region to amplify. As a rule of thumb, at their optimal temperature, most DNA polymerases polymerize a thousand bases per minute. Under optimal conditions (i.e., if there are no limitations due to limiting substrates or reagents), at each extension/elongation step, the number of DNA target sequences is doubled. With each successive cycle, the original template strands plus all newly generated strands become template strands for the next round of elongation, leading to exponential (geometric) amplification of the specific DNA target region.

The processes of denaturation, annealing and elongation constitute a single cycle. Multiple cycles are required to amplify the DNA target to millions of copies. The formula used to calculate the number of DNA copies formed after a given number of cycles is 2^n , where n is the number of cycles. Thus, a reaction set for 30 cycles results in 2^{30} , or 1,073,741,824, copies of the original double-stranded DNA target region.

(v) **Final elongation:** This single step is optional, but is performed at a temperature of 70–74 °C (158–165 °F) (the temperature range required for optimal activity of most polymerases used in PCR) for 5–15 min after the last PCR cycle to ensure that any remaining single-stranded DNA is fully elongated.

(vi) **Final hold:** The final step cools the reaction chamber to 4–15 °C (39–59 °F) for an indefinite time, and may be employed for short-term storage of the PCR products (Fig. 9.3).

To check whether the PCR successfully generated the anticipated DNA target region (also sometimes referred to as the amplicon or amplicon), agarose gel electrophoresis may be employed for size separation of the PCR products. The size (s) of PCR products is determined by comparison with a DNA ladder, a molecular weight marker which contains DNA fragments of known size run on the gel alongside the PCR products.

Stages

As with other chemical reactions, the reaction rate and efficiency of PCR are affected by limiting factors. Thus, the entire PCR process can further be divided into three stages based on reaction progress:

- (i) **Exponential amplification:** At every cycle, the amount of product is doubled (assuming 100% reaction efficiency). After 30 cycles, a single copy of DNA can be increased up to 1,000,000 copies. In a sense, then, the replication of a discrete strand of DNA is being manipulated in a tube under controlled

Polymerase chain reaction - PCR

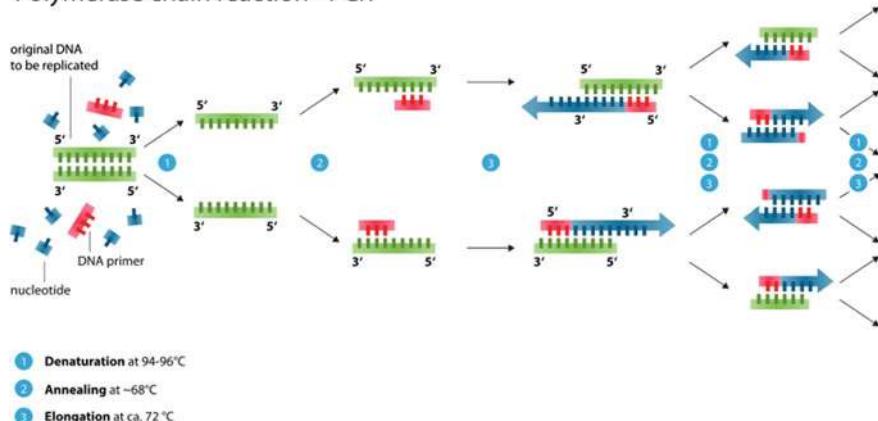


Fig. 9.3 Ethidium bromide-stained PCR products after gel electrophoresis. Two sets of primers were used to amplify a target sequence from three different tissue samples. No amplification is present in sample #1; DNA bands in sample #2 and #3 indicate successful amplification of the target sequence. The gel also shows a positive control, and a DNA ladder containing DNA fragments of defined length for sizing the bands in the experimental PCRs

conditions. The reaction is very sensitive, only minute quantities of DNA must be present.

- (ii) Leveling offstage: The reaction slows as the DNA polymerase loses activity and as consumption of reagents such as dNTPs and primers causes them to become limiting.
- (iii) Plateau: No more product accumulates due to exhaustion of reagents and enzyme.

PCR optimization

In practice, PCR can fail for various reasons, in part due to its sensitivity to contamination causing amplification of spurious DNA products. Because of this, a number of techniques and procedures have been developed for optimizing PCR conditions. Contamination with extraneous DNA is addressed with lab protocols and procedures that separate pre-PCR mixtures from potential DNA contaminants. This usually involves spatial separation of PCR-setup areas from areas for analysis or purification of PCR products, use of disposable plasticware, and thoroughly cleaning the work surface between reaction setups. Primer-design techniques are important in improving PCR product yield and in avoiding the formation of spurious products, and the usage of alternate buffer components or polymerase enzymes can help with amplification of long or otherwise problematic regions of DNA. Addition of reagents, such as formamide, in buffer systems may increase the specificity and yield of PCR. Computer simulations of theoretical PCR results (Electronic PCR) may be performed to assist in primer design.

Application

Selective isolation

PCR allows isolation of DNA fragments from genomic DNA by selective amplification of a specific region of DNA. This use of PCR augments many ways, such as generating hybridization probes for southern or northern hybridization and DNA cloning, which require larger amounts of DNA, representing a specific DNA region. PCR supplies these techniques with high amounts of pure DNA, enabling analysis of DNA samples even from very small amounts of starting material.

Other applications of PCR include DNA sequencing to determine unknown PCR-amplified sequences in which one of the amplification primers may be used in Sanger sequencing, isolation of a DNA sequence to expedite recombinant DNA technologies involving the insertion of a DNA sequence into a plasmid, phage, or cosmid (depending on size) or the genetic material of another organism. Bacterial colonies (e.g., *E. coli*) can be rapidly screened by PCR for correct DNA vector constructs. PCR may also be used for genetic fingerprinting; a forensic technique used to identify a person or organism by comparing experimental DNAs through different PCR-based methods.

Some PCR “fingerprints” methods have high discriminative power and can be used to identify genetic relationships between individuals, such as parent–child or between siblings, and are used in paternity testing (Fig. 9.4). This technique may also be used to determine evolutionary relationships among organisms when certain molecular clocks are used (i.e., the 16S rRNA and recA genes of microorganisms).

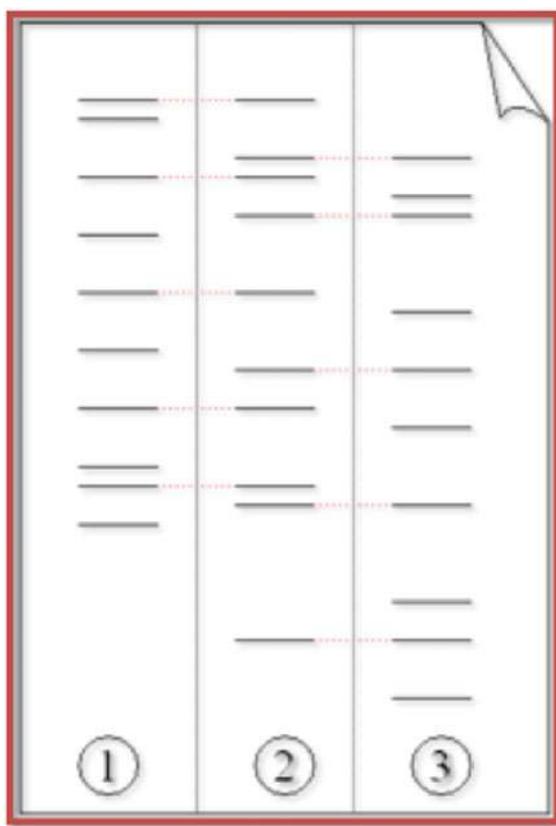
Amplification and quantification of DNA

Because PCR amplifies the regions of DNA that it targets, PCR can be used to analyze extremely small amounts of sample. This is often critical for forensic analysis, when only a trace amount of DNA is available as evidence. PCR may also be used in the analysis of ancient DNA that is tens of thousands of years old. These PCR-based techniques have been successfully used on animals, such as a forty-thousand-year-old mammoth, and also on human DNA, in applications ranging from the analysis of Egyptian mummies to the identification of a Russian tsar and the body of English king Richard III.

Quantitative PCR (qPCR) methods allow the estimation of the amount of a given sequence present in a sample—a technique often applied to quantitatively determine levels of gene expression. Quantitative PCR is an established tool for DNA quantification that measures the accumulation of DNA product after each round of PCR amplification.

qPCR allows the quantification and detection of a specific DNA sequence in real time since it measures concentration while the synthesis process is taking place. There are two methods for simultaneous detection and quantification. The first method consists of using fluorescent dyes that are retained nonspecifically in between the double strands. The second method involves probes that code for specific sequences and are fluorescently labeled. Detection of DNA using these methods can only be seen after the hybridization of probes with its complementary DNA takes place. An interesting technique combination is real-time PCR and

Fig. 9.4 Electrophoresis of PCR-amplified DNA fragments. (1) Father. (2) Child. (3) Mother. The child has inherited some, but not all of the fingerprints of each of its parents, giving it a new, unique fingerprint



reverse transcription (RT-qPCR). This sophisticated technique allows for the quantification of a small quantity of RNA. Through this combined technique, mRNA is converted to cDNA, which is further quantified using qPCR. This technique lowers the possibility of error at the end point of PCR, increasing chances for detection of genes associated with genetic diseases such as cancer. Laboratories use RT-qPCR for the purpose of sensitively measuring gene regulation.

Medical application

After the completion of sequencing of the first genome in 2000, the Human Genome Project, PCR has been applied to a large number of medical procedures:

- The first application of PCR was used for genetic testing, where a sample of DNA was analyzed for the presence of genetic disease mutations. Prospective parents can be tested for being genetic carriers, or their children might be tested for actually being affected by a disease. DNA samples for prenatal testing can be obtained by amniocentesis, chorionic villus sampling, or even by the analysis of rare fetal cells circulating in the mother's bloodstream. PCR analysis is also essential to preimplantation genetic

- diagnosis, where individual cells of a developing embryo are tested for mutations.
- (ii) PCR can also be used as part of a sensitive test for tissue typing, vital to organ transplantation. As of 2008, there is even a proposal to replace the traditional antibody-based tests for blood type with PCR-based tests.
 - (iii) Many forms of cancer involve alterations to oncogenes. Using PCR-based tests to study these mutations, therapy regimens can sometimes be individually customized to a patient. PCR permits early diagnosis of malignant diseases such as leukemia and lymphomas, which is currently the highest-developed in cancer research and is already being used routinely. PCR assays can be performed directly on genomic DNA samples to detect translocation-specific malignant cells at a sensitivity that is at least 10,000-fold higher than that of other methods.^[33] PCR is very useful in the medical field since it allows for the isolation and amplification of tumor suppressors. Quantitative PCR, for example, can be used to quantify and analyze single cells, as well as recognize DNA, mRNA, and protein confirmations and combinations.

Research applications

PCR has been applied to many areas of research in molecular genetics:

- (i) PCR allows rapid production of short pieces of DNA, even when not more than the sequence of the two primers is known. This ability of PCR augments many methods, such as generating hybridization probes for southern or northern blot hybridization. PCR supplies these techniques with large amounts of pure DNA, sometimes as a single strand, enabling analysis even from very small amounts of starting material.
- (ii) The task of DNA sequencing can also be assisted by PCR. Known segments of DNA can easily be produced from a patient with a genetic disease mutation. Modifications to the amplification technique can extract segments from a completely unknown genome or can generate just a single strand of an area of interest.
- (iii) PCR has numerous applications to the more traditional process of DNA cloning. It can extract segments for insertion into a vector from a larger genome, which may be only available in small quantities. Using a single set of “vector primers”, it can also analyze or extract fragments that have already been inserted into vectors. Some alterations to the PCR protocol can generate mutations (general or site-directed) of an inserted fragment.
- (iv) Sequence-tagged site is a process where PCR is used as an indicator that a particular segment of a genome is present in a particular clone. The Human Genome Project found this application vital to mapping the cosmid clones they were sequencing, and to coordinate the results from different laboratories.
- (v) An exciting application of PCR is the phylogenetic analysis of DNA from ancient sources, such as that found in the recovered bones of Neanderthals,

from frozen tissues of mammoths, or from the brain of Egyptian mummies. Have been amplified and sequenced. In some cases, the highly degraded DNA from these sources might be reassembled during the early stages of amplification.

- (vi) A common application of PCR is the study of patterns of gene expression. Tissues (or even individual cells) can be analyzed at different stages to see which genes have become active, or which have been switched off. This application can also use quantitative PCR to quantitate the actual levels of expression
- (vii) The ability of PCR to simultaneously amplify several loci from individual sperm^[40] has greatly enhanced the more traditional task of genetic mapping by studying chromosomal crossovers after meiosis. Rare crossover events between very close loci have been directly observed by analyzing thousands of individual sperms. Similarly, unusual deletions, insertions, translocations, or inversions can be analyzed, all without having to wait (or pay) for the long and laborious processes of fertilization, embryogenesis, etc.

References

- Akhtar MS, Athar MA, Yaqub M (1981) Effect of *Momordica charantia* on blood glucose levels of normal and alloxan diabetic rabbits. *Planta Med* 42:205–212
- Amarasingham RD, Bisset NG, Millard AH, Woods MC (1964) A phytochemical survey of Malaya III. Alkaloids and saponins. *Econ Bot* 18:270–278
- Aplin TEH, Cannon JR (1971) Distribution of alkaloids in some western Australian plants. *Eco Bot* 25(4):366–380
- Aquino R, De Feo V, De Simone F, Pizza C, Cirino G (1991) Plant metabolites. New compounds and anti-inflammatory activity of *Uncaria tomentosa*. *J Nat Prod* 54(2):453–459
- Eberhardt TL, Li X, Shupe TF, Hse CY (2007) Chinese Tallow Tree (*Sapium sebiferum*) utilization: characterization of extractives and cell-wall chemistry. *Wood Fiber Sci* 39:319–324
- Einhelling FA, Leather GR, Hobbs LL (1985) Use of *Lemna minor* L. as a bioassay in allelopathy. *J Chem Ecol* 11(1):65–72
- Farnsworth NR, Soejarto DD (1991) Global importance of medicinal plants. In: Akerele O, Heywood V, Syngle H (eds) *The conservation of medicinal plants*. Cambridge University Press, Cambridge, UK, pp 25–51
- Ferrigni NR, Putman JE, Anderson B, Jacobsen LB, Nichols DE, Moore DS et al (1982) Modification and evaluation of the potato disc assay and antitumor screening of Euphorbiaceae seeds. *J Nat Prod* 45:679–686
- Fings CS, Tatlioff CR, Dunn RT (1970) Glucose determination by *o*-toluidine method using acetic acid, ‘Clinical Chemistry’ by Toro C, Ackerman PG, vol 115. Little Browning and Company, Boston
- Goldstein A (1964) Bio-statistics, an introductory text. McMillan Co., New York, USA, pp 172–178
- Hamburger MO, Cordell GA (1987) A direct bioautographic TLC assay for compounds possessing antibacterial activity. *J Nat Prod* 50(1):19–22

- Harborne JB (1984) Phytochemical methods: a guide to modern techniques of plant analysis, 2nd edn. Chapman and Hall Ltd., London, New York, p 1984
- Hazra KM, Roy RN, Sen SK, Laska S (2007) Isolation of antibacterial pentahydroxy flavones from the seeds of *Mimusops elengi* Linn. Afr J Biotechnol 6(12):1446–1449
- Holstege DM, Seiber JN, Galey FD (1995) Rapid multiresidue screen for alkaloids in plant material and biological samples. J Agric Food Chem 43:691–699
- Hostettmann K, Kizu H, Tomimori T (1982) Molluscicidal properties of various saponins. Planta Med 44:34–35
- Jacobs RS, White S, Wilson L (1981) Selective compounds derived from marine organisms: effects on cell division in fertilized sea urchin eggs. Fed Proc 39:26–29
- Kavanagh F (1963) Analytical microbiology. In: Kavanagh F (ed) Academic Press, London, pp 125–141
- Kawashima K, Miwa Y, Kimura M, Mizutani K, Hayashi A, Tanaka O (1985) Diuretic action of paeonol. Planta Med 3:187–189
- Kawazu K (1981) Advances in natural products chemistry. In: Natori S, Itekawa N, Suzuki M, (eds) Wiley, New York, p 249
- Kazmi SU, Siddiqui R, Shekhani S (1990) Frontiers in natural products chemistry. In: Atta-ur-Rahman (ed), Shamil Printing Press, Karachi, pp 739–754
- Kumari S, Yasmin N, Hussain MR, Babuselvam M (2015) In vitro anti-inflammatory and anti-arthritis property of *Rhizophora mucronata* leaves. IJPSR 6:482–485
- Leelaprakash G, Dass SM (2011) In vitro anti-Inflammatory activity of methanol extract of *Ericostemma Axillare*. Int J Drug Dev Res 3(3):189–196
- Leven M, Vanden Berghe DA, Mertens F, Vlietinck A, Lammens E (1979) Screening of higher plants for biological activities. I. Antimicrobial activity. Planta Medica 36(4):311–321
- McLaughlin JL (1991). Methods of Plant Biochemistry. In: Hostettmann K (ed) vol. 6. Academic Press, London, pp 1–32
- Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL (1982) Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 45:31–34
- Mizushima Y, Kobayashi M (1968) Interaction of anti-inflammatory drugs with serum preoteins, especially with some biologically active proteins. J Pharma Pharmacol 20:169–173
- Rahman AU, Choudhary MI, Thomson WJ (2001) Bioassay techniques for drug development. Harwood academic publishers, Australia, Canada, France, Germany
- Sakat S, Juvekar AR, Gambhire MN (2010) In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharma Pharmacol Sci 2(1):146–155
- Schales O, Schales SS (1941) A simple and accurate method for the determination of chloride in biological fluids. J Biol Chem 140:879–884
- Shoyama Y, Tanaka H, Fukuda N (2003) Monoclonal antibodies against naturally occurring bioactive compounds. Cytotechnology 31:9–27
- Snedecor GW (1965) Statistical methods, 5th edn. The Iowa State University Press, Ames, Iowa, USA
- Srivastava OP (1984) Techniques for the evaluation of antimicrobial properties of natural products. In: Dhawan BN, Srimal RC (eds) The use of pharmacological techniques for the evaluation of natural products. UNESCO, New Delhi, pp 72–79
- Webb LJ (1949) An Australian phytochemical survey. I. Alkaloids and cyanogenic compounds in Queensland plants. CSIRO Bull. 260, Melbourne
- White SJ, Jacobs RS (1981) Inhibition of cell division and of microtubule assembly by elatone, a halogenated sesquiterpene. Mol Pharmacol 20:614–620
- Zarroug MA, Nugud AD, Bashir AK, Mageed AA (1988) Evaluation of Sudanese plant extracts as mosquito larvicides. Int J Crude Drug Res 26:77–80

Index

A

- Abatacept, 316, 317
Abciximab, 318
Abietic acid, 35, 191–193
Abscisic acid, 35, 95
Acetic acid (ethanoic acid), 125
Acetylcholine, 204, 205, 221, 222, 242, 337, 338, 433, 455, 551, 577, 579, 653, 782
Acetyl-CoA, 577
Acetyl coenzyme A, 281
Acetylsalicylic acid, 5, 122, 124, 383
Acids, 8, 11, 12, 18, 26, 30, 33, 35, 38, 45, 46, 48, 51, 53, 58, 60, 64, 71, 78, 85, 88, 95, 101, 107, 110, 111, 117–122, 125–127, 129, 132, 135, 148, 156, 167, 170, 178–180, 187, 191–195, 201, 203, 218, 224, 235, 243, 260, 267–269, 285, 292, 347, 348, 379, 421, 462, 468, 475, 476, 525, 552, 554, 558, 565, 575, 576, 578, 579, 588, 621, 655, 659, 706, 740, 742, 743, 756, 785, 793
Acquired immune deficiency syndrome, 758
Acrid taste, 27
Active drug constituents, 26
Active pharmaceutical ingredient, 492
Active specific immunotherapy, 636
Acute lymphocytic leukemia, 513
Acute Myeloid Leukemia (AML), 339
Acyl β-sitosterol, 110
Acyl groups, 105
Adacel, 315
Adenosine deaminase, 153, 155, 513, 661
Adenosine thiamine diphosphate, 425
Adenosine thiamine triphosphate, 425
ADT booster, 316
Advate, 317
Aerosol products, 319
Affects calcium and bone metabolism, 316, 317
Aflibercept, 317
Agalsidase beta, 317, 513
Age-related macular degeneration, 28, 182, 183
Aglycone, 9, 27, 212, 242–250, 253, 254, 257, 278, 281, 282, 545, 791, 792
Agrippal, 318
Agrochemicals, 12, 13, 586, 764
Alanine, 120, 127–132, 134, 135, 137, 138, 141, 357, 359–361
Albulin, 29
Albumen, 11, 28
Aldose sugars, 58, 62, 64, 355
Aldurazyme, 317, 513
Alemtuzumab, 316, 317
Aleurone grains, 11, 28
Algae, 4, 51, 53, 54, 63, 67, 70, 82, 84, 172, 182, 267, 275, 292, 335, 353, 355, 364, 366, 370–373, 438, 515, 522, 537–539, 559
Algic acid, 85, 353, 355, 356, 493, 496, 500
Alkaloids, 7–13, 18, 26–28, 31, 33, 38–41, 43, 45, 93, 95, 171, 202–225, 229–237, 239–242, 260, 267, 275, 281, 285, 287, 289, 292, 303, 305, 336, 338, 340–342, 355, 357, 515, 539, 540, 544–546, 550, 555, 558, 559, 561, 562, 564, 566, 586–597, 601, 606, 610, 618, 626–629, 703, 706, 724–726, 731, 738, 740, 742, 743, 785–787, 792, 793
Alkanes, 48, 49, 51, 54, 92, 93, 107
Alkenes, 48, 49, 51, 54, 92, 93
Alkynes, 48, 49

- Allergies, 186, 392, 449, 450, 545, 546, 563, 640, 641
 Allinin, 40
 Allophycocyanin, 372
 Allspice, 4
 Allyl sulfides, 4
 Almonds, 5, 132, 133, 254, 464, 487, 513
 Aloe, 27, 82, 233, 246, 247, 261, 263, 277, 285, 554, 589, 601, 606, 607, 609, 613
 Alpha- and beta-carotenes, 9
 Alpha-D-fructofuranose, 61
 Alpha-D-glucopyranose, 42, 270
 Alpha-D-glucosamine, 63, 322
 Alpha-D-mannopyranose, 86, 496
 Alpha-D-N-acetylglucosamine, 63
 Alpha-GAL- α -galactosidase, 68, 513
 Alpha-ketoglutaric acid, 118
 Alpha-L-guluronate(G) residues, 85
 Alpha-linolenic acid, 101
 Alpha-pinene, 34, 172
 Alpha-sedoheptitol, 59
 Alpha toxin, 539
 Alzheimer's disease, 13, 233, 337, 348, 476, 487, 783
 Amino acids, 8, 12, 14, 18, 26, 30, 32, 34, 120, 127–144, 146–148, 154, 155, 206–208, 210, 224, 241, 267, 292, 323, 357, 359, 425, 426, 437, 453, 467, 471, 488, 506–508, 512, 522, 526, 539, 541, 577, 578, 587, 646, 657, 669, 725, 738, 740, 741, 787, 788
 Amiodrone, 189, 190
 Amplified fragment length polymorphism, 673, 678, 679, 697, 698
 Amylase, 67, 244, 314, 355, 483, 493, 608
 Amylase inhibitors, 12, 39
 Amyotrophic lateral sclerosis, 137
 Anabolic pathway, 32
 Analgesic, 4, 9, 121, 122, 172, 179, 203–206, 209–211, 216, 217, 221, 224, 232, 234, 246, 335, 337, 339, 340, 344, 472, 515, 572, 581, 599, 601, 743
 Anatoxin, 331, 360, 361
 Anethole, 189, 190
 Angiotensin converting enzymes, 302
 Animal blood, 312
 Animal sources, 141, 175, 311, 312, 422, 430, 470, 483, 537, 554
 Anise, 4, 191, 269, 546
 Anise oil, 189
 Anterior pituitary, 312
 Anthelmintic, 13, 340, 514, 601, 623
 Anthocyanins, 4, 11, 27, 35, 37, 46, 271, 274, 445, 449, 450, 488, 724
 Anthraquinone derivatives, 11, 33, 247, 740
 Anthraquinones, 26, 244, 246, 276, 277, 586, 590, 592, 594, 621, 791
 Antianalgesic, 13
 Antiarrhythmic effect, 9
 Antibacterial, 5, 261, 270, 277, 279, 498, 601
 Antibiotics, 18, 26, 31, 63, 144, 203, 277, 285, 287, 312, 332, 345, 364–366, 371, 386, 389, 392, 452, 453, 456, 476, 523, 560, 633, 634, 649, 655, 703, 743, 752
 Antibody, 135, 140, 145, 562, 625, 649–653, 745, 802
 Anticancer, 4, 6, 8, 9, 11–13, 15, 150, 203, 232, 233, 256, 277, 279, 289, 290, 335–339, 343, 345, 353, 357, 363, 367–372, 388, 422, 456, 472, 514, 538, 549, 601, 602, 624, 626, 659, 743
 Anticancer actions, 9
 Anticancer phytonutrient, 5
 Anticancer properties, 5, 7, 264, 601
 Anticarcinogenic, 9, 53, 54, 335, 366, 449, 623
 Anticholinergic, 4, 203, 205, 234
 Anticoagulant, 29, 82, 253, 277, 314, 318, 322, 323, 345, 355, 371, 450, 514
 Anticoenzymes, 465
 Anti-diabetes herbal formulation, 298
 Antidiabetic, 12, 13, 507, 601, 605
 Anti-diarrhoeal, 315
 Antidiuretic hormone, 507, 603
 Antidisentery, 13
 Antifungal, 9, 12, 13, 35, 37, 156, 168, 174, 196, 198, 216, 276, 277, 279, 336, 337, 340, 341, 363, 364, 366, 510, 601, 634, 703, 757
 Antihypertensive effects, 9, 616
 Anti-inflammatories, 6, 269, 314, 450
 Anti-inflammatory, 4–6, 12, 15, 19, 27, 51–54, 68, 94, 156, 168, 173, 174, 178, 186, 198, 211, 216, 246, 257, 261, 275, 277, 279, 303, 306, 335, 337, 348, 351, 353, 355, 357, 363, 366–372, 380, 445, 472, 475, 487, 488, 513, 522, 525, 556, 587, 601, 602, 605, 623, 769, 780
 Antimalarial, 9, 11, 13, 173, 203, 205, 206, 232, 242, 291, 335–337, 340–343, 357, 375, 388, 599, 601, 646, 760, 761
 Antimalarial activity, 9, 342
 Antimalarial drug, 3, 12
 Antimicrobial, 9, 64, 156, 168, 172, 178, 196, 197, 205, 220, 256, 263, 279, 280, 282, 285, 287, 312, 318, 335–337, 363, 373, 475, 476, 518, 601, 602, 743, 751, 752
 Antimicrobial effect, 4, 6, 8, 481
 Antimicrobial peptide, 140, 336

- Antineoplastic, 224, 317, 318, 336, 337, 624
Antineoplastic agent, 316–318
Antisense oligodeoxynucleotides, 151
Antithrombin III, 314
Antithrombotics, 314
Antithymocyte globulin, 316
Anti-ulcer, 9
Antivenom, 316
Antiviral, 6, 9, 13, 51, 52, 54, 94, 150, 156, 168, 178, 198, 220, 233, 275, 335–338, 343, 355, 357, 363, 366, 375, 510, 601, 602, 625–630, 634, 645, 656, 758
Antiviral properties, 285, 287, 318, 628
Antivitamins, 409, 465, 467, 468
Aphidicolin, 175
Aphrodisiac, 13, 179, 205, 242, 347, 380
Apiole, 189, 190
ApoA, 116
ApoC, 116
Apocynaceae, 48, 206, 221, 248, 610
ApoE, 116
Apolipoproteins, 117
Aprotinin—Factor XIII, 315
Ara-A-9- β -D-arabinofuranosyladenine, 337
Arabinose, 9, 56, 58, 79, 80, 193, 244, 495, 496
Aranesp, 317
Arbitrarily primed PCR, 673, 680
Arginine, 128, 131, 132, 134, 135, 140, 141, 385, 507, 603
Aromatic amino acid, 10, 33
Aromatic hydrocarbons, 48, 49
Artemisia annua, 13, 173, 174, 232, 291, 340, 589, 701, 706
Artemisinin, 9, 12, 13, 173, 174, 291, 340, 386, 589, 602, 700, 701, 706
Ascaridole, 189, 190
Asclepiadaceae, 48
Ascorbic acid, 12, 123, 156, 409, 424, 441–443, 450, 465, 470, 485, 517, 518, 523, 526
Asiatic acid, 297
Asparagine, 127, 130–132, 135, 141, 385
Aspartame, 29, 139, 501, 503–506
Aspartic acid, 30, 118, 128, 132, 135, 140, 141, 156, 505
Assay plates, 20
Asthma, 13, 180, 182, 186, 378, 392, 450, 467, 472, 513, 553, 554, 563, 641, 700
Astringent, 27, 43, 125, 268, 271, 275, 472
ATGAM, 316
ATP-sensitive potassium channel, 59
Atropine, 3, 4, 26, 27, 31, 38, 40, 202–206, 208, 209, 214, 215, 225, 226, 232–235, 242, 545–548, 558, 559, 576, 792, 794
Autoimmune disease, 640, 641
Avastin, 312, 317, 318
Avaxim, 315
Avocado, 111, 133, 183, 185
Avonex, 317
Ayurvedic formulations, 17
Ayurvedic medicine, 17
Azadirachta indica, 35, 281, 518, 589, 601, 607, 608
Azadirachtin, 35, 589
- B**
- Barks, 8, 244, 246, 271, 274, 544
Barley, 5, 63, 72, 80, 81, 140, 273, 430
Basil, 4, 298, 616
Basiliximab, 318
Bay leaf, 4, 168, 492
Bee glue, 319
Bee pollen, 318
Bees, 35, 41, 67, 107, 312, 318, 632
Behind-the-counter medication, 527
BeneFIX, 317
Benzene, 48, 49, 54, 92, 123, 194, 201, 203, 218, 250, 254, 524, 553, 571, 756, 791
Benzoates, 29
Benzyl alcohol, 29
Benzyl isothiocyanate, 472
Beriberi, 7
Beta-carotene, 4, 28, 53, 54, 95, 156, 175, 177, 180, 182, 366, 412, 485
Beta-caryophyllene, 173
Beta-D-allopyranose, 61
Beta-D-fructofuranose, 75
Beta-D-fructopyranosyl-[D-fructofuranosyl] (n-1)-dfructofuranosides, 76
Beta-D-galactopyranose, 86, 88
Beta-N-methylamino-L-alanine, 357, 359
Beta-N-Oxaly-L- α , β -diaminopropionic acid, 12
Beta-pinene, 34
Beta-sitosterol, 4, 94, 110, 113, 186
Bevacizumab, 317, 318
Beverages, 4, 28, 67, 87, 279, 280, 372, 427, 469, 485, 491, 501, 588
Bicyclic monoterpenes, 170
Binding materials, 28

- Bioactive compounds, 10–13, 18, 26, 110, 155, 156, 290, 298, 305, 306, 311, 334–336, 343, 345, 346, 348, 353, 355–357, 359–362, 364–368, 373, 383, 388, 470, 471, 522, 586–588, 599–601, 610, 618, 621, 624, 625, 629, 630, 696, 724, 743, 744, 746, 747, 770
- Bioactive principles, 3, 10, 12, 13, 156
- Biochemistry, 3, 31, 245, 570, 598
- Biogenic amines, 12, 428
- Bioinformatics, 3, 587, 671, 691
- Biological macromolecules, 3
- Biomolecules, 17, 134, 366, 496, 569, 571, 576
- Bionutrients, 11
- Biotic and abiotic stresses, 3, 700
- Biotin, 366, 409, 411, 450, 451, 465
- Biotoxicity, 204
- Bisabolol, 9, 173
- Blackmores immunodefence capsules, 314
- Black snake antivenom, 316
- Black tea, 5
- Blueberries, 7, 52, 54, 89, 94, 111, 133, 297, 475, 488
- Bok choy, 4
- Boostrix, 315
- Borage, 185
- Botany, 3
- Botulinum* neurotoxins, 538
- Bovine aorta endothelial cell, 336
- Bovine colostrum, 314, 315
- Bovine products, 314
- Brassica campestris*, 185
- Brassicasterol, 113
- Brassica vegetables, 5, 7, 472, 623
- Brassinosteroids (BRs), 35
- Breast cancer, 154, 183, 197, 211, 353, 364, 475, 490, 622–625
- Brine shrimp toxicity, 747
- Broccoli, 4, 5, 8, 90, 111, 133, 180, 183, 374, 375, 423, 443, 461, 472, 475, 483, 487, 616, 622–624
- Brominated alkaloids, 336
- Bronchitis, 13, 138, 186
- Brown rice, 5, 133, 353, 453, 462, 487
- Brown snake antivenom, 316
- Brussels sprouts, 5, 68, 180, 472, 483, 623, 624
- Byakko-ka-ninjin-to, 298
- C**
- Cabbage, 5, 8, 42, 68, 89, 90, 118, 133, 250, 412, 420, 422, 425, 427, 436, 441, 443, 458, 459, 465, 472, 481, 483, 623
- Caesalpinia spinosa*, 88, 272
- Cafestol, 169, 175
- Caffeic acid, 4, 201, 285, 348, 706
- Caffeine, 9, 27, 31, 38, 40, 44, 156, 202–206, 210, 211, 230, 232, 234, 242, 554, 591, 614, 723, 726, 792, 793
- Calciferols, 97, 410, 415, 416
- Calcium-binding protein, 416
- Calcium chloride dihydrate - thrombin, 315
- Calcium oxalate, 11, 28, 545
- Calporo, 314
- Calvin-Basham pathway, 118
- Calvin cycle, 32, 48, 132
- CAM pathway, 118
- Campesterol, 109, 110, 185, 186, 350, 601
- Camphepane, 170, 172
- Camphor, 9, 95, 168, 170, 172, 191, 742
- Campesteryl ferulate, 109
- Camptotheca acuminata Decne, 13
- Camptothecin, 13, 15, 288, 595, 624, 625
- Cannabidiol, 43, 559, 560
- Cannabinol, 545, 559, 560
- Cannabis*, 3, 168, 172, 173, 191, 192, 206, 233, 285, 289, 516, 544, 545, 555, 559, 560, 678, 680
- Capillary electrophoresis, 19
- Capsaicin, 4, 31, 38, 40, 209, 221, 224, 233, 285, 298, 470, 475, 476, 487, 515, 516
- Capsules, 28, 312, 319, 327, 329, 486, 488, 492, 514, 569, 644
- Caraway oil, 189
- Carbohydrates, 7, 10, 11, 14, 18, 26, 33, 48, 56, 60, 71, 73, 76, 79, 94, 98, 114, 155, 156, 408, 426, 506, 510, 577, 788
- Carbon tetrachloride, 291, 553
- Carboxymethyl cellulose, 78, 499, 767
- Carboxymethyl group, 78
- Cardiac glycosides, 26, 27, 35, 212, 244, 248, 261, 282–284, 545, 621, 725, 727, 740
- Cardiac glycosidic phytosteroid, 6
- Cardioactive glycosides, 26, 283, 592
- Cardioactive steroids, 283, 284
- Cardiovascular diseases, 301, 507
- Carmine, 312, 319, 320, 519–521
- Carminic acid, 319, 320
- Carnitine, 136, 411, 442, 457, 459–461, 582
- Carnosol, 4
- Carob gum, 86, 88
- Carotenoids, 11, 12, 27, 31, 35, 48, 53, 54, 94, 175, 180–183, 185, 292, 336, 353, 362, 366, 367, 475, 485, 522
- Carrageenan, 82, 84, 85, 174, 337, 355, 356, 514, 769
- Carrots, 4, 8, 28, 89, 90, 133, 175, 182, 183, 189, 412, 422, 623
- Cartilag, 314

- Carvacrol, 170, 498, 515–517, 618, 621
Carvone, 170, 189, 190, 497, 498
Cascara, 27, 244, 246, 261
Catechins, 4, 303, 474, 479, 480, 490
Catechu, 11, 195, 225, 273, 500
Catharanthus roseus, 12, 13, 45, 204, 228, 232, 289, 590, 599, 601, 607, 701, 702, 706
Cats, 129, 280, 319
Cauliflower, 5, 90, 111, 133, 423, 451, 464, 472, 623
Cell culture, 45, 75, 145, 289, 332, 586–588, 600, 759
Cellulose, 10–12, 28, 29, 56, 66, 68, 70, 77, 78, 80, 81, 90, 126, 353, 474, 493, 496, 497, 499–502, 734, 740
Cellulose gum, 77, 78
Cembrene, 169, 175
Central nervous system, 71, 74, 134, 135, 172, 205, 207, 209, 215–218, 242, 261, 384, 435, 553, 554
Ceramides, 336
Ceratonia siliqua, 86, 88, 496
Cerebrosides, 92, 114, 337
Cerezyme, 317, 513
Cervical cancer, 186, 623
Cetuximab, 318
Chemistry, 2, 3, 21, 29, 47, 60, 85, 107, 154, 155, 305, 469, 492, 506, 510, 517, 558, 562, 568, 688, 704, 708, 730
Chemoprevention, 12, 475, 622–624
Chia, 6, 132, 133, 140
Chicken eggs products, 318
Chinese hamster ovary, 312, 314
Chinese tree, 13
Chirata, 27, 293, 602
Chitin, 29, 63, 64, 70, 80, 81, 318, 333, 493, 496, 632
Chives, 5
Chlorogenic acid, 4, 12, 201, 706
Chlorophyll, 8, 95, 156, 177, 182, 260, 292, 366
Chlorophyllum molybdites lectin, 507
Cho cells products, 317
Chocolate, 4, 5, 119, 210, 211, 275, 616
Cholelitholytics, 314
Cholera toxin, 331, 539
Cholesterol, 7, 76, 81, 87, 92, 94, 95, 97, 107, 109–113, 115, 117, 136, 179, 185–188, 212, 257, 279, 280, 282, 292, 350, 353, 357, 414, 420, 422, 424, 430, 442, 449, 450, 455, 456, 472, 475, 477, 484, 489, 491, 510, 606, 608, 614, 631
Cholesterol ester, 109
Cholesterol levels, 72, 112, 136, 263, 279, 318, 487
Choline, 113, 214, 261, 338, 411, 455, 459, 463, 464, 470, 551
Chondrodendron tomentosum, 5
Choriogonadotropin alfa, 316, 317
Chromium, 4, 8, 552
Chronic myelogenous leukemia, 339
Chymotrypsin, 136, 319, 483, 510, 512, 513, 775, 777
Ciguatoxin, 344, 345, 551
Cilantro, 4, 492
Cinchona alkaloids, 3
Cinchona bark, 3, 268, 291
Cinchonidine, 3, 204
Cinchonine, 3, 220
Cineole, 43, 189, 190, 497, 498
Cinnamaldehyde, 189, 190, 298, 497, 498
Cinnamon, 11, 28, 168, 188, 191, 252, 258, 273, 298, 492, 498, 602, 616
Cinnamon oil, 189
Cisaconic acid, 118
Cisatracurium, 5
Cis-diamminedichloroplatinum (II), 385
Citral, 9, 168, 170, 303, 601
Citric acid, 30, 118, 122, 135, 156, 523, 525
Citrus fruits, 4, 89, 170, 171, 443, 453
Cleaved amplified polymorphic sequences, 673
Clexane, 314
Clinical anesthesia, 5
Clove oil, 173, 189, 498, 516
Cluster beans, 87
CoA, 121, 281, 348, 410, 432, 433, 440, 485, 578, 579
CoA reductase, 34
Cocaine, 26, 27, 31, 203–206, 208, 214, 215, 222, 225, 230, 235, 242, 383, 384, 515–517, 545, 554, 555, 560, 576
Cochineal, 29, 319, 320, 519
Cochineal/carmine/carminic acid, 29, 312, 319, 320, 519–521
Coconut, 100, 102, 106, 119, 138, 185, 425, 427, 481, 517, 616, 723
Codeine, 3, 4, 27, 43, 203, 205, 206, 209, 216, 217, 227, 232, 234, 242, 383, 384, 515, 516, 545, 572, 595, 599
Cod fish liver, 312
Coenzyme Q, 410, 423
Coenzyme Q₁₀, 423, 424, 470
Coffee, 4, 5, 170, 180, 185, 186, 195, 201, 210, 253, 268, 456, 616, 623
Colchicine, 202, 203, 205–209, 221, 234, 264, 290, 547, 548, 588, 599, 792

- Collagen, 136, 137, 141, 275, 315, 321, 416, 441, 450, 510, 518, 519, 523, 525, 526, 557
 Collagen pentapeptide, 523
 Coloring matters, 29
 Column chromatography, 733–737, 742, 744
 Combined oral contraceptive pill, 387
 Combretaceae, 52, 54
 Common filler in tablets, 319
 Complementary DNA, 800
 Computational biology, 3
 Condiments, 4
 Conditional amino acids, 128, 141
 Coniine, 203, 204, 208, 209, 215, 216, 224, 545–548
 Conjugated Linoleic Acid (CLA), 470, 487, 490
 Conjunctivitis, 13, 337
 Continuous cell lines, 332
 Copper, 4, 203, 267, 313, 409, 441, 517–519, 525, 546, 554, 725, 788, 790
 CoQ, 97, 410, 423
 CoQ₁₀, 423, 424
 Coriander, 11, 28, 52, 492
 Corifollitropin alfa, 317
 Cork cells, 11
 Cortisol, 138, 185, 187, 188, 282
 Cortisone, 185, 187, 188, 276, 282
 Cosmeceuticals, 12, 28, 517, 522, 523, 525–527
 Cosmetics, 2, 3, 8, 67, 85, 107, 126, 172, 173, 177, 179, 190, 279, 280, 319, 320, 328, 329, 367, 372, 382, 451, 456, 499, 501, 514, 517–520, 523, 524, 527, 586, 599
 Costunolide, 34, 36
 Cottage cheese, 84
 Cottonseed, 185, 416, 422
 Coumarin, 39, 52, 53, 245, 250, 252–254, 560, 618
 Coupling processes, 32
 Cows, 312, 319, 345, 486
 Coxiella burnetii vaccine, 318
 C-Phycocyanin, 306, 372, 587
 Creatine, 135, 459, 463, 470
 Creon, 314
 Creon micro enteric coated granules, 314
 Crude drugs, 9, 28, 665–667, 689, 695, 703, 708, 709
 Cryptoxanthin, 4, 28, 53, 175, 180, 182, 412, 414
Cucurbita pepo, 186
 Cumin, 4, 282, 492, 616
 Curare (*d*-tubocurarine), 5
 Curcumines, 8
 Curosurf, 314
 Cutin, 29
Cyamopsis tetragonoloba, 86–88
 Cyanogenic glycosides, 12, 26, 27, 39, 40, 254, 256
 Cyclobutane, 48
 Cylindrospermopsin, 360, 361, 539, 541, 542
Cymbidium pendulum, 52
 Cysteine, 120, 128, 130–133, 135, 136, 141, 323, 471, 475, 571
 Cystic fibrosis, 150, 513, 646, 660
 Cytotoxicity, 145, 172, 201, 233, 280, 336, 337, 339, 363, 746–749, 770, 771
- D**
- Dalteparin, 314
 Danaparoid, 314
 Dandelion, 27, 180, 186, 253, 261, 298, 302, 412, 427, 562
 D-arabonic acid, 64
 Darbepoietin, 317
 Dark chocolate, 5
 Databases, 13, 53
 Daturin, 26
 Daunosamine, 63
 Death adder, 316
 Death adder antivenom, 316
 Decarbamoysaxitoxin, 543, 544
 Decasaccharide, 56
 Defensins, 39, 40
 Deflataluent, 13
 Degree of esterification, 89
 Degree of polymerization, 76
 Denaturation, 140, 321, 671, 672, 769, 770, 796–798
Dendrobium spp., 52
 Denosumab, 316–318
 Denthrysinin, 52
 Deoxyaurone (sulfuretin), 42
 Deoxy ribonucleic acid, 146, 148
 Deoxyribonucleoside triphosphates, 671
 Detoxifying agents, 11, 12
 Devil's claw, 27, 169, 261
 Dextromethorphan (DXM), 559
 Dextrose equivalent, 71
 D-galactaric acid, 64
 D-galactosamine, 63, 292, 293
 D-glucaric acid, 64
 D-gluconic acid, 64
 D-glucosamine, 63, 322

- D-glucose (G), 59, 65–68, 71, 72, 74, 81, 85, 90, 91, 130, 141, 146, 149–151, 201, 260, 261, 298, 312, 326, 327, 365, 371, 383, 478, 497, 651, 706
D-glucuronic acid, 64, 322, 496
D-glycero-d-manno-heptitol, 59
Diabetes, 11, 13, 133, 138, 139, 144, 150, 155, 246, 266, 283, 297, 298, 301, 312, 325, 353, 372, 384, 423, 447, 469, 472, 475, 484, 486, 487, 491, 506, 507, 513, 560, 602–606, 614, 640, 645
Diabetes mellitus, 297, 427, 462, 501, 602, 603, 655
Diagnostic agent, 316, 318
Diallyl disulfides, 475
Diastolic blood pressure, 298, 378
Dietary fiber, 4, 69, 76, 89, 91, 496
Digalactosyldiacylglycerol, 369
Digestive supplement, 314
Digitalis, 3, 27, 248, 261, 280, 283, 284, 383, 553, 555, 558, 601, 695
Digitalis purpurea, 5, 35, 248, 282, 285, 545, 592
Digitoxin, 5, 6, 35, 249, 261, 264, 284, 553
Digoxin, 3, 35, 243, 248, 249, 261, 283, 284, 536, 553, 554, 601
DIM-3, 3'-diindolylmethane, 5
Dimeric indoles, 9
Dimethylallyl pyrophosphate, 34, 220
Dimethylnitrosamine, 292
Dimethylsulfoniopropionate, 459
Dimethylsulfoxide, 748
Dimethyltryptamine, 559
Diode Array Detector (DAD), 19
Dioscoreaceae, 52, 54, 257
Dipeptidyl-peptidase-4, 355
Diphtheria toxoid, 315, 316, 514
Dipterocarpaceae, 48, 191
Direct amplification of length polymorphisms, 673
Direct Injection Mass Spectroscopy (DIMS), 19
Disaccharide, 42, 56, 66–68, 81–84, 322, 326, 356, 503
Diseases, 4, 6, 7, 11, 27, 35, 52, 135, 139, 144, 145, 150, 153–156, 174, 175, 183, 274, 283, 285, 291, 292, 330, 331, 351, 365, 378, 380, 383, 409, 416, 418, 420, 421, 423, 424, 429, 433, 442, 450, 463, 464, 467–472, 474, 486–488, 491, 501, 506, 507, 518, 524, 579, 601–603, 605, 612, 622, 625, 631, 632, 635–637, 641, 642, 645, 648, 652–655, 657, 658, 660, 662, 666, 669, 696, 700, 722, 757, 762, 763, 775, 780, 801, 802
Disodium inosinate, 319
Diterpenes, 35, 175, 176, 193, 241, 566, 618, 619
Dithiolthiones, 11
Diuretic, 9, 27, 214, 263, 282, 302, 303, 472, 601, 699, 768
Diversity arrays technology, 673
D-limonene, 171, 189, 190
D-lysergic acid amide, 559
D-mannosamine, 63
D-mannose (M), 85, 91, 107, 130, 141, 435, 437, 622, 732, 733, 759, 774, 776–781, 783, 784
Docetaxel, 12, 168, 288
Docosahexaenoic acid, 101, 343, 367, 474
Docosapentaenoic acid, 347, 368, 369
Dogs, 62, 280, 319, 332
Dopamine, 136, 137, 203, 204, 234
Dornase alfa, 316, 318
Double-stranded DNA, 153, 797, 798
Downstream sequence for pyrrolysine, 127
Dragendorff's, 203, 214, 726, 786, 787
Dried beans, 5
Drought, 4, 45, 76, 107
Drugs from Nature Targeting Inflammation (DNTI), 19
D-tubocurarine (DTC), 5, 6, 217, 696
Dyes, 28, 276, 492, 500, 523, 525, 586, 800
Dyspepsia, 13
- E**
- Ecdysone, 185, 186
Eicosapentaenoic acid, 101, 337, 338, 343, 347, 367, 368, 470, 474
Electronic PCR, 799
Element for selenocysteine, 127
Ellagic acid, 4, 28, 268–270, 272, 289, 474, 479–481, 601
Elonva, 317
Emollient, 279, 328, 525
Emulsifying, 56, 77, 278, 319, 469, 492, 501, 514, 522, 566
Emulsifying agent, 496, 501
Enbrel, 312, 317
Endothelial microparticles, 303
Engerix-B, 315
Enoxaparin, 314
Enzyme from pork pancreas, 319
Enzyme-linked immunosorbent assay, 744, 745

- Enzyme replacement therapy, 317, 513
 Enzymes, 4, 6–8, 11, 28, 33, 39–41, 44, 46, 71, 72, 76, 78, 136, 137, 140, 141, 144–146, 156, 224, 255, 276, 287, 290, 292, 303, 312, 336, 353, 355, 357, 362, 370, 381, 408, 409, 422, 427, 435, 438, 451, 452, 462, 471, 481, 483, 488, 506–508, 510–513, 522, 526, 569–573, 576–582, 623, 654, 655, 674, 679, 682, 700, 701, 704, 746, 775, 799
 Epicatechin, 4, 268, 273, 274, 303, 479, 613
 Epicatechin-3-gallate, 303
 Epigallocatechin, 289, 303, 479
 Epigallocatechin gallate, 479, 608, 613, 623
 Epoetin lambda, 316, 317
 Epoietin-alfa, 317
 Epoietin beta, 316, 317
 Eprex, 317
 Eptacog alfa, 316, 317
 Equine products, 316
 Erbitux, 318
 Ergosterol, 94, 113, 187
 Erythritol, 62, 98, 503, 504, 703
 Essential amino acids, 63, 128, 129, 132, 133, 140, 141, 156
 Essential and fixed oils, 4
 Essential fatty acids, 101, 408, 410, 418
 Essential medicine in WHO list, 6
 Essential nutrients, 7, 26, 486
 Estradiol, 185, 187, 188, 266, 329, 474, 475
 Ethane, 48
 Etanercept, 317
 Ethical nutrients digestion plus, 314
 Ethical nutrients inner health plus capsules, 314
 Ethnobotany, 3
 Ethnopharmacology, 3
 Ethylene, 41, 48, 54, 552, 566, 701, 702
 Ethyne, 48
 Etoposide, 12, 15, 266, 288, 601, 624
 Eucalyptol, 9, 43, 170
 Eudesmol, 9
 Eugenol, 43, 189, 190, 193, 194, 497, 498, 515, 516
Eulophia nuda, 52
 Euphorbiaceae, 35, 48, 51, 52, 54, 184, 246, 546
 Evaporative Light Scattering Detector (ELSD), 19
 Excipients, 28, 311, 321, 327, 334, 364, 469, 492, 493, 497, 499, 501
 Expectorant, 122, 222, 257, 261, 263, 264, 277, 688
 Expressed sequence tags, 673
 Extracellular polysaccharides, 371
 Exudates, 8, 26, 191, 277, 496, 649
 Eylea, 317
- F**
 Fabrazyme, 317, 513
 Fabric, 319
 Factor II, 314, 420
 Farnesenes, 173
 Farnesol, 95, 169, 173, 174, 178
 Fat extracted from sheep's wool, 319
 Fats, 11, 14, 33, 55, 73, 92, 94, 100, 102–106, 113, 116, 117, 126, 135, 172, 177, 183, 187, 319, 371, 408, 450, 451, 455, 476, 477, 491, 723, 788
 Fat-soluble vitamins, 409, 410, 422
 Fatty acid biosynthesis, 32, 700
 Fatty acids, 4, 29, 51, 90, 92–94, 98, 100–102, 104, 105, 107, 110, 111, 113, 119, 125, 136, 156, 189, 336, 343, 345, 347, 348, 351, 353, 355, 357, 366–370, 373, 418, 419, 422, 428, 451, 460, 469–472, 474, 476–478, 481, 484, 487, 488, 490, 500, 522, 523, 602, 724
 Fenugreek gum, 87
 Fern, 35
 Ferulic acid, 4, 110, 200, 201, 348, 523–525
 Fibrinogen, 315
 Fibrinolytic agent, 316, 317
 Fibroblast growth factor, 363, 645
 Fibromyalgia, 186
 Fish oil, 11, 102, 103, 484, 488
 Fixed oils, 11, 33, 106, 189, 723, 788
 Flash chromatography, 744
 Flavone, 37, 38, 267, 303, 349, 449, 622, 623
 Flavonics, 26
 Flavonoid glycosides, 26, 245, 250, 251, 544
 Flavonoids, 4, 7, 8, 12, 13, 26, 27, 31, 32, 35, 37, 41, 45, 46, 194, 201, 244, 250, 251, 271, 274, 285, 289, 292, 303, 348–351, 355, 443–445, 449, 450, 472, 515, 523, 586, 588, 593, 596–598, 601, 602, 608, 610, 618, 626–628, 703, 706, 708, 724, 725, 743, 785, 789, 791, 795
 Flavonoids (2-phenylchromans), 4
 Flavor, 4, 12, 13, 77, 84, 134–136, 168, 172, 492, 497, 498, 521, 588
 Flavoring agents, 28, 492, 497
 Flax seeds, 5, 188
 Flowers, 8, 9, 33, 34, 41, 42, 46, 52, 173, 188, 263, 320, 444, 517, 544, 565, 629
 Flow-mediated dilation, 303
 Fluarix, 316, 318
 Fluorine, 8, 379, 380, 546
 Fluvax, 318

- Folate, 4, 156, 435–437, 440, 457, 481, 485
Folic acid, 4, 366, 409–411, 424, 436–438, 440, 442, 443, 452, 453, 467, 468, 483, 515, 560, 561
Follitropin alfa, 316, 317
Follitropin beta, 316, 317
Food additives, 2, 13, 74, 77, 80, 126, 197, 356, 367, 469, 471, 492, 586, 588
Food color, 182, 429, 492
Food, Drug and Cosmetics Act, 527
Foods for Specified Health Uses, 487
Formic acid (methanoic acid), 118, 551
Forskolin, 175, 591, 608, 618, 619
Fourier Transform Mass Spectrometry, 18
Foxgloves, 27
Fragmin, 314
Fragrance, 9, 13, 41, 171, 177, 520
Frangula, 27, 246, 261, 592
Free radical, 12, 250, 488, 523
From cow's milk, 319
From cows or pigs, 319
From insets and crustaceans, 318
From meat extract, 319
From pressed tallow, 319
Fructooligosaccharides, 474, 478, 479
Fruits, 4, 7–10, 27, 55, 59, 62, 73, 89, 90, 97, 105, 106, 111, 118, 122, 141, 174, 180, 182, 183, 185, 188, 190, 192, 194, 195, 201, 206, 218, 250, 254, 261, 266, 274, 292, 297, 374, 414, 420, 422, 423, 436, 441, 443, 444, 465, 471, 475, 477, 482, 487, 491, 492, 503, 540, 546, 564, 588, 607, 608, 624, 668, 688
Fumaric acid, 122
Furostan, 9
- G**
- Galactocerebrosides, 115
Galactolipids, 369
Galactooligosaccharides, 479
Galactose, 9, 56, 59, 64–66, 68–70, 73, 82–84, 86–89, 113, 193, 211, 278, 326, 327, 355, 356, 369, 495, 496, 501, 503, 510, 613
Galanthamine, 13, 209, 232, 233
Galanthus nivalis L., 13
Gallic acid, 37, 38, 201, 267–270, 272, 348, 470, 602, 794, 795
Gama-aminobutyric acid, 139
Gama-aminobutyric acid type A, 515
Gamma-aminobutyric acid precursor, 135
Gamma-carotene, 9, 95, 180, 412
Gamma-terpinene, 189, 190
Garlic, 4, 5, 8, 42, 75, 263, 264, 278, 291, 298, 302, 375, 469, 470, 475, 479, 487–489, 516, 602, 617, 622, 699
Gas chromatography, 19, 185, 733, 741, 742
Gas chromatography–mass spectrometry, 19
Gas–liquid chromatography, 733
Gastric juices, 27, 136
Gastroesophageal reflux disorder, 377
G-blocks, 85
Gelatin, 84, 138, 140, 267, 275, 312, 319, 321, 330, 332, 500, 510, 789
Gelatin succinylated, 315
Gelofusine, 315
Genetic disorders, 150, 155, 423, 451, 660
Genetics, 3, 598, 660, 666, 668–670, 691, 696–698, 802
Genomics, 14, 16, 21, 587, 598, 668, 681, 690, 697, 703, 707, 708, 730
Gentian, 27, 298
Gentianose, 56
Geranylarnesol, 178
Germplasm, 7, 587, 681, 699
Gibberellins, 35, 566
Ginkgolides, 175
Glomerular filtration rate, 76
Glucitol, 62
Glucomannan, 79, 91
Glucosides, 8, 35, 38, 53, 110, 243, 472, 510, 589, 593, 594, 789
Glucosinolates, 39, 40, 256, 472, 475, 490
Glucuronic acid, 9, 71, 90, 193, 244, 496, 571, 577
Glutamic acid, 118, 128, 132, 133, 135, 140, 141, 421
Glutamine, 128, 130–132, 135, 141, 577, 579
Glutathione- GSH, 7, 40, 135, 292, 303, 418, 470, 586
Glycemic Index, 87
Glycerin, 312, 321
Glyceroglycolipids, 115
Glycerol, 30, 92, 94, 105, 113, 115, 156, 319, 321, 322, 327, 428
Glycerol monostearate, 501, 502
Glycetol, 62
Glycine, 128, 130–133, 135, 136, 140, 141, 302, 332, 385, 437, 577–579, 608, 618
Glycogen, 70, 73, 74, 435
Glycolipids, 71, 92, 94, 113–115, 369, 370
Glycolysis, 59, 121, 574
Glycosamino glycans, 71
Glycosides, 7, 10, 11, 13, 18, 26–28, 31, 33, 40, 68, 93, 95, 110, 167, 169, 203, 212, 214, 230, 233, 242–250, 253–258, 260–264, 278, 280, 281, 287, 292, 293,

- 303, 305, 306, 349, 501, 503, 513, 544, 545, 586, 587, 601, 608, 725, 727, 742, 743, 788, 791, 792
- Glycosphingolipids, 113–115
- Glycyrrhizin, 9, 118, 257, 258, 282, 293, 593
- Goitrogens, 12
- Gold chloride, 203
- Golimumab, 318
- Gonadal hormone, 316
- Gonadotropin-Releasing Hormone, 507, 508
- Gonal-f, 316, 317
- Gonyautoxins, 543, 544
- Good cholesterol lipoprotein, 117
- Gossypol, 12, 35
- Grain products, 4, 5, 422, 436
- Granocyte, 316, 317
- Granulocyte colony-stimulating factor, 632
- Green tea, 5, 156, 198, 250, 298, 490, 525, 622, 623
- G-residues, 85
- Guaiacol, 189, 190, 521
- Guar gum, 86–89, 91, 492, 493, 496
- Gum-resins, 11, 192
- Gums, 11, 12, 18, 26, 33, 56, 62, 71, 86, 90, 192, 380, 442, 496, 791
- Gymnosperms, 34, 205, 206, 559
- H**
- H1N1 pandemic influenza vaccine, 318
- Haemaccel, 315
- Haematological disorders, 13
- Haemophilus B conjugate vaccine, 315
- Haemopoietic agent, 316, 317
- Haemorrhoids, 13, 263
- Haemostatic agent, 314–318
- Hager's reagent, 203, 726, 786
- Hair loss, 186, 279, 456, 557
- Hallucinogenic effects, 204, 208, 559
- Hallucinogens, 556, 558, 559
- Hatch-Slack pathway, 118
- Havrix 1440, 315
- Havrix Junior, 315
- Healing wounds, 319, 520
- Heamopoietic agent, 317
- Heartburn, 87, 171, 377
- Helminthic therapies, 641
- Heme, 118, 292
- Hemicelluloses, 70, 78–80, 201, 470, 493
- Hemophilia, 150, 155, 312
- Heparin, 65, 71, 82, 312, 314, 322, 323, 510, 511, 514
- Heparinised saline, 314
- Heparinised saline injection, 314
- Heparin sodium, 314, 510
- Heparin sodium injection, 314
- Hepaticae, 52, 54
- Hepaticidal, 9
- Hepatitis A vaccine, 315
- Hepatitis B vaccine, 315, 646
- Herbal analgesics, 314
- Herbal daily supplements, 314
- Herbal drugs, 10, 15, 18, 26, 31, 33, 246, 667, 707
- Herbal gastrointestinal preparations, 314
- Herbalism, 3, 689
- Herbal medicine, 9, 15, 17, 210, 518, 602, 634, 635, 666, 667, 698, 701, 703, 705, 709
- Herbal teas, 5
- Herbivores, 4, 34–36, 39, 41, 42, 44, 138, 168, 183, 256, 702
- Herceptin, 145, 316–318
- Herpes simplex virus, 246, 338, 357, 625, 627, 648, 651
- Heterogeneous RNA, 146
- Heterosides, 18, 26, 168, 242, 243, 250
- Hexa pyra- and furanoses, 60, 61
- Hiberix, 315
- High-Density Lipoprotein (HDL), 477
- High fructose corn syrup, 72
- High-performance liquid chromatography, 733, 742
- High-throughput screening, 15, 18, 20, 21, 311, 725, 730
- Hiosciami, 26
- Hippocrates, 5, 487
- Histidine, 121, 127, 131–133, 136, 141, 206, 209, 222, 518, 519
- Hormone Replacements Therapy (HRT), 387
- Human chorionic gonadotropin, 312
- Human immunodeficiency virus, 627, 646, 758
- Human menopausal gonadotropin, 312
- Human papilloma virus, 146, 154, 182
- Human rotavirus live attenuated vaccine, 314
- Humulene, 169, 173, 174
- Hybridoma, 651, 744, 745
- Hydrocarbon plants, 48
- Hydrocarbons, 25, 48–54, 92–94, 98, 106–108, 167, 168, 189, 381, 382, 552, 553
- Hydrogenated glucose syrup, 72
- Hydrogenated starch hydrolysates, 62, 98
- Hydrogen cyanide, 39, 255, 551
- Hydrolyzable tannins, 2, 4, 166, 267, 268, 270, 274, 348
- Hydroxyl group, 60, 64, 110, 113, 130, 148, 150, 151, 180, 185, 194, 268, 270, 454, 574
- Hydroxypropyl methylcellulose, 501, 502

- Hyoscyamine, 3, 205, 206, 208, 214, 215, 225, 226, 233, 234, 545–548, 593, 706
- Hypertension, 254, 279, 298, 301, 312, 471, 472, 506, 547, 602, 610–612, 614–616, 621, 645, 660
- Hypoglycemic, 12, 135, 264, 297–299, 556, 601, 606, 607, 610, 766
- Hypoxanthine aminopterin thymidine, 745
- Hypurin isophane (NPH) injection, 315
- Hypurin neutral injection, 315
- I**
- Ibogaine, 205, 209, 220, 221, 242, 558, 559
- Ice cream, 76–78, 82, 84–86, 91
- Imiglucerase, 317, 513
- Immune supplement, 314
- Immune system, 4, 6, 8, 27, 28, 76, 90, 134, 135, 137, 182, 186, 188, 194, 250, 266, 279, 280, 318, 347, 375, 378, 422, 465, 481, 537, 538, 632–636, 639, 640, 642, 653, 656, 661, 702
- Immunity-potentiating agents, 11
- Immunoglobulin, 135, 312
- Immunomodifier, 316–318
- Immunomodulators, 632
- Immunophilins, 640
- Immunotherapy, 631, 632, 636, 638–640
- Indigestion, 136, 377, 518
- Indole alkaloids, 9, 205, 206, 209, 218, 220–222, 239, 242, 289, 291, 337, 546, 701
- Indole-3-carbinol, 4, 289, 623
- Indoles, 4, 12, 490, 559
- Inert constituents, 10, 28, 29
- Infections, 26, 155, 224, 248, 291, 312, 313, 319, 328, 332, 333, 335, 365, 375, 378, 450, 467, 468, 472, 481, 484, 489, 560, 561, 625, 626, 628, 633, 634, 649, 650, 656, 676, 758
- Infectious diseases, 145, 150, 155, 330, 335, 365, 491, 514, 638, 647
- Infestations, 26
- Inflammation, 6, 27, 138, 188, 211, 274, 318, 319, 335, 382, 427, 429, 430, 450, 487, 488, 512, 516, 522, 526, 547, 605, 623, 624, 780
- Inflammatory disease, 139, 174, 380
- Infliximab, 318
- Influenza virus vaccine, 316, 318
- Influvac, 318
- Injections, 28, 154, 174, 312, 330, 638, 640
- Injection, 11
- Inositolhexaphosphate, 455, 456
- Insect, 9, 34, 35, 37, 41, 52, 80, 93–95, 138, 174, 178, 180, 218, 264, 320, 500, 519, 520, 565, 566, 640, 646, 707, 760, 763
- Insect pheromone, 319
- Insect pollination, 4
- Insects, 33–36, 39, 41, 42, 52, 53, 63, 64, 105–107, 138, 167, 168, 174, 188, 190, 202, 204, 218, 234, 254, 261, 272, 276, 459, 514, 537, 555, 562, 565, 587, 669, 702, 746
- Insect secretion, 319
- Insulin, 59, 63, 135, 144, 146, 246, 297, 298, 312, 315, 323–326, 334, 355, 385, 388, 433, 488, 506, 507, 513, 603, 605–611, 613, 614, 637, 645, 655, 656, 658, 659
- Insulin-dependent diabetes mellitus, 603
- Insulin preparations, 315
- Intanza, 318
- Interferon beta-1a, 316–318
- Interleukin-1 β , 174
- Intermediate-Density Lipoprotein (IDL), 116
- International Society of Hypertension, 301
- International Union of Pure and Applied Chemistry (IUPAC), 48, 119
- Inter-simple sequence repeats, 673
- Inulins without glucose, 76
- Iodine, 4, 7, 353, 374, 381, 387, 409, 470, 554, 729, 756, 786
- Iodine in potassium iodide, 203, 726
- Iota, 84, 355, 356
- IPV suspension for injection, 315
- Iridoids, 26, 168–170, 258
- Iron, 4, 7, 45, 74, 75, 134, 135, 320, 347, 374, 375, 409, 440, 442, 443, 465, 484–486, 491, 549, 553, 554, 574
- Isocitric acid, 122
- Isoelectric point, 132
- Isoflavones (3-phenylchromans), 4
- Isoleucine, 133, 136
- Isomalt, 62, 98
- Isopentenyl Pyrophosphate (IPP), 34
- Isophane, 315
- Isoprene, 48, 92, 167–170, 173, 175, 178, 180, 183, 193, 239, 280, 412, 420
- Isothiocyanates, 4, 12, 472, 474, 475, 545, 622
- Isovaleric acid, 9, 169, 170
- J**
- Japanese Folk Medicine, 353
- Jasmonic acid, 40, 701, 702
- Jaundice, 13, 261, 292, 579, 582
- Juncaceae, 52, 54

- K**
K⁺-ATP channel, 59
 Kahweol, 169, 175
 Kale, 4, 5, 28, 180, 443, 472, 487, 489
 Kaposi sarcoma, 6
 Kappa, 84, 216, 355, 356, 472
 Kenacid blue, 748
 Ketamine, 559
 Ketodeoxyoctulosonic acid, 64
 Ketose sugars, 62
 King brown, 316
 Kogenate FS, 316, 317
 Krebs cycle, 118, 121, 572
- L**
 Lactitol, 61, 62, 98
 Lactitol (12C), 98
 Lactobacillus acidophilus, 314, 315, 471, 481, 482, 486
 Lactones, 4, 26, 168, 174, 258, 287, 564, 588, 594, 601, 628, 629, 724
 Lactose, 29, 64–66, 73, 312, 319, 326, 327, 332, 481, 500, 503, 504
 Lambda, 84, 355, 356
 Lanolin, 107, 312, 319, 327, 328
 Lanosterol, 9, 94, 107, 178, 179
 Laronidase, 317, 513
 Latex, 3, 8, 48, 51, 183, 184, 206, 216, 382, 512, 515
 Latex-producing plants, 48
 L-dihydroxyphenylalanine (L-DOPA), 134
 Leaves, 3, 8, 34, 37, 43–46, 54, 60, 94, 105–107, 118, 122, 184, 188, 189, 192, 198, 206, 209, 210, 214, 221, 222, 232, 242, 246, 248, 254, 256, 257, 261–263, 271, 272, 278, 282, 298, 302, 313, 340, 443, 449, 492, 503, 515–518, 544, 546, 564, 588, 607–609, 629, 708, 723, 793
 Leeks, 5, 75, 443
 Legumes, 4, 5, 8, 66, 68, 89, 111, 137, 138, 140, 186, 250, 266, 278, 374, 419, 422, 449, 465, 470, 475, 491
 Lenograstim, 316, 317
 Lentils, 5, 133, 140, 374
 Leucine, 120, 127, 130, 132, 136
 Leucodermia, 13
 L-galactaric acid, 64
 L-galactonic acid, 64
 L-glucaric acid, 64
 Lignans, 4, 8, 26, 201, 264–266, 292, 297, 474, 479, 480, 588
 Lignin, 29, 31, 35, 38, 39, 56, 70, 80, 264, 470, 521, 628, 629
 Lime juice, 7
- Limonene, 4, 9, 34, 169–171, 470, 497, 498
 Limulus Amebocyte Lysate, 313
 Linalool (enantiomer), 42
 Linoleic acid, 101, 102, 419
 Linolenic acids, 97
 Lipase, 116, 314, 355, 357, 483, 485
 Lipid peroxidation, 12
 Lipoid, 348, 349
 Lipopolysaccharides, 357, 360, 538, 539, 541, 542
 Lipoprotein, 92, 94, 111, 113, 115–117, 187, 357
 Liquid chromatography, 741, 742
 Liquid chromatography–mass spectrometry, 20
 Liquid hydrocarbons, 48
 Live, 32, 77, 88, 140, 312, 315, 316, 327, 331, 423, 481, 633, 636, 640, 642–644, 648
 L-limonene, 171, 189, 190
 Locust bean gum, 86, 88, 89, 493, 496
 Long-chained highly unsaturated fatty acids, 347
 Long-chained monounsaturated fatty acids, 347
 Long-chained polyunsaturated fatty acids, 347
 Long chain fatty acids, 101, 107, 112, 524
 Low-Density Lipoprotein (LDL), 7, 89, 90, 111, 112, 116, 117, 187, 357, 424, 477, 489, 606, 661
 Lung cancer, 12, 211, 337, 623
 Lutein, 4, 5, 28, 53–55, 156, 180, 182, 184, 302, 366–368, 485, 487, 489
 Luteinizing hormone-releasing factor, 507
 Lutropin alfa, 316, 317
 Luveris 75 IU, 316, 317
 Lycopene, 4, 7–9, 28, 54, 95, 180, 182, 183, 289, 469–471, 474, 487, 489, 525, 622, 623
 Lysergic acid, 26, 205, 218, 558
 Lysergic Acid Diethylamide (LSD), 27, 558, 559
 Lysine, 30, 127–129, 131–133, 136, 140, 141, 206, 235, 460, 519
- M**
 Mabcampath, 316, 317
 Mabthera, 316–318
 Macrolides, 287, 336, 357, 386, 389
 Macronutrients, 4
 Magnesium, 4, 7, 376, 377, 380, 409, 457, 458, 549, 621, 727, 789, 791
 Magnesium stearate, 312, 328, 329
 Malarial parasite, 761
 Malic acid, 118, 122, 124, 523, 525
 Malonic acid pathway, 32, 34
 Maltitol (12C), 98

- Maltotetraitol (24C), 98
Maltotriitol (18C), 98
Manganese, 4, 409, 546, 549, 551
Mango, 59, 291, 547
Mannan oligosaccharides, 479
Mannitol, 62, 73, 98
Mannoheptulose, 58, 59
Mannuronate, 85
Marme's, 203, 787
Mass Spectrometric Detector (MSD), 19
Mass Spectrometry (MS), 17–20, 589–598, 728, 731
Mass spectrometry techniques, 17, 19, 692
Mayer's, 203, 786
M-blocks, 85
Measles, 233, 315, 330–332, 514, 627
Measles vaccine, 315
Mechanically separated seal meat, 347
Mechanical ventilation, 217
Median effective dose, 746
Medicinal and Aromatic Plants (MAPs), 28, 168, 722
Medicinal phytochemistry, 8, 10, 19
Medicines and Healthcare products Regulatory Agency, 527
Medium chain fatty acids, 101
Melons, 4
Menthol, 9, 34, 43, 95, 168, 170, 172, 189, 190, 285, 472, 497, 498, 515
Mercuric chloride, 203, 786
Merieux-inactivated rabies vaccine, 315
Metabolic pathways, 7, 8, 26, 29, 30, 32, 33, 43, 44, 118, 348, 580, 587, 667, 700, 701, 705
Metabolome, 14, 15, 17
Metabolomics, 3, 14–19, 668, 703, 704, 707, 708
Metalysé, 316, 317
Methionine, 127, 130, 132, 133, 136, 137, 140, 141, 437, 440, 458, 459, 578
Methoxy polyethylene glycol-epoetin beta, 316, 317
Methylbenzoate, 41, 42
Methylenedioxymethamphetamine, 559
Methylerythritol phosphate, 32, 34
Methylerythritol phosphate pathway - MEP, 32, 34
Methylsulfoniopropionate, 459
Mevalonate pathway, 32, 34, 280
Mevalonic acid pathway, 34
Micronutrients, 4
Microplate, 20, 748, 778–780
MicroRNAs, 146, 155
Microsatellites, 673, 674, 677, 680, 681, 698
Microtiter plate, 761, 771
Migraine headache, 186
Milk, 11, 65, 66, 84, 119, 121, 126, 132, 140, 187, 188, 312, 313, 326–328, 412, 414, 422, 427, 429, 430, 432, 438, 450, 452, 459, 461, 464, 465, 470, 477, 482–485, 491, 503, 512, 513, 622, 633, 645
Millennia, 4, 5, 7
Mircera, 316, 317
Molecular biology, 3, 588, 598, 600, 660, 666–671, 690, 696, 796
Molluscicidal activity, 763
Mollusks, 335, 537, 551
Monoclonal antibodies, 646, 649, 651, 658, 659, 744, 745
Monocyclic aromatic hydrocarbon, 269
Monocyclic terpenes, 170
Monogalactosyl diacylglycerol, 369
Monoterpenes, 4, 34, 95, 168, 170, 171
Monounsaturated fat, 4
Moraceae, 48, 52, 191, 609, 613
Moroctocog alfa, 317, 318
Morphine, 3, 4, 9, 16, 27, 43, 202–206, 208, 209, 216, 217, 227, 232, 234, 242, 260, 383, 384, 515, 545, 553, 572, 595, 738
M-residues, 85
mRNA-messenger RNA, 146, 155
Mucilage, 18, 26, 56, 263
Mucopolysaccharidoses I, 513
Multicomponent formulation, 13
Multilocus sequence analysis, 673
Mumps, 315, 331, 332, 514, 636
Mumps vaccine, 315
Murine products, 318
Mycotoxin, 539, 540
Myrcene, 172
Myrecene, 34
Myristicin, 12, 189, 190
- N**
- N-acetyl-D-glucosamine, 63, 80, 780, 781
N-acetyl-glucosamine, 523, 526
Nanoparticles, 193
Naphthoquinones, 97, 276, 291, 468, 577
Nasunin, 4
National Biological Standards Board, 527
Natural product, 2, 3, 6, 10, 208, 270, 339, 566, 705, 707, 746–748, 750–754, 756, 757, 759, 761, 763–766, 768, 769, 772–774, 779, 781, 784

- Natural red 4 (red dye), 312, 319
 Natural rubber, 183
 Nauplii, 747, 749
 Neem, 35, 281
 NeoRecormon, 316, 317
 Neosaxitoxin, 543, 544
 Neurodegenerative diseases, 137, 150, 153, 155, 372
 Neuropharmacological agents, 11, 12
 Neurotoxin, 34, 209, 344, 345, 359, 360, 547, 548, 550, 551, 555
 Neurotransmitters, 129, 136, 204, 234, 455
 New chemical entities, 13, 15
 Next generation sequencing, 671, 673, 674, 688, 689
 Niacin, 4, 408–410, 424, 429–431, 435, 465, 467, 468, 515
 Nicotinamide adenine dinucleotide, 32, 651
 Nicotinamide adenine dinucleotide-phosphate, 32, 430, 431
 Nicotine, 9, 26, 27, 31, 38, 40, 203–209, 215, 221, 230, 233, 234, 242, 555, 564, 565, 586, 595, 700–702
Nidema boothii, 52
 Night blindness, 5, 409, 413, 450
 Nitric acid, 77, 786
 Nitrogen oxides, 552
 N-methyl-D-aspartate, 515
 N-methyl-D-aspartate receptors, 135
 Nonacog alfa, 317
 Non-communicable disease, 603
 Nonessential amino acids, 128, 132
 Nonesterified fatty acids, 347
 Noninsulin-dependent diabetes mellitus, 603
 Noniodide reagents, 203
 Nonprotein amino acids, 38, 42, 137, 138
 Non-starch polysaccharides, 12
 Nonsteroidal anti-inflammatory drugs, 385, 388
 Novicrit, 316, 317
 NovoSeven RT, 316, 317
 Nuclear magnetic resonance spectroscopy, 2, 17, 19, 20
 Nucleic acids, 8, 48, 92, 146–150, 457, 571, 588, 625, 683
 Nuts, 4, 5, 8, 10, 97, 111, 133, 141, 185, 187, 210, 218, 266, 379, 381, 419, 422, 436, 453, 462, 465, 470, 487, 492, 512
- O**
 Ochratoxin A, 540
 Ochratoxin B, 540–542
 Ochratoxin C, 540–542
 Octocog alfa, 316, 317
- Oestrogens, conjugated, 513
 Oils, 8, 10, 12, 26, 31, 34, 43, 51, 92, 94, 97, 100–102, 104, 105, 110, 111, 113, 119, 126, 168, 170–174, 178, 185–191, 194, 312, 328, 344, 348, 377, 382, 414, 416, 419, 422, 470, 491, 501, 517, 518, 522, 524, 624, 722, 723
 Oleic acid, 29, 102, 119, 156
 Oleic oil and oleostearin, 319
 Oleoresins, 4, 192
 Oligomeric flavonoids, 474
 Oligomeric proanthocyanidins, 273, 303
 Olive, 51, 94, 100, 102, 104–106, 169, 185, 298, 416, 470, 477, 484, 517, 525, 622, 634, 791
 Omalizumab, 317, 318
 Omega-3, omega-6 fatty acids, 4, 418, 422
 Omic-technique, 15
 One-dimensional NMR spectroscopy, 19
 Onion, 4, 37, 42, 75, 185, 263, 264, 479, 623
Ophiopogon japonicus, 187
 Ophthalmic medication, 317
 Opiates, 4, 383, 558
 Opium, 11, 16, 203, 205, 216, 217, 383, 515, 516, 555
 Opium poppy, 4, 217, 515
 Oral contraceptives, 387
 Orchidaceae, 52, 54, 253
 Orencia, 316, 317
 Organic chemistry, 2, 3, 144
 Organosulfures, 4
 Organar, 314
 Ornithine transcarbamylase, 155
 Orotic acid, 411, 457, 458, 460
 Osteoarthritis, 63, 186, 319, 467, 521
 Osteoporosis, 7, 63, 82, 380, 449, 450, 471, 472, 484, 491, 631, 645
 Other dermatological preparations, 315
 Other products, 11, 62, 190, 318, 635
 Other respiratory agent, 317, 318
 Ouabain, 27, 248, 249, 261, 283, 284, 545
 Ovarian cancer, 211, 339, 599, 624, 625
 Over-the-counter medication, 527
 Ovidrel, 316, 317
 Oxalic acid, 4, 118, 547, 548, 554
 Oxaloacetic acids, 118
 Ox pancreas, 319
- P**
 Pacific yew tree, 174
 Paclitaxel (taxol), 12, 15, 624
 Pain-relieving properties, 5
 Palivizumab, 318
 Palm, 102, 107, 119, 183, 185, 475

- p-Aminobenzoic acid (PABA), 128, 465
Pancreas, 59, 182, 261, 270, 312, 450, 460, 513, 623
Pancreatic cancer, 246, 623, 624
Pancrelipase, 314
Panitumumab, 317, 318
Pantothenic acid, 138, 409, 432, 433, 467, 469, 582
Panvax H1N1 vaccine, 318
Panzytrat 25000, 314
Papaverine, 4, 206, 209, 216, 217, 227, 546
Paralytic Shellfish Poisoning (PSP), 360, 541
Paralytic shellfish toxin, 360, 542
Parkinson's disease, 6, 137, 423
Pathogens, 4, 7, 26, 34, 35, 40, 41, 114, 138, 223, 256, 341, 383, 471, 481, 587, 626, 633, 637, 642, 644, 646, 649, 650, 652, 666, 669, 703
Peaches, 4, 55, 89, 254
Peanut, 102, 104, 119, 185, 451, 465
Peas, 5, 68, 133, 180, 183, 378, 436
Penta furanoses, 60, 61
Pentagalloyl Glucose (PGG), 269, 270
Pentose phosphate pathway, 32, 700
Pentose phosphate shunt, 118
Peppermint oil, 34, 189, 497
Peppers, 4, 133, 180, 182, 221, 260, 443, 465, 475, 487, 492, 515, 516
Pepsin, 312, 483, 512, 513
Peptides, 12, 128, 137, 138, 140, 145, 203, 218, 232, 287, 312, 336, 342, 343, 351, 357, 359, 372, 388, 474, 476, 477, 506–508, 510, 517, 518, 523, 526, 539, 549, 559, 610, 621, 632, 692, 741
Perfluorocarbons, 379
Perilla alcohol, 9
Perillyl alcohol, 4
Persea gratissima, 59
Pertussis toxin, 539
Pertussis vaccine, 315, 514
Pesticides, 13, 195, 242, 279, 450, 552, 562, 566, 567, 588, 738
Pests, 26, 33, 35, 107, 138, 168, 180, 263, 537, 562, 707
Pharmaceutical industry, 10, 20, 67, 89, 134, 276, 305, 319, 320, 327, 329, 372, 492, 722, 725
Pharmaceuticals, 29, 144, 145, 190, 192, 196, 285, 290, 305, 314, 319, 320, 329, 334, 335, 360, 367, 380, 382, 383, 385, 386, 499, 501, 506, 507, 517, 523, 582, 586, 587, 600, 616, 655, 669, 695, 703
Pharmacognosy, 665–668, 689, 708, 709
Pharmacognosy curriculum, 3
- Pharmacological activity, 18, 26, 146, 204
Pharmacophore analysis, 13
Phencyclidine (PCP), 291, 507, 553, 554, 559
Phenethyl isothiocyanate, 472
Phenolic glycosides, 26, 247, 248, 544, 619
Phenolics, 7, 8, 10, 12, 18, 27, 31, 32, 34, 35, 39, 41, 46, 194, 270, 274, 285, 305, 487, 586–588, 597
Phenols, 4, 11, 12, 26, 27, 33, 117, 191, 194, 195, 201, 232, 246, 275, 342, 348, 351, 355, 575, 577, 588, 601, 703, 734, 789
Phenylalanine, 121, 127, 129, 131–134, 136, 139–141, 206, 209, 210, 221–224, 505, 775–777
Phloroglucinol, 267, 268, 353, 355
Phosphatidylinositol, 456
Phosphatidylinositol phosphate, 455
Phospholipids, 92, 94, 101, 107, 113, 115, 136, 347, 421
Phosphomolybdenic acid, 203
Photo- and oxidative phosphorylation, 32
Photooxidation, 35
Photosynthesis, glycolysis, 32
Photosynthetes, 48
Phototsynthetically Active Radiation (PAR), 35
Phycobiliproteins, 364, 372
Phycocyanin, 156, 366, 367, 372
Phycoerythrin, 364, 366, 372
Phytic acid, 4, 455, 456
Phytoalexins, 9, 35, 37, 40, 41
Phytochemical arrays, 16
Phytochemicals, 2–8, 11, 12, 16, 19, 47, 302, 471, 472, 474–480, 484, 487–489, 515, 518, 522, 600, 601, 622, 624–626, 628, 629, 699, 724, 725, 743, 744, 792
Phytochemical screening assays, 18, 743
Phytochemistry, 2, 3, 18–20, 26
Phytoconstituents, 14–16, 26, 292, 587, 588, 599, 606, 610, 611, 625, 627
Phyo-drugs, 2
Phytoestrogens, 264–266, 474, 475, 622
Phytol, 95, 175, 177
Phytonutrients, 4–8
Phytostanol este, 109
Phytosterols, 4, 12, 30, 110, 113, 180, 185, 186, 188, 281, 282, 306, 366, 602, 621, 790
Phytotherapy, 13
Picric acid, 203, 726, 786
Picorrhiza, 27
Pine and fir, 34
Pinene, 9, 170, 172, 618, 621
Pituitary gonadotropins, 312

- Pituitary hormone, 316–318, 603
 Plant biochemistry, 2, 3, 47
 Plant-Made Pharmaceuticals (PMPs), 145
 Plant physiology, 47, 201
 Plant steroids, 8
 Plasma volume expander, 315
 Plasmid curing, 13
 Plastoquinone, 276
 Platelet aggregation, 4, 6, 8, 348
 Pneumococcal vaccine, 315
 Pneumocystis carinii pneumonia, 291
Podophyllum sp., 12
 Poisons, 26, 257, 260, 278, 429, 515, 536–539,
 541, 543–548, 554–558, 565, 570, 577,
 666, 770
 Poliomyelitis vaccine, 315
 Pollinators, 9, 30, 41, 42
 Pollution, 4, 450, 666, 667, 696, 707
 Polyalcohols, 62
 Polychlorinated biphenyls, 553, 561
 Polycyclic aromatic hydrocarbons, 54, 475
 Polyethylene glycol, 500–502, 635, 659, 745,
 753
 Polygeline, 315
 Polyglycerol (nC), 61, 98
 Polyhydric alcohols, 62
 Polyisoprene, 169, 183
 Polymerase chain reaction, 598, 669–673, 676,
 682, 684, 687, 796
 Polyols, 13, 30, 60, 62, 92, 93, 98, 99, 156, 270
 Polyphenols, 4, 8, 11, 12, 26, 194, 201, 267,
 268, 273, 353, 366, 479, 523, 577, 578,
 618, 622, 626–628, 724
Polyodium vulgare, 35
 Polysaccharides, 26, 56, 69, 70, 72, 78–82, 84,
 115, 156, 292, 336–338, 353, 355, 356,
 366, 367, 370, 371, 492, 493, 495, 496,
 500, 522, 607, 610, 626–628, 631, 659,
 741
 Polyurea, 13
 Polyvalent snake antivenom, 316
 Poractant alfa, 314
 Porcine, 314, 321, 322, 324, 332
 Positron Emission Tomography (PET), 379
 Potassium, 4, 7, 118, 135, 248, 256, 257,
 267, 302, 303, 374, 452, 457, 470,
 501, 503–505, 549, 552, 554, 578,
 614, 618, 726, 745, 768, 780, 782, 786,
 788, 790
 Potassium bismuth iodide, 203, 786
 Potassium cadmium iodide, 203, 787
 Prebiotics, 356, 471, 479, 484
 Predation by microbes, 26
 Predators, 4, 26, 34, 41, 42, 53, 138, 168, 201,
 373, 537, 550
 Premarin, 312, 316, 329, 330
 Premarin tablets, 316
 Prenol, 9, 169, 170
 Prescription-only medicine, 527
 Preservatives, 28, 119, 133, 197, 332, 492
 Prevenar, 315
 Prey, 4, 313, 516, 537, 550
 Primary constituents, 8
 Primary metabolic pathway, 30
 Primary metabolites, 10, 14, 30, 32, 155, 156,
 700
 Priorix, 315
 Priorix-tetra, 315
 Proanthocyanidin oligomers, 273
 Proanthocyanidins, 39, 45, 267, 268, 271, 273,
 274, 450, 473, 474, 489, 626, 628
 Probit analysis, 746, 748, 749
 Prolia, 316, 317
 Proline, 121, 128–132, 136, 137, 140, 141, 441
 Propolis, 319
 Propylene glycol, 29, 498, 760
 Prostate cancer, 5, 7, 28, 53, 54, 270, 475, 623,
 631
 Protease, 314, 483, 512, 775–778
 Protease inhibitors, 4, 12, 40, 628, 775
 Protein amino acids, 133, 134, 137
 Protein denaturation, 46, 770
 Protein kinase C, 359, 360
 Proteinogenic, 127, 128, 137
 Proteinogenic Amino Acids (PAAs), 127, 132,
 133, 137
 Proteins, 7, 8, 11, 14, 29, 33, 39, 41, 48, 63, 78,
 84, 92, 94, 115–117, 127, 132,
 134–138, 140–147, 153–156, 175, 203,
 214, 223, 267, 275, 280, 312, 313, 323,
 324, 330, 332, 336, 347, 351, 364, 366,
 372, 374, 385, 388, 408, 418, 425, 427,
 450, 456, 474, 506–508, 510, 512, 518,
 523, 538, 544, 549, 556, 571, 574, 577,
 581, 588, 599, 605, 625, 627, 628, 634,
 644–648, 653–657, 659, 669, 692–694,
 701–703, 705, 707, 725, 740, 741, 772,
 773, 785, 790
 Protein tyrosine phosphatase 1B, 355
 Proteomics, 14, 16, 668, 690, 703, 707
 Prothrombinex-VF, 314
 Protozoans, 9, 291
 Pseudolipid, 92
 Psoralea, 27, 52, 254, 596, 623
 Psoriasis, 52, 177, 186, 252, 253, 475, 481,
 484, 526

- Pugative, 13
Pulmozyme, 316, 318, 513
Punicalagins, 4
Puregon, 316, 317
Purines, 8, 148, 214, 437, 452, 458
Pyrethrin, 565
Pyridoxal, 410, 434, 435, 469
Pyridoxal 5'-phosphate, 434
Pyridoxamine, 434
Pyridoxamine 5'-phosphate, 434
Pyridoxine, 408, 410, 424, 431, 434, 435, 465, 469
Pyridoxine 5'-phosphate, 434
Pyrimidines, 8, 136, 148, 437, 458
Pyrroloquinoline quinine, 276
Pyrrolysine insertion sequence, 127
Pyruvate dehydrogenase complex, 653
- Q**
Quantitative PCR, 673, 684, 800, 802
Quassia, 27
Quercitin, 4
Quillaja saponaria, 257, 278
Quimidine, 3, 9, 203–206, 220, 227, 232, 242, 553, 696
Quinine, 3, 8, 9, 26, 27, 203–206, 220, 227, 232, 233, 242, 246, 291, 562, 591, 601, 695, 792
Q-Vax skin test, 318
- R**
Rabies immunoglobulin, 312
Rabies vaccine, 315, 318
Rabipur, 315, 318
Raffinose, 56, 65, 68, 69
Raffinose family oligosaccharides, 69
Random amplified polymorphic DNA, 673, 684, 697, 698
Ranunculosides, 26, 244
Rapid Amplification of cDNA Ends PCR, 155, 485, 686, 801
Reactive Oxygen Species (ROS), 202, 303, 474, 624
Real-Time PCR (RT-PCR), 673, 800
Rebif, 316, 318
Recombinant antihaemophilic factor, 316
Recombinate, 316, 318
Red pigment from crushed cochineal insects, 319
Reductive acids, 12
Refractive Index Detector (RID), 19
Rejuvenating tonic, 13
Remicade, 318
Reopro, 318
Repeating unit of D-glucose (G) and D-mannose (M), 68, 74, 91
Resin ducts, 34
Resins, 4, 11, 28, 31, 33, 107, 172, 191–193, 517, 544, 740, 742
Respiratory agent, 314, 316, 318
Restriction Fragment Length Polymorphism (RFLP), 673, 674, 676, 684, 697, 698
Resveratrol, 4, 51, 54, 94, 289, 290, 297, 484, 485, 515–517, 588, 598, 622, 624
Retention Factor (RF), 737
Retention time, 742
Retinal, 9, 175, 182, 410, 412
Retinol, 9, 95, 97, 175, 177, 410, 412–414, 422, 485
Reverse Transcription qPCR (RT-qPCR), 801
Rhamnose, 9, 211, 244, 249, 250, 278, 495, 496
Rheumatoid arthritis, 186, 319, 506, 507, 780
Rhubarb, 27, 89, 118, 246, 247, 277, 441
Riboflavin, 366, 409, 410, 424, 427–429, 431, 468, 582
Ribonucleic acid, 146, 148
Ribosomal RNA, 146, 686
Ribozyme, 151
Rituximab, 316–318
RNA interference, 154, 155
Rocuronium, 5
Romaine lettuce, 5
Roots, 8, 12, 69, 71, 88, 91, 169, 189, 192, 204, 206, 209, 214, 221, 246, 254, 257, 261, 263, 277, 278, 281, 289, 303, 382, 449, 475, 483, 492, 496, 524, 544, 546, 564–566, 587, 588, 606–608, 625, 629, 691, 705, 762
Rotarix, 314
Rota Teq, 314
Rotavirus vaccine live oral pentavalent, 314
R-phycoerythrin, 372
Rubella vaccine, 315
Rutin, 42, 43, 250, 303, 411, 445, 450, 592, 593, 608
- S**
Sabinene, 170
Saccharin, 29, 501, 503–505
S-adenosylmethionine, 138, 440, 578
Safer alternatives, 5
Safflower, 185, 422, 624, 637
Safrole, 189, 190
Saizen, 318
Salicin, 5, 15, 244, 245, 247, 383, 472

- Salicylic acid, 5, 31, 41, 122, 245, 383, 523, 526, 554
 Salinity, 4
 Saliva, 27, 222, 483, 760
Salix sp., 5
 S-allylcysteine, 475, 476
Salmonella typhi vaccine, 315
 Salvinorin A, 168, 175
 Saoproot, 27
 Saponins, 4, 8, 9, 26, 27, 47, 168, 244, 257, 263, 278–282, 292, 303, 337, 469, 471, 545, 586, 587, 589, 596, 598, 601, 602, 610, 618, 626, 628, 631, 724, 725, 729, 791
 Saponosides, 26
 Sapotaceae, 48, 183, 281
 Saxitoxin, 357, 358, 360, 361, 541, 542, 551
Scaphyglottis livida, 52
 Schizonticidal assay, 761
 Scrofula, 13
 Secondary constituents, 8
 Secondary metabolites, 2, 3, 6, 8, 10, 14, 15, 29–36, 38, 40–45, 48, 137, 155, 156, 201, 285–287, 302, 305, 334, 340, 341, 348, 357, 586–589, 600, 667, 674, 700–707, 725, 726, 742, 785
 Seeds, 4, 5, 8, 39, 52, 66, 67, 71, 86–88, 97, 105, 106, 111, 132, 133, 138, 140, 141, 145, 155, 185–188, 190, 206, 210, 222, 232, 248, 250, 254, 256, 261, 266, 274, 302, 419, 422, 449, 451, 455, 456, 462–465, 475, 492, 496, 507, 510, 544–547, 559, 562, 564, 566, 588, 607, 608, 629, 723, 794
 Selenium, 4, 7, 8, 381, 418, 470, 525, 546
 Selenium Sulfide (SeS), 381
 Selenocysteine Insertion Sequence (SECIS), 127
 Senna, 27, 246, 247, 261, 277, 510, 590
 Sephadex, 740
 Sephadex chromatography, 744
 Sequence characterized regions, 673
 Sequence Tag Sites (STSs), 673
 Serine, 114, 127, 132, 136, 141, 357, 437
 Serotonin, 27, 137, 203, 204, 209, 218, 220, 234, 298, 303, 455, 550, 551, 558
 Sesame, 5, 111, 132, 133, 185, 618, 723
 Sesquiterpene, 4, 35, 95, 169, 173, 258, 302, 340, 343, 564, 588, 594, 597
 Sesterterpenoid, 178
 Severe Combined Immunodeficiency (SCID), 153, 513
 Sheep, 106, 107, 147, 312, 319, 327, 520
 Sheep thyroid, 312
 Shellac, 191, 319, 500, 520
 Shikimic acid, 10, 118, 268, 269, 348, 601
 Shikimic acid pathway, 32, 34, 703
 Shikimic acid–phenylpropanoid pathways, 189
 Short-chained mono- and polyunsaturated fatty acids, 347
 Shortchained saturated fatty acids, 347
 Short chain fatty acids, 76, 101
 Short hairpin RNA (shRNA), 155
 Short Tandem Repeats (STRs), 674
 Shrimp larvae, 747–749
 Sialic acid, 63
 Silicon, 8, 686
 Silymarin, 4, 293, 597, 601, 622
 Simple Sequence Repeats (SSRs), 673, 674, 677, 684
 Simponi prefilled syringe solution, 318
 Simulect, 318
 Single nucleotide polymorphisms, 673, 681
 Single-stranded DNA (ssDNA), 147, 682, 797, 798
 Sitostanyl oleate, 187
 Skeletal muscle relaxation, 217
 Skin disorders, 13, 177, 452, 474
 Slippery elm, 27
 Small interfering RNAs (SiRNAs), 155
 Small nuclear RNA, 146
 S-Methylmethionine, 411, 457–460
 Soapbark, 27, 257, 278
 Soapberry, 27, 257, 278
 Soapwort, 27, 257, 278
 Solanine, 35, 45, 203, 233, 260, 261, 545, 547, 548
 Solubilizing agent, 319
 Soluble fiber, 4, 87, 302, 487, 489
 Soluble sugars, 8
 Somatropin, 318
 Sonnenschein's reagent, 203
 Sorbitan trioleate, 29
 Sorbitol, 62, 72, 73, 98, 501–504
 Source, 11, 13, 16, 27, 32, 34, 59, 67, 70, 82, 87–89, 91, 100–102, 104–107, 110, 119, 134–137, 140, 144, 172, 174, 195, 204, 225, 232, 233, 244, 264, 266, 272, 282, 284, 288, 293, 314, 318, 319, 321, 324–328, 334–337, 340, 343–345, 348, 351, 355–357, 359, 363, 367, 371, 373, 375, 379, 409, 414, 416, 418–420, 423–427, 429, 432, 434–436, 438, 441, 443, 450, 452, 453, 457–459, 461–464, 472, 474, 480, 483–485, 495, 497, 505, 514–516, 538, 549, 551, 552, 574, 577,

- 587, 588, 599, 600, 606, 622, 624, 625, 646, 655, 670, 672, 675, 684, 687, 701–704, 722, 749, 762
- Soya lecithin, 29
- Soybean, 94, 100, 104, 133, 138, 140, 185, 187, 422, 464, 483–486, 517, 608, 618, 622
- Spareien, 9
- Sparingly, 4
- Sparteine, 205, 209, 215, 216, 229, 545, 547, 548
- Spices, 4, 5, 8, 17, 195, 302, 492, 540
- Spinach, 4, 5, 28, 35, 133, 138, 175, 180, 183, 280, 374, 412, 416, 420, 423, 427, 436, 441, 461, 464, 487, 489, 618
- Spirostan, 9
- Sprouts, 4, 374, 419
- Squalene, 9, 94, 168, 169, 178–180, 624
- Squash, 4, 55, 133, 180, 183, 422
- Squill, 27, 261, 263, 284
- Stachyose, 56, 65, 69
- Stanol ester, 187
- Starch, 10, 11, 28, 29, 56, 65, 70–74, 76, 80, 89, 132, 156, 357, 373, 456, 484, 492, 493, 496, 499–502, 734, 740, 751
- Stearic acid, 102, 319, 328, 329, 520
- Stems, 8, 80, 106, 173, 192, 232, 257, 278, 516, 544, 566, 588
- Stereoisomers, 59, 60, 174, 189, 190, 454
- Sterids, 94, 109, 110, 113
- Steroid aglycones, 9
- Steroid alkaloids, 9, 95, 210
- Steroids, 8–10, 13, 31, 35, 92, 94–96, 113, 168, 178, 179, 185, 186, 207, 257, 278, 282, 283, 306, 329, 335, 336, 355, 414, 442, 577, 582, 586, 587, 608, 624, 626, 628, 659, 703, 706, 725, 738, 743, 792
- Stigmasterol, 94, 109, 110, 112, 113, 186, 187
- St. John's Wort, 46, 516
- Stock plates, 20
- Stomach tissue, 312
- Stresses, 4, 34, 60, 141
- Strophanthus, 27, 244, 248, 261, 545
- Strychnine, 27, 31, 203, 204, 206, 209, 220, 221, 228, 232, 546–548, 553, 558
- Suberin, 29
- Succinic acid, 118, 122
- Sucrose, 56, 62, 64–66, 68, 69, 73, 74, 249, 332, 500, 501, 503–506, 589, 591
- Sugar alcohols, 60–63, 72, 98, 471, 501, 503
- Sulfites, 29
- Sulfolipids, 92, 370
- Sulfo-quinovosyl-acyl-glycerol, 369, 370
- Sulforaphane, 4, 472, 622–624
- Sulfurides, 26
- Sulphated polysaccharides, 371
- Sunflower, 5, 67, 107, 111, 132, 133, 173, 185, 298, 416, 451, 464, 517, 618, 624, 723
- Supportive therapy, 316, 317
- Suppositories, 28, 328, 514
- Surgery, 28, 217, 313, 384, 516, 520, 622, 639
- Swallowing medicine, 11, 28
- Sweet potatoes, 4, 133, 182, 183, 422
- Swiss chard, 5
- Synagis, 318
- Syphomyeline, 92
- Systemic Lupus Erythematosus (SLE), 186, 450
- Systemic primary carnitine deficiency, 460
- Systolic blood pressure, 298, 378
- T**
- Tablet coating, 319, 492
- Tablets, 28, 68, 88, 312, 322, 327, 329, 488, 492, 496, 499, 500, 569
- Taipan, 316
- Talitol, 62
- Tannic acid, 4, 39, 203, 271, 272, 274, 289, 602, 726, 787
- Tannins, 11, 18, 26–28, 31, 33, 35, 37, 194, 201, 214, 250, 267, 268, 270–275, 285, 302, 348, 349, 355, 586, 601, 602, 621, 626, 628, 629, 703, 724, 725, 785, 789–791, 793, 795
- Taq polymerase, 671, 672
- Tara gum, 86, 88
- Target Compound Analysis (TCA), 15
- Tartaric acid, 4, 118, 122, 124, 517
- Taurine, 138
- Taxadiene, 169, 175
- Taxol, 5, 6, 9, 168, 174, 175, 210, 211, 263, 288, 289, 383, 384, 388, 586, 598, 599, 601, 655, 700, 701, 706
- Taxus brevifolia*, 5, 6, 12, 174, 211, 263, 601
- TCA cycle, 32
- Tea, 4, 5, 44, 138, 156, 172, 195, 210, 211, 242, 275, 298, 303, 379, 443, 450, 479, 487, 516, 517, 618, 623, 635, 723, 792, 793
- Temperature, 4, 44–46, 88, 94, 105, 172, 188, 189, 328, 375, 441, 451, 459, 512, 558, 581, 651, 671, 672, 726, 728, 736, 737, 747, 751, 752, 754, 758, 760, 762–765, 773–775, 786, 794–798

- Tenecteplase, 316, 317
 Teniposide, 12, 15, 266, 288, 601
 Teratogens, 558, 560–563
 Terpenes, 8, 9, 34, 36, 41, 54, 92–94, 96,
 167–169, 178, 188, 191, 192, 207, 292,
 305, 357, 498, 518, 626–628, 701, 725,
 743
 Terpenoids, 7–10, 12, 13, 18, 26, 31, 32, 41,
 45, 167–170, 185, 238, 257, 285, 289,
 305, 335, 336, 355, 515, 518, 522, 566,
 586–588, 596, 601, 608, 619, 625, 703,
 706, 724, 725, 790, 792
 Tetanus immunoglobulin, 312
 Tetanus toxoid, 315, 514
 Tetrachlorodibenzodioxin (TCDD), 553
 Tetracyclic Antidepressant (TeCA), 385, 388
 Tetrodotoxin, 344
 Tetrahydrocannabinol, 43, 559, 560
 Thalassiolins (A-C), 350, 352, 357, 358
 Theaflavin, 445
 Thebaine, 4, 43, 205, 209, 216, 217, 242, 545,
 595
 Therapeutic Nucleic Acids (TNAs), 150
 Theveti, 27
 Thiamin, 409, 424–426
 Thiamine diphosphate, 410, 425, 427
 Thiamine monophosphate, 425
 Thiamine pyrophosphate, 425, 466
 Thiamine triphosphate, 426
 Thin layer chromatography, 734–736, 738,
 739, 741
 Thioacetamide, 291, 293
 Thionins, 40
 Thiosulphonates, 4
 Threonine, 40, 120, 127, 130–134, 137, 141
 Thujene, 170, 172
Thunia alba, 52
 Thyme oil, 189
 Thymol, 170, 189, 190, 497, 498, 515–517,
 618, 621
 Thymoquinone, 303, 601
 Thyrogen, 316, 318
 Thyroid hormone, 7, 374, 381, 696
 Thyrotrophin alfa, 316, 318
 Thyrotropin-releasing hormone, 507, 508
 Thyroxin, 312, 513
 Tiger snakes, 316
 Tisseel VH S/D solution, 315
 Toad alkaloids, 312
 Tocoferols, 97, 410
 Tocopherols, 12, 366, 372, 417, 474,
 577, 624
 Toiletries, 277, 319, 320, 322, 328
 Toluene, 48, 511, 553, 756
 Tomatoes, 4, 8, 28, 37, 54, 118, 168, 182, 218,
 260, 261, 443, 465, 470, 471, 475, 487,
 489, 623
 Toxin, 63, 72, 195, 215, 331, 332, 373, 450,
 511, 536, 538–540, 545, 546, 550, 551,
 658, 661, 701
 Traditional Chinese Medicine (TCM), 52, 353,
 605, 634, 705, 707
 Transcriptomics, 14, 16, 703, 707
 Transdermal therapeutic system, 498
 Transfer RNA (tRNA), 146
 Transform Infra Red, 18, 743
 Trastuzumab, 145, 316–318
 Travelan, 315
 Tricarboxylic acid cycle, 122, 123
 Tri-carboxylic acids, 118
 Tricyclic Antidepressants (TCAs), 385, 553,
 554
Trigonella foenum-graecum, 86, 87, 225, 298,
 606, 613
 1,1,6-Trimethyl-1,2-Dihydronaphthalene
 (TDN), 184
 Triphala, 17
 TRNA, 138, 146, 148, 653
 True lipid, 113
 Trypsin, 136, 312, 319, 483, 510, 512, 513,
 573, 775, 777
 Tryptophan, 129, 131–134, 136, 137, 140, 141,
 206, 209, 218–220, 222, 430, 431, 435,
 441, 596, 703
 Tuberculosis, 107, 186, 383, 416, 468, 514,
 580, 637, 650, 651
 Tumor necrosis factor- α , 174
 Tumourogenesis, 12
 Turmeric, 6, 52, 298, 470, 475, 487, 488, 517,
 518, 622
 Two-dimensional NMR spectroscopy, 19
 Type II Diabetes Mellitus (T2DM), 355, 605
 Tyrosine, 33, 121, 127, 131, 132, 136, 141,
 206, 216, 237, 423, 778
- U**
 Ubiquinone, 97, 410, 423, 424, 523, 526, 586
 Ultraviolet 100–400nm, 44
 Ultraviolet B, 37, 44, 52, 201, 379, 425, 453,
 523, 526, 557
 Ultra Violet Radiation (UV radiation), 4, 33,
 44, 45, 182, 522, 526, 557
 Uracyl, adenine, adenine codon, 127
 Urinary discharges, 13

- Urine of pregnant women, 312
US Food and Drug Administration, 85, 91, 312, 506
UV-A, B radiation, 37, 45, 557
UVB-ultraviolet B 320–290 nm, 44
- V**
Vaccine, 314–316, 318, 330–332, 514, 631–633, 635, 636, 642, 644–648
Valine, 120, 127, 130–133, 137, 141
Vanillin, 189, 190
Varicella zoster vaccine, 315, 316
Varivax, 315, 316
Vascular endothelial growth factor, 290
Vasopressin, 507, 508, 513, 603
Vaxigrip, 318
Vectibix, 317, 318
Vegetables, 4, 7, 8, 10, 27, 45, 53, 59, 68, 75, 90, 97, 111, 118, 132, 141, 180, 182, 183, 185, 186, 194, 195, 201, 218, 250, 266, 374, 379, 412, 414, 416, 422, 436, 438, 441, 443, 453, 457, 458, 461, 465, 470–472, 475, 481–483, 487, 490, 491, 497, 503, 537, 566, 588, 622–624
Venoms, 218, 312, 507, 536, 547, 549
Veratridines, 12
Verbascose, 69, 70
Very fast death factor, 360
Very long chain fatty acids, 101
Very-Low-Density Lipoproteins (VLDL), 116, 117, 357
Vidarabine, 337, 338, 365
Vinblastine, 9, 11–13, 205, 220, 228, 242, 288, 289, 601, 624, 625, 696, 700–702, 759, 772–774
Vinca alkaloids, 12, 209, 220, 290, 625
Vincristine, 9, 11–13, 203, 205, 220, 228, 232, 242, 288, 289, 601, 624, 625, 696, 701
Virus-like particle, 332
Visnaga, 27, 252, 254, 589
Vitamin A, 5, 97, 98, 168, 175, 182, 183, 367, 409, 412–414, 418–420, 422, 467, 485, 491, 514, 517, 523, 526, 553, 554
Vitamin A and D, 312
Vitamin C, 4, 302, 408, 409, 424, 425, 441–443, 449, 450, 465–467, 487, 515, 517, 518, 523, 526
Vitamin D, 95, 97, 98, 179, 187, 248, 282, 319, 409, 414–416, 419, 422, 470, 484, 562, 577, 624, 631
Vitamin K, 95, 98, 177, 253, 276, 277, 409, 417, 420–422, 466, 468, 481
Vitaminoids, 97, 408–411, 423, 424, 443
Vitamins, 4, 5, 7, 9, 10, 12, 18, 26, 27, 30, 92, 93, 95, 97, 98, 111, 113, 156, 277, 302, 347, 362, 366, 373, 408–410, 414, 417, 420–422, 424–428, 430, 431, 433, 434, 436, 443, 452, 464–469, 471, 472, 474, 483, 485, 487, 488, 491, 514, 517, 518, 522, 523, 525, 536, 553, 594, 602, 655, 700
Vitamins B1, B6, E, K, 4, 7, 34, 353, 424, 481
Vivaxim, 315
Volatile oils, 11, 26, 28, 33, 41, 54, 188–192, 498, 586, 723, 742, 785
Volatile Organic Compounds (VOCs), 42, 46
Volemitol (7C), 98
Volemitol, 59, 60
Voltage operated calcium channels, 303
- W**
Wagner's, 203, 725, 726, 786
Walnuts, 5, 111, 451
Water-soluble vitamins, 408–410, 424, 425, 465
Waxes, 11, 31, 33, 92, 94, 105–108, 119, 382, 500, 501, 517
Weight loss, 72, 207, 216, 319, 325, 426, 479, 606
Wheat, 5, 8, 53, 63, 72, 75, 80, 111, 132, 133, 138, 140, 145, 178, 186, 187, 248, 416, 418, 422, 430, 452, 453, 464, 487, 492, 496, 562, 618, 624, 681
Wild rice, 5
Willow tree leaves, 5
Wood, 8, 10, 28, 91, 125, 168, 169, 172, 188, 191, 193, 269, 271, 274, 444, 465, 618, 632
Worm infestations, 13
Wormwood, 27, 174, 261
- X**
Xanthan gum, 90, 91, 493, 496
Xanthine, 204, 210, 211
Xanthomonas campestris, 497
Xanthones, 4
Xanthophylls, 48, 53–55, 180, 182, 487
Xenobiotics, 291, 568–582
Xgeva, 317, 318
Xolair, 317, 318
X-rays 0.01–10 nm, 45
Xylitol, 62, 73, 98, 501–504
Xylose, 9, 56, 58, 79, 80, 278, 495, 503
Xyntha, 317, 318

Y

Yerbe Mate—Guarana—Damiana, 298
Yogurt, 66, 77, 78, 89, 111, 132, 140, 470,
 471, 481, 484, 486
Yucca schidigera, 27, 278, 279, 281

Z

Zearalenone, 540–542
Zyoplast collagen implants, 315

Zeaxanthin, 4, 5, 28, 53, 55, 156, 180, 182,
 366, 368, 470, 485, 489
Ziconotide, 313, 339, 340
Zinc, 4, 328, 347, 378, 379, 409, 470, 525,
 549, 554, 702

Zostavax, 314

Zoster virus vaccine live, 314

Zyderm collagen implants, 315