Pharmacognosy

CHAPTERWISE NOTES

Analytical Pharmacognosy

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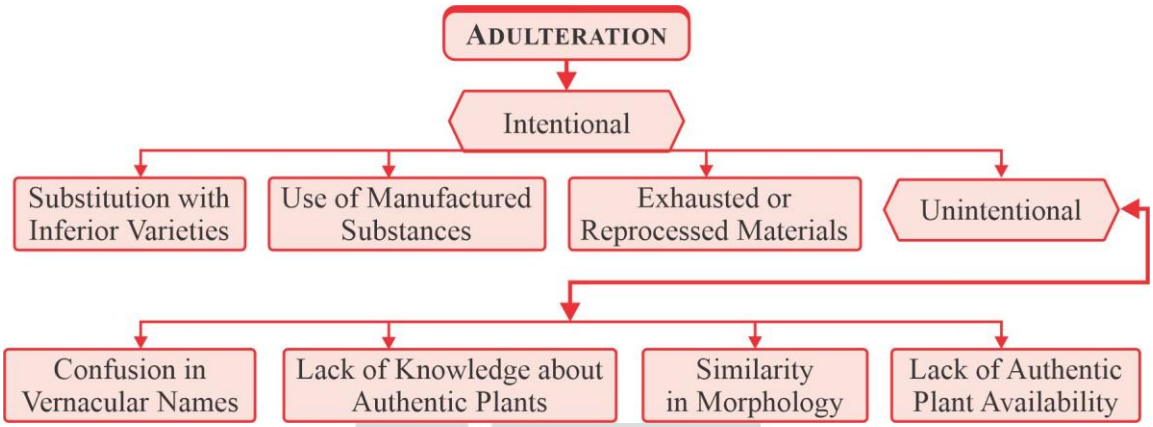
PHARMACOGNOSY

Analytical Pharmacognosy

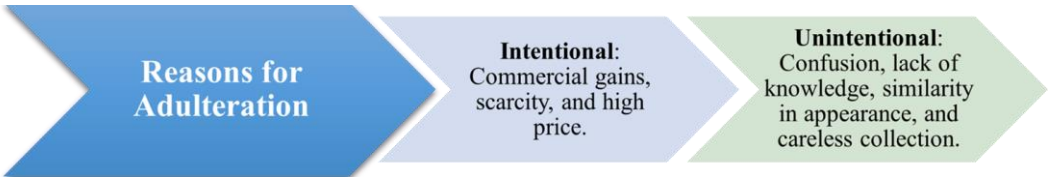
1. Drug Adulteration

Introduction: Herbal adulteration refers to the intentional or accidental substitution of authentic plant material with inferior or unrelated substances.

Types of Adulteration



Type of Drug Adulteration	Description	Examples
Intentional Adulteration 	Deliberate addition or substitution to increase quantity or deceive buyers	• **Substitution with inferior varieties:** Senna, Ginger • **Manufactured substances:** Chicory for coffee • **Exhausted drugs:** Reprocessed saffron, red rose petals • **Superficially similar substances:** Ailanthus leaves for Belladonna • **Addition of toxic substances:** Lead shot in opium • **Use of vegetative parts:** Excess stems in Lobelia • **Powder adulteration:** Powdered olive stones in gentian • **Synthetic principles:** Sildenafil in sexual enhancement drugs
Unintentional Adulteration 	Adulteration due to ignorance, carelessness, or misidentification	• **Confusion in vernacular names:** *Fumaria parviflora vs. Mollugo pentaphylla* • **Lack of knowledge:** *Calophyllum inophyllum* used as Nagakesar instead of Mesua ferrea • **Morphological similarity:** *Mucuna pruriens adulterated with M. deeringiana* • **Lack of availability:** Hypericum perforatum substituted with H. patulum • **Careless collection:** Parmelia species mixed with others



Impact of Adulteration

- Decreases faith in herbal medicine.
- Adulterated herbs may cause adverse reactions, as unintentional substances may be harmful.

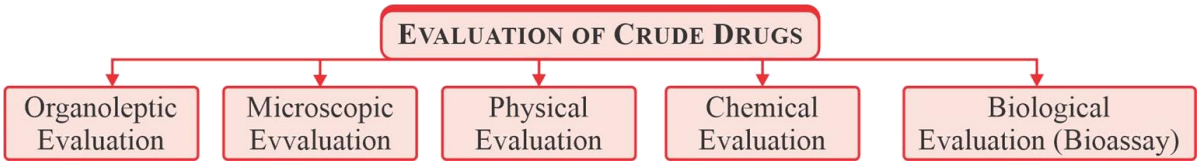
List of Drugs and Their Common Substituents and Adulterants

Sr No.	Drug Name	Adulterant/Substitute	Sr No.	Drug Name	Adulterant/Substitute
1.	**Aconite**	*Gloriosa superba*	20.	**Black pepper**	Papaya seeds/light berries
2.	**Aloe**	Natal aloes, Mocha aloe	21.	**Belladona**	*Althaea officinalis*, *Aliantus glandulosa*, *Phytolacca americana*
3.	**Asafoetida**	Soap stone or earthy matter	22.	**Casacara**	*Rhamnus fallax*
4.	**Chirata**	*Andrographis paniculata*, *Swertia angustifolia*, *Swertia decussate*	23.	**Cumin seeds (Black jeera)**	Grass seeds coloured with charcoal dust
5.	**Coffee**	Chicory	24.	**Cardamom big**	Cardamom small
6.	**Cinchona bark**	*Cuprea bark (Remijia purdiena)*	25.	**Cinnamon bark**	Jungle cinnamon, Saigon cinnamon, Seychelles cinnamon
7.	**Coffee**	Chicory	26.	**Clove buds**	Clove stalk, Exhausted clove, Blown clove, Mother clove
8.	**Coriander powder**	Dung powder	27.	**Digitalis**	*Verbascum thapsus*, *Inula racemosa*, Primrose leaves
9.	**Dill**	Indian dill (*Anethum sowa*)	28.	**Dioscorea**	*Dioscorea flouribunda*, *Dioscorea villosa*, *Costus* species
10.	**Datura**	Chestnut leaves, *Datura innoxia*	29.	**Ephedra**	*Ephedra equisetina*
11.	**Honey**	Sugar syrups, Pollen substitution	30.	**Gentian**	Kutki
12.	**Hyoscyamus**	*Hyoscyamus albus*, *Hyoscyamus muticus*	31.	**Ipecacuanha**	*Alangium hillia*, *Manettia psychotria*, *Borreria*, *Remijia*
13.	**Liquorice**	Manchurian, Manchurian liquorice, Sweetwood (Liquorice fern)	32.	**Kurchi**	*Wrightia tomentosa*, *Wrightia inctoria* bark
14.	**Mustard seeds**	Argemone seeds	33.	**Nux vomica**	*Strychnose potatorum*
15.	**Myrrh**	Scented bdellium	34.	**Paraffin Wax**	Bees wax
16.	**Red chilli powder**	Brick powder grit, sand, dirt, filth etc.	35.	**Saffron**	Colored dried tendrils of maize cob
17.	**Rauwolfia**	*Rauwolfia vomitoria* (African rauwolfia), *R. densiflora*	36.	**Senna**	Dog senna (*Cassia abovata*), Palthe senna (*Cassia auriculata*), Bombay senna
18.	**Turmeric powder**	Lead chromate	37.	**Tea**	Colored tea
19.	**Turmeric, chilly, curry powder etc.**	Colours	38.	**Thyme**	*Thymus zygis*

➤ 2. Evaluation of Crude Drugs:

Introduction

- Drug evaluation ensures ***identity, quality, purity, and detects adulterations.***
- Analytical methods have improved the harvesting, cultivation, storage, stability, and purity of herbal products.
- Evaluation methods include ***organoleptic, microscopic, physical, chemical, and biological tests.***

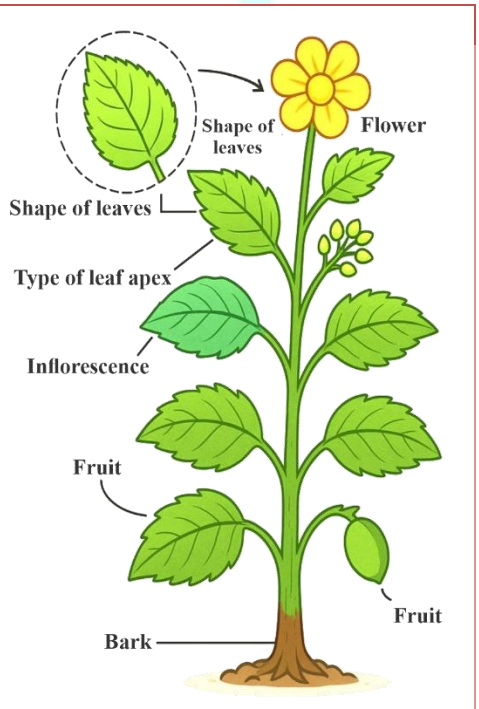


2.1 Organoleptic Evaluation

- **Definition:** Evaluation using the senses (sight, smell, taste, touch, texture).
- **Purpose:** Identifies drugs based on sensory characteristics.

Organoleptic Parameters	Purpose	Examples
Colour	Identifies variations in appearance	Talka gum vs. Acacia gum (Talka: brown, Acacia: white/yellow)
Odour	Distinguishes similar substances	Ginger vs. Capsicum (distinct smells)
Taste	Differentiates herbs by flavour	Ginger & Capsicum (pungent), Gentian & Chirata (bitter)
Shape/Size	Differentiates similar plants	Rhubarb vs. Chinese rhubarb (size/shape)
Texture	Identifies physical features	Cinnamon quills (texture)

Morphological Parameters		
Part	**Category**	**Types**
Seed	**Shape of seed**	Globular, Spherical, Oval, Fusiform
Fruit	**Type of fruit**	Simple fruit, Aggregate fruit, Compound fruit
Bark	**Shape of bark**	Flat, Curved, Recurved, Channelled quill, Double quill, Compound quill
Flowers	**Type of inflorescence**	Solitary, Raceme, Spike, Corymb, Capitulum
Leaves	**Type of leaf base**	Symmetrical, Asymmetrical, Cordate, Decurrent
	Type of leaf margin	Entire, Serrate, Crenate, Sinuate, Dentate
	Type of leaf apex	Acute, Acuminate, Obtuse, Recurved
	Shape of leaf	Oval, Oblong, Cordate, Linear, Lanceolate, Ovate, Obovate



2.2 Microscopic Evaluation

- Microscopic evaluation is crucial for identifying herbs, detecting adulterants (e.g., insects, mold), and confirming plant authenticity through tissue characteristics.
- **Techniques:** Staining and reagent used to identify features like cell walls, starch grains, trichomes, crystals, and fibers.

1. Purpose & Applications

- Identification of whole/powdered drugs.
- Detection of adulterants: molds, insect parts, faeces.
- Differentiation of closely related species or varieties.
- Used when physical/chemical tests are not feasible.

2. Diagnostic Structures & Stains

Feature	Staining Agent / Test	Staining Colour
Lignified tissues	Phloroglucinol + Conc. HCl	Red / Pink
Mucilage	Ruthenium Red	Pink
Starch, Hemicellulose	N/50 Iodine solution	Blue

Important Diagnostic Features

Drug	Key Diagnostic Traits
Surinam Quassia	No calcium oxalate; uniseriate medullary rays
Cascara Bark	Crystal fibres, wavy medullary rays
Frangula Bark	Stone cells absent
Nux Vomica	Lignified trichomes, plasmodesma
Senna (Alexandrian vs. Indian)	**Vein-islet number:** 25–29.5 vs 19.5–22.5 Stomatal index: 10–15 vs 14–20
Clove vs. Clove Stalk	**Stalks:** Contain sclereids & calcium oxalate; **Clove:** Absent
Rauwolfia spp.	Adulterants contain sclerenchyma; R. serpentina does not

3. Quantitative Microscopy

3.1 Leaf Constants

Parameter	Definition
Stomatal Number	Avg. number of stomata per mm² of leaf epidermis
Stomatal Index	**% of stomata to total epidermal cells: S.I. = S × 100 / (E + S)** *SI* = Stomatal Index *S* = Number of stomata per unit area *E* = Number of epidermal cells in the same unit area.
Vein-islet Number	No. of vein-islets per mm² (between midrib and margin)
Veinlet Termination No.	No. of ultimate free vein endings per mm²
Palisade Ratio	Avg. no. of palisade cells beneath one epidermal cell

4. Linear Measurements

- Used for differentiating species and detecting adulterants based on structural size.

Measurement	Example / Application
Starch grain diameter	Ipecacuanha varieties; Cassia vs Cinnamon
Vessel diameter	Clove stalks in powdered cloves
Fiber width	Detection of Cassia in cinnamon
Stomatal size	*Barosma betulina vs other species*

5. Lycopodium Spore Method (Quantitative Microscopy)

Purpose: Used to calculate the purity of powdered drugs by comparing microscopic particle counts.

Formula: % Purity = (N × W × 94,000 × 100) / (S × M × P)

Symbol	Meaning
N	No. of diagnostic structures (e.g., starch grains) in 25 fields
W	Weight of lycopodium powder (mg)
S	No. of lycopodium spores in same 25 fields
M	Weight of drug sample (mg, dried at 105°C)
P	Known no. of structures/mg in pure drug (e.g., 2,86,000 for ginger)
e.g.: ***Spent ginger detection:*** Count starch grains and compare with standard (286,000/mg for pure ginger).	

6. Stomata

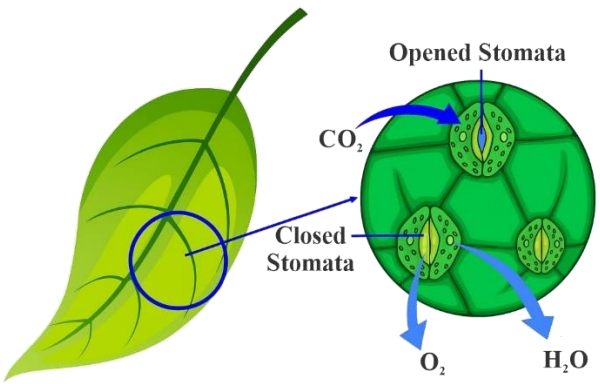
- ***Stomata are small openings in the epidermis of leaves, stems, flowers, and fruits.***
- Surrounded by guard cells (kidney-shaped).
- ***The opening and guard cells together form the stoma.***
- Neighbouring cells (subsidiary cells) surround guard cells, often differing from other epidermal cells.

Functions of Stomata:

1. **Gas Exchange** – Allow CO_2 in for photosynthesis and release O_2.
2. **Transpiration** – Help in water vapor loss, cooling the plant.
3. **Water Regulation** – Control water loss by opening and closing.
4. **Temperature Control** – Cool the plant through transpiration.

Nutrient Transport – Support mineral uptake via transpiration stream.

Stomata



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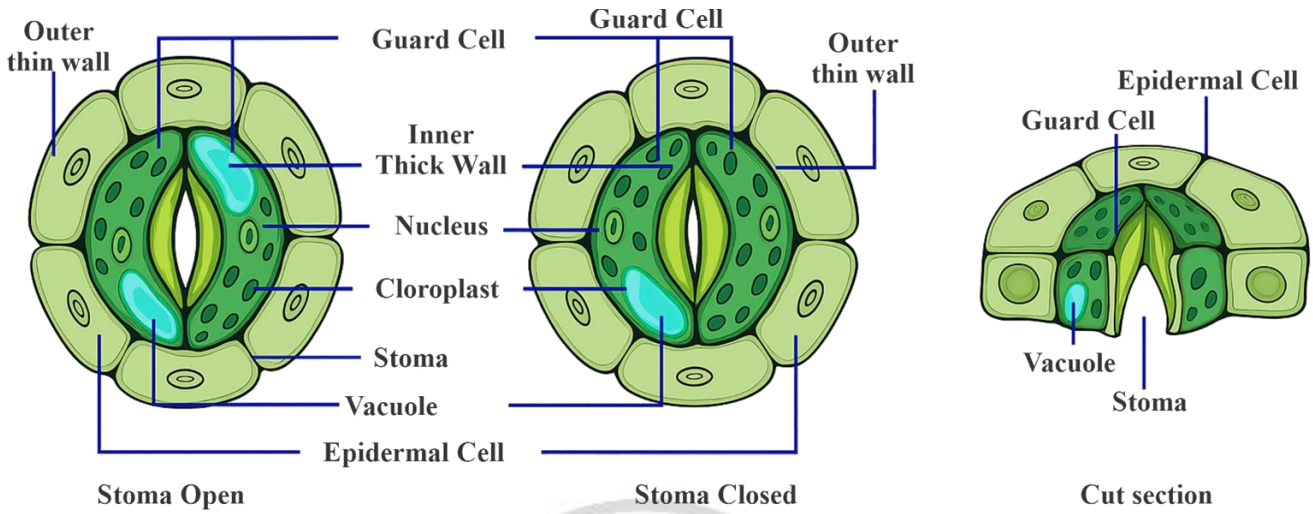
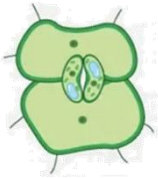
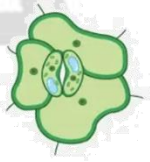
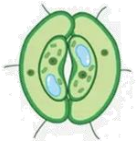
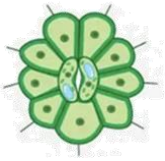
Part of Stomata



Stoma Open | Stoma Closed | Cut section

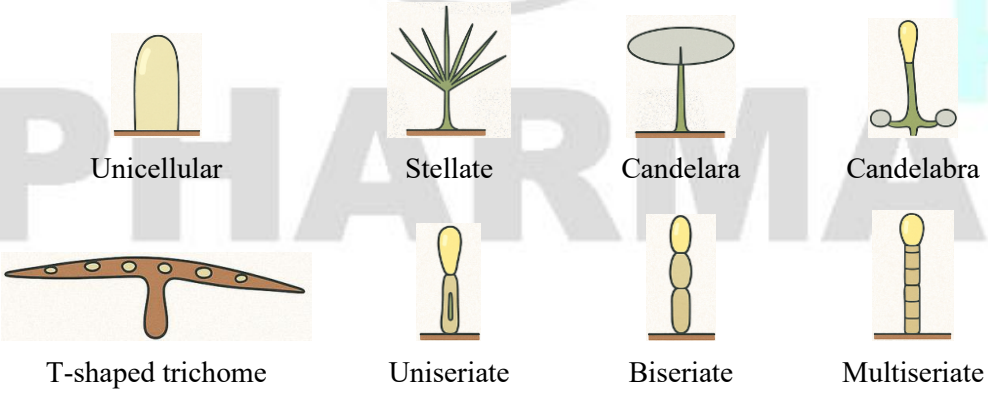
Types of Stomata in Crude Drugs

S.No.	Drug(s)	Type of Stomata	Characteristic Arrangement
1.	**Tulsi, Peppermint, Spearmint, Vasaka**	**Diacytic (Caryophyllaceous)**	**Cross-celled** – 2 subsidiary cells at right angles to guard cells  Diacytic
2.	**Hyoscyamus, Chirata, Duboisia, Vinca, Belladonna, Datura herb, Stramonium**	**Anisocytic (Cruciferous)**	**Unequal-celled** – 3 subsidiary cells, one smaller than the others  Anisocytic
3.	**Digitalis, Oleander, Eucalyptus, Apamarga, Punarnava, Clove, Buchu, Lobelia, Rue, Colchicum**	**Anomocytic (Ranunculaceous)**	**Irregular-celled** – Surrounded by cells similar to epidermal cells  Anomocytic

4.	**Senna, Coca**	**Paracytic (Rubiaceous)**	**Parallel-celled** – 2 subsidiary cells parallel to guard cells  Paracytic
5.	–	**Actinocytic**	**Radiate-celled** – Guard cells surrounded by a ring of radiating subsidiary cells  Actinocytic
6.	**Belladonna, Stramonium, Brahmi**	**Anisocytic + Some Anomocytic**	**Mixed** – Unequal + Irregular cells
7.	**Datura herb**	**Paracytic + Anisocytic**	**Mixed** – Parallel + Unequal cells

7. Trichomes

- ***Definition: Elongated outgrowths of epidermal cells, consisting of a foot embedded in the epidermis and a projecting body.***
- **Location:** Found mainly on leaves, but also on seeds (e.g., Nux vomica, Andrographis) and fruits (e.g., Lady's finger, Cummin).



Type	Subtypes
1. Covering Trichomes	**a. Unicellular:** Linear, conical, warty (e.g., Tea, Senna), large, lignified (e.g., Nux vomica). **b. Multicellular Unbranched:** Biseriate (e.g., Calendula), Multiseriate (e.g., Male fern). **c. Multicellular Branched:** Stellate (e.g., Hamamelis), Peltate (e.g., Cascarilla), T-shaped (e.g., Pyrethrum).
2. Glandular Trichomes	**a. Unicellular:** Sessile (e.g., Piper betle). **b. Multicellular:** Various forms (e.g., Digitalis, Hyoscyamus, Mentha).

8. Calcium oxalate crystals

- ***Calcium oxalate crystals are considered as excretory products of plant metabolism.***
- They occur in different forms and provide valuable information for identification of crude drugs in entire and powdered forms.
- *In **pharmacognosy**, **calcium oxalate crystals** are often studied as part of the **microscopic identification of crude plant drugs**.*
- These crystals are important **diagnostic features** used to help **identify plant materials**, especially in powdered or adulterated forms where macroscopic features are not visible.

Type (with Other Name(s))	Shape	Description
Raphides (*Needle crystals*)	Needle-like	Slender, elongated, often pointed crystals, found in bundles within idioblast cells. Known to irritate mucous membranes due to sharpness or toxins.
Acicular (*Fine needle crystals*)	Very thin, needle-shaped	Extremely slender and elongated crystals, even finer than raphides. Found individually or in small, scattered groups.
Prisms (*Prismatic crystals*)	Rectangular, square, polygonal	Sharp-edged, angular crystals with clear geometric forms (e.g., cubic, rhombic), usually found singly or in loose groups.
Styloids (*Columnar crystals*)	Elongated, rod-like, often pointed	Robust, thick crystals, usually longer than raphides, pointed at one or both ends. Occur singly in cells.
Rosettes (*Rosette aggregates*)	Star-like, radial, flower-like	Spherical or radial clusters of small crystals arranged like flower petals around a center. Appear decorative under the microscope.
Druses (*Cluster crystals*)	Spherical, globular, mulberry-like	Rounded ball-like clusters of tiny, interconnected crystals. Often look mulberry-shaped under a microscope.
Sand (*Crystal sand*)	Fine granular, amorphous	Minute, grain-like particles lacking distinct crystal shape. Appear powdery or sandy, often calcium oxalate.
Micro-sphenoidal (*Pyramidal microcrystals*)	Tiny wedge/pyramidal	Very small, symmetrical bipyramidal or wedge-shaped crystals, requiring high magnification. Characteristic of specific plant families.

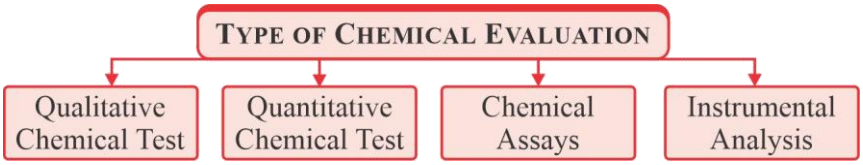
2.3. Chemical Evaluation

✶ ***The chemical evaluation includes qualitative chemical tests, quantitative chemical tests, chemical assays, and instrumental analysis.***

Purpose

✶ Chemical evaluation helps identify, quantify, and confirm the presence of key phytoconstituents.

✶ Ensures the quality, purity, and authenticity of the crude drug and detects adulteration or substitution.



Type of Chemical Evaluation	Description	Examples
Qualitative Chemical Tests 	Identify specific phytoconstituents	• **Copper acetate:** Detects colophony (adulterant in resins, waxes) • **Halphen's test:** Detects cottonseed oil in olive oil • **Baudouin's test:** Detects sesame oil in olive oil • **Iodine:** Detects starch • **Van Urk's reagent:** Detects ergot
Quantitative Chemical Tests 	Measure specific chemical values	• **Acid value:** For resins, balsams • **Saponification value:** For balsams • **Ester value:** For balsams, volatile oils • **Acetyl value:** For volatile oils
Chemical Assays 	Quantify specific active constituents	• **Alkaloids:** Total in belladonna, strychnine in nux vomica • **Resins:** In jalap • **Vitamins:** In cod-liver oil
Instrumental Analysis 	Advanced techniques for identification and quantification	• **Chromatographic Methods:** Paper Chromatography, Thin-layer Chromatography (TLC), Gas Chromatography (GC), High-Performance Liquid Chromatography (HPLC), High-Performance Thin-Layer Chromatography (HPTLC) • **Spectroscopic Methods:** Ultraviolet (UV) and Visible Spectroscopy, Infrared (IR) Spectroscopy, Mass Spectroscopy (MS), Nuclear Magnetic Resonance (NMR) Spectroscopy

2.4. Physical Evaluation

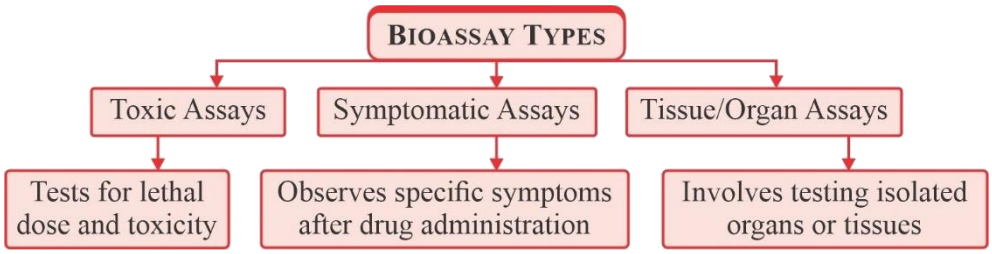
✶ In crude plant evaluation, physical methods are often used to determine the solubility, specific gravity, optical rotation, viscosity, refractive index, melting point, water content, degree of fiber elasticity, and other physical characteristics of the herb material.

S. No.	Physical Evaluation Parameter	Description	Examples
1.	**Solubility**	Examines the drug's behavior in different solvents	• Colophony in light petroleum; • Castor oil in alcohol (turbidity = adulteration); • Alkaloidal bases (organic solvents); • Alkaloidal salts (polar solvents)
2.	**Optical Rotation**	Measures how substances rotate plane-polarized light • **Dextrorotatory (d/+):** Rotates light clockwise. • **Levorotatory (l/-):** Rotates light counterclockwise.	• Eucalyptus oil (+0° to +10°) • Honey (+3° to +15°)
3	**Refractive Index**	Ratio of light speed in vacuum to speed in material; varies with frequency	• Castor oil (1.4758–1.527); • Clove oil (1.527–1.535)
4.	**Specific Gravity**	Ratio of mass to equal volume of water at 4°C	• Cottonseed oil (0.88–0.93); • Castor oil (0.95)
5.	**Viscosity**	Resistance of a fluid to flow; indicates composition	Pyroxylin (1100–2450 centistokes)
6.	**Melting Point**	Temperature at which a solid becomes liquid	Beeswax (62–65°C); Agar (85°C)
7.	**Moisture Content**	Indicates microbial spoilage risk; determined by heating at 105°C to constant weight	Digitalis (max 5%); Ergot (max 8%)
8.	**Ultraviolet Light**	Some drugs fluoresce under UV light; aids in identification	Rhubarb varieties show different UV fluorescence
9.	**Ash Values**	Indicates presence of impurities or minerals • **Total Ash:** Detects mineral adulteration. • **Acid-Insoluble Ash:** Detects calcium oxalate or earthy matter. • **Water-Soluble Ash:** Detects water-extracted material. • **Sulphated Ash:** Done at high temperature (~600°C) for sulphate salts.	**Guduchi:** Total ash ≤16%, Acid-insoluble ash ≤3%
10.	**Extractive Values**	Estimate of active constituents using different solvents • **Water-soluble extractive:** For glycosides, tannins, etc. • **Alcohol-soluble extractive:** For resins, glycosides, etc. • **Ether-soluble extractive:** For volatile constituents and fats.	• Aloe (Water-soluble ≥25%); • Glycyrrhiza (Water-soluble ≥20%)
11.	**Foreign Organic Matters**	Non-drug material such as soil, insects, moulds	• Garlic (max 2%); • Saffron (max 2%); • Satavari (max 1%)

2.5 Biological Evaluation:

- Determines pharmacological activity, potency, and toxicity of the plant or extract.
- Useful when physical and chemical methods cannot satisfactorily evaluate the drug.
- Biological effects are crucial for determining the value of crude drugs.
- Methods are less precise, time-consuming, and expensive.

1. Bioassay:



2. Assay Process:

- The amount of drug with known potency is tested on suitable test animals or organs under standard conditions.
- Reference standards are used to minimize errors.

3. Toxicity Studies:

- Performed on animal models to determine lethal and effective doses.
- Examples of animals used for testing:

Animal	Purpose / Test
Mice	Testing vaccines
Guinea pigs	Oxytocic activity of vasopressin
Chickens	Oxytocic activity
Pigeons	Testing lethal effects of digitalis glycosides
Cats	Testing depressor activities and mydriatic (pupil dilation) effects
Dogs	Evaluation of cardiac and gastrointestinal activities
Rabbits	Testing effects on eyes or ocular drug activity
Cock's comb, Rabbit's intestine or uterus	Testing pharmacological effects of ergot

4. Tested Biological Activities:

- Hepatoprotective (liver protection).
- Hypoglycemic (lowering blood sugar).
- Anti-inflammatory (reducing inflammation).
- Anti-ulcer (treating ulcers).
- Immunomodulatory (modulating immune response).

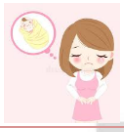
5. Microbiological Assays:

- Determine the effect of drugs on microorganisms.
- Used for identifying antimicrobial drugs.

- Living bacteria, yeast, and molds are used for assaying vitamins.

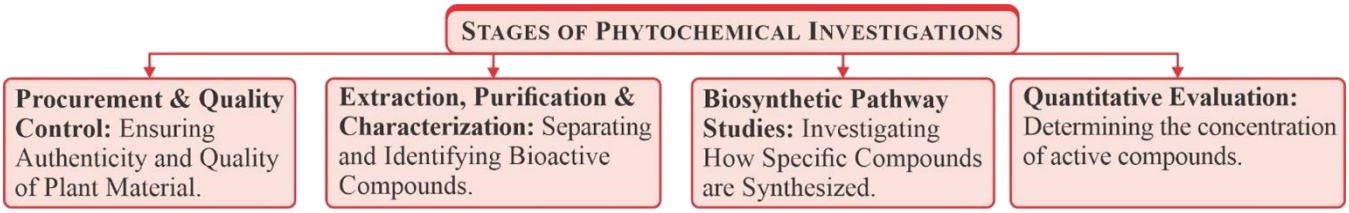
3. Biological Testing of Herbal Drugs:

- The biological testing of herbal drugs is essential for determining their efficacy and safety.
- It involves evaluating various biological activities that a herbal drug may possess, such as hepatoprotective, hypoglycemic, anti-fertility, anti-inflammatory, neuropharmacological, anti-ulcer, anti-insect, and microbiological effects.
- Below are some of the commonly used biological testing protocols for herbal drugs.

Activity	Animal Model/System	Method / Induction	Assessment Parameters
Hepatoprotective 	Rats (albino, male/female), hepatic cell culture	**Method:** After toxicity induction, drug treatment is given for 10-15 days, followed by an evaluation of recovery using the parameters.	Hexobarbital hypnosis, SGOT, SGPT, SOD, cholesterol, histopathology
Hypoglycemic 	Rats, mice, rabbits	**Induction:** Alloxan (80–180 mg/kg), Streptozotocin (80–150 mg/kg)	Fasting glucose, glucose tolerance test, insulin levels (RIA/ELISA)
Anti-fertility 	Rats (male and female)	**Female:** Estrogenicity, vaginal smears, ovulation **Male:** 60-day extract dosing	Uterus weight, implantation, sperm count, motility, histopathology
Anti-inflammatory 	Rats, mice	**Method:** Carrageenan-induced paw oedema in rats Croton oil-induced ear inflammation in mice.	Paw volume, ear thickness
Neuropharmacological 	Mice, guinea pig ileum, rabbit jejunum, rat diaphragm	Locomotor test, PTZ-induced convulsions, barbiturate sleep Isolated tissue studies	CNS depression/stimulation, cholinergic/adrenergic effects, neuromuscular activity
Anti-ulcer 	Wistar rats, guinea pigs	**Induction:** Alcohol, aspirin, serotonin, cold stress	Ulcer index, histopathology, sialic acid, DNA content
Anti-insect 	*Aedes aegypti, Tribolium castaneum*	Larvicidal, adulticidal exposure, arena ring test, mosquito repellency	Mortality rate, repellency duration
Microbiological 	Bacteria in culture (in vitro)	**Method:** Cylinder plate method, turbidimetric method	Zone of inhibition (mm), change in turbidity

➤ 4. Phytochemical Investigations:

Phytochemical investigations involve four stages:



Extraction Process:

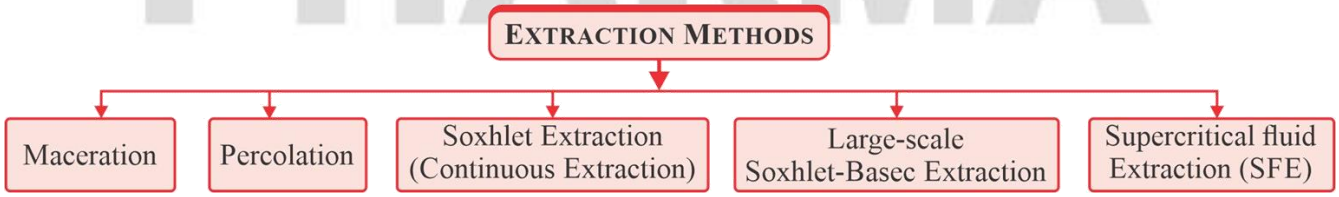
- **Authentication:** Plant material must be properly identified.
- **Solvents Used:**
  - **Alcohol:** For many constituents but may require removal of pigments/resins.
  - **Light Petroleum:** For fixed oils, essential oils, steroids, aglycones.
  - **Chloroform / Ether:** For alkaloids and quinines.
  - **Water / Alcohol:** For glycosides.
  - **Water, Alcohol, Ethyl Acetate:** For tannins.
- **Techniques:** Maceration, percolation, Soxhlet extraction.
- **Process:** Extraction is done successively with solvents (e.g., petroleum ether, benzene, chloroform, acetone, methanol). Extracts are concentrated, and yields are noted.

General Methods for Extraction, Isolation, and Identification of Herbal Drugs

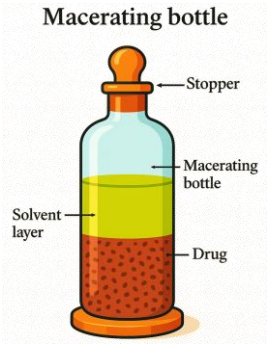
1. Introduction to Extraction

- **Extraction** = separation of soluble active principles from insoluble plant material using a suitable solvent.
- Controlled by **mass transfer** – movement of solutes from solid (plant cell) into solvent.
- **Key influencing factors**:
  - **Agitation** and **temperature** enhance diffusion.
  - **Particle size reduction** increases surface area.
  - **Solvent selection** and method type are critical for efficiency.

2. Extraction Methods



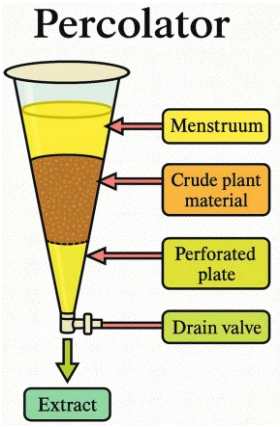
2.1 Maceration



- Crude drug soaked in solvent (menstruum) for 3–7 days with shaking.
- Standstill extraction → then filtered.
- ***Modified form*: Multiple stage maceration** using connected extractors, spray nozzles & circulation pumps.

2.2 Percolation

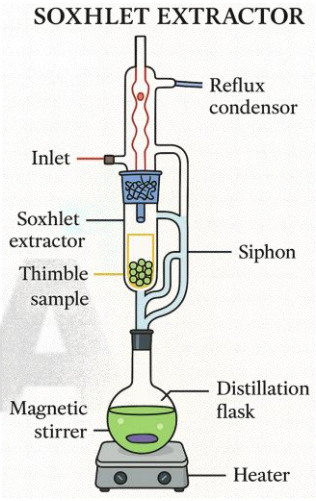
- Continuous passage of solvent through packed bed of powdered drug.
- **Steps:** moistening → packing → maceration (24 h) → slow percolation.
- **Modified percolation**:
  - ***Reserve percolate method*:** for alcohol-containing preparations to preserve alcohol strength.
  - ***Battery extractors*:** industrial semi-continuous systems.



2.3 Soxhlet Extraction (Continuous Extraction)

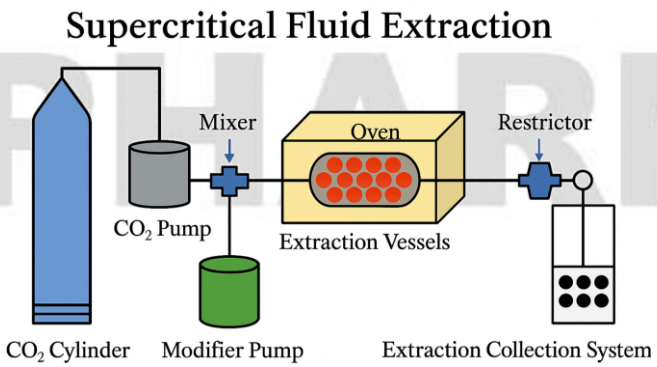
- ***Hot extraction using a siphon mechanism.***
- Solvent evaporates → condenses → trickles over drug → siphoned back → repeated.
- Useful for **exhaustive extraction** with small solvent volume.
- Limitation: Only **boiling point stable solvents** and non-azeotropes.

2.4 Large-scale Soxhlet-Based Extraction



- **Industrial scale setup:** separate drug loading chamber, heated distillation, vapor → condenser → spray onto crude drug.
- Automated cycle for recovery and reuse of solvent.

2.5 Supercritical Fluid Extraction (SFE)



- Uses **supercritical CO_2** at 31.1°C, 73.8 bar.
- Acts as a liquid with high diffusivity, penetrability, and solvating power.

Applications:

- Decaffeination, extracting pyrethrins, acorone, bisabolol, and volatile oils.

Advantages:

- Non-toxic, non-combustible, eco-friendly.
- Selective, reusable, suitable for heat-labile compounds.

Disadvantages:

- Not ideal for polar compounds.
- Needs **expensive setup**, expertise, and dry conditions.

3. Types of Extracts

Type	Description
Decoction	Boiling plant in water (15 min), then filter & cool.
Infusion	Soaking in cold/hot water (like tea) — for water-soluble, heat-sensitive compounds.
Digestion	Maceration with gentle heat — faster extraction.
Tincture	Alcoholic/hydroalcoholic extracts. E.g. Iodine, Belladonna tincture.
Liquid Extract	1g drug/ml alcohol. Used directly or preserved.
Soft Extract	Semi-solid, syrupy extracts. E.g. Glycyrrhiza soft extract.
Dry Extract	Powdered (spray/tray dried). E.g. Belladonna dry extract, used in tablets/capsules.

4. Isolation Techniques of Natural Products

Fractional Crystallization

- Used for **crystalline phytochemicals**: glycosides, alkaloids, flavonoids.
- Based on solubility differences, cooled or evaporated slowly.

Fractional Distillation

- For **volatile oils** (citral, eucalyptol, citronellal).
- Separates low/high boiling components.

Fractional Liberation

- Alkaloids are converted to salts or free bases to separate.
- Used industrially for **quinine**, **morphine**, etc.

Sublimation

- Few natural products (e.g., **camphor**, **caffeine**) directly vaporize and re-condense.
- Effective for volatile solids.

5. Chromatographic Techniques

Basics

- Based on partition between **mobile phase** and **stationary phase**.
- **Retention Time (Rt)** for GC/HPLC; **Retention Factor (Rf)** for TLC/PC.

Types of Chromatography

Type	Principle	Usage
Adsorption	Surface binding on solid	Classical TLC, PC
Partition	Solute distribution between two liquids	Paper, Column
Ion Exchange	Cation/anion exchange on resin	Protein, amino acid analysis
Gel Filtration	Size exclusion	Large biomolecules
Affinity	Lock-key specificity	Enzymes, antibodies

Paper Chromatography (PC)

- **Rf = Distance travelled by compound / solvent front.**
- **Uses:** spot detection via **UV**, **charring**, **sprays**.

Thin Layer Chromatography (TLC)

- **Stationary:** silica, alumina.
- **Carriers:** glass, aluminium foil.
- **Detection Reagents**:
  - Dragendorff's (alkaloids), Antimony chloride (steroids), Fast Blue B (cannabinoids), Iodine vapours.

High-Performance TLC (HPTLC)

- Automated spotting, scanning (densitometry).
- Smaller particle size (<1 μm), higher sensitivity.
- Used in **standardization of herbal extracts**.

Column & Flash Chromatography

- Vertical glass column, solvent flows by gravity or pressure.
- **Flash chromatography** = pressure/suction based faster separation.

HPLC

- High pressure (4000–5000 psi), fine particles, UV or MS detectors.
- Reversed phase (C18), normal phase (silica).
- **Highly reproducible**, versatile, allows compound recovery.

Gas Chromatography (GC)

- For **volatile compounds**.
- **Carriers:** nitrogen, helium.
- **Stationary:** WCOT/SCOT columns.
- **Detectors: FID**, **ECD**, **GC-MS**.
- High sensitivity but limited for non-volatile drugs.

6. Spectroscopic Techniques for Identification

UV-Visible Spectroscopy

- Detects **chromophores** (200–700 nm).
- λmax shifting (bathochromic/hypsochromic) indicates solvent effects.

IR Spectroscopy

- **IR Range:** 4000–400 cm^{-1}.
- Identifies **functional groups** based on vibrational frequency.

NMR Spectroscopy (^{1}H and ^{13}C)

- Detects **hydrogen environments** (^{1}H) and **carbon skeleton** (^{13}C).
- **Coupling constants**, **multiples**, and **chemical shifts** used for structural elucidation.
- **Advanced:** COSY, HMBC, HSQC, NOESY, TOCSY.

Mass Spectrometry

- Measures **molecular weight** and **fragmentation pattern**.
- **Ionization methods:** EI, CI, FAB, etc.
- **Modern combos: GC-MS**, **LC-MS**, **LC-NMR** for detailed analysis.

Preliminary Phytochemical Screening:

- **Plant Metabolism:** Produces primary metabolites (carbs, proteins) and secondary metabolites (glycosides, alkaloids) with medicinal effects.
- **Successive Solvent Extraction:** The plant is extracted in stages with increasing solvent polarity (e.g., petroleum ether → methanol).
- **Purification:** Extracts may contain impurities (e.g., chlorophyll, resins). Common purification methods include:
  - **Partitioning:** Using immiscible solvents.
  - **Precipitation:** Using reagents to separate impurities.
  - **Chromatography:** Modern technique for purification.
  - **Other Methods:** Sublimation, fractional distillation, fractional crystallization, & fractional liberation.

Purification Techniques:

- **Sublimation:** For certain crude extracts.
- **Fractional Distillation:** For separating volatile oils.
- **Steam Distillation:** For extracting volatile oils and hydrocyanic acid.
- **Fractional Liberation:** For alkaloidal mixtures by gradual base liberation.
- **Fractional Crystallization:** For separating components based on solubility differences.