A Textbook of
Pharmacognosy and Phytochemistry

(Theory)- BP205T

For B. Pharm 2nd Semester

As per the PCI New Syllabus 2026, NEP 2020

Authors

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Preface

Pharmacognosy and Phytochemistry form the scientific foundation for understanding medicinal plants, natural products, and their therapeutic applications. With the growing global interest in herbal medicines, phytopharmaceuticals, and evidence-based traditional healthcare, a thorough knowledge of natural drugs has become an essential component of pharmaceutical education and research. This textbook has been prepared in accordance with the **Pharmacy Council of India (PCI) B.Pharm Semester II syllabus (BP205T)** under the NEP-oriented curriculum.

The primary objective of this book is to provide students with a comprehensive yet easy-to-understand presentation of the fundamental concepts of pharmacognosy and phytochemistry. The contents are arranged systematically, beginning with the introduction to medicinal plants, cultivation, collection, processing, and quality control, followed by the study of primary and secondary metabolites, extraction, isolation, identification, characterization techniques, phytochemical screening, chromatographic fingerprinting, and detailed pharmacognostic studies of important medicinal plants. Recent advances in herbal drug standardization and modern analytical techniques have also been incorporated to bridge classical pharmacognosy with contemporary pharmaceutical sciences.

Every chapter has been written in a simple, student-friendly language while maintaining scientific accuracy. Appropriate tables, flowcharts, diagrams, and illustrations have been included to facilitate better understanding and quick revision. The book is designed to serve not only undergraduate pharmacy students but also teachers, researchers, and professionals engaged in pharmacognosy, phytochemistry, herbal drug research, and pharmaceutical sciences.

Although every effort has been made to ensure the accuracy and completeness of the information presented, constructive suggestions from teachers, students, and readers will always be welcome for improving future editions. We sincerely hope that this textbook will serve as a valuable academic resource and contribute to a better understanding of medicinal plants and their phytochemical constituents.

Authors

G. N. Pramodini**,** Smita Jain, Jiju V., Saif Mohammed Hassan, Shubham Ghosh

Acknowledgement

The authors express their sincere gratitude to all those who have directly or indirectly contributed to the successful completion of this book. We are deeply thankful to the **Pharmacy Council of India (PCI)** for designing a comprehensive curriculum that has guided the preparation of this textbook in accordance with the **BP205T Pharmacognosy and Phytochemistry (Theory)** syllabus.

We extend our heartfelt appreciation to our respected teachers, mentors, colleagues, and academic experts whose knowledge, guidance, and encouragement have inspired us throughout the development of this work. Their valuable suggestions and continuous support have significantly enhanced the quality of the manuscript.

We also acknowledge the contributions of researchers, scientists, and authors whose published work, standard textbooks, pharmacopoeias, and scientific literature have served as important references during the preparation of this book. Their dedication to advancing pharmaceutical and natural product sciences continues to inspire learners across the world.

Special thanks are due to our family members, friends, and well-wishers for their constant encouragement, patience, and moral support throughout the writing process. We are equally grateful to the editorial and publishing team for their professionalism, technical assistance, and commitment in bringing this book to publication.

Finally, we dedicate this book to the students of pharmacy, whose curiosity and enthusiasm for learning continue to motivate us to contribute to pharmaceutical education. We sincerely hope that this book will assist them in developing a strong conceptual understanding of pharmacognosy and phytochemistry and inspire future research in the field of natural products.

Authors

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UNIT – I

Metabolic Pathways and Biogenetic Studies

1. INTRODUCTION TO PLANT METABOLISM

1.1 Introduction

Metabolism is the sum total of all biochemical reactions occurring within living organisms that sustain life by converting nutrients into energy and essential biomolecules. In plants, metabolism not only supports growth, development, and reproduction but also enables adaptation to environmental stresses through the synthesis of a wide variety of chemical compounds. Plant metabolism is broadly categorized into primary metabolism and secondary (specialized) metabolism, which are closely interconnected through a network of enzymatic pathways.

Primary metabolism comprises the essential biochemical processes required for the survival of all plant cells, including photosynthesis, respiration, glycolysis, the tricarboxylic acid (TCA) cycle, and amino acid biosynthesis. These pathways produce fundamental biomolecules such as carbohydrates, proteins, lipids, and nucleic acids that are indispensable for cellular growth and maintenance.

In contrast, secondary metabolism involves the biosynthesis of specialized metabolites that are not directly required for basic cellular functions but play crucial ecological roles. These metabolites help plants defend themselves against herbivores and pathogens, attract pollinators, facilitate seed dispersal, and adapt to environmental stresses. Many of these compounds possess significant pharmacological activities and serve as valuable sources of medicines, flavors, fragrances, dyes, pesticides, and nutraceuticals.

Natural products such as alkaloids, flavonoids, terpenoids, phenolic compounds, glycosides, tannins, and essential oils are synthesized through well-organized metabolic pathways originating from common primary metabolites. The major biosynthetic routes responsible for the formation of these secondary metabolites include the Shikimic acid pathway, Acetate (Polyketide) pathway, Mevalonate (MVA) pathway, Methylerythritol phosphate (MEP/DOXP) pathway, and Amino acid-derived pathways.

Understanding these metabolic pathways is fundamental to pharmacognosy because they explain how medicinal plants synthesize bioactive compounds. Knowledge of plant metabolism also forms the basis for metabolic engineering, synthetic biology, plant biotechnology, and modern drug discovery.

1.2 Classification of Plant Metabolism

Plant metabolism can be divided into two major categories:

A. Primary Metabolism

Primary metabolism includes all biochemical reactions essential for plant survival, growth, and reproduction. The products of primary metabolism are universally present in all living cells and are directly involved in energy production, structural integrity, and cellular maintenance.

Major characteristics of primary metabolism include:

- Essential for normal growth and development.
- Conserved among most living organisms.

- Produces energy-rich molecules such as ATP.
- Generates precursors required for secondary metabolite biosynthesis.

Examples of primary metabolites include:

- Carbohydrates
- Amino acids
- Proteins
- Lipids
- Fatty acids
- Nucleic acids
- Organic acids
- Vitamins

Major primary metabolic pathways include:

- Photosynthesis
- Glycolysis
- Pentose Phosphate Pathway
- Tricarboxylic Acid (TCA) Cycle
- Amino Acid Biosynthesis
- Fatty Acid Biosynthesis

B. Secondary (Specialized) Metabolism

Secondary metabolism involves the synthesis of specialized compounds that are not directly required for plant survival but provide ecological and evolutionary advantages.

Characteristics include:

- Species-specific distribution.
- Produced in specialized tissues or developmental stages.
- Often synthesized in response to environmental stimuli.
- Derived from intermediates of primary metabolism.

Examples include:

- Alkaloids
- Flavonoids
- Phenolic acids
- Tannins
- Terpenoids
- Saponins
- Cardiac glycosides
- Essential oils
- Lignans

These metabolites are extensively used in pharmaceutical, cosmetic, food, agricultural, and biotechnology industries.

1.3 Relationship Between Primary and Secondary Metabolism

Secondary metabolites originate from intermediates generated during primary metabolism. Primary metabolic pathways provide the carbon skeletons, reducing power (NADPH), ATP, and precursor molecules required for specialized biosynthesis.

For example:

- Glycolysis generates phosphoenolpyruvate (PEP).
- Pentose phosphate pathway produces erythrose-4-phosphate (E4P).
- The TCA cycle produces acetyl-CoA.
- Amino acid biosynthesis forms phenylalanine, tyrosine, and tryptophan.

These intermediates serve as starting materials for various secondary metabolic pathways:

- PEP + E4P → Shikimic acid pathway → Aromatic amino acids → Phenolics, flavonoids, lignin, and many alkaloids.
- Acetyl-CoA → Acetate pathway → Fatty acids, polyketides, anthraquinones, and flavonoids.
- Amino acids → Amino acid pathways → Numerous alkaloids, cyanogenic glycosides, and glucosinolates.

Thus, primary and secondary metabolism function as integrated biochemical networks rather than independent processes.

1.4 Importance of Metabolic Pathways

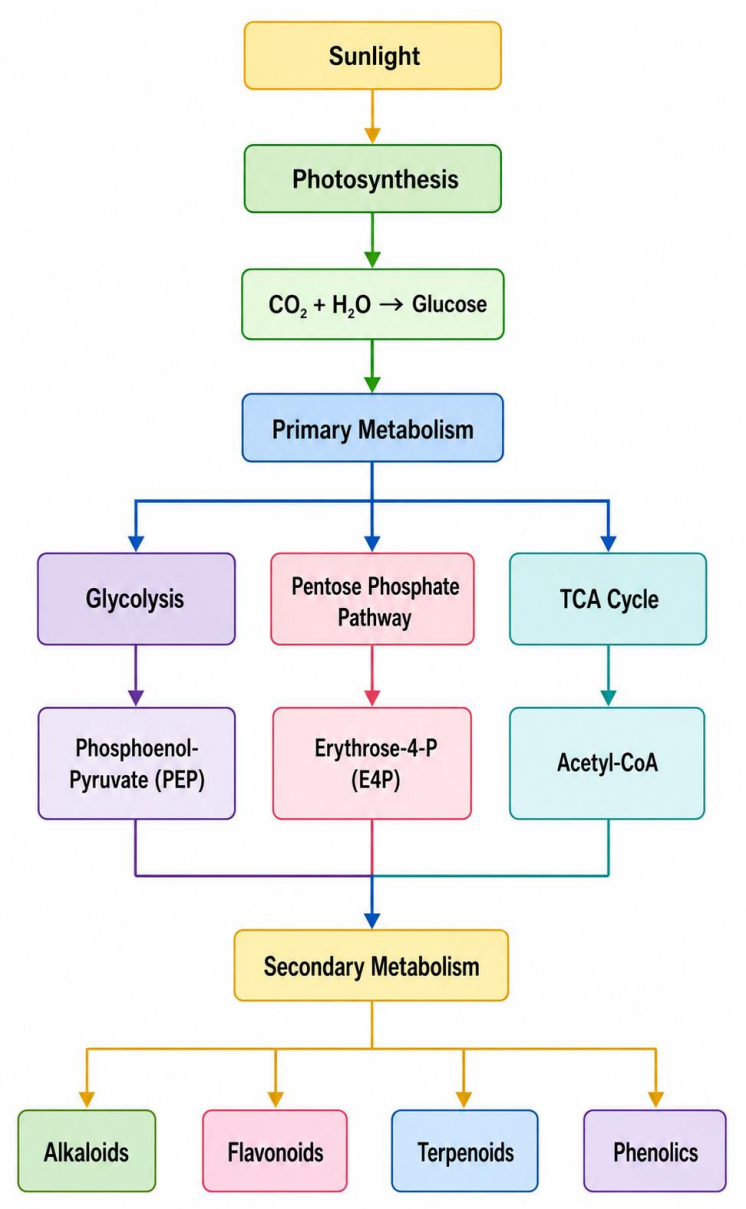
Metabolic pathways are sequential enzyme-catalyzed reactions that convert simple molecules into complex biologically active compounds. Each pathway is precisely regulated to maintain cellular homeostasis and efficient utilization of metabolic resources.

The study of metabolic pathways is important because it:

- Explains the biosynthesis of medicinally important phytochemicals.
- Helps identify key enzymes involved in natural product formation.
- Supports metabolic engineering to increase production of valuable compounds.
- Facilitates discovery of novel therapeutic agents.
- Assists in understanding plant adaptation to environmental stress.
- Provides a scientific basis for quality control and standardization of herbal medicines.
- Enables synthetic biology approaches for industrial-scale production of natural products.

1.5 Carbon Flow in Plant Metabolism

Plants capture atmospheric carbon dioxide through photosynthesis and convert it into carbohydrates. These carbohydrates enter central metabolic pathways, generating precursor molecules that ultimately give rise to a diverse array of secondary metabolites.



Flowchart: Overall Carbon Flow in Plant Metabolism

1.6 Comparison Between Primary and Secondary Metabolites

Feature	Primary Metabolites	Secondary Metabolites
Function	Essential for growth and survival	Ecological adaptation and defense
Distribution	Present in all living cells	Restricted to specific species or tissues
Quantity	Large amounts	Usually small amounts
Biosynthesis	Directly involved in energy metabolism	Derived from primary metabolites
Importance	Cell maintenance and reproduction	Defense, attraction, signaling, and pharmacological activity
Examples	Glucose, amino acids, proteins, lipids	Alkaloids, flavonoids, terpenoids, tannins

1.7 Significance in Pharmacognosy

Plant secondary metabolites constitute one of the richest sources of therapeutic agents used in modern medicine. Many clinically important drugs, including morphine, quinine, atropine, vincristine, digoxin, artemisinin, and paclitaxel, originate from specialized plant metabolic pathways.

A thorough understanding of plant metabolism enables researchers to identify biosynthetic routes, optimize phytochemical production, improve medicinal plant quality, and develop novel plant-based pharmaceuticals using biotechnology and metabolic engineering.

Key Points

- Plant metabolism comprises interconnected primary and secondary metabolic pathways.
- Primary metabolism generates energy and precursor molecules essential for life.
- Secondary metabolism synthesizes specialized compounds with ecological and medicinal significance.
- Most pharmacologically active natural products originate from a limited number of biosynthetic pathways.
- Knowledge of metabolic pathways is fundamental to pharmacognosy, phytochemistry, biotechnology, and natural product drug discovery.

2. BASIC METABOLIC PATHWAYS IN PLANTS

2.1 Introduction

Primary metabolism comprises essential biochemical reactions that provide energy, reducing power, and precursor molecules required for plant growth and the biosynthesis of secondary metabolites. The three major primary metabolic pathways are glycolysis, the pentose phosphate pathway (PPP), and the tricarboxylic acid (TCA) cycle. These pathways generate key intermediates such as phosphoenolpyruvate (PEP), erythrose-4-phosphate (E4P), pyruvate, and acetyl-CoA, which serve as precursors for alkaloids, flavonoids, terpenoids, and phenolic compounds.

2.2 Glycolysis (Embden–Meyerhof–Parnas Pathway)

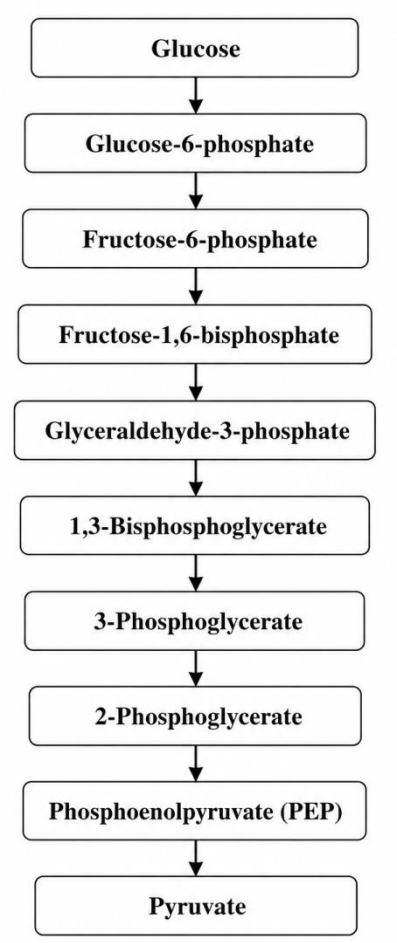
Definition

Glycolysis is the metabolic pathway in which one molecule of glucose is converted into two molecules of pyruvate, producing ATP and NADH. It occurs in the cytosol of plant cells and provides energy as well as intermediates for various biosynthetic pathways.

Overall Reaction

$$\text{Glucose} + 2\text{ADP} + 2\text{Pi} + 2\text{NAD}^+ \rightarrow 2\ \text{Pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}_2\text{O} + 2\text{H}^+$$

Simplified Pathway



Importance

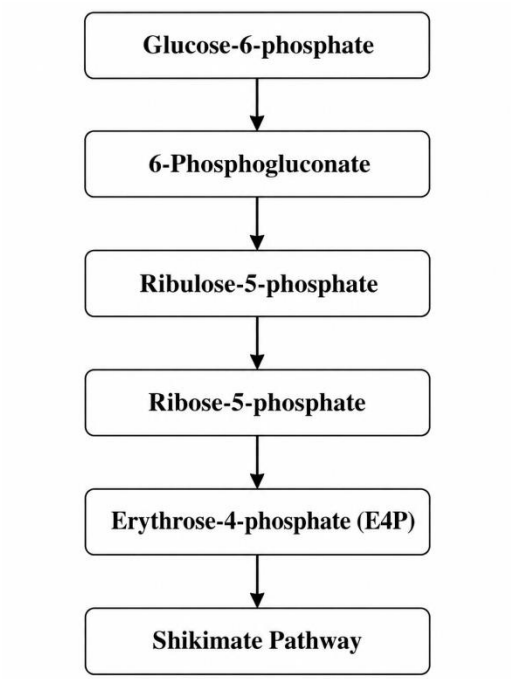
- Produces ATP and NADH.
- Generates **PEP**, a precursor of the shikimate pathway.
- Supplies pyruvate for acetyl-CoA formation and the TCA cycle.

2.3 Pentose Phosphate Pathway (PPP)

Definition

The Pentose Phosphate Pathway (PPP), also called the **Hexose Monophosphate (HMP) Shunt**, is an alternative pathway of glucose metabolism that produces **NADPH** and **pentose sugars** instead of ATP.

Simplified Pathway



Functions

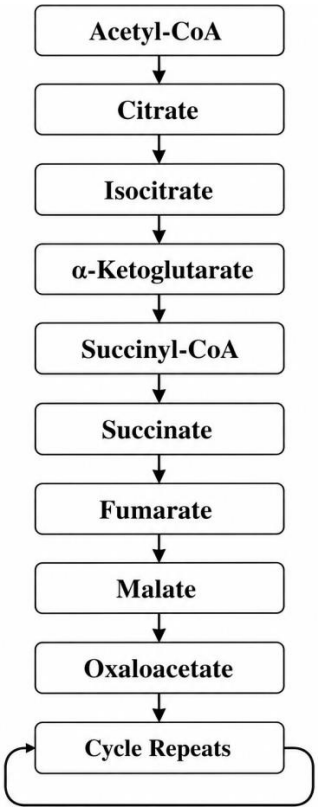
- Produces NADPH for reductive biosynthesis.
- Generates ribose-5-phosphate for nucleotide synthesis.
- Produces erythrose-4-phosphate (E4P), a precursor of the shikimate pathway.
- Protects cells against oxidative stress.

2.4 Tricarboxylic Acid (TCA) Cycle

Definition

The TCA cycle, also known as the **Citric Acid Cycle** or **Krebs Cycle**, oxidizes acetyl-CoA to carbon dioxide, producing NADH, $FADH_2$, and ATP (or GTP). It takes place in the mitochondrial matrix.

Simplified Pathway



Importance

- Produces NADH and $FADH_2$ for ATP synthesis.
- Provides intermediates for amino acid biosynthesis.
- Supplies precursors for secondary metabolites.

3. SHIKIMIC ACID PATHWAY

3.1 Historical Background

The shikimic acid pathway was first proposed in the early 20th century through studies on the biosynthesis of aromatic compounds in microorganisms and plants. The pathway was named after **shikimic acid**, which was first isolated from the fruits of the Japanese plant *Illicium anisatum* (Japanese star anise). Subsequent biochemical studies established shikimate as the central intermediate in the biosynthesis of aromatic amino acids and numerous secondary metabolites. Today, the shikimate pathway is recognized as one of the most important biosynthetic routes in plants, bacteria, fungi, and algae.

3.2 Importance of the Shikimic Acid Pathway

The shikimate pathway serves as the principal route for the biosynthesis of **aromatic amino acids** and a wide range of medicinally important secondary metabolites. Approximately 20–30% of the carbon fixed during photosynthesis is directed through this pathway in actively growing plant tissues.

Its major functions include:

- Biosynthesis of phenylalanine, tyrosine, and tryptophan.
- Formation of phenolic compounds, flavonoids, lignin, tannins, and several alkaloids.
- Production of compounds involved in plant defense, pigmentation, structural support, and stress tolerance.
- Supply of precursors for pharmaceuticals, nutraceuticals, flavors, fragrances, and agrochemicals.

3.3 Distribution in Plants

The shikimate pathway is widely distributed in:

- Higher plants
- Gymnosperms
- Angiosperms
- Algae
- Fungi
- Bacteria

In plants, the pathway is localized primarily in the plastids (chloroplasts). Importantly, this pathway is absent in animals, making its enzymes attractive targets for herbicides and antimicrobial drugs.

3.4 Starting Precursors

The shikimate pathway begins with two primary metabolic intermediates:

1. Phosphoenolpyruvate (PEP) – produced through glycolysis.
2. Erythrose-4-phosphate (E4P) – generated via the pentose phosphate pathway.

These precursors undergo enzyme-catalyzed condensation to form the first committed intermediate, 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP).

3.5 Overall Biosynthetic Pathway

3.6 Stepwise Biosynthesis

Step 1: Formation of DAHP

Reaction:

PEP + Erythrose-4-phosphate → 3-Deoxy-D-arabino-heptulosonate-7-phosphate (DAHP)

Enzyme: DAHP synthase

Significance: First committed and rate-regulating step of the pathway.

Step 2: Formation of 3-Dehydroquinate

Reaction:

DAHP → 3-Dehydroquinate

Enzyme: 3-Dehydroquinate synthase

Mechanism: Intramolecular cyclization and oxidation produce the first cyclic intermediate.

Step 3: Formation of 3-Dehydroshikimate

Reaction:

3-Dehydroquinate → 3-Dehydroshikimate

Enzyme: 3-Dehydroquinate dehydratase

Mechanism: Dehydration removes one molecule of water to form a ketone intermediate.

Step 4: Formation of Shikimic Acid

Reaction:

3-Dehydroshikimate + NADPH + H^+ → Shikimic acid + $NADP^+$

Enzyme: Shikimate dehydrogenase

Mechanism: Reduction of the keto group to produce shikimate.

Step 5: Formation of Shikimate-3-phosphate

Reaction:

Shikimic acid + ATP → Shikimate-3-phosphate + ADP

Enzyme: Shikimate kinase

Mechanism: ATP-dependent phosphorylation at the C-3 hydroxyl group.

Step 6: Formation of EPSP

Reaction:

Shikimate-3-phosphate + PEP → 5-Enolpyruvylshikimate-3-phosphate (EPSP)

Enzyme: EPSP synthase (EPSPS)

Mechanism: Transfer of the enolpyruvyl group from PEP to shikimate-3-phosphate.

Step 7: Formation of Chorismate

Reaction:

EPSP → Chorismate + Pi

Enzyme: Chorismate synthase

Mechanism: Elimination of phosphate forms chorismate, the universal branching intermediate.

3.7 Major Enzymes of the Shikimate Pathway

Step	Enzyme	Product
1	DAHP synthase	DAHP
2	3-Dehydroquinate synthase	3-Dehydroquinate
3	3-Dehydroquinate dehydratase	3-Dehydroshikimate
4	Shikimate dehydrogenase	Shikimic acid
5	Shikimate kinase	Shikimate-3-phosphate
6	EPSP synthase	EPSP
7	Chorismate synthase	Chorismate

3.8 Formation of End Products

Chorismate serves as the metabolic branch point for the synthesis of three aromatic amino acids:

- **Phenylalanine** → Phenylpropanoids, flavonoids, lignin, coumarins, tannins.
- **Tyrosine** → Isoquinoline alkaloids, tocopherols, betalains.
- **Tryptophan** → Indole alkaloids, auxin (IAA), serotonin, melatonin.

These compounds contribute to plant defense, structural integrity, pigmentation, signaling, and pharmaceutical activity.

3.9 Regulation of the Shikimate Pathway

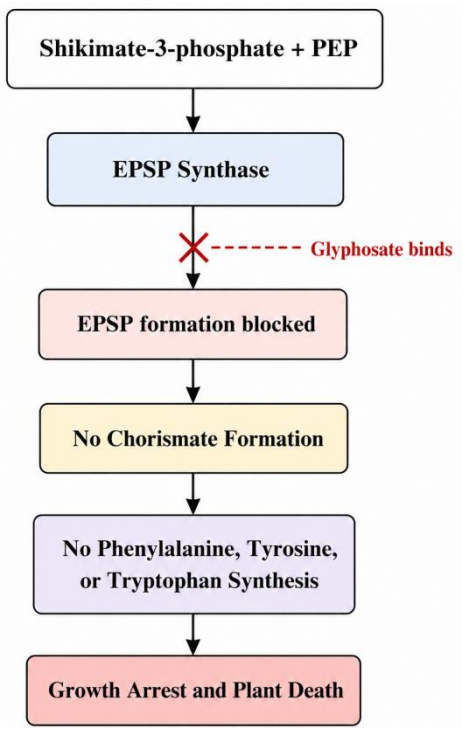
The pathway is tightly regulated to maintain metabolic balance.

- **DAHP synthase** is regulated by feedback inhibition from aromatic amino acids.
- **EPSP synthase** controls carbon flux toward chorismate.
- Environmental factors such as light, pathogen attack, nutrient availability, and abiotic stress enhance pathway activity by increasing enzyme expression.
- Transcriptional regulation coordinates the synthesis of enzymes involved in aromatic amino acid production.

3.10 Herbicide Target: Glyphosate Inhibition of EPSPS

Glyphosate is a broad-spectrum systemic herbicide that specifically inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS).

Mechanism of Action



Since animals lack the shikimate pathway, glyphosate selectively affects plants and many microorganisms.

3.11 Industrial and Pharmaceutical Importance

The shikimate pathway has significant industrial and biomedical applications:

- Shikimic acid is the key starting material for the synthesis of the antiviral drug oseltamivir (Tamiflu®).
- Aromatic amino acids produced through this pathway are used in food, pharmaceutical, and biotechnology industries.
- Metabolic engineering of the shikimate pathway enhances the production of flavonoids, phenolics, and medicinal alkaloids.
- The pathway is an important target for herbicide development, antimicrobial drug discovery, and synthetic biology.

Key Points

- The shikimate pathway links primary metabolism with the biosynthesis of aromatic amino acids and numerous secondary metabolites.
- PEP and erythrose-4-phosphate are the initial substrates.
- Chorismate is the central branching intermediate leading to phenylalanine, tyrosine, and tryptophan.
- The pathway is absent in animals, making it an ideal target for herbicides and antimicrobial agents.
- Shikimate-derived metabolites play essential roles in plant defense, growth, and the production of therapeutically important natural products.

4. ACETATE PATHWAY (POLYKETIDE PATHWAY)

4.1 Introduction

The Acetate Pathway, also known as the Polyketide Pathway, is one of the major biosynthetic routes responsible for the formation of numerous plant secondary metabolites. In this pathway, acetyl-CoA acts as the starter molecule, while malonyl-CoA serves as the chain-extending unit. Repeated condensation reactions catalyzed by polyketide synthases (PKSs) produce a variety of biologically active compounds such as fatty acids, anthraquinones, flavonoids, xanthones, and other polyketides.

The acetate pathway is widely distributed in plants, fungi, bacteria, and some marine organisms and plays a crucial role in the biosynthesis of medicinally important natural products.

4.2 Historical Background

The acetate pathway was established through isotope-labeling experiments using ^{14}C-labeled acetate, which demonstrated that acetate units are incorporated into the carbon skeletons of fatty acids and polyketides. These studies confirmed that many natural products are synthesized through the sequential condensation of two-carbon acetate units.

4.3 Importance of the Acetate Pathway

The acetate pathway is important because it:

- Produces a wide variety of secondary metabolites.
- Generates pharmacologically active compounds used in medicine.
- Provides precursors for pigments, antibiotics, and antioxidants.
- Supports plant defense against pathogens and herbivores.
- Serves as a target for metabolic engineering and synthetic biology.

4.4 Distribution in Plants

The acetate pathway occurs in:

- Higher plants
- Fungi
- Bacteria
- Algae

In plants, biosynthesis mainly occurs in the cytosol and plastids, depending on the type of metabolite being synthesized.

4.5 Starting Precursors

The pathway begins with two activated acetate derivatives:

- **Acetyl-CoA** – Starter molecule
- **Malonyl-CoA** – Chain extender

Malonyl-CoA is formed by the ATP-dependent carboxylation of acetyl-CoA.

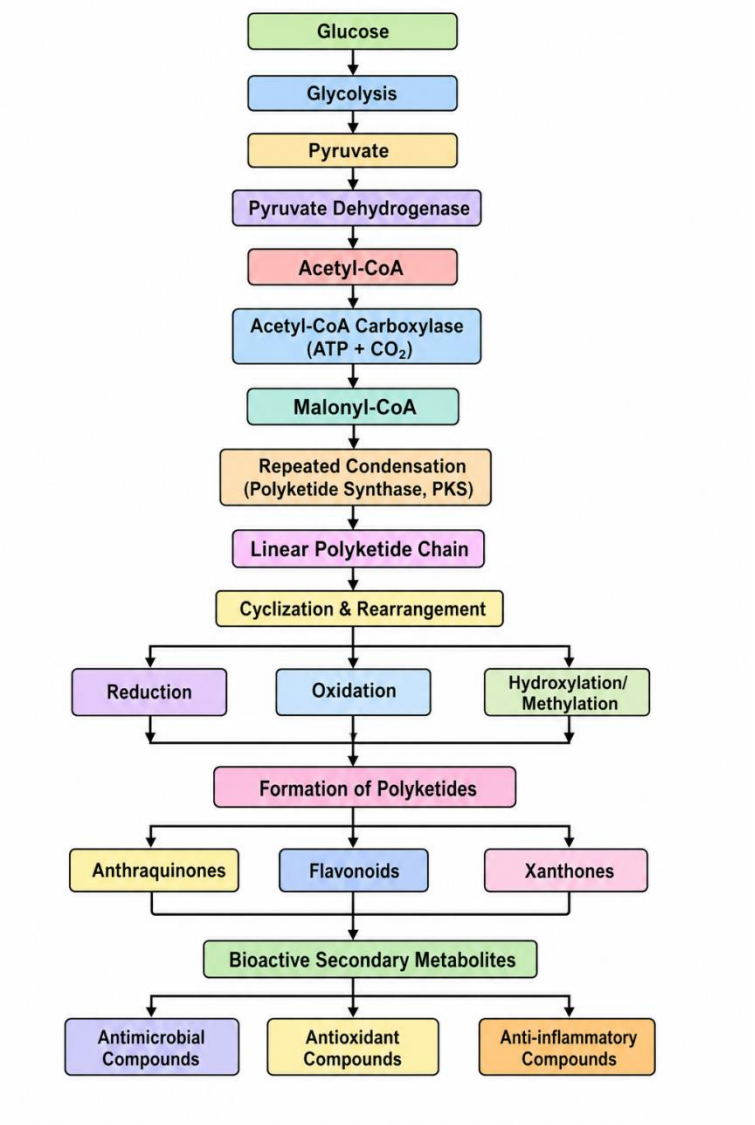
Reaction

Acetyl-CoA + CO_2 + ATP → Malonyl-CoA + ADP + Pi

Enzyme: Acetyl-CoA Carboxylase (ACC)

4.6 Overall Biosynthetic Pathway

Flowchart. Acetate (Polyketide) Pathway



4.7 Stepwise Biosynthesis

Step 1. Formation of Acetyl-CoA

Pyruvate produced during glycolysis is converted into acetyl-CoA by the pyruvate dehydrogenase complex.

Reaction

Pyruvate + CoA + NAD^+ → Acetyl-CoA + CO_2 + NADH

Step 2. Formation of Malonyl-CoA

Acetyl-CoA is carboxylated to produce malonyl-CoA.

Reaction

Acetyl-CoA + CO_2 + ATP → Malonyl-CoA + ADP + Pi

Enzyme: Acetyl-CoA Carboxylase

Step 3. Chain Elongation

One acetyl-CoA molecule combines with successive malonyl-CoA molecules through decarboxylative Claisen condensation reactions catalyzed by **Polyketide Synthase (PKS)**.

General Reaction

Acetyl-CoA + n Malonyl-CoA → Polyketide Chain + CO_2

Step 4. Cyclization

The linear polyketide chain undergoes:

- Cyclization
- Reduction
- Oxidation
- Hydroxylation
- Methylation
- Glycosylation

These reactions generate structurally diverse secondary metabolites.

4.8 Major Enzymes

Step	Enzyme	Function
1	Pyruvate Dehydrogenase	Formation of Acetyl-CoA
2	Acetyl-CoA Carboxylase (ACC)	Synthesis of Malonyl-CoA
3	Polyketide Synthase (PKS)	Chain elongation
4	Cyclases	Ring formation
5	Oxidases/Hydroxylases	Structural modification
6	Methyltransferases	Methyl group transfer
7	Glycosyltransferases	Glycoside formation

4.9 End Products of the Acetate Pathway

The acetate pathway gives rise to numerous biologically active natural products, including:

- Fatty acids
- Anthraquinones
- Flavonoids
- Xanthones
- Phloroglucinols
- Acetogenins
- Resorcinols
- Polyphenolic compounds

Examples include:

Compound	Plant Source	Biological Activity
Emodin	*Aloe vera*, *Cassia* spp.	Laxative, antimicrobial
Chrysophanol	*Rheum* spp.	Anti-inflammatory
Aloin	*Aloe vera*	Cathartic
Naringenin	Citrus species	Antioxidant
Phloroglucinol	*Hypericum* spp.	Antimicrobial

4.10 Regulation of the Acetate Pathway

The pathway is regulated at several levels:

- **Acetyl-CoA Carboxylase (ACC)** is the major rate-limiting enzyme.
- Availability of acetyl-CoA and malonyl-CoA determines metabolic flux.
- Gene expression of **Polyketide Synthases (PKSs)** increases in response to pathogen attack, ultraviolet radiation, wounding, and environmental stress.
- Plant hormones such as jasmonic acid and salicylic acid stimulate polyketide biosynthesis during defense responses.

4.11 Pharmaceutical and Industrial Importance

The acetate pathway is of considerable pharmaceutical and industrial significance because it produces numerous bioactive molecules with therapeutic applications.

Applications include:

- Production of antioxidant flavonoids.
- Biosynthesis of anthraquinone laxatives.
- Manufacture of antibiotics and antimicrobial agents.
- Development of anticancer and anti-inflammatory compounds.
- Metabolic engineering of medicinal plants for enhanced production of valuable phytochemicals.
- Industrial production of polyketides using microbial fermentation and synthetic biology.

4.12 Comparison Between the Shikimate and Acetate Pathways

Feature	Shikimate Pathway	Acetate Pathway
Starting precursor	PEP + Erythrose-4-phosphate	Acetyl-CoA + Malonyl-CoA
Initial enzyme	DAHP Synthase	Acetyl-CoA Carboxylase
Key intermediate	Chorismate	Polyketide Chain
Major products	Aromatic amino acids, phenolics	Fatty acids, anthraquinones, flavonoids
Distribution	Plants, fungi, bacteria	Plants, fungi, bacteria
Pharmacological significance	Alkaloids, lignin, flavonoids	Anthraquinones, flavonoids, polyketides

Key Points

- The acetate pathway is also known as the polyketide pathway.
- Acetyl-CoA is the starter molecule, whereas malonyl-CoA serves as the chain extender.
- Polyketide synthases (PKSs) catalyze repeated condensation reactions to form diverse carbon skeletons.
- The pathway is responsible for the biosynthesis of numerous medicinally important compounds, including anthraquinones, flavonoids, and fatty acids.
- The acetate pathway is widely exploited in pharmacognosy, biotechnology, and drug discovery for the production of valuable natural products.

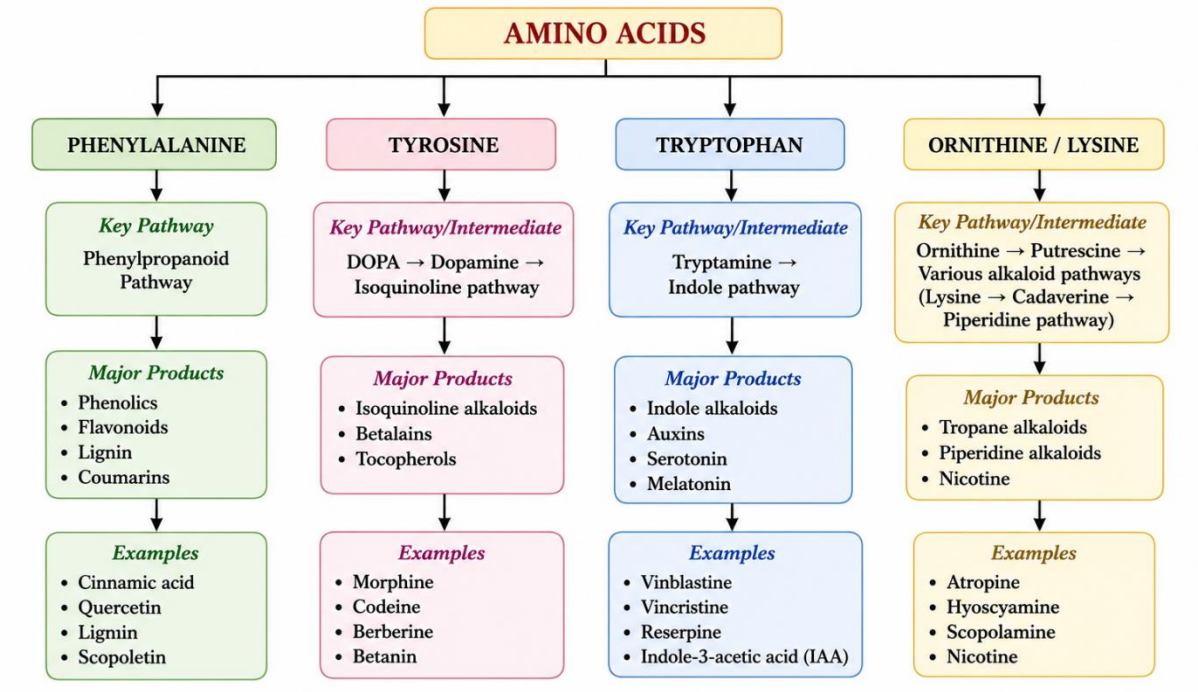
5. AMINO ACID PATHWAYS

5.1 Introduction

Amino acid pathways are major biosynthetic routes in plants that produce numerous nitrogen-containing secondary metabolites. Besides serving as the building blocks of proteins, amino acids act as precursors for alkaloids, phenolic compounds, cyanogenic glycosides, glucosinolates, phytohormones, and other biologically active molecules. Among the twenty proteinogenic amino acids, phenylalanine, tyrosine, tryptophan, ornithine, lysine, and histidine are particularly important in secondary metabolite biosynthesis.

Most aromatic amino acids are synthesized through the shikimate pathway, while other amino acids originate from intermediates of glycolysis and the tricarboxylic acid (TCA) cycle.

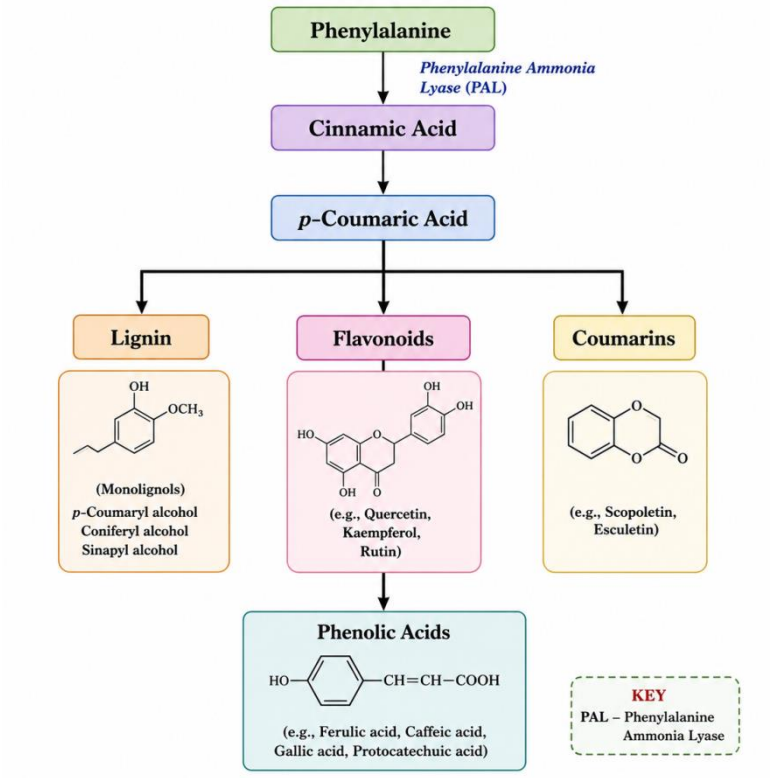
5.2 Major Amino Acid-Derived Biosynthetic Pathways



5.3 Phenylalanine Pathway

Phenylalanine is synthesized through the **shikimate pathway** and serves as the precursor of the **phenylpropanoid pathway**, which produces several important phenolic compounds.

Biosynthetic Pathway



Important Enzyme

- **Phenylalanine Ammonia Lyase (PAL)** – Catalyzes the deamination of phenylalanine to cinnamic acid and is the key regulatory enzyme of the phenylpropanoid pathway.

Major Products

- Phenolic acids
- Flavonoids
- Lignin
- Coumarins
- Tannins

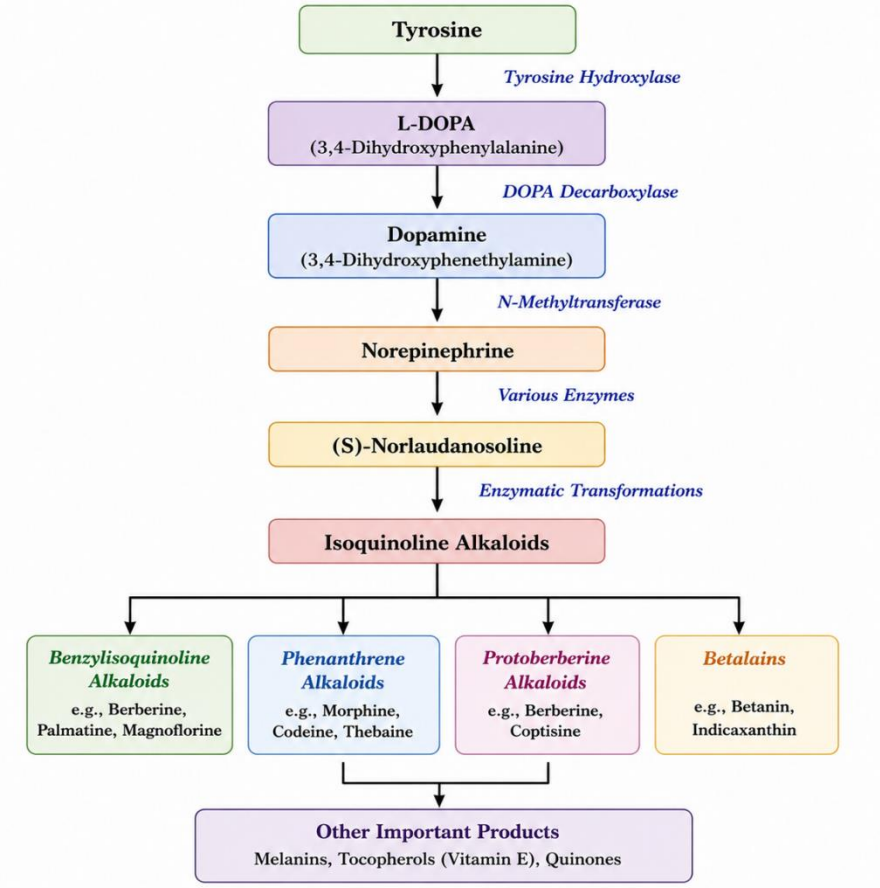
Significance

These compounds contribute to structural support, UV protection, antioxidant activity, and plant defense.

5.4 Tyrosine Pathway

Tyrosine is an aromatic amino acid that serves as a precursor for several alkaloids, pigments, and signaling molecules.

Biosynthetic Pathway



Major Products

- Dopamine
- Isoquinoline alkaloids
- Morphine
- Codeine
- Betalains
- Tocopherols (Vitamin E)

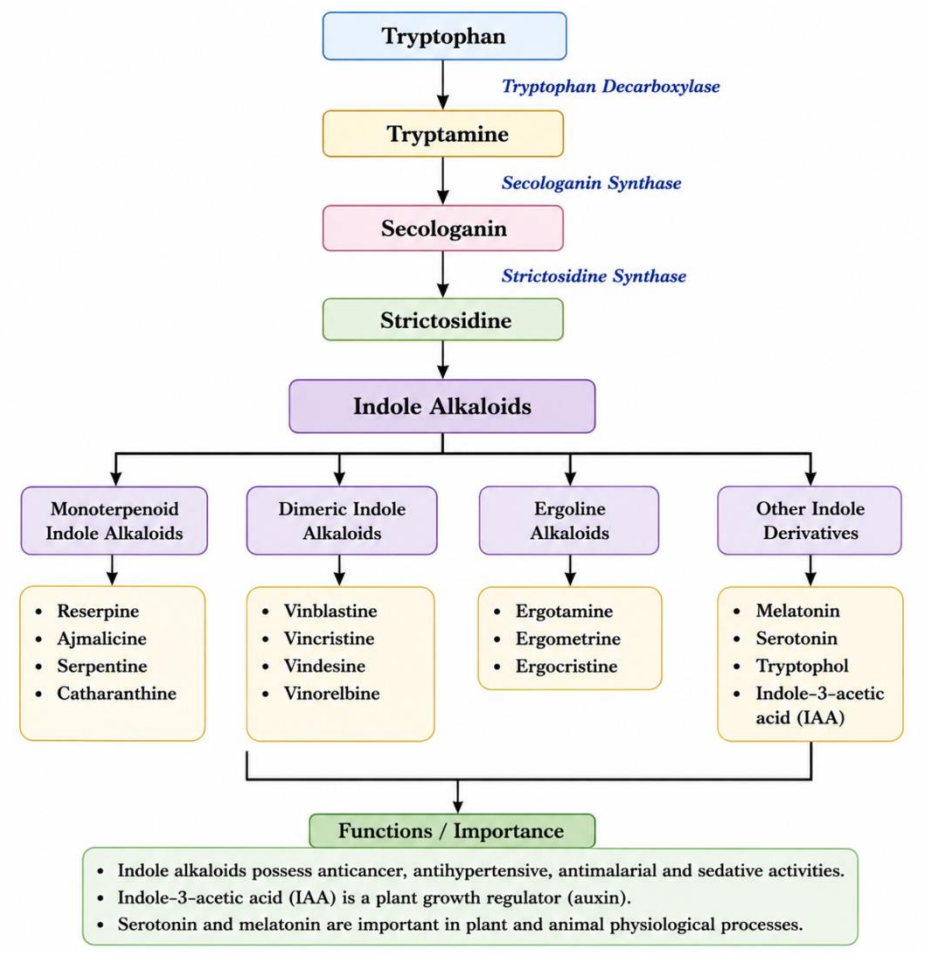
Importance

Tyrosine-derived metabolites possess analgesic, antimicrobial, antioxidant, and pharmaceutical activities.

5.5 Tryptophan Pathway

Tryptophan is an important precursor for indole-containing compounds and plant growth regulators.

Biosynthetic Pathway



Importance

Tryptophan-derived alkaloids exhibit anticancer, antihypertensive, antimalarial, and neuropharmacological properties.

5.6 Ornithine and Lysine Pathways

Ornithine and lysine are important amino acid precursors for the biosynthesis of several alkaloids involved in plant defense and medicine.

Ornithine pathway:
Ornithine → Putrescine → Nicotine and Ornithine → Putrescine → Tropane alkaloids (Atropine, Hyoscyamine, Scopolamine).

Lysine pathway:
Lysine → Cadaverine → Piperidine alkaloids.

Major Products

- Nicotine
- Atropine
- Hyoscyamine
- Scopolamine
- Piperidine alkaloids

Importance

These alkaloids play vital roles in plant defense and are widely used as antispasmodic, bronchodilator, insecticidal, and pharmaceutical agents.

5.7 Histidine Pathway

Histidine serves as a precursor for **imidazole alkaloids**.

Histidine pathway:
Histidine → Imidazole alkaloids → Pilocarpine and histamine-related metabolites.

5.8 Regulation of Amino Acid Pathways

The biosynthesis of amino acid-derived secondary metabolites is regulated by:

- Feedback inhibition of amino acid biosynthesis.
- Activity of key enzymes such as Phenylalanine Ammonia Lyase (PAL), Tyrosine Hydroxylase, and Tryptophan Decarboxylase.
- Environmental factors including light, temperature, pathogens, and nutrient availability.
- Plant hormones such as jasmonic acid, salicylic acid, and ethylene.

5.9 Pharmaceutical Importance

Amino Acid	Biosynthetic Products	Therapeutic Applications
Phenylalanine	Phenylalanine → Cinnamic acid → Flavonoids, lignin, coumarins	Antioxidant, anti-inflammatory
Tyrosine	Tyrosine → L-DOPA → Dopamine → Isoquinoline alkaloids	Analgesic, antimicrobial
Tryptophan	Tryptophan → Tryptamine → Indole alkaloids	Anticancer, antihypertensive
Ornithine	Ornithine → Putrescine → Atropine, Scopolamine, Nicotine	Antispasmodic, bronchodilator
Lysine	Lysine → Cadaverine → Piperidine alkaloids	Antimicrobial, insecticidal

Key Point

Amino acid pathways provide essential precursors for numerous medicinally important secondary metabolites and play a central role in pharmacognosy, natural product chemistry, and drug discovery.

6. UTILIZATION OF RADIOACTIVE ISOTOPES IN BIOGENETIC STUDIES

6.1 Introduction

Biogenetic studies investigate the biosynthetic origin and metabolic fate of natural products in living organisms. One of the most powerful approaches for elucidating biosynthetic pathways is the use of **radioactive isotopes (radioisotopes)** and **stable isotopes** as tracers. These labeled atoms are incorporated into precursor molecules and followed through metabolic reactions to determine the sequence of biochemical transformations.

The tracer technique helps identify precursor–product relationships, reaction intermediates, enzyme functions, and the complete biosynthetic pathway of primary and secondary metabolites.

6.2 Principle of Isotope Tracer Technique

In isotope labeling experiments, a metabolite containing a radioactive or stable isotope is supplied to a plant or microorganism. The labeled precursor is incorporated into newly synthesized metabolites, which are subsequently isolated and analyzed using autoradiography, liquid scintillation counting, mass spectrometry (MS), or nuclear magnetic resonance (NMR).

6.3 Commonly Used Isotopes in Biogenetic Studies

1. Carbon-14 (^{14}C)

- **Type:** Radioactive isotope
- **Half-life: 5,730 years**
- **Radiation:** β (Beta)
- **Applications:**
  - Most widely used tracer in biosynthetic studies.
  - Traces carbon atoms during the synthesis of carbohydrates, alkaloids, terpenoids, flavonoids, steroids, and phenolic compounds.
  - Establishes precursor–product relationships.

Example: Feeding **^{14}C-acetate** demonstrates the biosynthesis of fatty acids and polyketides.

2. Tritium (^{3}H)

- **Type:** Radioactive isotope of hydrogen
- **Half-life: 12.32 years**
- **Radiation:** β (Beta)
- **Applications:**
  - Labels hydrogen atoms in metabolites.
  - Studies enzyme kinetics and hydrogen transfer reactions.
  - Investigates receptor binding and drug metabolism.

Example: ^{3}H-labeled amino acids are used to study protein biosynthesis.

3. Sulfur-35 (^{35}S)

- **Type:** Radioactive isotope
- **Half-life: 87.5 days**
- **Radiation:** β (Beta)
- **Applications:**
  - Traces sulfur-containing amino acids such as cysteine and methionine.
  - Studies sulfur metabolism and sulfur-containing natural products.
  - Investigates protein synthesis.

4. Phosphorus-32 (^{32}P)

- **Type:** Radioactive isotope
- **Half-life: 14.3 days**
- **Radiation:** β (Beta)
- **Applications:**
  - Studies phosphate metabolism.
  - Labels nucleotides, nucleic acids, ATP, and phosphorylated intermediates.
  - Investigates DNA replication, RNA transcription, and signal transduction.

5. Oxygen-18 (^{18}O)

- **Type:** Stable isotope (Non-radioactive)
- **Half-life:** Stable (does not undergo radioactive decay)
- **Applications:**
  - Traces oxygen incorporation into metabolites.
  - Studies oxidation–reduction reactions and photosynthesis.
  - Used with isotope-ratio mass spectrometry (IRMS) to investigate metabolic pathways.

6.4 Commonly Used Isotopes in Biogenetic Studies

Isotope	Type	Half-life	Major Uses
^{14}C (Carbon-14)	Radioactive	5,730 years	Carbon tracing, biosynthetic pathway studies
^{3}H (Tritium)	Radioactive	12.32 years	Hydrogen transfer, enzyme kinetics, protein synthesis

^{35}S (Sulfur-35)	Radioactive	87.5 days	Sulfur metabolism, sulfur-containing amino acids
^{32}P (Phosphorus-32)	Radioactive	14.3 days	DNA, RNA, ATP, phosphorylation studies
^{18}O (Oxygen-18)	Stable isotope	Stable	Photosynthesis, oxygen incorporation, metabolic flux analysis

6.5 Applications of Radioactive Isotopes in Pharmacognosy

Radioactive isotope techniques are widely employed to:

- Determine biosynthetic pathways of alkaloids, flavonoids, terpenoids, lignans, and glycosides.
- Identify precursor molecules and metabolic intermediates.
- Study enzyme-catalyzed reactions and reaction mechanisms.
- Measure metabolic turnover rates.
- Investigate plant growth regulators and secondary metabolite production.
- Improve metabolic engineering and synthetic biology strategies.

6.6 Advantages

- Highly sensitive and accurate.
- Enables tracing of metabolic pathways in living systems.
- Detects minute quantities of metabolites.
- Facilitates identification of biosynthetic intermediates.
- Supports pathway validation and metabolic engineering.

6.7 Limitations

- Requires specialized laboratory facilities and radiation safety measures.
- Radioactive waste disposal is essential.
- Short half-life of some isotopes limits long-term experiments.
- Stable isotope studies often require sophisticated analytical instruments such as GC-MS, LC-MS, or NMR.

Key Points

- Radioactive and stable isotopes are indispensable tools in biogenetic investigations.
- **^{14}C** is the most extensively used isotope for tracing carbon skeletons in natural product biosynthesis.
- **^{3}H, ^{35}S, and ^{32}P** are widely employed for studying hydrogen, sulfur, and phosphorus metabolism, respectively.
- **^{18}O** is a stable isotope used to investigate oxygen incorporation and metabolic flux.
- Isotope tracer techniques have greatly advanced the understanding of plant secondary metabolite biosynthesis and continue to play a crucial role in pharmacognosy, phytochemistry, and biotechnology.

7. MODERN PATHWAY PREDICTION TOOLS

7.1 Introduction

Advances in genomics, bioinformatics, metabolomics, and artificial intelligence (AI) have revolutionized the identification and prediction of biosynthetic pathways in plants and microorganisms. Modern pathway prediction tools integrate genomic sequences, enzyme databases, metabolic networks, and computational algorithms to identify genes, enzymes, intermediates, and metabolic products involved in primary and secondary metabolism.

These tools are extensively used in pharmacognosy, phytochemistry, biotechnology, synthetic biology, and drug discovery for the exploration of novel natural products and metabolic engineering.

7.2 Genome Mining

Definition

Genome mining is the computational analysis of whole-genome sequences to identify genes and gene clusters responsible for the biosynthesis of natural products.

Applications

- Discovery of biosynthetic gene clusters (BGCs).
- Identification of novel secondary metabolites.
- Prediction of enzyme functions.
- Metabolic engineering of medicinal plants and microorganisms.
- Drug discovery and synthetic biology.

7.3 KEGG (Kyoto Encyclopedia of Genes and Genomes)

Overview

KEGG is one of the most widely used biological databases for studying metabolic pathways, genomes, enzymes, diseases, and drugs. It provides interactive pathway maps linking genes, enzymes, metabolites, and biochemical reactions.

Features

- Comprehensive metabolic pathway maps.
- Enzyme Commission (EC) numbers.
- Gene–enzyme–metabolite relationships.
- Drug and disease pathways.
- Pathway reconstruction from genomic data.

Applications

- Identification of metabolic pathways.
- Functional annotation of genes.
- Drug target identification.
- Comparative genomics.

7.4 MetaCyc

Overview

MetaCyc is a curated database containing experimentally verified metabolic pathways and enzymes from diverse organisms.

Features

- Experimentally validated pathways.
- Detailed enzyme information.
- Reaction mechanisms.
- Metabolite structures.
- Cross-references with other biological databases.

Applications

- Biosynthetic pathway analysis.
- Comparative metabolism.
- Enzyme function prediction.
- Natural product research.

7.5 PlantCyc

Overview

PlantCyc is a specialized metabolic pathway database dedicated to plant metabolism and secondary metabolite biosynthesis.

Features

- Plant-specific pathways.
- Secondary metabolite biosynthesis.
- Plant enzymes and metabolites.
- Comparative pathway analysis among plant species.

Applications

- Phytochemical biosynthesis.
- Plant metabolic engineering.
- Crop improvement.
- Functional genomics.

7.6 BioCyc

Overview

BioCyc is a collection of organism-specific pathway/genome databases integrating genomic information with metabolic pathways.

Features

- Genome annotation.
- Enzyme databases.
- Metabolic pathway visualization.
- Regulatory network analysis.
- Comparative genomics.

Applications

- Genome-scale metabolic reconstruction.
- Functional annotation.
- Pathway visualization.
- Systems biology.

7.7 BLAST (Basic Local Alignment Search Tool)

Overview

BLAST is a sequence similarity search algorithm developed by the National Center for Biotechnology Information (NCBI). It compares DNA or protein sequences with known databases to identify homologous genes and predict biological functions.

Features

- DNA and protein sequence alignment.
- Gene identification.
- Functional annotation.
- Evolutionary relationship analysis.

Applications

- Identification of biosynthetic genes.
- Prediction of enzyme functions.
- Comparative genomics.
- Discovery of homologous metabolic pathways.

7.8 AntiSMASH (Antibiotics & Secondary Metabolite Analysis Shell)

Overview

AntiSMASH is a specialized bioinformatics platform used to identify and analyze **biosynthetic gene clusters (BGCs)** responsible for natural product biosynthesis in bacteria and fungi.

Features

- Detection of biosynthetic gene clusters.
- Prediction of secondary metabolites.
- Identification of polyketide synthases (PKS).
- Identification of non-ribosomal peptide synthetases (NRPS).
- Comparative cluster analysis.

Applications

- Natural product discovery.
- Antibiotic research.
- Genome mining.
- Metabolic engineering.

7.9 AI-Based Pathway Prediction

Artificial Intelligence (AI) and Machine Learning (ML) have become powerful tools for predicting metabolic pathways by integrating genomic, transcriptomic, proteomic, and metabolomic datasets.

AI Applications

- Enzyme function prediction.
- Biosynthetic pathway reconstruction.
- Metabolic network modeling.
- Drug target identification.
- Prediction of novel natural products.
- Synthetic biology and metabolic engineering.

7.10 Comparative Features of Major Pathway Prediction Tools

Tool	Primary Purpose	Major Applications
Genome Mining	Gene cluster identification	Novel natural products
KEGG	Metabolic pathway mapping	Functional annotation
MetaCyc	Experimentally validated pathways	Biosynthetic studies
PlantCyc	Plant metabolism	Phytochemical biosynthesis
BioCyc	Genome-pathway integration	Systems biology
BLAST	Sequence similarity search	Gene and enzyme prediction
AntiSMASH	Biosynthetic gene cluster analysis	Secondary metabolite discovery
AI-Based Tools	Computational pathway prediction	Drug discovery and metabolic engineering

7.11 Advantages

- Rapid identification of biosynthetic pathways.
- Discovery of novel bioactive compounds.
- Accurate gene and enzyme annotation.
- Supports metabolic engineering and synthetic biology.
- Reduces experimental time and cost.
- Facilitates genome-scale analysis.

7.12 Limitations

- Dependent on the quality of genome annotation.
- Computational predictions require experimental validation.
- Incomplete databases may limit prediction accuracy.
- AI models require large, high-quality datasets.

Key Points

- Modern pathway prediction tools combine bioinformatics, genomics, and AI to identify biosynthetic pathways.
- KEGG, MetaCyc, PlantCyc, and BioCyc are major metabolic pathway databases.
- BLAST identifies homologous genes and predicts enzyme functions.
- AntiSMASH is widely used for detecting biosynthetic gene clusters involved in secondary metabolite production.
- AI-based tools are increasingly used for pathway reconstruction, metabolic engineering, and the discovery of novel natural products.
- These computational resources have become indispensable in pharmacognosy, natural product chemistry, biotechnology, and precision drug discovery.

8. CRISPR/CAS9 IN METABOLIC ENGINEERING

8.1 Introduction

CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9) is a revolutionary genome-editing technology that enables precise modification of DNA sequences in plants, animals, and microorganisms. Originally discovered as an adaptive immune system in bacteria, CRISPR/Cas9 has become a powerful tool for metabolic engineering, allowing researchers to modify genes involved in primary and secondary metabolite biosynthesis.

In pharmacognosy and plant biotechnology, CRISPR/Cas9 is used to improve the production of medicinal compounds such as alkaloids, flavonoids, terpenoids, phenolics, and glycosides, as well as to enhance disease resistance and stress tolerance in medicinal plants.

8.2 Components of the CRISPR/Cas9 System

The CRISPR/Cas9 system consists of three essential components:

1. Cas9 Protein

Cas9 is a DNA endonuclease that acts as molecular scissors, introducing a **double-stranded break (DSB)** at a specific genomic location.

2. Single Guide RNA (sgRNA)

The **single guide RNA (sgRNA)** is an engineered RNA molecule (~20 nucleotides) that directs Cas9 to the target DNA sequence through complementary base pairing.

3. Protospacer Adjacent Motif (PAM)

The **PAM** is a short DNA sequence located immediately downstream of the target site. For the commonly used *Streptococcus pyogenes* Cas9 (SpCas9), the PAM sequence is:

5′-NGG-3′

where **N** represents any nucleotide.

8.3 Mechanism of CRISPR/Cas9

The CRISPR/Cas9 editing process involves target recognition, DNA cleavage, and DNA repair.

Step 1. Target Recognition

The sgRNA binds to a complementary DNA sequence adjacent to the PAM sequence.

Step 2. Cas9 Binding

Cas9 associates with the sgRNA to form a ribonucleoprotein complex, which scans genomic DNA for the PAM motif.

Step 3. DNA Cleavage

After target recognition, Cas9 introduces a **double-stranded DNA break (DSB)** approximately three nucleotides upstream of the PAM sequence.

Step 4. DNA Repair

The DNA break is repaired by one of two endogenous repair pathways:

- **Non-Homologous End Joining (NHEJ)**
- **Homology-Directed Repair (HDR)**

These repair mechanisms generate gene knockouts or precise gene modifications.

8.4 DNA Repair Pathways

A. Non-Homologous End Joining (NHEJ)

NHEJ directly rejoins broken DNA ends without using a repair template. It is error-prone and frequently introduces insertions or deletions (**indels**), leading to **gene knockout**.

Applications

- Gene inactivation
- Functional genomics
- Elimination of competing metabolic pathways

B. Homology-Directed Repair (HDR)

HDR utilizes a homologous DNA template to repair the break accurately, enabling precise nucleotide substitutions or insertion of new genes.

Applications

- Gene replacement
- Precise genome editing
- Introduction of beneficial biosynthetic genes

8.5 Applications of CRISPR/Cas9 in Metabolic Engineering

CRISPR/Cas9 has transformed plant metabolic engineering by enabling targeted modification of genes involved in secondary metabolite biosynthesis.

A. Increased Alkaloid Production

Editing regulatory genes can enhance the biosynthesis of medicinal alkaloids such as:

- Morphine
- Codeine
- Scopolamine
- Hyoscyamine
- Vinblastine
- Vincristine

B. Enhanced Flavonoid Biosynthesis

Overexpression or activation of key enzymes (e.g., **Chalcone Synthase, Chalcone Isomerase, Flavonol Synthase**) increases the accumulation of flavonoids with antioxidant and anti-inflammatory properties.

C. Improved Disease Resistance

CRISPR-mediated editing of susceptibility genes enhances resistance against:

- Bacterial pathogens
- Fungal diseases
- Viral infections
- Insect pests

This reduces dependence on chemical pesticides and improves crop productivity.

D. Metabolic Engineering of Medicinal Plants

CRISPR/Cas9 is employed to:

- Redirect metabolic flux toward desired compounds.
- Eliminate competing biosynthetic pathways.
- Increase yield of valuable phytochemicals.
- Develop improved medicinal plant varieties.

Examples include enhanced production of:

- Artemisinin (*Artemisia annua*)
- Tropane alkaloids (*Atropa belladonna*)
- Flavonoids in medicinal herbs
- Ginsenosides (*Panax* spp.)
- Paclitaxel precursors (*Taxus* spp.)

8.6 Advantages of CRISPR/Cas9

- High editing precision.
- Simple guide RNA design.
- Rapid and cost-effective.
- Applicable to diverse plant species.
- Multiplex genome editing is possible.
- Accelerates metabolic engineering and crop improvement.

8.7 Limitations

- Off-target mutations may occur.
- HDR efficiency is generally low in plants.
- Delivery of CRISPR components into plant cells can be challenging.
- Regulatory and ethical concerns exist for genome-edited crops.

8.8 Future Perspectives

Integration of CRISPR/Cas9, artificial intelligence (AI), genome mining, metabolomics, and synthetic biology is expected to accelerate the discovery and production of novel natural products. Future developments such as base editing, prime editing, and CRISPR activation/interference (CRISPRa/CRISPRi) will enable even more precise regulation of biosynthetic pathways, facilitating sustainable production of high-value pharmaceuticals.

Key Points

- CRISPR/Cas9 is a versatile genome-editing technology used for precise modification of genes involved in metabolic pathways.
- The system comprises Cas9 protein, single guide RNA (sgRNA), and a PAM sequence for target recognition.
- Cas9 generates a double-stranded DNA break, which is repaired by NHEJ or HDR, resulting in gene knockout or precise gene editing.
- CRISPR/Cas9 has broad applications in increasing alkaloid and flavonoid production, enhancing disease resistance, and engineering metabolic pathways in medicinal plants.

- The integration of CRISPR with genomics, metabolomics, and AI is driving the next generation of plant metabolic engineering and natural product drug discovery.

UNIT – II

Primary Metabolites

1: PRIMARY METABOLITES

1.1 Introduction

Primary metabolites are naturally occurring organic compounds that are indispensable for the growth, development, reproduction, and survival of living organisms. These metabolites are synthesized during the normal metabolic processes of plants, animals, microorganisms, and humans and are directly involved in fundamental physiological functions. Unlike secondary metabolites, which are produced mainly for ecological advantages such as defense and attraction, primary metabolites are universally present and are essential for cellular metabolism.

Plants produce primary metabolites through complex biochemical pathways involving photosynthesis, respiration, nitrogen assimilation, and lipid metabolism. These compounds serve as the building blocks for cellular structures, provide energy for metabolic activities, regulate enzymatic reactions, and participate in biosynthetic pathways leading to the formation of secondary metabolites.

The three major classes of primary metabolites are carbohydrates, proteins (including enzymes), and lipids. Each class performs unique physiological and biochemical functions while collectively maintaining cellular integrity and normal metabolic activities.

In pharmacognosy, primary metabolites are of considerable importance because many crude drugs obtained from plants and animals are rich sources of these compounds. Natural carbohydrates such as acacia and agar are widely used as pharmaceutical excipients, proteins such as gelatin and casein serve as stabilizers and capsule materials, while enzymes like papain and bromelain possess therapeutic properties. Lipids, including castor oil and cocoa butter, are valuable ingredients in pharmaceutical formulations, cosmetics, and nutraceutical products.

Definition of Primary Metabolites

Primary metabolites are organic compounds synthesized during the normal metabolic processes of living organisms that are directly involved in growth, development, reproduction, and maintenance of cellular functions. They are produced during the active growth phase (trophophase) and are essential for the survival of the organism.

These metabolites participate in primary metabolic pathways such as glycolysis, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway, photosynthesis, amino acid biosynthesis, protein synthesis, and lipid metabolism.

Characteristics of Primary Metabolites

- Produced by all living organisms.
- Essential for normal growth and development.
- Synthesized during active growth (trophophase).
- Present in relatively large quantities.
- Highly conserved among species.
- Serve as precursors for secondary metabolites.
- Participate in energy production and biosynthetic reactions.

Importance in Living Organisms

Primary metabolites are fundamental to every aspect of cellular life. They provide the energy and molecular components required for growth, reproduction, and physiological functioning.

1. Energy Production

Carbohydrates are the principal source of metabolic energy. During cellular respiration, glucose is oxidized to generate ATP, which powers numerous biochemical reactions.

2. Structural Components

Proteins and carbohydrates contribute to the structural organization of cells. Cellulose forms plant cell walls, while proteins constitute cellular membranes, cytoskeletal elements, and connective tissues.

3. Growth and Development

Primary metabolites provide the building materials necessary for cell division, tissue differentiation, and organ development.

4. Enzymatic Functions

Many proteins function as enzymes that catalyze metabolic reactions, increasing reaction rates without being consumed.

5. Storage of Energy

Lipids and polysaccharides act as energy reserves. Plants store starch, whereas animals store glycogen and triglycerides.

6. Genetic Expression

Amino acids derived from primary metabolism are essential for synthesizing proteins involved in DNA replication, transcription, and translation.

7. Transport Functions

Certain proteins transport oxygen, nutrients, hormones, and metabolites throughout the organism.

8. Cellular Communication

Proteins and lipids participate in signal transduction pathways, allowing cells to respond to environmental stimuli.

Biosynthesis of Primary Metabolites

Primary metabolites are synthesized through interconnected biochemical pathways that utilize carbon dioxide, water, minerals, and sunlight as raw materials.

1. Photosynthesis

Photosynthesis is the primary process responsible for carbohydrate synthesis in plants.

Equation

$$6CO_2 + 6H_2O \xrightarrow{Light, Chlorophyll} C_6H_{12}O_6 + 6O_2$$

Glucose formed during photosynthesis serves as the precursor for numerous carbohydrates, proteins, and lipids.

2. Glycolysis

Glucose is converted into pyruvate through glycolysis, generating ATP and NADH. The intermediates also serve as precursors for amino acid and lipid biosynthesis.

3. Tricarboxylic Acid (TCA) Cycle

Pyruvate enters the mitochondria and is oxidized to produce ATP while generating intermediates required for amino acid synthesis.

4. Pentose Phosphate Pathway

This pathway produces ribose sugars required for nucleic acid synthesis and NADPH required for lipid biosynthesis.

5. Amino Acid Biosynthesis

Plants synthesize amino acids from carbohydrate intermediates and inorganic nitrogen absorbed from the soil.

6. Protein Biosynthesis

Amino acids are polymerized by ribosomes into proteins according to the genetic code.

7. Lipid Biosynthesis

Fatty acids are synthesized from acetyl-CoA and subsequently esterified with glycerol to produce triglycerides and phospholipids.

Role in Plant Growth and Development

Primary metabolites regulate nearly every physiological process occurring within plants.

Photosynthesis

Carbohydrates synthesized during photosynthesis provide energy for plant metabolism.

Respiration

Glucose is oxidized to release ATP needed for cellular activities.

Cell Division

Proteins and nucleotides synthesized from primary metabolites support DNA replication and mitosis.

Cell Wall Formation

Cellulose and hemicellulose strengthen plant tissues.

Protein Synthesis

Amino acids are assembled into structural and enzymatic proteins.

Storage

Plants accumulate starch and oils in seeds, roots, and fruits as reserve food.

Transport

Sucrose transports photosynthetic products from leaves to growing tissues.

Stress Adaptation

Some primary metabolites, such as proline and soluble sugars, help plants tolerate drought, salinity, and temperature stress.

Difference Between Primary and Secondary Metabolites

Feature	**Primary Metabolites**	**Secondary Metabolites**
Definition	Essential compounds required for growth	Non-essential specialized compounds
Occurrence	Present in all living organisms	Present in selected organisms
Quantity	Large amounts	Small amounts
Growth Phase	Produced during trophophase	Produced during idiophase
Function	Growth, development, reproduction	Defense, signaling, adaptation
Examples	Carbohydrates, proteins, lipids	Alkaloids, glycosides, tannins, flavonoids, volatile oils
Distribution	Universal	Restricted
Pharmaceutical Use	Excipients, nutrients, enzymes	Active therapeutic agents

Pharmaceutical Importance of Primary Metabolites

Primary metabolites have extensive applications in pharmaceutical science because of their biocompatibility, safety, and multifunctional properties.

1. Pharmaceutical Excipients

Natural gums such as acacia and tragacanth are used as suspending, emulsifying, and binding agents.

2. Capsule Manufacturing

Gelatin is extensively used for hard and soft gelatin capsules.

3. Therapeutic Enzymes

Papain, bromelain, serratiopeptidase, urokinase, streptokinase, and pepsin are employed for anti-inflammatory therapy, wound debridement, thrombolysis, digestive disorders, and enzymatic treatment.

4. Ointment and Suppository Bases

Cocoa butter serves as a suppository base due to its suitable melting characteristics.

5. Laxatives

Castor oil is used as a stimulant laxative.

6. Emollients

Olive oil, lanolin (wool fat), and beeswax are incorporated into creams, ointments, lotions, and cosmetics.

7. Controlled Drug Delivery

Natural polysaccharides are widely used in sustained-release, controlled-release, and mucoadhesive drug delivery systems.

8. Biotechnology

Enzymes are utilized in recombinant DNA technology, diagnostics, and industrial pharmaceutical manufacturing.

1.2 Classification of Primary Metabolites

Primary metabolites are broadly classified into three major groups based on their chemical composition and biological functions:

1. Carbohydrates
2. Proteins and Enzymes
3. Lipids

Each class differs in chemical structure, biosynthesis, physiological role, and pharmaceutical application.

1.2.1 Carbohydrates

Definition

Carbohydrates are polyhydroxy aldehydes or ketones, or compounds that yield such molecules upon hydrolysis. They are the most abundant organic compounds in nature and constitute the primary source of energy for living organisms.

General Formula

$$(CH_2O)_n$$

where **n = 3–7** for most naturally occurring sugars.

Classification

A. Monosaccharides

- Simplest carbohydrates
- Cannot be hydrolyzed further
- Examples: Glucose, Fructose, Galactose, Ribose

B. Disaccharides

- Composed of two monosaccharide units
- Examples: Sucrose, Maltose, Lactose

C. Oligosaccharides

- Contain 3–10 monosaccharide units
- Examples: Raffinose, Stachyose

D. Polysaccharides

- Long chains of monosaccharides
- Examples: Starch, Cellulose, Glycogen, Acacia Gum, Agar, Tragacanth

Functions

- Primary source of energy
- Storage of energy

- Structural support (cellulose)
- Cell recognition and signaling
- Components of nucleic acids
- Maintenance of osmotic balance

Pharmaceutical Applications

- Tablet binders
- Suspending agents
- Emulsifying agents
- Thickening agents
- Controlled-release matrices
- Stabilizers
- Culture media
- Dietary fibers
- Plasma expanders

1.2.2 Proteins and Enzymes

Definition

Proteins are high-molecular-weight biological macromolecules composed of amino acids linked by peptide bonds. Enzymes are specialized proteins that act as biological catalysts, accelerating biochemical reactions without being consumed.

Classification of Proteins

Based on Composition

- Simple proteins
- Conjugated proteins
- Derived proteins

Based on Shape

- Fibrous proteins
- Globular proteins

Based on Function

- Structural proteins
- Enzymatic proteins
- Transport proteins
- Hormonal proteins
- Defensive proteins
- Storage proteins

Classification of Enzymes

According to the International Union of Biochemistry and Molecular Biology (IUBMB), enzymes are classified into seven major classes:

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases
7. Translocases

Properties

- High molecular weight
- Specific amino acid sequence
- Complex three-dimensional structure
- Catalytic activity (enzymes)
- Substrate specificity
- Sensitive to pH and temperature
- Denatured by heat and chemicals
- Biodegradable and biocompatible

Pharmaceutical Importance

- Therapeutic enzymes (e.g., papain, bromelain, streptokinase)
- Capsule shell production (gelatin)
- Nutritional supplements
- Diagnostic reagents
- Vaccine and biotechnology manufacturing
- Controlled drug delivery systems
- Tissue engineering and regenerative medicine

1.2.3 Lipids

Definition

Lipids are a diverse group of hydrophobic or amphipathic organic compounds that are insoluble in water but soluble in non-polar organic solvents such as ether, chloroform, benzene, and hexane. They serve as concentrated energy reserves, structural components of biological membranes, and signaling molecules.

Classification

A. Fixed Oils

- Liquid at room temperature
- Mainly of plant origin
- Examples: Castor oil, Olive oil, Sesame oil

B. Fats

- Solid or semi-solid at room temperature
- Mainly of animal origin
- Examples: Cocoa butter, Lard

C. Waxes

- Esters of long-chain fatty acids and long-chain alcohols
- Examples: Beeswax, Wool fat (Lanolin), Carnauba wax

Physical and Chemical Properties

Physical Properties

- Insoluble in water
- Soluble in organic solvents
- Lower density than water
- Greasy texture
- Characteristic melting point
- Variable color and odor depending on source

Chemical Properties

- Hydrolysis
- Saponification
- Hydrogenation
- Oxidation (rancidity)
- Halogenation
- Esterification

Pharmaceutical Applications

- Ointment and cream bases
- Suppository bases
- Emollients and moisturizers
- Solvents for lipophilic drugs
- Controlled and sustained drug delivery systems
- Nanoemulsions and lipid nanoparticles
- Cosmetic formulations
- Nutraceutical products
- Soft gelatin capsule filling materials
- Lubricants and protective coatings in pharmaceutical formulations

Primary metabolites comprise carbohydrates, proteins (including enzymes), and lipids, all of which are essential for the growth, development, and survival of living organisms. They are synthesized through fundamental metabolic pathways and play crucial roles in energy production, structural organization, enzymatic catalysis, and storage. In pharmacognosy and pharmaceutical sciences, these

metabolites are indispensable as therapeutic agents, excipients, formulation aids, and industrial raw materials, making them foundational to both natural product research and modern drug development.

2. GENERAL IDENTIFICATION TESTS OF PRIMARY METABOLITES

2.1 Introduction

Primary metabolites such as carbohydrates, proteins, enzymes, and lipids constitute the major biochemical components of living organisms. Their qualitative and quantitative identification is essential in pharmacognosy, pharmaceutical analysis, food chemistry, biotechnology, and clinical diagnostics. Identification tests help confirm the presence of specific metabolites based on characteristic chemical reactions, color changes, precipitate formation, or enzymatic activity.

Qualitative tests are simple laboratory procedures widely employed for preliminary identification of biomolecules before advanced instrumental analysis. These tests rely on the interaction of specific functional groups with chemical reagents, producing distinctive visual observations.

The objectives of identification tests are to:

- Confirm the identity of primary metabolites.
- Differentiate one class of compounds from another.
- Assess purity and quality of crude drugs.
- Detect adulteration.
- Support pharmacopoeial quality control.
- Provide preliminary biochemical characterization.

2.1 Identification Tests for Carbohydrates

Carbohydrates possess aldehyde, ketone, or hydroxyl functional groups that undergo characteristic chemical reactions. Various qualitative tests are available to identify reducing sugars, non-reducing sugars, monosaccharides, ketoses, and polysaccharides.

2.1.1 Molisch Test (General Test for Carbohydrates)

Principle

Concentrated sulfuric acid dehydrates carbohydrates to form furfural (from pentoses) or hydroxymethylfurfural (from hexoses). These compounds react with α-naphthol (Molisch reagent) to produce a violet-purple ring.

Reagents

- Molisch reagent (5% α-naphthol in ethanol)
- Concentrated sulfuric acid (H_2SO_4)

Procedure

1. Place **2 mL** of the test solution in a test tube.

2. Add **2–3 drops** of Molisch reagent and mix.
3. Carefully pour **1–2 mL** of concentrated sulfuric acid along the side of the test tube to form two separate layers.
4. Do not shake the tube.
5. Observe the junction between the two layers.

Observation

- Violet or purple ring at the interface.

Inference

- Presence of carbohydrates.

Applications

- General screening test for all carbohydrates.
- Identification of sugars in plant extracts and food samples.

2.1.2 Benedict's Test (Reducing Sugars)

Principle

Reducing sugars reduce cupric ions (Cu^{2+}) to cuprous oxide (Cu_2O) under alkaline conditions, forming a colored precipitate.

Reagent

Benedict's reagent containing:

- Copper sulfate
- Sodium carbonate
- Sodium citrate

Procedure

1. Take **5 mL** Benedict's reagent.
2. Add **0.5 mL** sample solution.
3. Heat in a boiling water bath for **2–5 minutes**.
4. Allow to cool.

Observation

Colour	**Interpretation**
Blue	Negative
Green	Trace reducing sugar
Yellow	Low concentration
Orange	Moderate concentration
Brick-red precipitate	High concentration

Inference

Presence of reducing sugars such as glucose, fructose, lactose, and maltose.

2.1.3 Fehling's Test

Principle

Reducing sugars reduce alkaline copper tartrate solution to brick-red cuprous oxide.

Reagents

- Fehling's Solution A (Copper sulfate)
- Fehling's Solution B (Alkaline sodium potassium tartrate)

Procedure

1. Mix equal volumes of Fehling's A and B.
2. Add test solution.
3. Heat gently for several minutes.

Observation

Brick-red precipitate.

Inference

Presence of reducing sugars.

2.1.4 Barfoed's Test

Principle

Monosaccharides rapidly reduce copper acetate in acidic medium, whereas disaccharides react slowly.

Reagent

Barfoed's reagent (Copper acetate in dilute acetic acid)

Procedure

1. Mix equal volumes of sample and reagent.
2. Heat for **2 minutes**.
3. Observe immediately.

Observation

Red precipitate within **2–3 minutes**.

Inference

Presence of monosaccharides.

2.1.5 Seliwanoff's Test

Principle

Ketoses are rapidly dehydrated by concentrated hydrochloric acid to form hydroxymethylfurfural, which reacts with resorcinol to produce a cherry-red color.

Reagent

Seliwanoff reagent (Resorcinol in concentrated HCl)

Procedure

1. Add reagent to the test solution.
2. Heat gently.

Observation

Cherry-red color.

Inference

Presence of ketose sugars (e.g., fructose).

2.1.6 Iodine Test

Principle

Iodine molecules are trapped within the helical structure of starch, producing a characteristic blue-black color.

Reagent

Dilute iodine solution (I_2/KI)

Procedure

1. Add a few drops of iodine solution to the sample.
2. Mix gently.

Observation

Sample	Colour
Starch	Blue-black
Glycogen	Reddish-brown
Dextrin	Red

Inference

Identification of polysaccharides.

2.1.7 Anthrone Test

Principle

Carbohydrates are dehydrated by concentrated sulfuric acid to form furfural derivatives, which react with anthrone to produce a blue-green complex.

Reagent

Anthrone reagent.

Procedure

1. Mix sample with anthrone reagent.
2. Heat in boiling water for **10 minutes**.
3. Cool.

Observation

Blue-green color.

Inference

Presence of carbohydrates.

2.1.8 Osazone Formation Test

Principle

Reducing sugars react with phenylhydrazine to form characteristic crystalline osazones.

Reagents

- Phenylhydrazine hydrochloride
- Sodium acetate
- Acetic acid

Procedure

1. Add reagents to sugar solution.
2. Heat in boiling water.
3. Cool and observe crystals under microscope.

Observation

Characteristic yellow crystals.

Inference

Identification of specific reducing sugars based on crystal morphology.

2.2 Identification Tests for Proteins

Proteins contain peptide bonds and amino acid side chains that react specifically with certain chemical reagents.

2.2.1 Biuret Test

Principle

Peptide bonds form a violet-colored complex with copper ions in alkaline medium.

Reagents

- Sodium hydroxide
- Copper sulfate

Procedure

1. Add NaOH to the protein solution.
2. Add a few drops of $CuSO_4$.

Observation

Violet or purple color.

Inference

Presence of proteins.

2.2.2 Ninhydrin Test

Principle

Free amino groups react with ninhydrin to produce Ruhemann's purple.

Reagent

Ninhydrin solution.

Procedure

1. Add ninhydrin reagent.
2. Heat gently.

Observation

Blue-violet color.

Inference

Presence of amino acids or proteins.

2.2.3 Millon's Test

Principle

Phenolic amino acid (tyrosine) reacts with Millon's reagent to produce a red color upon heating.

Reagent

Millon's reagent.

Procedure

1. Add Millon's reagent.
2. Heat gently.

Observation

Brick-red coloration.

Inference

Presence of tyrosine-containing proteins.

2.2.4 Xanthoproteic Test

Principle

Nitric acid nitrates aromatic amino acids, producing yellow nitro derivatives that become orange in alkaline medium.

Procedure

1. Add concentrated nitric acid.

2. Heat.
3. Cool.
4. Add sodium hydroxide.

Observation

Yellow changing to orange.

Inference

Presence of aromatic amino acids.

2.2.5 Hopkins-Cole Test

Principle

Tryptophan reacts with glyoxylic acid in the presence of concentrated sulfuric acid to produce a violet ring.

Observation

Violet ring at the junction.

Inference

Presence of tryptophan.

2.2.6 Sakaguchi Test

Principle

Arginine reacts with α-naphthol and sodium hypobromite in alkaline medium to produce a red color.

Observation

Bright red color.

Inference

Presence of arginine.

2.3 Identification Tests for Enzymes

Enzymes are identified primarily by measuring their catalytic activity rather than simple color reactions.

2.3.1 Enzyme Activity Tests

Principle

The presence of an enzyme is confirmed by its ability to catalyze a specific biochemical reaction under optimum conditions.

Procedure

- Incubate the enzyme with its specific substrate.
- Measure the formation of product or disappearance of substrate.

Observation

Product formation indicates enzyme activity.

Inference

Presence of active enzyme.

2.3.2 Proteolytic Activity Test

Principle

Proteolytic enzymes hydrolyze proteins into peptides and amino acids.

Procedure

- Incubate enzyme with casein or gelatin.
- Observe protein digestion.

Observation

Clear hydrolyzed zone or disappearance of protein.

Inference

Presence of protease enzymes such as papain, bromelain, pepsin, or serratiopeptidase.

2.3.3 Milk Clotting Test

Principle

Certain enzymes coagulate milk proteins by hydrolyzing casein.

Procedure

- Add enzyme solution to fresh milk.
- Incubate at **37°C**.

Observation

Milk coagulates or curdles.

Inference

Presence of milk-clotting enzymes.

2.3.4 Gelatin Liquefaction Test

Principle

Gelatinase-producing enzymes hydrolyze gelatin into soluble peptides.

Procedure

1. Prepare gelatin medium.
2. Inoculate enzyme.
3. Incubate.
4. Refrigerate.

Observation

Medium remains liquid after refrigeration.

Inference

Presence of gelatin-hydrolyzing enzyme.

2.4 Identification Tests for Lipids

Lipids are identified based on their solubility, staining properties, and characteristic chemical reactions.

2.4.1 Sudan III Test

Principle

Sudan III is a fat-soluble dye that stains lipids orange-red.

Procedure

Add Sudan III solution to the sample.

Observation

Orange-red stained lipid droplets.

Inference

Presence of lipids.

2.4.2 Sudan IV Test

Principle

Sudan IV selectively stains lipids deep red.

Observation

Bright red lipid globules.

Inference

Presence of lipids.

2.4.3 Grease Spot Test

Principle

Lipids produce a translucent spot on absorbent paper that persists after drying.

Procedure

Rub the sample onto filter paper and allow it to dry.

Observation

Permanent translucent greasy spot.

Inference

Presence of lipids.

2.4.4 Acrolein Test

Principle

Glycerol in fats and oils is dehydrated by potassium bisulfate to form acrolein, which has a characteristic pungent odor.

Procedure

Heat the sample with potassium bisulfate.

Observation

Sharp irritating odor of acrolein.

Inference

Presence of glycerides.

2.4.5 Saponification Test

Principle

Lipids react with alkali to form soap and glycerol.

Procedure

Boil the sample with alcoholic sodium hydroxide.

Observation

Soap formation.

Inference

Presence of saponifiable lipids.

2.4.6 Iodine Value

Principle

The iodine value measures the degree of unsaturation in fats and oils by determining the amount of iodine absorbed by double bonds.

Significance

- Higher iodine value indicates greater unsaturation.
- Used for characterization and quality control of oils.

2.4.7 Acid Value

Principle

The acid value is the number of milligrams of potassium hydroxide (KOH) required to neutralize the free fatty acids present in **1 g** of fat or oil.

Significance

- Indicates hydrolytic rancidity.
- Reflects the quality and freshness of oils.

2.4.8 Ester Value

Principle

The ester value represents the number of milligrams of potassium hydroxide required to saponify the esterified fatty acids in **1 g** of fat or oil.

Significance

- Used to evaluate ester content.
- Helps characterize fats and waxes.

2.4.9 Peroxide Value

Principle

The peroxide value measures the concentration of peroxides and hydroperoxides formed during the initial stages of lipid oxidation.

Significance

- Indicates oxidative rancidity.
- Essential quality control parameter for edible oils and pharmaceutical lipids.

Qualitative identification tests provide a rapid and reliable means of detecting primary metabolites based on their characteristic chemical and biochemical properties. Carbohydrates are identified through colorimetric and reduction reactions, proteins through reactions involving peptide bonds and specific amino acid residues, enzymes through their catalytic activity, and lipids through staining methods and physicochemical quality indices such as iodine, acid, ester, and peroxide values. These tests form the foundation of pharmacognostic evaluation, quality assurance, pharmaceutical analysis, and routine laboratory investigations.

3: PHARMACOGNOSTIC STUDY OF CARBOHYDRATES

3.1 Acacia (Gum Arabic)

Introduction

Acacia is a natural dried gummy exudate widely used as a pharmaceutical excipient.

Biological Source

Dried gummy exudate from the stems and branches of *Acacia senegal* and *Acacia seyal*.

Family

Fabaceae (Leguminosae)

Distribution

Sudan, Senegal, Nigeria, Chad, and India.

Identifying Characters

- Pale yellow to amber tears.
- Odourless, tasteless.
- Brittle; forms a viscous mucilage with water.

Chemical Constituents

- Arabinogalactan
- Arabin
- Calcium, magnesium, and potassium salts

Identification Tests

- Positive Molisch test.
- Forms viscous mucilage with water.
- Precipitated by lead acetate.

Therapeutic Uses

- Demulcent
- Mild emulsifier
- Soothing agent

Commercial Applications

- Tablet binder
- Suspending and emulsifying agent
- Food stabilizer
- Cosmetic formulations

3.2 Agar

Introduction

Agar is a gelatinous polysaccharide obtained from red marine algae.

Biological Source

Prepared from *Gelidium*, *Gracilaria*, and related red algae.

Family

Gelidiaceae / Gracilariaceae

Distribution

Japan, China, India, Indonesia, Korea.

Identifying Characters

- White or yellowish translucent strips or flakes.
- Odourless and tasteless.
- Swells in water and forms a firm gel on cooling.

Chemical Constituents

- Agarose
- Agaropectin

Identification Tests

- Positive Molisch test.
- Forms a firm gel after boiling and cooling.

Therapeutic Uses

- Bulk laxative
- Culture medium
- Demulcent

Commercial Applications

- Microbiological media
- Pharmaceutical suspending agent
- Food gelling agent
- Biotechnology

3.3 Tragacanth

Introduction

Tragacanth is a natural gum used as a stabilizer and suspending agent.

Biological Source

Dried gummy exudate from *Astragalus gummifer* and related species.

Family

Fabaceae

Distribution

Iran, Turkey, Iraq, Syria.

Identifying Characters

- White or cream ribbon-like flakes.
- Odourless.
- Swells in water to produce a thick mucilage.

Chemical Constituents

- Bassorin
- Tragacanthin

Identification Tests

- Positive Molisch test.
- Swells in water without dissolving completely.

Therapeutic Uses

- Demulcent
- Bulk-forming laxative

Commercial Applications

- Tablet binder
- Suspending agent
- Thickener
- Cosmetic formulations

3.4 Honey

Introduction

Honey is a natural sweet substance produced by honeybees from flower nectar.

Biological Source

Prepared by the honeybee *Apis mellifera* and other *Apis* species from floral nectar.

Family

Apidae

Distribution

Worldwide; major producers include India, China, Argentina, Turkey, and New Zealand.

Identifying Characters

- Thick, viscous liquid.
- Golden yellow to dark brown.
- Sweet taste with characteristic aroma.

Chemical Constituents

- Fructose
- Glucose
- Sucrose (small amount)
- Enzymes (Invertase, Diastase)
- Organic acids
- Vitamins and minerals

Identification Tests

- Positive Molisch test.
- Positive Benedict's and Fehling's tests (reducing sugars).

Therapeutic Uses

- Nutritional supplement
- Demulcent
- Wound healing
- Cough suppressant
- Antioxidant

Commercial Applications

- Pharmaceutical syrups
- Nutraceuticals
- Food industry
- Cosmetics
- Traditional medicine

Carbohydrate drugs such as **Acacia, Agar, Tragacanth, and Honey** are important natural products used in pharmacy and medicine. They serve as pharmaceutical excipients, demulcents, suspending agents, gelling agents, and nutritional supplements. Their identification is based on characteristic morphology, chemical constituents, and qualitative carbohydrate tests such as the Molisch, Benedict's, and Fehling's tests.

4. PHARMACOGNOSTIC STUDY OF PROTEINS AND ENZYMES

4.1 Gelatin

Biological Source

Obtained by partial hydrolysis of collagen from the skin, bones, and connective tissues of cattle, pigs, and fish.

Distribution

Worldwide; major producers include the USA, Brazil, China, India, and European countries.

Preparation

Collagen-rich tissues are cleaned, treated with acid or alkali, extracted with hot water, filtered, concentrated, sterilized, and dried.

Identifying Characters

- Pale yellow, translucent sheets or granules
- Odourless and tasteless
- Swells in cold water and dissolves in hot water to form a gel

Chemical Constituents

- Collagen-derived proteins
- Glycine, proline, and hydroxyproline

Identification Tests

- Positive Biuret test
- Positive Ninhydrin test

Therapeutic Uses

- Protein supplement
- Wound dressing
- Plasma expander

Commercial Applications

- Hard and soft gelatin capsules
- Tablet coating
- Food and cosmetic industries

4.2 Casein

Biological Source

Major protein obtained from cow's milk.

Distribution

Worldwide in dairy-producing countries.

Preparation

Prepared by acidification or enzymatic coagulation of skimmed milk followed by washing and drying.

Identifying Characters

- White or cream-colored powder
- Odourless
- Insoluble in water

Chemical Constituents

- Casein phosphoproteins
- Calcium phosphate

Identification Tests

- Positive Biuret test
- Positive Millon's test

Therapeutic Uses

- Nutritional supplement
- Protein fortification

Commercial Applications

- Pharmaceutical excipient
- Food products
- Adhesives and coatings

4.3 Papain

Biological Source

Dried latex obtained from the unripe fruits of *Carica papaya*.

Family

Caricaceae

Distribution

India, Sri Lanka, Brazil, Mexico, and tropical regions.

Collection

Latex is collected by making shallow incisions on unripe fruits and dried.

Identifying Characters

- White to cream-colored powder
- Characteristic odour
- Soluble in water

Chemical Constituents

- Papain
- Chymopapain
- Protease enzymes

Identification Tests

- Positive proteolytic activity test
- Gelatin digestion test

Therapeutic Uses

- Digestive enzyme
- Wound debridement
- Anti-inflammatory agent

Commercial Applications

- Pharmaceutical enzyme preparations
- Meat tenderizer
- Food processing

4.4 Bromelain

Biological Source

Proteolytic enzyme obtained from the stem and fruit of *Ananas comosus* (Pineapple).

Family

Bromeliaceae

Distribution

India, Thailand, Philippines, Brazil, and tropical countries.

Collection

Extracted from pineapple stems and fruits followed by purification and drying.

Identifying Characters

- Yellowish amorphous powder
- Slight characteristic odour
- Water soluble

Chemical Constituents

- Bromelain enzyme complex
- Proteases
- Peroxidases

Identification Tests

- Proteolytic activity test
- Casein digestion test

Therapeutic Uses

- Anti-inflammatory
- Digestive aid
- Wound healing

Commercial Applications

- Enzyme supplements
- Food industry
- Meat tenderizer

4.5 Serratiopeptidase

Biological Source

Proteolytic enzyme produced by *Serratia marcescens*.

Production

Produced by microbial fermentation.

Identifying Characters

- White to pale yellow powder
- Water soluble

Chemical Constituents

- Serratiopeptidase enzyme

Identification Tests

- Proteolytic enzyme assay
- Casein hydrolysis test

Therapeutic Uses

- Anti-inflammatory
- Reduction of edema
- Mucolytic agent

Commercial Applications

- Tablets
- Capsules
- Combination enzyme formulations

4.6 Urokinase

Biological Source

Originally isolated from human urine; now produced by recombinant cell culture technology.

Production

Microbial fermentation and recombinant DNA technology.

Identifying Characters

- White lyophilized powder
- Water soluble

Chemical Constituents

- Urokinase enzyme (serine protease)

Identification Tests

- Fibrinolytic activity assay
- Enzyme activity test

Therapeutic Uses

- Thrombolytic agent
- Pulmonary embolism
- Deep vein thrombosis

Commercial Applications

- Injectable thrombolytic preparations

4.7 Streptokinase

Biological Source

Produced by β-hemolytic *Streptococcus* species.

Production

Microbial fermentation followed by purification.

Identifying Characters

- White lyophilized powder
- Water soluble

Chemical Constituents

- Streptokinase protein

Identification Tests

- Fibrinolytic assay
- Clot lysis test

Therapeutic Uses

- Acute myocardial infarction
- Pulmonary embolism
- Deep vein thrombosis

Commercial Applications

- Injectable thrombolytic formulations

4.8 Pepsin

Biological Source

Proteolytic enzyme obtained from the gastric mucosa of pigs or cattle.

Production

Extracted from gastric mucosa, purified, and dried.

Identifying Characters

- White to light yellow powder
- Slight characteristic odour
- Soluble in water

Chemical Constituents

- Pepsin enzyme

Identification Tests

- Protein digestion test
- Gelatin liquefaction test

Therapeutic Uses

- Digestive enzyme
- Management of gastric hyposecretion
- Indigestion

Commercial Applications

- Digestive enzyme preparations
- Pharmaceutical formulations
- Biotechnology applications

Proteins and enzymes such as Gelatin, Casein, Papain, Bromelain, Serratiopeptidase, Urokinase, Streptokinase, and Pepsin are valuable natural and biological products with significant pharmaceutical importance. They are widely used as nutritional supplements, pharmaceutical excipients, digestive aids, anti-inflammatory agents, thrombolytic drugs, and biotechnology products. Their identification is based on protein-specific chemical tests (Biuret, Ninhydrin, Millon's) and enzyme activity assays such as proteolytic, gelatin liquefaction, and fibrinolytic tests.

5. PHARMACOGNOSTIC STUDY OF LIPIDS

5.1 Castor Oil

Introduction

Castor oil is a fixed vegetable oil widely used in pharmaceutical and cosmetic preparations.

Biological Source: Fixed oil obtained from the seeds of *Ricinus communis* L.

Family: Euphorbiaceae

Distribution: India, Brazil, China, and tropical regions.

Identifying Characters

- Clear, pale yellow, viscous liquid
- Characteristic odor
- Mild taste
- Soluble in alcohol

Chemical Constituents

- Ricinoleic acid (≈85–90%)
- Oleic acid
- Linoleic acid
- Stearic acid

Identification Tests

- Positive Acrolein test
- Positive Saponification test
- Soluble in alcohol

Physicochemical Constants

- Specific Gravity: 0.958–0.969
- Refractive Index: 1.477–1.481
- Iodine Value: 82–90
- Saponification Value: 176–187

Therapeutic Uses

- Stimulant laxative
- Emollient
- Anti-inflammatory

Commercial Applications

- Pharmaceutical formulations
- Cosmetics
- Lubricants
- Soap manufacture

5.2 Olive Oil

Introduction

Olive oil is a fixed oil valued for its nutritional and medicinal properties.

Biological Source: Fixed oil obtained from the ripe fruits of *Olea europaea* L.

Family: Oleaceae

Distribution: Mediterranean countries, Spain, Italy, Greece, Turkey, and India.

Identifying Characters

- Pale yellow to greenish-yellow liquid
- Characteristic pleasant odor
- Bland taste

Chemical Constituents

- Oleic acid
- Linoleic acid
- Palmitic acid
- Tocopherols

Identification Tests

- Positive Sudan III test
- Positive Acrolein test

Physicochemical Constants

- Specific Gravity: **0.910–0.916**
- Refractive Index: **1.467–1.470**
- Iodine Value: **75–94**
- Saponification Value: **184–196**

Therapeutic Uses

- Mild laxative
- Emollient
- Cardioprotective dietary oil

Commercial Applications

- Pharmaceutical preparations
- Cosmetics
- Food industry
- Massage oils

5.3 Cocoa Butter

Introduction

Cocoa butter is a natural fat commonly used as a suppository base.

Biological Source: Fat obtained from the seeds of *Theobroma cacao* L.

Family: Malvaceae (formerly Sterculiaceae)

Distribution: Ghana, Ivory Coast, Brazil, Indonesia, and India.

Identifying Characters

- Pale yellow solid
- Pleasant chocolate odor
- Melts near body temperature (30–35°C)

Chemical Constituents

- Stearic acid
- Palmitic acid
- Oleic acid
- Triglycerides

Identification Tests

- Positive Grease Spot test
- Positive Acrolein test

Physicochemical Constants

- Melting Point: **30–35°C**
- Iodine Value: **32–42**
- Saponification Value: **188–198**

Therapeutic Uses

- Suppository base
- Skin moisturizer

Commercial Applications

- Pharmaceutical suppositories
- Cosmetics
- Chocolate industry

5.4 Wool Fat (Lanolin)

Introduction

Lanolin is a purified wax obtained from sheep wool and is extensively used in topical formulations.

Biological Source: Purified wool fat obtained from the wool of *Ovis aries* (Sheep).

Family: Bovidae

Distribution: Australia, New Zealand, United Kingdom, and India.

Identifying Characters

- Yellowish, waxy mass
- Characteristic odor
- Sticky and unctuous

Chemical Constituents

- Cholesterol esters
- Lanosterol
- Long-chain fatty acid esters

Identification Tests

- Positive Sudan IV test
- Positive Saponification test

Physicochemical Constants

- Melting Point: **38–44°C**
- Acid Value: **1–8**
- Saponification Value: **90–110**

Therapeutic Uses

- Emollient
- Skin protectant
- Moisturizer

Commercial Applications

- Ointments
- Creams
- Cosmetic products

5.5 Beeswax

Introduction

Beeswax is a natural wax widely used in pharmaceutical and cosmetic formulations.

Biological Source: Purified wax secreted by the worker honeybee, *Apis mellifera*.

Family: Apidae

Distribution: Worldwide.

Identifying Characters

- Yellow or white solid
- Pleasant honey-like odor
- Brittle when cold, soft when warm

Chemical Constituents

- Myricyl palmitate
- Cerotic acid
- Hydrocarbons
- Free fatty acids

Identification Tests

- Positive Grease Spot test
- Positive Saponification test

Physicochemical Constants

- Melting Point: **62–65°C**
- Acid Value: **17–24**
- Ester Value: **70–80**
- Saponification Value: **87–104**

Therapeutic Uses

- Protective agent
- Emollient
- Ointment stiffening agent

Commercial Applications

- Ointments
- Creams
- Lipsticks
- Polishes
- Candles

Lipids such as Castor Oil, Olive Oil, Cocoa Butter, Wool Fat (Lanolin), and Beeswax are important natural products used in pharmacy due to their emollient, laxative, protective, and formulation-enhancing properties. They serve as ointment bases, suppository bases, excipients, moisturizers, and carriers for lipophilic drugs. Their quality is evaluated using physicochemical constants such as specific gravity, refractive index, melting point, iodine value, acid value, ester value, and saponification value, which help ensure purity and compliance with pharmacopoeial standards.

UNIT – III

Secondary Metabolites

1. INTRODUCTION TO SECONDARY METABOLITES

1.1 Definition

Secondary metabolites are naturally occurring organic compounds synthesized by plants, microorganisms, fungi, and some animals that are not directly involved in their normal growth, development, reproduction, or primary metabolic processes. Unlike primary metabolites such as carbohydrates, proteins, lipids, and nucleic acids, secondary metabolites are produced through specialized metabolic pathways and perform ecological, physiological, and protective functions.

These compounds are usually synthesized from primary metabolites through complex enzymatic reactions. Although they are not essential for the immediate survival of the organism, they significantly enhance its ability to adapt to environmental stress, defend against herbivores and pathogens, attract pollinators, and compete with neighboring organisms. In medicinal plants, secondary metabolites constitute the major bioactive constituents responsible for therapeutic activities.

Secondary metabolites have immense pharmaceutical significance because many clinically important drugs are derived from them. Examples include morphine from *Papaver somniferum* (Opium), quinine from *Cinchona* species, vincristine and vinblastine from *Catharanthus roseus* (Vinca), digoxin from *Digitalis* species, artemisinin from *Artemisia annua*, and reserpine from *Rauwolfia serpentina*. These compounds have contributed substantially to modern medicine and continue to serve as lead molecules in drug discovery and development.

The synthesis of secondary metabolites is influenced by various environmental and genetic factors such as light, temperature, soil nutrients, altitude, water availability, developmental stage, and biotic stress. Consequently, the concentration and quality of these metabolites may vary depending on geographical location, cultivation practices, harvesting season, and post-harvest processing.

1.2 Characteristics of Secondary Metabolites

Secondary metabolites possess several distinguishing features that separate them from primary metabolites.

General Characteristics

- They are not directly involved in primary metabolic activities such as growth and reproduction.
- They are synthesized from primary metabolites through specialized biosynthetic pathways.
- Their production is often species-specific or restricted to certain plant families.
- They are generally produced in relatively small quantities.
- Their concentration varies with plant age, season, environmental conditions, and developmental stage.
- They accumulate in specialized plant tissues such as glandular trichomes, secretory ducts, resin canals, laticifers, vacuoles, bark, seeds, roots, flowers, and fruits.
- They contribute to plant color, aroma, bitterness, pungency, and taste.
- They play an essential role in plant defense against insects, microorganisms, fungi, herbivores, and environmental stresses.

- They possess significant pharmacological and biological activities in humans.
- They serve as valuable raw materials for pharmaceuticals, cosmetics, food additives, dyes, perfumes, pesticides, and nutraceuticals.

1.3 Difference Between Primary and Secondary Metabolites

Feature	Primary Metabolites	Secondary Metabolites
Definition	Compounds essential for growth and survival	Compounds mainly involved in ecological and protective functions
Requirement	Essential	Generally non-essential for immediate survival
Occurrence	Present in all living organisms	Restricted to specific species or groups
Quantity	Produced in large amounts	Produced in relatively small amounts
Distribution	Universal	Species-specific
Biosynthesis	Primary metabolic pathways	Specialized secondary metabolic pathways
Function	Growth, respiration, reproduction, energy production	Defense, attraction, competition, signaling
Examples	Glucose, amino acids, proteins, nucleic acids, lipids	Alkaloids, glycosides, flavonoids, tannins, volatile oils, terpenoids, resins

1.4 Importance in Plants

Secondary metabolites play indispensable roles in the survival and ecological success of plants.

1. Protection Against Herbivores

Many alkaloids, tannins, and terpenoids possess bitter taste or toxic properties that discourage grazing animals and insects.

Examples

- Nicotine acts as an insect deterrent.
- Caffeine protects young leaves from herbivores.
- Tannins reduce digestibility of plant tissues.

2. Protection Against Pathogens

Several secondary metabolites exhibit antimicrobial activity against bacteria, fungi, viruses, and nematodes.

Examples include:

- Phenolic compounds
- Essential oils
- Phytoalexins
- Flavonoids

3. Attraction of Pollinators

Colored pigments and aromatic volatile compounds attract insects, birds, and other pollinators.

Examples:

- Anthocyanins impart bright flower colors.
- Essential oils produce characteristic floral fragrances.

4. Seed Dispersal

Pigments and flavor compounds encourage animals to consume fruits, facilitating seed dispersal.

5. Allelopathy

Some plants release secondary metabolites into the surrounding environment to inhibit the germination and growth of competing plant species.

Example:

- Juglone produced by walnut trees.

6. Protection Against Environmental Stress

Secondary metabolites protect plants from:

- UV radiation
- Oxidative stress
- Drought
- High temperature
- Salinity

7. Storage of Waste Products

Certain metabolites accumulate in vacuoles and specialized tissues, reducing cellular toxicity.

1.5 Importance in Pharmacy and Medicine

Secondary metabolites constitute one of the most important sources of therapeutic agents used in modern medicine.

Source of Drugs

Numerous drugs are directly isolated from medicinal plants.

Drug	Source	Therapeutic Use
Morphine	Opium	Analgesic
Atropine	Belladonna	Antispasmodic
Quinine	Cinchona	Antimalarial
Vincristine	Vinca	Anticancer
Digoxin	Digitalis	Cardiotonic

Artemisinin	Artemisia	Antimalarial
Reserpine	Rauwolfia	Antihypertensive

Lead Molecules for Drug Discovery

Many synthetic drugs have been developed by modifying naturally occurring secondary metabolites.

Pharmaceutical Excipients

Several secondary metabolites are used as:

- Flavoring agents
- Coloring agents
- Preservatives
- Antioxidants
- Stabilizers

Nutraceutical Applications

Polyphenols and flavonoids are incorporated into dietary supplements because of their antioxidant and health-promoting properties.

Herbal Medicines

Secondary metabolites form the active principles of many herbal formulations used in Ayurveda, Unani, Siddha, Traditional Chinese Medicine (TCM), and other traditional systems.

1.6 Role in Plant Defense

Secondary metabolites represent the plant's chemical defense system.

Chemical Defense Against Herbivores

Many compounds are toxic or unpalatable.

Examples:

- Nicotine
- Morphine
- Strychnine
- Capsaicin

Antimicrobial Defense

Plants synthesize antimicrobial compounds that inhibit pathogen invasion.

Examples:

- Phytoalexins
- Flavonoids
- Essential oils

Insect Repellents

Essential oils such as citronella, lemongrass, and eucalyptus repel insects.

Antioxidant Defense

Phenolic compounds neutralize reactive oxygen species generated during environmental stress.

UV Protection

Flavonoids absorb ultraviolet radiation, protecting plant tissues from DNA damage.

Wound Healing

Resins and gums seal damaged tissues and prevent microbial invasion.

1.7 Biosynthetic Pathways

Secondary metabolites originate from primary metabolites through specialized enzymatic pathways.

1. Shikimate Pathway

Produces:

- Phenolic compounds
- Flavonoids
- Lignans
- Aromatic amino acids

2. Mevalonate (MVA) Pathway

Produces:

- Sesquiterpenes
- Triterpenes
- Steroids

3. Methylerythritol Phosphate (MEP/DOXP) Pathway

Produces:

- Monoterpenes
- Diterpenes
- Carotenoids

4. Acetate–Malonate (Polyketide) Pathway

Produces:

- Anthraquinones
- Fatty acid derivatives
- Polyketides

5. Amino Acid-Derived Pathways

Responsible for biosynthesis of numerous alkaloids from:

- Tyrosine
- Tryptophan
- Phenylalanine
- Lysine
- Ornithine
- Histidine

1.8 Industrial Importance

Secondary metabolites have extensive applications in pharmaceutical, food, cosmetic, agricultural, and biotechnology industries.

Pharmaceutical Industry

- Drug discovery
- Herbal formulations
- Antibiotics
- Anticancer agents
- Antimalarial drugs
- Cardiovascular drugs

Food Industry

- Natural flavors
- Food preservatives
- Natural antioxidants
- Beverages
- Functional foods

Cosmetic Industry

- Perfumes
- Essential oils
- Skin-care products
- Hair-care formulations
- Aromatherapy products

Agriculture

- Botanical pesticides
- Insect repellents
- Fungicides
- Herbicides
- Plant growth regulators

Biotechnology

- Metabolic engineering of medicinal plants
- Cell and tissue culture for metabolite production
- Biotransformation of natural products
- Production of high-value phytochemicals using microbial and plant cell systems

Chemical Industry

- Natural dyes
- Resins
- Adhesives
- Fragrances
- Specialty chemicals

Secondary metabolites are specialized organic compounds produced through distinct biosynthetic pathways from primary metabolites. Although they are not directly required for basic physiological processes, they play crucial roles in plant defense, ecological interactions, and environmental adaptation. Major classes include alkaloids, glycosides, terpenoids, volatile oils, tannins, flavonoids, resins, iridoids, and naphthoquinones. Their remarkable biological activities have made them indispensable in pharmacy, medicine, agriculture, food technology, cosmetics, and biotechnology. Consequently, secondary metabolites remain a cornerstone of pharmacognosy, natural product chemistry, and modern drug discovery.

2. GENERAL CLASSIFICATION OF SECONDARY METABOLITES

Secondary metabolites are naturally occurring organic compounds synthesized by plants, microorganisms, and certain animals through specialized metabolic pathways. Unlike primary metabolites, these compounds are not directly involved in growth, development, or reproduction but perform essential ecological functions such as defense against herbivores and pathogens, attraction of pollinators, protection from environmental stress, and interspecies communication. From a pharmacognostic perspective, secondary metabolites constitute the principal bioactive constituents responsible for the medicinal value of crude drugs. Based on their chemical structure, biosynthetic origin, and biological activity, secondary metabolites are classified into several major groups, including alkaloids, glycosides, volatile oils, tannins, resins, flavonoids, terpenoids, steroids, saponins, lignans, coumarins, and quinones. Each group possesses characteristic chemical properties and pharmacological activities, making them important in medicine, pharmacy, food technology, agriculture, and cosmetics.

2.1 Alkaloids

Definition

Alkaloids are naturally occurring basic nitrogen-containing organic compounds, predominantly of plant origin, that exhibit significant physiological and pharmacological activities. The term *alkaloid* is derived from the Arabic word *Al-Qali*, meaning "plant ash" or "alkali," reflecting their alkaline nature. Most alkaloids contain one or more nitrogen atoms within a heterocyclic ring and are biosynthesized from amino acids such as lysine, tyrosine, tryptophan, ornithine, and phenylalanine. They generally occur in plants as salts of organic acids, including citric, malic, oxalic, tartaric, and acetic acids.

Alkaloids are among the most therapeutically valuable classes of secondary metabolites and exhibit diverse pharmacological activities, including analgesic, antihypertensive, antimalarial, anticancer, bronchodilator, antispasmodic, and central nervous system stimulant effects. Examples include morphine, quinine, atropine, vincristine, vinblastine, reserpine, and caffeine.

Classification of Alkaloids

Alkaloids are commonly classified according to their biosynthetic origin and the position of the nitrogen atom.

1. True Alkaloids

True alkaloids are derived from amino acids and contain nitrogen within a heterocyclic ring. They are strongly basic and exhibit marked physiological activity.

Examples: Morphine, Atropine, Quinine, Nicotine, Hyoscyamine, Reserpine.

2. Protoalkaloids

Protoalkaloids originate from amino acids, but their nitrogen atom is not incorporated into a heterocyclic ring. They generally possess simpler structures than true alkaloids.

Examples: Ephedrine, Mescaline, Colchicine.

3. Pseudoalkaloids

Pseudoalkaloids are not directly synthesized from amino acids. Their carbon skeleton is derived from terpenoids, steroids, or purines, while nitrogen is incorporated later during biosynthesis.

Examples: Caffeine, Theobromine, Solanidine, Conessine.

Distribution

Alkaloids are widely distributed throughout the plant kingdom and have been identified in more than 20% of flowering plant species. They occur predominantly in dicotyledonous plants, particularly in families such as Apocynaceae, Solanaceae, Papaveraceae, Rubiaceae, Fabaceae, Berberidaceae, Loganiaceae, and Rutaceae. Alkaloids may accumulate in roots, rhizomes, bark, stems, leaves, flowers, fruits, seeds, latex, or tubers depending on the species. Their concentration is influenced by environmental conditions, geographical location, climatic factors, soil nutrients, plant age, and

harvesting season. Small quantities of alkaloids have also been reported in fungi, microorganisms, marine organisms, and certain animals.

Biosynthesis

Alkaloids are synthesized through specialized metabolic pathways originating from amino acids. Lysine serves as the precursor for piperidine and quinolizidine alkaloids, ornithine produces tropane alkaloids, phenylalanine and tyrosine give rise to isoquinoline alkaloids, while tryptophan is the precursor for indole alkaloids. These amino acids undergo decarboxylation, oxidation, methylation, cyclization, reduction, and condensation reactions to produce structurally diverse alkaloids. The biosynthesis occurs mainly in actively growing tissues, after which the alkaloids are transported and stored in vacuoles or specialized secretory cells.

Chemical Properties

Alkaloids are generally colorless, crystalline solids, although some, such as nicotine, are volatile liquids. Most alkaloids possess a bitter taste and alkaline reaction. They are sparingly soluble in water in the free base form but readily soluble in organic solvents such as chloroform, ether, and alcohol. Their salts are usually water-soluble. Alkaloids readily form salts with mineral and organic acids and produce precipitates with alkaloidal reagents such as Dragendorff's, Mayer's, Wagner's, and Hager's reagents.

General Identification Tests

The presence of alkaloids can be confirmed using general alkaloidal reagents.

Test	Positive Observation
Dragendorff's Test	Orange or reddish-brown precipitate
Mayer's Test	Cream-colored precipitate
Wagner's Test	Brown or reddish-brown precipitate
Hager's Test	Yellow precipitate

Pharmaceutical Importance

Alkaloids constitute one of the most important groups of therapeutic agents. Morphine is employed as a potent opioid analgesic, quinine is used in malaria treatment, atropine serves as an anticholinergic agent, reserpine acts as an antihypertensive drug, vincristine and vinblastine are valuable anticancer agents, while caffeine functions as a central nervous system stimulant. Numerous modern medicines have been developed directly from naturally occurring alkaloids or their semisynthetic derivatives.

2.2 Glycosides

Definition

Glycosides are naturally occurring organic compounds composed of two structural components: a sugar portion known as the **glycone** and a non-sugar portion called the **aglycone** or **genin**. These two components are joined by a glycosidic bond, which may be hydrolyzed enzymatically or chemically to release the sugar and aglycone. The pharmacological activity of glycosides largely depends on the

nature of the aglycone. Glycosides occur abundantly in higher plants and represent one of the most important classes of secondary metabolites used in pharmacotherapy.

Classification

Anthraquinone Glycosides

These glycosides contain anthraquinone derivatives as aglycones and exhibit stimulant laxative activity.

Examples: Senna, Aloe, Cascara, Rhubarb.

Cardiac Glycosides

These compounds contain steroidal aglycones and exert powerful effects on cardiac muscle by increasing myocardial contractility.

Examples: Digitalis, Strophanthus, Squill.

Saponin Glycosides

Saponins possess soap-like foaming properties due to their amphiphilic structure. They may contain either steroidal or triterpenoid aglycones.

Examples: Liquorice, Dioscorea, Ginseng.

Cyanogenetic Glycosides

Upon enzymatic hydrolysis, these glycosides liberate hydrogen cyanide, which protects plants from herbivores.

Examples: Bitter almond, Wild cherry, Linseed.

Thioglycosides

These sulfur-containing glycosides release isothiocyanates after hydrolysis and are responsible for the pungent odor of several cruciferous plants.

Examples: Black mustard, White mustard.

Flavonoid Glycosides

These glycosides contain flavonoids as aglycones and exhibit antioxidant, anti-inflammatory, and cardioprotective activities.

Examples: Rutin, Hesperidin, Quercetin glycosides.

Distribution

Glycosides are widely distributed in medicinal plants and are commonly found in leaves, bark, roots, rhizomes, seeds, flowers, and fruits. They accumulate in vacuoles where they remain stable until hydrolysis occurs. Their concentration depends on species, environmental conditions, maturity, and harvesting season.

General Identification Tests

Test	Indicates
Keller-Killiani Test	Cardiac glycosides
Borntrager's Test	Anthraquinone glycosides
Modified Borntrager's Test	C-glycosides
Legal Test	Cardiac glycosides
Baljet Test	Cardiac glycosides
Foam Test	Saponin glycosides

Importance

Glycosides possess significant medicinal value. Cardiac glycosides are used in heart failure, anthraquinone glycosides serve as stimulant laxatives, saponins exhibit expectorant and anti-inflammatory properties, flavonoid glycosides act as antioxidants, while thioglycosides and cyanogenetic glycosides contribute to plant defense. Glycosides also play important roles in pharmaceutical manufacturing, nutraceuticals, cosmetics, and herbal medicine.

2.3 Volatile Oils

Definition

Volatile oils, also known as essential oils or ethereal oils, are aromatic, volatile, and odorous plant constituents that readily evaporate at room temperature without leaving a permanent greasy stain. They are complex mixtures of monoterpenes, sesquiterpenes, phenylpropanoids, alcohols, aldehydes, ketones, esters, ethers, and oxides. These oils are synthesized in specialized secretory structures such as glandular trichomes, oil cells, resin ducts, and secretory cavities.

Classification

Volatile oils are classified according to their predominant chemical constituents into monoterpene oils, sesquiterpene oils, phenylpropanoid oils, sulfur-containing oils, and mixed essential oils.

Occurrence

Volatile oils occur in flowers, fruits, leaves, bark, roots, rhizomes, wood, and seeds of aromatic plants. Major plant families containing essential oils include Lamiaceae, Apiaceae, Rutaceae, Myrtaceae, Lauraceae, and Zingiberaceae.

Physical Properties

Volatile oils possess characteristic pleasant odors and pungent tastes. Most are colorless liquids, although some become darker upon oxidation. They are volatile, possess high refractive indices, are

immiscible with water but soluble in alcohol and organic solvents, and generally have densities lower than water except for a few oils such as clove oil and cinnamon oil.

Chemical Properties

Chemically, volatile oils readily undergo oxidation, polymerization, esterification, reduction, and resinification during storage. Exposure to air, moisture, and sunlight gradually deteriorates their quality; therefore, they should be stored in tightly closed, light-resistant containers.

Extraction Methods

The principal methods employed for extraction include steam distillation, hydro-distillation, expression (cold pressing), solvent extraction, enfleurage, and supercritical carbon dioxide extraction. Steam distillation remains the most widely used method in pharmaceutical industries.

Identification Tests

Test	Observation
Filter Paper Test	Temporary translucent spot disappears on drying
Sudan III Test	Red coloration
Solubility Test	Soluble in alcohol and organic solvents

Uses

Volatile oils are extensively utilized as flavoring agents, perfumes, antiseptics, antimicrobial agents, carminatives, expectorants, analgesics, aromatherapy products, cosmetics, food preservatives, and pharmaceutical preparations.

2.4 Tannins

Definition

Tannins are naturally occurring, high-molecular-weight polyphenolic compounds capable of precipitating proteins, gelatin, alkaloids, and heavy metal ions. They impart an astringent taste to plant materials and play an important role in plant defense. Tannins are widely distributed in bark, fruits, leaves, roots, and galls of numerous medicinal plants.

Classification

Hydrolysable Tannins

Hydrolysable tannins are esters of gallic acid or ellagic acid with glucose or other polyhydric alcohols. They are readily hydrolyzed by acids, alkalis, or enzymes.

Examples: Myrobalans, Pomegranate, Galls.

Condensed Tannins

Condensed tannins, also called proanthocyanidins, consist of polymerized flavan-3-ol units and are resistant to hydrolysis.

Examples: Catechu, Cocoa, Tea.

Occurrence

Tannins are abundant in bark, fruits, seeds, roots, leaves, and galls. They accumulate mainly in vacuoles and provide protection against herbivores, insects, and microbial infection.

Chemical Properties

Tannins are generally amorphous, non-nitrogenous, water-soluble compounds possessing strong astringent properties. They form insoluble complexes with proteins and alkaloids and produce characteristic colored reactions with ferric salts.

Identification Tests

Test	Positive Observation
Ferric Chloride Test	Blue-black or green coloration
Gelatin Test	White precipitate
Lead Acetate Test	White precipitate
Goldbeater's Skin Test	Brown or black coloration

Pharmaceutical Uses

Tannins are widely employed as astringents, antidiarrheal agents, hemostatic agents, antioxidants, wound-healing agents, antimicrobial agents, and anti-inflammatory drugs. They are also utilized in leather tanning, dye manufacturing, beverage processing, cosmetics, and food preservation.

2.5 Resins

Definition

Resins are amorphous, non-volatile, solid or semi-solid plant exudates composed mainly of diterpenes, triterpenes, resin acids, resin alcohols, esters, and neutral resin compounds. They are insoluble in water but readily soluble in organic solvents such as alcohol, ether, chloroform, and acetone. Resins are secreted naturally or produced following mechanical injury to plant tissues.

Classification

Resins are classified as true resins, oleoresins, gum resins, oleo-gum-resins, balsams, and glycoresins, depending on their chemical composition and associated constituents.

Formation

Resins are synthesized through terpenoid biosynthetic pathways and accumulate in specialized resin ducts, secretory canals, and cavities. Their production increases following injury or microbial attack, where they function as protective barriers by sealing wounds and preventing pathogen invasion.

Identification Tests

Test	Positive Observation
Turbidity Test	Milky turbidity with water
Copper Acetate Test	Green coloration
Acetone Test	Complete dissolution

Therapeutic Uses

Resins possess antiseptic, antimicrobial, anti-inflammatory, expectorant, cathartic, carminative, wound-healing, and antioxidant activities. They are widely used in pharmaceutical formulations, incense, varnishes, adhesives, perfumes, cosmetics, and traditional herbal medicines. Common medicinal resins include Guggul, Asafoetida, Benzoin, and Colophony.

2.6 Phenylpropanoids and Flavonoids

Introduction

Phenylpropanoids and flavonoids constitute one of the largest and most diverse groups of plant secondary metabolites. They are biosynthesized primarily through the shikimate and phenylpropanoid pathways and are widely distributed throughout the plant kingdom. These compounds contribute significantly to plant growth, pigmentation, aroma, structural integrity, and defense mechanisms. In medicinal plants, they are responsible for numerous pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, hepatoprotective, neuroprotective, and antidiabetic effects.

Phenylpropanoids are characterized by a C6–C3 carbon skeleton, consisting of an aromatic benzene ring attached to a three-carbon side chain. Important members include cinnamic acid, caffeic acid, ferulic acid, coumarins, lignans, and eugenol. Flavonoids are polyphenolic compounds possessing a C6–C3–C6 carbon framework, consisting of two aromatic benzene rings connected through a heterocyclic pyran ring. More than 10,000 flavonoids have been identified from plants, making them one of the largest classes of natural phenolic compounds.

These metabolites occur in leaves, flowers, fruits, bark, seeds, roots, and stems, where they contribute to flower coloration, UV protection, attraction of pollinators, defense against microorganisms, and adaptation to environmental stress. Owing to their remarkable biological properties, phenylpropanoids and flavonoids are extensively utilized in pharmaceuticals, nutraceuticals, cosmetics, food preservation, and functional foods.

Classification

Phenylpropanoids and flavonoids are classified according to their chemical structures and biosynthetic origin.

A. Phenylpropanoids

Phenylpropanoids are divided into several subclasses, including simple phenylpropanoids, hydroxycinnamic acids, coumarins, lignans, neolignans, stilbenes, and phenolic acids.

Examples: Cinnamic acid, Ferulic acid, Eugenol, Vanillin, Sinapic acid.

B. Flavonoids

Flavonoids are classified into flavones, flavonols, flavanones, flavanonols, flavanols (catechins), anthocyanidins, isoflavones, chalcones, aurones, and biflavonoids.

Examples: Quercetin, Kaempferol, Rutin, Catechin, Hesperidin, Naringenin, Genistein.

Biosynthesis

The biosynthesis of phenylpropanoids begins with the amino acid phenylalanine, which is converted into cinnamic acid by the enzyme phenylalanine ammonia-lyase (PAL). Subsequent hydroxylation, methylation, reduction, and condensation reactions produce numerous phenylpropanoid derivatives.

Flavonoids are synthesized through the combined action of the phenylpropanoid pathway and the acetate–malonate pathway. Chalcone synthase catalyzes the formation of chalcones, which are subsequently converted into flavones, flavonols, flavanones, anthocyanins, and other flavonoid subclasses through enzyme-mediated reactions. The accumulation of flavonoids is influenced by light intensity, temperature, nutrient availability, developmental stage, and environmental stress.

Identification Tests

Test	**Positive Observation**
Shinoda Test	Pink, red, or magenta coloration
Alkaline Reagent Test	Yellow color that disappears upon acidification
Lead Acetate Test	Yellow precipitate
Ferric Chloride Test	Green or bluish-green coloration for phenolic compounds

Biological Importance

Phenylpropanoids and flavonoids play indispensable roles in plant physiology and ecology. They protect plants against ultraviolet radiation by absorbing harmful UV light and preventing cellular damage. These compounds act as natural antioxidants by scavenging reactive oxygen species generated during environmental stress. They also provide defense against bacteria, fungi, viruses, insects, and herbivores through antimicrobial and deterrent activities.

Flavonoid pigments impart attractive colors to flowers and fruits, thereby facilitating pollination and seed dispersal. Certain flavonoids function as signaling molecules during symbiotic nitrogen fixation in legumes. Lignin, a phenylpropanoid derivative, strengthens plant cell walls and provides mechanical support.

From a pharmaceutical perspective, flavonoids exhibit antioxidant, anti-inflammatory, antihypertensive, cardioprotective, hepatoprotective, neuroprotective, anticancer, antiviral, and antidiabetic activities. Consequently, they are widely incorporated into herbal medicines, dietary supplements, cosmetics, and functional foods.

2.7 Iridoids

Introduction

Iridoids are naturally occurring **monoterpenoid glycosides** possessing a cyclopentane ring fused to a six-membered oxygen-containing heterocyclic ring. They are synthesized through the terpenoid biosynthetic pathway and are widely distributed in medicinal plants belonging to the families Gentianaceae, Rubiaceae, Scrophulariaceae, Plantaginaceae, Lamiaceae, and Oleaceae. Most iridoids possess a distinctly bitter taste, which contributes to plant defense against herbivores.

Iridoids exhibit numerous pharmacological activities, including anti-inflammatory, hepatoprotective, antioxidant, antimicrobial, immunomodulatory, gastroprotective, cardioprotective, and anticancer properties. Because of these biological activities, iridoid-containing medicinal plants have been extensively employed in traditional systems of medicine and modern phytotherapy.

Classification

Iridoids are broadly classified into:

- Iridoid glycosides
- Secoiridoids
- Non-glycosidic iridoids
- Bis-iridoids
- Esterified iridoids

Examples: Gentiopicroside, Amarogentin, Aucubin, Catalpol, Harpagoside.

Chemical Nature

Iridoids are monoterpenoid derivatives generally occurring as glycosides. They are colorless crystalline compounds with a characteristic bitter taste and are readily soluble in water and alcohol due to the presence of sugar moieties. The aglycone portion contains an oxygenated cyclopentanoid monoterpene skeleton. Hydrolysis liberates the sugar and aglycone components. Their chemical structures frequently contain hydroxyl, aldehyde, ester, epoxide, and lactone functional groups, contributing to their biological activity.

Identification Tests

Test	**Positive Observation**
Trim-Hill Test	Blue coloration
Vanillin-Hydrochloric Acid Test	Pink to red coloration
Keller-Killiani Test (selected iridoid glycosides)	Characteristic colored ring

Uses

Iridoids are extensively utilized in herbal medicine because of their diverse pharmacological properties. They are used as bitter stomachics to stimulate appetite and improve digestion. Many iridoid-containing drugs exhibit anti-inflammatory and analgesic activities useful in rheumatic disorders. They also possess hepatoprotective, antioxidant, antimicrobial, antipyretic, gastroprotective, immunomodulatory, and cardioprotective properties. In pharmaceutical research,

iridoids are investigated as potential anticancer and neuroprotective agents and serve as valuable lead compounds for new drug development.

2.8 Other Terpenoids

Introduction

Terpenoids, also known as **isoprenoids**, represent the largest and most structurally diverse class of plant secondary metabolites. They are synthesized from five-carbon **isoprene units** through the mevalonate (MVA) and methylerythritol phosphate (MEP/DOXP) pathways. More than 80,000 terpenoids have been identified from plants, microorganisms, and marine organisms.

Terpenoids perform numerous biological functions, including plant growth regulation, defense against herbivores and pathogens, attraction of pollinators, protection from environmental stress, and intercellular communication. Many volatile oils, plant hormones, carotenoids, steroids, and natural rubber belong to this group. Terpenoids also constitute valuable pharmaceutical agents with antimicrobial, anticancer, anti-inflammatory, antiviral, antimalarial, and antioxidant activities.

Classification

Terpenoids are classified according to the number of isoprene units present in their structures.

Class	Number of Carbon Atoms	Examples
Hemiterpenoids	C5	Isoprene
Monoterpenoids	C10	Menthol, Limonene
Sesquiterpenoids	C15	Artemisinin
Diterpenoids	C20	Taxol, Phytol
Sesterterpenoids	C25	Ophiobolins
Triterpenoids	C30	Guggulsterone, Squalene
Tetraterpenoids	C40	β-Carotene, Lycopene
Polyterpenoids	>C40	Natural Rubber

Chemical Nature

Terpenoids are hydrocarbons or oxygenated derivatives composed of repeating isoprene units linked mainly in a head-to-tail fashion. Depending on their structures, they may exist as alcohols, aldehydes, ketones, esters, epoxides, oxides, lactones, or acids. They may be volatile liquids, crystalline solids, or viscous resins and are generally soluble in organic solvents but sparingly soluble in water. Their structures exhibit considerable diversity because of cyclization, oxidation, rearrangement, and glycosylation reactions.

Identification Tests

Test	Positive Observation
Salkowski Test	Reddish-brown coloration at the interface
Liebermann-Burchard Test	Green or bluish-green coloration
Vanillin-Sulfuric Acid Test	Violet or purple coloration

Uses

Terpenoids possess broad pharmaceutical and industrial applications. They serve as antimicrobial, anti-inflammatory, anticancer, antioxidant, antimalarial, antiviral, expectorant, bronchodilator, and analgesic agents. Many terpenoids are used as flavoring agents, fragrances, food additives, cosmetic ingredients, insect repellents, pesticides, and perfume constituents. Diterpenoids such as paclitaxel have revolutionized cancer chemotherapy, whereas sesquiterpenoids like artemisinin remain among the most effective antimalarial drugs.

2.9 Naphthoquinones

Introduction

Naphthoquinones are aromatic quinone derivatives containing a naphthalene ring with two ketone groups. They constitute an important class of phenolic secondary metabolites and are widely distributed in higher plants, fungi, bacteria, and lichens. In plants, they participate in defense mechanisms by exhibiting antimicrobial and insecticidal activities. Many naphthoquinones possess bright yellow, orange, red, or purple pigments and contribute to the coloration of roots, bark, and heartwood.

Pharmacologically, naphthoquinones exhibit antimicrobial, antifungal, antiviral, antiparasitic, antioxidant, anti-inflammatory, wound-healing, and anticancer properties. Their ability to undergo oxidation-reduction reactions makes them biologically active and valuable in medicinal chemistry.

Classification

Naphthoquinones are classified according to the position of carbonyl groups and structural modifications.

- 1,4-Naphthoquinones
- 1,2-Naphthoquinones
- Glycosylated naphthoquinones
- Prenylated naphthoquinones
- Dimeric naphthoquinones

Examples: Lawsone, Juglone, Plumbagin, Shikonin, Lapachol.

Distribution

Naphthoquinones occur mainly in the families Bignoniaceae, Plumbaginaceae, Boraginaceae, Juglandaceae, Ebenaceae, and Droseraceae. They are commonly found in roots, bark, wood, leaves, and fruits. Important medicinal plants containing naphthoquinones include Henna (*Lawsonia inermis*), Plumbago (*Plumbago zeylanica*), Walnut (*Juglans regia*), and Alkanet (*Alkanna tinctoria*).

Identification Tests

Test	Positive Observation
Alkali Test	Development of red or violet coloration
Ferric Chloride Test	Characteristic colored complex
Thin-Layer Chromatography (TLC)	Characteristic fluorescent or colored spots under UV light

Therapeutic Importance

Naphthoquinones possess considerable therapeutic significance because of their broad spectrum of biological activities. They exhibit potent antibacterial, antifungal, antiviral, antiparasitic, antioxidant, anti-inflammatory, anticancer, and wound-healing properties. Compounds such as plumbagin demonstrate promising anticancer activity, whereas lawsone is widely used in traditional medicine and cosmetic preparations. Certain naphthoquinones also function as natural dyes, food colorants, and antimicrobial preservatives. Their diverse pharmacological effects continue to make them attractive candidates for drug discovery and the development of novel therapeutic agents.

Phenylpropanoids, flavonoids, iridoids, terpenoids, and naphthoquinones represent major classes of plant secondary metabolites with immense pharmacognostic and pharmaceutical significance. These compounds are synthesized through specialized biosynthetic pathways and perform essential ecological functions, including plant defense, stress tolerance, pigmentation, signaling, and pollinator attraction. Their diverse biological activities have led to widespread applications in pharmaceuticals, nutraceuticals, cosmetics, agriculture, and food industries, making them indispensable components of modern pharmacognosy and natural product research.

3. GENERAL IDENTIFICATION TESTS OF SECONDARY METABOLITES

Introduction

Secondary metabolites are naturally occurring organic compounds produced by plants through specialized metabolic pathways. These compounds are responsible for the therapeutic efficacy of most medicinal plants and serve as important markers in pharmacognostic evaluation. Before a crude drug is used for medicinal purposes, it is essential to identify the presence of its major secondary metabolites. Various qualitative phytochemical tests have been developed to detect different classes of secondary metabolites based on characteristic color changes or precipitate formation after reaction with specific chemical reagents.

These identification tests are simple, rapid, economical, and widely employed in pharmacognosy laboratories for preliminary screening of medicinal plants. Although modern analytical techniques such as HPLC, GC-MS, LC-MS, and NMR provide precise identification and quantification, classical phytochemical tests remain valuable for routine laboratory analysis, quality control, and educational purposes.

3.1 Identification Tests for Alkaloids

Alkaloids are basic nitrogen-containing compounds that readily react with alkaloidal reagents to produce colored precipitates. Before performing these tests, the powdered drug is generally extracted with dilute hydrochloric acid, filtered, and the filtrate is used for analysis.

3.1.1 Dragendorff's Test

Dragendorff's reagent contains potassium bismuth iodide, which reacts with alkaloids to form insoluble complexes.

Procedure: A few drops of Dragendorff's reagent are added to the acidic extract of the drug.

Observation: Formation of an orange or reddish-brown precipitate indicates the presence of alkaloids.

Principle: Bismuth-potassium iodide reacts with basic nitrogen atoms of alkaloids to form insoluble alkaloidal complexes.

3.1.2 Mayer's Test

Mayer's reagent consists of potassium mercuric iodide.

Procedure: Add a few drops of Mayer's reagent to the acidic extract.

Observation: Appearance of a **cream or white-colored precipitate** confirms alkaloids.

Principle: Mercuric ions combine with alkaloids to form insoluble mercury-alkaloid complexes.

3.1.3 Wagner's Test

Wagner's reagent contains iodine dissolved in potassium iodide.

Procedure: Treat the acidic extract with Wagner's reagent.

Observation: A reddish-brown or brown precipitate indicates alkaloids.

Principle: Iodine forms insoluble complexes with alkaloids.

3.1.4 Hager's Test

Hager's reagent is a saturated solution of picric acid.

Procedure: Add Hager's reagent to the acidic extract.

Observation: Formation of a yellow crystalline precipitate confirms alkaloids.

Principle: Picric acid reacts with alkaloids to produce insoluble picrate salts.

Summary of Alkaloid Tests

Test	Positive Observation
Dragendorff's Test	Orange or reddish-brown precipitate
Mayer's Test	Cream or white precipitate
Wagner's Test	Brown or reddish-brown precipitate

Hager's Test	Yellow crystalline precipitate

3.2 Identification Tests for Glycosides

Different glycosides require different identification tests depending on the nature of their aglycone.

3.2.1 Keller-Killiani Test

This test is specific for **cardiac glycosides** containing deoxy sugars.

Procedure : The extract is mixed with glacial acetic acid containing ferric chloride and carefully layered with concentrated sulfuric acid.

Observation: Formation of a **brown ring** at the junction with a bluish-green upper layer indicates cardiac glycosides.

Principle: Deoxy sugars react with ferric ions under acidic conditions to produce characteristic colored complexes.

3.2.2 Legal Test

This test detects cardenolide-type cardiac glycosides.

Procedure: The extract is treated with sodium nitroprusside followed by pyridine and sodium hydroxide.

Observation: Development of a **pink to deep red color** confirms cardiac glycosides.

Principle: Unsaturated lactone rings react with sodium nitroprusside under alkaline conditions.

3.2.3 Baljet Test

Baljet reagent consists of alkaline picric acid.

Procedure: Add Baljet reagent to the extract.

Observation: An orange to red coloration indicates cardiac glycosides.

Principle: The lactone ring reacts with alkaline picrate solution.

3.2.4 Borntrager's Test

This test identifies free anthraquinone glycosides.

Procedure: The drug extract is hydrolyzed, extracted with benzene, and shaken with ammonia solution.

Observation: A pink or cherry-red color in the ammoniacal layer confirms anthraquinone glycosides.

Principle: Anthraquinone aglycones dissolve in alkaline solution and develop characteristic colors.

3.2.5 Modified Borntrager's Test

This test detects C-glycosides that are resistant to ordinary hydrolysis.

Procedure: The extract is first treated with ferric chloride and hydrochloric acid before performing Borntrager's test.

Observation: A pink to red color in the ammoniacal layer indicates C-glycosides.

Principle: Ferric chloride facilitates oxidation and cleavage of C-glycosidic bonds.

3.2.6 Foam Test

Foam test is employed for identifying **saponin glycosides**.

Procedure: The aqueous extract is shaken vigorously for several minutes.

Observation: Formation of a stable honeycomb-like foam persisting for at least 10–15 minutes indicates saponins.

Principle: Saponins reduce surface tension, producing stable froth.

Summary of Glycoside Tests

Test	Positive Observation
Keller-Killiani Test	Brown ring with bluish-green layer
Legal Test	Pink to red color
Baljet Test	Orange or red color
Borntrager's Test	Pink or cherry-red color
Modified Borntrager's Test	Pink or red color
Foam Test	Persistent froth

3.3 Identification Tests for Volatile Oils

Volatile oils possess characteristic physical and chemical properties that assist in their identification.

3.3.1 Sudan III Test

Procedure: Add Sudan III solution to the oil sample.

Observation: The oil becomes bright red or orange-red.

Principle: Sudan III is a fat-soluble dye that stains volatile oils.

3.3.2 Filter Paper Test

Procedure: Place one drop of oil on filter paper.

Observation: A temporary translucent spot appears and disappears completely on drying.

Principle: Volatile oils evaporate without leaving permanent greasy stains.

3.3.3 Solubility Test

Procedure: Mix the oil separately with water, alcohol, and organic solvents.

Observation: Volatile oils are insoluble in water but readily soluble in alcohol, ether, chloroform, and other organic solvents.

Principle: Essential oils possess non-polar characteristics.

Summary of Volatile Oil Tests

Test	Positive Observation
Sudan III Test	Red coloration
Filter Paper Test	Temporary translucent spot
Solubility Test	Soluble in organic solvents

3.4 Identification Tests for Tannins

Tannins are polyphenolic compounds capable of precipitating proteins and heavy metals.

3.4.1 Ferric Chloride Test

Procedure: Add neutral ferric chloride solution to the extract.

Observation: Hydrolysable tannins produce a blue-black color, whereas condensed tannins produce a greenish-black color.

Principle: Phenolic hydroxyl groups form colored complexes with ferric ions.

3.4.2 Gelatin Test

Procedure: Add gelatin solution containing sodium chloride to the extract.

Observation: Formation of a white precipitate indicates tannins.

Principle: Tannins precipitate proteins.

3.4.3 Goldbeater's Skin Test

Procedure: Goldbeater's skin is soaked in hydrochloric acid, washed, treated with the extract, and then immersed in ferric sulfate.

Observation: A brown or black coloration confirms tannins.

Principle: Tannins combine with collagen proteins present in the membrane.

3.4.4 Lead Acetate Test

Procedure: Treat the extract with lead acetate solution.

Observation: Formation of a white precipitate confirms tannins.

Principle: Lead ions precipitate tannin molecules.

3.4.5 Potassium Dichromate Test

Procedure: Add potassium dichromate solution to the extract.

Observation: A yellow or brown precipitate indicates tannins.

Principle: Potassium dichromate reacts with phenolic compounds to form insoluble complexes.

Summary of Tannin Tests

Test	Positive Observation
Ferric Chloride Test	Blue-black or green-black color
Gelatin Test	White precipitate
Goldbeater's Skin Test	Brown or black color
Lead Acetate Test	White precipitate
Potassium Dichromate Test	Yellow or brown precipitate

3.5 Identification Tests for Resins

Resins are amorphous, non-volatile plant exudates that are insoluble in water but soluble in organic solvents.

3.5.1 Acetone Test

Procedure: Dissolve the sample in acetone.

Observation: Complete dissolution indicates the presence of resins.

Principle: Most resins are readily soluble in acetone.

3.5.2 Copper Acetate Test

Procedure: Add copper acetate solution to the resin extract.

Observation: Development of a green coloration confirms resin acids.

Principle: Copper ions react with resin acids to form colored complexes.

3.5.3 Turbidity Test

Procedure: Prepare an alcoholic solution of the resin and pour it into distilled water.

Observation: Formation of milky turbidity indicates resins.

Principle: Resins are insoluble in water and precipitate upon dilution.

Summary of Resin Tests

Test	Positive Observation
Acetone Test	Complete dissolution
Copper Acetate Test	Green coloration
Turbidity Test	Milky turbidity

3.6 Identification Tests for Flavonoids

Flavonoids are polyphenolic compounds responsible for many antioxidant properties of medicinal plants.

3.6.1 Shinoda Test

Procedure: Add magnesium turnings followed by concentrated hydrochloric acid to the extract.

Observation: A pink, crimson, or magenta color confirms flavonoids.

Principle: Magnesium reduces flavonoids under acidic conditions to form colored compounds.

3.6.2 Alkaline Reagent Test

Procedure: Treat the extract with sodium hydroxide solution and then add dilute acid.

Observation: A yellow color develops in alkali and disappears after acidification.

Principle: Flavonoids form colored ionized salts in alkaline medium.

3.6.3 Lead Acetate Test

Procedure: Add lead acetate solution to the extract.

Observation: Formation of a yellow precipitate confirms flavonoids.

Principle: Lead ions form insoluble complexes with flavonoids.

Summary of Flavonoid Tests

Test	Positive Observation
Shinoda Test	Pink, crimson, or magenta color
Alkaline Reagent Test	Yellow color disappearing on acidification
Lead Acetate Test	Yellow precipitate

3.7 Identification Tests for Terpenoids

Terpenoids are structurally diverse isoprenoid compounds with important medicinal properties.

3.7.1 Salkowski Test

Procedure: Mix the extract with chloroform and carefully add concentrated sulfuric acid along the side of the test tube.

Observation: A reddish-brown ring appears at the interface.

Principle: Sulfuric acid reacts with unsaturated terpenoid molecules to produce characteristic colored products.

3.7.2 Liebermann-Burchard Test

Procedure: Treat the extract with acetic anhydride followed by concentrated sulfuric acid.

Observation: Development of a green, bluish-green, or emerald-green color confirms terpenoids or steroids.

Principle: Acetic anhydride and sulfuric acid produce conjugated colored complexes with terpenoids.

Summary of Terpenoid Tests

Test	Positive Observation
Salkowski Test	Reddish-brown ring
Liebermann-Burchard Test	Green or bluish-green color

3.8 Identification Tests for Iridoids

Iridoids are bitter monoterpenoid glycosides found in many medicinal plants.

3.8.1 Trim-Hill Test

Procedure: Treat the extract with Trim-Hill reagent and warm gently.

Observation: Appearance of a blue coloration indicates iridoids.

Principle: Iridoid glycosides react with acidic reagents to produce colored derivatives.

3.8.2 Vanillin-HCl Test

Procedure: Add vanillin solution followed by concentrated hydrochloric acid to the extract.

Observation: Development of a pink, red, or reddish-violet color confirms iridoids.

Principle: Vanillin condenses with iridoid compounds in acidic medium to form colored complexes.

Summary of Iridoid Tests

Test	Positive Observation
Trim-Hill Test	Blue coloration
Vanillin-HCl Test	Pink to red coloration

General identification tests of secondary metabolites provide a rapid and economical approach for the preliminary phytochemical screening of medicinal plants. Each class of secondary metabolites reacts specifically with certain reagents, producing characteristic colors or precipitates that aid in their identification. Alkaloids are detected by Dragendorff's, Mayer's, Wagner's, and Hager's tests; glycosides by Keller-Killiani, Legal, Baljet, Borntrager's, Modified Borntrager's, and Foam tests; volatile oils by Sudan III, Filter Paper, and Solubility tests; tannins by Ferric Chloride, Gelatin, Goldbeater's Skin, Lead Acetate, and Potassium Dichromate tests; resins by Acetone, Copper Acetate, and Turbidity tests; flavonoids by Shinoda, Alkaline Reagent, and Lead Acetate tests; terpenoids by Salkowski and Liebermann-Burchard tests; and iridoids by Trim-Hill and Vanillin-HCl tests. These qualitative tests remain fundamental tools in pharmacognostic evaluation, quality control, and authentication of crude drugs before advanced instrumental analysis.

4. ALKALOID DRUGS

Alkaloids are one of the most important classes of secondary metabolites in pharmacognosy due to their remarkable physiological and therapeutic activities. They are naturally occurring basic nitrogen-containing organic compounds that are primarily synthesized from amino acids and are widely distributed in higher plants. Most alkaloids occur in plants as salts of organic acids and are stored in specialized tissues such as roots, bark, leaves, seeds, fruits, latex, and rhizomes. Alkaloids play a significant role in plant defense against herbivores, insects, and microorganisms because of their bitter taste and toxic properties. In medicine, alkaloids have contributed immensely to modern therapeutics, with drugs such as morphine, quinine, atropine, vincristine, vinblastine, and reserpine serving as valuable agents in the treatment of pain, malaria, hypertension, cancer, and numerous neurological disorders. This chapter discusses the pharmacognostic study of three important alkaloidal drugs—Vinca, Rauwolfia, and Opium.

4.1 Vinca

Introduction

Vinca is one of the most important medicinal plants containing indole alkaloids with potent anticancer activity. The drug is obtained from *Catharanthus roseus* (formerly *Vinca rosea*), commonly known as Madagascar periwinkle. More than 130 alkaloids have been isolated from this plant, among which vincristine and vinblastine are of outstanding pharmaceutical importance. These alkaloids interfere with cell division by inhibiting microtubule formation and are extensively used in cancer chemotherapy. Besides its anticancer properties, the plant contains alkaloids exhibiting antihypertensive, hypoglycemic, antimicrobial, and antioxidant activities.

Biological Source

Vinca consists of the dried leaves, stems, roots, and entire plant of ***Catharanthus roseus* (L.) G. Don**.

Family

Apocynaceae

Distribution

Vinca is native to Madagascar but is now cultivated extensively throughout tropical and subtropical regions of the world. It is widely grown in India, Sri Lanka, China, Brazil, the United States, South Africa, and Southeast Asian countries. In India, commercial cultivation is carried out in Tamil Nadu, Karnataka, Andhra Pradesh, Uttar Pradesh, West Bengal, and Maharashtra.

Cultivation

Vinca grows well under warm tropical climates with moderate rainfall and abundant sunlight. It prefers fertile, well-drained sandy loam soils having a pH of 6.0–7.5. Propagation is generally carried out by seeds, although stem cuttings may also be used. The crop requires regular irrigation during dry periods and responds well to organic manure and balanced fertilizers. Proper weed control and pest management improve biomass and alkaloid content.

Collection

Leaves are harvested after flowering when alkaloid concentration is highest. Roots are collected after approximately one year of cultivation, washed thoroughly, shade dried, and stored in moisture-free containers.

Macroscopy

The leaves are opposite, simple, ovate to elliptic, smooth, glossy, dark green above, and pale green beneath with a distinct white midrib. Flowers are pink, white, or purple with five petals arranged in a salver-shaped corolla. The roots are slender, cylindrical, light brown externally, and cream-colored internally with a slightly bitter taste.

Microscopy

The leaf shows a single-layered upper and lower epidermis covered by a thin cuticle. Both anisocytic and paracytic stomata are present, predominantly on the lower surface. The mesophyll is differentiated into palisade and spongy parenchyma. Numerous calcium oxalate crystals, laticifers, and collateral vascular bundles are observed. The root possesses cork, cortex, secondary phloem, cambium, and well-developed xylem with lignified vessels.

Powder Study

The powder is greenish-brown with a characteristic odor and bitter taste. Diagnostic characters include fragments of epidermis with stomata, spiral and pitted vessels, lignified fibers, calcium oxalate crystals, starch grains, and laticiferous tissues.

Chemical Constituents

Vinca contains more than 130 indole alkaloids. The principal alkaloids include vincristine, vinblastine, vindoline, catharanthine, ajmalicine, serpentine, lochnericine, and leurosine. The plant also contains flavonoids, tannins, phenolic acids, steroids, triterpenoids, sugars, amino acids, and volatile constituents.

Identification Tests

Test	Observation
Dragendorff's Test	Orange precipitate
Mayer's Test	Cream precipitate
Wagner's Test	Brown precipitate
Hager's Test	Yellow precipitate

Evaluation

Evaluation includes determination of foreign matter, moisture content, total ash, acid-insoluble ash, alcohol-soluble extractive, water-soluble extractive, and chromatographic estimation of vincristine and vinblastine using TLC or HPLC.

Therapeutic Uses

Vinca alkaloids are widely used in the treatment of acute lymphoblastic leukemia, Hodgkin's lymphoma, breast cancer, lung cancer, neuroblastoma, and non-Hodgkin's lymphoma. The plant also possesses antidiabetic, antihypertensive, antimicrobial, antioxidant, and wound-healing activities.

Pharmaceutical Applications

Vincristine and vinblastine are indispensable anticancer drugs used in combination chemotherapy. Other alkaloids such as ajmalicine are utilized in antihypertensive and vasodilator formulations.

Commercial Applications

Vinca is cultivated commercially for extraction of pharmaceutical alkaloids used by the oncology industry. The ornamental value of the plant also contributes significantly to the horticultural market.

Storage

The dried drug should be stored in tightly closed, moisture-proof containers protected from excessive heat, light, and humidity to preserve alkaloid content.

Vinca is a valuable medicinal plant belonging to the family Apocynaceae and represents one of the most important natural sources of anticancer alkaloids. Its principal constituents, vincristine and vinblastine, have revolutionized cancer chemotherapy and continue to remain indispensable in modern medicine.

4.2 Rauwolfia

Introduction

Rauwolfia is an important medicinal drug containing indole alkaloids, chiefly reserpine, which possesses antihypertensive and tranquilizing properties. The drug is obtained from the dried roots of *Rauwolfia serpentina*, commonly known as Indian snakeroot or Sarpagandha. It has been used for centuries in Ayurveda for the treatment of hypertension, insomnia, anxiety, epilepsy, and snakebite. More than fifty alkaloids have been isolated from Rauwolfia, making it one of the richest natural sources of indole alkaloids.

Biological Source

Rauwolfia consists of the dried roots of *Rauwolfia serpentina* (L.) Benth. ex Kurz.

Family

Apocynaceae

Distribution

The plant is distributed throughout India, Nepal, Bangladesh, Sri Lanka, Myanmar, Thailand, and other Southeast Asian countries. In India, it is cultivated mainly in Uttar Pradesh, West Bengal, Assam, Kerala, Karnataka, and Tamil Nadu.

Cultivation

Rauwolfia grows best in warm, humid climates with fertile, well-drained loamy soils rich in organic matter. Propagation is carried out by seeds, root cuttings, or stem cuttings. The crop requires partial shade, adequate irrigation, and regular weeding for optimum root development.

Collection

Roots are harvested after two to three years of cultivation, thoroughly cleaned, cut into suitable lengths, shade dried, and stored in airtight containers.

Macroscopy

Roots are cylindrical, tapering, slightly curved, grayish-brown externally, yellowish internally, and possess a characteristic odor with a bitter taste.

Microscopy

The transverse section shows cork, cortex containing starch grains, secondary phloem, cambium, secondary xylem with lignified vessels, medullary rays, and abundant calcium oxalate crystals.

Powder Study

The powder is light brown and contains cork cells, lignified vessels, fibers, starch grains, calcium oxalate crystals, and parenchymatous cells.

Chemical Constituents

The chief alkaloids include reserpine, rescinnamine, ajmaline, ajmalicine, serpentine, yohimbine, rauwolscine, and deserpidine.

Identification Tests

Test	Observation
Dragendorff's Test	Orange precipitate
Mayer's Test	Cream precipitate
Wagner's Test	Brown precipitate
Hager's Test	Yellow precipitate

Evaluation

Quality evaluation includes determination of total alkaloid content, ash values, extractive values, moisture content, foreign matter, and chromatographic fingerprinting by TLC or HPLC.

Therapeutic Uses

Rauwolfia is employed in the treatment of hypertension, anxiety, psychotic disorders, insomnia, and certain cardiac conditions. Traditionally, it has also been used in the management of snakebite and mental illness.

Pharmaceutical Applications

Reserpine is used as an antihypertensive and tranquilizer, while ajmaline is an important antiarrhythmic agent.

Commercial Applications

The roots serve as a commercial source of indole alkaloids utilized by the pharmaceutical industry for manufacturing cardiovascular and neurological medications.

Storage

Store in airtight, light-resistant containers in a cool and dry place to prevent deterioration of alkaloids.

Rauwolfia is one of the most important medicinal plants used for the production of antihypertensive alkaloids. Its principal constituent, reserpine, has played a significant role in cardiovascular medicine and neuropharmacology.

4.3 Opium

Introduction

Opium is the dried latex obtained from the unripe capsules of the opium poppy, *Papaver somniferum*. It is one of the oldest medicinal drugs known to mankind and has been used for thousands of years as a powerful analgesic and sedative. Opium contains more than forty alkaloids, among which morphine, codeine, thebaine, papaverine, and noscapine are therapeutically important. Morphine remains one of the most potent natural analgesics and is extensively employed in the management of severe pain.

Biological Source

Opium is the dried latex obtained by incision of the unripe capsules of ***Papaver somniferum* L.**

Family: Papaveraceae

Distribution

The plant is cultivated in India, Turkey, Afghanistan, Iran, China, Australia, France, and several European countries. In India, licensed cultivation is mainly carried out in Madhya Pradesh, Rajasthan, and Uttar Pradesh.

Cultivation

Opium poppy grows well in cool climates with fertile, well-drained loamy soil. Seeds are sown during winter, and irrigation, thinning, and fertilizer application are carried out throughout the growing season.

Collection

When the capsules become fully developed but remain green, shallow longitudinal incisions are made in the evening. The milky latex exudes, coagulates overnight, and is scraped off the following morning. The collected latex is kneaded into masses and dried.

Macroscopy

Opium occurs as irregular rounded masses or flattened cakes, dark brown to black externally, with a characteristic narcotic odor and intensely bitter taste. It becomes sticky on warming.

Microscopy

The powdered drug contains latex fragments, parenchyma cells, laticifer tissues, starch grains, and occasional calcium oxalate crystals.

Powder Study

The powder is dark brown with a characteristic odor and bitter taste. Microscopic examination reveals latex cells, parenchyma, starch grains, and vascular tissue fragments.

Chemical Constituents

Major alkaloids include morphine, codeine, thebaine, papaverine, noscapine (narcotine), narceine, and laudanine. The drug also contains sugars, proteins, gums, organic acids, and resins.

Identification Tests

Test	**Observation**
Dragendorff's Test	Orange precipitate

Mayer's Test	Cream precipitate
Wagner's Test	Brown precipitate
Hager's Test	Yellow precipitate
Ferric Chloride Test (Morphine)	Deep blue coloration

Evaluation

Evaluation includes determination of morphine content, moisture content, total ash, extractive values, foreign matter, and chromatographic analysis using TLC or HPLC.

Therapeutic Uses

Opium and its alkaloids are used as potent analgesics, antitussives, antidiarrheal agents, sedatives, and antispasmodics. Morphine is widely used for severe postoperative pain and cancer pain, whereas codeine serves as an effective cough suppressant.

Pharmaceutical Applications

Morphine, codeine, papaverine, and noscapine are extensively employed in modern pharmaceutical formulations for pain management, cough suppression, gastrointestinal disorders, and smooth muscle relaxation.

Commercial Applications

Licensed cultivation of opium provides raw material for the pharmaceutical industry for the manufacture of opioid analgesics and other alkaloidal medicines under strict governmental regulation.

Storage

Opium should be stored in well-closed, light-resistant containers in a cool, dry place with strict security measures because of its narcotic nature.

Opium is one of the most valuable alkaloidal drugs in pharmacognosy and remains an indispensable source of opioid analgesics. Its major constituent, morphine, continues to be the standard drug for the management of severe pain, while codeine, papaverine, and noscapine contribute significantly to respiratory and gastrointestinal therapeutics.

5. VOLATILE OIL DRUGS

Volatile oils, also known as essential oils or ethereal oils, are aromatic, volatile secondary metabolites obtained from different parts of plants such as leaves, flowers, fruits, seeds, bark, roots, and rhizomes. They are complex mixtures of monoterpenes, sesquiterpenes, phenylpropanoids, alcohols, aldehydes, ketones, esters, and oxides. Unlike fixed oils, volatile oils evaporate completely on exposure to air without leaving a permanent greasy stain. They possess characteristic aroma and are widely used in pharmaceutical, cosmetic, food, perfumery, and aromatherapy industries. Important volatile oil drugs included in the B.Pharm syllabus are Lemongrass, Clove, Cinnamon, and Fennel.

5.1 Lemongrass

Introduction

Lemongrass is one of the most important aromatic medicinal plants cultivated for its essential oil, which is rich in **citral** (geranial and neral). The pleasant lemon-like fragrance and potent antimicrobial properties make lemongrass valuable in pharmaceuticals, cosmetics, perfumery, and food industries. The oil also possesses antioxidant, anti-inflammatory, analgesic, insect-repellent, and digestive properties.

Biological Source

Lemongrass consists of the fresh or dried leaves of ***Cymbopogon citratus*** or ***Cymbopogon flexuosus***.

Family

Poaceae (Gramineae)

Distribution

Lemongrass is native to tropical Asia and is extensively cultivated in India, Sri Lanka, China, Thailand, Brazil, Indonesia, and several African countries. In India, major cultivation occurs in Karnataka, Kerala, Tamil Nadu, Uttar Pradesh, Assam, and West Bengal.

Cultivation

The crop grows well in warm tropical climates with annual rainfall of 100–250 cm. It requires fertile, well-drained loamy soil and is propagated mainly by root slips or divisions. Regular irrigation, organic manure, and periodic harvesting improve essential oil yield.

Collection

Leaves are harvested 4–6 months after planting and subsequently every 2–3 months. Fresh leaves are immediately subjected to steam distillation for extraction of essential oil.

Macroscopy

Leaves are long, narrow, linear, and bluish-green with rough margins and a characteristic lemon-like odor. The taste is aromatic and slightly bitter.

Microscopy

The leaf shows a single-layered epidermis covered with cuticle, numerous silica cells, stomata, vascular bundles, sclerenchyma fibers, and abundant oil-containing secretory cells.

Powder Study

The powder is greenish with fragments of epidermis, silica bodies, fibers, vascular elements, and parenchymatous cells exhibiting characteristic aroma.

Chemical Constituents

The essential oil mainly contains **citral (65–85%)**, geraniol, nerol, citronellal, limonene, myrcene, linalool, citronellol, and other terpenoids.

Identification Tests

Test	Positive Observation
Sudan III Test	Red coloration
Filter Paper Test	Temporary translucent spot
Solubility Test	Soluble in alcohol and organic solvents

Evaluation

Evaluation includes determination of volatile oil content, refractive index, specific gravity, optical rotation, acid value, and chromatographic analysis (GC-MS).

Therapeutic Uses

Lemongrass is used as a carminative, antimicrobial, antifungal, antioxidant, anti-inflammatory, antipyretic, digestive stimulant, and insect repellent.

Pharmaceutical Applications

Its essential oil is incorporated into herbal formulations, antiseptic creams, massage oils, aromatherapy preparations, mouthwashes, soaps, and liniments.

Commercial Applications

Widely used in perfumes, cosmetics, beverages, confectionery, flavoring agents, mosquito repellents, and household cleaning products.

Storage

Store in tightly closed, amber-colored containers protected from light, heat, and moisture.

5.2 Clove

Introduction

Clove is one of the oldest and most valuable spice drugs obtained from the dried flower buds of *Syzygium aromaticum*. It is an excellent source of **eugenol**, a phenolic compound possessing strong antiseptic, analgesic, antioxidant, and antimicrobial activities. Clove has been extensively employed in dentistry, food preservation, traditional medicine, and perfumery.

Biological Source

Clove consists of the dried unopened flower buds of ***Syzygium aromaticum* (L.) Merr. & L.M. Perry**.

Family

Myrtaceae

Distribution

Native to Indonesia, clove is cultivated commercially in India, Sri Lanka, Madagascar, Tanzania, Zanzibar, Malaysia, and Brazil.

Cultivation

Clove thrives in humid tropical climates with fertile, well-drained soils and abundant rainfall. It is propagated through seeds and begins commercial flowering after 6–8 years.

Collection

Flower buds are harvested when they turn pink and are subsequently dried in the sun until they acquire a dark brown color.

Macroscopy

Cloves are nail-shaped flower buds measuring 10–17 mm in length, dark brown in color, possessing a strong aromatic odor and pungent taste.

Microscopy

The bud contains oil glands, thick-walled epidermal cells, vascular bundles, sclerenchyma fibers, and abundant volatile oil cells.

Powder Study

The powder is dark brown and contains oil cells, stone cells, pollen grains, fibers, and fragments of epidermis.

Chemical Constituents

Major constituents include **eugenol (70–90%)**, eugenyl acetate, β-caryophyllene, tannins, flavonoids, and triterpenoids.

Identification Tests

Test	**Positive Observation**
Sudan III Test	Red coloration
Filter Paper Test	Temporary translucent spot
Solubility Test	Soluble in alcohol

Evaluation

Evaluation involves determination of volatile oil content, refractive index, specific gravity, eugenol content, and chromatographic fingerprinting.

Therapeutic Uses

Clove is used as an antiseptic, local anesthetic, carminative, antimicrobial, antioxidant, and anti-inflammatory agent.

Pharmaceutical Applications

Widely employed in dental preparations, mouthwashes, toothpaste, cough syrups, herbal oils, and antiseptic formulations.

Commercial Applications

Used extensively in spice industries, perfumes, cosmetics, flavoring agents, food preservation, and aromatherapy.

Storage

Store in airtight containers away from moisture, heat, and direct sunlight.

5.3 Cinnamon

Introduction

Cinnamon is an important aromatic spice obtained from the dried inner bark of *Cinnamomum verum*. It possesses a pleasant aroma due to **cinnamaldehyde**, which exhibits antimicrobial, antioxidant, hypoglycemic, anti-inflammatory, and digestive properties. Cinnamon has been used for centuries as both a culinary spice and medicinal drug.

Biological Source

Cinnamon consists of the dried inner bark of ***Cinnamomum verum* J. Presl** (Syn. *Cinnamomum zeylanicum*).

Family

Lauraceae

Distribution

It is cultivated in Sri Lanka, India, Madagascar, Indonesia, China, and Seychelles. In India, cultivation occurs mainly in Kerala and Tamil Nadu.

Cultivation

The plant grows well in tropical climates with fertile, well-drained soils and adequate rainfall. Bark is collected from young shoots after 2–3 years of growth.

Collection

After harvesting, the outer bark is removed and the inner bark is peeled, dried, and rolled into characteristic quills.

Macroscopy

The bark occurs as thin, smooth, yellowish-brown quills with a pleasant aromatic odor and sweet, warm taste.

Microscopy

The bark contains cork cells, sclerenchyma fibers, stone cells, oil cells, parenchyma, and calcium oxalate crystals.

Powder Study

The powder is light brown with cork fragments, stone cells, fibers, starch grains, and volatile oil cells.

Chemical Constituents

The principal constituents are **cinnamaldehyde**, eugenol, cinnamic acid, coumarin (trace), tannins, mucilage, and sugars.

Identification Tests

Test	**Positive Observation**
Sudan III Test	Red coloration
Filter Paper Test	Temporary translucent spot
Solubility Test	Soluble in alcohol

Evaluation

Evaluation includes volatile oil estimation, ash values, extractive values, moisture content, and GC analysis.

Therapeutic Uses

Cinnamon acts as a carminative, antimicrobial, antioxidant, hypoglycemic, anti-inflammatory, and digestive stimulant.

Pharmaceutical Applications

Used in syrups, digestive formulations, herbal capsules, mouth fresheners, and flavoring preparations.

Commercial Applications

Extensively employed in bakery products, beverages, confectionery, cosmetics, perfumes, and food industries.

Storage

Store in tightly closed containers in a cool, dry place away from sunlight.

5.4 Fennel

Introduction

Fennel is an aromatic medicinal plant widely used as a spice and digestive remedy. The fruits contain volatile oil rich in **anethole**, which imparts the characteristic sweet aroma and exhibits carminative, expectorant, antispasmodic, and antimicrobial properties. Fennel has been used in traditional medicine for digestive disorders, respiratory ailments, and infant colic.

Biological Source

Fennel consists of the dried ripe fruits of ***Foeniculum vulgare* Mill.**

Family

Apiaceae (Umbelliferae)

Distribution

Fennel is cultivated in India, Egypt, Turkey, China, Italy, Iran, and Mediterranean countries. Major Indian producing states include Rajasthan, Gujarat, Uttar Pradesh, and Punjab.

Cultivation

The crop requires cool climatic conditions with fertile, well-drained loamy soil. It is propagated through seeds and harvested when fruits become fully mature.

Collection

Mature umbels are cut, dried, threshed, cleaned, and stored under dry conditions.

Macroscopy

The fruits are elongated, cylindrical, greenish-yellow, aromatic, and sweet with prominent longitudinal ridges.

Microscopy

The fruit contains epidermis, vittae (oil ducts), vascular bundles, endosperm, aleurone grains, and fixed oil.

Powder Study

The powder is yellowish-green with oil ducts, parenchyma, sclerenchyma fibers, and fragments of endosperm.

Chemical Constituents

The volatile oil mainly contains **anethole**, fenchone, estragole, limonene, α-pinene, and fixed oil.

Identification Tests

Test	Positive Observation
Sudan III Test	Red coloration
Filter Paper Test	Temporary translucent spot
Solubility Test	Soluble in alcohol

Evaluation

Evaluation includes volatile oil determination, moisture content, ash values, refractive index, and chromatographic analysis.

Therapeutic Uses

Fennel is used as a carminative, digestive stimulant, expectorant, antispasmodic, galactagogue, and mild antimicrobial agent.

Pharmaceutical Applications

Included in digestive formulations, pediatric gripe water, cough syrups, herbal teas, mouth fresheners, and gastrointestinal preparations.

Commercial Applications

Widely utilized in spice blends, confectionery, bakery products, beverages, cosmetics, perfumes, and flavoring industries.

Storage

Store in well-closed containers in a cool, dry place protected from moisture and direct sunlight.

Lemongrass, Clove, Cinnamon, and Fennel are important volatile oil drugs that contain essential oils responsible for their characteristic aroma and therapeutic properties. Their principal constituents include citral (Lemongrass), eugenol (Clove), cinnamaldehyde (Cinnamon), and anethole (Fennel). These drugs are widely used as carminatives, antimicrobial agents, antioxidants, flavoring agents, and pharmaceutical excipients, while also having extensive applications in the food, cosmetic, perfumery, and aromatherapy industries.

6. TANNIN DRUGS

Tannins are naturally occurring high-molecular-weight polyphenolic compounds capable of precipitating proteins, gelatin, and alkaloids. They possess a characteristic astringent taste and play an important role in plant defense against insects, microorganisms, and herbivores. Tannin-containing drugs are widely used as astringents, antidiarrheal agents, antioxidants, antimicrobial agents, anti-inflammatory drugs, wound-healing agents, and hemostatics. The important tannin drugs included in the B.Pharm syllabus are Myrobalans, Catechu, and Pomegranate.

6.1 Myrobalans

Introduction

Myrobalans is an important tannin-containing crude drug obtained from the dried fruits of *Terminalia chebula*. It is commonly known as Haritaki and is regarded as one of the most valuable medicinal plants in Ayurveda. The fruits contain abundant hydrolysable tannins responsible for their strong astringent, antioxidant, antimicrobial, laxative, and wound-healing properties. Myrobalans is also an important constituent of the famous Ayurvedic formulation Triphala.

Biological Source

Myrobalans consists of the dried mature fruits of ***Terminalia chebula* Retz.**

Family

Combretaceae

Distribution

The plant is widely distributed throughout India, Nepal, Sri Lanka, Bangladesh, Myanmar, Thailand, and other Southeast Asian countries. In India, it grows abundantly in forests of Madhya Pradesh, Uttar Pradesh, Tamil Nadu, Karnataka, Kerala, and West Bengal.

Cultivation

The plant grows well in tropical and subtropical climates with well-drained fertile soils. It is propagated mainly through seeds and requires moderate rainfall and sunlight for optimum growth.

Collection

Fully mature fruits are collected during winter, cleaned, shade dried, and stored in moisture-free containers.

Macroscopy

The fruits are oval to oblong, yellowish-brown to dark brown, hard, wrinkled, and possess a characteristic astringent taste.

Microscopy

The fruit shows epidermis, parenchymatous cells, stone cells, sclerenchyma fibers, vascular bundles, and numerous tannin-containing cells.

Powder Study

The powder is brownish-yellow and contains stone cells, fibers, starch grains, parenchyma, and tannin cells.

Chemical Constituents

Major constituents include chebulagic acid, chebulinic acid, gallic acid, ellagic acid, tannic acid, flavonoids, amino acids, and sugars.

Identification Tests

Test	Positive Observation
Ferric Chloride Test	Blue-black coloration
Gelatin Test	White precipitate
Lead Acetate Test	White precipitate

Evaluation

Evaluation includes determination of tannin content, ash values, moisture content, extractive values, and chromatographic fingerprinting.

Therapeutic Uses

Used as an astringent, mild laxative, antioxidant, antimicrobial, antidiarrheal, digestive tonic, and wound-healing agent.

Pharmaceutical Applications

Included in Ayurvedic formulations, herbal capsules, digestive preparations, mouthwashes, and antioxidant formulations.

Commercial Applications

Used in leather tanning, dye manufacturing, herbal medicines, nutraceuticals, and cosmetic preparations.

Storage

Store in tightly closed containers protected from moisture and insects.

6.2 Catechu

Introduction

Catechu is a tannin-rich drug obtained from the heartwood of *Acacia catechu*. It is a well-known natural astringent widely used in traditional medicine for the treatment of diarrhea, sore throat, mouth ulcers, and bleeding disorders. The drug possesses antimicrobial, antioxidant, anti-inflammatory, and wound-healing activities.

Biological Source

Catechu is the dried aqueous extract prepared from the heartwood of ***Acacia catechu* (L.f.) Willd.**

Family

Fabaceae (Leguminosae)

Distribution

The tree is widely distributed in India, Nepal, Pakistan, Bangladesh, and Myanmar. Commercial production occurs mainly in Uttar Pradesh, Madhya Pradesh, Rajasthan, and Bihar.

Cultivation

The plant grows well in dry tropical climates and is propagated by seeds. It prefers well-drained sandy or loamy soils.

Collection

The heartwood is cut into small pieces, boiled in water, filtered, concentrated, and dried to obtain catechu.

Macroscopy

Catechu occurs as irregular dark brown or black brittle masses with a shiny fractured surface and strongly astringent taste.

Microscopy

The powder contains lignified fibers, vessels, stone cells, medullary ray cells, and abundant tannin deposits.

Powder Study

Brown powder containing fibers, vessels, sclereids, tannin cells, and parenchyma.

Chemical Constituents

Major constituents include **catechin, catechutannic acid, quercetin, tannins, flavonoids**, and gums.

Identification Tests

Test	Positive Observation
Ferric Chloride Test	Greenish-black coloration
Gelatin Test	White precipitate
Lead Acetate Test	White precipitate

Evaluation

Determination of tannin content, moisture, ash values, extractive values, and chromatographic profile.

Therapeutic Uses

Used as an astringent, antidiarrheal, antioxidant, antimicrobial, anti-inflammatory, and wound-healing agent.

Pharmaceutical Applications

Used in throat lozenges, mouthwashes, herbal preparations, and dental formulations.

Commercial Applications

Utilized in leather tanning, textile dyeing, food industry, and traditional medicines.

Storage

Store in airtight containers away from moisture.

6.3 Pomegranate

Introduction

Pomegranate is an important medicinal plant rich in hydrolysable tannins, particularly in its fruit rind. The rind possesses excellent antioxidant, antimicrobial, anthelmintic, and astringent properties. Besides medicinal uses, pomegranate is widely consumed as a nutritious fruit rich in vitamins and polyphenols.

Biological Source

The drug consists of the dried fruit rind of ***Punica granatum* L.**

Family

Lythraceae

Distribution

Pomegranate is cultivated throughout India, Iran, Afghanistan, Mediterranean countries, Turkey, Spain, and the Middle East.

Cultivation

It grows well in warm climates with well-drained fertile soils and moderate irrigation.

Collection

Fully mature fruits are harvested, the rind is separated, shade dried, and stored.

Macroscopy

The rind is hard, reddish-brown, leathery, rough externally, yellowish internally, and strongly astringent.

Microscopy

Contains epidermis, sclerenchyma, stone cells, vascular bundles, tannin cells, and calcium oxalate crystals.

Powder Study

Brown powder containing stone cells, fibers, tannin cells, and parenchymatous tissue.

Chemical Constituents

Contains **punicalagin, punicalin, ellagic acid, gallic acid, tannins, flavonoids, anthocyanins**, and organic acids.

Identification Tests

Test	**Positive Observation**
Ferric Chloride Test	Blue-black coloration
Gelatin Test	White precipitate
Lead Acetate Test	White precipitate

Evaluation

Evaluation includes tannin estimation, ash values, extractive values, and chromatographic analysis.

Therapeutic Uses

Used as an astringent, antioxidant, antimicrobial, antidiarrheal, anthelmintic, and gastroprotective agent.

Pharmaceutical Applications

Used in herbal syrups, antidiarrheal formulations, antioxidant supplements, and oral care products.

Commercial Applications

Extensively utilized in food processing, beverages, nutraceuticals, cosmetics, and natural preservatives.

Storage

Store in dry, airtight containers protected from moisture.

7. RESIN DRUGS

Resins are amorphous, non-volatile, solid or semi-solid plant exudates composed mainly of diterpenes, triterpenes, resin acids, esters, and neutral resin compounds. They are secreted naturally or after injury to protect plants against insects and microorganisms. Resin drugs possess anti-inflammatory, antimicrobial, expectorant, antiseptic, carminative, antioxidant, and wound-healing activities. Two important medicinal resin drugs are Guggul and Asafoetida.

7.1 Guggul

Introduction

Guggul is an oleo-gum-resin obtained from *Commiphora wightii*. It has been used extensively in Ayurveda for the treatment of obesity, arthritis, hyperlipidemia, inflammation, and cardiovascular disorders. The biologically active compounds, known as **guggulsterones**, exhibit hypolipidemic and anti-inflammatory activities.

Biological Source

Guggul is the oleo-gum-resin obtained from ***Commiphora wightii* (Arn.) Bhandari**.

Family

Burseraceae

Distribution

Found in India, Pakistan, and parts of Arabia. In India, it occurs mainly in Rajasthan and Gujarat.

Cultivation

The plant grows in dry arid climates with sandy soils and is propagated by stem cuttings or seeds.

Collection

Resin is collected by making incisions in the bark, after which the exuded oleo-gum-resin is allowed to harden and is collected.

Macroscopy

Occurs as irregular yellowish-brown or dark brown masses with aromatic odor and bitter taste.

Microscopy

Contains resin ducts, gum cells, oil cells, and parenchymatous tissue.

Powder Study

Brown powder containing resin fragments, gum particles, fibers, and oil cells.

Chemical Constituents

Contains guggulsterones (E & Z), resin, volatile oil, gum, steroids, diterpenes, and lignans.

Identification Tests

Test	Positive Observation
Turbidity Test	Milky turbidity
Copper Acetate Test	Green coloration

Evaluation

Determination of resin content, volatile oil content, ash values, and chromatographic fingerprint.

Therapeutic Uses

Used as an anti-inflammatory, hypolipidemic, antiarthritic, antioxidant, anti-obesity, and cardioprotective agent.

Pharmaceutical Applications

Used in lipid-lowering formulations, antiarthritic medicines, herbal capsules, and Ayurvedic preparations.

Commercial Applications

Widely used in nutraceuticals, cosmetics, incense, perfumes, and traditional medicine.

Storage

Store in tightly closed containers away from heat and moisture.

7.2 Asafoetida

Introduction

Asafoetida is an oleo-gum-resin obtained from the roots of *Ferula asafoetida*. It possesses a strong characteristic odor due to sulfur-containing volatile compounds and has been used traditionally as a digestive stimulant, carminative, expectorant, and antispasmodic.

Biological Source

Asafoetida is the oleo-gum-resin obtained from the roots and rhizomes of ***Ferula asafoetida* L.**

Family

Apiaceae (Umbelliferae)

Distribution

Native to Iran and Afghanistan and widely imported into India for medicinal and culinary purposes.

Cultivation

The plant grows in dry temperate climates and sandy soils. Resin is obtained from mature plants by tapping the roots.

Collection

Deep incisions are made at the root crown, and the exuded resin is collected periodically until secretion ceases.

Macroscopy

Occurs as irregular yellowish-brown masses with a characteristic strong garlic-like odor and bitter acrid taste.

Microscopy

Contains resin cells, oil ducts, parenchyma, fibers, and gum particles.

Powder Study

Yellowish-brown powder containing resin masses, oil cells, parenchyma, and starch grains.

Chemical Constituents

Contains resin (40–65%), gum (20–25%), volatile oil (4–20%), ferulic acid, sulfur compounds, and sesquiterpene coumarins.

Identification Tests

Test	Positive Observation
Turbidity Test	Milky turbidity
Acetone Test	Complete dissolution
Copper Acetate Test	Green coloration

Evaluation

Evaluation includes determination of resin content, volatile oil content, ash values, and moisture content.

Therapeutic Uses

Used as a carminative, antispasmodic, expectorant, antimicrobial, digestive stimulant, and anthelmintic agent.

Pharmaceutical Applications

Used in digestive formulations, cough syrups, antispasmodic preparations, and Ayurvedic medicines.

Commercial Applications

Widely used as a culinary spice, flavoring agent, food preservative, incense ingredient, and herbal medicine.

Storage

Store in airtight containers in a cool, dry place away from light and moisture.

Myrobalans, Catechu, and Pomegranate are important tannin-containing drugs possessing strong astringent, antioxidant, antimicrobial, and antidiarrheal properties, while Guggul and Asafoetida are therapeutically important resin drugs used mainly for anti-inflammatory, hypolipidemic, digestive, expectorant, and antimicrobial purposes. These crude drugs continue to play a vital role in modern pharmaceuticals, Ayurveda, nutraceuticals, cosmetics, and food industries due to their diverse pharmacological activities and commercial significance.

8. GLYCOSIDE DRUGS

Glycosides are naturally occurring secondary metabolites composed of a sugar (glycone) linked to a non-sugar (aglycone or genin) through a glycosidic bond. They are widely distributed in higher plants and exhibit diverse pharmacological activities depending on the nature of the aglycone. Glycoside-containing drugs are extensively used as laxatives, cardiotonics, anti-inflammatory agents, expectorants, hepatoprotective agents, and antioxidants. The important glycoside drugs included in the B.Pharm syllabus are Senna, Liquorice, and Digitalis.

8.1 Senna

Introduction

Senna is one of the most important natural stimulant laxatives obtained from the dried leaflets and pods of *Cassia angustifolia* and *Cassia acutifolia*. The drug contains **anthraquinone glycosides**, mainly sennosides, which stimulate intestinal peristalsis and promote bowel evacuation. Senna is widely used for the treatment of constipation and bowel preparation before surgical and diagnostic procedures.

Biological Source

Senna consists of the dried leaflets and pods of ***Cassia angustifolia* Vahl** and ***Cassia acutifolia* Delile**.

Family

Fabaceae (Caesalpiniaceae)

Distribution

Cultivated mainly in India, Sudan, Egypt, Saudi Arabia, and Pakistan. In India, it is extensively grown in Tamil Nadu and Rajasthan.

Cultivation

Grows well in warm, dry climates with sandy loam soil. It is propagated through seeds and harvested after 3–4 months.

Collection

Fully developed leaves and pods are collected, shade dried, cleaned, and stored.

Macroscopy

Leaflets are lanceolate, yellowish-green, brittle, and slightly bitter with a faint characteristic odor.

Microscopy

Shows epidermal cells, paracytic stomata, calcium oxalate crystals, vascular bundles, and palisade tissue.

Powder Study

Contains epidermal fragments, fibers, vessels, calcium oxalate crystals, and stomata.

Chemical Constituents

Major constituents include Sennoside A, Sennoside B, Rhein, Aloe-emodin, flavonoids, mucilage, and resin.

Identification Tests

Test	Positive Observation
Borntrager's Test	Pink to cherry-red color
Modified Borntrager's Test	Red coloration

Evaluation

Determination of sennoside content, ash values, extractive values, and TLC/HPLC analysis.

Therapeutic Uses

Used as a stimulant laxative in constipation and bowel cleansing.

Pharmaceutical Applications

Used in tablets, syrups, granules, herbal teas, and laxative formulations.

Commercial Applications

Widely used in herbal medicine and pharmaceutical industries.

Storage

Store in airtight containers away from moisture and light.

8.2 Liquorice

Introduction

Liquorice is obtained from the roots and stolons of *Glycyrrhiza glabra*. It is one of the oldest medicinal plants known for its sweet taste due to **glycyrrhizin**, a triterpenoid saponin glycoside. Liquorice exhibits anti-inflammatory, expectorant, anti-ulcer, hepatoprotective, antiviral, and immunomodulatory activities.

Biological Source

Dried roots and stolons of ***Glycyrrhiza glabra* L.**

Family

Fabaceae

Distribution

Cultivated in India, Iran, Afghanistan, China, Russia, Spain, and Mediterranean countries.

Cultivation

Prefers fertile sandy loam soil and moderate climatic conditions. Propagated by root cuttings.

Collection

Roots are harvested after 3–4 years, cleaned, peeled if necessary, and dried.

Macroscopy

Roots are cylindrical, yellowish-brown externally, fibrous internally, with sweet taste and faint odor.

Microscopy

Contains cork, cortex, medullary rays, vessels, fibers, starch grains, and calcium oxalate crystals.

Powder Study

Contains fibers, vessels, starch grains, cork cells, and parenchyma.

Chemical Constituents

Major constituents include Glycyrrhizin, Liquiritin, Isoliquiritigenin, Flavonoids, Couma**rins**, and sugars.

Identification Tests

Test	Positive Observation
Foam Test	Stable persistent froth

Evaluation

Determination of glycyrrhizin content, ash values, moisture, extractive values, and chromatography.

Therapeutic Uses

Used as an expectorant, anti-ulcer, anti-inflammatory, antiviral, demulcent, and hepatoprotective agent.

Pharmaceutical Applications

Used in cough syrups, throat lozenges, gastric formulations, herbal preparations, and flavoring agents.

Commercial Applications

Widely used in confectionery, beverages, tobacco industry, cosmetics, and pharmaceuticals.

Storage

Store in well-closed containers protected from moisture.

8.3 Digitalis

Introduction

Digitalis is a well-known cardiotonic drug obtained from the dried leaves of *Digitalis purpurea* and *Digitalis lanata*. It contains **cardiac glycosides** that increase the force of cardiac contraction and improve cardiac efficiency. Digitalis preparations remain valuable in the treatment of congestive heart failure and certain cardiac arrhythmias.

Biological Source

Dried leaves of ***Digitalis purpurea* L.** and ***Digitalis lanata* Ehrh.**

Family

Plantaginaceae (formerly Scrophulariaceae)

Distribution

Native to Europe and cultivated in India, Germany, England, Hungary, and the United States.

Cultivation

Requires cool climate, fertile well-drained soil, and moderate rainfall. Propagated through seeds.

Collection

Leaves are harvested during the flowering stage and carefully dried.

Macroscopy

Leaves are ovate, wrinkled, grayish-green, hairy, bitter, and slightly odorless.

Microscopy

Contains multicellular covering hairs, glandular trichomes, anisocytic stomata, calcium oxalate crystals, and vascular bundles.

Powder Study

Shows trichomes, vessels, fibers, epidermal fragments, and calcium oxalate crystals.

Chemical Constituents

Contains **Digitoxin, Digoxin, Gitoxin, Lanatosides A, B & C**, flavonoids, and steroids.

Identification Tests

Test	**Positive Observation**
Keller-Killiani Test	Brown ring with bluish-green layer
Legal Test	Pink-red coloration
Baljet Test	Orange-red color

Evaluation

Evaluation includes glycoside assay, ash values, moisture, extractive values, and HPLC analysis.

Therapeutic Uses

Used in congestive heart failure, atrial fibrillation, and supraventricular arrhythmias.

Pharmaceutical Applications

Used in tablets, injections, and cardiac formulations.

Commercial Applications

Cultivated commercially for isolation of cardiac glycosides.

Storage

Store in airtight, light-resistant containers.

9. PHENYLPROPANOIDS AND FLAVONOIDS

Phenylpropanoids and flavonoids are naturally occurring polyphenolic secondary metabolites synthesized through the shikimate and phenylpropanoid pathways. These compounds possess antioxidant, anti-inflammatory, cardioprotective, antimicrobial, neuroprotective, hepatoprotective, and anticancer properties. Important drugs include Green Tea, Ginkgo, and Flax Seed.

9.1 Green Tea

Introduction

Green tea is obtained from the leaves of *Camellia sinensis* and is rich in catechins, particularly **epigallocatechin gallate (EGCG)**, a powerful antioxidant.

Biological Source

Dried leaves of ***Camellia sinensis* (L.) Kuntze**.

Family

Theaceae

Distribution

India, China, Japan, Sri Lanka, and Kenya.

Cultivation & Collection

Cultivated in cool humid regions; young leaves are hand-plucked and dried without fermentation.

Macroscopy

Green, curled leaves with aromatic odor and slightly bitter taste.

Chemical Constituents

Catechins, **EGCG**, epicatechin, caffeine, flavonoids, tannins.

Identification Tests

Test	Positive Observation
Shinoda Test	Pink-red color
Ferric Chloride Test	Green coloration

Therapeutic Uses

Antioxidant, cardioprotective, anti-obesity, antidiabetic, anticancer.

Pharmaceutical & Commercial Applications

Used in herbal capsules, beverages, nutraceuticals, cosmetics, and functional foods.

Storage

Store in airtight containers away from moisture.

9.2 Ginkgo

Introduction

Ginkgo is obtained from the leaves of *Ginkgo biloba* and is one of the most extensively studied herbal medicines for improving cerebral circulation and memory.

Biological Source

Leaves of ***Ginkgo biloba* L.**

Family

Ginkgoaceae

Distribution

Native to China and cultivated worldwide.

Cultivation & Collection

Cultivated in temperate climates; mature leaves are harvested and dried.

Macroscopy

Fan-shaped leaves with dichotomous venation.

Chemical Constituents

Ginkgolides, Bilobalide, Quercetin, Kaempferol, flavonoids.

Identification Tests

Test	**Positive Observation**
Shinoda Test	Pink coloration
Lead Acetate Test	Yellow precipitate

Therapeutic Uses

Memory enhancer, antioxidant, neuroprotective, peripheral vasodilator.

Pharmaceutical & Commercial Applications

Used in capsules, tablets, herbal extracts, and nutraceuticals.

Storage

Store in dry, airtight containers.

9.3 Flax Seed

Introduction

Flax seed is obtained from *Linum usitatissimum* and is an excellent source of lignans, omega-3 fatty acids, mucilage, and flavonoids.

Biological Source

Seeds of ***Linum usitatissimum* L.**

Family

Linaceae

Distribution

India, Canada, China, Russia, and Europe.

Cultivation & Collection

Cultivated in cool climates and harvested after seed maturation.

Macroscopy

Small, flat, shiny brown seeds with mucilaginous taste.

Chemical Constituents

Secoisolariciresinol diglucoside (SDG), lignans, α-linolenic acid, mucilage, proteins.

Identification Tests

Test	**Positive Observation**
Shinoda Test	Pink coloration

Therapeutic Uses

Cardioprotective, antioxidant, laxative, hypolipidemic, anti-inflammatory.

Pharmaceutical & Commercial Applications

Used in nutraceuticals, bakery products, dietary supplements, and functional foods.

Storage

Store in cool, dry, airtight containers.

10. IRIDOIDS, OTHER TERPENOIDS AND NAPHTHOQUINONES

Iridoids and terpenoids are important plant secondary metabolites exhibiting anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, digestive, anticancer, and antimalarial activities. Important drugs included in this category are Gentian and Artemisia.

10.1 Gentian

Introduction

Gentian is a bitter medicinal drug obtained from the roots and rhizomes of *Gentiana lutea*. It is rich in iridoid glycosides that stimulate gastric secretion and improve digestion.

Biological Source

Roots and rhizomes of ***Gentiana lutea* L.**

Family

Gentianaceae

Distribution

Europe, Himalayan regions, and temperate countries.

Cultivation

Grown in cool mountainous regions with fertile soil.

Collection

Roots are harvested from mature plants, cleaned, and dried.

Macroscopy

Cylindrical yellowish-brown roots with intensely bitter taste.

Chemical Constituents

Gentiopicroside, Amarogentin, Gentisin, xanthones.

Identification Tests

Test	Positive Observation
Trim-Hill Test	Blue coloration

Therapeutic Uses

Bitter tonic, digestive stimulant, appetizer, hepatoprotective.

Pharmaceutical Applications

Used in digestive tonics and herbal formulations.

Commercial Applications

Used in herbal medicine and bitter beverages.

Storage

Store in airtight containers.

10.2 Artemisia

Introduction

Artemisia is an important medicinal plant containing the sesquiterpenoid **artemisinin**, one of the most effective natural antimalarial agents. Besides antimalarial activity, the plant possesses antioxidant, anti-inflammatory, antimicrobial, and anticancer properties.

Biological Source

Leaves and flowering tops of ***Artemisia annua* L.**

Family

Asteraceae

Distribution

China, India, Vietnam, Europe, Africa, and South America.

Cultivation

Cultivated in temperate climates with fertile, well-drained soils.

Collection

Leaves and flowering tops are harvested before full flowering and shade dried.

Macroscopy

Green, aromatic, deeply divided leaves with characteristic odor.

Chemical Constituents

Artemisinin, Artemisinic acid, Artemisinin derivatives, Flavonoids, Essential oil.

Identification Tests

Test	Positive Observation
Liebermann–Burchard Test	Green coloration
Salkowski Test	Reddish-brown ring

Therapeutic Uses

Antimalarial, antimicrobial, anti-inflammatory, antioxidant, anticancer.

Pharmaceutical Applications

Used in antimalarial tablets, capsules, injections, and combination therapies.

Commercial Applications

Widely cultivated for pharmaceutical extraction of artemisinin and herbal products.

Storage

Store in tightly closed, light-resistant containers.

Senna, Liquorice, and Digitalis are important glycoside drugs used respectively as laxative, expectorant, and cardiotonic agents. Green Tea, Ginkgo, and Flax Seed are rich in phenylpropanoids and flavonoids, providing strong antioxidant and cardioprotective benefits. Gentian is a valuable iridoid-containing bitter tonic, whereas Artemisia is an important terpenoid-containing medicinal plant that serves as the primary natural source of the life-saving antimalarial drug artemisinin. Together, these drugs have significant pharmaceutical, nutraceutical, and commercial importance in modern pharmacognosy.

UNIT – IV

Extraction Methods for Medicinal Plants

1. INTRODUCTION OF EXTRACTION METHODS FOR MEDICINAL PLANTS

Extraction is the process of separating biologically active chemical constituents (phytoconstituents) from crude plant materials using suitable solvents and extraction techniques. It is one of the most important steps in pharmacognosy because the therapeutic efficacy of herbal drugs largely depends on the quality and quantity of the constituents extracted. Medicinal plants contain numerous primary and secondary metabolites such as alkaloids, glycosides, flavonoids, tannins, terpenoids, phenolic compounds, volatile oils, saponins, steroids, and polysaccharides, which are responsible for their pharmacological activities. These compounds are usually present in complex plant matrices and cannot be utilized directly; therefore, extraction is essential to isolate them in a concentrated and usable form.

The extraction process involves the transfer of soluble constituents from plant tissues into a suitable solvent through diffusion, osmosis, and dissolution. Different extraction techniques are selected depending on the nature of the plant material, chemical properties of the active constituents, desired yield, purity, and intended pharmaceutical application. Traditionally, extraction has been carried out using conventional methods such as infusion, decoction, maceration, percolation, digestion, reflux, distillation, and Soxhlet extraction. In recent years, advanced extraction technologies such as supercritical fluid extraction, microwave-assisted extraction, ultrasonic-assisted extraction, enzyme-assisted extraction, and pressurized liquid extraction have gained considerable attention because they provide higher extraction efficiency, reduced solvent consumption, shorter extraction time, and better preservation of heat-sensitive compounds.

Extraction is widely employed in the pharmaceutical, nutraceutical, cosmetic, food, and herbal industries for the preparation of extracts, tinctures, essential oils, standardized herbal formulations, and phytopharmaceutical products. The quality of the final extract depends not only on the extraction method but also on several operational parameters including solvent selection, extraction temperature, extraction time, particle size of the plant material, moisture content, pH, solvent-to-solid ratio, and agitation. Proper optimization of these parameters ensures maximum recovery of bioactive compounds while minimizing degradation or contamination.

With the growing global demand for herbal medicines and natural products, extraction technology has become an indispensable component of medicinal plant research and industrial production. Modern extraction techniques are increasingly replacing conventional methods because they are more efficient, environmentally friendly, and suitable for large-scale manufacturing. Therefore, understanding the principles and factors influencing extraction is essential for obtaining high-quality herbal extracts with consistent therapeutic efficacy.

Importance of Extraction in Pharmacognosy and Herbal Medicine

Extraction plays a vital role in pharmacognosy because it enables the isolation of therapeutically active phytochemicals from crude medicinal plants. It improves the concentration, stability, and bioavailability of herbal constituents, making them suitable for pharmaceutical formulations. Extraction also facilitates phytochemical screening, quality control, standardization, and identification of medicinal compounds. In herbal medicine, standardized extracts ensure consistent potency, safety, and therapeutic effectiveness. Furthermore, extraction serves as the first step in the discovery of new natural drugs and the development of phytopharmaceuticals.

Objectives of Plant Extraction

- To isolate bioactive phytoconstituents from medicinal plants.
- To obtain concentrated and standardized herbal extracts.
- To remove unwanted or inert plant materials.
- To improve the stability and shelf life of herbal preparations.
- To facilitate phytochemical analysis and quality control.
- To prepare extracts suitable for pharmaceutical, cosmetic, food, and nutraceutical applications.
- To maximize extraction yield while preserving active constituents.

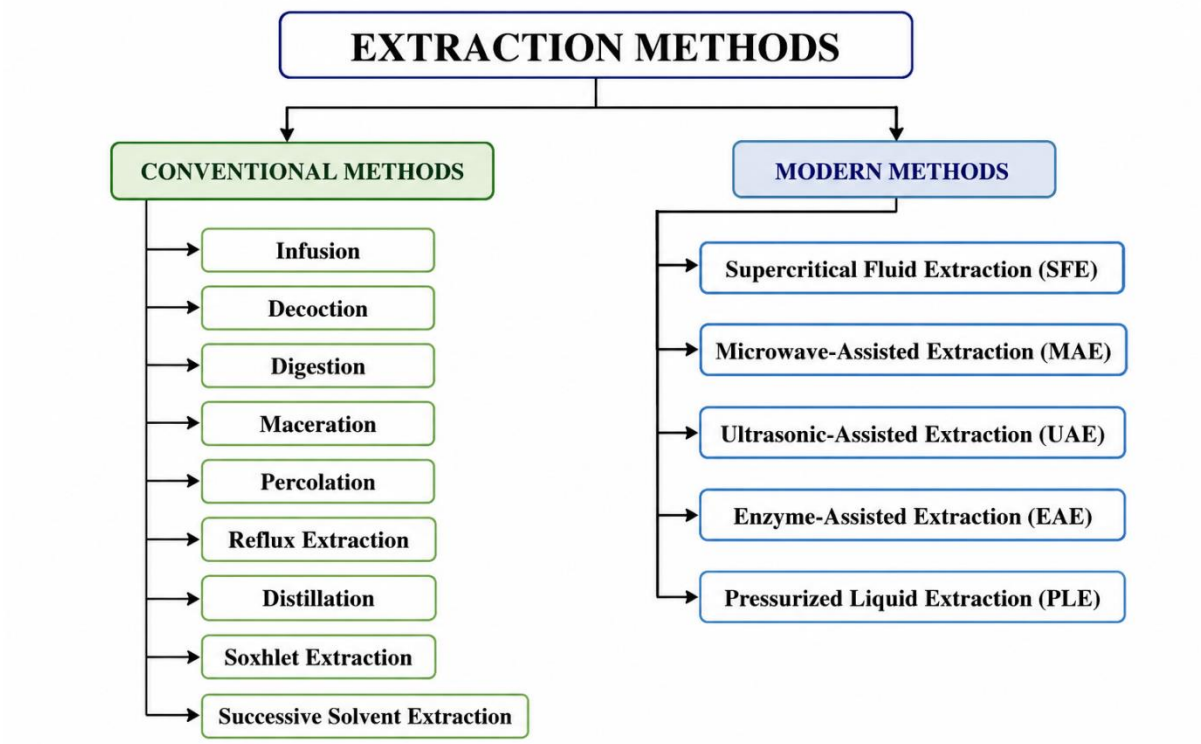
Factors Affecting Extraction Efficiency

Several physical and chemical factors influence the efficiency of plant extraction. Proper optimization of these parameters improves extraction yield, reduces solvent consumption, and enhances the quality of the final extract.

Factor	Effect on Extraction
Nature of plant material	Fresh or dried plant material, hardness, and plant tissue structure influence solvent penetration and extraction efficiency.
Particle size	Smaller particles provide a larger surface area, increasing extraction rate and yield. Excessively fine powder may obstruct solvent flow.
Moisture content	Appropriate moisture softens plant tissues and facilitates solvent penetration, whereas excessive moisture may dilute the solvent and reduce extraction efficiency.
Solvent polarity	Solvents should possess polarity similar to the target phytochemicals to achieve maximum extraction efficiency.
Temperature	Higher temperature generally accelerates diffusion and solubility of constituents but may degrade thermolabile compounds.
Extraction time	Adequate extraction time ensures maximum recovery, while prolonged extraction may lead to degradation or unnecessary impurity extraction.
pH	The pH affects the ionization and stability of many phytochemicals, thereby influencing extraction yield.
Agitation	Continuous stirring or shaking improves solvent penetration and enhances mass transfer between plant material and solvent.
Solvent-to-solid ratio	An optimum solvent volume ensures complete immersion of plant material and efficient extraction without excessive solvent consumption.

The selection of an appropriate extraction method and optimization of these influencing factors are essential for obtaining high-quality herbal extracts with maximum recovery of biologically active compounds. Standardized extraction procedures not only improve the therapeutic efficacy of herbal products but also ensure reproducibility, quality assurance, and regulatory compliance in pharmaceutical and herbal industries.

2. CLASSIFICATION OF EXTRACTION METHODS



3. CONVENTIONAL METHODS OF EXTRACTION

Conventional extraction methods are the oldest and most widely used techniques for isolating bioactive constituents from medicinal plants. These methods primarily rely on the principle of diffusion, osmosis, and solvent penetration to dissolve the desired phytochemicals from plant tissues. Conventional techniques are simple, economical, require minimal instrumentation, and are suitable for both laboratory-scale and small-scale industrial applications. Although these methods often require longer extraction times and higher solvent consumption than modern extraction techniques, they remain indispensable in pharmacognosy because of their reliability and ease of operation. The choice of a conventional extraction method depends on the nature of the plant material, the chemical properties of the active constituents, and the intended pharmaceutical application.

3.1 Infusion

Introduction

Infusion is one of the simplest and oldest extraction methods used for obtaining water-soluble constituents from soft plant materials such as leaves, flowers, and tender stems. In this method, boiling or hot water is poured over the plant material and allowed to stand for a specified period so that the soluble constituents diffuse into the solvent. The process is similar to the preparation of herbal tea and is particularly suitable for extracting heat-sensitive compounds that require only mild heating. Infusion is widely employed in traditional medicine as well as in the preparation of household herbal remedies because it is convenient, inexpensive, and does not require specialized equipment.

Principle

Infusion is based on the diffusion of soluble phytochemicals from plant tissues into hot water under atmospheric pressure. Heat increases the solubility of active constituents and enhances solvent penetration into plant cells, resulting in efficient extraction.

Procedure

Fresh or dried plant material is cleaned and coarsely powdered. The material is placed in a suitable container, and freshly boiled water is poured over it. The container is covered to prevent the loss of volatile constituents and allowed to stand for approximately 10–20 minutes. The mixture is then filtered to separate the extract from the plant residue, and the filtrate is used immediately or stored under suitable conditions.

Equipment Required

Equipment	**Purpose**
Beaker or infusion vessel	Extraction container
Hot water source	Heating
Measuring cylinder	Solvent measurement
Stirring rod	Mixing
Filter paper or muslin cloth	Filtration

Advantages

- Simple and inexpensive technique.
- No sophisticated equipment required.
- Suitable for heat-sensitive compounds.
- Rapid preparation.
- Ideal for domestic herbal preparations.

Limitations

- Suitable only for water-soluble constituents.
- Short shelf life of the extract.
- Low extraction efficiency for poorly soluble compounds.
- Not suitable for woody plant materials.

Applications

- Herbal teas
- Ayurvedic formulations
- Traditional medicinal preparations
- Extraction of flavonoids and phenolics

Examples

- Chamomile flowers
- Peppermint leaves
- Green tea leaves
- Tulsi leaves

3.2 Decoction

Introduction

Decoction is a conventional extraction method used mainly for hard and woody plant materials such as roots, rhizomes, bark, seeds, and stems. Unlike infusion, decoction involves boiling the crude drug in water for a prolonged period to extract active constituents that are not easily released under mild conditions. This technique has been extensively practiced in Ayurveda, Traditional Chinese Medicine, and other herbal systems for centuries. Decoction provides higher extraction efficiency for water-soluble constituents present in hard plant tissues but may not be suitable for volatile or heat-sensitive compounds.

Principle

Decoction operates on the principle that prolonged boiling softens rigid plant tissues, allowing water to penetrate deeply and dissolve active constituents. Continuous heating increases mass transfer and facilitates the extraction of bioactive compounds.

Procedure

The coarse plant material is placed in a suitable vessel with the required quantity of water. The mixture is heated to boiling and maintained at a gentle boil for 15–60 minutes depending on the nature of the drug. After cooling slightly, the extract is filtered to remove insoluble plant residue, and the filtrate is collected for further use.

Equipment Required

Equipment	Purpose
Stainless steel vessel	Boiling
Heating mantle or burner	Heating
Measuring cylinder	Solvent measurement
Filter paper	Filtration
Stirring rod	Mixing

Advantages

- Efficient for hard plant materials.
- High extraction yield of water-soluble constituents.
- Simple and economical.
- Suitable for large-scale traditional preparations.

Limitations

- Not suitable for volatile oils.
- Heat-sensitive compounds may degrade.
- Longer extraction time.
- Higher energy consumption.

Applications

- Ayurvedic decoctions (Kwath)
- Herbal syrups
- Traditional medicinal formulations
- Extraction of tannins and polysaccharides

Examples

- Liquorice root
- Cinnamon bark
- Ginger rhizome
- Ashwagandha root

3.3 Digestion

Introduction

Digestion is a modified form of maceration in which extraction is performed at moderately elevated temperatures, usually between **40°C and 60°C**, to accelerate the dissolution of phytoconstituents. Gentle heating increases solvent penetration and diffusion without causing significant degradation of

most active compounds. Digestion is commonly used when room-temperature maceration is insufficient to achieve satisfactory extraction efficiency. This method is suitable for extracting constituents that are moderately heat stable and readily soluble in the selected solvent.

Principle

Digestion is based on the combined effect of solvent penetration and mild heating, which enhances the solubility of phytochemicals and accelerates diffusion from plant tissues into the extraction solvent.

Procedure

The powdered crude drug is placed in a closed extraction vessel, and the selected solvent is added. The mixture is maintained at **40–60°C** with occasional stirring for several hours. After completion of extraction, the mixture is filtered, and the extract is concentrated if required.

Equipment Required

Equipment	**Purpose**
Digestion vessel	Extraction
Water bath	Controlled heating
Thermometer	Temperature monitoring
Stirring rod	Agitation
Filter paper	Filtration

Advantages

- Faster than maceration.
- Improved extraction efficiency.
- Mild heating minimizes degradation.
- Suitable for moderately heat-stable compounds.

Limitations

- Not suitable for highly volatile constituents.
- Requires temperature control.
- Some sensitive compounds may still degrade.

Applications

- Herbal tinctures
- Pharmaceutical extracts
- Medicinal plant research
- Laboratory-scale extraction

Examples

- Senna leaves
- Gentian root
- Digitalis leaves
- Liquorice root

3.4 Maceration

Introduction

Maceration is one of the most widely employed conventional extraction methods in pharmacognosy. It involves soaking powdered medicinal plant material in a suitable solvent at room temperature for a predetermined period, allowing the solvent to diffuse into plant tissues and dissolve the active constituents. The method is particularly suitable for extracting thermolabile compounds because no external heating is required. Maceration is extensively used in pharmaceutical industries for preparing tinctures, fluid extracts, and herbal formulations due to its simplicity and versatility.

Principle

Maceration is based on the diffusion of soluble constituents from plant cells into the surrounding solvent until equilibrium is established between the concentration inside and outside the plant tissues.

Types of Maceration

Type	Description
Simple maceration	Single soaking of plant material in solvent.
Double maceration	Extraction performed in two successive stages for improved yield.
Triple maceration	Extraction carried out in three stages to maximize recovery of phytoconstituents.
Modified maceration	Incorporates occasional stirring or controlled conditions to improve efficiency.

Procedure

The crude drug is dried, coarsely powdered, and placed in a clean extraction vessel. A suitable solvent is added in sufficient quantity to completely immerse the plant material. The mixture is sealed and allowed to stand for several days with intermittent shaking to enhance solvent penetration. After completion of extraction, the liquid is filtered, and the marc is pressed to recover the remaining solvent. The combined extract is clarified and concentrated when required.

Equipment Required

Equipment	**Purpose**
Maceration vessel	Extraction
Glass stopper	Prevent solvent evaporation
Stirring rod	Mixing
Measuring cylinder	Solvent measurement
Filter paper	Filtration
Hydraulic press (optional)	Recovery of extract from marc

Advantages

- Simple and economical.
- No heating required.
- Suitable for thermolabile compounds.
- Minimal equipment requirement.
- Applicable to various solvents.

Limitations

- Long extraction time.
- Large solvent requirement.
- Lower extraction efficiency than modern techniques.
- Possible microbial contamination during prolonged extraction.

Applications

- Preparation of tinctures.

- Herbal extracts.
- Pharmaceutical research.
- Cosmetic formulations.
- Nutraceutical products.

Examples

- Vanilla pods
- Cinchona bark
- Digitalis leaves
- Belladonna leaves
- Calendula flowers

The above conventional extraction methods continue to be widely employed in pharmacognosy laboratories and herbal industries because of their simplicity, reproducibility, and suitability for a broad range of medicinal plants. Although modern extraction techniques offer greater efficiency and reduced processing time, conventional methods remain the foundation of herbal extraction and are extensively used for teaching, research, quality control, and the preparation of traditional herbal medicines.

3.5 Percolation

Introduction

Percolation is a continuous extraction technique widely employed in pharmacognosy and pharmaceutical industries for obtaining soluble constituents from powdered medicinal plants. Unlike maceration, where the plant material remains immersed in a fixed volume of solvent, percolation involves the slow and continuous passage of fresh solvent through a packed bed of powdered crude drug. As the solvent moves downward under the influence of gravity, it dissolves the phytoconstituents and carries them into the collecting vessel. Continuous replacement of saturated solvent with fresh solvent results in higher extraction efficiency and improved recovery of bioactive compounds. Percolation is extensively used in the preparation of tinctures, fluid extracts, and standardized herbal formulations because it provides better extraction with comparatively less solvent and shorter processing time than maceration.

Principle

Percolation is based on the continuous diffusion and dissolution of soluble phytochemicals by the downward movement of fresh solvent through a compact bed of powdered plant material. Continuous contact between fresh solvent and plant tissues maintains the concentration gradient, resulting in efficient extraction.

Types of Percolation

Type	Description
Simple Percolation	Extraction performed once using fresh solvent.
Reserved Percolation	A portion of concentrated extract is reserved and mixed with later fractions.
Modified Percolation	Includes soaking or controlled flow rate to improve extraction efficiency.

Procedure

The medicinal plant is dried, coarsely powdered, and uniformly moistened with the selected solvent. The moistened material is allowed to stand for several hours to permit swelling and complete wetting. It is then transferred into a percolator and packed uniformly without excessive compression. Additional solvent is added until the powder is completely covered, and the system is allowed to macerate for approximately 24 hours. The outlet of the percolator is then opened, allowing the solvent to flow slowly through the drug bed. Fresh solvent is continuously added to maintain a constant level until complete extraction is achieved. The collected percolate is filtered and concentrated if necessary.

Equipment Required

Equipment	**Purpose**
Percolator	Continuous extraction
Measuring cylinder	Solvent measurement
Collection flask	Collection of extract
Filter paper	Filtration
Glass rod	Packing of plant material

Advantages

- Higher extraction efficiency.
- Continuous supply of fresh solvent.
- Shorter extraction time than maceration.
- Suitable for large-scale production.
- Produces concentrated extracts.

Limitations

- Not suitable for very fine powders.
- Requires careful packing of the drug.
- Channel formation may reduce extraction efficiency.
- Less suitable for mucilaginous materials.

Applications

- Preparation of tinctures.
- Fluid extract manufacturing.
- Herbal pharmaceutical production.
- Pharmacognostic laboratories.

Examples

- Cinchona bark
- Belladonna leaves
- Digitalis leaves
- Senna leaves

3.6 Reflux Extraction

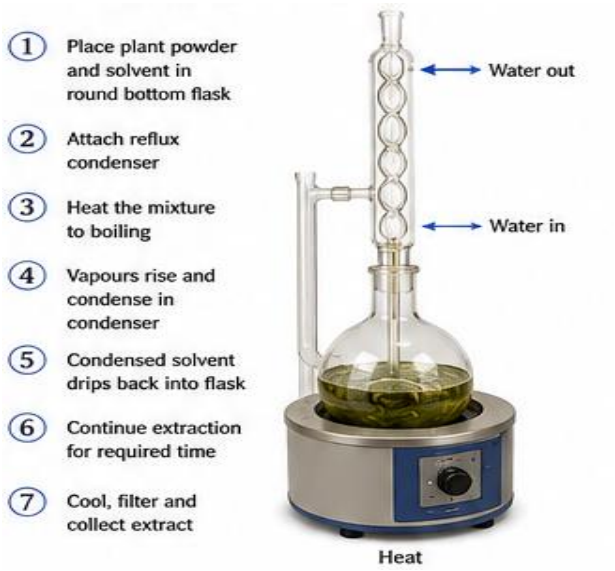
Introduction

Reflux extraction is a conventional extraction technique in which plant material is continuously heated with a solvent under reflux conditions. During heating, the solvent evaporates, condenses in a condenser, and returns to the extraction vessel, thereby preventing solvent loss while maintaining constant extraction conditions. Continuous recycling of the solvent enhances the dissolution of phytoconstituents and significantly reduces extraction time compared with maceration or percolation. Reflux extraction is commonly employed in phytochemical investigations and laboratory-scale extraction of alkaloids, glycosides, flavonoids, and phenolic compounds.

Principle

Reflux extraction is based on continuous boiling and condensation of the extraction solvent, allowing repeated contact between fresh hot solvent and plant material without solvent loss.

Procedure



The powdered plant material is placed in a round-bottom flask with the selected solvent. The flask is connected to a reflux condenser and heated gently. Solvent vapors rise into the condenser, where they cool and return to the extraction flask. This process continues for the desired extraction period. After completion, the mixture is cooled, filtered, and concentrated.

Equipment Required

Equipment	Purpose
Round-bottom flask	Extraction vessel
Reflux condenser	Condensation of solvent vapors
Heating mantle	Heating
Thermometer	Temperature monitoring
Filter paper	Filtration

Advantages

- Rapid extraction.
- Minimal solvent loss.
- High extraction efficiency.
- Suitable for moderately heat-stable compounds.
- Easy laboratory operation.

Limitations

- Not suitable for volatile constituents.
- Heat-sensitive compounds may degrade.
- Requires continuous heating.

Applications

- Herbal extract preparation.
- Pharmaceutical research.
- Phytochemical isolation.
- Natural product chemistry.

Examples

- Turmeric rhizome
- Liquorice root
- Green tea
- Neem leaves

3.7 Distillation

Introduction

Distillation is an important conventional extraction technique used primarily for the isolation of volatile and aromatic compounds such as essential oils from medicinal plants. The process involves vaporization of volatile constituents followed by condensation to obtain purified liquid extracts.

Distillation is extensively employed in pharmaceutical, cosmetic, food, and perfumery industries for the production of essential oils. The selection of an appropriate distillation method depends on the thermal stability, volatility, and chemical nature of the constituents.

Principle

Distillation is based on differences in the volatility and boiling points of plant constituents. Volatile compounds evaporate upon heating and are subsequently condensed into liquid form for collection.

Types of Distillation

1. Water Distillation

The plant material is completely immersed in boiling water. Volatile oils evaporate with water vapor, condense, and are collected. This method is suitable for dried plant materials that are not damaged by prolonged boiling.

2. Water and Steam Distillation

The plant material is placed above boiling water and comes into contact with steam generated from the water. It reduces overheating and is widely used for aromatic herbs.

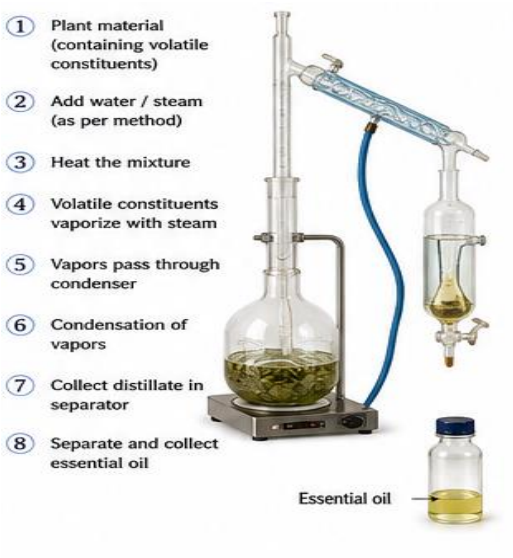
3. Steam Distillation

Steam generated in a separate boiler passes through the plant material. The volatile constituents vaporize with the steam and are subsequently condensed. This is the most widely used industrial method for essential oil extraction.

4. Vacuum Distillation

Distillation is carried out under reduced pressure, thereby lowering the boiling point of the solvent and volatile compounds. This method is suitable for heat-sensitive phytochemicals.

Procedure



The medicinal plant material is placed in the distillation apparatus according to the selected method. Heat or steam is applied to volatilize the essential oils. The vapor passes into a condenser, where cooling converts it back into liquid form. The condensate is collected in a separator, allowing essential oil to separate from water because of density differences.

Equipment Required

Equipment	**Purpose**
Distillation flask	Heating plant material
Condenser	Condensation
Steam generator	Steam production
Receiver	Collection of distillate
Oil separator	Separation of essential oil

Advantages

- Produces high-purity essential oils.
- Solvent-free extraction.
- Suitable for industrial production.
- Easy purification.

Limitations

- Limited to volatile compounds.
- High energy requirement.
- Heat-sensitive constituents may degrade.
- Expensive equipment for large-scale production.

Applications

- Essential oil production.
- Aromatherapy.
- Cosmetic industry.

- Pharmaceutical preparations.
- Food flavoring.

Examples

- Eucalyptus oil
- Clove oil
- Peppermint oil
- Cinnamon oil
- Lavender oil

3.8 Soxhlet Extraction

Introduction

Soxhlet extraction is a continuous hot extraction technique developed by Franz von Soxhlet in 1879. It is one of the most widely used laboratory methods for exhaustive extraction of phytochemicals from dried medicinal plants. The method allows repeated washing of plant material with fresh hot solvent, ensuring maximum extraction without frequent solvent replacement. Soxhlet extraction is particularly suitable for compounds with limited solubility in cold solvents but high solubility in hot solvents.

Principle

Soxhlet extraction is based on repeated extraction by continuous reflux and siphoning of hot solvent through the plant material until complete extraction is achieved.

Components of Soxhlet Apparatus

Component	Function
Round-bottom flask	Holds extraction solvent
Soxhlet extractor	Contains extraction chamber
Extraction thimble	Holds powdered plant material
Siphon tube	Transfers extract back to flask
Condenser	Condenses solvent vapors
Heating mantle	Provides heat

Working Procedure

The dried powdered plant material is placed inside a cellulose thimble and inserted into the Soxhlet extractor. Solvent contained in the round-bottom flask is heated until it boils. Solvent vapors rise into the condenser, condense, and drip onto the plant material. Once the extraction chamber becomes full, the siphon automatically empties the solvent back into the flask carrying dissolved phytochemicals. This cycle repeats continuously until exhaustive extraction is achieved. Finally, the solvent is evaporated to obtain the concentrated extract.

Advantages

- High extraction efficiency.
- Automatic continuous extraction.
- Fresh solvent repeatedly contacts plant material.
- Suitable for exhaustive extraction.

Limitations

- Long extraction time.
- High solvent consumption.
- Not suitable for thermolabile compounds.
- Continuous heating required.

Applications

- Phytochemical analysis.
- Herbal drug standardization.
- Pharmaceutical research.
- Isolation of alkaloids, flavonoids, and lipids.

Examples

- Turmeric
- Neem
- Black pepper
- Fenugreek
- Ginger

3.9 Successive Solvent Extraction

Introduction

Successive solvent extraction, also known as sequential solvent extraction, is a widely used technique in pharmacognosy for separating phytoconstituents according to their polarity. In this method, the same plant material is extracted successively using solvents arranged in increasing order of polarity. Each solvent selectively dissolves compounds having similar polarity, resulting in better separation and easier phytochemical analysis. This technique is extensively employed for phytochemical screening, isolation of natural products, and standardization of herbal drugs.

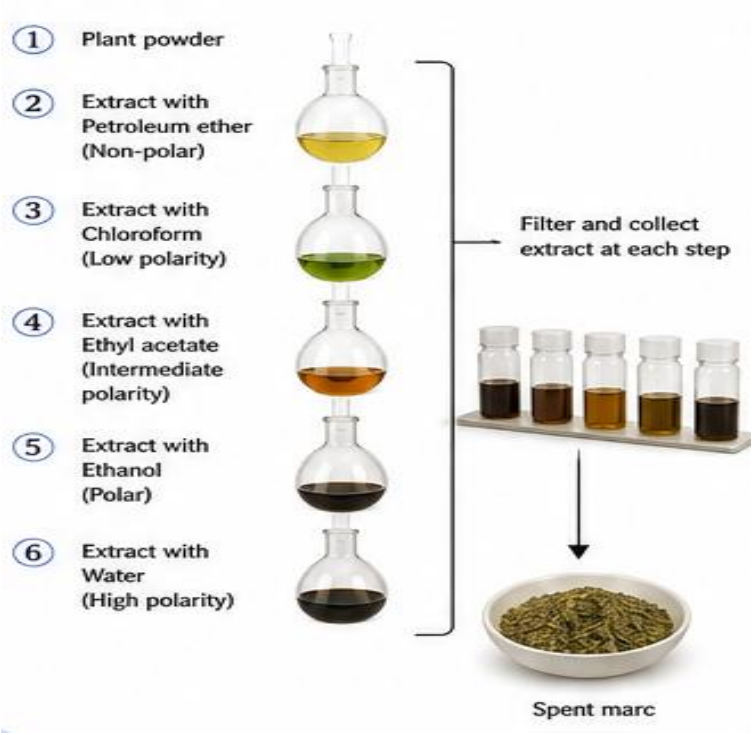
Principle

Successive solvent extraction is based on the principle of "like dissolves like," whereby compounds dissolve preferentially in solvents possessing similar polarity.

Solvent Selection Based on Polarity

Solvent	Polarity	Common Constituents Extracted
Petroleum ether	Non-polar	Fats, waxes, fixed oils
Chloroform	Low polarity	Steroids, terpenoids
Ethyl acetate	Intermediate	Flavonoids, phenolics
Ethanol	Polar	Alkaloids, glycosides, tannins
Water	Highly polar	Sugars, proteins, polysaccharides

Procedure



The powdered medicinal plant is first extracted with the least polar solvent. After completion, the plant residue is dried and extracted with the next solvent of higher polarity. The process continues sequentially until the most polar solvent is used. Each extract is filtered, concentrated separately, and stored for phytochemical evaluation.

Advantages

- Efficient fractionation of phytochemicals.
- Better separation based on polarity.
- Facilitates phytochemical screening.
- High extraction efficiency.

Limitations

- Time-consuming.
- Requires multiple solvents.
- Increased operational cost.
- Multiple concentration steps required.

Applications

- Phytochemical investigations.
- Herbal drug standardization.
- Isolation of bioactive compounds.
- Pharmaceutical research.
- Natural product chemistry.

Examples

- Neem leaves
- Turmeric rhizome
- Green tea
- Ginkgo leaves
- Liquorice root

The above conventional extraction methods constitute the fundamental techniques of pharmacognosy and continue to play a significant role in herbal drug research, quality control, phytochemical analysis, and industrial production of standardized medicinal plant extracts. Their simplicity, reproducibility, and broad applicability make them indispensable despite the emergence of modern extraction technologies.

4. MODERN METHODS OF EXTRACTION

Modern extraction methods have revolutionized the isolation of bioactive constituents from medicinal plants by improving extraction efficiency, reducing solvent consumption, minimizing processing time, and preserving thermolabile phytochemicals. Unlike conventional extraction techniques, which rely primarily on prolonged solvent contact and heating, modern methods utilize advanced technologies such as supercritical fluids, microwave energy, ultrasonic waves, enzymes, and pressurized solvents to enhance mass transfer between plant tissues and extraction solvents. These techniques provide higher extraction yields, improved selectivity, reduced environmental impact, and greater reproducibility. Consequently, they are increasingly employed in pharmaceutical, nutraceutical, cosmetic, food, and herbal industries for the production of high-quality standardized plant extracts. Among the various advanced techniques, Supercritical Fluid Extraction (SFE), Microwave-Assisted Extraction (MAE), and Ultrasonic-Assisted Extraction (UAE) are the most extensively used owing to their efficiency, speed, and ability to preserve sensitive phytochemicals.

4.1 Supercritical Fluid Extraction (SFE)

Introduction

Supercritical Fluid Extraction (SFE) is an advanced extraction technique that employs fluids maintained above their critical temperature and critical pressure, where they exhibit properties intermediate between those of gases and liquids. In the supercritical state, fluids possess gas-like diffusivity and liquid-like solvent power, enabling them to penetrate plant tissues rapidly and dissolve bioactive constituents efficiently. Carbon dioxide (CO_2) is the most commonly used supercritical solvent because it is non-toxic, non-flammable, inexpensive, chemically inert, and can be easily removed from the final extract. SFE is widely applied for extracting essential oils, lipids, alkaloids, terpenoids, and other valuable phytochemicals while avoiding thermal degradation and solvent residues.

Principle

Supercritical fluid extraction is based on the enhanced solvent power of fluids above their critical point, allowing rapid diffusion into plant matrices and efficient dissolution of target phytoconstituents under controlled pressure and temperature.

Supercritical Fluids

Fluid	Characteristics
Carbon dioxide (CO_2)	Most widely used, non-toxic, inexpensive, chemically inert
Nitrous oxide	Moderate solvent strength
Propane	Suitable for non-polar compounds
Ethane	Used for specialized extractions

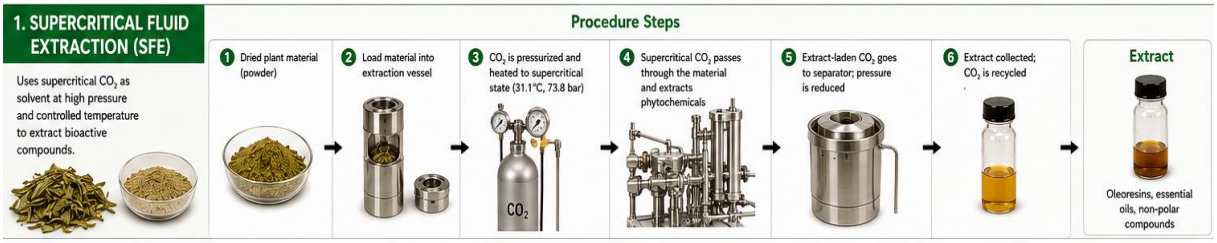
Carbon Dioxide as Solvent

Carbon dioxide is the preferred supercritical solvent because its critical temperature (31.1°C) and critical pressure (73.8 bar) are relatively low, making it suitable for extracting thermolabile phytochemicals. After extraction, CO_2 returns to the gaseous state upon depressurization, leaving solvent-free extracts with excellent purity.

Instrumentation

Component	Function
CO_2 cylinder	Supplies compressed carbon dioxide
High-pressure pump	Maintains extraction pressure
Extraction vessel	Holds plant material
Heater	Controls extraction temperature
Pressure regulator	Maintains operating pressure
Separator vessel	Collects extracted compounds

Working Procedure



The dried and powdered medicinal plant is loaded into the extraction vessel. Liquid carbon dioxide is compressed and heated beyond its critical temperature and pressure to produce supercritical CO_2. The supercritical fluid passes through the plant material, dissolving the desired phytochemicals. The extract-laden CO_2 then enters a separator where pressure is reduced, causing dissolved compounds to precipitate while CO_2 returns to the gaseous state and can be recycled for subsequent extraction cycles.

Advantages

- High extraction efficiency.
- Solvent-free final extract.
- Suitable for thermolabile compounds.
- Environmentally friendly process.
- Selective extraction by pressure adjustment.

Limitations

- High equipment cost.
- Requires skilled operation.
- Not suitable for highly polar compounds without modifiers.

Applications

- Essential oil extraction.
- Nutraceutical production.
- Pharmaceutical industries.
- Food flavor extraction.
- Cosmetic ingredients.

Examples

- Ginger oil
- Turmeric oleoresin
- Black pepper extract
- Rosemary extract
- Ginseng

4.2 Microwave-Assisted Extraction (MAE)

Introduction

Microwave-Assisted Extraction (MAE) is a rapid and efficient extraction technique that utilizes microwave energy to heat both the solvent and plant tissues simultaneously. Microwave irradiation causes rapid oscillation of polar molecules, producing internal heating that disrupts plant cell walls and facilitates the release of intracellular phytochemicals into the extraction solvent. Compared with conventional extraction methods, MAE significantly reduces extraction time, solvent consumption, and energy requirements while improving extraction yield. The technique has become increasingly important in pharmaceutical research, phytochemical investigations, and quality control laboratories.

Principle

Microwave-assisted extraction is based on dielectric heating, where microwave energy causes rapid rotation of polar molecules and ionic conduction, resulting in efficient cell rupture and accelerated extraction of bioactive constituents.

Instrumentation

Component	Function
Microwave generator	Produces microwave energy
Extraction vessel	Holds sample and solvent
Temperature controller	Maintains desired temperature
Pressure controller	Controls extraction pressure
Condenser	Prevents solvent loss

Working Procedure

The powdered medicinal plant is mixed with a suitable solvent inside a microwave extraction vessel. Microwave irradiation is applied for a predetermined period under controlled temperature conditions. Rapid internal heating ruptures plant cells and enhances solvent penetration, resulting in efficient release of phytochemicals. After extraction, the mixture is cooled, filtered, and concentrated to obtain the desired extract.

Advantages

- Very short extraction time.
- Reduced solvent consumption.
- High extraction yield.
- Energy efficient.
- Suitable for heat-sensitive compounds.

Limitations

- High initial equipment cost.
- Limited sample size.
- Unsuitable for non-polar solvents alone.
- Careful temperature control required.

Applications

- Herbal drug extraction.
- Natural product research.
- Pharmaceutical analysis.
- Food industry.
- Cosmetic manufacturing.

Examples

- Green tea polyphenols
- Curcumin
- Flavonoids
- Aloe vera
- Ginkgo biloba

4.3 Ultrasonic-Assisted Extraction (UAE)

Introduction

Ultrasonic-Assisted Extraction (UAE) is a green extraction technology that employs high-frequency ultrasonic waves to improve the extraction of phytochemicals from medicinal plants. The ultrasonic waves generate microscopic bubbles in the extraction solvent, which grow and collapse violently in a phenomenon known as cavitation. This process disrupts plant cell walls, increases solvent penetration, and enhances mass transfer between plant tissues and the solvent. UAE is widely recognized for its rapid extraction, low energy consumption, and ability to preserve heat-sensitive compounds because extraction is usually performed at relatively low temperatures.

Principle

Ultrasonic-assisted extraction operates on the principle of acoustic cavitation, in which ultrasonic waves produce microscopic bubbles whose collapse generates localized pressure and temperature, resulting in disruption of plant tissues and accelerated diffusion of phytochemicals into the solvent.

Cavitation Mechanism

During ultrasonic irradiation, alternating compression and expansion cycles create microscopic bubbles within the extraction solvent. These bubbles enlarge and eventually collapse violently, producing localized shock waves and microjets that rupture plant cell walls. The disrupted tissues permit greater solvent penetration and facilitate rapid release of intracellular phytochemicals, thereby increasing extraction efficiency.

Instrumentation

Component	Function
Ultrasonic generator	Produces ultrasonic waves
Ultrasonic bath/probe	Delivers ultrasonic energy
Extraction vessel	Holds sample and solvent
Temperature controller	Prevents overheating
Filtration unit	Separates extract

Working Procedure

The powdered medicinal plant is mixed with an appropriate extraction solvent in a suitable vessel. The vessel is placed in an ultrasonic bath or treated using an ultrasonic probe for a predetermined

period. Cavitation produced by ultrasonic waves disrupts plant cells and accelerates solvent penetration. After extraction, the mixture is filtered and concentrated to obtain the final extract.

Advantages

- Rapid extraction.
- Low solvent consumption.
- High extraction efficiency.
- Low operating temperature.
- Environmentally friendly.

Limitations

- Limited industrial scalability.
- Excessive sonication may degrade sensitive compounds.
- Equipment cost higher than conventional methods.

Applications

- Herbal extract preparation.
- Essential oil extraction.
- Pharmaceutical research.
- Food processing.
- Cosmetic industry.

Examples

- Garlic extract
- Turmeric
- Green tea
- Neem leaves
- Grape seed extract

Modern extraction techniques such as **SFE, MAE, and UAE** provide significant advantages over conventional methods by improving extraction efficiency, reducing extraction time, minimizing solvent consumption, and preserving thermolabile phytoconstituents. These technologies have become integral to contemporary pharmacognosy and phytopharmaceutical research, facilitating the production of high-quality, standardized herbal extracts for pharmaceutical, nutraceutical, cosmetic, and food applications.

4.4 Enzyme-Assisted Extraction (EAE)

Introduction

Enzyme-Assisted Extraction (EAE) is an eco-friendly and innovative extraction technique that utilizes specific enzymes to degrade the structural components of plant cell walls, thereby facilitating the release of intracellular bioactive compounds into the extraction solvent. Plant cell walls are primarily composed of cellulose, hemicellulose, pectin, lignin, and proteins, which often act as barriers to

solvent penetration during conventional extraction. Enzymatic hydrolysis weakens these structural polymers, increasing cell permeability and enhancing the extraction efficiency of valuable phytoconstituents. EAE is performed under mild operating conditions, making it particularly suitable for heat-sensitive compounds such as polyphenols, flavonoids, essential oils, polysaccharides, and proteins. Owing to its high extraction efficiency, reduced solvent requirement, and environmentally friendly nature, EAE has gained considerable importance in pharmaceutical, nutraceutical, food, cosmetic, and biotechnology industries.

Principle

Enzyme-assisted extraction is based on the enzymatic degradation of plant cell wall components, which enhances solvent penetration and promotes the release of intracellular phytochemicals into the extraction medium under controlled conditions.

Commonly Used Enzymes

Enzyme	**Function**
Cellulase	Degrades cellulose in plant cell walls
Pectinase	Hydrolyzes pectin substances
Hemicellulase	Breaks down hemicellulose
Protease	Digests proteins associated with cell walls
Amylase	Hydrolyzes starch components

Instrumentation

Equipment	**Purpose**
Reaction vessel	Enzymatic extraction
Water bath/Incubator	Temperature control
pH meter	Adjustment of pH
Mechanical stirrer	Uniform mixing
Filtration unit	Separation of extract

Working Procedure

The medicinal plant material is cleaned, dried, and finely powdered before being mixed with a suitable extraction solvent. Appropriate enzymes are added according to the nature of the plant material, and the mixture is incubated under controlled temperature and pH conditions for a predetermined period. During incubation, the enzymes hydrolyze the structural components of the cell wall, increasing solvent penetration and releasing intracellular phytoconstituents. After enzymatic treatment, the mixture is filtered to remove plant residue, and the filtrate is concentrated to obtain the desired extract.

Advantages

- High extraction efficiency.
- Mild extraction conditions.
- Reduced solvent consumption.
- Environmentally friendly process.
- Suitable for thermolabile compounds.
- Improved yield of bioactive constituents.

Limitations

- High cost of enzymes.
- Requires strict pH and temperature control.
- Longer extraction time than some advanced techniques.
- Enzyme activity may decrease during storage.

Applications

- Extraction of polyphenols and flavonoids.
- Herbal drug manufacturing.
- Food and beverage industry.
- Cosmetic formulations.
- Nutraceutical production.

Examples

- Grape seed polyphenols.
- Aloe vera polysaccharides.
- Citrus pectin.
- Green tea catechins.
- Ginseng extract.

4.5 Pressurized Liquid Extraction (PLE)

Introduction

Pressurized Liquid Extraction (PLE), also known as Accelerated Solvent Extraction (ASE), is an advanced extraction technique that utilizes liquid solvents at elevated temperatures and high pressures to rapidly extract phytochemicals from medicinal plants. The application of high pressure keeps the solvent in the liquid state even above its normal boiling point, while elevated temperature increases the solubility and diffusion rate of phytoconstituents. As a result, extraction is completed in a much shorter time with significantly lower solvent consumption than conventional methods. PLE is widely used in pharmaceutical research, phytochemical analysis, environmental testing, and quality control laboratories because it provides rapid, reproducible, and efficient extraction of a wide range of bioactive compounds.

Principle

Pressurized liquid extraction is based on the use of solvents maintained at high temperature and pressure, which enhances solvent penetration, increases the solubility of phytochemicals, and accelerates mass transfer from plant tissues to the extraction solvent.

Instrumentation

Equipment	Purpose
Solvent reservoir	Stores extraction solvent
High-pressure pump	Delivers solvent under pressure
Extraction cell	Holds plant material
Heating system	Maintains extraction temperature
Pressure regulator	Controls operating pressure
Collection vessel	Collects extract

Working Procedure

The dried and powdered medicinal plant is packed into the extraction cell of the PLE system. A suitable solvent is pumped into the extraction chamber under high pressure while the temperature is increased above the solvent's atmospheric boiling point. Under these conditions, the solvent rapidly penetrates the plant matrix and dissolves the desired phytoconstituents. After the predetermined extraction time, the extract is flushed into a collection vessel, filtered if necessary, and concentrated for further analysis or formulation.

Advantages

- Rapid extraction.
- High extraction yield.
- Low solvent consumption.
- Automated operation.
- Good reproducibility.
- Suitable for a wide range of phytochemicals.

Limitations

- Expensive instrumentation.
- Requires high-pressure operation.
- Not suitable for extremely heat-sensitive compounds.
- Higher maintenance cost.

Applications

- Pharmaceutical quality control.
- Phytochemical analysis.
- Herbal drug standardization.
- Food and nutraceutical industries.
- Environmental sample extraction.

Examples

- Curcumin from turmeric.
- Ginsenosides from ginseng.
- Green tea polyphenols.
- Rosemary antioxidants.
- Flavonoids from medicinal herbs.

Enzyme-Assisted Extraction (EAE) and Pressurized Liquid Extraction (PLE) are important modern extraction techniques that offer higher extraction efficiency, improved selectivity, reduced solvent consumption, and better preservation of bioactive constituents compared with conventional methods. Their ability to produce high-quality, standardized plant extracts has made them valuable tools in pharmacognosy, pharmaceutical research, herbal medicine, food technology, and the development of phytopharmaceutical products.

UNIT – V

Overview of Isolation, Identification and Characterization Techniques–

1. SEPARATION AND ISOLATION TECHNIQUES

1.1 Introduction to Separation and Isolation

Definition

Separation and isolation are fundamental processes in pharmacognosy used to obtain individual chemical constituents from complex mixtures present in medicinal plants, microorganisms, and other natural sources. **Separation** refers to the process of dividing a mixture into its individual components based on differences in their physicochemical properties, whereas **isolation** involves obtaining a pure compound or group of compounds after separation. These processes are essential for identifying biologically active constituents, evaluating their therapeutic potential, ensuring quality control, and developing herbal medicines and phytopharmaceuticals. Modern separation techniques provide high efficiency, reproducibility, and purity, making them indispensable in natural product research.

Principle

The principle of separation and isolation is based on the differential distribution of compounds between two phases, commonly referred to as the **stationary phase** and the **mobile phase**. Components of a mixture migrate at different rates depending on their polarity, molecular size, adsorption capacity, solubility, ion-exchange properties, or affinity toward the stationary phase. These differences enable the selective separation of compounds, which can subsequently be collected and purified for structural characterization and biological evaluation.

Importance in Pharmacognosy

Separation and isolation techniques play a vital role in pharmacognosy by facilitating the purification of bioactive phytoconstituents such as alkaloids, glycosides, flavonoids, terpenoids, tannins, saponins, steroids, and phenolic compounds. They are widely used in herbal drug standardization, phytochemical investigations, quality control, authentication of medicinal plants, isolation of marker compounds, impurity profiling, and the discovery of new natural products. These techniques also support pharmacological, toxicological, and analytical studies by providing pure compounds required for research and industrial applications.

Selection Criteria

Parameter	Selection Basis
Nature of sample	Plant extract, oil, resin, powder
Polarity	Polar or non-polar compounds
Quantity	Analytical or preparative scale
Purity required	Crude fraction or pure compound
Cost	Laboratory budget
Time	Rapid or conventional separation
Sensitivity	Trace or major constituents

1.2 Planar Chromatography

Introduction

Planar chromatography is one of the simplest and most widely employed chromatographic techniques for separating phytochemicals. In this technique, the stationary phase is coated as a thin uniform layer on a flat surface such as a glass, aluminum, or plastic plate, while the mobile phase moves across the stationary phase through capillary action. The separated compounds appear as distinct spots that can be visualized under ultraviolet light or after spraying suitable detecting reagents. Because of its simplicity, low cost, and rapid analysis, planar chromatography is extensively used in pharmacognostic laboratories for qualitative analysis and herbal drug identification.

Principle

The separation of compounds in planar chromatography depends on their differential adsorption and partition between the stationary phase and the moving solvent. Components having greater affinity for the stationary phase migrate slowly, whereas those with higher affinity for the mobile phase migrate faster, resulting in effective separation. The migration of compounds is expressed by the Retention Factor (Rf value), which is characteristic for a particular compound under fixed experimental conditions.

Types

Type	**Application**
Paper Chromatography	Polar compounds
Thin Layer Chromatography (TLC)	Routine phytochemical analysis
High-Performance Thin Layer Chromatography (HPTLC)	Advanced fingerprinting and quantification

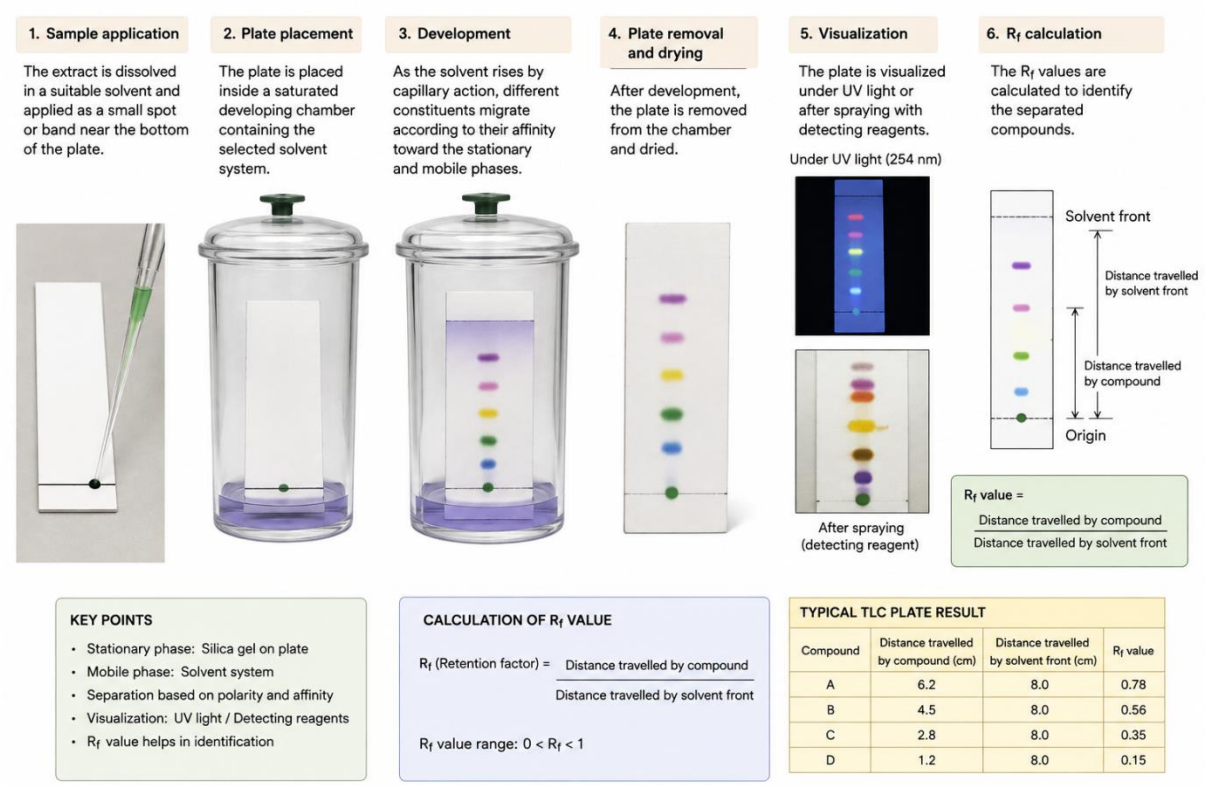
Instrumentation

- TLC/HPTLC plates
- Sample applicator
- Developing chamber
- UV cabinet (254 and 366 nm)
- Spray reagents
- Densitometer (HPTLC)

Materials Required

- Silica gel plate
- Plant extract
- Mobile phase solvents
- Capillary tubes or applicator
- UV chamber
- Spray reagents

Procedure



Factors Affecting Separation

Factor	Effect
Solvent composition	Determines separation efficiency
Plate quality	Influences resolution
Sample concentration	Excess causes tailing
Chamber saturation	Improves reproducibility
Temperature	Alters solvent migration
Humidity	Affects adsorption

Applications

- Herbal drug authentication
- Phytochemical screening
- TLC fingerprinting
- Detection of adulteration
- Marker compound identification
- Quality control of herbal medicines

Advantages

- Simple and economical
- Rapid analysis
- Requires small sample volume
- Simultaneous analysis of multiple samples
- Minimal solvent consumption

Limitations

- Lower resolution than HPLC
- Limited quantitative accuracy
- Manual operation
- Not suitable for highly volatile compounds

1.3 Column Chromatography

Introduction

Column chromatography is a preparative chromatographic technique used for separating and purifying individual phytoconstituents from crude plant extracts. It is based on the differential adsorption of compounds on a stationary phase packed inside a glass or stainless-steel column. This technique is extensively employed for the isolation of alkaloids, flavonoids, glycosides, terpenoids, steroids, and other natural products in research laboratories and pharmaceutical industries.

Principle

The sample mixture is loaded onto a column packed with a stationary phase such as silica gel or alumina. A suitable solvent or solvent mixture is passed through the column, causing different compounds to migrate at different rates according to their adsorption affinity. Less strongly adsorbed compounds elute first, whereas strongly adsorbed compounds require more polar solvents for elution.

Types of Columns

Type	Use
Glass column	Laboratory separation
Flash column	Rapid purification
Gravity column	Conventional isolation

Stationary Phase

- Silica gel
- Alumina
- Cellulose
- Sephadex

Mobile Phase

- Hexane
- Chloroform
- Ethyl acetate
- Methanol
- Acetone

Instrumentation

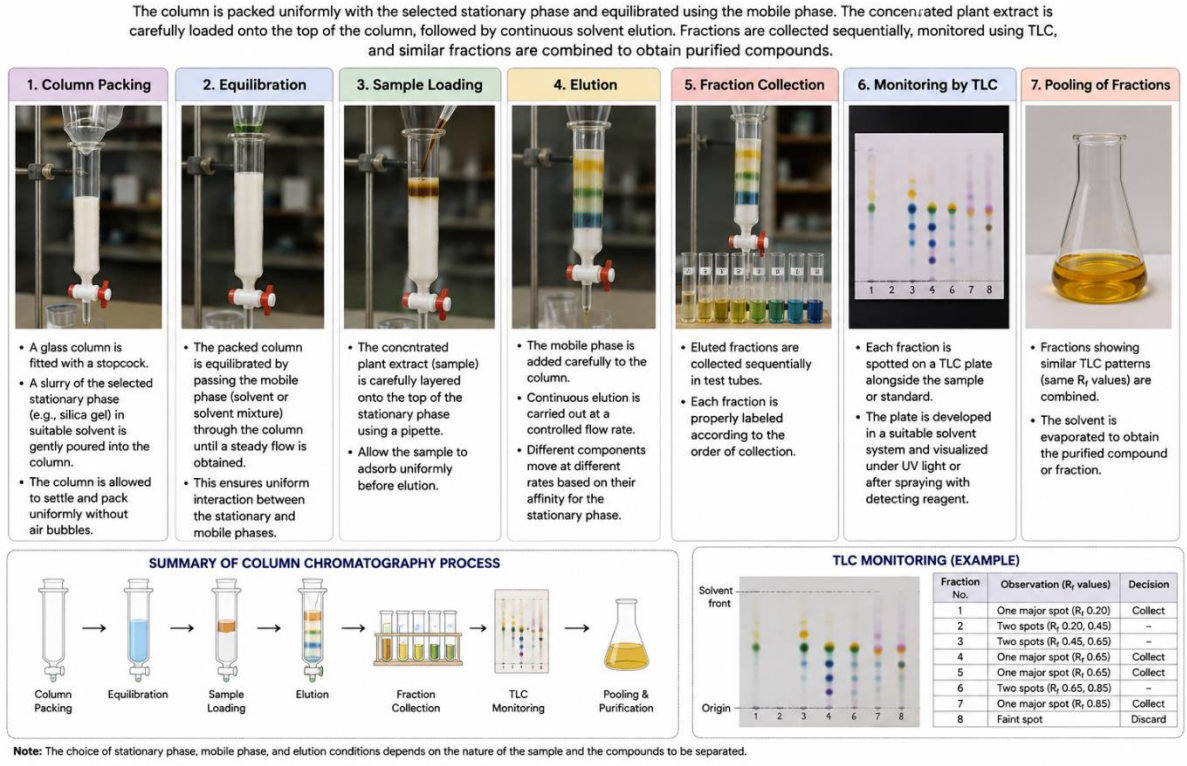
- Glass column
- Solvent reservoir

- Fraction collector
- Flow controller
- Collection tubes

Packing Methods

Method	Description
Dry packing	Dry adsorbent packed first
Wet packing	Slurry packed with solvent

Working Procedure



Elution Techniques

- Isocratic elution
- Gradient elution
- Stepwise gradient elution

Applications

- Isolation of pure phytochemicals
- Fractionation of plant extracts
- Natural product research
- Herbal drug standardization
- Pharmaceutical purification

Advantages

- High sample loading capacity
- Good purification efficiency
- Suitable for preparative work
- Flexible solvent selection

Limitations

- Time-consuming
- Large solvent requirement
- Manual operation
- Lower efficiency than automated systems

1.4 Preparative Thin Layer Chromatography (Preparative TLC)

Introduction

Preparative Thin Layer Chromatography (Preparative TLC) is an advanced form of TLC designed for the isolation and purification of compounds rather than merely analytical identification. Thick-layer silica gel plates permit the separation and recovery of milligram to gram quantities of purified phytochemicals.

Principle

Compounds are separated on a thick adsorbent layer based on differential adsorption. After chromatographic development, the separated bands are physically scraped from the plate, and the compounds are extracted using suitable solvents to obtain purified constituents.

Materials Required

- Preparative TLC plates
- Silica gel
- Plant extract
- Developing chamber
- UV cabinet
- Scraper
- Collection vessel

Instrumentation

- Sample applicator
- Developing chamber
- UV visualization chamber
- Glass scraper
- Rotary evaporator

Plate Preparation

A thick layer (0.5–2 mm) of silica gel is uniformly coated on glass plates and activated by heating before use.

Sample Application

A concentrated sample is applied as a narrow band using a syringe or applicator to maximize separation efficiency.

Development

The plate is developed in a saturated solvent chamber until the solvent reaches the desired distance.

Band Collection

Separated bands are identified under UV light, carefully scraped from the plate, and transferred into extraction vessels.

Purification

The adsorbent is extracted with an appropriate solvent, filtered, and concentrated to obtain purified compounds.

Applications

- Isolation of phytochemicals
- Purification of marker compounds
- Natural product research
- Structural characterization

Advantages

- Simple procedure
- Low operational cost
- Good recovery
- Suitable for small-scale purification

Limitations

- Limited sample capacity
- Manual recovery
- Loss of material during scraping
- Lower reproducibility

1.5 Flash Chromatography

Introduction

Flash chromatography is a rapid form of column chromatography in which compressed gas or positive pressure is used to force the solvent through a finely packed adsorbent column. Compared with conventional column chromatography, flash chromatography provides faster separation, improved resolution, and higher recovery of phytochemicals. It is widely employed in pharmaceutical research for the purification of natural products and synthetic compounds.

Principle

Separation is based on differential adsorption between the stationary phase and the mobile phase. Positive pressure accelerates solvent flow, thereby reducing analysis time while maintaining efficient separation of compounds.

Instrumentation

- Flash chromatography system
- Compressed air or nitrogen source
- Pre-packed silica column
- Solvent reservoir
- UV detector (optional)
- Fraction collector

Types

Type	Description
Manual flash chromatography	Pressure applied manually
Automated flash chromatography	Computer-controlled purification

Working Procedure

The column is packed or connected with a pre-packed cartridge and equilibrated using the selected solvent. The sample is loaded onto the column, and compressed gas drives the mobile phase through the stationary phase. Fractions are collected automatically or manually and monitored by TLC or UV detection. Desired fractions are pooled and concentrated to obtain purified compounds.

Solvent Selection

Solvent	Common Use
Hexane	Non-polar compounds
Ethyl acetate	Moderately polar compounds
Chloroform	Medium polarity separation
Methanol	Polar compounds

Applications

- Rapid purification of plant extracts
- Isolation of marker compounds
- Drug discovery research
- Natural product chemistry
- Pharmaceutical quality control

Advantages

- Rapid separation
- Higher resolution
- Reduced solvent consumption
- Good recovery
- Easy scalability

Limitations

- Higher equipment cost
- Requires compressed gas
- Limited column life
- Skilled operation required

2. IDENTIFICATION TECHNIQUES

2.1 Introduction

Identification techniques are analytical methods used to determine the identity, purity, and chemical composition of phytoconstituents present in medicinal plants and herbal formulations. These techniques enable researchers to confirm the authenticity of crude drugs, detect adulterants and contaminants, identify marker compounds, and establish quality standards. Identification involves both preliminary phytochemical screening and advanced analytical techniques such as chromatography and spectroscopy. Modern identification methods provide accurate, reproducible, and sensitive analysis, which is essential for herbal drug standardization, pharmacological research, and regulatory compliance.

Need for Identification

The identification of medicinal plants and their constituents is essential to ensure the safety, efficacy, and quality of herbal medicines. Many medicinal plants possess similar morphological characteristics, increasing the possibility of substitution and adulteration. Identification techniques help distinguish genuine drugs from inferior or counterfeit materials, confirm the presence of therapeutically active constituents, monitor batch-to-batch consistency, evaluate raw materials before formulation, and ensure compliance with national and international pharmacopoeial standards.

Importance in Herbal Drug Standardization

Identification techniques form the foundation of herbal drug standardization by providing scientific evidence regarding the identity and chemical composition of herbal products. These methods facilitate authentication of medicinal plants, quantitative estimation of marker compounds, quality control during manufacturing, detection of impurities and adulterants, stability evaluation, and regulatory approval of herbal formulations. They also contribute significantly to natural product research, drug discovery, and phytopharmaceutical development.

2.2 Phytochemical Tests

Introduction

Phytochemical tests are preliminary qualitative chemical tests performed to detect the presence of different classes of secondary metabolites in medicinal plants. These tests employ specific reagents that produce characteristic color changes or precipitates in the presence of particular phytochemicals. Although qualitative in nature, phytochemical screening provides valuable information regarding the chemical profile of medicinal plants and guides further chromatographic and spectroscopic investigations.

Principle

The principle of phytochemical testing is based on the reaction between specific phytochemicals and selective chemical reagents. When the target constituent is present, the reagent produces a characteristic color, precipitate, foam, or ring, allowing rapid identification of different groups of natural products.

Qualitative Screening

Qualitative screening involves systematic testing of plant extracts using standard reagents to identify major classes of metabolites including alkaloids, glycosides, flavonoids, phenolics, tannins, saponins, steroids, terpenoids, resins, proteins, carbohydrates, and fixed oils. The results are expressed as present (+), absent (−), or strongly positive (++).

General Procedure

The dried plant material is powdered and extracted using suitable solvents such as water, ethanol, methanol, or hydroalcoholic mixtures. The extract is filtered and subjected to individual phytochemical tests using specific reagents under controlled laboratory conditions. The appearance of characteristic colors, precipitates, or other reactions indicates the presence of the corresponding phytochemical group.

Interpretation

Positive phytochemical reactions indicate the presence of the corresponding class of secondary metabolites, whereas negative reactions suggest their absence or concentration below the detection limit. The intensity of color or precipitate often provides an approximate indication of the relative abundance of the phytochemical.

Applications

- Preliminary phytochemical screening
- Herbal drug authentication
- Selection of extraction methods
- Quality control
- Standardization of medicinal plants
- Natural product research

Advantages

- Simple and economical
- Rapid analysis
- Minimal instrumentation
- Suitable for routine screening
- Requires small sample quantity

Limitations

- Qualitative rather than quantitative
- Lower sensitivity
- Possible interference from impurities
- Confirmation requires instrumental analysis

Common Phytochemical Tests

Phytochemical	Common Test	Positive Observation
Alkaloids	Dragendorff's Test	Orange precipitate
Glycosides	Keller–Killiani Test	Brown-green ring
Flavonoids	Shinoda Test	Pink or red color
Phenolics	Ferric Chloride Test	Blue-green color
Tannins	Ferric Chloride Test	Dark blue/green color
Saponins	Foam Test	Stable froth
Steroids	Liebermann–Burchard Test	Green color
Terpenoids	Salkowski Test	Reddish-brown layer
Resins	Acetone-Water Test	Turbidity
Proteins	Biuret Test	Violet color
Carbohydrates	Molisch Test	Violet ring
Fixed Oils	Spot Test	Permanent translucent spot

Common Reagents Used

Reagent	Application
Dragendorff's reagent	Alkaloids
Mayer's reagent	Alkaloids
Wagner's reagent	Alkaloids
Keller–Killiani reagent	Cardiac glycosides
Ferric chloride	Phenolics and tannins
Shinoda reagent	Flavonoids
Liebermann–Burchard reagent	Steroids
Salkowski reagent	Terpenoids
Molisch reagent	Carbohydrates
Biuret reagent	Proteins

2.3 Chromatographic Identification Techniques

Chromatographic techniques are widely used for the identification, purification, and quantification of phytoconstituents. These methods separate compounds based on their differential distribution between stationary and mobile phases and provide reliable information regarding the chemical composition of herbal drugs.

Thin Layer Chromatography (TLC)

Principle

TLC separates compounds according to their adsorption and partition behavior between a silica-coated stationary phase and a moving solvent. Each compound migrates with a characteristic Retention Factor (Rf value), which aids identification.

Instrumentation

- TLC plate
- Developing chamber
- Sample applicator
- UV cabinet
- Spray reagents

Procedure

The sample is applied near the base of the TLC plate, developed in an appropriate solvent system, dried, and visualized under UV light or after spraying with detecting reagents. The Rf values are calculated and compared with reference standards.

Applications

- Herbal drug identification
- Fingerprinting
- Purity assessment
- Detection of adulteration
- Marker compound identification

High-Performance Thin Layer Chromatography (HPTLC)

Principle

HPTLC is an advanced form of TLC using finer particle stationary phases and automated sample application for improved resolution and reproducibility.

Instrumentation

- Automatic sampler
- HPTLC plates
- Developing chamber
- Scanner
- Densitometer
- Software

Procedure

Samples are applied as bands, chromatograms are developed, scanned by densitometry, and compared with authenticated reference standards for qualitative and quantitative analysis.

Densitometric Analysis

A densitometer measures the intensity of separated bands at selected wavelengths, producing chromatographic peaks proportional to compound concentration.

Applications

- Herbal fingerprinting
- Quantification of marker compounds
- Quality control
- Stability studies

High-Performance Liquid Chromatography (HPLC)

Principle

HPLC separates compounds under high pressure through a packed analytical column according to differences in polarity and interaction with the stationary phase.

Instrumentation

- Solvent reservoir
- Pump
- Injector
- HPLC column
- Detector
- Computer system

Procedure

Filtered samples are injected into the HPLC system, separated within the column, detected using UV or PDA detectors, and analyzed using chromatographic software.

Applications

- Quantitative estimation
- Marker analysis
- Purity evaluation
- Pharmaceutical quality control

Gas Chromatography (GC)

Principle

GC separates volatile compounds based on their distribution between an inert carrier gas and a stationary phase inside a capillary column.

Instrumentation

- Carrier gas cylinder
- Injector
- GC column
- Oven
- Detector
- Data processor

Procedure

The volatile sample is injected, vaporized, separated in the heated column, detected, and recorded as chromatographic peaks.

Applications

- Essential oil analysis
- Volatile phytochemicals
- Aroma profiling
- Residual solvent analysis

Liquid Chromatography–Mass Spectrometry (LC–MS)

Principle

LC–MS combines liquid chromatographic separation with mass spectrometric detection to identify compounds based on their mass-to-charge (m/z) ratio.

Instrumentation

- LC system
- Mass spectrometer
- Ion source
- Detector
- Computer software

Procedure

Compounds are first separated by LC, ionized, analyzed according to m/z values, and identified by comparing spectra with reference databases.

Applications

- Metabolite profiling
- Drug impurity analysis

- Biomarker identification
- Herbal medicine research

Gas Chromatography–Mass Spectrometry (GC–MS)

Principle

GC–MS integrates gas chromatography with mass spectrometry for simultaneous separation and structural identification of volatile compounds.

Instrumentation

- Gas chromatograph
- Mass spectrometer
- Ion source
- Detector
- Data system

Procedure

Separated GC components enter the mass spectrometer, where they are ionized, fragmented, and identified from characteristic mass spectra.

Applications

- Essential oil characterization
- Natural product analysis
- Forensic investigations
- Environmental analysis

2.4 Spectroscopic Identification Techniques

Spectroscopic techniques identify compounds by measuring their interaction with electromagnetic radiation. They provide detailed information about molecular structure, functional groups, and chemical composition, making them indispensable in phytochemical characterization.

UV–Visible Spectroscopy

Principle

UV–Visible spectroscopy is based on the absorption of ultraviolet or visible light by molecules containing chromophoric groups, resulting in electronic transitions.

Instrumentation

- Light source
- Monochromator
- Sample holder

- Detector
- Computer

Working

A monochromatic beam passes through the sample solution, and the absorbed light is measured as absorbance at specific wavelengths.

Applications

- Quantitative estimation
- Marker compound analysis
- Purity testing

Advantages

- Rapid
- Sensitive
- Simple operation
- Low cost

Limitations

- Limited structural information
- Unsuitable for compounds without chromophores

Fourier Transform Infrared (FTIR) Spectroscopy

Principle

FTIR identifies functional groups by measuring infrared radiation absorbed during molecular vibrations.

Instrumentation

- IR source
- Interferometer
- Sample holder
- Detector
- Computer

Working

Infrared radiation passes through the sample, generating a spectrum that represents characteristic molecular vibrations.

Functional Group Identification

Functional Group	Approximate Range (cm^{-1})
O–H	3200–3600
N–H	3300–3500
C=O	1650–1750
C=C	1600–1680
C–O	1000–1300

Applications

- Functional group identification
- Herbal drug authentication
- Structural characterization

Nuclear Magnetic Resonance (NMR)

Principle

NMR spectroscopy is based on the absorption of radiofrequency energy by atomic nuclei placed in a strong magnetic field.

Instrumentation

- Superconducting magnet
- RF transmitter
- Probe
- Detector
- Computer

Types

- **^{1}H NMR:** Provides information about hydrogen atoms.
- **^{13}C NMR:** Provides information about carbon atoms.

Interpretation

Chemical shifts, signal splitting, integration, and coupling constants are interpreted collectively to determine molecular structure.

Applications

- Structure elucidation
- Purity determination
- Natural product characterization

Mass Spectrometry (MS)

Principle

Mass spectrometry identifies compounds by measuring the mass-to-charge ratio (m/z) of ionized molecules.

Instrumentation

- Ion source
- Mass analyzer
- Detector
- Vacuum system
- Computer

Ionization Techniques

- Electron Ionization (EI)
- Electrospray Ionization (ESI)
- Atmospheric Pressure Chemical Ionization (APCI)
- MALDI

Fragmentation Pattern

Characteristic fragmentation produces diagnostic ions that assist in molecular identification and structural confirmation.

Applications

- Molecular weight determination
- Structural elucidation
- Drug metabolite identification
- Phytochemical profiling

Hyphenated Techniques

Hyphenated analytical techniques combine chromatographic separation with spectroscopic detection, providing highly accurate qualitative and quantitative analysis of complex herbal extracts. These methods significantly improve sensitivity, specificity, and structural characterization.

Common Hyphenated Techniques

- LC–MS/MS
- GC–MS
- LC–NMR
- LC–FTIR

Applications

- Herbal fingerprinting
- Marker compound identification
- Metabolomics

- Drug impurity profiling
- Pharmacokinetic studies
- Quality control

Advantages

- High sensitivity
- Excellent selectivity
- Structural confirmation
- Simultaneous qualitative and quantitative analysis
- Suitable for complex herbal matrices

Limitations

- Expensive instrumentation
- High maintenance cost
- Requires skilled personnel
- Complex data interpretation

Comparison of Identification Techniques

Technique	Major Use	Main Advantage	Major Limitation
TLC	Qualitative identification	Simple and inexpensive	Lower sensitivity
HPTLC	Fingerprinting	High resolution	Moderate cost
HPLC	Quantitative analysis	High accuracy	Expensive
GC	Volatile compounds	Excellent separation	Limited to volatile samples
LC–MS	Structural identification	High sensitivity	High instrument cost
GC–MS	Volatile compound identification	Accurate spectral library matching	Requires volatile analytes
UV–Visible	Quantification	Rapid and economical	Limited structural information
FTIR	Functional group analysis	Non-destructive	Cannot fully determine molecular structure
NMR	Structure elucidation	Detailed structural information	Very expensive
MS	Molecular weight determination	Highly sensitive	Complex instrumentation

3. FINGERPRINTING OF MEDICINAL PLANTS USING TLC AND HPTLC

Introduction

Fingerprinting of medicinal plants is a modern analytical approach used to establish the identity, quality, consistency, and authenticity of herbal drugs by generating a characteristic chromatographic profile of their chemical constituents. Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) are among the most widely employed fingerprinting techniques because they are simple, rapid, economical, and capable of analyzing multiple samples simultaneously. Each medicinal plant possesses a unique phytochemical composition, and the

chromatographic pattern produced by these techniques acts as a "chemical fingerprint" that can be used for authentication and quality control. TLC is mainly employed for qualitative analysis, whereas HPTLC provides both qualitative and quantitative information with higher sensitivity, reproducibility, and resolution. Fingerprinting has become an essential requirement in the standardization of herbal medicines and is widely accepted by pharmacopoeias and regulatory agencies worldwide.

Concept of Fingerprinting

Chemical fingerprinting refers to the generation of a characteristic chromatographic profile representing the complete chemical composition of a medicinal plant or herbal formulation. Instead of identifying only a single active constituent, fingerprinting evaluates the overall phytochemical pattern, including major and minor constituents. The chromatographic profile obtained from an authentic sample serves as a reference against which unknown or commercial samples can be compared for authentication and quality assessment.

Importance

Fingerprinting plays a significant role in ensuring the quality, safety, and efficacy of herbal medicines. It helps authenticate medicinal plants, distinguish closely related species, detect adulteration and substitution, evaluate batch-to-batch consistency, monitor manufacturing quality, standardize herbal formulations, identify marker compounds, and comply with national and international regulatory requirements. It is also extensively used in pharmacognostic research, phytochemical investigations, and quality assurance laboratories.

Principle

The principle of TLC and HPTLC fingerprinting is based on the differential migration of phytochemicals between a stationary phase (usually silica gel) and a mobile phase (solvent system). Individual constituents travel at different rates depending on their polarity, molecular size, and affinity for the stationary phase, producing characteristic bands or spots with specific Retention Factor (Rf) values. In HPTLC, densitometric scanning further measures the intensity of each band, providing quantitative information about the chemical constituents.

Materials Required

Material	**Purpose**
Plant extract	Sample analysis
TLC/HPTLC plate	Stationary phase
Mobile phase solvents	Separation
Sample applicator	Sample loading
Developing chamber	Chromatogram development
UV cabinet	Spot visualization
Spray reagents	Detection of compounds

Instrumentation

Instrument	**Function**
Automatic sample applicator	Uniform sample application
TLC/HPTLC chamber	Plate development

UV cabinet (254 & 366 nm)	Visualization
Densitometer/Scanner	Peak analysis
Computer with software	Data processing

Sample Preparation

Fresh or dried medicinal plant material is cleaned, shade dried, and finely powdered. The powdered sample is extracted using suitable solvents such as methanol, ethanol, water, or hydroalcoholic mixtures depending on the nature of the phytochemicals. The extract is filtered, concentrated if necessary, and diluted to an appropriate concentration before application onto the chromatographic plate.

Method Development

Method development involves selecting a suitable stationary phase, optimizing the mobile phase composition, determining the sample concentration, selecting visualization methods, and optimizing development conditions to achieve maximum separation of phytochemical constituents. Several solvent systems may be evaluated before selecting the most suitable one for fingerprint generation.

Plate Development

The prepared chromatographic plate is placed vertically inside a saturated developing chamber containing the optimized mobile phase. The solvent ascends by capillary action, separating the phytochemicals into distinct bands. After the solvent reaches the desired height, the plate is removed, dried, and prepared for visualization.

Detection

Separated phytochemicals are detected under ultraviolet light at 254 nm and 366 nm or after spraying with suitable chromogenic reagents such as anisaldehyde-sulfuric acid, vanillin-sulfuric acid, Dragendorff's reagent, ferric chloride, or NP/PEG reagent. Different phytochemicals produce characteristic colored spots or fluorescent bands that facilitate identification.

Densitometric Analysis

In HPTLC, the developed chromatographic plate is scanned using a densitometer at a selected wavelength. The scanner converts individual bands into chromatographic peaks, and the peak area and peak height are proportional to the concentration of the respective compounds. This allows accurate qualitative and quantitative evaluation of marker compounds and overall fingerprint comparison.

Data Interpretation

The chromatographic fingerprint is interpreted by comparing Rf values, number of bands, band color, fluorescence characteristics, peak area, peak height, and overall chromatographic pattern with those of authenticated reference standards. A close similarity between the test sample and reference fingerprint confirms the identity and quality of the medicinal plant.

Applications

- Authentication of medicinal plants
- Herbal drug standardization
- Detection of adulteration and substitution
- Identification of marker compounds
- Quality control of herbal formulations
- Batch-to-batch consistency evaluation
- Stability studies
- Pharmacognostic and phytochemical research

Advantages

- Simple and rapid analysis
- Cost-effective technique
- Simultaneous analysis of multiple samples
- High reproducibility (HPTLC)
- Requires small sample quantity
- Suitable for qualitative and quantitative analysis
- Accepted by pharmacopoeias and regulatory authorities

Limitations

- TLC has lower sensitivity than HPLC
- Careful optimization of solvent systems is required
- Manual errors may affect reproducibility
- Quantitative accuracy is lower in conventional TLC
- Complex herbal mixtures may require advanced analytical techniques for complete characterization

WHO Guidelines

The **World Health Organization (WHO)** recommends TLC and HPTLC fingerprinting as important tools for the quality control and standardization of herbal medicines. According to WHO guidelines, authenticated reference materials should be used, chromatographic conditions should be standardized, marker compounds should be identified whenever possible, and chromatographic fingerprints should be documented and compared to ensure consistency, authenticity, and safety of herbal products.

Case Studies

Medicinal Plant	**Marker Compound**	**Fingerprinting Technique**
Turmeric (*Curcuma longa*)	Curcumin	HPTLC
Senna (*Senna alexandrina*)	Sennosides	TLC/HPTLC
Liquorice (*Glycyrrhiza glabra*)	Glycyrrhizin	HPTLC
Ginkgo (*Ginkgo biloba*)	Ginkgolides	HPTLC
Aloe (*Aloe vera*)	Aloin	TLC

4. TYPES AND SIGNIFICANCE OF MARKERS (PHYTOCHEMICAL REFERENCE STANDARDS)

Introduction

The quality, safety, and therapeutic efficacy of herbal medicines depend largely on the presence of specific bioactive phytochemicals in appropriate concentrations. Since medicinal plants are complex mixtures containing hundreds of chemical constituents, it is often difficult to evaluate their quality by analyzing every compound. Therefore, specific phytochemicals known as marker compounds or phytochemical reference standards are selected for the identification, authentication, and standardization of herbal drugs. Marker compounds serve as reliable indicators of the identity, purity, consistency, and quality of medicinal plants and herbal formulations. They are extensively used in pharmacognostic studies, chromatographic fingerprinting, quantitative analysis, quality assurance, and regulatory evaluation. Modern analytical techniques such as TLC, HPTLC, HPLC, GC–MS, LC–MS, and spectroscopic methods are commonly employed for the detection and estimation of marker compounds.

Definition of Marker Compounds

Marker compounds are chemically characterized constituents present in medicinal plants that are used as reference standards for the identification, authentication, standardization, and quality control of herbal drugs and herbal formulations. A marker compound may or may not be responsible for the therapeutic activity of the plant, but it should be consistently present in authentic plant material and measurable using suitable analytical techniques. Marker compounds provide a scientific basis for ensuring batch-to-batch consistency and maintaining the quality of herbal products throughout manufacturing and storage.

Characteristics of an Ideal Marker

An ideal phytochemical marker should possess several desirable characteristics to ensure accurate and reliable quality evaluation. It should be chemically stable during extraction, processing, and storage, present in sufficient concentration for easy detection, and specific to the medicinal plant under investigation. The marker should be easily isolated, purified, and quantified using validated analytical methods. It should exhibit minimal variation due to environmental or seasonal factors and should remain unaffected by normal manufacturing processes. Whenever possible, the marker should also correlate with the pharmacological activity of the herbal drug and be commercially available as a certified reference standard.

Classification of Marker Compounds

1. Active Markers

Active markers are biologically active phytochemicals that are directly responsible for the therapeutic effects of a medicinal plant. The concentration of these compounds is closely related to the efficacy of the herbal medicine, making them valuable for both quality control and pharmacological evaluation. Examples include curcumin in turmeric, glycyrrhizin in liquorice, and withanolides in Ashwagandha.

2. Analytical Markers

Analytical markers are selected primarily for analytical and quality control purposes rather than therapeutic activity. These compounds are chosen because they can be accurately identified and

quantified, allowing reliable assessment of herbal drug quality and consistency. They are particularly useful when the actual active constituents are unknown or difficult to estimate.

3. Negative Markers

Negative markers are undesirable or potentially toxic constituents that should be absent or present only within acceptable limits in herbal preparations. Their determination ensures the safety of herbal medicines by detecting harmful substances, degradation products, contaminants, or adulterants during quality control testing.

4. Biomarkers

Biomarkers are characteristic chemical constituents that indicate the authenticity, origin, or biological activity of a medicinal plant. They are widely used in phytochemical research, metabolomic studies, herbal authentication, and pharmacological investigations to distinguish genuine medicinal plants from substitutes or adulterants.

5. Chemical Markers

Chemical markers are naturally occurring phytochemicals selected because they are characteristic of a particular medicinal plant or herbal formulation. They may or may not possess pharmacological activity but serve as reliable indicators for identification, standardization, and routine quality evaluation.

6. Taxonomic Markers

Taxonomic markers are compounds unique to a particular botanical family, genus, or species and are useful in plant taxonomy and botanical authentication. These markers help differentiate closely related species that exhibit similar morphological characteristics but differ chemically.

7. Quality Control Markers

Quality control markers are routinely estimated during the manufacturing of herbal medicines to ensure uniformity, consistency, purity, and compliance with pharmacopoeial standards. Their quantitative estimation is an essential part of quality assurance programs in herbal pharmaceutical industries.

Selection Criteria of Marker Compounds

The selection of an appropriate marker compound is a critical step in herbal drug standardization. The selected marker should be specific to the medicinal plant, chemically stable, and present in measurable quantities. It should be easily extractable and detectable using validated analytical techniques such as HPTLC, HPLC, GC–MS, or LC–MS. The marker should demonstrate reproducible analytical behavior, remain stable during storage and processing, and preferably have a documented pharmacological or taxonomic significance. Certified reference standards should be available to facilitate accurate qualitative and quantitative analysis.

Significance of Marker Compounds

Marker compounds play a central role in modern pharmacognosy and herbal drug standardization by providing objective criteria for evaluating medicinal plant quality. They enable authentication of crude drugs, detection of adulteration and substitution, assessment of batch-to-batch consistency, and monitoring of manufacturing quality. Marker-based standardization ensures that herbal products contain consistent levels of important phytochemicals, thereby improving their therapeutic reliability and patient safety. These compounds are also valuable in phytochemical research, stability studies, pharmacokinetic investigations, formulation development, and regulatory approval of herbal medicines. International organizations such as the World Health Organization (WHO) and various pharmacopoeias recommend the use of marker compounds as reference standards for quality control of herbal products.

Applications of Marker Compounds

- Authentication of medicinal plants and crude drugs.
- Standardization of herbal formulations.
- Detection of adulteration and substitution.
- Quantitative estimation of phytochemicals.
- Chromatographic fingerprinting using TLC, HPTLC, and HPLC.
- Quality assurance during manufacturing.
- Stability and shelf-life evaluation.
- Pharmacognostic and phytochemical research.
- Regulatory compliance and pharmacopoeial quality control.
- Development of phytopharmaceuticals and herbal medicines.

Examples of Important Marker Compounds

Medicinal Plant	**Marker Compound**	**Major Therapeutic Significance**
Senna alexandrina (Senna)	Sennosides	Stimulant laxative
Glycyrrhiza glabra (Liquorice)	Glycyrrhizin	Anti-inflammatory, expectorant
Curcuma longa (Turmeric)	Curcumin	Antioxidant, anti-inflammatory
Camellia sinensis (Green Tea)	Epigallocatechin gallate (EGCG)	Antioxidant
Ginkgo biloba (Ginkgo)	Ginkgolides	Neuroprotective, circulatory support
Withania somnifera (Ashwagandha)	Withanolides	Adaptogenic, immunomodulatory
Aloe vera (Aloe)	Aloin	Laxative
Digitalis purpurea (Digitalis)	Digoxin	Cardiotonic

Marker compounds, also known as phytochemical reference standards, are indispensable tools for the scientific evaluation of medicinal plants and herbal formulations. They provide a reliable basis for authentication, quality control, standardization, and regulatory compliance by ensuring consistent phytochemical composition in herbal products. Depending on their role, marker compounds are classified as active, analytical, negative, biomarkers, chemical markers, taxonomic markers, and quality control markers. Modern chromatographic and spectroscopic techniques facilitate their accurate identification and quantification, thereby supporting the development of safe, effective, and standardized herbal medicines. The use of well-characterized marker compounds has become an

essential component of pharmacognosy, phytochemistry, pharmaceutical quality assurance, and evidence-based herbal drug research.

5. SCREENING AND ANALYSIS OF MAJOR METABOLITES

Introduction

Secondary metabolites are naturally occurring organic compounds synthesized by plants that are not directly involved in their normal growth and development but play essential roles in defense, adaptation, and survival. These metabolites possess a wide range of pharmacological activities and are responsible for the medicinal properties of herbal drugs. Screening and analysis of major metabolites are fundamental steps in pharmacognosy for the identification, authentication, standardization, and quality control of medicinal plants. Preliminary phytochemical screening provides qualitative information regarding the presence or absence of different classes of metabolites, whereas advanced chromatographic and spectroscopic techniques enable their separation, identification, characterization, and quantitative estimation. The major groups of metabolites commonly investigated include alkaloids, glycosides, saponins, tannins, resins, flavonoids, phenolics, and steroids.

Importance

Screening and analysis of plant metabolites are essential for the scientific evaluation of medicinal plants. These studies facilitate the identification of biologically active constituents, detection of adulteration, selection of suitable extraction methods, isolation of marker compounds, standardization of herbal formulations, quality assurance, pharmacological investigations, and development of phytopharmaceutical products. They also provide valuable information for drug discovery, toxicological evaluation, and regulatory approval of herbal medicines.

General Screening Procedure

The general phytochemical screening process begins with the collection and authentication of plant material, followed by cleaning, drying, and pulverization. The powdered material is extracted using suitable solvents such as water, ethanol, methanol, chloroform, or hydroalcoholic mixtures depending on the polarity of the desired constituents. The extract is filtered, concentrated, and subjected to preliminary qualitative tests using specific chemical reagents. Positive samples are further analyzed by chromatographic techniques such as TLC, HPTLC, HPLC, GC–MS, or LC–MS and confirmed using spectroscopic methods including UV–Visible spectroscopy, FTIR, NMR, and Mass Spectrometry.

Sample Preparation

Plant materials are collected from authenticated sources and dried under shade to preserve thermolabile constituents. The dried material is finely powdered and extracted using appropriate extraction methods such as maceration, Soxhlet extraction, ultrasonic extraction, or microwave-assisted extraction. The crude extract is filtered and concentrated under reduced pressure before phytochemical screening and instrumental analysis.

5.1 Alkaloids

Definition

Alkaloids are naturally occurring nitrogen-containing organic compounds, usually basic in nature, that exhibit significant pharmacological activities. They are mainly derived from amino acids and occur in numerous medicinal plants.

Classification

Type	Example
True alkaloids	Morphine
Protoalkaloids	Ephedrine
Pseudoalkaloids	Caffeine

Chemical Nature

Most alkaloids contain one or more nitrogen atoms within heterocyclic rings and occur as salts of organic acids in plant tissues. They are generally bitter, crystalline, and physiologically active.

General Tests

- Dragendorff's Test
- Mayer's Test
- Wagner's Test
- Hager's Test

Chromatographic Analysis

TLC, HPTLC, HPLC, and LC–MS are commonly employed for qualitative and quantitative estimation of alkaloids.

Spectroscopic Analysis

UV–Visible spectroscopy, FTIR, NMR, and Mass Spectrometry are used for structural characterization and identification.

Applications

- Drug discovery
- Herbal drug standardization
- Pharmaceutical quality control
- Pharmacological research

Examples

Morphine, Quinine, Atropine, Berberine, Caffeine.

5.2 Glycosides

Definition

Glycosides are compounds consisting of a sugar (glycone) linked to a non-sugar (aglycone) through a glycosidic bond. Hydrolysis releases the sugar and aglycone components.

Classification

Type	Example
Cardiac glycosides	Digoxin
Anthraquinone glycosides	Sennosides
Saponin glycosides	Dioscin
Cyanogenic glycosides	Amygdalin

Chemical Nature

They contain carbohydrate and non-carbohydrate portions linked by glycosidic bonds and exhibit diverse pharmacological activities.

Identification Tests

- Keller–Killiani Test
- Borntrager's Test
- Legal's Test

Chromatographic Analysis

TLC, HPTLC, HPLC, and LC–MS.

Spectroscopic Analysis

FTIR, NMR, UV, and MS.

Applications

- Herbal standardization
- Cardiotonic drugs
- Laxative preparations
- Quality control

Examples

Digoxin, Sennosides, Glycyrrhizin.

5.3 Saponins

Definition

Saponins are glycosidic compounds characterized by their ability to produce stable foam in aqueous solutions due to their surfactant properties.

Types

Type	Example
Steroidal saponins	Dioscin
Triterpenoid saponins	Glycyrrhizin

Foam Test

Persistent froth lasting for several minutes indicates the presence of saponins.

Hemolysis Test

Saponins produce hemolysis of red blood cells because of their interaction with membrane sterols.

Chromatographic Analysis

TLC, HPTLC, and HPLC.

Spectroscopic Analysis

FTIR, NMR, and MS.

Applications

- Vaccine adjuvants
- Expectorants
- Anti-inflammatory agents
- Herbal formulations

Examples

Dioscin, Glycyrrhizin, Ginsenosides.

5.4 Tannins

Definition

Tannins are high molecular weight polyphenolic compounds capable of precipitating proteins and forming stable complexes with metal ions.

Classification

Type	Example
Hydrolysable tannins	Tannic acid
Condensed tannins	Catechin tannins

Ferric Chloride Test

Formation of blue-black or green coloration indicates the presence of tannins.

Gelatin Test

White precipitate formation confirms tannins.

Chromatographic Analysis

TLC, HPTLC, HPLC.

Spectroscopic Analysis

UV, FTIR, NMR, and MS.

Applications

- Antioxidants
- Antidiarrheal preparations
- Wound healing
- Herbal quality control

Examples

Tannic acid, Catechin.

5.5 Resins

Definition

Resins are amorphous, non-volatile, solid or semi-solid plant exudates composed mainly of terpenoid derivatives and oxidation products.

Classification

Type	Example
True resins	Colophony
Gum resins	Asafoetida
Oleoresins	Turpentine

Identification Tests

- Acetone-water turbidity test
- Solubility test
- Alcohol precipitation test

Chromatographic Analysis

TLC, HPTLC, GC–MS.

Spectroscopic Analysis

FTIR, NMR, and MS.

Applications

- Pharmaceutical excipients
- Antimicrobial preparations
- Incense and varnishes
- Herbal medicines

Examples

Colophony, Benzoin, Asafoetida.

5.6 Flavonoids

Definition

Flavonoids are naturally occurring polyphenolic compounds possessing a characteristic C6–C3–C6 carbon skeleton and exhibiting strong antioxidant activity.

Classification

Type	Example
Flavones	Apigenin
Flavonols	Quercetin
Flavanones	Hesperidin
Anthocyanins	Cyanidin

Shinoda Test

Development of pink, red, or orange color confirms flavonoids.

Alkaline Reagent Test

Yellow coloration that disappears upon acidification indicates flavonoids.

Chromatographic Analysis

TLC, HPTLC, HPLC, LC–MS.

Spectroscopic Analysis

UV, FTIR, NMR, and MS.

Applications

- Antioxidants
- Anti-inflammatory agents
- Cardioprotective drugs
- Nutraceuticals

Examples

Quercetin, Rutin, Kaempferol, Catechin.

5.7 Phenolics

Definition

Phenolic compounds are aromatic compounds containing one or more hydroxyl groups attached directly to a benzene ring. They constitute one of the largest groups of plant secondary metabolites.

Classification

Type	Example
Simple phenolics	Gallic acid
Phenolic acids	Caffeic acid
Polyphenols	Ellagic acid

Ferric Chloride Test

Blue, green, or violet coloration indicates phenolic compounds.

Chromatographic Analysis

HPTLC, HPLC, LC–MS.

Spectroscopic Analysis

UV, FTIR, NMR, and MS.

Applications

- Antioxidants
- Food preservatives
- Herbal quality assessment
- Pharmaceutical formulations

Examples

Gallic acid, Caffeic acid, Ellagic acid.

5.8 Steroids

Definition

Steroids are naturally occurring compounds possessing the cyclopentanoperhydrophenanthrene nucleus and are widely distributed in plants and animals.

Classification

Type	Example
Phytosterols	β-Sitosterol
Steroidal glycosides	Diosgenin
Steroidal alkaloids	Solasodine

Liebermann–Burchard Test

Formation of a green or bluish-green color confirms steroids and sterols.

Salkowski Test

Appearance of a reddish-brown ring at the interface indicates steroids.

Chromatographic Analysis

TLC, HPTLC, HPLC, GC–MS.

Spectroscopic Analysis

FTIR, NMR, UV–Visible spectroscopy, and Mass Spectrometry.

Applications

- Steroidal drug synthesis
- Hormonal preparations
- Anti-inflammatory agents
- Herbal standardization

Examples

β-Sitosterol, Diosgenin, Stigmasterol, Solasodine.

Screening and analysis of major plant metabolites are essential components of pharmacognostic evaluation and herbal drug standardization. Preliminary phytochemical tests provide rapid qualitative identification of major metabolite classes, while chromatographic techniques such as TLC, HPTLC, HPLC, GC–MS, and LC–MS enable accurate separation and quantification. Spectroscopic techniques including UV–Visible spectroscopy, FTIR, NMR, and Mass Spectrometry provide structural characterization and confirmation of phytochemicals. Together, these analytical approaches ensure the authenticity, quality, safety, efficacy, and consistency of medicinal plants and herbal formulations, thereby supporting pharmaceutical research, quality control, and the development of evidence-based herbal medicines.

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