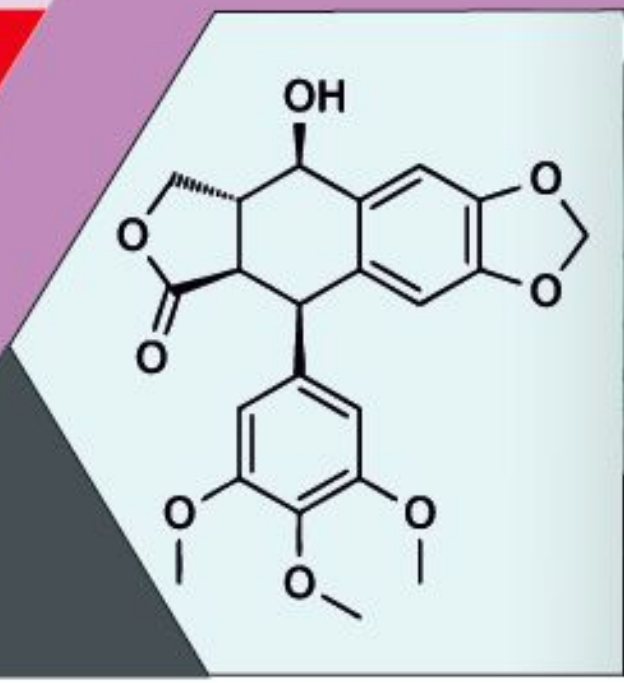
As Per PCI Regulations
Third Year B. Pharm.

Semester-V

PHARMACOGNOSY AND PHYTOCHEMISTRY-II



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Dr. SHASHI ALOK
Dr. PADMINI SHUKLA

NIRALI PRAKASHAN
ADVANCEMENT OF KNOWLEDGE

A Text Book of

PHARMACOGNOSY AND PHYTOCHEMISTRY - II

As Per PCI Regulations

Third Year B. Pharm

Semester V

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Preface

The subject Pharmacognosy is very old and mankind evolution. Herbal field is evolved as multidisciplinary area especially focusing on may modern disciplines i.e. Phytochemistry, production and utilization of phytoconstituents etc. The updated syllabus of Pharmacognosy and phytochemistry –II (Semester V) prescribed by Pharmacy council of India (PCI) for B. Pharm is of multidisciplinary characters. The syllabus covers different aspects of the subject and no single book can meet the needs of the prescribed syllabus. A variety of pharmacognosy books are available in market and colleges libraries. The teachers and students facing difficulties to collect the requisite material as the books available on the subject partially meet the requirement of the course content. So from this attempt students will find useful information, concise literature collected from various sources in one place in the form of this textbook named as 'Pharmacognosy and phytochemistry-II'

The few salient features of the book are, it is easy to understand by the students and teachers due to its simple language, by the diagrammatic representation the content becomes easier to understand, point wise content makes it more easier to understand and each new concept has been introduced through day to day problem of interest to the students which makes the matter more interesting.

The book comprises following five units covers all the content in nearly 14 chapters divided unit wise. The different contents covered under in this book are Basic metabolic pathways in Higher plants, Introduction about Plant Secondary Metabolites, Industrial Production and Utilization of Phytoconstituents, Basics of Phytochemistry and Isolation, Identification and analysis of Phytoconstituents.

The authors highly appreciate the work of publishing staff of Nirali Prakashan, Pune, especially Mr. Amit Jha, Ms Roshan Khan and Mr. Jignesh Furia for their co-operation in bringing out this book.

Finally, we express our deep gratitude to our family members for being supportive in times of stress in bring out this book. No major professional project can be completed without cooperation and encouragement of family.

At last, the authors heartily welcome the valuable suggestions by the readers of this book so that we can improve the content more precisely and could come up with another better edition later on.

Authors

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Syllabus

Unit I **[07 Hours]**

Metabolic Pathways in Higher Plants and their Determination

(a) Brief study of Basic Metabolic Pathways and Formation of different Secondary Metabolites through these pathways: Shikimic Acid Pathway, Acetate Pathways and Amino Acid Pathway.

(b) Study of Utilization of Radioactive Isotopes in the Investigation of Biogenetic studies.

Unit II **[14 Hours]**

General Introduction, Composition, Chemistry and Chemical Classes, Biosources, Therapeutic uses and Commercial Applications of following Secondary Metabolites:

- **Alkaloids:** Vinca, Rauwolfia, Belladonna, Opium.
- **Phenylpropanoids and Flavonoids:** Lignans, Tea, Ruta.
- **Steroids, Cardiac Glycosides and Triterpenoids:** Liquorice, Dioscorea, Digitalis.
- **Volatile oils:** Mentha, Clove, Cinnamon, Fennel, Coriander.
- **Tannins:** Catechu, Pterocarpus.
- **Resins:** Benzoin, Guggul, Ginger, Asafoetida, Myrrh, Colophony.
- **Glycosides:** Senna, Aloes, Bitter Almond.
- **Iridoids, Other Terpenoids and Naphthaquinones:** Gentian, Artemisia, Taxus, Carotenoids.

Unit III **[06 Hours]**

Isolation, Identification and Analysis of Phytoconstituents:

(a) Terpenoids: Menthol, Citral, Artemisin.

(b) Glycosides: Glycyrhetinic acid and Rutin.

(c) Alkaloids: Atropine, Quinine, Reserpine, Caffeine.

(d) Resins: Podophyllotoxin, Curcumin.

Unit IV **[10 Hours]**

Industrial Production, Estimation and Utilization of the following Phytoconstituents: Forskolin, Sennoside, Artemisinin, Diosgenin, Digoxin, Atropine, Podophyllotoxin, Caffeine, Taxol, Vincristine and Vinblastine.

Unit V **[08 Hours]**

Basics of Phytochemistry

Modern methods of Extraction, Application of latest techniques like Spectroscopy, Chromatography and Electrophoresis in the Isolation, Purification and Identification of Crude Drugs.

■■■

Contents

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Unit I

Chapter ... 1

Basic Metabolic Pathways

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *Brief study of basic metabolic pathways and formation of different secondary metabolites through these pathways:*
  - *Shikimic acid pathway*
  - *Acetate pathways*
  - *Amino acid pathway*
- *Study of utilization of radioactive isotopes in the investigation of biogenetic studies.*

1.1 INTRODUCTION

All living beings need various number of organic molecules for survival purposes like respiration, formation of building block, protection etc. For the synthesis of these molecules a series of chain reaction occurs inside the organism. The long chain of these reactions or path of these reactions is called as metabolic pathways.

Though the organisms are very much different in their characteristic but in general the pathway for the production of fat, carbohydrate, protein, nucleic acid is very much similar. These common pathways in all organisms are collectively called as primary metabolism and the compound formed by these are known as primary metabolite.

Apart from primary metabolic pathways there are some more compounds which have less distribution in nature and these compounds are called as secondary metabolites. These are specific for particular organism and they are not produce in cell condition. Some secondary metabolites have obvious reason for their production like for defense mechanism for pollination by insect etc. these are the secondary metabolite which shows the vast pharmacological activity.

1.2 SHIKIMIC ACID PATHWAY

- Erythrose-4-phosphate and phosphoenol pyruvate interact each other and synthesize the 2-keto-3-deoxy-7-phosphoglucoheptonic acid and enzyme which mediate this reaction is DAPH synthase.
- DHQ (dehyroquinate) synthase catalyse the further reaction and form the 3-dehydroquinate from the 2- keto-3-deoxy-7-phosphoglucoheptonic acid.
- NADC (Nicotinamide adenine dinucleotide) used as a cofactor in this reaction.

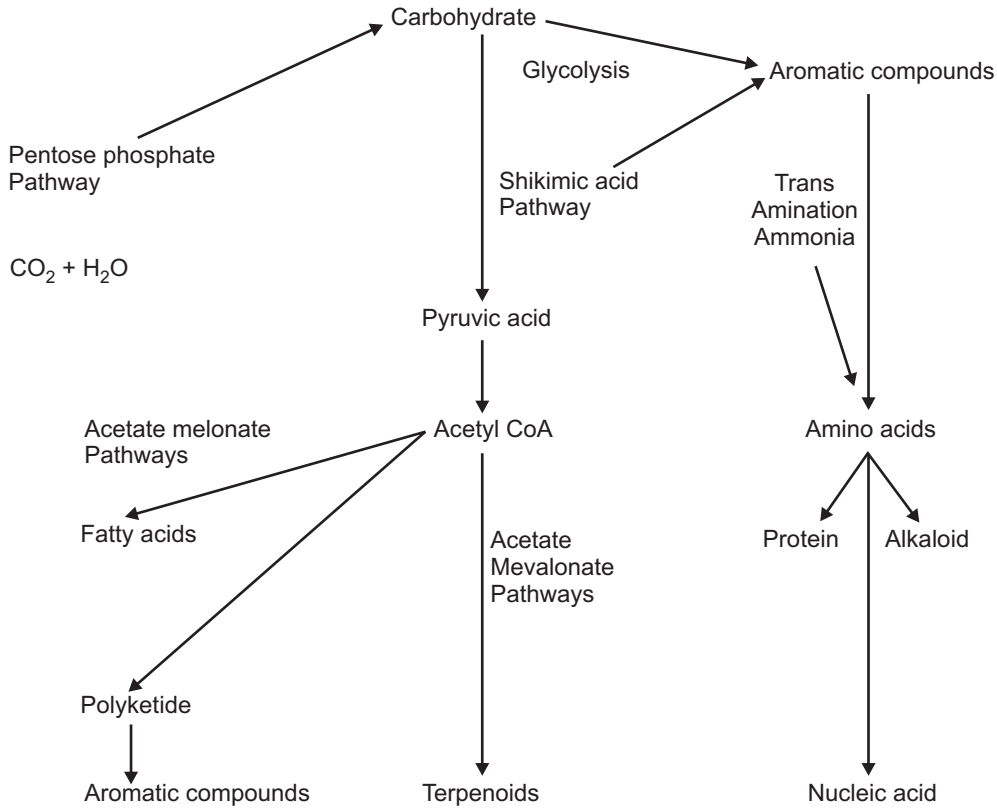


Fig. 1.1: Production of primary and secondary metabolites in Plants

- In the presence of another enzyme 3-dehydroquinate dehydratase the DHQ remove the water and form 3-dhydroshikimic acid.
- ATP base phosphorylation occurs of the product dehydroshikimic acid and form the shikimate- 3- phosphate in the presence of enzyme shikimate kinase. Phosphoenol pyruvate reacts with this shikimate- 7- phosphate to form 5-enol pyruvyl shikimate-3-phosphate with the help of enzyme 5-enol pyruvyl shikimate-3-phosphate (EPSP) synthase.
- The next product chorismate is formed from the product 5-enol pyruvyl shikimate-3 phosphate via the enzyme chorismate synthase.
- Clasien rearrangement reaction occurs with chorismate in the presence of enzymes chorismate mutase and prephenic acid was formed.
- From chorismic acid various intermediate product like Anthranillic acid, phosphoribosyl anthranillic acid were formed which will last produce tryptophan.
- While prephenic acid converted into phenyl pyruvic acid or 4-hydroxy phenyl pyruvic acid which in last produce the amino acid phenyl alanine or tyrosine.

Importance:

- Phenyl propanoids (Phenyl alanine and Tyrosine) is the main precursor for the synthesis of coumarin, lignin, tannins and flavonoids.
- Gallic acid is also biosynthesized from shikimic acid pathway (rearrangement of 3-5 didehydro shikimate in the presence of enzyme shikimate dehydrogenase).
- Aromatic metabolite and alkaloids are also formed by this cycle.
- Indole, Indole derivatives are also formed from this pathway.

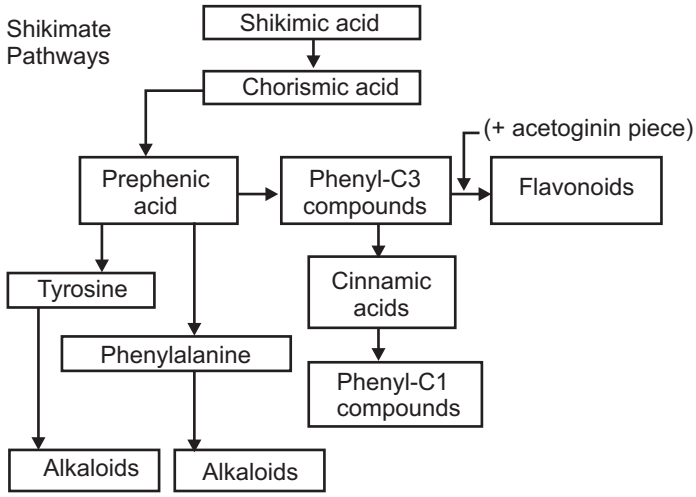


Fig. 1.2: Production of secondary metabolites from Shikimic acid pathway in Plants

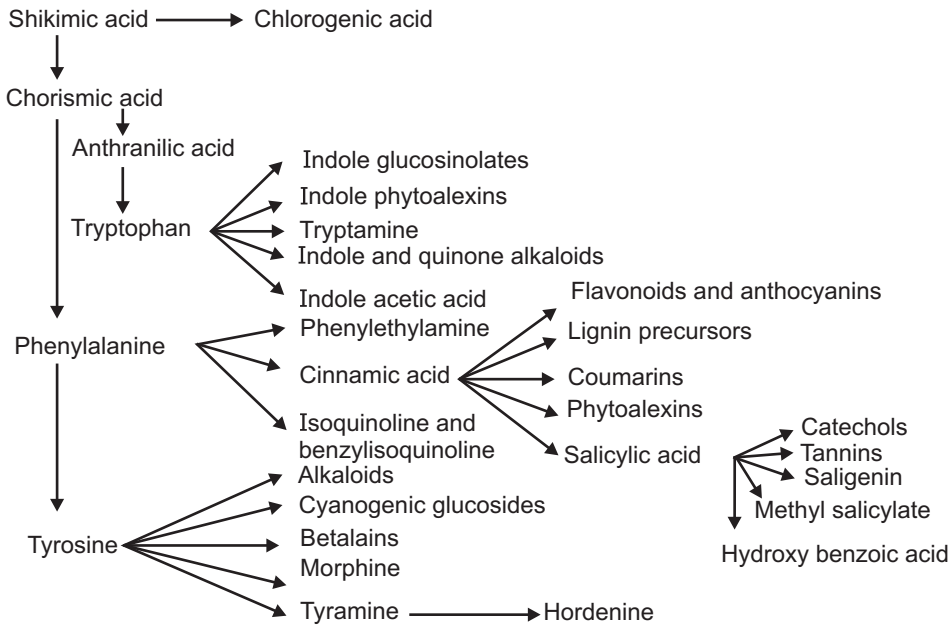


Fig. 1.3: Shikimic acid pathway leading to the biosynthesis of diverse bioactive principles

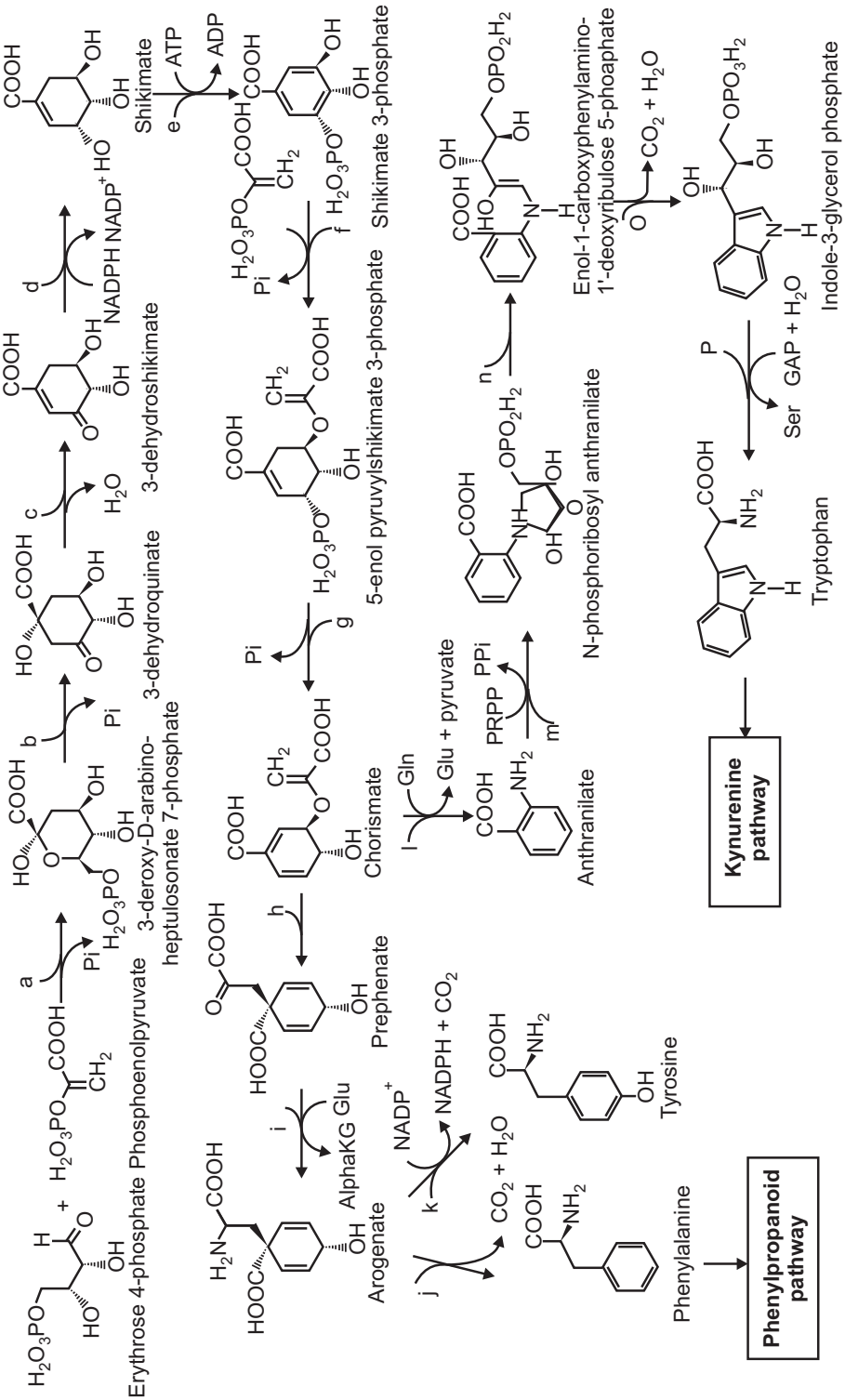


Fig. 1.4: Production of Amino acids by Shikimic acid pathway

Biosynthesis of Glycosides:

The metabolic process of glycoside formation occurs in two steps. In first step various types of aglycone are formed by biosynthetic reactions whereas in second step coupling of aglycone with sugar moiety occurs. In different types of glycosides interaction of nucleotide glycoside occurs between UDP-glucose with alcoholic or phenolic group of secondary compound aglycone (called O-glycosides), through linkage with carbon (C-glycosides), nitrogen (N-glycosides) or sulphur (S-glycosides).

The following two steps are involved in this process:

1. In first step, the uridine triphosphate (UTP) transferred an uridylyl group to sugar-1-phosphate and forms UDP-sugar and inorganic pyrophosphate. The enzyme which catalyzes this reaction is uridylyl transferases.

$$\text{UTP + Sugar-1-PO}_4 \xleftrightarrow{\text{Uridylyl transferases}} \text{UDP- sugar + PP}_1$$

2. In second step, transfer of the sugar moiety from UDP to a suitable acceptor (aglycone) occurs. This reaction is mediated by enzyme glycosyl transferases and forms glycoside.

$$\text{UDP-sugar + Acceptor (aglycone)} \xleftrightarrow{\text{Glycosyl transferases}} \text{Acceptor-sugar (glycoside) + UDP}$$

The sugars of glycosides are monosaccharides (like Rhamnose, Glucose, Frucose or deoxy sugars i.e. digitoxose or cymarose).

The aglycone moieties of cardiac glycosides are steroidal in nature. These are derivatives of cyclopentenophenanthrene ring which contains unsaturated lactone ring attached with C_{17}, a 14-alpha hydroxyl group and a cisjucture of ring C and D.

The anthraquinone glycosides are biosynthesized from shikimic acid pathway in Rubiaceae family. The alizarin biosynthesis shows ring A is derived from shikimic acid whereas mevalonic acid is included in ring C.

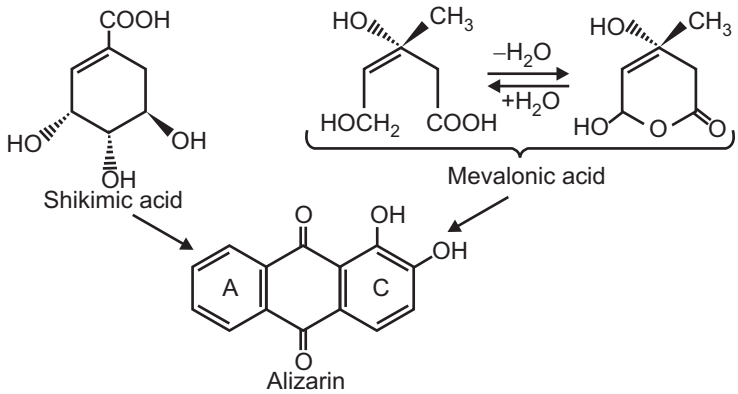


Fig. 1.5: Biosynthesis of anthraquinone glycosides

It is very important to understand the biosynthesis of flavonoids due to their diversity. These flavonoidal molecules are biosynthesized by their precursor which is three molecules of acetic acid and phenyl propane moiety.

It mainly involves the interaction of five different pathways which are named as:

1. The Glycolytic pathway.
2. The Pentose phosphate pathway.
3. The Shikimate pathway that synthesizes phenylalanine (An amino acid).
4. The phenylpropanoid metabolism that produces activated cinnamic acid derivatives i.e. 4-coumaroyl-CoA and lignin (also the plant structural component).
5. The diverse specific flavonoidal pathway.

The flavonoids are biosynthesized via condensation of the shikimic acid and acylpolymalonate pathways. The phenyl propane (cinnamic acid derivative) synthesized from shikimic acid which acts as a precursor in a polyketide synthesis. In this scheme additional three acetate residues are incorporated into the structure and followed by ring closure. The plants biosynthesizes different classes of flavonoids like flavonols, flavanones, flavones, flavanols or catechins, iso-flavones, dihydro-flavonols, anthocyanidins, and chalcones through subsequent hydroxylation and reduction.

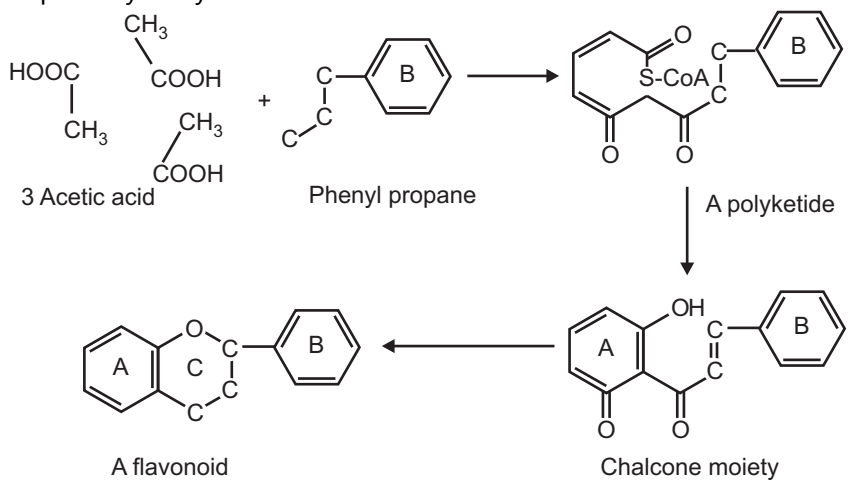


Fig. 1.6: Biosynthesis of Flavonoid molecule

In the biosynthesis of Cyanogenetic or Cyanophoric glycosides (e.g. Prunasin) the amino acid phenylalanine acts as a precursor. In this biosynthetic pathway an aldoxime, a nitrile and a cyanohydrin are involved as intermediate. The chiral centre in the mandelonitrile provides the opportunity for 2 β–glucosides to form. D-mandelonitrile glucoside is formed in *Prunus serotina* (wild cherry) whereas L-mandelonitrile glucoside (isomeric samburgrin) is found in *Sambucus nigra*. These compounds are not found in same species.

The aglycone moiety of cardiac glycosides is basically steroidal in nature. These are derivatives of cyclopentenophenanthrene ring which contains unsaturated lactone ring. This ring is attached to C_{17} a 14-β hydroxyl group and at cis position of ring C and D. The steroidal biosynthesis is derived from cholesterol production pathway. The biosynthesis of cholesterol involves cyclization of squalene, an aliphatic triterpene.

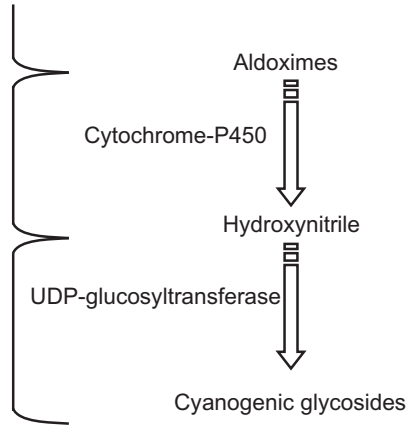


Fig. 1.7: Biosynthesis of Cyanogenetic glycosides

Acetate
↓
Mevalonate
↓
Isopentenyl pyrophosphate
↓
Squalene

The sapogenins occurs in glycosidic form of saponins. The neutral saponins are steroidal derivatives possessing spiroketal side chain and acid saponins have triterpenoidal structure. The pathway is similar for the biosynthesis of sapogenins. The triterpenoid hydrocarbon squalene is formed after cyclisation of triterpenoids in one direction and spiroketal steroids in other direction. The squalene, cholesterol and other steroidal compounds including aglycone are formed in the following manner.

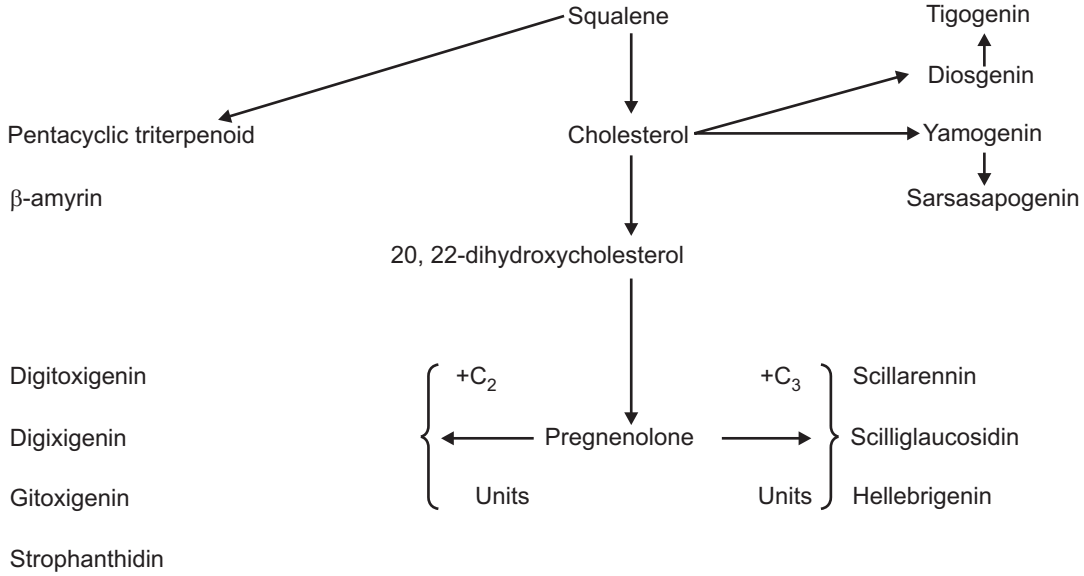


Fig. 1.8: Secondary metabolites obtained by Cholesterol metabolism

The aglycone or isothiocyanate glycosides may consist of either aliphatic derivative biosynthesized via acetate pathway or aromatic derivatives produced biosynthetically via shikimic acid.

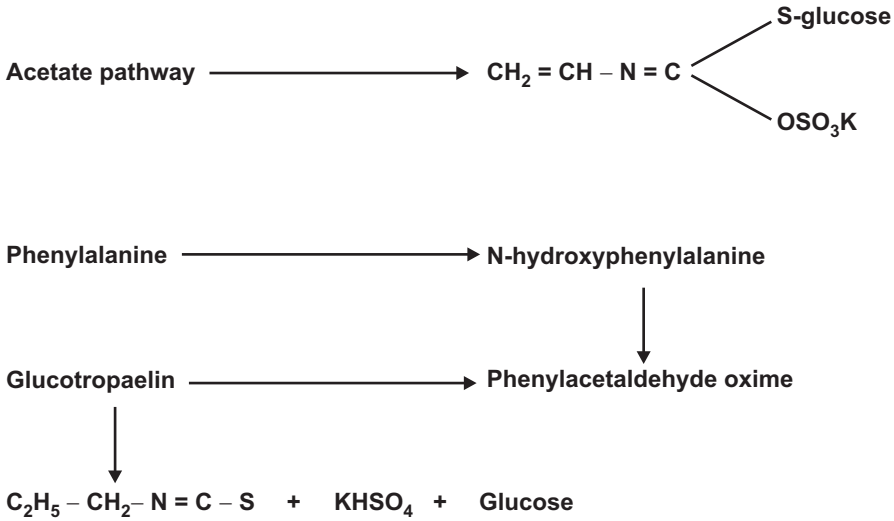
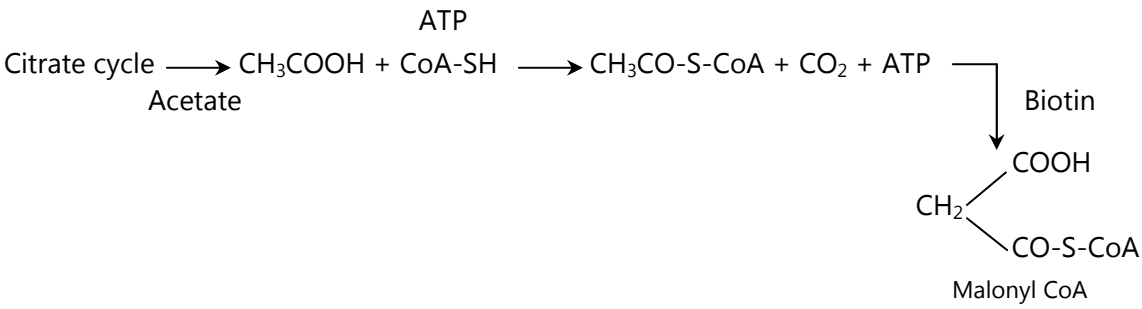


Fig. 1.9: Biosynthesis of isothiocyanate aglycone

1.3 THE ACETATE PATHWAYS

The acetate pathway is important in the formation of various important phytoconstituents like fatty acids, polyketides, prostaglandins, aflatoxin, tetracycline and other various important phytoconstituents. For the biosynthesis of fatty acid, the acetyl CoA carboxylated to form malonyl CoA by the presence of enzyme named acetyl CoA carboxylase. The energy requisite for this carboxylation is supplied by ATP and loss of CO_2 occurs. After this step reduction, dehydration and again reduction will occur. During both reductions process the electron is provided by $NADH^+$ and H^+ and the formation of butyryl ALP will occur. The coupling between malonyl ALP and butyryl ALP will occur and their reduction is repeated again for whole chain. Malonyl CoA bind again with the fatty acid residue by increase the chain with two carbon unit. The first end product is palmitic acid which has 16 carbon atoms. The chain is further elongated by various mechanisms.



The chain continuously increases by addition of malonyl (acp).

Malonyl (acp) + Butyryl (acp) ⟶⟶ Hexanyl (acp)

Malonyl (acp) + Hexanoyl (acp) ⟶⟶ Octanoyl (acp)

Malonyl (acp) + Octanoyl (acp) ⟶⟶ Decanoyl (acp)

Malonyl (acp) + Decanoyl (acp) ⟶⟶ Dodecanol (acp)

Malonyl (acp) + Dodecanol (acp) ⟶⟶ Tetra-decanoyl (acp)

Malonyl (acp) + Tetra-decanoyl (acp) ⟶⟶ Hexa-decanoyl (acp)

Hexa-decanoyl (acp) ⟶⟶ Hexadecanoate ⟶⟶ Fatty acid

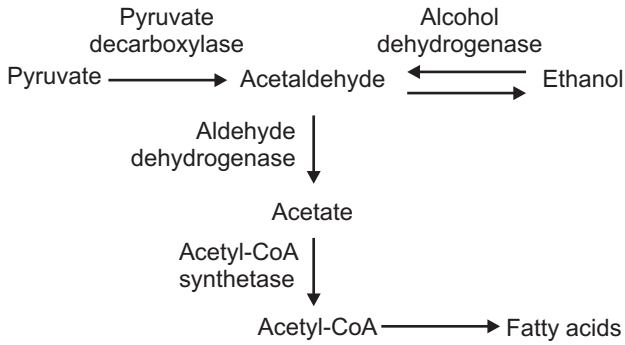


Fig. 1.10: Fatty acid metabolism

1.4 AMINO ACID FORMATION

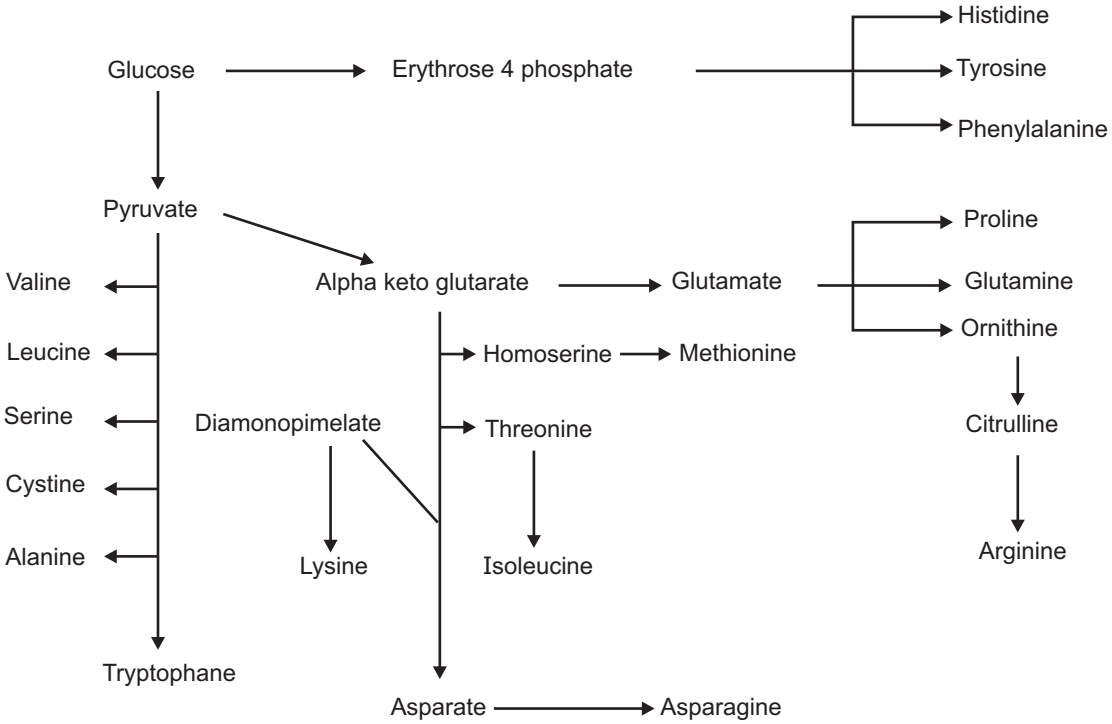


Fig. 1.11: General scheme for amino acid formation

Around 20 amino acids are important for the synthesis of protein in the plant. The transamination reaction along with other like acids, alanine, glutamic acid and aspartic acid with amino transferase enzyme transfer the amino group.

$$\alpha\text{-ketoglutamic acid} + NH_3 + NADPH \longleftrightarrow \text{Glutamic acid} + NAD$$

The amino acid can be differentiated into five different groups:

1. The Glutamate family-α-ketoglutarate is the precursor for this family. Glutamate, glutamine, arginine, and proline are the main amino acids belonging to this family.
2. Aspartate family- Oxaloacetate is the starting material for this group. The main examples of amino acid of this group are aspargine, isoleucine, threonine, aspartate and methionine.
3. Another example is Alanine-valine and leucine group.
4. The amino acid of serine glycine group.
5. Aromatic amino acids with pyruvate 3-phosphoglycerate phosphoenol pyruvate and erythrose 4-phosphate as starting material.

The amino acids synthesized from the intermediate of pentose phosphate pathways, glycolysis and citric acid cycle. Around ten of the amino acids synthesized through few of the enzymatic steps with precursor but rest (like aromatic amino acids) are complex in synthesis. Around all 20 amino acids can synthesize the bacteria and plants but only half by the mammals.

Formation of Ornithine, Proline, Hydroxyproline and Arginine:

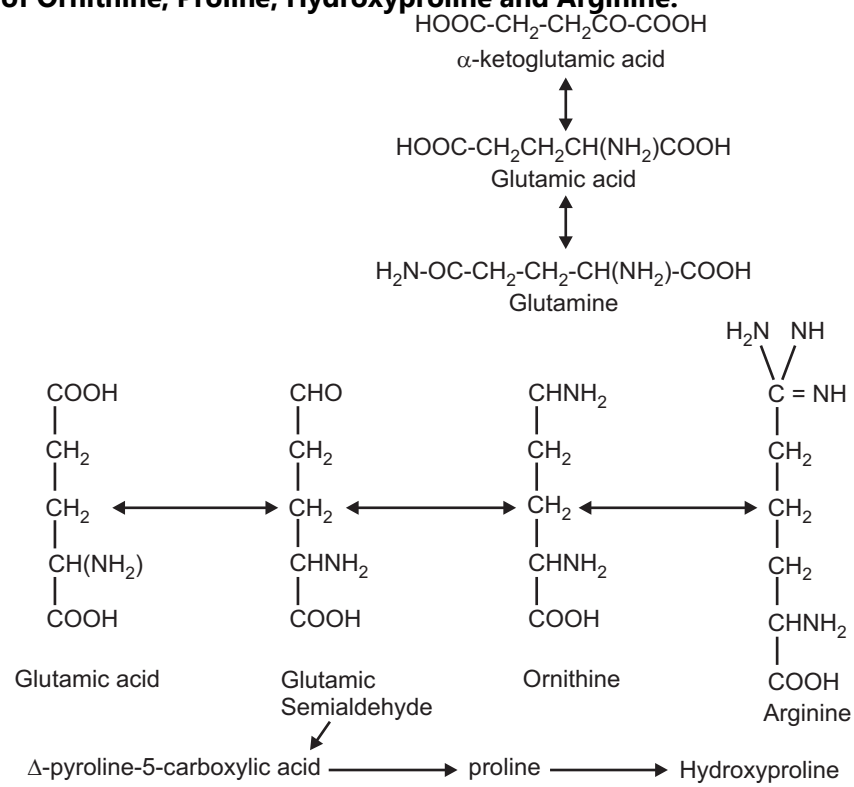


Fig. 1.12: Biosynthesis of Amino acids (ornithine, proline, hydroxyproline and arginine)

The amino acids of this group are the precursor of various important glycosides. The precursors used for the synthesis are glutamic acid. By the help of enzyme N-acetyl derivative ornithine is formed. Arginine is synthesized by ornithine through urea cycle.

Formation of Serine and Glycine:

In the formation of serine, NAD^+ oxidizes the hydroxyl group of 3-phosphohydroxy pyruvate. 3 phosphorine is generated through transamination from glutamate. Hydrolysis of 3-phosphoserine phosphatase gives free serine. Serine (3-carbon amino acids) is the precursor for the synthesis of glycine (two carbon atom) by the presence of enzyme serine hydroxyl methyl transfer.

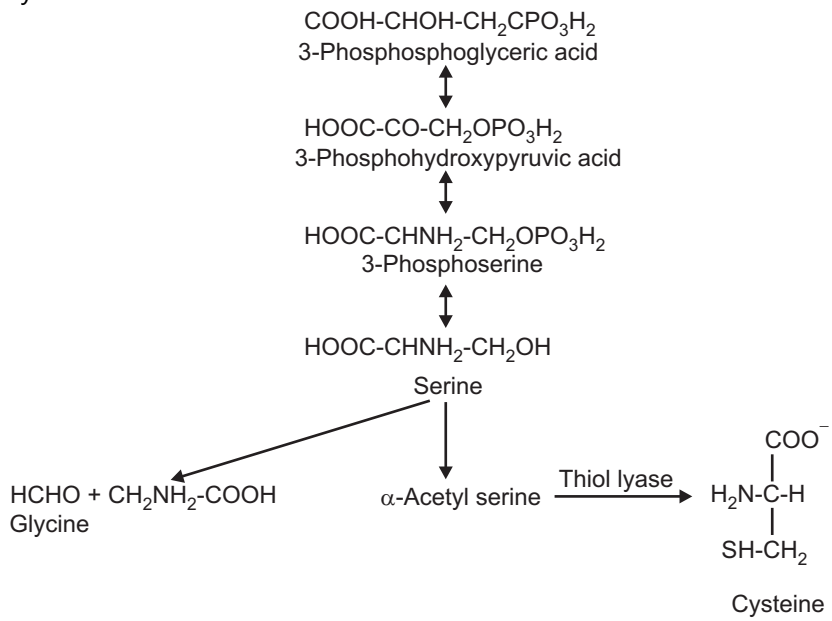
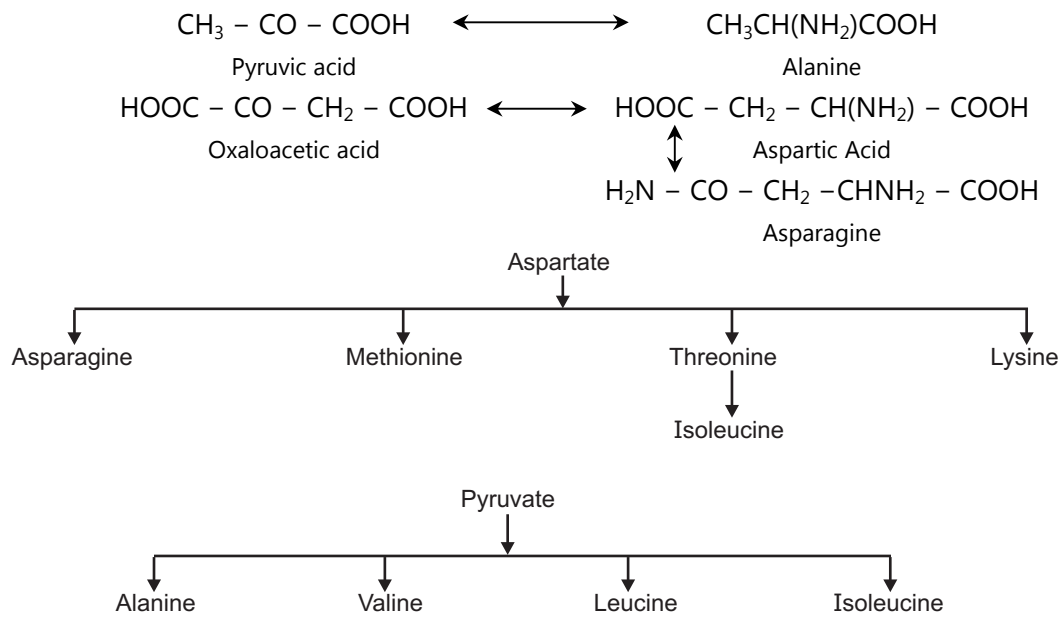


Fig. 1.13: Biosynthesis of Serine and Glycine

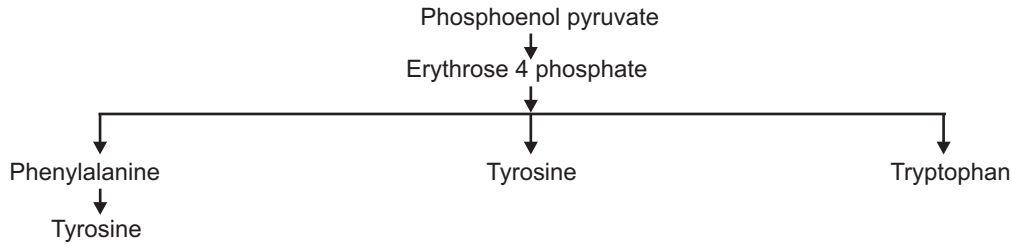
Formation of three Non-essential and Six essential Amino Acids:



Aspartate and alanine made from oxaloacetate and pyruvate respectively through transamination of glutamate. Amidation of aspartate aspargine is formed and NH_4^+ donated by glutamine. Threonine, isoleucine, methionine, lysine, leucine and valine are among the essential amino acids. The biosynthesis of these amino acids is very complex and interconnected. Threonine, methionine and lysine are generated by aspartate. Valine, isoleucine, leucine and alanine are generated by pyruvate. Valine and isoleucine involve enzyme for their biosynthesis.

In the formation of valine an intermediate is formed known as ketoisovalerate which is another important precursor for the synthesis of leucine.

Formation of Tyrosine, Tryptophan and Phenylalanine



Cohorismate is a major compound for the formation of phenylalanine, tryptophan and tyrosine.

Amino acid Biosynthetic families group by Metabolic Precursor:

- α-ketoglutarate – Glutamate, Glutamine, Proline, Arginine
- 3-phosphoglycerate- Serine, Glycine, Cystine
- Oxaloacetate- Aspartate, Aspargine, Methionine, Threonine, Lysine
- Pyruvate- Alanine, Valine, Leucine, Isoleucine
- Ribose-5-phosphate- Histidine
- Phosphoenol pyruvate and erythrose 4-phosphate-Tryptophan, Phenylalanine, Tyrosine

1.5 UTILIZATION OF RADIOACTIVE ISOTOPES IN BIOGENETIC INVESTIGATION

The living material which is used in biochemical studies varies greatly. Some research can be done which uses the whole organism with minute disturbances like bacteria, moulds and fungi. These can be cultivated and studied biochemically but in animals test material is added into feed and blood and fecal material is analyzed. In the case of plants the destruction of plant is compulsory for analysis purpose. Few examples like minces, breis and homogenates are the preparations in which the cell wall and tissues are destroyed but intracellular particles remains intact. The components of these mixtures can be separated by centrifugation and biological activities of these fractions are examined. The penultimate stage (before last stage) of a biogenetic study is the enzymatic isolation involved in the pathway and in-vitro demonstration of their properties. So it is becoming very possible to locate and clone the gene which is responsible for the enzyme synthesis. The tracer techniques uses for the study of secondary metabolism.

Tracer techniques are widely used in all branches of science. It was originated in beginning of last century. It was observed that elements exist with same chemical properties but they have different atomic weights. These isotopes may be stable like ^{2}H, ^{13}C, ^{15}N, ^{18}O or the nucleus may be unstable e.g. ^{1}H, ^{14}C and decays with the emission of radiation. If these isotopes are detected by suitable method then they should be incorporated in starting material or precursors of phytoconstituents and used as markers in biogenetic pathways study.

Radioactive Tracers:

The radioactive carbon and hydrogen are used mostly in biological investigation. Others like sulphur, phosphorus, alkali and alkaline earthy metals are also used but they should be used in specific purposes. These compounds enable the compound metabolism in living organisms. In the study of proteins, alkaloids and amino acids the labeled nitrogen atom gives more specific information in comparison to labeled carbon. The available nitrogen isotopes are stable and necessitate the utilization of mass spectrometer for their use as tracers.

The naturally occurring carbon isotopes are stable having mass number 12 and 13. The latter isotope is most abundant. Other radioactive isotopes of carbon having mass number 10, 11 and 14 have varied half life (^{10}C half life is 8.8s, ^{11}C half life is 20 min and ^{14}C half life is about more than 5000 years). The ^{10}C and ^{11}C has usefulness in biological research. The use of organic compound with prelabeled carbon atoms lead to the compound synthesis from inorganic carbon compounds which is produced in the pile by routes not previously commercially utilized. In this synthesis the compound will be more pure because strong radioactive impurity even in less proportion causes serious risk or damage of the experimental result.

Many compounds which are produced by natural sources like certain amino acid produced by the hydrolysis of proteins are prepared by developing *Chlorella* in $^{14}CO_2$ rich environment. All the carbon compounds of this organism are prelabelled.

The tritium ^{3}H labeled compounds are available commercially. Its labeling is effected by catalytic exchange in water or aqueous media or by irradiation of organic compounds by tritium gas and by hydrogenation of unsaturated compounds with tritium gas. Tritium is a pure beta emitter of less toxicity having half life 12.43 years and radiation energy is less than ^{14}C.

Detection and Assay of Radioactively Compounds:

The adequate methods are essential for the detection and estimation of labeled compounds. The liquid scintillation counter instrument is preferred for the soft and easily absorbed radiation from ^{3}H and ^{14}C labeled compounds. It mainly depends on the conversion of kinetic energy of a particle into a fleeting pulse of light as result of its penetration in a suitable luminescent material. Liquid scintillation media consists of solvent where excitation occurs and a fluorescent solute emits the light to actuate the photomultiplier. This have been devised the purpose of sample enabling incorporated into the same solute and attains optimum balance between sample and scintillator.

Today's modern instruments are fully automatic. Nearly 100 samples can be run at a time and measurement of mixed radiations is also possible e.g. ^{3}H and ^{14}C both is beta emitter and possess different energies. The instrument containing all counters is connected to a ratemeter which counts in a given time and records the readings. With ^{14}C isotope no decay correction is essential for normal biogenetic experiments because ^{14}C has long half life. So the half life is important in carbon dating of old materials.

'Curie' is the unit for radioactivity. It is defined as 'Quantity of any radioactive nuclide which has $3.7X10^{10}$ numbers of disintegrations per second.

Autoradiography:

Autoradiography is a technique used for locating the radioisotope in biological and other material. In autoradiography the sample or specimen is placed in contact with suitable emulsion for example X-ray sensitive film, and after exposure the emulsion media is developed in normal manner. The resultant autoradiograph shows the distribution pattern of radioactive material in the sample or specimen. This method can be applied to whole morphological part of plant or its section. The resultant negative is observed under microscope. The radioactive compounds can also be detected on paper chromatogram or thin layer chromatogram. The different spots determined by densitometer or by calibrated films.

Precursor-product Sequence:

In this a prelabelled precursor is fed into the plant and after a definite period of time the compound is isolated and purified. After purification the radioactivity is determined. If specific atom of the precursor is labeled, there may be the chances of isolated metabolite degradation. Radioactivity of the isolated constituent is not sufficient proof that the particular compound fed is direct precursor. The substances enter the metabolic pathway of plant and are distributed throughout the whole plant and its products. For solving this problem of none providing the complete evidence of precursor double or triple labeling experiments are developed.

The multiple labeling simplifies the procedure for determination of particular label of molecule. It provides tremendous information in comparison to the information provided by single experiment. In multiple labeling process atleast two or more than two positions are labeled at a time by using same isotope or different isotope. If we take an example of ergot alkaloids it is seen that ergot alkaloids are formed by precursor amino acid tryptophan and mevalonic acid. The cis-trans isomerism take place when chanoclavine-I is converted into agroclavine and elymoclavine. For much information 4R and 4S were fed in parallel experiments and percentage of labeled compounds in alkaloid is determined. It was observed from the results that 4S hydrogen of mevalonate was eliminated but 4R was retained.

Biosynthesis of Ergot Alkaloids:

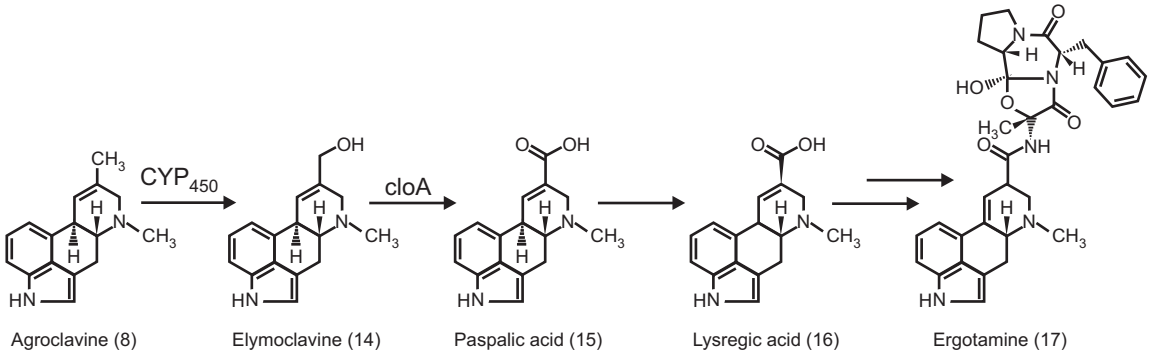
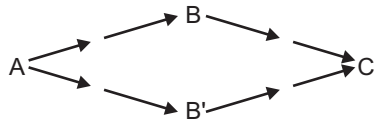


Fig. 1.14: Biosynthesis of Ergot alkaloids

Competitive Feeding:

Competitive feeding experiments are useful in determination of which two possible intermediates normally used by plants in normal biosynthetic pathway. In simple form competitive feeding can differentiate whether B or B' was the normal intermediate in the formation of C from A as below:



Of the three groups of plant, one is fed with labeled A along with inactive B and the other with labeled A and inactive B'. The third group i.e. control group is fed only with labeled A. if the product C obtained is inactive in the plant receiving B and the product is active in the plant receiving B' indicates the intermediate is B and not B'.

Administration of Precursors:

Sometimes the administered precursor may not reach the required site of synthesis in plant or at the time of experiment gives negative results. Sometimes the radioactivity of labeled compound can be destroyed because of the contamination of solution by microorganisms during infiltration. So for this problem broad spectrum antibacterial agents are used. The various methods uses for the administration of labeled substance into plants are as follows:

1. Stem feeding
2. Floating method
3. Root feeding
4. Infiltration
5. Direct injection

The root feeding technique use in those plants which synthesizes the compounds in roots. It can also be implemented for those plants which can grow in hydroponic culture solutions. The infiltration is mainly for rigid stems. In this method the stem wick is dipped into labeled solution or a flap cutted in the stem is dipped in the solution for infiltration. The direct injection of labeled substance is possible in hollow stems and capsules of plants.

Sequential Analysis:

Sequential analysis method is utilized for the sequential analysis of related compounds which are labeled. This method uses in carbon elucidation in photosynthesis and for the determination of sequential formation of plant constituents. For example- exposure period to $^{14}CO_2$ for only 5 minutes have been used to obtain evidence of the biosynthetic sequence in *Mentha piperita* plant.

Piperitone $\rightarrow$ (-)-Menthone $\rightarrow$ (-)-Menthol

The stable isotopes ^{2}H, ^{13}C, ^{15}N and ^{18}O have low natural occurrence. They can be used in the same way as radioactive elements for labeled compounds which uses as possible intermediates in biogenetic pathways. The mass spectroscopy (^{15}N and ^{18}O) and nuclear magnetic resonance (NMR) spectroscopy (^{1}H and ^{13}C) are the usual methods for detection.

QUESTIONS

1. What do you understand by study of basic metabolic pathways and formation of different secondary metabolites?
2. How secondary metabolites are produced from biosynthetic pathways?
3. What is Shikimic acid pathway? Draw its pathway.
4. How glycosides are biosynthesized? Draw its pathway.
5. Draw biosynthetic pathway of Flavonoids.
6. Draw biosynthetic pathway of Isothiocyanate aglycones.
7. Draw biosynthetic pathway of Cyanogenetic glycosides.
8. How secondary metabolites are obtained by Cholesterol metabolism?
9. Draw Acetate pathway.
10. Draw Amino acid pathway.
11. Draw general scheme for amino acid production through pathway.
12. Write a detailed note on utilization of radioactive isotopes in the investigation of biogenetic studies.
13. What is the role of radioactive tracers? How they are detected?
14. What is autoradiography? Explain precursor product sequence.
15. What is competitive feeding? How precursors are administered in any pathway?
16. What is sequential analysis? Describe briefly.

■■■

Unit II

Chapter ... 2

Alkaloids

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *General introduction and brief description about Alkaloids*
- *Pharmacognostic profile of alkaloidal containing drugs*
  - *Vinca*
  - *Rauwolfia*
  - *Belladonna*
  - *Opium*

2.1 INTRODUCTION

The term alkaloid was introduced by W. Meissener at the beginning of the 19th century to designate natural substances reacting like base, in other words like alkalis (from the Arabic alkaly = soda and from the Greek word eiods = appearance).

The definition of alkaloids is not simple and precise and sometimes it is difficult to distinguish the thin line between alkaloids and other natural nitrogen containing metabolites.

Natural nitrogen containing compound are:

- Amino acids
- Amines
- Alkaloids
- Indole
- Purines, pyrimidines and cytokinins
- Cyanogenetic glycosides
- Chlorophylls

An alkaloid is an organic compound of natural origin which contains nitrogen atom which is more or less basic, is of limited distribution and has at low doses marked pharmacological properties.

This grouping has a sound basis which is confirmed by the fact that these compounds have in common some reactions of precipitation with the general reagent for alkaloids. Their nitrogen atom is a part of heterocyclic system and causes a significant pharmacological activity.

According to some authors, they occur only in the vegetable kingdom. They are found as salt and it can also say that they are form biosynthetically from an amino acid.

Pseudo alkaloids most often have all of the characteristics of the true alkaloid but they are not derived from amino acids. Some of the most known examples are Isoprenoid, Aconite, Aconine, Hypoaconitine etc.

Steroidal alkaloids like Conessine, Solanidine are few examples. They are also known as heterocyclic nitrogen containing substances arising from the metabolism of Acetate. Example is coniine, the toxic principle of Hemlock.

Proto alkaloids are simple amines in which the nitrogen atom is not part of heterocyclic ring. They are basic in nature and derived from amino acids. Few examples of these are Colchicine, Ephedrine and Pseudo ephedrine.

2.2 DISTRIBUTION

Alkaloids occur exceptionally in bacteria (*Pseudomonas aeruginosa*) and rather rarely in fungi (Psilocin from the hallucinogenic mushroom).

The pteridophyta rarely contain alkaloids. Thus alkaloids are compounds essentially found in the angiosperm.

Alkaloid concentrations have a wide range of variation from a few ppm (for example: *Catharanthus roseus* contain 3 gm alkaloid in one metric ton leaves) to more than 15% in the bark of the trunk of *Cinchona ledgeriana*.

Sometimes they do contain only one constituent (for example Hyoscyamine from the leaves of belladonna) but must often they yield a complex mixture which may be dominated by one major constituent.

As a general rule all of the alkaloids of a given plant have a common biogenetic origin even if their structure may at first seen quite different.

The conc. of alkaloids can vary from part to part and some part may contain none. For example Quinine accumulates in the trunk bark of cinchona but is completely absent from leaves.

For a long time alkaloids used to be considered products of metabolism of plant only. In fact alkaloids also occur in animals (Arthopods who secrete them in very small quantities in their exocrine glands).

Sometimes they are volatile enough to act as chemical signals defense compound known as allomones or communication compounds called pheromones.

Localization

In the plant, alkaloid occurs as soluble salt (citrate, Malate, tartarate, melonates, benzoates, isobutyrates) or in combination with tannins.

They are often localized in peripheral tissue external layer of the bark and root or seed tegument. Alkaloids synthesis takes place at specific sites (growing root, chloroplast, laticiferous cell).

2.3 FUNCTIONS OF ALKALOIDS

Alkaloids are poisonous in nature but when used in small quantities they exert useful physiological effects on animals and humans beings.

Their exact role in plant is still a topic of research. Some of the predicted roles are:

(a) They are reserve substances which can supply nitrogen.
(b) They might be the defensive mechanism for plant growth in dry region to protect from grazing animals, herbivores and insects.
(c) They may be end product of detoxification mechanism in plant and by this way check formation of substance which may be prove to harmful to the plant.
(d) The possible role as growth regulatory factor in the plant.
(e) They are present normally in conjugation with plant acid like mercuric acid, cinchotannic acid etc. Therefore alkaloids could be acting as carriers within plant for transportation of such acids.

2.4 PHYSICOCHEMICAL PROPERTIES

- Alkaloids have molecular weight from 100-900.
- Although most of the bases that do not contain oxygen atom are liquid at ordinary temperature (examples: nicotine, coniine). Those that do contain oxygen atom are normally crystallisable solids and in rare cases they are coloured compound (example berberine).
- Almost all of the crystallized bases rotate the plane of polarized light and have melting points without decomposition especially below 200^{o}C.
- As a general rule alkaloids as bases are not soluble in water. They are soluble in polar or slightly polar organic solvents.
- The basicity of alkaloids varies greatly. Since this property depends entirely on the availability of the lone pair of electron on the nitrogen atom.
- Electron withdrawing group in close proximity to the nitrogen atom decrease the basicity, whereas electron donating group enhances it.
- The basic character allows the formation of salts with mineral acids (i.e. hydrochloride, sulphates, and nitrates) and organic acids (i.e. tartarate, sulfonate).
- Alkaloidal salts are generally soluble in water and in dilute alcohols and they are except in rare cases not soluble in organic solvent.
- Pseudo alkaloids and proto alkaloids show higher solubility in water while free bases of alkaloids are soluble in organic non polar immiscible solvent.

2.5 DETECTION AND CHARACTERIZATION

The different reagents used for the detection of alkaloids are:

- Mayer reagent (Potassium mercuric iodide solution) gives cream coloured precipitate.
- Dragendorff reagent (Potassium bismuth iodide solution) shows reddish brown precipitate.
- Wagner reagent (Iodine potassium Iodide solution) yields reddish brown precipitate.
- Hagers reagent (saturated Picric acid solution) gives yellow colour precipitate.

- ρ-dimethyl amino benzaldehyde uses for ergot alkaloids and pyrrolizidine alkaloids.
- Cerium and ammonium sulfate for different indole (yellow), dihydro indole (red) and β-amilino acelates (blue).
- Caffeine, purine derivatives does not precipitate like most alkaloids. It is detected by mixing with a very small amount of potassium chlorate and a drop of HCl, evaporating to dryness and exposing the residue to ammonia vapour. A purple colour is produced with caffeine and other purine derivatives (Murexide test).

Care must be taken in the application of these alkaloidal tests, as the reagent also gives precipitate with proteins. During the extraction of alkaloids from the plant and subsequent evaporation, some proteins will not be extracted and other will be made insoluble (denatured) by the evaporation process and may be filtered out. If the original extract has been concentrated to low bulk and the alkaloids extracted from an alkaline solution by means of an organic solvent and then transferred into dilute acid (e.g. Tartaric acid) the latter solution should be protein free and ready to test for alkaloids.

2.6 EXTRACTION

The extraction of alkaloids is based as a general rule on the fact that they normally occur in the plant as salts and on their basicity, in other words on the differential solubility of the bases and salt in water and organic solvent.

[A] Solvent extraction in alkaline medium

Step 1:

- The powdered defatted drug is mixed with an alkaline aqueous solution which displaces the alkaloids with acids tannins and other phenolic substances, the free base are then extracted with an organic solvent.
- Alkalization is very often achieved with aqueous ammonia. If the structure of the alkaloids to be extracted contains a fragile element for example ester or lactone fraction aqueous ammonia must be replaced by an alkaline carbonate solution.
- In some cases a mixture of calcium hydroxide and sodium hydroxide is used i.e. in cinchona bark in which the alkaloids are combined with tannins.
- When using sodium hydroxide, it will turn phenolic alkaloids into phenolates which will remain in aqueous solution, if necessary this can be used to fractionate the total alkaloids.
- The organic solvent can be a chlorinate solvent (dichloromethane or chloroform) depend on cost, toxicity, safety and recovery.

Step 2:

- The organic solvent containing the alkaloids as bases is separated from the residue and if necessary partially concentrated by distillation under reduced pressure.
- The solvent is then stirred with an acidic aqueous solution. The alkaloids go into the solution in the aqueous phase as salts, whereas neutral impurities remain in the organic phase. Repeat the process till the organic phase no longer contain any alkaloids. Acids may be HCl, H_2SO_4, sulfanic, citric and tartaric acid.

Step 3:

- The aqueous solution of the alkaloidal salt combines and if necessary, washed with non polar solvent (hexane or diethyl ether), are alkalinized with a base in the presence of an organic solvent not miscible with water.
- The alkaloids as bases precipitate and dissolve in the organic phase.
- The extraction of organic phase continues until the totality of the alkaloids has gone into the organic phase.
- Finally the organic solvent containing the alkaloids, as bases is decanted, freed from possible traces of water by drying over an anhydrous salt and evaporated under reduced pressure.

[B] Extraction in Acidic Medium:

- Two approaches are possible, in the first one the pulverized drug is extracted directly with acidified water.
- In the second case it is extracted with an acidified alcoholic or hydro alcoholic solution.
- In the latter case, the extraction is followed by a distillation under vacuum which eliminate the alcohol and leave behind as acidic aqueous solution of the alkaloidal salts.
- In both cases the result is an aqueous solution of an alkaloidal salt requiring purification. This can be accompanied by:
  - Alkalizing the solution and extracting the bases with an immiscible organic solvent which leads back to the above steps.
  - Selectively absorbing of the alkaloids in the solution of an ion exchange resin, then eluting with a strong acid.
- Precipitating the alkaloids are iodomercurals. The resulting complex is recovered by filtration dissolved in a mixture of water alcohol and acetone and decomposed by passing it through an ion exchange resin. This technique can be used to extract quaternary ammonium salts.

2.7 ISOLATION

By extraction we cannot yield pure compound but total alkaloids which are complex mixture of bases that must be separated. The method for isolation is following:

(a) Direct crystallization from solvent: This method is very simple and cannot be used for the isolation of complex mixtures. One of the alkaloid should be in major constituent and can be obtained by direct crystallization. For example- quinine which is crystallized as basic sulfate by simple neutralizing the acidic extraction medium with sodium carbonate to pH 6.

(b) Steam distillation: This method is specially employed for volatile liquid alkaloids like coniine, spartine and nicotine but otherwise this process is not suitable for alkaloids of high molecular weights.

(c) Chromatographic techniques: Sometimes it is necessary to follow the classical methods of isolation for complex mixtures, mainly chromatographic techniques. In research and development labs these technique as well as high performance liquid chromatography and preparative thin layer chromatography are most often used.

(d) Gradient pH technique: Generally it is known that alkaloids are basic in nature and extent of basicity variation exist among various alkaloids of the same plant. For this case the crude alkaloidal mixture is dissolve in 2% tartaric acid solution and then extracted with benzene. The first fraction contains neutral or very weakly basic alkaloids. pH of aqueous solution is increased gradually by 0.5 increments up to pH 9 and extraction can be done with organic solvents. By this way the alkaloid of different basicity are extracted at the end.

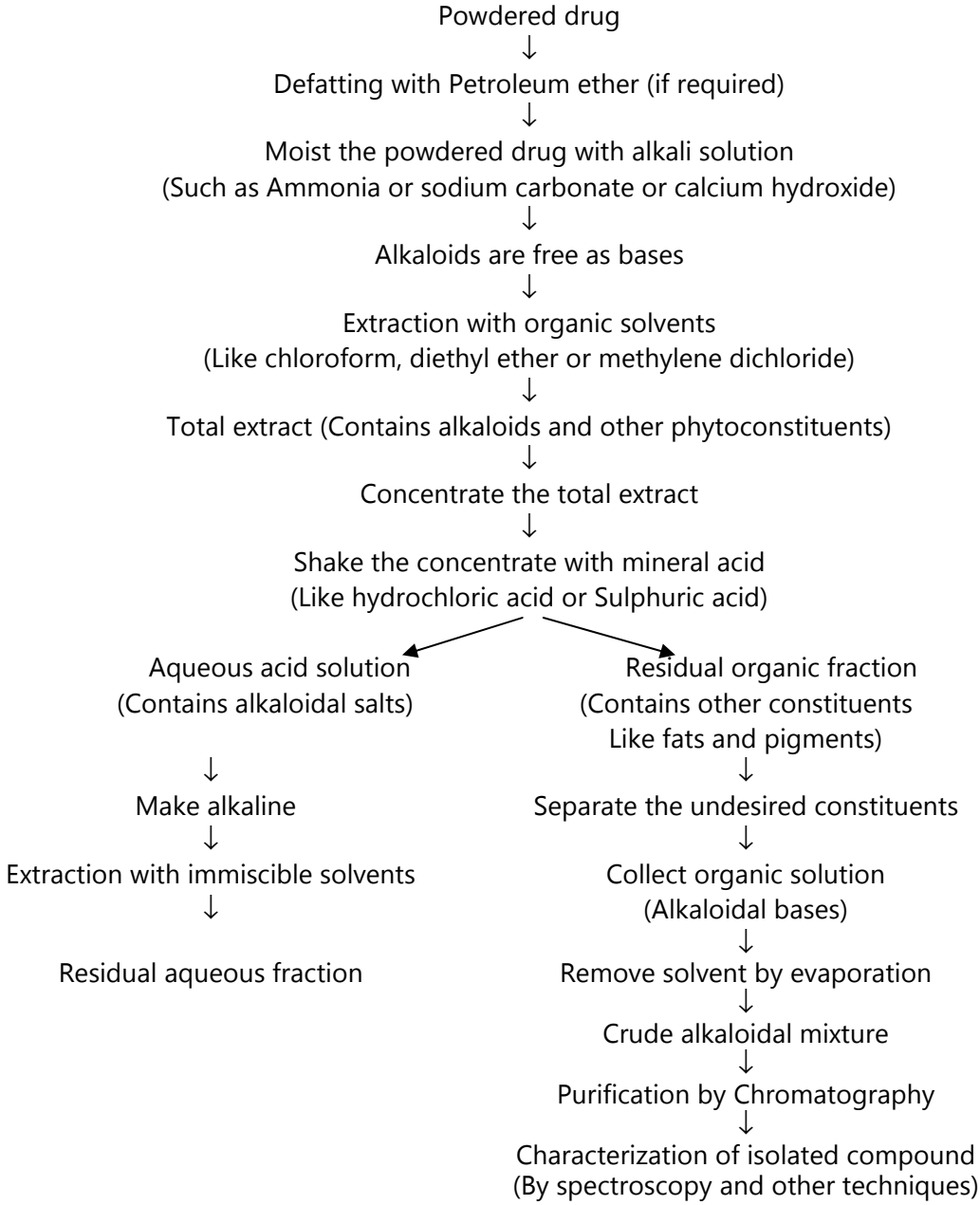


Fig. 2.1: General extraction procedure of Alkaloids

2.8 CLASSIFICATION OF ALKALOIDS

The classification is mainly based on pharmacological activity, taxonomical distribution, their biogenetic origination and presence of chemical entities.

2.8.1 Pharmacological Classification

Depending on the physiological response the alkaloids are classified under various pharmacological categories like CNS stimulants or depressant, sympathomimetics, analgesics, purgatives etc. Main drawback of this system is that it does not take into consideration about chemical nature of crude drug. Within the same drug the individual alkaloids may exhibit different action. Example- 1) Morphine is narcotic and analgesic while codeine is mainly antitussive. 2) Cinchona quinine is antimalarial whereas quinidine is cardiac depressant.

2.8.2 Taxonomic Classification

This method classifies the vast number of alkaloids based on their distribution in various plant families like solanaceous alkaloids in solanaceae family or papillionaceous alkaloids in papillionaceae family. The grouping of alkaloids are done as per the name of genus in which they occur e.g. Ephedra, cinchona etc. The chemotaxonomic classification has been further derived from this classification.

2.8.3 Biosynthetic Classification

This method gives the significance to the precursor from which the alkaloids are biosynthesized in the plant. Hence the variety of alkaloid with different taxonomic distribution and physiological activities can be brought under some group if they are derived from same precursor i.e. all indole alkaloid from tryptophan are grouped together. Alkaloidal drugs are categorised on the fact whether they are derived from amino acids precursor as ornithine, lysine, phenylalanine, tryptophan etc.

2.8.4 Chemical Classification

This is the most accepted way of classification of alkaloids which basically depends on ring structure present in the alkaloid. The alkaloidal drugs are broadly categorized into two divisions:

- True alkaloids (subdivided into 12 groups).
- Proto alkaloids or biological amines and pseudo alkaloids.

(a) True alkaloids

1. Pyrrole and pyrrolidine: Hygrine, coca species.
2. Pyridine and piperidine: Arecoline, Anabasine, coniine, trigonelline.
3. Pyrrolizidine: Echimidine, symphitine.
4. Tropane (piperidine/N-methyl pyrrolidine): Atropine, hyoscine.
5. Quinoline: Quinine, Quinidine, Chinchonine.

6. Isoquinoline: Morphine, codeine.
7. Aporphine (reduced isoquinoline - Naphthalene): boldine.
8. Indole (Benzpyrole): Vincristine, Ergometrine, Reserpine.
9. Imidazole: Pilocarpine, Isopilocarpine.
10. Norlupiname: Cytisine, Spartine.
11. Purine (pyrimidine/ imidazole): Caffine/ theofronine, theophylline.
12. Steroidal (cyclo pentano per hydro phenathrene ring): Solanidine, Conessine.

(b) Pseudo alkaloids

Diterpenes- Aconitine, Aconine

(c) Proto alkaloids

Alkylamines (amino alkaloids) – Ephedrine, colchinine

[I] VINCA

Synonyms: Catharanthus, Periwinkle

Biological source: It consists of dried whole plant of *Catharanthus roseus* L or *Vinca rosea*.

Family: Apocynaceae.

Geographical source: It is indigenous to Madagascar and cultivated in South Africa, India, USA, Europe and Australia.

Cultivation and Collection:

It grows well at an altitude upto 500 mt. This plant is found all over India but grown well in tropical and subtropical areas (like southern and north eastern part) of India. It does not require any particular type of soil. It grows well in light sandy soil which should be rich in humus content. The 100 cm rainfall is most preferable for its cultivation. The fresh seeds are used for its propagation. The seeds should be shown in nursery or direct sowing can also be done. The seeds are mixed with sand (1:10) and sown in rows having 45 cm distance in between two rows. The sowing should be in monsoon season. The plants are thinned out upon sufficiently growth. In other case nursery sowing is more economical. They are sown in nursery in February or March season and transplanted in open field after 2 months or when they achieve 6-7 cm height. The plants are drought resistant and do not require much water supply. The plants also not require any type of fertilizers. A mixture of nitrogen, phosphorus and potassium gives better results. Farmyard manures are used and weeding is done time to time periodically. The stems are cut after 1 year of growth. The leaves, stems and seeds are separated and dried in air. The roots are collected by dugging out method followed by ploughing. The roots are washed properly, shade dried and finally packed into bales. The yield is about 1 to 5 ton per hectare (roots), 1 to 2 ton per hectare (stem) and 3 to 4 ton per hectare (leaves).

Fig. 2.2: Vinca (Catharanthus roseus), *C. roseus* Var Major and *C. roseus* Var Alba.

Macroscopical characters:

Colour	**:**	Green (Leaves), Pale grey (Roots), Purple or pinkish white or caramine red (Flowers)
Odour	**:**	Characteristic
Taste	**:**	Bitter
Other features	**:**	The plant is an erect, pubescent herb having branched tap root.
Leaves	**:**	Simple, petiolate, ovate or oblong and glossy.
Flowers	**:**	Bracteate, pedicellate, complete and hermaphrodite.
Fruits	**:**	Follicles with many black seeds.

Constituents:

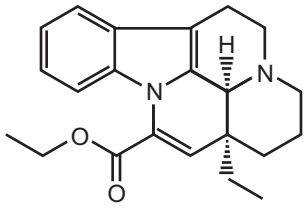
- About 150 alkaloids have now been isolated from *Catharanthus roseus* e.g. ajmalicine, serpentine, tetrahydrolalstonin and lochnerine etc.
- The plant contain large number of indole alkaloids, out of them about 20 dimeric indole dihydroindole alkaloids contains antineoplastic activity including vincristine and vinblastine. These two alkaloids are much significant.
- Vinblastine is produced by coupling of the indole alkaloid catharanthine (indole alkaloid part) and vindoline (dihydro indole alkaloid part).
- About 500kg of drug gives 1 gm of vincristine. Vincristine concentration is very low in plant extract (about 0.0002 percent) which makes it very costly. So attempts are made for its synthesis.
- The plant contains alkaloids in very low concentration; by tissue culture technique its production can be increased.

Uses:

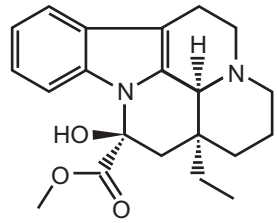
Vinca plant is used to extract alkaloids like vincristine, vinblastine and ajmalicine. Vincristine sulphate acts on mitotic cell division of metaphase and arrest the cell for further division; hence used as an antineoplastic drug whereas vinblastine sulphate acts on mitosis of metaphase and interferes in amino acid metabolism. It suppresses the immunity and uses in hodgkin's disease, lymphoma and choriocarcinoma treatment. Vincristine is applied by

intravenous route of administration in leukemia, hodgkin's disease, sarcoma of reticulum cells, lymphosarcoma and myosarcoma treatment. Vinca alkaloids also uses in diabetes and high blood pressure treatment. The maximum dose of vincristine sulphate is up to 2 mg but 10 to 30 µg/kg body weight administered intravenously whereas the dose of vinblastine sulphate is about 100 µg/kg body weight administered intravenously.

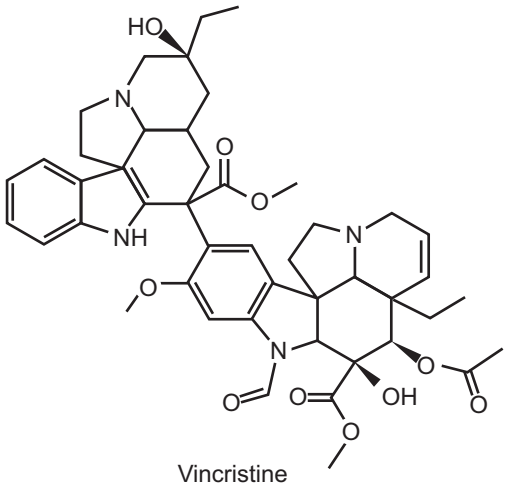
Other species: *Catharanthus lengifolius, C. Trichophyllus.*



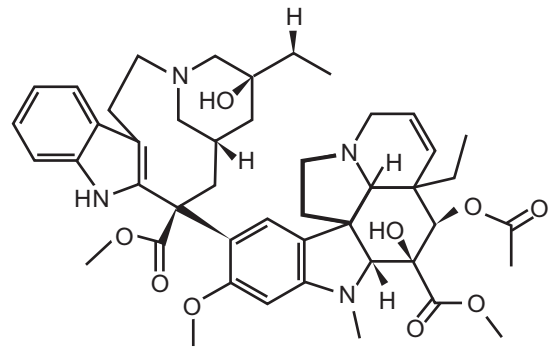
Vinpocetine



Vincamine



Vincristine



Vinblastine

Fig. 2.3: Structure of Vinca alkaloids

[II] RAUWOLFIA

Synonym: Rauwolfia root, Sarpagandha

Biological source: It consists of the dried roots and rhizomes of *Rauwolfia serpentina* Benth.

Family: Apocynaceae.

The name *Rauwolfia serpentina* has been taken from the name of scientist Dr. Rauwolf and serpentina means snake like structured root.

Geographical source: Several species of plant is distributed in the tropical region of Asia, America and Africa. It is commercially cultivated in India (mainly in Uttar Pradesh, Bihar, Orissa, Tamil Nadu, West Bengal, Karnataka, Maharashtra and Gujarat), Srilanka, Myanmar, Thailand and America.

Cultivation and collection: Rauwolfia develops luxuriantly under wide range of climatic condition. It flourishes well in hot humid climate and grows quite well in shade. The clay loamy soil with handsome amount of humus is most recommended. Good drainage is supposed to be ideal for its cultivation. The acidic soil having pH 4 favors the growth of plant. The other requirements for its cultivation are temperature 10-38℃, Rainfall in the range of 250-500 cm. Sandy soil makes the plant more susceptible towards diseases. The propagation is mainly done by seeds, roots, cutting root stump etc. Propagation from seeds is the method of choice. For this method, the seeds are sown in nursery beds in the month of May-July. The developed seedlings are transplanted in the month of August-September into open field at a distance of 15 to 30 cm. The fertilizers (like Ammonium sulphate, Urea) and manures (generally bone meal) are supplied to the plants. The plants should be kept free from weeds and they are uprooted when the plants are about 3 to 4 years old. The roots are cut properly and washed with water to remove adhering impurities like sand or other earthy matter. Then it is dried in air. About 5 kg of seeds is sufficient to cover one hectare area. The average yield is about 1200 kg/ hectare but the yield can vary with type of soil, climatic condition and age of plant.

Fig. 2.4: Rauwolfia plant and their roots

Macroscopical characters

Colour : Greyish yellow to brown (root bark) and pale yellow (wood).

Odour : Odorless

Taste : Bitter

Size : 10-80cm in length and 1-3cm in diameter.

Shape : Roots are sub-cylindrical, slightly tapered, tortuous.

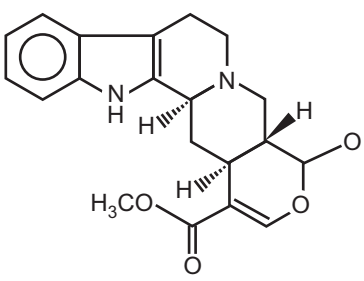
Fracture : Short and irregular.

Chemical constituents

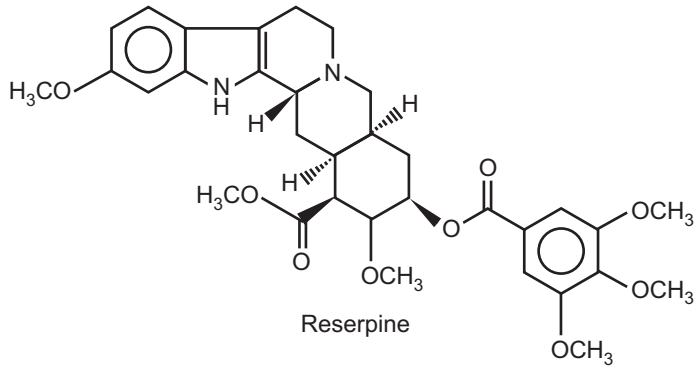
Rauwolfia contains at least 30 indole alkaloids. The total alkaloidal range is in 0.7-3 percent (depends upon the source). Alkaloids are concentrated mostly in the base of the root and can be classified into:

1. Indole alkaloid
2. Indoline alkaloid
3. Indolenine alkaloid
4. Oxyindole alkaloid
5. Pseudo indoxyl alkaloid

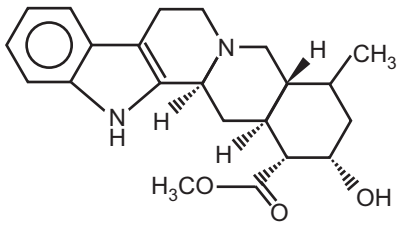
Reserpine is the main alkaloid in Rauwolfia. It also contains oleo resin, phytosterol, fatty acids, alcohols and sugars. The other alkaloids are Ajmaline, Ajmalicine, Rauwolfinine, Rescinnamine, reserpinine, yohimbine, serpentine, serpentinine. Reserpine alkaloid is colorimetrically determined by the reaction in between acidic solution of alkaloid and sodium nitrite.



Ajmalicine



Reserpine



Yohimbine

Fig. 2.5: Structure of Rauwolfia alkaloids

Chemical Test:

1. When fractured surface is treated with concentrated nitric acid, a red coloration observed.
2. When reserpine is treated with solution of vanillin in acetic acid, it shows violet red colouration.

Uses:

It has antihypentensive activity. The most clinically important compounds are reserpine, rescinnamine and ajmalicine. Reserpine depletes the catecholamines stored at nerve endings and lowers the blood pressure. The drug is used in mild anxiety condition and neuropsychiatric disorders (due to its tranquillising effect). Deserpidine and Rescinnamine used as antihypertensive. Deserpidine shows very less side effects and rescinnamine causes mental depression in higher dose concentration. Ajmalicine uses in the treatment of circulatory diseases and in cerebral blood flows obstruction.

Allied Drugs:

R. tetraphylla
R. nitida
R. densiflora
R. *vomitoria*

[III] BELLADONNA

Synonym: Belladonna leaves, Belladonnae folium, Deadly night shade leaf, European belladonna

Biological source: It consist of dried leaves, flowering tops or other aerial parts of European belladonna *Atropa belladona* Linn or Indian belladonna *Atropa acuminata* Royle. It is also collected in mixture form of both species when the plants exist in flowering condition. It contains not less than 0.3 to 0.5 percent of total alkaloid hyoscyamine.

Traditionally the British pharmacopoeia drug has consisted of all the aerial part (belladona herb) but under the European requirement there is a limit (3%) of stem with a diameter exceeding 5mm.

Family: Solanaceae

Geographical source: It is indigenous to England and other European countries. It also found in India (western Himalayan region from Shimla to Kashmir and Himachal Pradesh). It mainly occurs in Jammu and Sind forest and valley of Chinab.

The juice of this plant was used as a cosmetic in olden time due to its dilatory effect on the pupil of eye.

Cultivation and collection: It was introduced in London pharmacopoeia (1809) but the root was not used in Britain until a liniment prepared from it.

The Plant of deadly night shade *Atropa belladona* is a perennial herb which attains a height of about 1.5m. The flowers appear about the beginning of June. They are solitary, shortly stalked, drooping and about 2.5 cm long. A yellow variety of plant lacks the anthocyanin pigmentation, the leaves and stem are yellowish green and flowers and berries yellow in colour.

Belladonna is grown from seed. For this, the berries are crushed to collect seeds. Washing and sieving should be done to obtain good quality of seeds which are going to be used for cultivation purpose. The broadcasting method is applied for sowing of seeds in well ploughed field in the month of May to July. The foliar spray of fungicides encourages the growth of plants. The developed seedlings are transplanted in September or October at a certain distance between two plants. The seedlings are carefully irrigated. Fertilizers (Urea, Potash, Superphosphate etc) and insecticides are applied time to time as per the requirement. The leaves are said to be richest in alkaloidal content at the end of June or July. A sunny position is said to give more active leaves than a shady ones. The leaves and flowering parts are cut and dried in sun or shade dried. During drying care should be taken to retain green colour of leaves. Plants about 3yr old are sufficiently large to give a good yield of leaves.

If roots are being collected, it would seem to be best to repellant about every third year. Two or more crops of leaves are collected annually. Leaves left in an imperfectly dry state deteriorate and gives ammonia like odour. They should therefore be dried immediately after collection and be carefully stored. A good colored leaves may be obtained by drying in thin layer starting with a moderate heat which is gradually increased about 60^{o}C and then gradually decreased.

Fig. 2.6: Belladonna leaves with flower

Morphological characters: The drug consists of leaf and the other smaller stems, the latter seldom exceeding 5mm diameter, together with flowers and fruits.

Colour:

- Leaf- Greenish or brownish green
- Flowers- Purplish to Yellowish brown
- Fruits- Greenish to brownish in colour

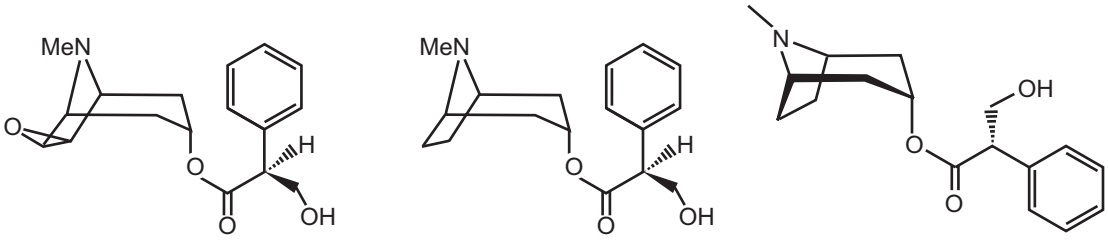
Odour **:** Slight and characteristics

Taste **:** Bitter and acrid

Size **:** Leaves are 5 − 25 cm × 2.5 − 12 cm
Flower corolla 2.5 cm long and 1.5 cm wide
Fruits about 10 cm in diameter

Shape **:** Leaves: Ovate, lanceolate to broadly ovate, brittle
Flowers: Campanulate, 5 small reflexed lobes of corolla
Fruits: Berries, sub-globular in shape with numerous flat seeds

Chemical constituents: The drug from *Atropa belladona* contains 0.3-0.6 percent alkaloids. The chief alkaloid is Hyoscyamine, small quantities of volatile bases such as pyridine and N-methyl pyrroline are present and if not removed during the assay of drug by heating, increases the titration and appear in the result as hyoscyamine. The leaf also contain a fluorescent substance, β-methyl esculitine and calcium oxalate. They yield about 14 percent of ash and not more than 4 percent acid insoluble ash. Root contain 0.6 percent, stem 0.05 percent, leaves 0.4 percent, unripe and ripe berries 0.19-0.2 percent and seeds 0.33 percent alkaloids. The main alkaloids are l-hyoscyamine and its racemic form atropine. The drug also contains belladonin, hyoscine etc.



(1) (–)-Hyoscine (scopolamine) (3) (–)-Hyoscyamine Atropine

Fig. 2.7: Structure of belladonna alkaloids

Dose: 0.6 to 1ml of belladona tincture 4 times a day.

Adulterant and substitute: The adulteration mainly done by leaves of *Phytollacca americana*, *Solanum nigrum* and *Ailanthus glandulosa*.

Allied drugs: Indian belladona from *Atropa acuminata*, *Atropa batrica*.

Uses: Belladona leaves are mainly used for internal preparations which uses as sedative and to check secretion. The root preparation mainly used externally as an parasympatholytic drug whose anticholinergic properties are used to reduce secretion such as sweat, saliva and gastric juice and reduces spasm in intestinal griping due to strong purgative action. It is also used as an antidote in opium and chloral hydrate poisoning.

Pharmacological activity: Atropine and hyoscyamine have the same activity. Atropine acts by competitive and reversible inhibition of acetylcholine binding on its receptor. In the heart and after temporary bradycardia atropine increases the heart rate by suppressing vagal inhibition.

In the blood vessels, the effect on blood pressure is not marked.

Belladonna (fruits, roots, leaves) are toxic. The ingestion of these plants induces turn red, the mouth and mucosal membranes turn dry and an intense thirst and muscular weakness develops.

The heart rate increases substantially, mydriasis and hyperthermia are always observed. Hallucination and delirium follow accompanied by agitation sometimes convulsions, sleepiness or comma is next. Recovery takes times (1-3 days). The patient must be monitored and may be treated with charcoal or sedatives.

[IV] OPIUM

Synonym: Raw opium.

Biological source: The latex is collected from unripe capsules upon incision of *Papaver somniferum* Linn. The latex is dried fully or partly by heat or spontaneous evaporation. The irregular shaped masses or moulds of uniform sizes and shapes are found. It contains not less than 9.5 percent of morphine calculated as anhydrous morphine.

Family: Papaveraceae

Geographical source- India, Pakistan, Afghanistan, Turkey, Russia, China.

Cultivation, Collection and Preparation

There are more than 50 different species exists in genus *Papaver*. Out of which six species are found in India like *P. nudicaule, P. rhoeas, P. argemone, P. dubium P. somniferum and P. orientale*.

It is an erect plant having 60-110 cm height approximately. It is branched having linear oblong or ovate oblong leaves, bluish white, violet or purplish flowers. Cultivation of opium is mainly done in certain regions like Madhya Pradesh, Punjab, Rajasthan and Uttar Pradesh under government supervision. The yield depends upon weather conditions. It grows well in temperate climate. It can also grow well in winter season but extreme cold and frost adversely affects the plant and yield of opium. The cool weather (without freezing temperature and fog or cloudiness) with full sunshine favors the growth of the plant and gives better yield. It grows in November to March months. The propagation mainly done by seeds sowing (generally 3-4 kg seeds per hectare are required). The seeds are mixed with sand (1:3) and sown in soil. The well drained loamy soil is preferred for cultivation. The soil should have pH 7 for better cultivation. Organic matters and fertilizers are supplied for cultivation. The distance should be 25 cm in between two plants. The plant reaches up to one meter height.

Fig. 2.8: Opium capsule (incised) and its field.

During the maturity period, the capsule exudes maximum latex which shows a colour change from dark green to light green. Such capsules are incised vertically in the afternoon

with the help of specific needle shape apparatus called Nushtur. Latex exudes out and thickens due to cold weather in night which is eventually scrapped and collected next morning by an iron scoop called enarpala. Incision is repeated 4 times. Latex is collected in plastic container. The capsules are collected and dried in open areas and further the seeds are separated by beating.

Macroscopical characters:

Odour : Strong, characteristics

Taste : Bitter taste

Table 2.1: Commercial varieties of opium and their appearances

Sr. No.	Commercial variety	Appearance
1.	Indian opium	Colour: Dark brown Form: Cubical pieces Fracture: Brittle
2.	Persian opium	Colour: Dark brown Form: Brick shaped masses Nature: Hygroscopic, granular, smooth
3.	Turkish opium	Colour: Brown or Dark brown Form: Conical, rounded or flattened masses
4.	Chinese opium	Colour: Dark brown Form: Globular cake

Chemical Constituent:

Opium contains more than 30 alkaloids which are largely combined with the organic acid meconic acid. The drug also contains sugar salts, albuminous substances, colouring matter and water. Six principle alkaloids are Morphine, Codeine, Thebaine, Noscapine (also known as narcotine), Narceine, Papaverine. The first group consist phenanthrene nucleus (morphine groups) whereas these of papaverine group have benzyl isoquinolene structure. Morphine molecule have both phenolic and alcoholic hydroxyl group and when acetylated forms diacetyl morphine or heroin. Codeine is an ether form of morphine (methyl morphine). Meconic acid is dibasic and easily detected either in Free State as meconate by the formation of a deep red colour on addition of ferric chloride. Less important opium alkaloids (protopine and hydrocotarine) are different structural type.

Chemical test:

- Opium is dissolved in water and filtered. To the filtrate add ferric chloride solution which gives deep reddish purple colour. This purple colour persists even on addition of hydrochloric acid indicates the presence of meconic acid which ensures the detection of opium. This test is general for its detection.

- Morphine gives orange red colour when sprinkled on nitric acid whereas codeine does not gives this test.
- Morphine solution develops bluish green colour upon addition of potassium ferricyanide and ferric chloride.
- Papaverine solution develops lemon yellow colour with hydrochloric acid and potassium ferricyanide solution.

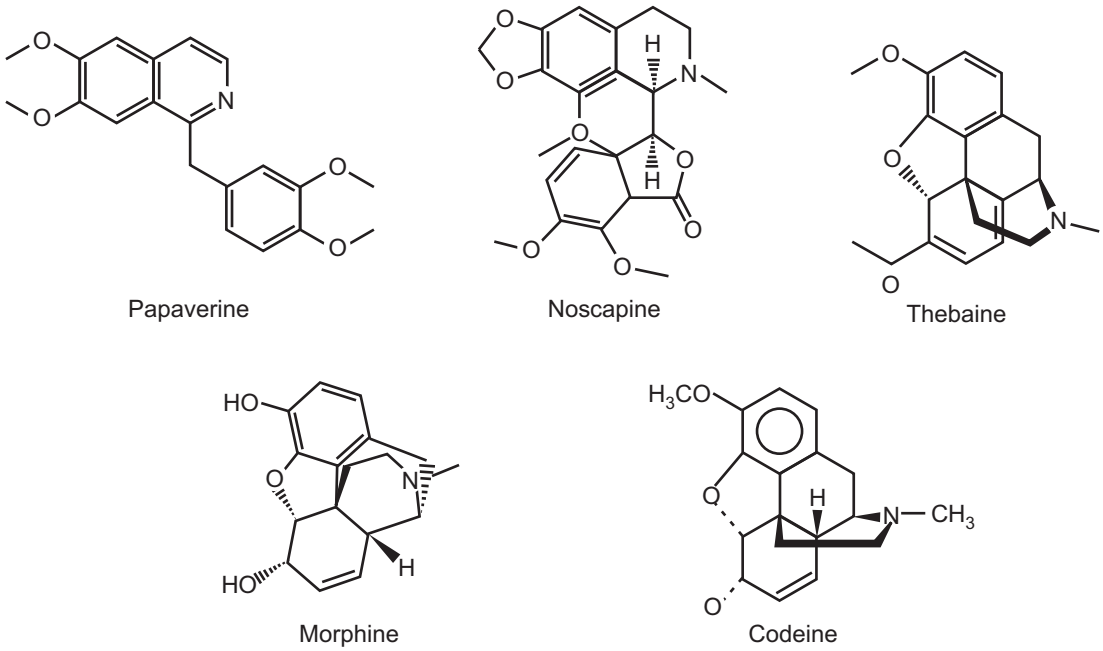


Fig. 2.9: Structure of Opium alkaloids

Uses:

It is used as hypnotic, sedative and analgesic but have addiction property so given in severe pain or if patient does not show response to other analgesics. It stimulates the chemoreceptor zone in medulla which leads to nausea and vomiting. Codeine (mild analgesic which is potent to aspirin) relieves local irritation in the bronchial tract and as an antitussive. Papaverine has smooth muscle relaxant activity. Narcotine (or Noscapine) used in the cough preparations due to its depressant action on cough reflex. Diacetyl morphine also known as Heroin has more narcotic analgesic effect than morphine.

Adulterants:

The adulterated forms show presence of Opium capsules in powdered form, gum and sugary fruits. Generally opium is not found to be adulterated due to the production done under Governmental control.

Allied drugs:

Papaver dubium, *P. orientate*, *P. argemone*, hybrids of *P. somniferum* and *P. orientate* (these species does not possess morphine content).

QUESTIONS

1. Define alkaloids. Mention the extraction method of alkaloids.
2. What is the role of alkaloids? Write in short.
3. What are 'Proto', 'Pseudo' and 'True' alkaloids? Classify alkaloids on the basis of their chemical structure.
4. What are the different techniques for the isolation of alkaloids?
5. What are the different chemical tests for alkaloids?
6. Write biological source, chemical constituents and uses of Vinca.
7. How Vinca alkaloids act on cancerous cells? Explain.
8. How the vinca plant is cultivated? Write its geographical source as well.
9. Write biological source, chemical constituents and uses of Rauwolfia.
10. What are the requirements for the cultivation and collection of Rauwolfia plant?
11. What are the main chemical constituents of Rauwolfia?
12. What are the chemical tests for Rauwolfia constituents? Draw chemical structure of its constituents.
13. Write biological source, chemical constituents and uses of Belladonna
14. What are the chemical tests for Belladonna constituents? Draw chemical structure of its constituents.
15. Write synonym, biological source, chemical constituents and uses of Opium.
16. What are the chemical tests for opium constituents? Draw chemical structure of its constituents.

■■■

Chapter ... 3

Phenylpropanoids and Flavonoids

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *General introduction and brief description about:*
  - *Phenylpropanoids*
  - *Flavonoids*
  - *Lignans*
- *Pharmacognostic profile of following drugs*
  - *Tea*
  - *Ruta*

3.1 INTRODUCTION

Flavonoids come under the class of phenolic compounds. Phenolic constituents are present in the vast group of plants. Phenolic groups consist of at least one benzene ring with one or more hydroxyl group. Though they are present as glycoside or with sugar, they have aqueous solubility. Generally they are located in cell vacuole.

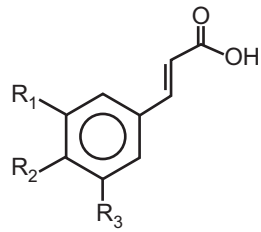
Phenolic compounds can be categorized in following groups.

- Phenyl propanoids
- Phenol and phenolic acids
- Flavonoids
- Flavonols and flavones
- Xanthone and stillbene
- Anthocyanins
- Quinones
- Tannins

Phenylpropanoids are naturally occurring phenolic compounds which contain three carbon side chains attached aromatic ring. They are biosynthetically derived from phenylalanine (amino acid) which contains one or more C_6-C_3 residues. Hydroxycinnamic acid is most widely occurring compound which provides building block of lignins, growth regulation and disease resistance. Hydroxycoumarins, phenylpropenes and lignans are other phenylpropanoids existing in this category. Generally four hydroxycinnamic acids named as ferulic acid, caffeic acid, ρ-coumaric acid and sinapic acid are found abundantly in nature.

They gets separated and detected easily on paper chromatogram because they produce blue and green fluorescence under UV light. Other six cinnamic acids are well known but they are rare in occurrence like isoferulic, o-coumaric and ρ-methoxycinnamic acids.

Hydroxycinnamic acids



R_2 = OH : p-Coumaric acid

R_2 = R_3 = OH : Caffeic acid

R_3 = OCH_3, R_2 = OH : Ferulic acid

R_1 = OCH_3, R_2 = OH, R_3 = OCH_3 : Sinapic acid

Hydroxycinnamic acids occurrs in plants in combined ester form and yielded by mild alkaline hydrolysis. Caffeic acid is found in the ester form of quinic acid and chlorogenic acid. Coumarin is the widely spread parent compound which occurs in twenty seven plant families. It is sweet smelling volatile matter released mown hay. The other plant families also contain hydroxycoumarin. Common compounds are umbelliferon (7-hydroxy), aesculetin (6,7-dihydroxy) or scopoletin (6-methoxy-7-hydroxycoumarin) etc. Daphnetin from daphne and fraxetin from Fraxinus tree are few example of rarer hydroxycoumarin.

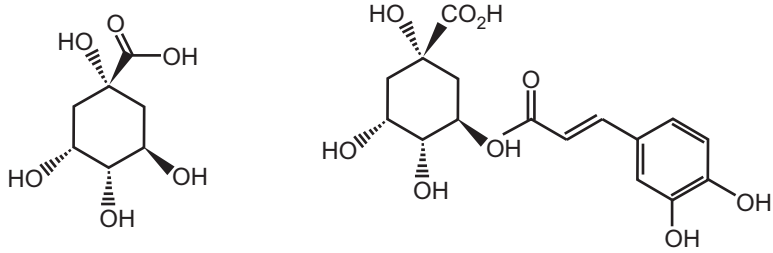


Fig. 3.1: Structure of Quinic acid and Chlorogenic acid

The other group of phenylpropanoids is the phenylpropenes which contributes in the volatile flavor and odour of plants. These phenylpropenes are isolated from plants in essential oil fraction along with volatile terpenes. Hydroxycinnamic acids and hydroxyl-coumarins are detected together by acid or alkaline treatment of plant extract. The extraction can be done by ether and ethyl acetate solvents. The extract is washed and dried properly. Chromatographic studies should be done on paper and microcrystalline cellulose plates.

Table 3.1: R_f value and spectral data (for hydroxycinnamic acids)

Cinnamic acid	R_f value in				Colour		EtOH λ_{max}	EtOH-NaOH λ_{max}
	BAW	BN	BEW	Water	UV	UV + NH_3		
ρ-Coumaric acid	92	16	88	42	None	Mauve	227, 310	335
Caffeic acid	79	04	79	26	Blue	Light blue	243, 326	decomposition
Ferulic acid	88	12	82	33	Blue	Bright blue	235, 324	344
Sinapic acid	84	04	88	62	Blue	Blue green	239, 325	350
o-Coumaric acid	93	21	85	82	Yellow	Yellow green	227, 275, 325	390
ρ- Methoxy cinnamic acid	95	17	87	23	Dark absorbing	--	274, 310	298
Isoferulic acid	89	12	67	37	Mauve	Yellow	295, 323	345

Indications:

BAW = n butanol : Acetic acid : Water (4 : 1 : 5)

BN = n butanol : Ammonium hydroxide (1 : 1)

BEW = n butanol : Ethanol : Water (4 : 1 : 2.2)

These compounds give characteristic fluorescent colour in UV light which gets intensified by ammonia vapor treatment. Paper chromatogram is more advantageous than thin layer chromatogram because the colour change can easily observed on paper and identification can be done by spectral measurements. Hydroxycoumarins absorbs longer wavelength than cinnamic acids.

The flavonol glycosides and their aglycones are known as flavonoids. They occur in nature abundantly and widely distributed throughout in higher plants. In the above classification flavonoids are one of the biggest class along with phenyl propanoids, phenols and phenolic quinones. The most common flavonoidal constituents are rutin, quercitin, citrus bioflavonoids like hesperidin, hesperetin, naringin, doismin etc. Rutin and hesperidin are commonly known as Vitamin P (or permeability factor) which is used in the treatment of capillary bleeding and capillary fragility.

Flavonoids are generally the compound which gives colour to the plant or responsible for plant pigmentation in the fruits, leaves and flowers. The yellow pigmentation of the plant is responsible for various flavonoids like aurones, chalcones and yellow flavonols.

There are different (around 4000) flavonoids known which have some common basic skeleton (C_6-C_3-C_6).

3.2 CLASSIFICATION

The flavonoids are classified as:

Table 3.2: Classification of flavonoid

Sr. No.	Flavonoid class	Distribution
1.	Anthocyanins	Scarlet, red, mauve and blue colour pigments (flower, leaf and other tissues).
2.	Proanthocyanidins	Colourless (leaves of woody plants and heartwoods).
3.	Flavonols	Colourless co-pigment (in cyanic and acyanic flowers, leaves).
4.	Flavones	As flavonols.
5.	Glycoflavones	As flavonols.
6.	Biflavonyls	Colourless (confined to gymnosperms).
7.	Chalcones and aurones	Yellow pigment (flower and other tissues).
8.	Flavonones	Colourless (mainly in citrus fruits and leaves).
9.	Isoflavones	Colourless (root and leguminosae family).

3.3 PHYSICO-CHEMICAL PROPERTIES

- Flavonoids generally found in glycosidic form.
- They are water as well as alcohol soluble but some numbers are sparingly soluble like hesperidine or rutin.
- Aglycone part of the flavonoid glycosides are more frequently solubilize in polar solvent. They may be solubilized in alkaline hydroxide solution if one or more free phenolic group are present.
- The flavonoids which have lipophilic characteristic generally found in superficial tissue i.e. leaf can be separated with the help of medium polarity solvent like dichloromethane then the other impurities like fats and waxes should be separated from this extract (generally a prewash with n-hexane are performed but sometime it also dissolves some lipophilic flavonoids.

3.4 BIOLOGICAL PROPERTY

- Flavonoids or polyphenols are also well known for their free radicals scavenge activity which is formed under different conditions.
- Flavonoids also act as enzyme inhibitor for example histidine decarboxylase inhibits the naringenin or quercitin.
- Flavonoids are also useful as antiallergic, anti-inflammatory, hepatoprotective, maintains blood cholesterol level, diuretic, antispasmodic, antiviral or antibacterial.
- Some of them inhibit the growth of tumor or anticancer activity.

- One of the important activities of flavonoid is venoactivity which means the decrease permeability of capillary and their fragility. Due to this important property it is also termed as 'Vitamin P factor' or 'P factor'.

3.5 EXTRACTION OF FLAVONOIDS

Plant material

↓ Extracted with boiling water

Crude extract

↓ Addition of lead acetate

Tannins removed (in the form of lead salts)

↓ Filter

Filtrate

1. Add water
2. Acidified with HCl
3. Boil for few hours

Precipitate of sugar free flavones or flavonones

↓ Add alcohol for extraction

Extract

↓

Purification of compound (fractional crystallization or by recrystallisation from organic solvents like benzene, carbon disulphide, alcohol etc.)

↓

Characterization of purified compound by using various spectroscopic techniques

Fig. 3.2: Extraction procedure of Flavonoids

3.6 CHEMICAL TESTS

1. **Shinoda test:** Upon addition of few magnesium turnings and conc. Hydrochloric acid (drop by drop) to the test solution, a pink scarlet, crimson red or sometimes green to blue colour develops after some time.
2. **Alkaline reagent test:** When few drops of sodium hydroxide solution are added in the test solution, first sharp yellow colour develops which upon addition of dil. acid becomes colourless.
3. **Zinc hydrochloric acid test:** A mixture of zinc dust and concentrated hydrochloric acid is added in the test solution, it develops red colour after some time.

LIGNANS

The lignans comprises a large group of polyphenols which mainly occurs in plants (e.g. enterolignans, enterodiol and enterolactone). Plant lignans are polyphenolic substances which derived from phenylalanine (an amino acid) via dimerization of substituted cinnamic

alcohols which is known as monolignols to a dibenzylbutane skeleton. This reaction is controlled by originated proteins and catalysed by oxidative enzymes. Phenylpropanoids are built up of C_6-C_3 units (n-propylbenzene skeleton) which are derived from cinnamyl units (like as terpene chemistry builds on isoprene units). Four groups of compounds which are formed by condensation of phenylpropane units are a) lignin b) neolignan c) oligomers and d) norlignans.

(a) Lignans are those compounds whose skeleton results from bonding between the beta carbons of the side chains of two units derived from 1-Phenylpropane (8-8' bond).

(b) Neolignan is a structure formed by condensation of phenylpropanoid units. The actual bond varies which involves only one beta carbon (e.g. 8-3', 8-1', 3-3', 8-o-4').

(c) The oligomers are improper resulted from condensation of two to five phenylpropanoid units.

(d) Norlignans are specific to gymnosperm which have C_{17} skeleton.

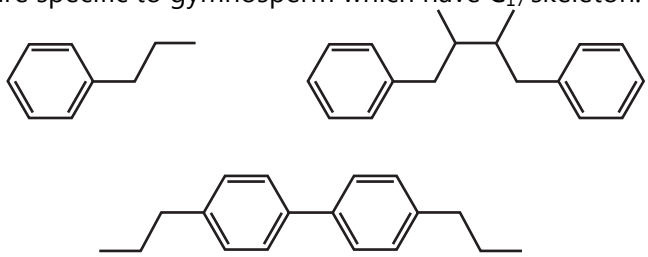


Fig. 3.3: Chemical structures of Lignan and Neolignan

Lignans are widely distributed among several families. Many compounds have been isolated in about 70 families. They are mainly found in wood in gymnosperms and in angiosperm they are present in all tissues. Neolignans have limited distribution (mainly in Magniolales and Piperales).

The plant lignans, if taken in diet form gets metabolized by intestinal bacteria to mammalian lignans named enterodiol and enterolactone. Lignans that can be metabolized to mammalian lignans are pinoresinol, lariciresinol, secoisolariciresinol, matairesinol, hydroxymatairesinol, syringaresinol and sesamin. Lignans is the largest class of phytoestrogens (estrogen-like substance) which act as antioxidant.

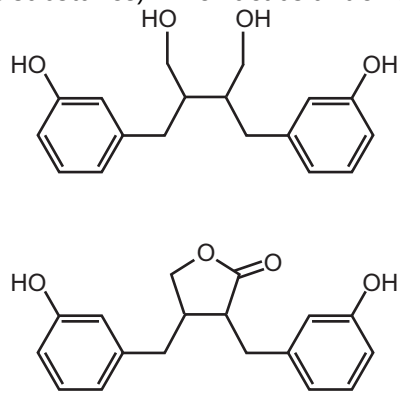


Fig. 3.4: Chemical structures of Enterodiol and enterolactone

[I] TEA

Synonym: Camellia thea.

Biological source: Tea contains prepared leaves and leaf buds of Thea sinensis Linn.

Family: Theaceae.

Geographical source: Tea is cultivated in India, Srilanka, Indonesia, China and Japan. In India it is cultivated in North-Eastern India (mainly in Assam and Bengal), South India (in Nilgiri, Palni, Annamalai hills in Tamilnadu, Kerala and Karnataka states) and North West India (in Dehradun, Almora and Garhwal district of Uttaranchal, Kangra valley and Mandi district of Himanchal Pradesh).

Cultivation and Collection:

The tea is available in two forms:

1. Black tea
2. Green tea

Black tea is available from India and Srilanka whereas green tea is available from China and Japan. Black tea is obtained by fermentation of fresh tea leaves which further dried artificially.

Green tea is obtained by keeping tea leaves in copper vessel and then dried artificially.

Tea is a dried leaf of bush which contains theine which gives cheap and stimulation drink upon addition of boiling water with sugar and milk. So tea is most important beverage crop of India. The basic requirement for the cultivation of tea leaves are deep, light, well drained acidic soil (pH 5.8 to 5.4 or less), humid climate with annual rainfall (100 cm), altitude (2100 meter above sea level), temperature is in the range of 21°C to 29°C is ideal for its cultivation. Tea is shade loving plant and it develops more vigorously when planted along with shady trees.

The propagation is mainly done by seeds which are sown in germination beds and developed saplings are transplanted into the open fields. The garden or field is regularly hoed and weeded for the better quality of crop. Pruning is done time to time for maintaining the proper shape of tea bush. The yield can be increase by the use of nitrogenous fertilizers like ammonium sulphate. Tea does not favor water logging because roots of tea plant gets leached and deteriorated. Hence it is grown in hilly areas where water drains out easily.

The tea leaves along with bud are plucked by the skilled labour preferably female labourers and dried by artificial method. Tea needs to be stored in heat, light, air and moisture proof packing to remain fresh and mold free.

Preparation of Green Tea:

The freshly collected tea leaves are exposed to air until most of the moisture gets removed. Then it is roasted and stirred continuously so that the leaves become moist and flaccid. These are passed through rolling table to make rolling balls which should be moisture free. The leaves are shaken and roasted in copper vessel. The leaves turned dull green in colour. The leaves are winnowed, screened and graded into different varieties.

Fig. 3.5: Tea plant (Image)

Macroscopic Characters:

The cultivated tea is small evergreen shrub which is 1 to 1.5 meter in height. The wild plants are up to 6 meter in height. The leaves are dark green in colour, lanceolate, elliptical, blunted at apex, serrate, hairy and glabrous in nature. Flowers are solitary in nature and generally in group of 2 to 3 leaf axils. It possesses characteristic odour and bitter taste.

Chemical Constituents:

Tea leaves contain caffeine (1 to 3 percent), theobromine and theophylline (in trace amount), gallotannic acid (15 percent) and an enzyme mixture known as thease. The colour of tea leaves is due to the presence of gallotannic acid.

Caffeine is a white powder which is odourless and bitter in taste. It is weakly basic in nature. It has feeble solubility in water, alcohol, chloroform and ether.

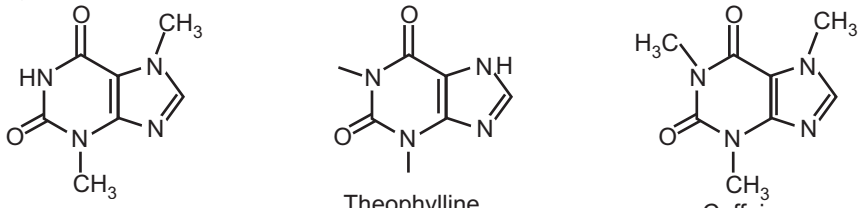
Caffeine is an example of purine group of alkaloids which gives positive Murexide test.

Chemical Test:

1. **Murexide test:** Take caffeine in a petridish and add hydrochloric acid with potassium chlorate. Heat it upto dryness. Then this obtained residue is exposed to ammonia vapours, a purple colour develops which disappears upon the addition of alkali solution.
2. When caffeine is treated with tannic acid solution it produces white precipitate.

Uses:

Tea is used as central nervous system stimulant and as diuretic. Caffeine possess cerebral vasoconstrictor action hence it uses as CNS stimulant. Caffeine also uses in migraine (given along with ergotamine tartarate).



Theobromine Theophylline Caffeine

Fig. 3.6: Structures of Theobromine, Theophylline and Caffeine

[II] RUTA

Synonyms: Ruta graveolens, Rue, Herby grass, Garden rue

Biological source: It is a perennial under shrub which consists of dried herb of *Ruta graveolens*.

Family: Rutaceae.

Geographical source: It is indigenous to Southern Europe and cultivated in Britain and India.

Cultivation of plant: It grows well in well drained in any variety of soil but prefers rocky and dry soil. It needs full sunshine to grow well. It is drought resistant and needs very less watering.

Care should be taken during handling of plant because the sap is often irritating and leaves burn like rashes on people's skin.

Rue can be harvested and used in the houses as an insect repellant. Simply cut the leaves and dry them properly. Then keep the dried leaves in cloth bags and place them where ever needed to repel insects.

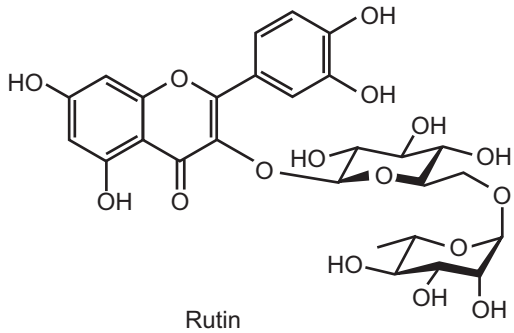
Fig. 3.7: Rue plant (Image)

Macroscopic characters:

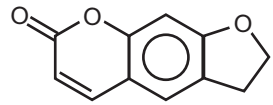
It is an erect, glabrous herb which is about 30 to 40 cm in height. Its leaves are bluish green in colour. It has characteristic and strong aromatic odour and pungent taste. Flowers have green yellow colour, fruits are sub cylindrical which is brownish green in colour and rough on external surface.

Chemical constituents:

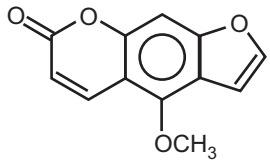
It contains glycosides, alkaloids, volatile oils and fixed oils. Rue contains rutin, psoralen, bergapten, graveolin, rutarin and naphthoharniarin. Rutin is pale yellow crystalline compound which belongs to flavonosoidal glycoside category. The alkaloids like rutalinium, dictamine, rutamine, rubalinium and 1-methyl-2-nonyl-4-quinoline are present in drug. The fixed oils which are present in drug are linoleic, stearic and palmitic acid where as volatile oils are limonene, cineole, ethyl valerate, methyl salicylate, caryophylline and myrecine.



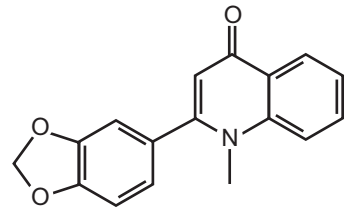
Rutin



Psoralen



Begrapten



Graveolin

Fig. 3.8: Chemical structure of Rutin, Psoralen, Bergapten, Graveolin

Uses:

It is used therapeutically as capillary protectant, antitussive, spasmolytic, emmenogogue etc. It is mainly used in liquid extract form or infusion.

Rue extract are mutagenic and hepatotoxic in nature. Its large doses can cause gastric pain, vomiting, systemic complications and even death.

It can cause severe phytophotodermatitis upon exposure to rue herbal preparations which results in burn like blisters.

Allied species: *Peganum harmala* known as 'Syrian rue'.

QUESTIONS

1. What are 'Polyphenols'? Explain flavonoids in detail with some examples.
2. Define 'Flavonoids'. Classify them.
3. How flavonoids are extracted? Draw flowchart.
4. What are the various physicochemical properties of flavonoids?
5. Write biological properties of flavonoids.
6. What are the various chemical tests for flavonoids?
7. What are 'Phenylpropanoids'? Write in short.
8. What are 'Lignans'? Explain briefly.
9. Write a detailed pharmacognostic note on Tea.
10. Write method of cultivation and collection of Tea.
11. How green tea is produced?
12. What is the main constituent of Tea? Draw its chemical structure.
13. Write about Murexide test and other test for purine group of alkaloid.
14. Write biological source, family and geographical source of Ruta.
15. What are the chemical constituents and uses of drug 'Rue'?

■■■

Chapter ... 4

Steroids and Triterpenoids

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

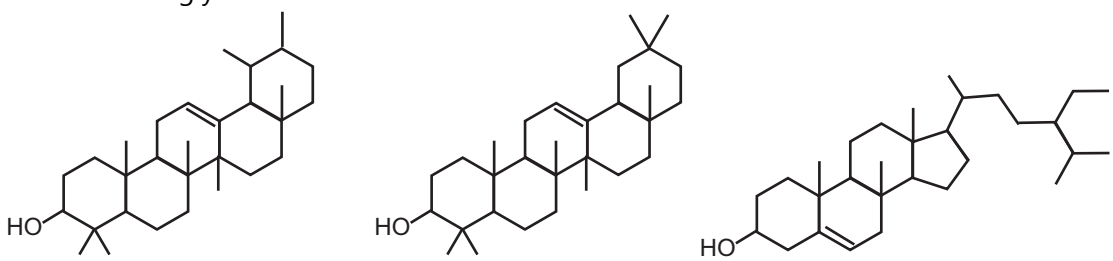
- *General introduction and brief description about Triterpenoids and steroids.*
- *Pharmacognostic profile of following drugs:*
  - *Liquorice*
  - *Digitalis*
  - *Dioscorea*

4.1 INTRODUCTION

Triterpenoids are compounds with a carbon skeleton possessing 6-isoprene units which are biogenetically derived from squalene (acyclic C_{30} hydrocarbon). They are generally complex cyclic structures of alcohols, aldehydes or carboxylic acids. They generally have high melting point and optically active. Along with these they are colourless compounds which are crystalline in nature. These compounds are hard to characterize because of their less chemical reactivity. The Liebermann burchard reagent contains acetic anhydride with concentrated sulfuric acid produces bluish green colour with triterpenes and sterols.

Triterpenoids comprises four groups of compounds:

1. True triterpenes
2. Steroids
3. Saponins
4. Cardiac glycosides



α-Amyrin β-Amyrin γ-Sitosterol

Fig. 4.1: Chemical structures of α-Amyrin, β-Amyrin and γ-sitosterol

Some triterpenes are well known and newer compounds are regularly being discovered and characterized. α-amyrin and β-amyrin and their derived acids, ursolic and oleanolic acids are some examples of pentacyclic triterpenes which exist in nature widely. These compounds

occur in the waxy coating of leaves and fruits like apple and pear. They may have protective function in insect repelling and microbial attack. Some triterpenes (*Euphorbia, Hevea* etc.) are found in resins; bark of tree and in latex.

Certain triterpenes have bitter taste e.g. limonin (lipid soluble bitter principle of *Citrus* fruits). It belongs to pentacyclic triterpenes category, known as limonoids and quassinoids, occurs in Rutaceae, Meliaceae and Simaroubaceae family. Other example of this category is cucurbitacin which is present in seeds of Cucurbitaceae and Cruciferae.

Sterols and triterpenes contain cyclopentane per hydro phenanthrene ring. Primarily, sterols were considered as animal product (as sex hormone, bile acids etc.) but later there occurrence reported in plant tissues also. The three phytosterols i.e. sitosterol (also known as β- sitosterol), stigmasterol and campesterol occurs in higher plants abundantly. These sterols occur in free form and as simple glucosides. Some examples of less common sterols are α-spinasterol (found in spinach, alfalfa and senega), ergosterol (found in yeast and fungi), fucosterol (found in brown algae and coconut).

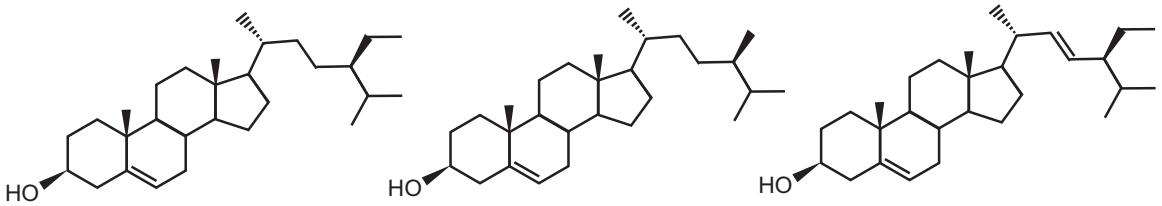


Fig. 4.2: Chemical structure of plant derived sterols (β-sitosterol, Campesterol and Stigmasterol)

Saponins, are glycosides of triterpenes and sterols, occurs in more than seventy families of plants. They have soap like properties and act as surface active agents. They have the ability to haemolyse the blood cells. Few examples of saponins are hecogenin from *Agave* and yamogenin from *Dioscorea* species, saponins from *alfalfa* and glycyrrhizin of *Liquorice* root etc. Saponins have occasional toxicity in cattles and possess sweet taste so they are economically beneficial. The glycosidic pattern of saponins are quite complex. They have as many as five sugar units attached and glucuronic acid.

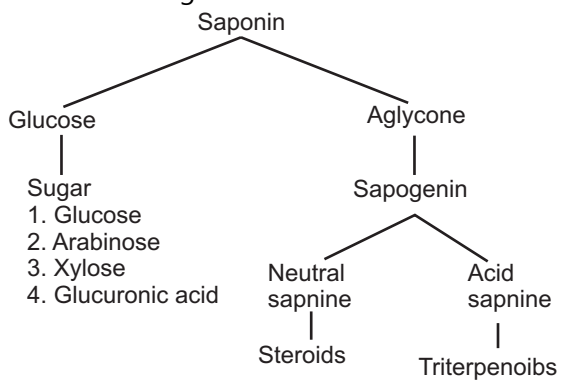


Fig. 4.3: Hydrolysis of Saponin glycosides

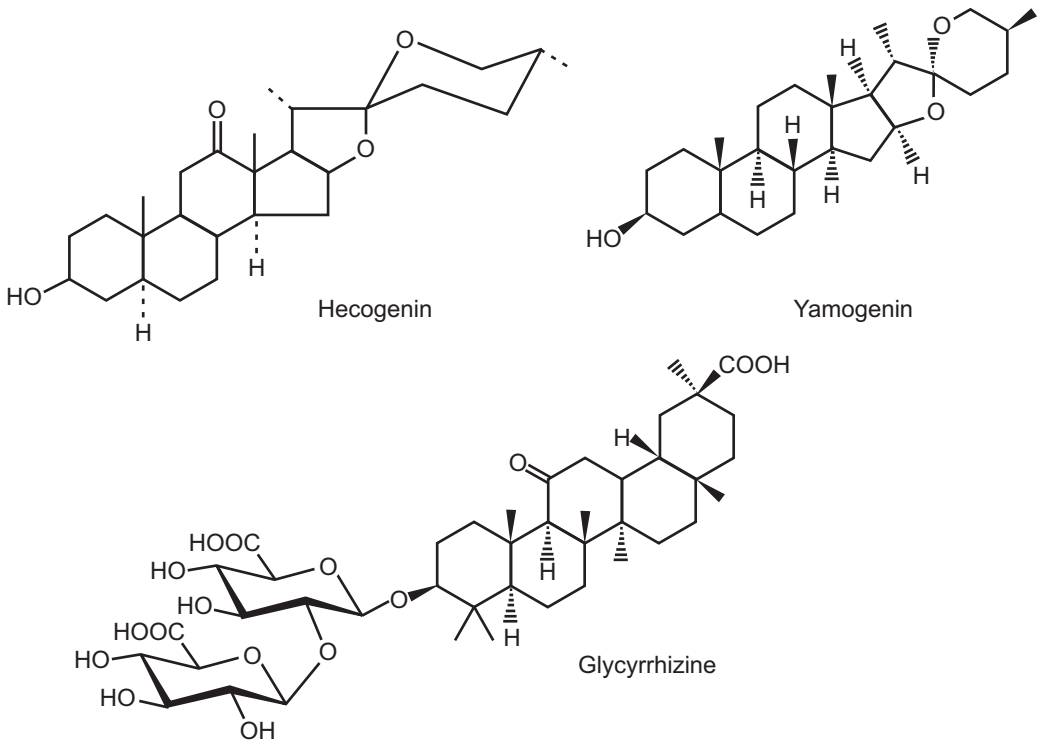


Fig. 4.4: Chemical structure of Hecogenin, Yamogenin and Glycyrrhizin

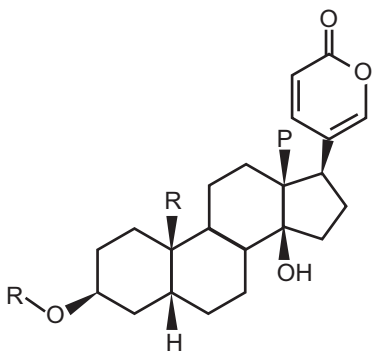
The last group of triperpenoids are cardiac glycosides or cardenolides. A typical cardiac glycoside is oleandrin (obtained from *Nerium oleander*, Apocynaceae). A considerable number of plants scattered throughout the plant kingdom contain C_{23} and C_{24} steroidal glycosides which exert on the failing heart a slowing and strengthening effect. Cardiac glycosides have a fairly limited distribution in several dozen genera unevenly scattered in about 15 families. The majority of the saccaharides found in the Cardiac glycosides are highly specific. They are 2, 6 di deoxy hexose such as D-digitoxose. In addition to these specific saccaharides 6- deoxy hexose also occurs. The cardiac activity is linked to the aglycone. The sugar moiety enhances the activity but does not participate directly in the activity and modulates it by modifying the polarity of the compound. The lactone at C-17, the presence of a X=C-C= function (where X is a hetero atom) is required and it must be in β- configuration. The activity is maximized when the A, B and C rings are in the cis, Trans, cis configuration. The activity is greatly diminished when the A and B ring are trans fused. The C and D ring must be cis fused. As a general rule the glycosides are fairly soluble in water and slightly soluble in C_2H_5OH and $CHCl_3$, the lactone ring likely to open in an alkaline medium.

The cardiac glycosides are mainly classified into two groups:

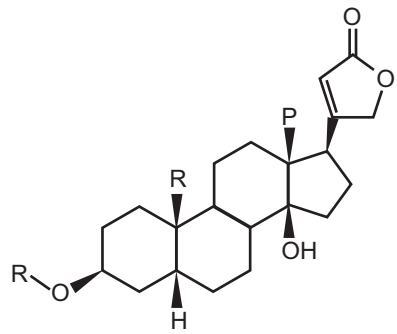
1. Cardenolides
2. Bufadienolides

Chemically the aglycone part of cardiac glycosides is a steroidal moiety. They are either C_{23} or C_{24} steroids because of either five membered or six membered lactone ring respectively. Those with five membered lactone rings are called as cardenolide while six membered lactone rings are termed as bufadienolides. The lactone ring of cardenolide contains only one double bond and is attached to steroidal nucleus through C-17 position. In bufadienolides the lactone ring contain 2 double bonds and is attached to steroidal nucleus through 17 β-position.

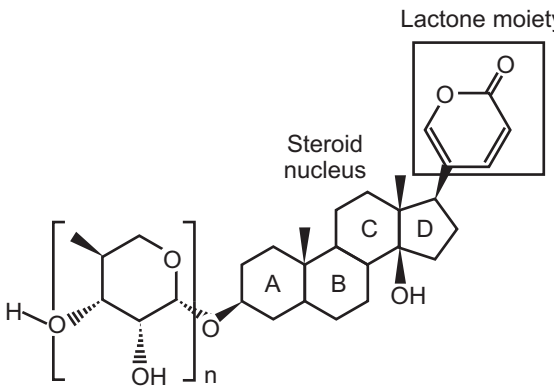
Cardenolides vs Butadienolides	
Cardenolide It contains five membered ring. It is butyrolactone ring.	Bufadienolides It contains six membered ring. It is a pyrone ring.



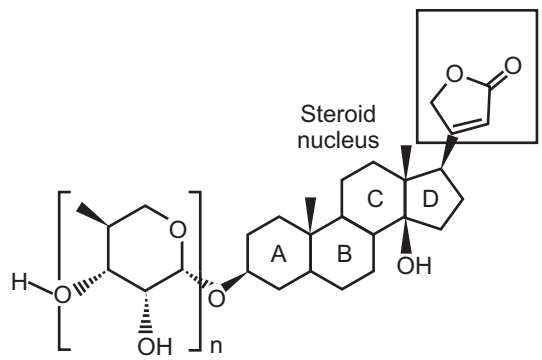
Bufadienolide



Cardenolide



Sugar moiety bufadienolides



Sugar moiety cardenolides

Fig. 4.5: Basic chemical structures of Bufadienolide and Cardenolide

4.2 EXTRACTION AND ISOLATION

Dried powdered drug
↓
Defatted with ether
↓
Extracted with hot methanol
↓
Methanolic extract
↓
Concentrated
↓
Add inorganic acid
↓
Liberates aglycones or hydrolysates
↓
Isolation of compound by TLC
↓
TLC on silica gel, in solvent system
[Such as hexane: ethyl acetate (1:1) or chloroform: methanol (10:1) or n-butanol-2M NH_4OH]
↓
Characterization of isolated compounds by spectroscopy

Fig. 4.6: Extraction procedure of Triterpenes

Isolation techniques to be employed depend on the structural complexity of the glycosides present in any plant source. Thus the cardiac glycosides of *Strophanthus* species can be separated by one dimensional TLC on silica gel in the upper layer of ethyl acetate: pyridine: water (5:1:4). On the other hand separation of the 28 or 50 cardenolide in the foxglove *Digitalis purpurea* requires two dimensional TLC in ethyl acetate: methanol: water (16:1:1) and Chlororform: Pyridine (6:1).

4.3 CHEMICAL TEST FOR TRITERPENOIDS AND STEROIDS

(a) Libermann-Burchard test: The extract is boiled with acetic anhydride (few ml) and cooled. Then concentrated sulphuric acid is added from the side wall of test tube (precaution should be taken during addition otherwise bumping happens) brown ring forms at the junction of two liquids. The upper layer turns green (due to the presence of steroids) and deep red colour develops (indicates the presence of triterpenoids).

(b) Salkowski test: When extract is treated with concentrated sulphuric acid (few drops), a red colour develops in lower layer (due to the presence of steroids) or yellow colour develops (indicates the presence of triterpenoids).

(c) Sulfur powder test: In this test sulphur powder is added in test solution which sinks at the bottom of test tube indicates the presence of phytoconstituents.

4.4 EXTRACTION PROCEDURE OF CARDIAC GLYCOSIDES

Powdered drug
↓
Extraction with 50 percent ethanol at low temperature
↓
Add lead acetate solution
↓
Removal of impurities
↓
Centrifugation
↓
Removal of precipitate
↓
Supernatant is extracted with chloroform
↓
Chloroform extract
↓
Evaporate the extract
↓
Residue contains cardiac glycosides
↓
Purification by chromatography
↓
Characterization from spectroscopy

Fig. 4.7: Extraction procedure of Cardiac glycosides

4.5 CHEMICAL TEST FOR CARDIAC GLYCOSIDES

(a) Baljet reaction: When test solution is treated with picric acid (or sodium picrate) yields a stable orange colour. These reactions are negative with saponin and either negative or much weaker with bufadenolides.

(b) Raymond test: When test solution is added in hot methanolic alkali solution, a violet colour develops.

(c) Legal test: When aqueous or alcoholic extract is treated with pyridine (1 ml) and sodium nitroprusside (1 ml) solution, pink to blood red colour appears.

(d) Killer-killani test: Take chloroform extract and dry it. Then add glacial acetic acid (0.4 ml) along with traces of ferric chloride. Transfer the content in small test tube and add concentrated sulphuric acid (0.5 ml) by the sidewall of the test tube. Acetic acid solution slowly turns bluish green in colour indicates the presence of deoxy sugars.

(e) Xanthydrol test: When extract is heated with Xanthydrol solution (5 percent) in glacial acetic acid and hydrochloric acid (1 percent), a red colour develops indicates the presence of 2-deoxysugar.

(f) Antimony trichloride test: Add antimony trichloride and trichloro acetic acid into a solution of glycoside and heat the solution, blue or violet colour appears indicates the presence of cardenolides and bufadenolides.

[I] LIQUORICE

Synonyms: Glycyrrhiza, Liquorice root, Mulethi

Biological source: It consists of dried roots and stolons, whole or cut, peeled or unpeeled of *Glycyrrhiza glabra* Linn and other species of *Glycyrrhiza*.

Family: Leguminosae (Fabaceae)

Geographical source: Liquorice obtained from wild plants and from semi wild plants cultivated in Iraq, Syria, Afghanistan, Spain, Sicily and England.

Table 4.1: Varieties of Liquorice

Sr. No.	Variety	Biological source	Characters
1.	Spanish liquorice	*Glycyrrhiza glabra* var. typica	• It is about 1.5cm high bearing typical papillion-aceous flowers of a purplish blue colour. • The underground portion consists of a long root and thin rhizome or stolons. • The principal root divided just below the crown into several branches which penetrate the soil to the depth of 1m or more. • A considerable number of stolons are also given off which attain a length of 2m but run nearer the surface then the root. • The plant is grown in Spain, Italy, England, France, Germany and USA.
2.	Russian liquorice	G. glabra var. glandulifera	• It is obtained in the wild state in Southern Russia. • The underground portion consists of a large root stock. • It bears numerous long roots but no stolons.
3.	Persian liquorice	G. glabra var. violaceae	• It is collected in the Iran and Iraq. • It bears violet flowers.

Cultivation and Collection:

The liquorice is cultivated in Western Europe but the Russian variety and Persian varieties are obtained from wild plants Russia and Iran. Spanish variety is cultivated abundantly in Spain and Italy. The plant usually grows well in deep sandy soil but the soil should be fertile and near streams. The soil should be well prepared and added with farmyard manures. Liquorice is usually propagated by replanting young pieces of stolons in the month of March.

The stolons may be grown from seeds. The roots are harvested 3 to 4 years after plantation or when they are sufficiently grown. The underground organs are developed to a sufficient extent by the end of the third or fourth year. Then they are dug out in the month of October and washed with water. Some roots are peeled and cut into short length before drying. The drug is sun dried and then shade dried. Much part of drug is now used unpeeled. Some time stick or blocks of liquorice roots are made. This is prepared by the process of decoction, the liquid being subsequently clarified and evaporated to the consistency of the soft extracts. The latter is made into block or sticks.


Fig. 4.8: Glycyrrhiza glabra plant and its roots (Image)

Macroscopical Character:

Colour: Unpeeled drug is yellowish brown or dark brown in color externally and yellowish colored internally but peeled liquorice is pale yellow in colour.

Odour: Typical odour, faint and characteristics.

Taste: Sweet.

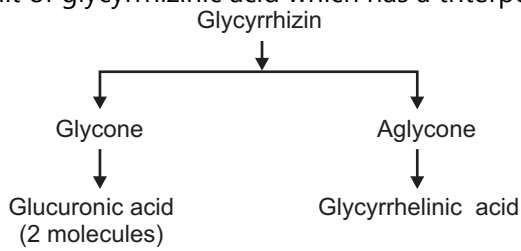
Size: Stolons are several meter in length and are cut in pieces having 20-50 cm length and 1 to 2 cm diameter fragments.

Shape: Cylindrical pieces sometimes straight or irregular peeled or unpeeled.

Fracture: Fibrous (bark), splintery (wood).

Chemical Constituents:

Liquorice owes most of its sweet taste due to glycyrrhizin or glycyrrhizic acid which is potassium and calcium salt of glycyrrhizinic acid which has a triterpenoid structure.



Other hydroxyl and deoxy triterpenoid acid related to glycyrrhetinic acid or glycyrrhetic acid have been isolated; the C-20 epimer of glycyrrhetinic acid is named liquiritic acid. The yellow colour of liquorice is due to flavonoids which also having antigastric effect. Flavonoid rich fraction included liquiritin, isoliquertin (a chalcone) which occurs as a glycoside and during drying partly converted into liquiritin, liquiritigenin and isoliquiritigenin.

Isoliquiritigenin is reported to be an aldose reductase inhibitor and may be effective in preventing diabetic complication. Other active constituent of liquorice are polysaccharide with a pronounced activity on the reticulo endothelial system. The root also contains 5-15 percent of sugars about 1-2 percent of asparagine (amide of aspartic or amino succinic acid), 0.04 to 0.06 percent volatile compound, β-sitosterol, starch, protein, bitter principles (glycyramarin). The latter are particularly abundant in the outer tissue and are therefore largely removed in the peeled variety of liquorice. Carbenoxolone is an oleandane derivative prepared from glycyrrhiza and posses significant minerocorticoid activity.

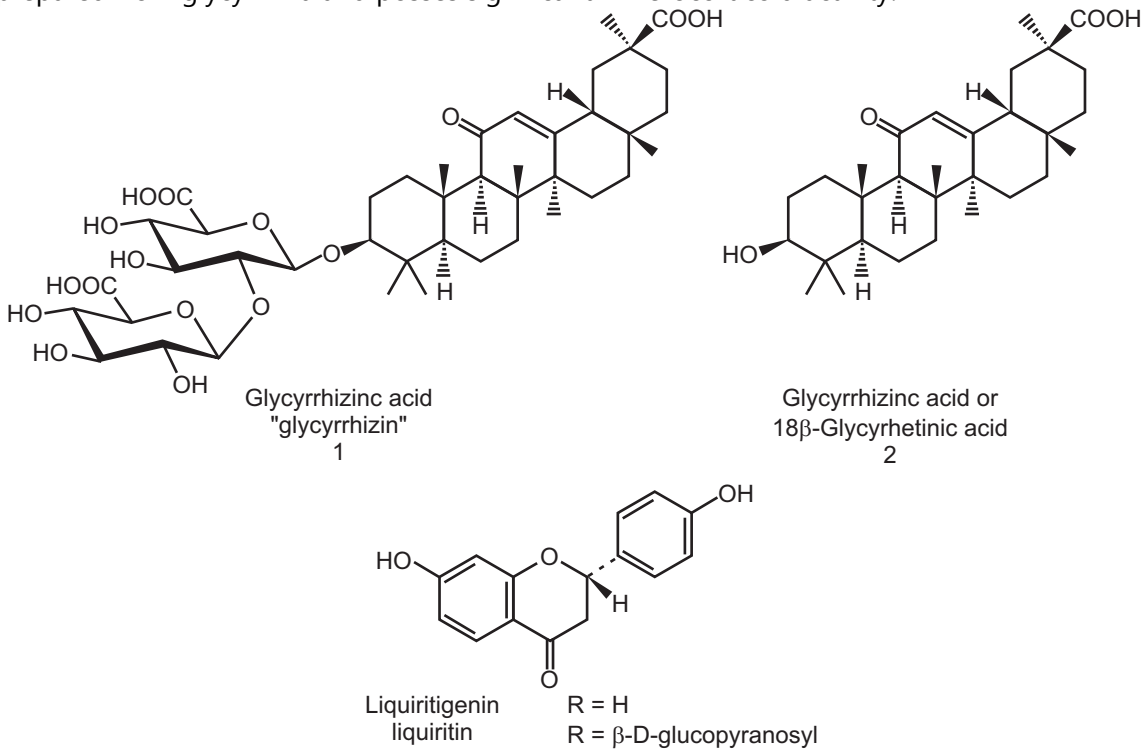


Fig. 4.9: Chemical structures of liquorice compounds

Chemical Test:

1. When 80 percent sulphuric acid is added on thick section of drug or on powdered drug it shows deep yellow colour.
2. A dark colour appears when aqueous extract of drug is treated with 5 percent ferric chloride solution (due to phenolic compounds).
3. Take aqueous extract and treat it with 5ml dilute potassium permagnate solution. Decolourisation of potassium permagnate indicates the presence of reducing sugars.
4. Aqueous extract of drug is added in lead acetate reagent, white precipitate formed (due to the presence of phenolic and flavonoidal compounds).
5. When aqueous extract of drug is added with mineral acid a reddish orange colour appears which decolorizes upon addition of alkali.
6. When powdered drug is shaken with water a stable foam produces which indicates the presence of saponins.

Uses:

Traditionally it is used as flavouring agent, demulcent and mild expectorant. It is used in the preparation of cough syrups. The flavonoidal content of liquorice i.e. Isoliquiritin possess antigastric activity so used in peptic ulcer treatment for healing purpose (in deglycyrrhized liquorice form known as DGL). The Isoliquiritin also have antispasmodic activity. The glycyrrhetinic acid possesses minerocorticoid activity so it is also used in the treatment of rheumatoid arthritis, inflammation addison's disease but at high dose it causes hypertension (due to sodium retention, water retention and electrolyte imbalance). Glycyrrhizin have anti-inflammatory effect so uses in the treatment of swelling. Ammoniated glycyrrhiza used as flavouring agent in beverages, confectionaries and pharmaceutical industries. Other use of liquorice is as foam stabilizer in fire extinguishers.

Adulterant and Substituent:

Manchurian liquorice derived from *Glycyrrhiza uralensis* which is chocolate brown in colour and contains glycyrrhizin. *Glycyrrhiza glabra* var *glandulifera* also known as Russian liquorice is uses as substituent. It contains long roots and no stolons. It is purple in colour.

[II] DIGITALIS

Synonym: Digitalis leaf, Foxglove leaves

Biological source: Digitalis (purple foxglove leaves) consist of the dried leaves of *Digitalis purpurea*. It is dried at a temperate below 60℃ immediately after collecting the leaves. It should not contain more than 5 percent of moisture.

Family: Scrophulariaceae

Geographical Source:

Digitalis is native of Western Europe, British Island and USSR countries. Crop is being cultivated in France, Germany, United Kingdom and Hungary. It has been introduced as an ornamental plant throughout North America, Canada, Mexico, Central America and Asia. In India it is cultivated in Northern states like Jammu and Kashmir, Himachal Pradesh and hilly areas of South India.

Cultivation and Collection:

It requires well drained calcarious, sandy loam soil which should be rich in organic matter. Lime rich soil is essential for proper growth and glycosidic content of *Digitalis lanata* but harmful for *Digitalis purpurea* which require acidic soil. The climatic conditions which are required for luxuriant growth are well distributed rainfall (30-40 cm per annum), good sunshine and temperature range between 20-30°C. For cultivation of Digitalis the seeds can be sown directly or first nursery rising and then transplanted in the field. Harvesting depends on some factors like age of leaves, time of day and length of leaves. These factors are very important for glycosidic content. Cardioactivity of leaves is higher when the leaves are harvested during the hotter part of the day i.e. midday and lowest at midnight. The leaves are collected when they are well developed and attain 8-10 m length. The collected leaves should dry at 50-60^{o}C.

Fig. 4.10: Digitalis purpurea plant (Image)

Macroscopic Characters:

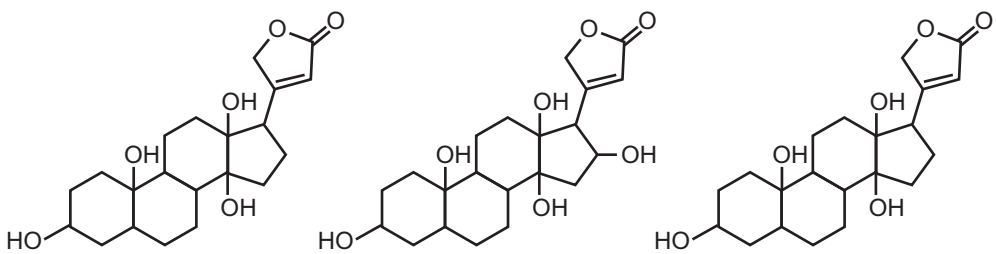
It is erect branched biennials attaining a height of 60-90 cm. Leaves are simple alternate opposite. Digitalis is an insect pollinated plant, bees being the main pollinator. The colour of leaves is dark greyish green having slight odour or odorless, bitter taste. The leaves are ovate lanceolate to broadly ovate having size is about 10-40 cm in length and 4-20 cm in width.

Chemical Constituent:

Purpuria glycoside A (Primary metabolite) —Glucose, Enzyme hydrolysis→ Digitoxin → Digitoxigenin + Digitoxose (Secondary metabolite)

Purpuria glycoside B (Primary metabolite) —Glucose, Enzyme hydrolysis→ Gitoxin → Gitoxigenin + Digitoxose (Secondary metabolite)

Odoroside —Enzyme hedrolysis→ Dixiloxigenin + Digitalose



Digiloxigenin Giloxigenin Digixigenin

Fig. 4.11: Chemical structures of Digitoxigenin, Gitoxigenin and Digixigenin

Digitalis contains 0.2-0.45 percent mixture of both primary and secondary cardiac glycoside (cardenolide). Purpurea glycoside A & B and glucogitaloxin are primay glycoside

possessing at C-3 of the aglycone, a linear chain of 3 digitoxose moieties terminated by glucose. Digitalis also contains many other glycosides viz odoroside H, gitaloxin, verodoxin and glucoverodoxin. The primary glycosides are less absorbed and less stable than secondary glycoside such as digitoxin, gitoxin and gitaloxin. The products of hydrolysis of purpurea glycoside A and purpurea glycoside B are shown in Fig. 4.11.

Additionally it also contains 2 saponins glycoside i.e. digitonin and gitonin. The total number of glycoside reported in the drug is about 30. *Digitalis purpurea* leaves also contain anthraquinone derivative which include 1-methoxy 2 methyl anthraquinone 3 methylalizarin.

Chemical Test:

Legal test: Extract is dissolved in pyridine and sodium nitro prusside solution is added to it and made alkaline. Pink or red colour is produced.

Baljet test: To a section of digitalis leaf, sodium picrate solution is added. It shows yellow to orange colour.

Keller: killani test: Boil 1gm of finely powdered digitalis with 10 ml alcohol (70%) for 2-3 min. Extract is filtered. To the filtrate add 5ml of water and 0.5 ml strong solution of lead acetate. Shake well and separate the filtrate. Filterate is treated with equal volume of chloroform and evaporated to yield extractive. Then extractive is dissolved in glacial acetic acid and after cooling 2 drops ferric chloride solution are added to it. These content are transferred to test tube contain 2 ml conc. sulphuric acid. A reddish brown layer acquiring bluish green colour after standing is observed due to the presence of digitoxose.

Uses:

Most frequent use of digitalis is the treatment of congestive heart failure. Another major use of digitalis is slowing ventricular rate in atrial fibrillation, artrial flutter, supraventricular tachycardia and premature extra systole. Digitalis has a cumulative effect in body and is slowly eliminated. Hence dosage is important aspects. Dose- initial dose 1-2 gm in 24-48 hrs. Maintenance dose - 100 mg daily.

The drug should be stored in such container where moisture content is less than 5 percent otherwise destruction of the glycoside and loss of cardiac activity occurs.

Adulterants:

1. *Primula vulgaris* (belonging to the Family- Primulaceae) leaves are added to digitalis leaves.
2. *Symphytum officinale* (belonging to the Family- Boraginaceae) leaves are added for adulteration purpose.
3. *Verbascum thapsus* (belonging to the Family- Scrophulariaceae) leaves are mixed with genuine drug of digitalis.

Allied Drug: *Digitalis lanata*

[III] DIGITALIS LANATA

Synonyms: Woolly fox glove leaves, Australian digitalis.

Biological source: It consists dried leaves of *Digitalis lanata* Ehrhart.

Family: Scrophulariaceae

Geographical source: Southern Europe and Central Europe, USA, Holland and Equador

Macroscopic characters: The plant is biennial in nature and having about 1 meter height. Leaves are sessile, oblong with entire margin and about 20 cm in length and 5 to 6 cm in width.

Fig. 4.12: Digitalis lanata plant (Image)

Chemical Constituent:

It mainly contains five primary glycosides and about 70 cardiac glycosides. The primary glycosides are identified as lanatosides A, B, C, D & E. The aglycone digoxigenin and diginatigenin are specific to Digitalis lanata while others are present in Digitalis purpurea.

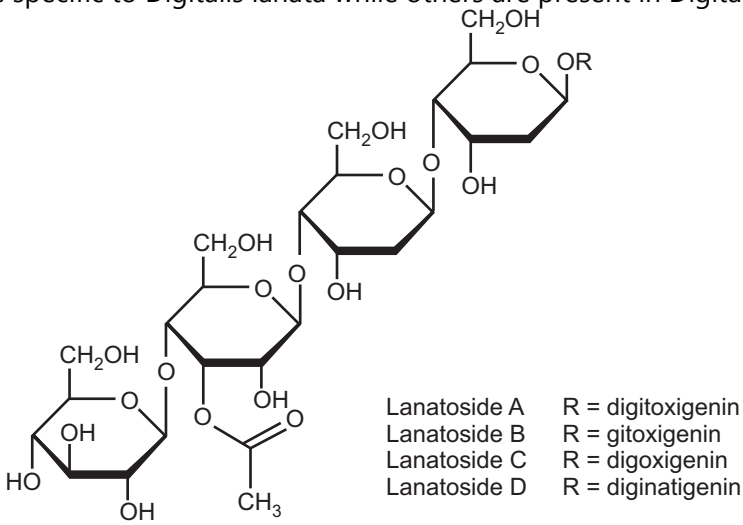


Fig. 4.13: Chemical structure of Lanatosides A, B, C and D

Uses:

It is used as commercial source for Digoxin, lanatoside C, lanatoside A and mixture of lanatosides. Lanatoside and digoxin have same action like digitalis. Digoxin used for auricular fibrillation treatment and congestive cardiac failure. It is most preferred because of its less cumulative action.

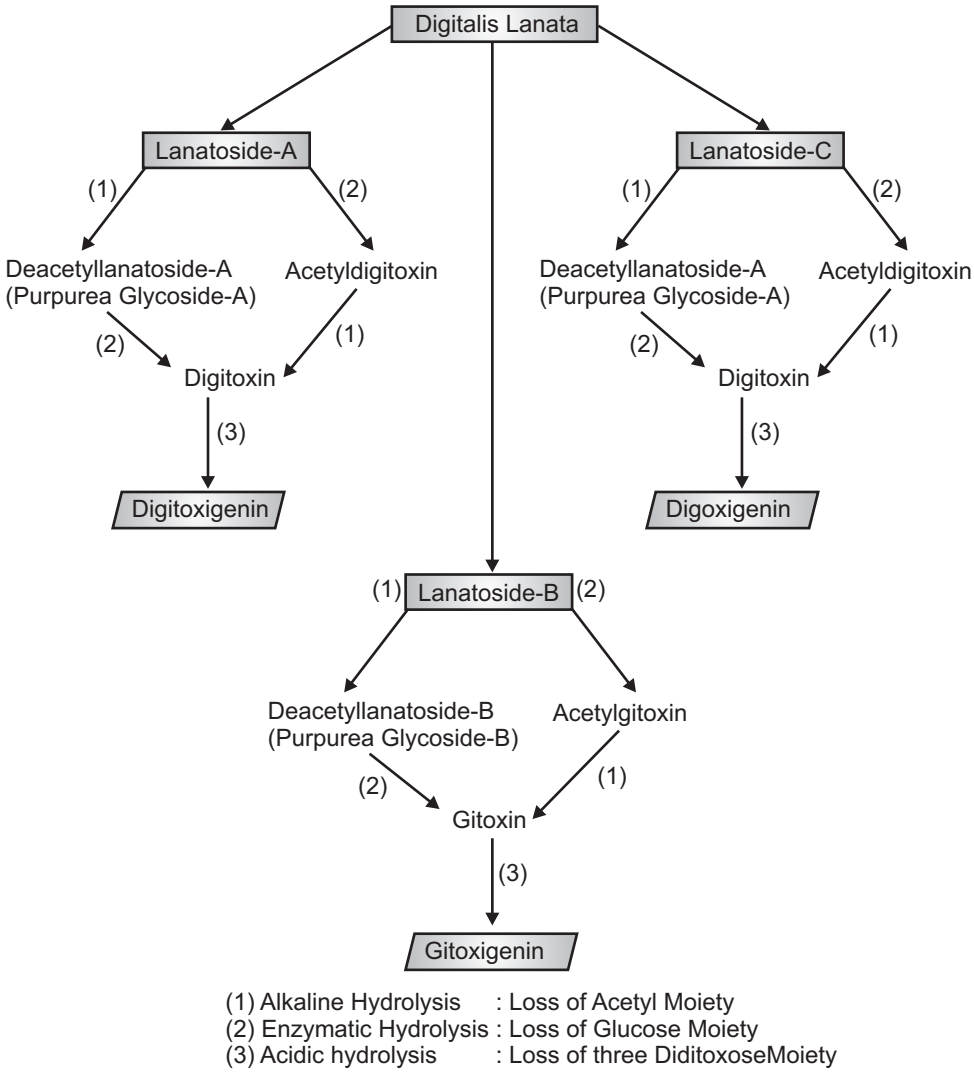


Fig. 4.14: Interconversion of lanatosides into their derivatives

[IV] DIOSCOREA

Synonyms: Yam, Rheumatism root, Greater Yam, Asiatic Yam.

Biological source: It consist of dried tubers of the plants *Dioscorea deltoidea*, *D. composita*, *D. floribunda*.

Family: Dioscoreaceae

Geographical source: It is found in Africa, Asia, Europe, Mexico, South America, USA, China and India. In India it is found in North Western Himalaya from Kashmir and Punjab to Nepal. It is also cultivated in Jammu and Kashmir, Himachal Pradesh, Tamilnadu, West Bengal, Maharashtra and Karnataka.

Cultivation and Collection:

The best yield is obtained in the medium loam and deep soils which should be rich in organic matter or humus. Red soil is best for *D. floribunda* and *D. composita*. The cultivation of *D. floribunda* and *D. composita* is more suited to the climate of tropical countries (tropics) whereas *D. deltoidea* suitable climate is of temperate regions. The propagation is mainly done by seeds, rhizome and stem cuttings. In India commercial plantation are raised from tuber cuttings. Seed progeny is variable and takes a larger time to start yielding tubers compared to plants raised from tubers. There are three types of pieces from tuber pieces:

1. Crown (Stem ends)
2. Medians (Middle portion)
3. Tips (Distal end)

Crown produces new shoots within 30 days but contains less diosgenin. The other tuber parts take nearly 100 days for development. In Karnataka February to March is the best time while June to July is also fine for plantation. *D. floribunda* and *D. composita* can also be propagated by seeds. Harvesting is done after three years of germination in the month of February to March. The drug is collected and dried. The rhizome loses about 50 percent of its weight upon drying.

Fig. 4.15: Dioscorea plant (Image) and its tubers

Macroscopic Character:

Colour: Slightly brown

Odour: Odourless or none

Taste: Bitter

Size: Variable (depending upon age of rhizomes)

Chemical Constituents:

Dioscorea have long been used for food as they are rich in starch. It contains about 75% starch but many species are non edible because they possess bitter taste. Diosgenin is the chief active constituent of dioscorea which is the hydrolytic product of saponin dioscin. Other constituents are steroidal sapogenin (4-6 percent) and its glycosides, smilagenin, epismilagenin and β-isomer yammogenin. Rhizomes also contain an enzyme sapogenase. Tubers are also rich in glycosides and phenolic compounds. For the isolation of diosgenin the

tubers are dried, powdered and hydrolysed with mineral acids. The liberated diosgenin is extracted with the help of non polar solvents like benzene or solvent ether. After isolation diosgenin is degraded to 16-dehydro pregnenelane acetate. This latter molecule is used as precursor for synthesis of various steroidal drugs.

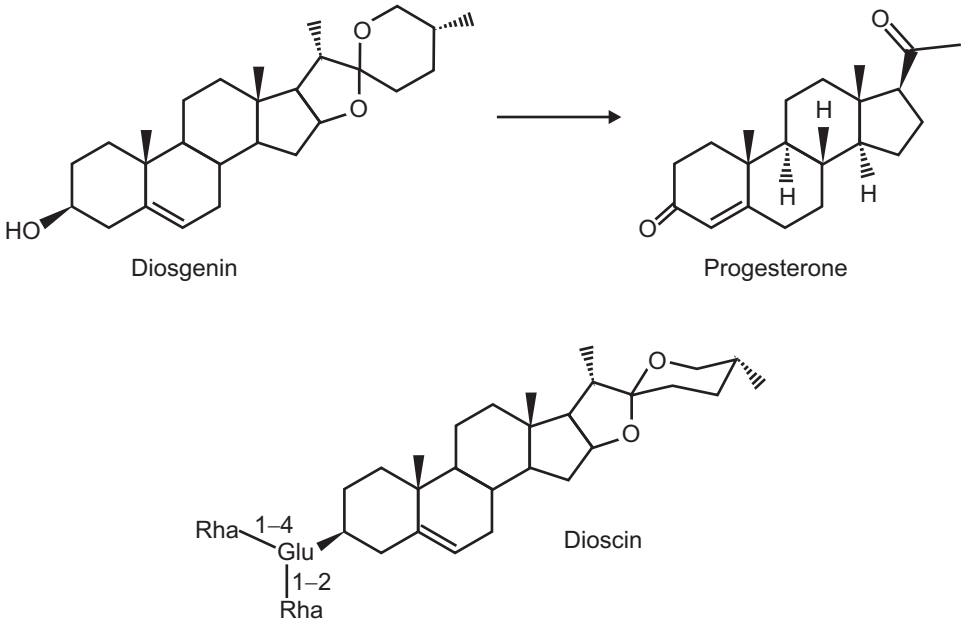


Fig. 4.16: Active constituents of Dioscorea

Uses:

Diosgenin, a steroidal sapogenin obtained from the drug is the major base chemical for the synthesis of several steroidal hormones, cortisone, corticosteroids and oral contraceptive pills. Dioscorea is used in the treatment of rheumatic arthritis.

Allied Drugs:

1. *Dioscorea villosa* Linn is a twin perennial plant which has yellow flowers and triangular capsule. It contains rich amount of diosgenin. It is mainly found in Virginia and Carolina in USA.
2. *Dioscorea deltoidae* wall var. *sikkimensis* Prain found in eastern Himalaya, Nepal, Sikkim, Assam, Bihar and Bengal. It contains less amount of diosgenin (2 to 2.5 percent).
3. *Costus speciosus* also used as substituent for genuine drug. It contains 1.5 percent diosgenin.

QUESTIONS

1. What are Triterpenoids? Classify them with examples.
2. How triterpenes are extracted? Explain in flowchart.
3. What are the chemical tests for triterpenes and steroids?
4. Define cardiac glycosides. Classify them.

5. How cardiac glycosides are extracted?
6. What are the chemical tests for cardiac glycosides?
7. What are saponins? Write in short.
8. Write biological source, family and geographical source of liquorice.
9. Write a detailed pharmacognostic note on Glycyrrhiza.
10. Write method of cultivation and collection of liquorice.
11. Write biological source, family and geographical source of Digitalis.
12. Write method of cultivation and collection of Digitalis.
13. What is the main constituent of Digitalis? Draw it chemical structure.
14. Write about Killer killani test and other test of Digitalis.
15. Write about the allied drug of Digitalis.
16. Draw an interconversion flowchart of Digitalis constituents (lanatosides).
17. Write biological source, family and geographical source of Dioscorea.
18. What are the chemical constituents and uses of drug Dioscorea?
19. Write method of cultivation and collection Dioscorea.
20. Which are the allied drugs of Dioscorea?

■■■

Chapter ... 5

Volatile Oils (Terpenoids)

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *General introduction and brief description about Volatile oils or Terpenoids*
- *Pharmacognostic profile of following drugs*
  - *Mentha*
  - *Clove*
  - *Cinnamon*
  - *Fennel*
  - *Coriander*

5.1 INTRODUCTION

Terpenoids are the hydrocarbons of plant origin having general formula $(C_5H_8)_n$. They are also oxygenated, hydrogenated and dehydrogenated derivatives. Basically terpenoids are volatile substances which give fragrance or aroma to plants. They are also known as essential oils because they represent essence. They are mainly found in leaves and fruits of plants like conifers, citrus and eucalyptus. The term 'terpene' was assigned to the compounds after isolation of volatile liquid turpentine from pine trees. Volatile oils get vaporized or evaporates upon exposure to air at normal or ordinary temperature, so they are termed as etherel oil. Volatile oils generally occurs in plant families like Umbelliferae (examples Coriander, Fennel), Zingiberaceae (Ginger), Myrtacea (Clove), Piperaceae (black pepper), Rutaceae (Lemon, Orange) etc.

5.2 GENERAL PROPERTIES OF VOLATILE OILS OR TERPENOIDS

1. They are colourless, fragrant liquids lighter than water and volatile in nature.
2. A few of them are solids e.g. camphor.
3. They are soluble in organic solvents, alcohol and fixed oils and usually insoluble in water.
4. They are open chain or cyclic unsaturated compounds having one or more double bonds.
5. They give addition reaction with hydrogen, halogen, acids, etc and addition products possess antiseptic properties.
6. They undergo polymerization and dehydrogenation.
7. They are easily oxidized nearly by all the oxidizing agents.

8. On thermal decomposition, most of the terpenoids yields isoprene as one of the product.
9. They are optically active.
10. It becomes darker in colour upon long standing in air or sunlight.
11. Volatile oils should be stored in well closed air tight umbered colour containers or bottles.

5.3 CLASSIFICATION OF TERPENOIDS

The terpenoids have general formula $(C_5H_8)_n$. The classification mainly done on the basis of value of n or number of carbon atoms present in the structure.

Table 5.1: Classification of Terpenoids

Sr. No.	Number of carbon atoms	Class	Value of n
1.	10	Monoterpenoids ($C_{10}H_{16}$)	2
2.	15	Sesquiterpenoinds ($C_{15}H_{24}$)	3
3.	20	Diterpenoids ($C_{20}H_{32}$)	4
4.	30	Triterpenoids ($C_{30}H_{48}$)	6
5.	40	Tetraterpenoids ($C_{40}H_{64}$)	8
6.	50	Pentaterpenes ($C_{50}H_{80}$)	10
7.	>40	Polyterpenoids $(C_5H_8)_n$	>8

Depending upon the type and number of rings present in terpenoids, they are named as Acyclic terpenoids, Monocyclic terpenoids, Bicyclic terpenoids and Tricyclic terpenoids which contain open chain structure, one ring structure, two ring structure and three rings structure respectively.

Table 5.2: Types of Volatile Oils

Sr. No.	Type	Examples
1.	Alcohol volatile oils	Mentha, Cardamom, Coriander, Rose oil, Sandalwood oil (Santalol).
2.	Aldehyde volatile oils	Cinnamon, Lemon peel, Orange peel, Citronella oil (Geraniol, Citronellal, Farnesol), Lemon grass oil, Bitter almond oil.
3.	Ester volatile oils	Gaultheria, Lavender, Mustard.
4.	Hydrocarbon volatile oils	Turpentine, Black pepper.
5.	Ketone volatile oils	Caraway (Carvone, Limonene), Spearmint, Buchu, Camphor, Musk, Civet oil.
6.	Oxide volatile oils	Chenopodium, Eucalyptus (Cineole).
7.	Phenolic ether volatile oils	Anise, Fennel, Nutmeg.
8.	Phenol volatile oils	Clove (Eugenol), Thyme.

5.4 CHEMICAL TEST

1. Volatile oil produces temporary stains when smeared on paper or cloth. The stain disappears after some time because the oil volatilizes.
2. Upon addition of Sudan III (alcoholic solution) to the thin section of volatile oil containing drug, red colour forms.
3. When few drops of Tincture alkana added on thin section of drug, red colour develops which indicates the presence of volatile oil.

5.5 EXTRACTION OF VOLATILE OILS

Steam distillation, solvent extraction or mechanical means like ecuelle and enfleurage methods are employed for extraction purpose of volatile oils. These methods are as follows:

1. **Hydro-distillation method:** This method comprises of water distillation, water and steam distillation and steam distillation. These techniques are used for extraction of volatile oils from crude drugs. Hydro-distillation technique is used for fresh leaves and steam distillation uses for subterranean parts.
2. **Enfleurage method:** This method uses for the extraction of flavouring agents and perfumery. In this method, the fresh flowers (mainly petals) are taken and spreaded over layer of fatty material. Then it kept over fatty material for imbibition. After the imbibition the fresh petals are placed on fat layer and exhausted petals are removed. This process continues until saturation of fat layer with volatile constituents. This saturated fatty layer is extracted with lipid solvents.
3. **Ecuelle method:** This method is employed for citrus fruits which contains volatile principles. Here the oil containing ducts which contain oil cells are ruptured by mechanical means or manually. For this purpose special vessel are taken which contain pointed projections. The raw material is twisted over the projections in clockwise direction which rupture the cell and oil comes out.

5.6 ISOLATION OF VOLATILE OILS

The oil obtained by distillation contains number of mono and sesqueterpenes. The monoterpenes hydrocarbons are distilled out first into their oxygenated derivatives by fractional distillation. Other methods like physical methods, chemical methods and chromatography also uses for their isolation. The chemical methods like treating the oil with sodium bisulphate, Phenylhydrazine, Tilden's reagent and Grignard reagent isolates the compounds. Suitable adsorbents are used in chromatography for the elution of chromatogram of isolated compound.

Uses:

The utilization of terpenoids is mainly in perfumery, cosmetic industries, soaps manufacturing, incense-sticks manufacturing, food industries, pharmaceutical industries and beverage industries.

They are also used as antiseptic, carminative, anthelmintic, stimulant, analgesic, antirheumatic, diuretic, aromatic, counter-irritant effect, insecticide, pesticide, insect repellant, and deodorant.

[I] MENTHA (MENTHA OIL)

Synonyms: Peppermint oil, Oleum mentha piperita, Mint oil.

Biological source: Mentha oil is obtained by steam distillation of flowering tops of *Mentha piperita* Linn.

Family: Labiatae.

Geographical source: It is cultivated in Japan, England, France, Italy, USA, Bulgaria, USSR and India (Jammu and Uttar Pradesh).

Cultivation and Collection:

The basic requirement for the cultivation of mentha are well drained fertile sandy loam soil (pH = 7), rainfall (95 to 105 cm), temperature (15 to 25°C), altitude (250 to 400 meter). The suckers are vegetatively propagated for cultivation purpose. The use of fungicides like mercury compounds enhances the better sprouting. The suckers are placed in field in January or February month by keeping distance of 10 cm. The distance between rows should be 50 to 60 cm. The foliar spray of urea and other fertilizers like superphosphate or potash are found advantageous. The harvesting should be done when plants reaches flowering stage. Thiodan (1: 700), endrine (1: 700), BHC (10 per cent), Sulphur (0.5 per cent) and malathion (5 per cent) are used for pest control.

Production of Mentha Oil:

The plants are dried in air. Sun drying causes the loss of active principles i.e. volatile oils. The air dried material is kept in iron or steel vessel which has a false perforated bottom. The steam under pressure is passed through the drug for about 3 to 4 hours for complete distillation. Near about 80 percent of oil is distilled off in first half of distillation. The medicinally important menthol distilled out in the latter half of distillation. The mentha oil is collected in separating vessel where it is decanted and filtered. The average yield of oil is approximately 0.5 to 1 percent v/w.

Fig. 5.1: *Mentha* plant (Image)

Description:

Colour: Yellowish in colour or colourless

Odour: Agreeable, pleasant and characteristic

Taste: Aromatic, Pungent (followed by cooling sensation)

Solubility: Soluble in alcohol (70 percent), alcohol, ether and chloroform insoluble in water.

Weight per ml: 0.9 to 0.912 gm.

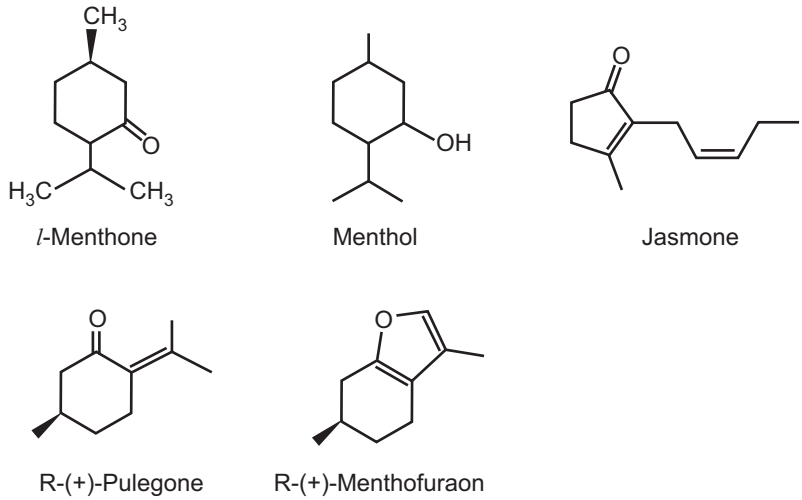
Optical rotation: At 25°C −16° to −30°.

Refractive index: 1.4590 to 1.4650.

Chemical nature: Neutral to litmus paper.

Chemical Constituents:

It mainly contains l-menthol (either in free form or ester form). There are three varieties of peppermint i.e. American variety (contain 80 per cent menthol), Japanese variety (contain 70 to 90 per cent menthol) and Indian variety which contain 70 per cent of menthol. Other important constituents are menthofuran, menthone, menthyl acetate, menthyl isovalerate, jasmone and other derivatives like cineole, l-limonene, isopulegone, camphene, pinene, jasmine and esters (possess pleasant odour). Menthofuran is responsible for bad odour due to resinification.



l-Menthone Menthol Jasmone

R-(+)-Pulegone R-(+)-Menthofuraon

Fig. 5.2: Chemical structure of Mentha plant

Chemical Test:

Take few ml of oil and mix it with 5 ml nitric acid solution. The solution should be made by adding 1 ml nitric acid into 300 ml of glacial acetic acid. Heat the solution on waterbath for 5 to 10 minutes. The liquid develops bluish colour which gets deep in colour upon more heating and shows copper coloured fluorescence and after few minutes it turns golden yellow.

Uses:

The oil is used as carminative, stimulant, flavouring agent and antiseptic. It is used in pharmaceutical preparations, cosmetic industries, tooth paste, tooth powder, shaving cream, chewing gum, candies, jellies, perfumery and essence manufacturing.

The other therapeutical uses of mentha oil are as spasmolytic, smooth muscle relaxant, digestant, anti-inflammatory, anti-ulcer, nasal decongestant, antitussive etc. It is used in the preparation of topical preparation and lozenges.

The oil should be stored in well closed air tight umber coloured container because it becomes dark and viscous upon storage.

[II] CLOVE

Synonyms: Clove bud, Laung, Lavang, Caryophyllum.

Biological source: It consists of dried flower buds of *Eugenia caryophyllus* (Sprengel) Bullock & Harrison *(Syzygium aromaticum* Linn).

Family: Myrtaceae.

Geographical source: It is native from the Mollucca Island and traditionally cultivated in Tanzania (Zinziber), Madagascar, Indonesia, Srilanka and India (mainly in Nilgiri hills, Kanyakumari, Kottayam and Quilon hills of Kerala).

Cultivation and Collection:

The deep rich loamy soil is good for clove cultivation but it can also grow in sandy loam and laterite soil. It favors warm humid climate with annual rainfall in the range of 150 to 250 cm. It grows well at an altitude of 900 meter from sea level. The seeds are sown in august to October for cultivation purpose. The seeds are sown in nursery bed at a distance of 10 cm. the seeds germinates in four or five weeks and these seedlings are transplanted to pots after six months. These seedlings are kept in pots for nearly about 12 months. Then they are transferred into open field. The shade is provided in the initial stage because the plants cannot bear the full sunshine. It can also be cultivated along with other plants like areca nut, coconut or nutmeg. The suitable fertilizers like ammonium sulphate, super phosphate, potash etc are provided to the plants in two doses. The first dose should be given in May or June and second dose is in October. The plants started bearing after 7 to 8 years and gives abundant yield per hectare after 15 to 20 years of growth. Generally the clove plant produces about 3 to 4 kg of drug per year.

The clove buds are handpicked or by beating with bamboo. Sometimes platform ladders are also used in case of tall trees. The collection starts when clove buds changes its colour from green to light pink. The cloves are sun dried and kept free from foreign matter. After drying the colour of cloves changes and becomes crimson or brownish black.

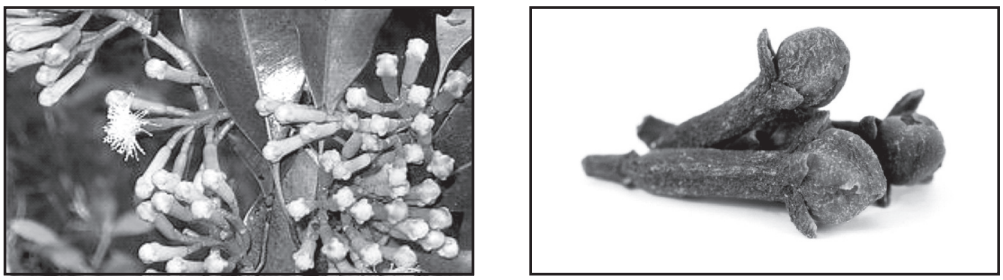


Fig. 5.3: *Clove* plant and Clove buds (Image)

Macroscopic Characters:

Colour: Crimson red to brown.

Odour: Characteristic or aromatic.

Taste: Aromatic and pungent.

Size: Length (10 to 18 mm), width (3 to 4 mm) and thickness (2 mm).

Shape: Flower bud is nail-shaped, Hypanthium - Quadrangular (length 10 to 12 mm × diameter 2 to 3 mm), Head – Globulous.

Hypanthium corresponds to the inferior ovary, and a globulous head, surrounded by four divergent sepal lobe consist of four imbricated petals wrapped numerous incurved stamens. The pulverized drugs shows the presence of large secretory cavities, calcium oxalate crystals, pollen grains, fibres with thick, lignified punctate walls.

Chemical Constituents:

It mainly contains volatile oil (15-20 percent), gallotannins (10 to 15 percent), resin, chromone and eugenin. Eugenol (70 to 90 percent), eugenol acetate, caryophyllenes, traces of esters, ketones and alcohols are the constituents of volatile oil which is present in oil ducts of clove.

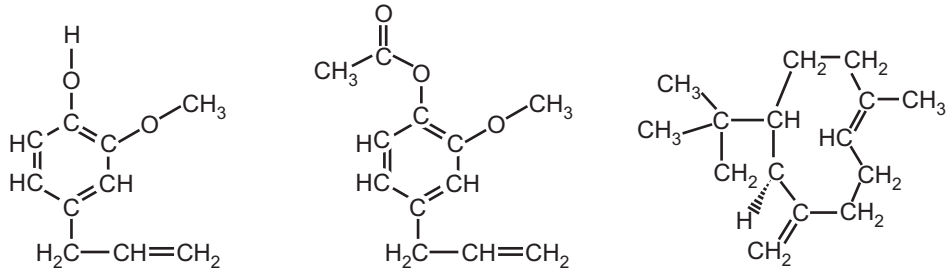


Fig. 5.4: Chemical structure of clove constituents

Properties of Clove Oil:

Colour: Clove oil is colourless to pale yellow.

Specific gravity: 1.038 to 1.06

Refractive index: 1.527 to 1.535

Boiling point: 250°C

It should be stored in proper conditions because it becomes dark and thick on storage.

Chemical Test:

Needle shaped crystals are seen when section of clove is treated with strong potassium hydroxide solution.

Uses:

Clove based phytomedicines are used locally to treat minor wounds after cleansing, as an analgesic (headache, toothache), as an analgesic in disease of mouth, pharynx, or both (in the form of lozenges), in mouthwashes for oral hygiene. Internally it is used to treat symptoms of gastrointestinal disturbances like epigastric bloating, improper digestion and flatulence.

In Germany, clove based products are used in mouthwashes for swelling of mouth and throat. In Indonesia, it is consumed as 'Kretek' cigarettes.

Table 5.3: Adulterants of Cloves

Sr. No.	Adulterant	Characters
1.	Exhausted cloves	Oil free or contain very less amount of oil, darker in colour, shrinked, floats on water.
2.	Clove stalks	Generally used for adulteration of powdered cloves, ash value is high, fibre content is high, calcium oxalate prism are present, contains 5 percent of oil.
3.	Blown cloves	Expanded flowers of clove tree, stamens free, contains less volatile oil.
4.	Mother clove	Dark brown in colour, oval in shape, slight aromatic, starchy, contains less volatile oil.

[III] CINNAMON

Synonyms: Dalchini, Ceylon Cinnamon, Cinnamon bark.

Biological source: Cinnamon consist of dried bark, freed from the outer cork and from the underlying parenchyma, from the shoots growing on the cut stumps of *Cinnamomum zeylanicum* Nees.

Family: Lauraceae.

Geographical source: Srilanka, Malabar Coast of India, Jamaica and Brazil.

Cultivation and Collection:

It is generally cultivated by seed propagation method but sometimes plant cuttings are also preferred. It mainly needs sandy or siliceous soil which should be rich in humus. The other requirements for its better cultivation are altitude (800 to 1000 meter) and annual rainfall (200 to 250 cm). It is shade loving plant. The seeds are propagated in nursery beds in the month of June and July. The distance should be 10 cm in between two plants. The plants should be watered time to time. Generally the seeds are germinated within 20 days. Shading is provided to the plants and allowed to grow for about 1 year. Then transplantation in open field should be done in the month of October or November or in rainy season. The distance should be kept atleast 2 meter in between two plants. Weeding should be done 2 to 4 times in a year. The plants should be manured in the first year and subsequently increased depending upon the age of plant. The fertilizers are applied first in monsoon and second in October-November. It will encourage the growth of shoots. Coppicing should be done to induce the formation of shoots. Harvesting should be done in rainy season because in this season peeling of bark from shoots is easy. The peeled strips are made into bundles, wrapped in coir mats and allow to ferment for 24 hours. This will loosen the outer cork and cortex which should be removed from curved brass knife. The collected bark contracts and converted into quill form after drying. The smaller quills are placed in between larger quills

and forms compound quills. The soft and fresh quills are rolled by hand and lightly pressed so it will avoid the splitting of bark into pieces. Then the drug should be shade dried and dried quills are packed into bundles of different grades and marketed. The small pieces and debris are used for the production of Cinnamon oil. The average yield of bark is about 200-300 kg per hectare and 2-3 kg leaves per hectare, annually.

Fig. 5.5: *Cinnamon* plant and Cinnamon bark (Image)

Description:

Cinnamon Bark:

Colour: Externally dull yellowish brown, internally dark yellowish brown

Odour: Aromatic

Taste: Warm and very refined (Sweetish and aromatic followed by warm sensation)

Fracture: Splintery

Size: Length is about 1 meter, diameter is nearly 1 cm and thickness is approximately 0.5 mm.

Shape: Compound quill form.

The wavy longitudinal striations are present on external and internal surfaces of bark (bark freed from cork).

Cinnamon Oil:

Colour: Yellow to reddish in colour.

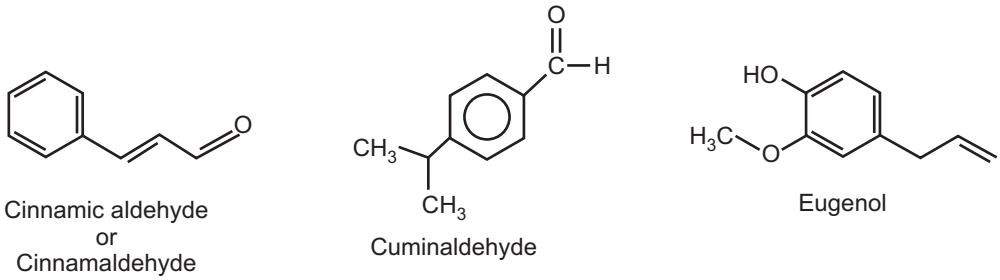
Specific gravity: 1.00 to 1.030.

Optical rotation: 0 to − 2.

Refractive index: 1.562 to 1.582.

Chemical Constituents:

Cinnamon bark contains polycyclic diterpenes and proanthocyanidinoid oligomers. It contains volatile oils (0.5 to 1 percent), phlobatannins (1.2 percent), mucilage, calcium oxalate, starch and mannitol (responsible for sweetish taste). The cinnamon oil obtained from distillation method which is light yellow in colour and upon storage changes to reddish in colour. The essential oil (5 to 20 ml/kg) is composed of phenylpropane derivatives. Cinnamon oil mainly contains cinnamaldehyde (60 to 70 percent), eugenol (5 to 10 percent), benzaldehyde, cuminaldehyde and other terpenes such as phellandrene, pinene, cymene, caryophyllene.



Cinnamic aldehyde or Cinnamaldehyde — Cuminaldehyde — Eugenol

Fig. 5.6: Chemical structure of *Cinnamon* constituents.

Chemical Test:

To a drop of volatile oil add a drop of ferric chloride solution, a pale green colour develops (cinnamaldehyde produces brown colour and eugenol gives blue colour which results in the formation of pale green colour).

Uses:

The drug is used as aromatic stimulant, antibacterial, antifungal, antiseptic, carminative, stomachic and astringent. Commercially, it is also used as spice, condiment, in candy preparation, dentrifices and perfumery. Cinnamon oil is used in urinary infection and food technology.

Cinnamon oil and cinnamaldehyde are irritating to skin and mucous membranes. They cause allergic reactions like urticaria or edema of the face and lips.

Table 5.4: Substituent and Adulterants of Cinnamon

Sr. No.	Adulterant	Characters
1.	Java Cinnamon	Obtained from *Cinnamomum burmanii*, Family- Lauraceae, less aromatic, peeled, occurs in double quill form, contains cinnamaldehyde (74 percent)
2.	Saigon Cinnamon	Obtained from *Cinnamomum burmanii*, Family- Lauraceae, Bark greyish brown in colour, sweet taste, contain volatile oil (2.5 percent)
3.	Cinnamon chips	Pieces of unpeeled and untrimmed bark, contain cork cells, poor yield to alcohol (90 percent)
4.	Jungle Cinnamon	Obtained from wild grown trees, darker in colour, less aromatic, slightly bitter

[IV] CASSIA CINNAMON

Synonym: Chinese Cinnamon.

Biological source: Dried stem bark of *Cinnamomum cassia* Blume.

Family: Lauraceae.

Geographical source: Native of China also found in Myanmar, Srilanka.

Macroscopic Characters:

Colour: Earthy brown on both surfaces.

Odour: Sweet, aromatic.

Taste: Aromatic and slight sweetish.

Size: Length 5 to 40 mm, width 12 to 18 mm and thickness 1 to 3 mm.

Shape: Single quill.

Fracture: Short and granular.

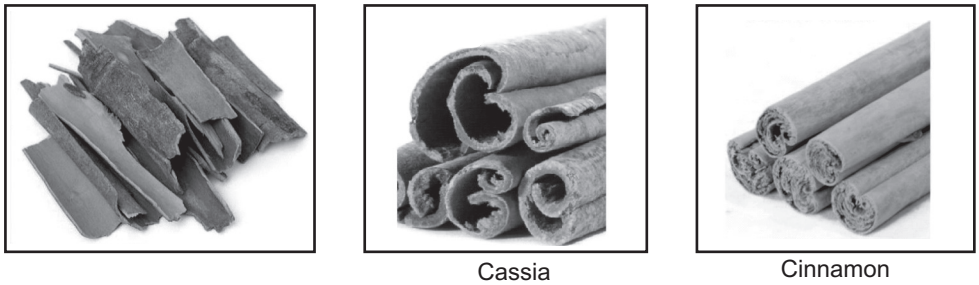


Fig. 5.7: *Cassia cinnamon* and *Cinnamomum zeylanicum* bark (Image)

Chemical Constituents:

It contains volatile oil (1 to 2 percent) known as cassia oil, mucilage, starch, calcium oxalate and tannins. Volatile oil contains nearly 85 percent cinnamaldehyde, traces of eugenol, cinnamyl acetate and coumarin.

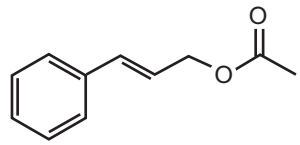


Fig. 5.8: Chemical structure of Cinnamyl acetate

Uses: It is used as carminative, stimulant, flavouring agent, aromatic and spices.

[V] FENNEL

Synonyms: Foeniculum species, Saunf, Fennel fruits.

Biological source: It consist of dried ripe fruits obtained from cultivation of *Foeniculum vulgare* Miller.

Family: Umbelliferae.

Geographical source: Fennel is native of Mediterranean countries. It is cultivated in Romania, Russia, Germany, France, Japan and India. In India, it is cultivated in many states like Gujarat, Maharashtra, Punjab, Rajasthan, Uttar Pradesh and West Bengal.

Cultivation and Collection:

The cultivation is mainly done by dibbling method. The good quality fruits are sown in just before spring season. Four or five seeds are put into holes by keeping distance 25 cm in between two holes. The specific arrangement of leaves (umbel inflorescence) and free

branching of herb requires enough space between two plants and rows. The seeds are sown in well drained calcareous soil in sunny days. The crop should be provided with suitable fertilizers. The crop kept free from weeds by the use of weedicide. When the crop ripens, it is harvested and sun dried. After drying, the fruits are separated by thrashing. Gujarat is the only state which supplies nearly 90 percent of fennel demand in market.

Fig. 5.9: *Fennel* plant and its fruits (Image)

Macroscopic Characters:

Colour: Green to yellow brown.

Odour: Sweet, aromatic.

Taste: Aromatic and slight sweetish.

Size: Length 5 to 10 mm and width 2 to 4 mm.

Shape: Straight or slightly curved, cremocarpus fruit, glabrous, straight with 5 primary ridges and bifid stylopod.

Extra characters- Transverse section shows 2 commissural vittae and 4 dorsal vittae.

Fennel oil is pale yellow in colour with specific gravity 0.953 to 0.973, refractive index of 1.526 to 1.538 and optical rotation of +12° to +24°.

Chemical Constituents:

It contains volatile oil (3 to 7 percent), protein (20 percent) and fixed oil. Volatile oil contains nearly 20 percent fenchone (ketone) and phenolic ether anethole (about 50 percent). The other constituents are limonene, phellandrene, anisic aldehyde and methyl chavicol. Fenchone possess aromatic odour and is a colourless pungent liquid whereas anethole is sweet in taste and smell.

Sweet fennel oil contains 80 percent or more of *E*-anethole, 1 to 5 percent methylchavicol (estragole) and less than 5 percent (+) fenchone. Fennel fruits also contain furanocoumarins including bergapten, imperatorin and xanthotoxol.

Bitter fennel oil contains 50 to 80 percent *E*- anethole, 3 to 20 percent estragole and upto 25 percent (+) fenchone.

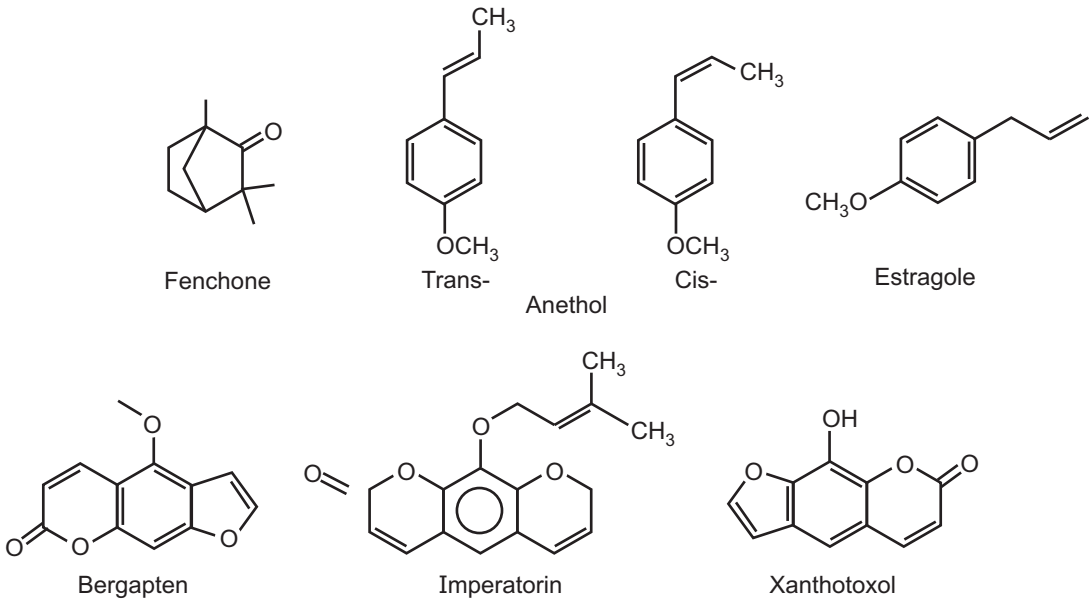


Fig. 5.10: Chemical structure of Fenchone, Anethole (its isomer), Estragole, Bergapten, Imperatorin and Xanthotoxol.

Uses:

It is used as carminative, stimulant, flavouring agent, aromatic and expectorant. Traditionally it is used for the symptomatic treatment of gastrointestinal disturbances such as epigastric bloating, impaired digestion, eructations, flatulence and as an adjuctive treatment of the painful components of functional dyspepsia. Fennel roots are used to enhance urinary and digestive elimination functions and also enhance the renal excretion of water.

Adulterant and Substituent:

It is generally adulterated with exhausted fennel fruits. The exhausted fruits contain less percentage of volatile oil. The oil is removed by treating with alcohol which develops a characteristic odour of fusel oil. The fruits can also be exhausted by the steam treatment. They become darker greenish brown in colour and contain traces of volatile oil. They sink in water.

Table 5.5: Substituent of Fennel with their characteristic features

Sr. No.	Variety	Size (in mm)	Taste	Volatile oil (%)	Fenchone content (%)
1.	Japanese	3-4 × 2-3 mm	Sweet and camphor like	2.7	10.2
2.	Indian	4-7 mm	Aromatic	0.72	6.7
3.	Roman or French sweet	7-8 × 2-3 mm	Sweet	2.1	Nil
4.	Russian or Romanian	4-6 × 1-2 mm	Camphor like	4.5	18.1
5.	Saxony	9-10 × 3-4 mm	Aromatic	4.8	22.0

[VI] CORIANDER

Synonyms: Dhaniya fruits, Coriander fruits, Coriandrum.

Biological source: It consist of dried ripe fruits of *Coriandrum sativum* Linn.

Family: Umbelliferae.

Geographical source: It is cultivated in Europe (mainly in Russia, Hungary and Holland), Egypt, Morocco and India (mainly in some states like Andhra Pradesh, Maharashtra, West Bengal, Uttar Pradesh, Rajasthan and Jammu and Kashmir).

Cultivation and Collection:

Coriander is cultivated as both Ravi and Kharif crop in India. It is grown as mixed crop with wheat, grain, jowar, onion, sugarcane, cotton, brinjal etc. For cultivation purpose it needs near about 15 to 20 kg of fruits per hectare area. It needs light to heavy black soil and calcareous soil for cultivation. Coriander fruits are sown by drilling method and within 100 days the crop is ready for harvesting. Few varieties like J-16, J-214, K-45 and New Pusa give better yield per hectare. The fruits are sown in March or soon. The plants achieves height upto 45 to 60 cm and larger. The plants are grown in rows by keeping distance about 50 cm apart. Harvesting should be done in August. The umbels may be cut off with scissors when they are ripened. For larger fields 'Scythe' or 'Mower' may be used. The cutting may be done early in morning to avoid loss of fruits. The collected fruits are spreaded over sheets for drying in sun. After about 48 hours thrashing should be done. After thrashing the fruits are dried in warm air in shade. After drying the fruit loses their pungent foetid smell and acquires a warm agreeable odour and sweet taste. After drying the fruits are stored in well closed containers because they are highly susceptible for deterioration from insects. About 70 to 75 per cent world requirement of coriander is fulfilled from India. The Rajasthan state produces nearly 70 per cent of crop demand from India.

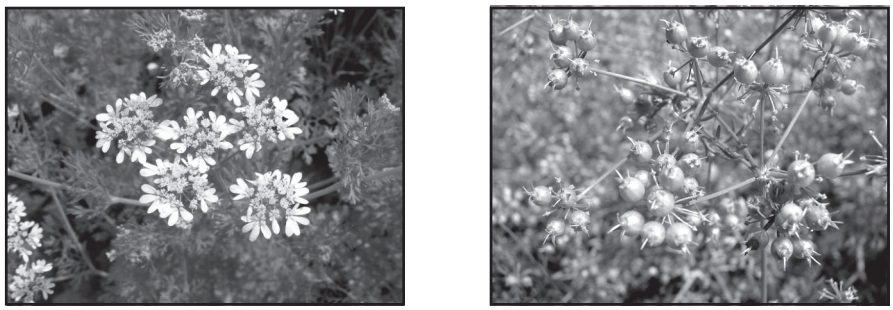


Fig. 5.11: *Coriandrum sativum* plant and its fruits (Image)

Macroscopic Characters:

Colour: Brownish yellow.

Odour: Sweet, aromatic.

Taste: Spicy.

Size: 3 to 5 mm in diameter.

Shape: Subspherical, cremocarpous fruit.

Extra features: Each mericarp has five wavy inconspicuous primary ridges and four straight prominent secondary ridges. The seed is coelospermous. The oil possesses specific gravity 0.870 to 0.885, optical rotation +8° to +14° and refractive index 1.462 to 1.472.

Chemical Constituents:

It contains volatile oil (0.8 to 1 percent), fixed oil (13 percent) and proteins (20 percent). Volatile oil contains nearly 90 per cent D-linalool (Coriandrol), coriandryl acetate, traces of L-borneol, geraniol, pinene etc. The other constituent is Vitamin A.

The fruits contain up to 14 ml/kg essential oil with (+)- linalool, camphor (4-6 percent), geranyl acetate (1 to 3.5 percent), γ-terpinene (2 to 7 percent) and other monoterpenoid hydrocarbons.

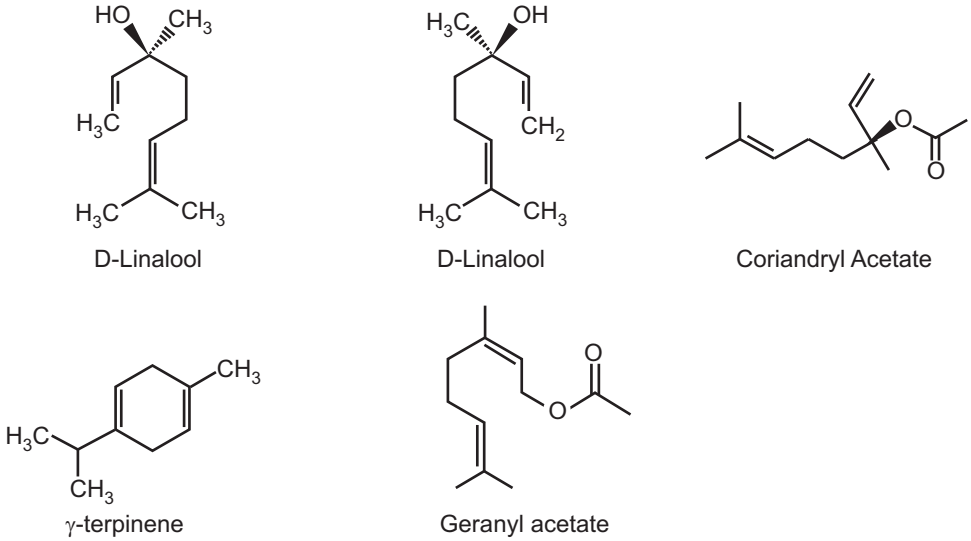


Fig. 5.12: Chemical structure of D-Linalool and L- Linalool, Coriandryl acetate, γ-terpinene, Geranyl acetate

The fruits and fruit oil is used as aromatic, carminative, stimulant, flavouring agent, along with purgative uses in prevention of gripping effect. It is used as antiaspasmodic.

Adulterants

Bombay coriander, Fenugreek seeds, cereal fruits are used as adulterant. The Bombay coriander fruits are 5 to 8 mm in length and 3 to 4.5 mm in width, ellipsoidal in shape and contain less volatile oil.

Varieties of Coriander: There are three varieties which can be best distinguished by the determination of number of fruits per gram.

1. **English coriander:** These have the finest flavour contains nearly 98 fruits per gram and not less than 80 fruits.
2. **Russian and German coriander:** These are richest in oil (up to 1 percent) contains average 160 fruits per gram and not less than 130.
3. **Mogadore coriander:** These are the largest, contain less oil. Nearly contain 66 fruits in one gram and not less than 80 fruits.

QUESTIONS

1. Define Volatile oil. Write its uses in therapy.
2. Classify terpenes with examples.
3. What are the chemical tests for Volatile oil identification?
4. What are the methods for extraction of volatile oil?
5. Write physicochemical properties of volatile oil.
6. Write synonym, biological source, family and geographical source of Mentha.
7. Write a detailed pharmacognostic note on Mentha.
8. What are the main constituents of Mentha oil? Draw it chemical structure.
9. Write chemical test and uses of Mentha.
10. Name out the adulterants of Mentha.
11. Write biological source, family and geographical source of Clove.
12. Write a note on cultivation and collection of Clove.
13. What are the main constituents of Clove? Draw it chemical structure.
14. Write chemical test and uses of Clove.
15. Name out the adulterants of Clove.
16. Write biological source, family and geographical source of Cinnamon.
17. What is the main constituent of Cinnamon? Draw it chemical structure.
18. Write chemical test and uses of Cinnamon.
19. How Cinnamon is adulterated?
20. Write biological source, family and geographical source of Cassia cinnamon.
21. What are the chemical constituents of Cassia cinnamon? Draw its chemical structure.
22. Write biological source, family and geographical source of Fennel.
23. Write method of cultivation and collection of Fennel.
24. Write in short about uses of Fennel.
25. What are the adulterants and substituent for Fennel?
26. Write synonym, biological source, family and geographical source of Coriander.
27. Write method of collection and preparation of Coriander.
28. What are the chemical constituents of Coriander? Draw it chemical structure.
29. Write in brief about uses of Coriander.
30. What are the adulterants and varieties for Coriander?

■■■

Chapter ... 6

Tannins

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *General introduction and brief description about Tannins.*
- *Pharmacognostic profile of following drugs:*
  - *Catechu*
  - *Pterocarpus*

6.1 INTRODUCTION

These are complex natural organic compounds which are polyphenolic, polyhydroxy benzoic acid derivatives or flavanol derivatives, having astringent action. They are widely distributed in the nature (leaves, barks, immature fruits but, they disappear during ripening process). They provide protection to the plants especially during growth from herbivorous animals, insects, bacteria and others. They have large molecular weight but some have low molecular weight. The general uses of tannins are:

1. Anti-diarrheal
2. Anti-bacterial
3. Antidote in alkaloidal and heavy metal poisoning
4. Haemostatic
5. Mild diuretic
6. Leather industry
7. Astringent for inflamed mucous membrane
8. Stomachic etc.

6.2 CLASSIFICATION

Tannins are classified into:

1. True tannins:

(a) Hydrolysable group:

- Hydrolyzed by acids or enzymes (Tannase).
- They are esters of a sugar (β-D-glucose), with one or more trihydroxy-benzene carboxylic acid.
- The tannins which is derived from Gallic acid called Gallo-tannin or gluco-gallin like in Rhubarb rhizomes, Clove buds etc.

- Tannis which is derived from ellagic acid called ellagotannin or glucoellagin like in Pomegranate roots bark and Eucalyptus leaves (which also contain Tannic acid which is Gallotannin).
- They form dark blue color with $FeCl_3$.

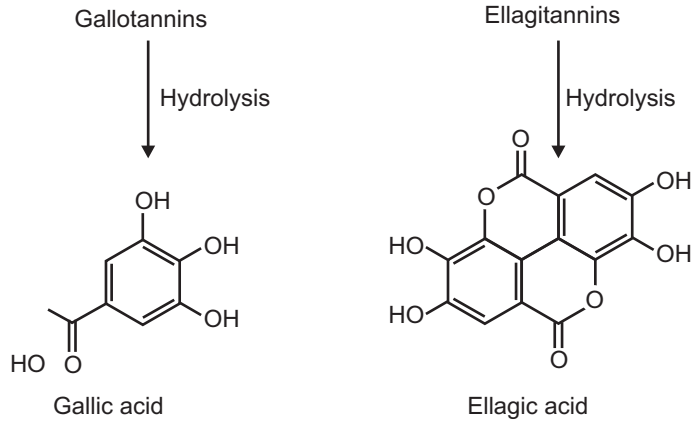


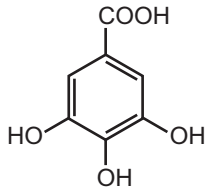
Fig. 6.1: Chemical structures of Gallic acid and Ellagic acid

(b) Non hydrolysable group of tannin:

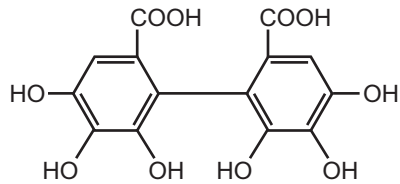
- They are known as condensed tannins or proanthocyanidins.
- They have resistance to acid or enzymatic hydrolysis.
- They are Flavanol derivatives:
  1. Flavan-3-ol like catechin.
  2. Flavan-4-ol like leucocyanidin.
- They are founded:
  - In barks like: Cinnamon (Phlobatannins), Cinchona (Cinchotannin), Wild cherry.
  - In seeds: Cacao, Cola (Colacatechin).
  - In leaves: Tea.
- They produce dark green color with $FeCl_3$.
- They produce Catechol with conc. HCl and vanillin.

2. Pseudo tannins:

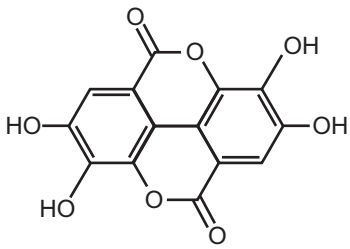
- They have low molecular weight.
- They occur:
  - As Gallic acid like in Rhubarb rhizomes.
  - As catechin like in Cacao, Acacia.
  - As chlorogenic acid like in coffee.



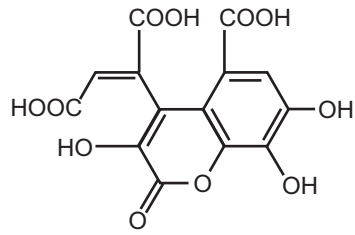
Gallic acid



Hexahydroxydiphenic acid



Ellagic acid



Chebulic acid

Fig. 6.2: Chemical structures of Gallic acid, Hexahydroxydiphenic acid, Ellagic acid, Chebulic acid

6.3 PHYSICO-CHEMICAL PROPERTIES

1. They form colloidal solutions with water.
2. They are non crystalline substances.
3. Their aqueous solution is acidic.
4. They have sharp puckering taste.
5. They have astringent property.
6. They are precipitated by: alkaloids, gelatin, salts of heavy metals, proteins (enzymes).

6.4 CHEMICAL TESTS

1. With $FeCl_3$: Upon addition of ferric chloride solution in tannins aqueous solution:
   (a) Hydrolysable Tannins gives dark blue colour.
   (b) Non Hydrolysable tannins develops dark green colour.
2. It forms precipitate with alkaloids, gelatin, proteins, and salts of heavy metals like lead acetate, copper acetate.
3. Tannins extract yields yellow colour with sulphuric acid.
4. When vanillin hydrochloride reagent is added in tannin solution, red to pink colour is seen.

6.5 EXTRACTION AND ISOLATION OF TANNINS

Tannins (hydrolysable and condensed both) are highly soluble in water and alcohol but insoluble in non polar solvents like ether, chloroform and benzene. So tannins can be easily extracted by water or alcohol.

Powdered crude drug
↓
Extracted with water-saturated ether
Or mixture of water, alcohol and ether
↓ Gallic, ellagic acid, chlorophyll or
↓ Pigments extracted in ether
Tannins gets extracted in water
↓
Separate aqueous and ethereal layers
↓
Concentrate and drying
↓
Isolation and purification
(Various separation techniques of chromatography)

Fig. 6.3: Extraction and isolation procedure of tannins

[I] CATECHU (BLACK CATECHU)

Synonyms: Kattha, Cutch, Catechu.

Biological Source: Black catechu or cutch is an aqueous extract of the heartwood of *Acacia catechu* Willdenow, concentrated by boiling.

Family: Leguminosae.

Geographical Source: The plants used for preparation of catechu are grown in Myanmar and India.

Catechu was described in 1574 by a Portuguese writer 'Garcia de Orta' and Dr Wrath used the scientific process first time to extract catechu. According to him catechu consist of two parts i.e. kattha and cutch. Catechu is used in India as a masticatory from ancient time. The female used catechu for colouring purpose of their feet.

Preparation:

The trees are felled; bark and sapwood are stripped off from trunk. Then the dark red heartwood is cutted into chips and boiled with water into earthen vessels. The decoction obtained which is strained. This strained liquid is again boiled in iron vessels with continuous stirring until it gets syrupy consistency. The syrupy liquid is allowed to cool. It is spreaded over leaves of *Dipterocarpus tuberculatus* Roxburgh, *Cassia fistula* Linn etc which are arranged in a wooden frame or mould. It is kept for overnight. Next morning the cutch will be dry and forms brick like masses having weight about 20 kg. These bricks are broken up for the market.

In another method, the red wood obtained from tree is cut into chips and kept into extractor. Then steam is passed through the drug for complete extraction. The extract is allowed to concentrate under vacuum and cooled. The obtained concentrated extract is centrifuged. The kattha cakes will separate and is moulded into different shapes and dried. By this method good quality of kattha is ready for market. The cutch is obtained by concentrating the mother liquor left after centrifugation. The cutch is allowed to cool.

Fig. 6.4: *Acacia catechu* plant and Kattha pieces

Description:

Colour: Dull brownish to black.

Odour: Odourless.

Taste: Astringent and subsequently sweet.

Size: Near about 2.0 cm × 5.0 cm.

Shape: Cube like or brick shaped pieces.

Solubility: A brown magma forms with cold water but with boiling water it dissolves entirely and crystalline sediment is deposited upon cooling.

Chemical Constituents:

The chief constituents are catechutannic acid (25-33%) and acacatechin (10-12%). Other constituents are catechu red, gum, quercitin and quercitrin.

Chemical Test:

1. When drug is treated with vanillin and hydrochloric acid, it produces pink or red colour due to the presence of catechin.
2. Add ferric ammonium sulphate to the aqueous solution of drug, green colour develops which turns to purple by the addition of sodium hydroxide.
3. To the aqueous solution of drug, add lime water brown colour produced which turns into reddish precipitate after some time.
4. Test for catechin: Dip a matchstick in the test solution, dry it and moist it with concentrated hydrochloric acid. Then warm the stick near flame. The colour of the wood changes to pink due to the formation of phloroglucinol.

Uses:

Catechu is used as an astringent, cooling and digestive action whereas cutch is not used medicinally. It is used for dyeing and tanning purposes like fishing nets etc.

Chemical Structure:

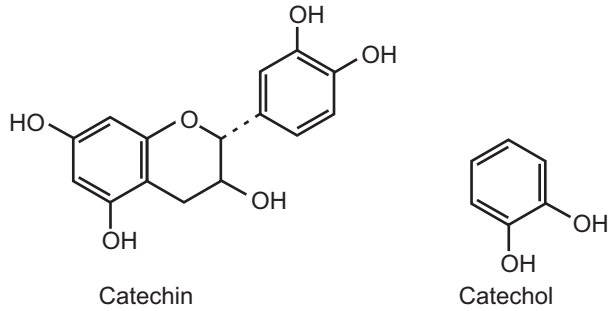


Fig. 6.5: Chemical structures of Catechin and Catechol

Substituents:

Similar extracts are prepared from other substances and called as cutch. Thus mangrove cutch is obtained from the bark of *Rhizophora mangle* Linn and of *Ceriops candolleana* Arnold, family Rhizophoraceae which contains 42 percent tannins.

[II] PALE CATECHU

Synonyms: Gambier, Catechu

Biological source:

It is an aqueous extract prepared from the leaves and young shoots of *Uncaria gambier* Roxburgh.

Family: Rubiaceae.

Geographical source: It is a climbing shrub native to Malay Archipelago and largely cultivated on islands of Singapore and Sumatra. This drug was used in India for the chewing purpose with *Piper betle* (betel) leaf.

Cultivation and Collection:

Plantation is done in damp soil and at an altitude of 500 ft above the sea level. The nursery raised seedlings of about 9 months are planted at about 3 metre distances in between. When the plant reaches height of about 2 meter coppicing has been done. From about eight years after plantation to twenty years age of plant, the yield is at its maximum. The shoots are cut down with a broad bladed knife and put it in special type of vessel known as 'Cauldron' which is made of hard wood with an iron bottom. The leaves and young shoots are boiled in 'Cauldron' for about three hours with continuous stirring of the content. The decoction is evaporated till it becomes thick and pasty with yellowish green colour. The contents transferred into wooden tubs and allow cooling. After about ten minutes the semi crystallised magma is poured into wooden trays to settle down. It is cut into cubes with a wooden knife while the preparation is still moist and sun dried. Much amount of Gambier is filled into kerosene tins to solidify to obtain large blocks which are marketed. The cube shape of Gambier is preferred for pharmaceutical purpose.

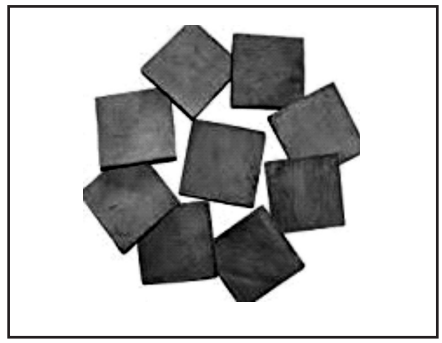


Fig. 6.6: *Uncaria gambier* plant and pale catechu pieces

Description:

Form: Cubes, rectangular blocks or irregular broken pieces.

Colour: Dark reddish brown.

Odour: None.

Taste: First bitter and astringent afterwards sweetish.

The surfaces of cubes are even with minute cavities, slightly concave, breaks easily and are friable.

Chemical Constituent:

The drug mainly contains (+)-catechin (7-33%), catechutannic acid (22-50%). Other constituents are catechu red, quercitin and gambier fluorescin, a fluorescent substance.

Chemical Test:

1. Dip a matchstick in the test solution, dry it and moist it with concentrated hydrochloric acid. Then warm the stick near flame. The colour of the wood changes to pink due to the formation of phloroglucinol.
2. When drug is treated with vanillin and hydrochloric acid, it produces pink or red colour due to the presence of catechin.
3. Add sodium hydroxide to the alcoholic extract of drug and add few drops of petroleum ether in it, stirr the solution and kept it aside for some time. Green fluorescence observed in layer of petroleum ether.

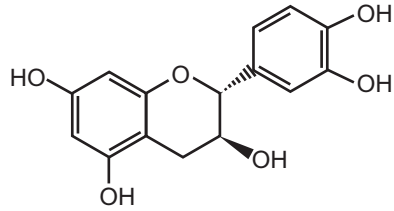
Uses:

It is used medicinally as an astringent in the treatment of diarrhoea and a local astringent in the form of lozenges. It is also used for tanning and dyeing purposes.

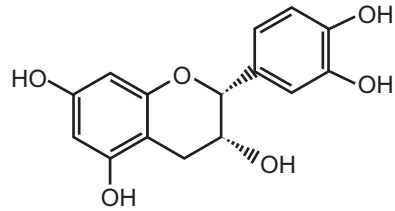
Adulterants:

The starch, astringent extracts and mineral matters like clay, ferric hydroxide etc has been added as an adulterant.

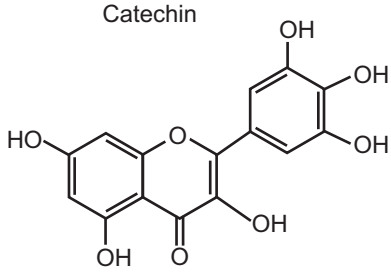
Chemical Structure:



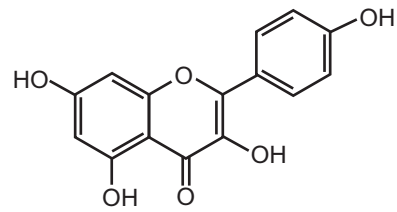
Catechin



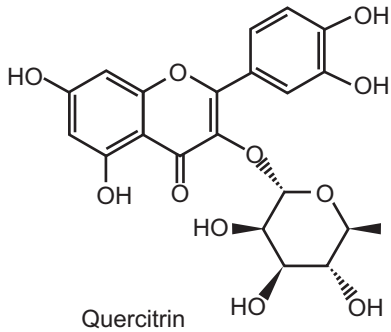
Epicatechin



Quercetin



Kaempfero



Quercitrin

Fig. 6.7: Chemical structures of Catechin, epicatechin, Quercitin, Kaempferol and Quercitrin

[III] PTEROCARPUS

Synonyms: Malbar Kino, Indian kino tree, Cochin Kino.

Biological Source

It is unorganized drug obtained in dried juice of *Pterocarpus marsupium* Linn. The juice is collected by making incision on the stem bark.

Family: Leguminosae.

Geographical source: It is found in Ceylon and India (forest of Karnal, Kerala, West Bengal, Assam, Gujarat, Madhya Pradesh, Uttar Pradesh, Bihar and Orissa).

Collection and Preparation:

Many wide and short tubular cells are arranged in rows in phloem of tree. These cells are filled with red astringent fluid which flows upon incision. Vertical incisions are made in the bark. The filled juice oozes out which is collected into small cups of leaves or other convenient material. This fluid dries in the sun and become dark mass which breaks into the form of smaller grains. Sometimes it is boiled before evaporation because this operation modifies the subsequent behavior of the drug.

Fig. 6.8: *Pterocarpus* plant (Image)

Description:

Form: Small, glistering, angular grain.

Size: Grains are 3 to 5 mm in diameter and sometimes more than 10 mm.

Colour: Transparent or dark ruby red.

Odour: None or odourless.

Taste: Astringent.

Solubility: Partially soluble in cold water, soluble in hot water and alcohol.

Constituents:

The chief constituent of kino is kinotannic acid (70 to 80 percent). Other constituents are kino red, Catechol (κ-pyrocatechin), tannin (gallic acid) and resins.

Chemical Test:

1. When drug solution is treated with ferrous sulphate, it produces green colour.
2. Drug solution is added in alkali solution like potassium hydroxide, purple or violet colour develops.
3. A precipitate is formed with mineral acid treatment.

Uses:

It is used as astringent, in the treatment of diarrhea and dysentery, bleeding gums and toothache and diabetes. It is also used in dyeing, painting and tanning industries.

Substituents:

Kinos have been obtained from numerous plants belonging to various families e.g. Leguminosae, Myrtaceae, Polygonaceae, Myristicaceae and saxifragaceae.

1. **Botany Bay kino:** It is obtained from various species of *Eucalyptus* (Australia), the most suitable being *E. calophylla* R. Brown, Family Myrtaceae, the tannin of which does not gelatinise. The drug occurs in irregular dark red pieces.
2. **African kino:** It is obtained from *Pterocarpus erinaceus* Poiret, family Leguminosae in West Africa. It contains about 50 percent of kinotannic acid and resembles Malabar kino.
3. **Jamaica kino:** It is an extract obtained from decoction of leaves, wood and bark of *Coccoloba uvifera* Linn, family Polygonaceae.

QUESTIONS

1. Define tannins. Write its uses in therapy.
2. Classify tannins with examples.
3. What are the chemical tests for tannins identification?
4. How tannins are extracted and isolated?
5. Write physicochemical properties of tannins.
6. Write synonym, biological source, family and geographical source of Black Catechu.
7. Write a detailed pharmacognostic note on Catechu.
8. Write method of preparation of 'Kattha'.
9. What is the main constituent of Black catechu? Draw it chemical structure.
10. Write chemical test and uses of Black catechu.
11. Write biological source, family and geographical source of 'Gambier'.
12. Write method of cultivation and collection of Pale catechu.
13. What are the chemical constituents of Pale catechu? Draw it chemical structure.
14. Write chemical test and uses of Pale catechu.
15. What are the distinguish features of black and pale catechu? Explain.
16. Write synonym, biological source, family and geographical source of 'Kino'.
17. Write method of collection and preparation of Pterocarpus juice.
18. What are the chemical constituents of Pterocarpus? Draw it chemical structure.
19. Write chemical test and uses of Pterocarpus.
20. What are the substituents for pterocarpus?

■■■

Chapter ... 7

Resins

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *General introduction and brief description about Resins.*
- *Pharmacognostic profile of following drugs*
  - *Benzoin*
  - *Guggul*
  - *Ginger*
  - *Asafoetida*
  - *Myrrh*
  - *Colophony*

7.1 INTRODUCTION

Resins are amorphous mixtures of essential oils and oxygenated products of terpenes, transparent or translucent solids, semi solid or liquid substances. They have complex chemical nature and contains large no of carbon atoms. Resins are insoluble in water and heavier than water but they are soluble in non polar solvents like benzene or ether, volatile oils, fixed oils and alcohol. Resins are hard, non conductor of electricity and combustible in nature. They soften and finally melt upon heating. They are usually formed in schizogenous glands, lysigenous glands or ducts as end product of metabolism. The pharmaceutical applications of resins are local irritant, local cathartic (e.g. Jalap, Ipomoea), as anticancer (podophyllum), in bronchial asthma (Cannabis), used externally as mild antiseptic in the form of tinctures (Benzoin), ointment and plasters (Turpentine and Colophony) and used in the preparation of emulsion and sustained release formulations.

7.2 CLASSIFICATION

Depending upon the type of the constituents:

Resins are of three types:

1. Acid resins examples Colophony contains abietic acid, Copaiba (copaivic and oxycopaivic acid), Myrrh (Commiphoric acid) etc
2. Ester resins examples Benzoin (Coniferyl benzoate), Storax (Cinnamyl cinnamate) etc.
3. Resin alcohols examples Peru balsam (Peruresinotannol), Guaiacum resin (Guaic resinol).

Depending upon combination with other Constituents:

1. **Gum resin:** Gum resins are in homogenous combination of gum and resin. These are always associated with small quantities of other substances like bitter principle, enzymes and volatile oils etc. It may consist of two or more glycosidal acids in various proportions and contains trace amount of nitrogen e.g. Myrrh.
2. **Oleo resin:** When resins are in homogenous combination with volatile oils or oily liquids, are called oleo resin. They are secreted in schizogenous or schizolysigenous ducts. Ginger, Capsicum, Turpentine oil.
3. **Oleo gum resin:** These resins are in homogenous combination with volatile oil and gum. e.g. Asafoetida.
4. **Balsam resin:** Those oleo resins which contain aromatic acids like benzoic acid or Cinnamic acid are known as balsam resin e.g. Benzoin.
5. **Glycoresin:** These are made up of resin along with sugars e.g. Jalap, Ipomoea.

Some resins are complex natural substances not having transpose any specific chemical property, chemically inert and do not get hydrolysed are known as resenes. Few examples are asafoetida, colophony etc.

Properties of resins:

1. These are amorphous and brittle in nature.
2. They occur in translucent hard solid form.
3. The resin softens and finally melted upon heating.
4. They have specific gravity ranges from 0.9 to 1.25.
5. When burnt, they produce smoky flame.
6. They are bad conductor of electricity.
7. They are soluble in organic solvents like alcohol, ether and chloroform.
8. They are insoluble in water.
9. The resin film formed upon drying becomes hard and transparent which is unaffected by moisture and air.
10. Majority of resins undergo slow atmospheric oxidation which darkens its colour and impaired solubility.

7.3 DISTRIBUTION

Resins are abundantly distributed in plants and rarely in insects (Example- Shellac). Resins are present in ducts or cavities which are called schizolysigenous ducts. There are 2 types of resin which exist in nature.

1. **Normal or Physiological resin:** These are preformed in the plants and their yield increases upon injury or incision. Example- Resin of Pinus.
2. **Abnormal or Pathological resin:** This type of resin is formed only upon injury or incision made to the plant. Example- Benzoin, Tolu Balsam.

7.4 CHEMICAL TEST

1. **Solubility test:** Resin dissolves when treated with organic solvents like alcohol, ether or chloroform etc.
2. **Ignition test:** They produces smoky flame upon burning.
3. **HCl test:** Drug is treated with hydrochloric acid which forms pink colour, ensures the presence of resins.
4. **Ferric chloride test:** The greenish blue colour develops when drug is treated with ferric chloride solution. This indicates the presence of resins.

7.5 EXTRACTION AND ISOLATION OF RESINS

Method A:

Powdered drug
↓
Extract the resin with alcohol
↓
Filter
↓
Concentrate
↓
Concentrate extract an excess of water; shake
↓
Resins get precipitated

Method B:

Powdered drug containing Oleo-resin
↓
Percolate the drug with non-polar solvents e.g. Acetone, Chloroform
↓
Non-polar solvent
↓
Steam distillation
↓

Oleo-resin | Volatile oils

[I] BENZOIN

Synonyms: Loban, Sumatra Benzoin.

Biological Source: It is a balsamic resin obtained by Styrax species. There are two types of benzoin available in market

1. **Sumatra benzoin:** It is obtained from *Styrax benzoin* Dryand or *Styrax paralleloneurus* Perkins (Family - Styraceae).
2. **Siam benzoin:** It obtained from *Styrax tonkinesis* (Family - Styraceae).

Geographical source:

Sumatra benzoin is produced in trees grown in South Eastern Asia whereas siam benzoin found in trees grown in Thailand and Vietnam.

Collection and Preparation:

It belongs to pathological resin category. It is collected by making the incision on the trunk part near the base of the tree. It is collected from 5 to 6 year old plants gown wildly or cultivated. The resin is formed only after incision. The initial product obtained after incision is discarded into medicines. The second or next flow is collected which oozes out of the plant and uses medicinally. The collected resinous matter is allowing to sun dry and becomes solidify. Approximately 10 kg of benzoin is collected per tree. Benzoin trees are not found in India hence drug is imported from other countries like Indonesia.

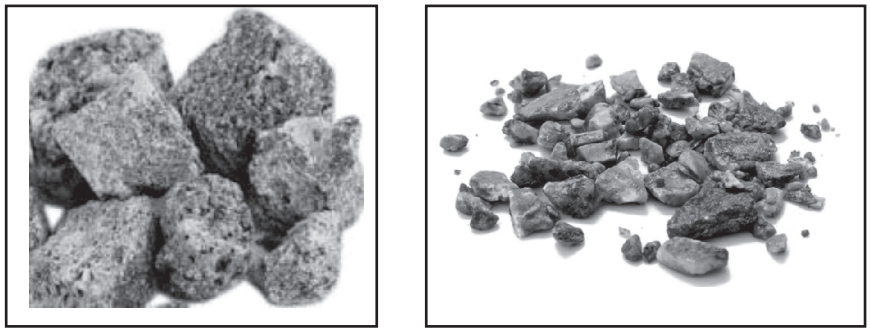


Fig. 7.1: Sumatra benzoin and Siam Benzoin

Macroscopical characters:

Sumatra Benzoin:

Colour: Grey or greyish brown.

Odour: Characteristic and aromatic.

Taste: Sweet and slight acrid.

Form: Lump or tear form.

Size: Varying size.

Texture: Uneven.

Siam Benzoin:

Colour: Rusty- brown or Yellowish-brown.

Odour: Pleasant and vanilla flavor.

Taste: Sweet and slight acrid.

Form: Hard and brittle masses.

Size: Varying size.

Fracture: Brittle.

Chemical constituent:

It contains free balsamic acids i.e. benzoic acid and cinnamic acids and its derived esters. It also contains triterpenoid acids like summaresinolic acid and siaresinolic acids.

Siam benzoin contains esters of coniferyl benzoate (76 percent). Other constituents are styrol, vanillin and phenyl propyl cinnamate. Siam benzoin contains inadequate amount of cinnamic acid so it does not give odour of benzaldehyde when heated with potassium permagnate solution.

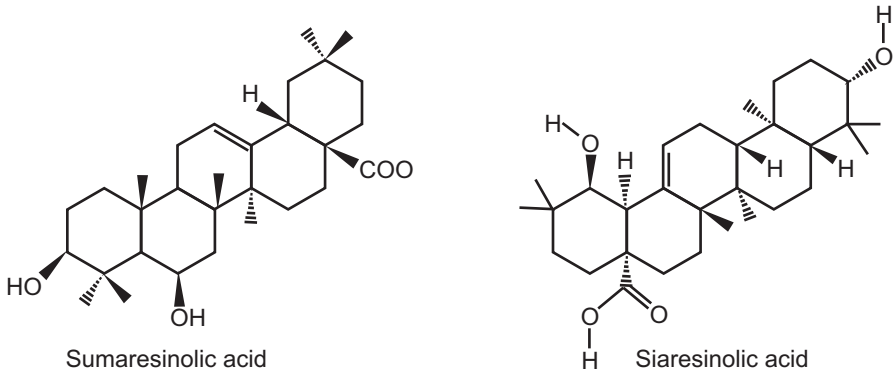


Fig. 7.2: Chemical structure of Sumaresinolic acid and Siaresinolic acid

Chemical test:

1. To 1 gm benzoin add 4 ml potassium permagnate solution and warm it. Sumatra benzoin will produce odour of benzaldehyde.
2. Take 2.5 gm benzoin and add 10 ml ether, shake it well and pour few ml (2 to 3 ml) of extract into porcelain dish. Now add 2 to 3 drops of sulphuric acid into dish. Sumatra benzoin produces deep brown colour whereas siam benzoin produces deep purple colour.
3. Take small quantity of benzoin in a test tube and heat it. Cover the opening of test tube with a glass plate and allow to cool. Crystals of cinnamic acid are observed under microscope.
4. Alcoholic solution of drug produces milky white solution with water.

Uses:

It is used as irritating expectorant, diuretic and carminative. Externally it is applied as an antiseptic and protective. It also uses in the form of inhalant in the treatment of upper respiratory tract infection. It is also used in tincture form. Industrially, it is used in odour fixation of incenses, soaps, perfumes, cosmetics and other preparation. It also masks the taste of pharmaceutical preparations. It retards the rancidity of fats and oils (in benzoated lard preparation).

[II] GUGGAL

Synonym: Gum guggul, Scented bdellium.

Biological source: Guggul belongs to the the oleogum resin category and obtained from *Commiphora weightii*, *Commiphora mukul* by making deep incision at the basal part of stem bark.

Family: Burseracae.

Geographical source: It is small to medium size tree, native to African arid zones like Ethopia, Somalia, Kenya, Zaire and Zimbabwe and cultivated in Rajasthan, Haryana and Gujarat.

Cultivation and Collection:

The cultivation is mainly done by seeds and stem cuttings but seeds are more preferable. It grows well in sandy loam soil with high amount of gypsum (pH 7 to 9). It is up to 2-4 meter in height occurs as a woody tree and shows spines cut branches on pale yellow to brownish stem. The peels of bark are silvery and papery. In the arid and semiarid zones sloppy well drained highly degraded lands are most preferred for cultivation. The seeds are collected from fully developed red berries in July to September because at this time the viability is more. The plant are raised through nursery beds and transplanted into field after six month. The 25-30 cm long stem cuttings are planted in June or October, November for vegetative propagation. The resin is collected by making deep circular incision on the stem of at least 5 years old plant. It is tapped and guggal secreted out as yellowish white aromatic latex like matter. The average yield is about 0.5-1 kg guggul per tree per year.


Fig. 7.3: *Commiphora weightii* plant and oleo gum resin guggul

Description:

Colour: Brown or pale yellow or dull green.

Odour: Aromatic, balsamic and Pleasant.

Taste: Bitter and Characteristic.

Size: 1 to 2.5 cm in diameter.

Shape: Circular, irregular masses or agglomerated tears.

Solubility: Forms white emulsion with water, partly soluble in alcohol.

Chemical Constituent:

Major constituents are Guggulsterone E and Z, resin, gum, volatile oil and minor constituents are oleogum resin which is a complex mixture of various classes of chemical compounds such as lignans, lipids, diterpene, steroids etc. Other constituent like Guggulsterol I, II, III and mukulol have been isolated from the drug. Quercitin, linoleic acid, oleic acid, stearic acid, palmitic acid, stigmasterol are other compounds. Upon steam distillation Guggal yield volatile oil which contain myrcene and caryophylline. Pentosan, pentose and furfural are the constituents of purified gum.

Chemical structure:

Guggulsterone Z, Guggulsterone E

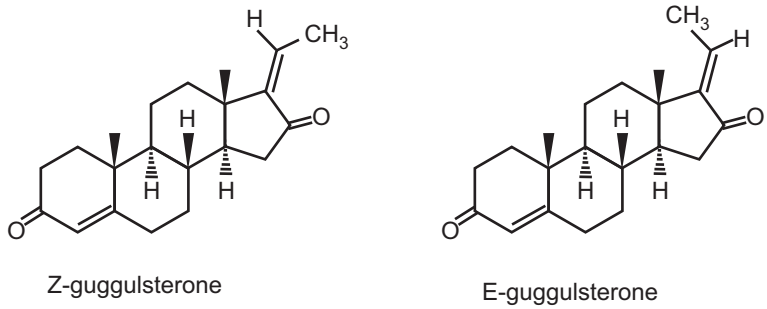


Fig. 7.4: Chemical structure of Z-guggulsterone and E-guggulsterone

Chemical test:

Add acetic anhydride to the ethyl acetate extract of guggul and boil it. After cooling add 2 ml of sulphuric acid which gives green colour at the junction of two liquid which confirms the presence of sterols.

Uses:

Guggul used as anti inflammatory, hypolipidemic, antirheumatic and hypocholesteremic drug. Guggul extract lowers total lipid, serum cholesterol and triglyceride level and reduces the serum p- lipoprotein level and to alter the lipoprotein ratio significantly. The Oleogum portion shows antiarthritic and anti-inflammatory activities. It also shows anti obesity, Antiinflammatory, antiacne, platelet aggregration inhibition and immunomodulatory effect.

Crude gum, oleo gum resin, alcohol extract and petroleum ether extract have showed some side effect like skin rashes and diarrhea. It enhances the menstrual discharge so it should not be taken during pregnancy.

Adulterant:

Commiphora species like *C. abyssinica, C. roxburghii, C. molmol* and *Boswellia serrata.*

[III] GINGER

Synonyms: Adarak, Zingiber.

Biological source

Ginger belongs to oleo gum resin category and is obtained from the rhizomes of *Zingiber officinale* Roscoe.

Family: Zingiberaceae.

Geographical source: It is indigenous to South East Asia and cultivated in Caribbean Island, Jamaica, Taiwan, Africa, Australia, Mauritius and India. In India it is cultivated in nearly all states like Kerala, Assam, Himanchal Pradesh, Orissa, West Bengal and Karnataka.

Cultivation and Collection:

The basic requirement of soil for the cultivation of ginger is sandy loam, clay or red loam soil. It needs warm humid climate and heavy rainfall. It grows well at an altitude of 1000 to 1500 meter from sea level. The propagation is mainly done from rhizomes in the month of June. The seed rhizome having bud are sowed into soil and supplemented with adequate amount of manures and fertilizers viz. Superphosphate, potash and ammonium sulphate. If

rainfall is not enough than proper irrigation arrangements are done. Ginger is ready for harvesting in six months or when leaves become yellowish. Harvesting is done by digging method. Rhizomes are washed properly and then dried. Then rhizomes are scrapped, dried and coated with inert material such as calcium sulphate. The average yield of green ginger is about 1500 kg per hectare.

Macroscopical Characters:

Colour: Buff or earthy brown.

Odour: Characteristic, Agreeable and Aromatic.

Taste: Pungent.

Size: Rhizomes are 5 to 15 cm in length and 2 to 6 cm in width.

Shape: Rhizomes are laterally compressed, bears short, flat, ovate branches and bud at apex.

Fracture: Fibrous, short.

Chemical Constituents:

It contains volatile oil, fat, starch, moisture, resinous matter, fibre and inorganic material etc. The oil contains hydrocarbons (monoterpene and sesquiterpene), oxygenated derivatives of terpenes and phenyl propanoids. Along with these compounds α–zingiberene, β-sesquiphellandrene, α-curcumene, β-bisabolene, α-farnesene, geranial, citral are also present in the drug. The specific aroma of ginger is due to the presence of volatile oils whereas the pungency and therapeutic action is due to the presence of phenolic ketones which includes gingerols like Zingerone, gingediols, paradols, shogaols, o-methyl ethers and hexahydrocurcumin.

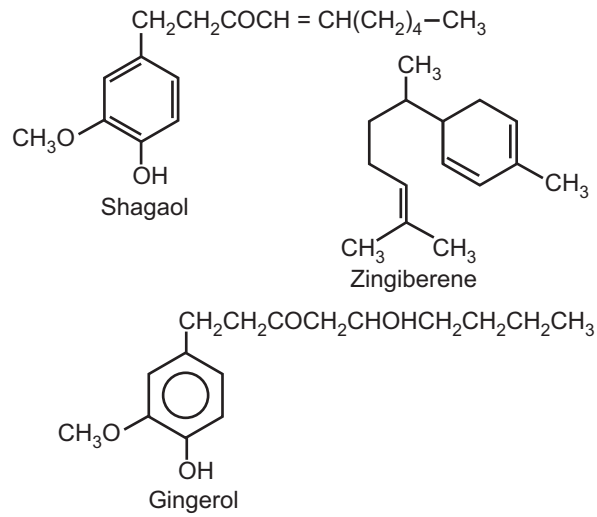


Fig. 7.5: Ginger plant and Chemical structure of ginger constituents

Chemical test:

To identify the adulterant, the tincture of ginger is heated with caustic alkali at 90-100°C and evaporates the liquid. Then it is dissolved in dilute HCl and extracted with ether. The ethereal layer is evaporated and left residue should have no pungency (indicates the presence of gingerol).

Uses:

It is used as stomachic, carminative, aromatic, stimulant and flavouring agent. Its oil is used in mouth washes, beverages and liquor preparation. Ginger has molluscicidal effects and controls parasitic infections. It also blocks the gastro intestinal reactions and nausea feeling. It causes adsorption of toxins and increases the gastric motility. It is also effective in the control of nausea and vomiting in *Hyperemesis gravidarum* as well as postoperative nausea and vomiting.

Ginger also possess cardiovascular activity, antiplatelet aggregation property, analgesic, antipyretic, antitussive, antibacterial, anthelmintic, fungicidal and antiulcer activity.

Limed ginger is the coated ginger with lime which improves its colour and quality. It is mainly done for long storage of ginger.

Allied Drugs:

Japanese ginger obtained from *Z. mioga* contains volatile oil which gives bergamot odour and less pungency than the *Z. officinale*.

Galangal rhizome obtained from *Aplinia officinarum*.

Adulterants:

It is adulterated with exhausted ginger (detected by determination of water soluble ash value, volatile oil content and extractive values).

The 'Spent ginger' is also uses for adulteration purpose. In this type, the aroma has been extracted which yields low quality of ginger.

Ginger is often contaminated with capsicum or grains of paradise to enhance the pungency.

[IV] ASAFOETIDA

Synonyms: Hing, Ferula.

Biological source

It is an oleo gum resin obtained from the roots of *Ferula foetida* Regel, *F. Rubricaulis* Boissier.

Family: Umbelliferae.

Geographical source: These plants are large and grow in Afganistan, Persia and Central Asia.

Collection and Preparation:

There are numerous large schizogenous ducts which are filled with whitish, gummy resinous emulsion in the cortex of the stem and root. After about five years the roots has stored sufficient reserve and is about thick at the crown. In the end of March, the stems close to the crown are cut off. The exudate flows out from the cut surface and hardened. The cut surface which oozes out the exudates is covered with dome like covering of sticks and leaves. The hardened gum resin is scrapped off after few weeks and again a cut is made to allow the juice to flow out. This process is repeated many times with a time interval of about ten days until the plant ceases to produce latex. The yield is about 1000 gm per plant. The collected drug is dried properly, packed in tin containers and marketed.

Fig. 7.6: Asafoetida plant and its tear form

Description:

Forms: Paste, tear (pure form) and mass bulk (block or lump).

Shape: Tears are separate, rounded or flattened.

Size: 0.5-4 cm in diameter.

Colour: Dull yellow or dingy grey colour which changes into reddish brown on storage.

Odour: Intense, penetrating, persistent, alliaceous smell.

Taste: Bitter, acrid and alliaceous.

Mass asafoetida consist of tears agglutinated into less uniform mass and mixed with extraneous substances like stones, earthy matter, calcium carbonate and root slices etc.

Chemical Constituents:

The main constituents are volatile oil (10-17%), resin (40-65%) and gum (1.5-10%). Resin consists of resene (asaresene A) and volatile oil. It also contains 1.5 percent of free ferulic acid and 16 percent of unstable ester of ferulic acid with asaresinol. Volatile oil contains pinene and various disulphides ($C_7H_{14}S_2$, $C_{11}H_{20}S_2$, $C_{10}H_{16}S_2$). Ferulic acid yields umbellic acid, when it is treated with hydrochloric acid, loses water molecule and forms umbelliferone. Free umbelliferone is absent in asafoetida.

Chemical Test:

1. Reddish brown colour develops upon addition of sulphuric acid on fractured surface of drug.
2. A yellowish orange emulsion forms when asafoetida is triturated with water.
3. When drug is treated with nitric acid green colour appears.
4. Take 0.5 gm of drug and triturate it with sand and hydrochloric acid, add little quantity of water and filter it. To the filtrate add ammonia blue fluorescence develops which shows the presence of umbelliferone.

Uses:

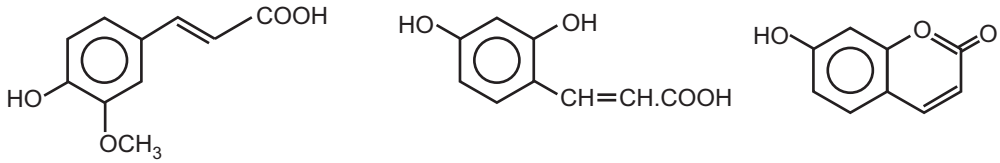
It is a powerful nervine tonic, used in the treatment of Hysteria, bowel stimulant, expel flatulence, relieves constipation and flavouring agent.

Allied Drug:

Galbanum (*Ferula galbaniflua*) is an oleo gum resin used as allied drug. The distinction between galbanum and asafoetida is the presence of free umbelliferone. Galbanum contains free umbelliferone whereas asafoetida does not contain umbelliferone in free form.

Asafoetida is also adulterated with red clay, wheat flour, chalk and gum acacia.

Chemical Structure of Chemical Constituents:



trans-Ferullic acid Umbellic acid Umbelliferone

Fig. 7.7: Chemical structure of Asafoetida constituents

[V] MYRRH

Synonyms: Gum Myrrh, Commiphora, Bissabol

Biological Source: It is a gum resin obtained from the stem of *Commiphora molmol* Engier.

Family: Burseracae.

Geographical source: It is mainly collected from Somaliland in the north east of Africa and south of Arabia.

Collection and Preparation:

The oleo gum resin is collected into lysigenous cavities. The schizogenous ducts form lysigenous cavity by the breakdown of bark. The gum exudes out upon wounding of bark and changes its colour from yellowish white to reddish brown and forms hard mass. The gum is collected on goat skin and marketed.

Description:

Shape: Irregular rounded tears

Size: 2.5 to 10 cm in diameter.

Texture: Rough, dull and dusty surface.

Fracture: Brittle, granular fracture.

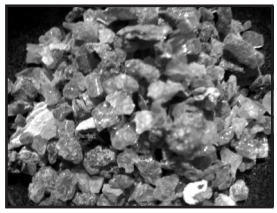
Colour: Reddish brown.

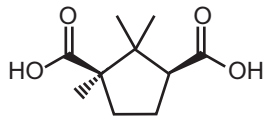
Odour: Agreeable aromatic.

Taste: Unpleasant, aromatic, bitter, acrid.

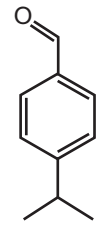
Chemical Constituent:

The drug contains mixture of resin (25%), volatile oil (2.5-6.5%) and gum (60%). Along with these compounds, three free resin acids α, β and γ-Commiphoric acids, esters of resin acid, commiphorinic acid, two phenolic resins α and β-heerabomyrrhol, volatile oil consist terpene, cuminic aldehyde and eugenol etc.





Commiphoric acid



Cumminaldehyde

Fig. 7.8 Myrrh tears and Chemical structure of Commiphoric acid and Cuminaldehyde

Chemical Test:

1. A yellowish brown emulsion forms upon trituration with water.
2. Take 0.1gm of drug and 0.5 gm sand, triturate it with ether and filter it. Evaporate the filterate and add bromine vapour, violet colour develops.

Uses:

It has stimulant, antiseptic property, uterine stimulant, emmenagogue. Due to its astringent property to mucous membrane it is also used for mouth wash and gargles.

Substituent and Adulterant:

The substituents of myrrh are fadhli or Arabian myrrh, Yemen myrrh, perfumed bdellium or bissabol, Indian bdellium, African bdellium and Gum hotai etc.

[VI] COLOPHONY

Synonyms: Rosina, Long needle pine, Colophonium, Pine resin

Biological source: Colophony is the solid residue of turpentine oil left after distilling the crude oleo resin obtained from *Pinus* species like *Pinus palustris*, *P. echinata, P. maritime, P. longifolia*.

Family: Pinaceae.

Geographical source: It is mainly prepared in Pakistan, South East USA, North America, South West France, Europe and India (in Himalayan territories).

Collection and Preparation:

Nearly 80 species of Pinaceae family are available from which the oleo resin, a mixture of turpentine oil and colophony is collected. The trees contains normal oleo resin but in small quantity. After incision the new oleo resin containing ducts are formed which produces good amount of oleo resin. This oleo resin is collected and kept in copper distilling vessels with water and heated. Turpentine oil is distilled oil and colophony left as residue forms and collected.

Fig. 7.9: Colophony plant and its tear form (Image)

Macroscopical Characters:

Colour: Amber coloured or sometimes Yellowish to yellowish brown.

Odour: Faint.

Taste: Angular, Translucent masses.

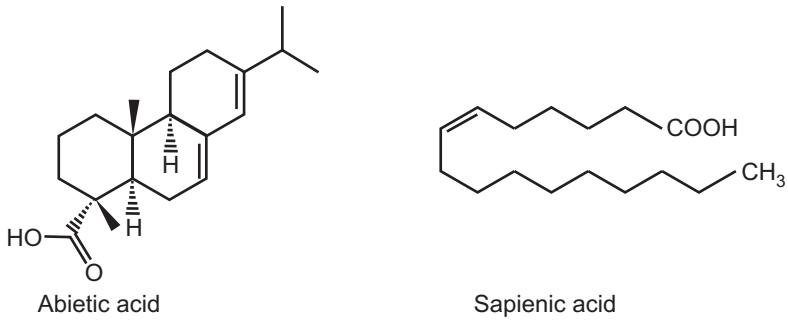
Size: Varies in size.

Appearance: Glossy appearance with brittle nature.

It has solubility in alcohol, chloroform, ether, fixed oil, essential oils, light petroleum and glacial acetic acid but insoluble in water.

Chemical Constituents:

It mainly contains unsaturated resin acids principally abietic acid (nearly 90 percent), esters of oleic acids, volatile oil (0.5 percent), resenes (5 to 6 percent), sapenic acid, pimaric acid etc. The unsaponifiable matter of colophony contains high molecular weight alcohols and hydrocarbons.



Abietic acid Sapienic acid

Fig. 7.10: Chemical Structure of Abietic acid and Sapenic acid

Chemical Test:

1. Dissolve 1 gm of drug into 10 ml acetic anhydride and heat gently. Now add drop of sulphuric acid which gives bright red colour changes to violet colour.
2. Dissolve drug in light petroleum ether and make up the double volume with dilute copper acetate solution. The petroleum ether layer shows emarald green colour which shows the presence of copper salt of abeitic acid.

Uses:

It is used as diuretic, stimulant, ointment preparation, varnishes manufacturing, insulators, soaps, ink preparations. It is also used in flooring preparation, soldering compounds, mastics and pressure sensitive adhesive manufacturing.

Adulterant:

It is adulterated with black resin and apic resin.

QUESTIONS

1. Define resins. Write its uses in therapy.
2. Classify resins with examples.
3. What are the chemical tests for resin identification?
4. How resins are extracted and isolated?
5. Write physicochemical properties of resins.
6. Write synonym, biological source, family and geographical source of Benzoin.
7. What are the distinguish features of Sumatra and Siam benzoin? Explain.
8. Write a detailed pharmacognostic note on Styrax.
9. Write biological source, family and geographical source of Guggul.
10. What are the main constituents of Guggul? Draw it chemical structure.
11. Write chemical test and uses of Guggul.
12. Name out the adulterants of guggul.
13. Write biological source, family and geographical source of ginger.
14. What is the main constituent of Ginger? Draw it chemical structure.
15. Write chemical test and uses of Ginger.
16. How ginger is adulterated?
17. Write biological source, family and geographical source of 'Asafoetida'.
18. Write method of cultivation and collection of Asafoetida.
19. What are the chemical constituents of Asafoetida? Draw its chemical structure.
20. Write chemical test and uses of asafoetida.
21. What are the adulterants for asafoetida?
22. Write biological source, family and geographical source of 'Myrrh'.
23. Write method of collection of Myrrh.
24. Write chemical test and uses of Myrrh.
25. Write synonym, biological source, family and geographical source of 'Colophony'.
26. Write method of collection and preparation of Colophony.
27. What are the chemical constituents of colophony? Draw it chemical structure.
28. Write chemical test and uses of colophony.
29. What are the adulterants for colophony?

■■■

Chapter ... 8

Glycosides

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *General Introduction and brief description about Glycosides.*
- *Pharmacognostic Profile of Glycosidal containing Drugs:*
  - *Senna*
  - *Aloes*
  - *Bitter Almond*

8.1 INTRODUCTION

Glycosides are the naturally occurring organic compounds found in plants and some animals, which upon hydrolysis (either acid or enzymatic) gives one or more sugar (glycone) moiety and non sugar (aglycone) moiety. The non sugar or aglycone moiety is called genin. The pharmacological activity of any glycoside is mainly due to the presence of genin part whereas glycone part facilitates the transportation of genin part to the site of action. Most frequently occurring sugar is β-D-glucose, although rhamnose, digitoxose, cymarose and other sugars are components of glycosides. When sugar part is glucose then it is known as glucoside. Other sugars may be developed during hydrolysis then term glycoside is applied.

Glycosides are acetals in which hydroxyl group of sugar is attached with hydroxyl group of non sugar component. The secondary hydroxyl group is condensed within the sugar molecule itself and forms an oxide ring. In other way, glycosides are considered as sugar ethers. Both alpha and beta glycosides are possible which depends upon the stereoconfiguration of the glycosidic linkage. The glycosidic linkage is the link between glycone (sugar) and aglycone (non sugar) part of glycoside. The beta glycosides widely occur in plants.

Biologically, glycosides have role in plant life include regulatory, protective and sanitary functions. They have many functions like cardioactive (digitalis, stropanthus, squill, convallaria, apocynum etc), laxative (senna, aloe, rhubarb, cascara, frangula etc.), analgesic (methyl salicylate from wintergreen), local irritant (allyl isothiocynate from black mustard).

It's very difficult to classify the glycosides because if the classification is based on sugar group then a number of rare sugars are involved and the structures of which are not well known. In other hand, if the aglycone group is used as classification base, one encounters groups from probably all classes of plant constituents like tannins, sterols, carotenoids, anthocyanins and many others whose structures are still unknown. A therapeutic classification is good from a pharmaceutical point of view which omits many glycosides of pharmacognostic interest.

8.2 CLASSIFICATION OF GLYCOSIDES

(a) On the Basis of the Linkage:

1. **O-glycosides:** In these glycosides the sugar part is linked with alcoholic or phenolic hydroxyl or carboxyl group.
2. **S-glycosides:** In these glycosides the sugar is attached to a sulfur atom of aglycone such as in sinigrin.
3. **N-glycosides:** In these glycosides the sugar linked with nitrogen atom of ($-NH_2$,-NH-) amino group of aglycone like in nucleosides DNA, RNA.
4. **C-glycosides:** In these glycosides the sugar linked (condensed) directly to carbon atom of aglycone like in aloin.

Note: N & C-glycosides are not hydrolyzed by acids or alkalis or by enzymes.

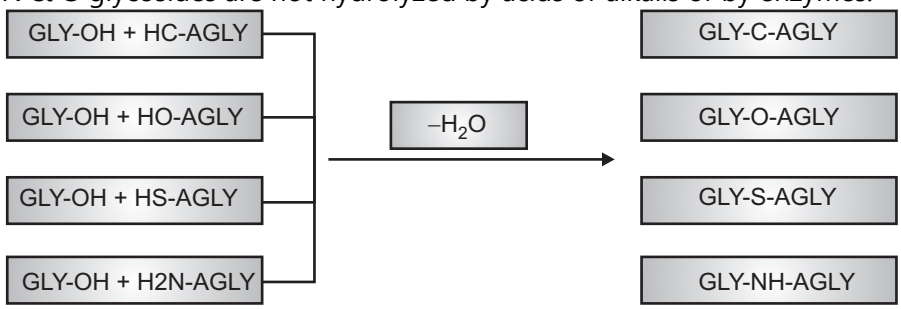


Fig. 8.1: Schematic representation of various types of glycosides

(b) According to aglycone part:

1. If aglycone part alcohol: This group called alcoholic group like Salicin.
2. If aglycone part aldehyde: This group called aldehydic group like glucovanillin.
3. If aglycone part phenol, it is called phenolic group like arbutin.
4. If aglycone part cyanone, it is called cyanogenic or cyanophoric or cyanoside like amygdalin.
5. If aglycone part thio, it is called isothiocyanate glycoside like sinigrin or sinalbin.
6. If aglycone part anthracene, it is called anthraquinone glycoside. example sennoside.
7. If aglycone part steroid, it is known as steroidal glycoside (cardiac) e.g. Digoxin.
8. If flavone, flavonol, flavanone known, it is as flavonoid glycoside.
9. If triterpenoid it is called saponin glycoside e.g. glycyrrhizin, melanthin, ginsenoside.

(c) According to glycone part:

1. Glucose: Glucoside group like in Sennoside.
2. Rhamnose: Rhamnoside like in frangulin.
3. Digitoxose: Digitoxoside like in digoxin.
4. Glucose and Rhammnose: Glucorhamnoside - glucofrangulin.
5. Rhamnose and glucose: Rhamnoglucoside – Rutin

8.3 PHYSICO-CHEMICAL PROPERTIES

- They are colorless, amorphous, solid, non-volatile compounds.
- They give positive test with Molisch and Fehling's solution test.
- They have solubility in water but insoluble in organic solvents.
- Most of them possess bitter taste but some exceptions are populin, glycyrrhizin, stevioside.
- They are odorless compounds except saponin (glycyrrhizin).
- Glycosides gets hydrolyzed by mineral acids and temperature or by enzymes like
  - **(a) Emolsin:** Bitter almond seeds.
  - **(b) Myrosin or Myrosinase:** Black mustard seeds.
  - **(c) Rhamnase:** Glycosides containing rhamnose as sugar part.

8.4 TESTS FOR IDENTIFICATION

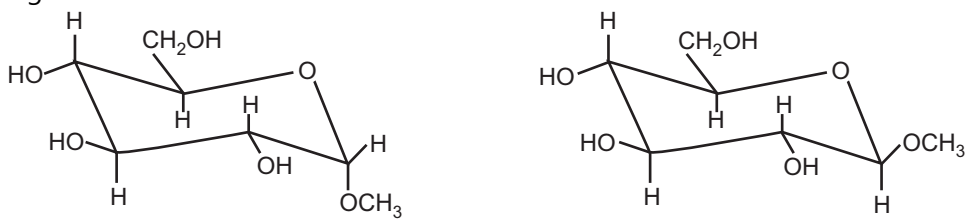
General Test:

Test A: In this test 200 mg drug is extracted with 5 ml sulphuric acid (dilute), warm it on waterbath and filter. Take the acidic extract and neutralize it by adding sodium hydroxide solution (5 percent). After neutralization Fehling solution A and B (0.1 ml) is added and heated it on waterbath for few minutes (2-3 minutes). Red precipitate produced, note the quantity of precipitate and compare it with red precipitate formed in test B.

Test B: Here 200 mg drug extracted with water (5ml), boil it then add water in same volume as used for sodium hydroxide in the previous test. After that add Fehling solution A and B (0.1ml) and heat it on waterbath for 2 to 3 minutes. Red precipitate forms. Note the quantity of precipitate and compare it with precipitate formed in test A.

Note: If test A precipitate ≥ test B precipitate----------Glycoside may be present.

Where test B indicates amount of free reducing sugar already present in drug and test A shows the presence of free reducing sugar which forms upon acid hydrolysis of glycoside in crude drug.



Alpha-methyl glucoside Beta-methyl glucoside

Fig. 8.2: Structure of alpha and beta methyl glucoside

8.5 EXTRACTION METHOD OF GLYCOSIDES- STAS-OTTO METHOD

- The drug should be coarsely powdered for extraction purpose.
- The temperature should not exceed 45°C for thermolabile drugs.

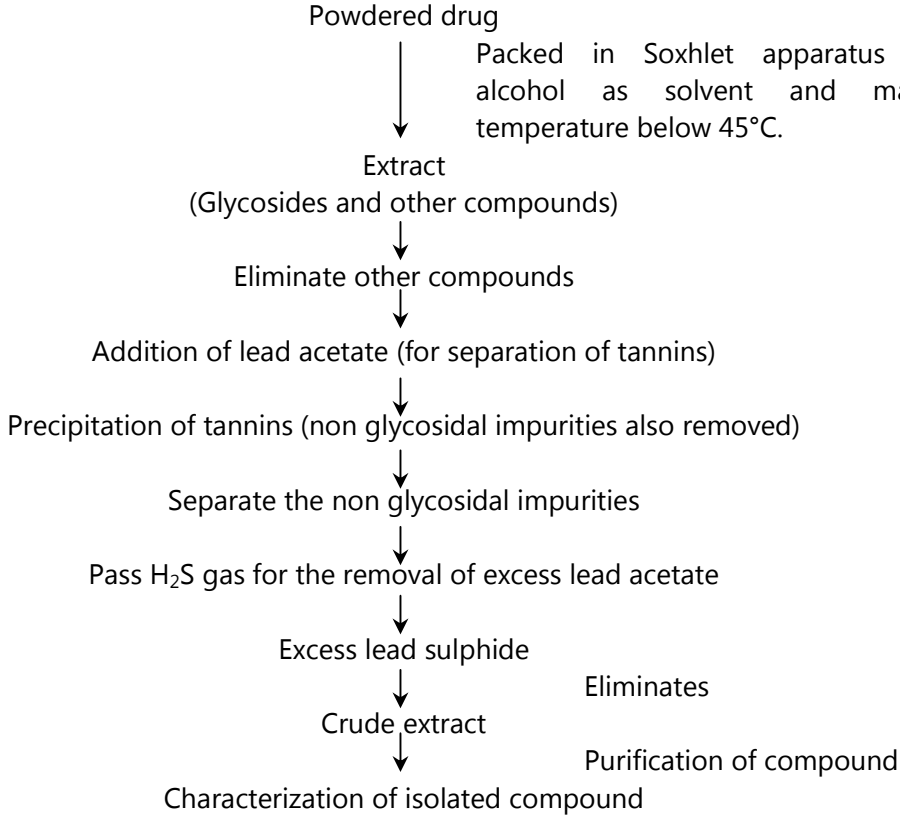


Fig. 8.3: Stas - Otto method for extraction of Glycoside

[I] SENNA

Senna is an example of anthraquinone glycoside. Anthraquinone glycosides are mainly found in dicot families like Euphorbiaceae, Ericaceae, Polygonaceae, Rhamnaceae, Rubiaceae, Leguminosae, Verbenaceae etc. The monocot family liliaceae also shows the presence of this glycoside. The aglycone part of these glycosides is formed by head to tail condensation of acetate units. This glycoside comprises the aglycone part like anthaquinone, anthrone, anthranol, dianthranol, oxanthrone and dianthrone. The anthraquinones are more biologically active in reduced forms. In fresh drugs these are present in reduced forms but gets oxidized and hydrolyzed upon storage.

Synonyms: Senna leaf, Tinnevelly senna, Alexandrian senna, Cassia senna, Indian senna.

Biological source: It consists of dried leaflets of *Cassia angustifolia* or *Cassia senna* Vahl (Tinnevelley senna) and *Cassia acutifolia* Delile (Alexandrian Senna).

Family: Leguminosae


Fig. 8.4: *Cassia acutifolia* and *C. angustifolia* plant

Geographical source: *C. angustifolia* (Indian senna) is cultivated in Tinnevelley, Madurai, Tamilnadu (Ramanathapuram district), Andhra Pradesh, Gujarat and Rajasthan. *Cassia acutifolia* (Alexandrian senna) is indigenous to tropical Africa and is cultivated in Sudan, Middle and upper Nile territory.

Cultivation and Collection:

Senna is used as cathartic from ancient times and it also included in Unani system of medicine. Near about 26 species are well known which contain anthracene derivatives in free form or in glycosidic state. Among them *Cassia angustifolia* and *Cassia acutifolia* are in official monograph of pharmacopoeias. They are available abundantly. For the cultivation of senna near about 25 thousand hectares land used in India which produces 22 thousand ton of leaves and 75 thousand tones fruits per year. It is a small shrub having 1.5 meter height contains compound paripinnate leaves. Generally the red loamy or coarse gravelly soil is required for its better cultivation but it can also grow in alluvial loamy soil. The land is ploughed twice before sowing of seeds. The sowing is done by broadcasting method. First sowing should be done in February or march whereas second sowing can be done in the month of October or November. Seeds have hard and tough seed coats. Certain amount of abrading of its surface is necessary to induce quick germination. It is achieved by pouring the seeds lightly with coarse sand in a mortar. About 20 to 25 kg/hectare seeds are required. The plants favor light irrigation for better development. Water logging or heavy irrigation should be avoided. It is sun loving crop, grown as an early summer (February to March) or a winter (October to November). Leguminous fertilizers are applied time to time. The plants are allowed to grow for about 5 months till flower stalk gets developed.

The harvesting can be done after 2-3 months in three stages. In the first stage, the leaves are plucked out when leaflets are thick, fully grown and green in color. The second plucking should be done after one month and third plucking should be done after 4-6 weeks. After third plucking the plants are uprooted. The sennosidal content is maximum when leaves are fully grown. It is observed that the senna yield is good if it is cultivated after rice crop on the same field. After collection the leaflets are dried under shade by spreading them in thin layers. The leaves are dried after 10-12 days and turns yellowish green in color. The leaves are separated and packed into bales under vacuum and stored in dark and cool place. The leaves can be used upto 5 years because it does not loses the potency.

Macroscopic Character:

Colour: Yellowish green.

Odour: Slight.

Taste: Mucilaginous and bitter.

Size: 20- 60 mm in length and 7 to 8 mm in width.

Shape: Leaves are lanceolate, entire apex is acute with spine at the top

Other characteristics are small, perennial, under shrub below 1m in height with ascending branches; flowers are bright yellow in colour. The full grown leaflet emits characteristics smell when crushed. Flowers are not season bound and are brome between 35 to 70 days of age. The other species is very similar to *C. angustifolia* in morphology and growth except in the dimension of its leaflets and pods which are shorter and narrow.

Chemical Constituents:

The composition of the follicles and pods of the two official species is very similar and the differences are quantitative rather than qualitative. Both species contain flavanoids, a polyol (pinitol) acidic polysaccharide anthra-quinones. Alexandrian senna is characterized by 6-hydroxy-musizin glucoside and Tinnevelley senna is characterized by Tinnevellin glucoside. The major components of the dried drug are sennosides which are glycosides of dianthrone type aglycone in other words Sennidins. Sennoside A and B are major components and they are 8, 8' diglucoside of a symmetrical homodianthropic aglycone, dirhein anthone. The dianthrone derivatives do not exist in fresh senna which mainly contains the 8 glucoside of rhein-anthrone and of aloe-emodin anthrone. Senna leaf also contains other anthraquinone glycoside in small amount. They are sennosides C and D, rhein- 8- glucoside, rhein 8-2 Diglucoside and aloe-emodin. It also contains phytosterol, mucilage, resin, myricyl alcohol, chrysophonic acid, salicylic acid.

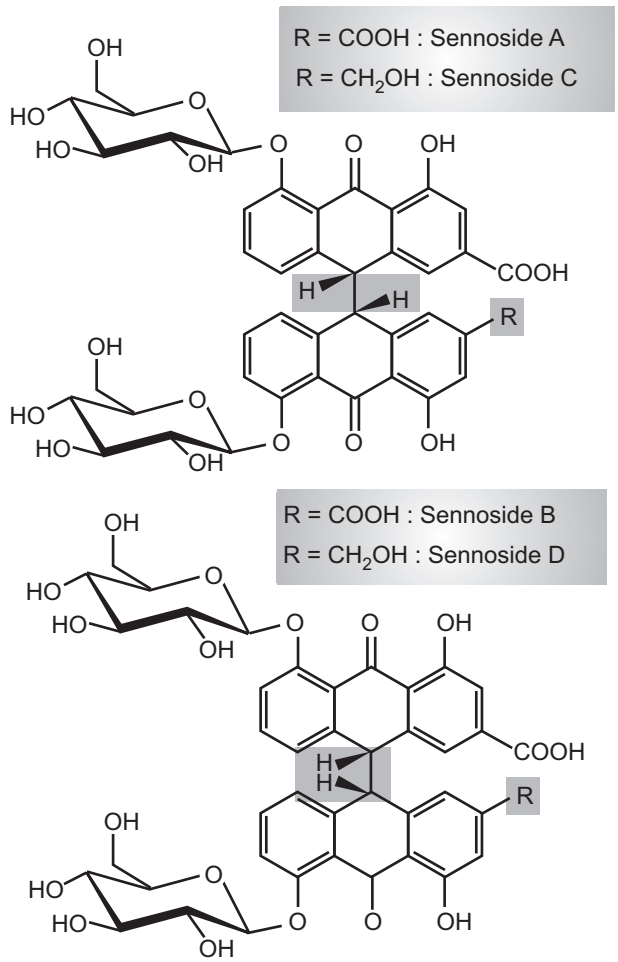


Fig. 8.5: Structure of Sennoside A, B, C and D

Chemical Test:

Borntrager's test: The drug is boiled with dilute sulphuric acid and filtered. To the filtrate add benzene or ether or chloroform and shake well. Separate the organic layer and slowly add ammonia into it. Pink to red color showed in ammoniacal layer which confirms the presence of anthraquinone glycosides.

Allied drug: *Cassia podocarpa*, *Cassia auriculata* (Palthe senna) and *Cassia obovata* (Dog Senna)

Table 8.1: Differences between Alexandrian Senna and Tinnevelley Senna

Sr. No.	Alexandrian senna	Tinnevelly senna
1.	Seldom exceed 40 mm length	Seldom exceed 50mm length
2.	Greyish green	Yellowish green
3.	More asymmetric at base	Less asymmetric at base
4.	More broken and curled at edges	Seldom broken and usually flat
5.	Stomatal index is about 10-15 usually 12.5	About 14-20 usually 17.5
6.	Vein islet no is about 25-29.5	About 19.5- 22.5
7.	Most stomata have two subsidiary cell	Two or three subsidiary cells
8.	Hair more numerous	Less numerous
9.	Ether extract of hydrolyzed acidic solution of drug gives with methanolic magnesium acetate solution:	
(a)	Pink colour daylight,	Orange colour in daylight
(b)	Pale greenish orange in filtered UV	Yellowish green
10.	TLC Test	
(a)	6-hydroxy musigin	Tinnevellin glycoside

Uses:

Senna and its preparation uses as purgative in habitual constipation. The anthraquinone part of senna gets absorbed in intestinal tract after which the aglycone part is separated and excreted in the colon. These excreted anthraquinones irritates and stimulates the colon which increases the movements of colon due to local action. The increased peristalsis causes reduction in the water absorption and results in soft and bulky faeces. The griping effect of

senna is due to its resin or emodin content. The drug is dispensed along with carminative to counteract the gripping effect. The drug given by parenteral way is secreted in colon which causes the pharmacological action.

[II] ALOES

Synonyms: Aloe, Musabbar, Kumari.

Biological source: Aloes are the dried juice obtained by transversely cut leaves of various species of *Aloe barbedensis* Miller (known as Curacao aloes), *Aloe perryi* Baker (Socotrine aloes), *Aloe spicata* Baker and *Aloe Africana* Miller (Cape aloes).

Family: Liliaceae.

Aloe is an example of anthraquinone glycoside.

Geographical source: It is indigenous to eastern and southern Africa and cultivated for commercial purpose in West Indian islands off the north coast of South America.

Fig. 8.6: *Aloe barbedensis* plant

Cultivation and collection: The root suckers are planted at 50 cm distance to get better growth. The aloe plant can grow in low grade soil and even in dry climate. Water logging should be avoided to obtain better mucilage content. For the manuring purpose, a mixture of nitrogen, potassium and phosphorous is used. The drug is collected from two year old plant and collection continued up to twelve years old plant. After twelve years, the plants are uprooted and replantation occurs. An incision made near leaves base to draw all the juice from pericyclic cells of leaves.

Various types of Aloe:

1. **Preparation of Curacao aloes:** The transverse incision made near the base of fleshy leaves (Aloe barbedensis). The cut leaves are kept in sides of V-shaped wooden troughs. The cutted spiny leaves are put into kerosene tins immediately and kept in tilted position in wooden troughs to drain out all the juicy material. The juicy material boiled in copper vessels. The juice gets thickened by the evaporation of latex. The thickened juice is poured into metal containers to become harden. Then it is marketed under the name of Curacao aloes. It is mainly prepared in islands of Aruba and Bonaire in West Indies.
2. **Preparation of Socotrine aloes:** This type of aloe is marketed in semi-solid form. It is prepared in island of Socotra and mainland of east Africa. In this method, the juice of leaves gets collected in goat skin and allows solidifying in semi solid form.

3. **Preparation of Cape aloes:** This type of aloe is mainly prepared in South Africa. For the collection of juice, a pit is dugout which is lined by goat skin. The transversely incised leaves are arranged in circular manner, so as to overlap the cut ends, for 5-6 hours till all the juicy material comes out and collected in goat skin. Then collected juicy material is boiled and stirred continuously in a large iron vessel. When the boiling juice gets a desired consistency, it is poured into wooden moulds to solidify and marketed under the name of Cape aloes.

Description:

- Curacao aloes of good quality varies in colour from yellowish or reddish brown to chocolate brown, lower grades are black in colour and burnt. It has an intensely bitter taste, strong penetrating odour like iodoform and has density about 1.33.
- Socotrine aloes are opaque, pasty or semisolid in nature, having brownish yellow colour. It separates on standing into clear, dark brown, supernatant liquid and dark yellow sediment which shows minute crystals of aloin under microscope.
- Cape aloes are dark reddish brown or black in colour and breaks with a clean glassy fracture. It shows no crystals under microscope. The splinters are thin, transparent, reddish brown or yellow coloured. It has sour distinct odour and nauseating bitter taste.

Chemical Constituent:

Aloe is the main source of glycosides i.e. anthraquinone glycosides. The chief constituent of all the varieties of aloes is aloin which mainly contains barbaloin (a pale yellow, crystalline substance). Other constituents are resin and aloe emodin, which is a hydrolytic decomposition product of barbaloin. It occurs in small proportion.

Curacao aloes contains barbaloin with isomeric and crystalline isobarbaloin, barbal-oresinotannol combined with cinnamic acid whereas Socotrine aloes contain no isobarbaloin and cape aloes contain in trace amount. Cape aloes consist of capaloresinotannol combined with paracumaric acid.

Other constituents of this drug are aloetic acid, aloesone, choline, choline salicylate, chrysophanic acid, chrysamminic acid, homonataloin, galactouronic acid, mucopoly-saccharides, glucosamine, saponins and coniferyl alcohol etc.

Preparation of Aloin:

For the preparation of aloin, Curacao aloes are dissolved in boiling water, acidified with sulphuric acid, a precipitate of resinous matter obtain, cool and filter. Then neutralise the filtrate and evaporate under reduced pressure. The weight of the liquid will be twice with that of the aloes used. Add few crystals of aloin, cool and keep aside for crystallisation, filter it and wash it with dilute alcohol. Then recrystallise the aloin from dilute alcohol. The final yield is about 10-20 percent of the weight of the aloes used.

Commercially, Aloin occurs in two varieties a) crystalline and b) amorphous. Research shows that the amorphous aloin has less cathartic property than crystalline aloin (about one third less potency).

Chemical Test:

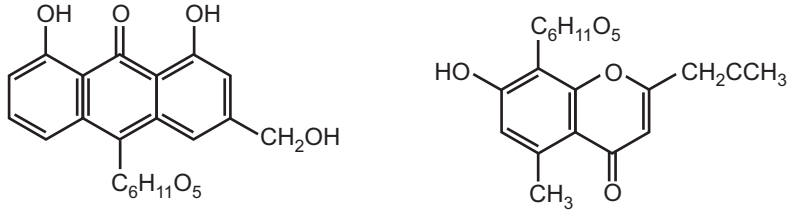
1. **Borax test:** Aloe solution in boiling water (1%), cool it and add kiesselguhr to clear it. Take 10 ml clear filtrate, add 0.25gm borax and heat it till it dissolves. Then pour some dark fluid into water till green fluorescence produce. The green fluorescence is formed due to the hydrolysis of barbaloin which produces aloe emodinanthranol.
2. **Bromine test:** To some clear solution of aloes, as prepared earlier, add freshly prepared saturated bromine solution. A yellow precipitate observes due to the formation of tetrabromaloin.
3. **Nitric acid test:** To 5 ml of 1% clarified solution add 2 ml nitric acid. A vivid green colour produced (in Cape aloes), a deep brownish red colour develops (in Curacao aloes) or pale brownish yellow colour seen (in Socotrine aloes).

Uses: All the varieties of aloes have more or less purgative action. It improves the digestion and does not lose its activity by repetition. It mainly affect on colon and have much potent purgative activity than other anthraquinone glycosidal drugs.

Aloe gel is used in topical preparations and cosmetics. It possess good moisturing property, anti-inflammatory property, anti wrinkle property, protective etc. Fresh gel has a role in burns and wounds.

Substitutes and Adulterant: Natal aloes- Natal aloes are opaque, dull greenish black or dull brown colour and have characteristic odour resembles with cape aloes. Natal aloes are weak purgative.

Mocha aloes: It is black in colour, strong odour, brittle and inferior drug.



Barbaloin Aloc-resin B

Fig. 8.7: Structure of Aloe constituents

[III] BITTER ALMOND

Bitter almond is the example of cyanogenetic glycosides. Cyanogenetic glycosides are also known as cyanophoric glycosides because they contain hydrocyanic acid as an aglycone moiety. These glycosides are derivatives of mandelonitrile which yield hydrocyanic acid and benzaldehyde upon hydrolysis. These glycosides also referred as aldehyde glycosides. About 110 families contain cyanogenetic glycosides among these rosaceae is the most prominent family which contain cyanogenetic glycosides. These glycosidal containing drugs possess flavouring property. They do not have any specific pharmacological property.

Synonym: Amygdala amara.

Biological source: It consists of dried ripe seeds of plant *Prunus amygdalus* Batsch var amara.

Family: Rosaceae.

Geographical source: It is indigenous to Iran and Asia and cultivated in Sicily, Italy, Protugal, Morocco and South France.

Fig. 8.8: *Bitter almond* plant

Cultivation and Collection:

The almond trees require hot and dry climate. They do not tolerate wet soils and frosts. They are sun-loving trees and flowers well in full sunlight. Although they can tolerate partial shade but could not flower or fruit. They require well-drained, deep loamy soil. They need sufficient rainfall around 500-600mm or irrigation to produce better yields and well-filled nuts. They can also survive with less water. They especially benefitted from irrigation in early spring, during summer and sometimes during the first months of autumn. Almonds are generally not self-pollinating. Generally cross-pollination with another variety is required for better quality fruit production. The distance should be kept around 4 to 6 meter in between two plants. Before planting, the roots should be watered. The dugged out hole should be deep enough for the whole root system. The root should not be bent in shape. Like many nut trees, almonds are especially sensitive to tampering with their tap root, so they should never be trimmed or forced into a hole not big enough to accommodate it. The nuts should be dried well before consumption. This can be done by leaving them on the ground for a few days after shaking them or stored safely at dried place.The average healthy and mature almond tree can produce nearly 23-30 kg of nuts per tree.

Macroscopical Characters:

Colour: Brown.

Odour: None.

Taste: Bitter.

Size: 20 mm length, 120 mm width and 10 mm thickness.

Shape: Flat, Oblong, Ovoid having marks on testa.

Chemical Constituents:

Bitter almond contains fixed oil (40-50 percent), Protein (20 percent), enzyme emulsin and bitter glycoside amygdalin (1-3 percent). It also contains volatile oil (0.5 percent). Amygdalin gives benzaldehyde and hydrocyanic acid upon hydrolysis. Bitter almond oil contains 80 percent benzaldehyde and 2-6 percent hydrocyanic acid.

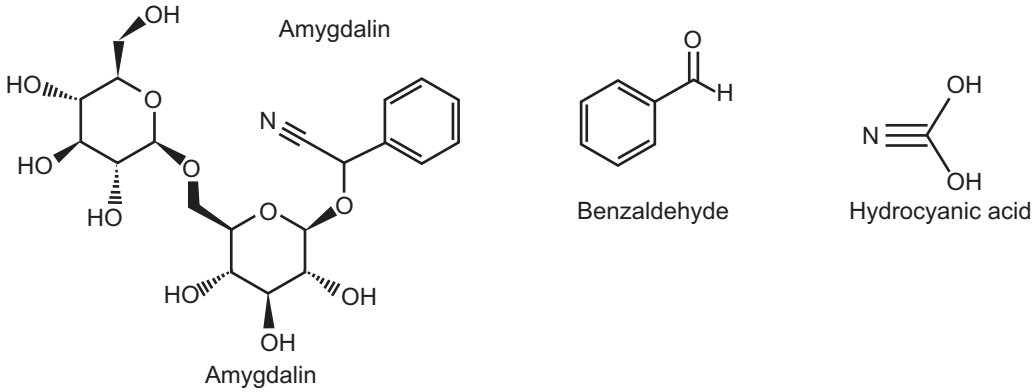


Fig. 8.9: Structure of amygdalin, benzaldehyde and hydrocyanic acid

Chemical Tests:

1. **Ferriferrocyanide Test:** 1 g of the powdered drug macerated with 5 ml of alcoholic KOH (5% w/v) for five minutes. Then transfer it to an aqueous solution which contain ferrous sulphate (2.5% w/v) and ferric chloride (1% w/v). Maintain the temperature at 60-70°C for 10 minutes. Now, transfer the contents to hydrochloric acid (20%), appearance of a distinct prussian blue colour confirms the presence of HCN.
2. **Grignard Reaction Test:** Dip a strip of white filter paper into a solution of picric acid (1 % w/v in water) and drain it and then dip into a solution of sodium carbonate (10% w/v in water) and again drain it. Place the crushed and moistened drug material in a small Erlenmeyer flask. Subsequently place the strip of the prepared sodium picrate paper above the material. Maintain the flask warm for about 1 hour. The liberated HCN will turn the sodium picrate paper original yellow colour to brick red colour (due to the formation of sodium isopurpurate).

Uses:

It is used as sedative. The oil is used in skin lotion as demulcent. It is also used for the preparation of amygdalin, bitter almond water, in perfume industry and liquors preparation, due to the presence of hydrocyanic acid it is not used as flavoring agent in food industry.

QUESTIONS

1. Define Glycosides. Classify them with example.
2. What is the role of glycosides in plants? Write in short.
3. What are ‘C’, ‘N’, ‘O’ and ‘S’ glycosides?
4. Classify glycosides on the basis of their chemical structure.
5. Classify glycosides on the basis of their sugar moiety.
6. Classify glycosides on the basis of their glycosidic linkage.

7. What is Stas-otto method? Explain in detail.
8. What is the general chemical test for glycoside?
9. Write biological source, chemical constituents and uses of Senna.
10. How will you differentiate between Tinnevelley senna and Alexandrian senna? Explain.
11. How the Senna plant is cultivated? Write its geographical source as well.
12. Write biological source, chemical constituents and uses of Aloe.
13. What are the requirements for the cultivation and collection of *Aloe barbedensis* plant?
14. What are the main chemical constituents of Aloe?
15. What are the chemical tests for aloe constituents? Draw chemical structure of its constituents.
16. Write biological source, chemical constituents and uses of bitter almond.
17. What are the chemical tests for bitter almond constituents? Draw chemical structure of its constituents.
18. Write cultivation and collection method of bitter almonds.

■■■

Chapter ... 9

Iridoids and Naphthaquinones

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *General introduction and brief description about Iridoids and Naphthaquinones.*
- *Pharmacognostic profile of following drugs:*
  - *Gentian*
  - *Artemisia*
  - *Taxus*

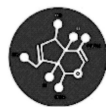
9.1 IRIDOIDS AND OTHER TRITERPENES

Iridoids are monoterpenes characterized by a cyclopenta pyranoid skeleton. It is also known as iridane skeleton (cis-2-oxabicyclo-4, 3, 0-nonane) included in secoiridoids group which arises from cleavage of 7, 8 bond of cyclopentane ring. This group contains about 500 known structures which chiefly comprise more than 300 iridoid glycosides, more than 100 secoiridoid glycosides and near about 100 non glycosidic compounds.

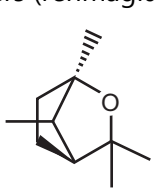
The name iridoids arises from ants of iridomirmex genus. The isolated compounds like iridodial, iridomyrmecin of this genus were involved in the defense mechanism of these insects. Some simple structures like nepetalactone obtained from *Nepeta cataria* L., Family-Lamiaceae or teucriumlactone C from *Teucrium marum* L. exists in plants. The simple structure containing iridoids are rarer in nature. The structure containing ten carbon atoms have more occurrence in nature. The gamopetalous plants of Dipsacales, Gentianales, Lamiales, Scrophulariales order contains iridoids abundantly and makes them important chemotaxonomic markers.

Iridoids: How derived?

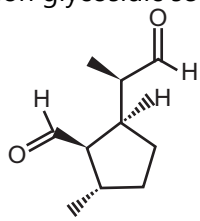
- Named after ants of the Iridomirmex genus, from which were isolated compounds involved in the degence mechanism of the insects: iridoidal, iridomyrmecin and related compuonds.
- Exists in palnts as well: nepetalactone from **Nepeta cataria** (lamiaceae), o**r teucriumlactone C from Teucrium marum** haave marked properties.
- **Nepeta cataria**: effects on cats, so termed as Catnip, katzenmelisse, herbe-aux-chats
- Iridoid: **generally 10 carbons,** may even have more or multiple structure variation up to polyclinic structures.



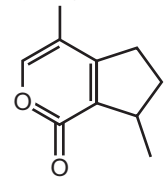
Mostly iridoid glycosides are formed by the glycosidic linkage in between hydroxyl group on the anomeric carbon of D-glucose and hydroxyl group of 1-position of the aglycone. Few structures are known in which the sugar portion of molecule is an oligosaccharide, example Rehmaniosides, whereas Ebuloside in Caprifoliaceae are the structures which have linkage in between glucose is linked with 11-hydroxymethyl group. Non glycosidic iridoids are alkaloids (skytanthine), polycyclic compounds (plumericin), polyesters (valepotriates) or intramolecular ethers (rehmaglutin B). Non glycosidic secoiridoids (Syringa sp., Olea sp.) are exceptional.



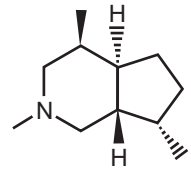
Iridane



Iridodial



Nepetalactone



Skytanthine

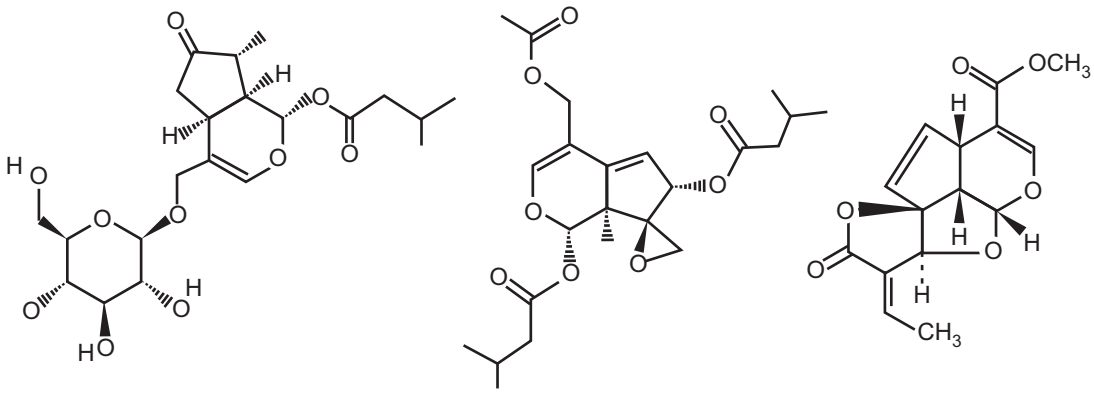


Fig. 9.1: Structures of iridane, iridodial, nepetalactone, skytanthine, ebuloside, valtrate, plumericin

These compounds have role in the defensive function of ants, involves in plant animal interaction, anti-inflammatory activity (weaker by oral route and stronger by topical application) etc.

9.2 EXTRACTION AND CHARACTERIZATION

These glycosides have great instability so the extraction of these glycosides is very delicate. The darkening of iridoid containing plants after collection is also due to this instability. Extraction mainly done by polar solvents like alcohol of various concentration. Initial separation is obtained by redissolving the extract in water. Then re-extract with immiscible solvents of increasing polarity. The fractionation done by chromatography on alumina, on charcoal, on porous polymers like XAD-2 with polar eluent. The purification is done by classic procedures e.g. TLC, HPLC. The detection of iridoids is often accomplished by Trim and Hill colour reagent (dilute solution of copper sulfate with hydrochloric acid). For visualization of TLC a non specific reagent used which contains vanillin with sulfuric acid or hot hydrochloric acid.

Other triterpenes are Cucurbitacin and Bryony (found in *Bryonia cretica* L, Family Cucurbitaceae). Cucurbitacins are tetracyclic triterpenes formed by the rearrangement of protostane cation. Cucurbitacins are unsaturated in nature, polyfunctionalized (may include upto 8-9 oxygen atoms) and occurs in glycoside form. It mainly occurs in Cucurbitaceae family. They are toxic in nature, bitter, cytotoxic and purgative in action. Colocynth seeds (*Citrullus colocynthis* L), wild cucumber (*Ecballium elaterium* L) and bryony roots (*Bryonia cretica*) uses as purgative. Bryony still used in homeopathic medicines but it is abandoned in other systems of medicine like allopathy and phytotherapy.

9.3 NAPHTHOQUINONES

Naphthoquinones are widely occurring natural phenolic compounds. Naphthoquinone are structurally naphthalene like. Two common isomers of parent naphthoquinones are1, 2-Naphthoquinone and 1,4- Naphthoquinone. Few examples of naturally occurring naphthoquinones are Alkannin, Juglone, Lapachol, Lawsone, Menatetrenone, Nigrosporin B, Phylloquinone, Plumbagin, Vitamin K etc whereas synthetic naphthoquinones are Menadione, Atovaquone, Buparvaquone, Diazonaphthoquinone etc.

Naphthoquinones are yellow or orange coloured pigments, mainly found in Angiosperm families such as Ebanaceae, Droseraceae and Bignoniaceae. They are generally 1,4-naphthoquinones and rarely 1,2-naphthoquinones.

They are bacterial and fungal product and also obtained from secondary metabolism of higher plants. Few examples are lawsone, juglone and plumbagin occurs most widely in nature. Naphthoquinones are cytotoxic, antibacterial, antifungal, antiviral, insecticidal, anti-inflammatory and antipyretic in nature. It also possesses pharmacological effect on cardiovascular and reproductive system. Their mechanism is quite large and complex. They bind with DNA and inhibit the replication process. They interact with enzymes and create disturbance in cell and mitochondrial membranes. They also interfere with electrons of respiratory chain in mitochondrial membranes. The plants containing naphthaquinone widely used in China and South America for malignant and parasitic disease treatment.

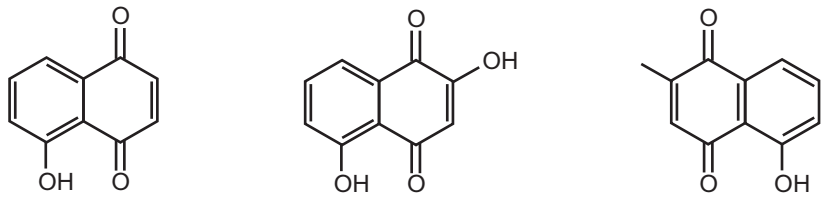


Fig. 9.2: Structure of juglone, lawsone, plumbagin

Free quinones are water insoluble. It can be extracted with common organic solvents and can be separated by chromatography. Naphthoquinones are obtained from steam distillation. They are fairly stable but artefacts are formed e,g. 7-methyljuglone upon oxidation by silica gel yields methylnaphtharizin and its dimer or methoxylation of naphthaquinones by using methanol.

Chemical Test:

1. **Juglone test:** Take chloroform extract (2 ml) and treat it with ethyl ether (2 ml) with dilute ammonia solution. Pink colour develops which indicates the presence of naphthoquinones.
2. **Dam-karrer test:** Add potassium hydroxide solution (10 percent) into chloroform extract (2 ml) of drug, blue colour appears, indicates the presence of naphtha-quinones.

[I] GENTIAN

Synonyms: Gentian root, Gentiana, Radix Gentianae.

Biological source: Gentian consists of the dried fermented roots and rhizomes of *Gentiana lutea*, the yellow gentian.

Family: Gentianaceae.

Geographical source: Gentian is indigenous to hilly areas in Southern and Central Europe (like Jura, Vosges mountains), Yugoslavia and Turkey.

Collection and Preparation:

It is a perennial herbaceous tree and when the plants are 2 to 5 yrs old the turf i.e. a surface layer of earth containing grass plant with their matted root is carefully stripped off. The rhizomes and roots are dug up. This usually takes place from May to October. Collection in the autumn being more difficult because hardness of the soil but preferable from the medicinal point of view. At this stage the pieces of roots and rhizomes are white in colour without any odour which is unfermented gentian and have no demand in market. Then the drug is made into heaps which are allowed to lie on the hill side for some time and may even be covered with earth. After it is washed and cut into suitable length, the drug is dried first in the open air and then in shades. Prepared in this way the drug became much darker in colour loses some of its bitterness and acquires a very distinctive odour.

Fig. 9.3: Gentian plant and roots (Image)

Macroscopical Character:

Colour: Rhizomes are yellowish brown.

Odour: Special odour.

Taste: Sweet taste followed by intensely bitter taste.

Shape: Cylindrical rhizome.

Size: Diameter is about 4 cm and more than 1m in length.

Fracture: Brittle, tough.

Constituents:

Gentian contains bitter glycosides, alkaloids, yellow colouring matters, sugars, pectin and fixed oil. The bitter glycosides mainly contain gentiopicrin (also called gentiopicroside) which is water soluble crystalline compound with bitter value of 12,000. During fermentation and drying, it breaks down into gentiogenin and glucose. A biphenolic acid ester of gentiopicroside, amarogentin which occur in small amount (0.025-0.05 percent) has a bitterness value some 5000 times greater than that of gentiopicroside and is therefore an important constituent of the root. Other bitters isolated are sweroside and swertiamarin. The yellow colour of fermented gentian root is due to xanthones and gentisin (also known as gentiamarin) isogentisin and gentioside. Gentian also contains gentisic acid and about 0.03 percent of the alkaloids gentianine and gentialutine. Gentian is rich in sugars which include the trisaccharide gentianose, the disaccharide gentiabiose and sucrose which on fermentation can convert on glucose and fructose and for very long fermentation can convert into alcohol and CO_2.

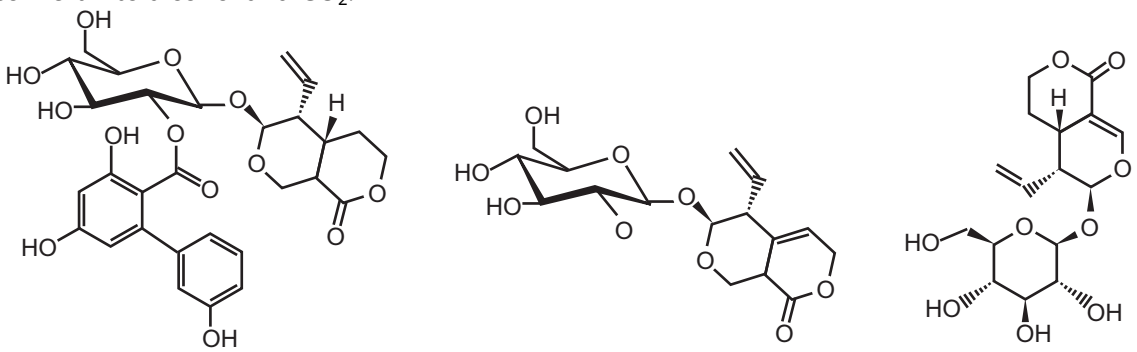


Fig. 9.4: Chemical structure of Gentian constitutents

Chemical test: The extract shows blue fluorescence under UV light.

Uses: Bitter tonic, stimulates gastric secretion, appetite enhancer. Gentian is used in herbal medicines for digestive problems, fever, hypertension, muscle spasm, parasitic worms, wounds, cancer, sinusitis, and malaria.

Adulterant and Substituent:

1. Rhizome of *Rumex alpines*
2. *Veratrum album*,
3. *Gentiana purpurea*
4. *Gentiana pannonica*

[II] ARTEMISIA

Synonyms: Worm seeds, Santonica.

Biological source: Artemisia is unexpanded flowering heads of *Artemisia brevifolia* Wall, *Artemisia cina* Berg and *A. maritima* Linn and its other species.

Family: Compositae.

Geographical source: It wildly grows in Pakistan, India West Tibet and Turkey. In India, it is found in Punjab, Haryana, Uttar Pradesh, Kashmir, Kumaon.

Cultivation and Collection:

Artemisia mainly requires sandy loam to loamy soil for cultivation. The soil should not have water logging property. A well drained light loam soil rich in organic matter is good for cultivation. It can grow in sub tropical areas as a winter crop. It requires cold winter and moderate summer. The cultivation is mainly done by seed propagation. The seedlings are first raised in nursery beds and then transplanted to open field. The seeds are mixed with sand, spreaded uniformly over the nursery beds and covered with thin layer of soil. The beds are kept moist by water sprinkler. The seeds germinate within 5 to 8 days. The seedlings are ready for transplantation into land after 6 to 8 weeks. The land should be ploughed and well manured. The seedlings are transplanted at a spacing of 30 to 60 cm between rows and 45 to 60 cm between two plants. Transplanting is mainly done in evening time. The field should light irrigate after transplantation of seedlings. The crop nutrition is provided by the use of manures and fertilizers. A fertilizer dose of nitrogen, phosphorus pentaoxide and potassium oxide gives good yield. Nearly 3 to 4 irrigation are required upto harvesting. The crop is prone to insects so insecticides are used. The crop is ready for harvesting in 4 to 5 months after transplantation. The crop is harvested at bloom stage to give good yield. The crop is harvested by cutting the plants 15 to 30 cm above the ground level. The crop is dried and packed for market.

Macroscopical Characters:

Colour: Flowers are yellowish, other parts are whitish grey.

Odour: Aromatic, sweet.

Taste: Bitter, Camphor like.

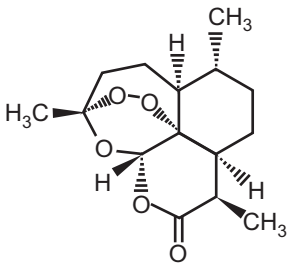
Shape: The flower heads are yellow or brown in colour and oval in shape. The flowers are fertile and have tubular corolla, short cylindrical tube, narrow limb and no calyx.

Chemical constituents:

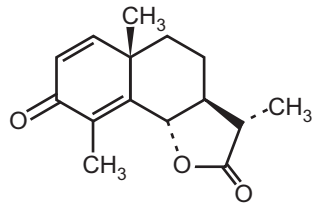
It contains volatile oil (1 to 2 percent) and crystalline substance santonin and artemisin. Other constituents are cineole, pinene and resin. Santonin is anhydride of santonic acid belongs to sesquiterpene lactone. Its amount varies upon the time of collection and type of species.

Chemical Test:

Take powdered drug (1gm) and boil it with alcohol (10 ml), filter it. Add sodium hydroxide to the filtrate and warm it until red colour appears.



Artemisinin



α-Santonin

Fig. 9.5: Structure of Artemisinin, Santonin and *Artemisia* plant

Uses:

It is used as strong anthelmintic especially for round worms. It has no effect or less effect against hook worm and tape worms.

Substituents: *Artemisia vulgaris* Linn.

[III] TAXUS

Synonyms: Yew, Himalayan Yew, Talispatra.

Biological source: Taxus consists of dried roots, bark and leaves of various Taxus species i.e. *Taxus brevifolia*, *Taxus baccata*, *Taxus cuspidata, Taxus Canadensis*.

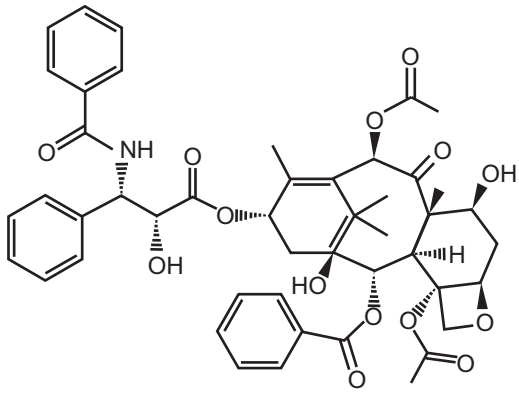
Family: Taxaceae.

Geographical Source: It is mainly found in India, America and Canada. In India it is found in temperate Himalayan region (up to an altitude 2000 to 3500 meters).

Cultivation and Collection:

It is very slow developing evergreen tree which belongs to gymnosperm. Taxus plant grows well in any kind of soil. It can bear bitter cold and grows well in shade as well as in sun. Taxus plants are planted as ornamental plant in parks and gardens, especially as hedges. They lend themselves to clipping.

Taxol isolated from the trunk of *Taxus brevifolia* but the yield was very low (0.01%). That means a plant with 100 years old produce 3 kg bark (or 300 mg taxol). Generally I Kg taxol will be isolated from seven tons dried bark. The content of taxol in the leaves is comparatively more (as much as 0.1%). Drying process of the plant is very much related to the content of taxol. The drying should be done at 40-50°C.



Taxol

Fig. 9.6: Structure of Taxol, *Taxus brevifolia* plant

Description:

The Plant: There is eight species in this genus, which is the only one in the Taxaceae family, are all found in the northern hemisphere. They are characterized by leaves that are flattened limp needles, almost in a single plane, with two yellowish green stomata bands on the inner side. Male flower have 6 to 14 anthers shaped shields and female part is reduced to an ovule surrounded by scales.

Chemical Constituents:

Different metabolites are present in the leaves and stems like saccharides, polysaccharides and cyclitols, sterols, proanthocyanidins, bisflavonoids, fatty acids, cyanogenetic glycosides and lignans.

The most important constituents are tricyclic diterpenes with taxane nucleus viz. taxusines, baccatin III, taxagifin, taxine, taxol, pseudoalkaloids.

Uses:

Taxol inhibits the polymerization of tubulin similar to podophylotoxin and vincristine. It was used to treat the ovarian tumors and other cancer. Taxol can be dispensing at the rate of 135-175mg/m^2 depending on the indication. Infuse the drug after every three week with proper premedication. The toxicity produce by the treatment of taxol is neutropenia, peripheral neuropathy, cardiovascular problems, vomiting, alopecia, nausea, hypersensitivity etc.

Taxol prevents spreading of metastatic cancerous cells. Taxol is approved by USFDA for the treatment of ovarian cancer. It is also used in lung cancer, gastric and cervical cancer, head, neck, colon and prostate cancer.

QUESTIONS

1. Define iridoid and naphthaquinones. Write its uses in therapy.
2. What are the chemical tests for identification of Naphathaquinones?
3. How iridoid and naphthaquinones are extracted and isolated?
4. Write physicochemical properties of iridoid and naphthaquinones.

5. Write synonym, biological source, family and geographical source of Gentian.
6. What are the main constituents of Gentian? Draw its chemical structure.
7. Write chemical test and uses of Gentian.
8. Name out the adulterants of Gentian.
9. Write biological source, family and geographical source of Artemisia.
10. What is the main constituent of Artemisia? Draw its chemical structure.
11. Write chemical test and uses of Artemisia.
12. How artemisia is substituted?
13. Write biological source, family and geographical source of 'Taxus'.
14. Write method of cultivation and collection of Taxus.
15. What are the chemical constituents of Taxus? Draw its chemical structure.
16. Write uses of Taxus.

■■■

Chapter ... 10

Carotenoids

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *General introduction about carotenoids.*

10.1 INTRODUCTION

Carotenoids include many tetraterpenoid compounds which consists chain of eight isoprene units. The colour and oxidizing tendency of carotenoids mainly depends on the presence of characteristic chromophore which contain at least ten conjugated double bonds. The yellow or orange colour mainly depends on the presence of particular chromophore. 'Carotenes' are the hydroxylated derivatives as Xanthophylls. Carotenoids are acyclic like lycopene or comprises one or two pentacyclic or hexacyclic rings at one end or other end i.e. β, ψ-carotene, or both ends like β, β-carotene. These pigments widely occur and accumulate in the chloroplast of photosynthetic tissues. β-carotene, neoxanthine and lutein are few examples which accumulates in leaf part of plant whereas some chloroplastic carotenoids or their derivatives are accumulated in fruits (e.g. capsanthine found in capsicum, lycopene found in tomato, apocarotenoids found in citrus fruits), flower petals (e.g. marigold, French marigold), roots (carotene found in carrot roots) and seeds (e.g. zeaxanthine found in corn).

Carotenoid involved in photosynthesis, absorbs and transmits photo radiation (450-500 nm), provides protection against harmful radiations (prevents photo oxidation by reacting with singlet oxygen), act as antioxidants (reacts with peroxy free radicals).

Retinol or Vitamin A is produced by the degradation of β-carotenes in human intestinal mucosa and fulfills the requirement of body. Carotenoids also prevents from degenerative disorders. They are also used in porphyria photosensitization (because interferes in photo oxidation processes), dermatitis of phototoxic origin, urticaria, lupus erythematosus etc.

In pharmaceutical industries, it is used as natural, efficacious, non toxic coloring agent (e.g. annatto extract, lycopene, carotene, xanthophylls etc).

Pure carotenoid occurs in two forms- (a) As in the form of microcrystalline suspension in vegetable oil (b) In the form of powder dispersed in water. The application of carotenoids in food industries, other than pharmaceutical industries, are in dairy products, pastries, soup and candies preparations, liquors and beverages preparations etc. In animal feed industries the pigmentation of poultry meat and color of eggs can be enhanced by the use of carotenoids. The natural carotenoids occur in extract form like paprika extract. It has no acceptable daily intake limit but others have limit (5 mg/Kg).

10.2 STRUCTURE OF LYCOPENE, CAROTENE, XANTHOPHYLLS, CAPSANTHIN, LUTEIN

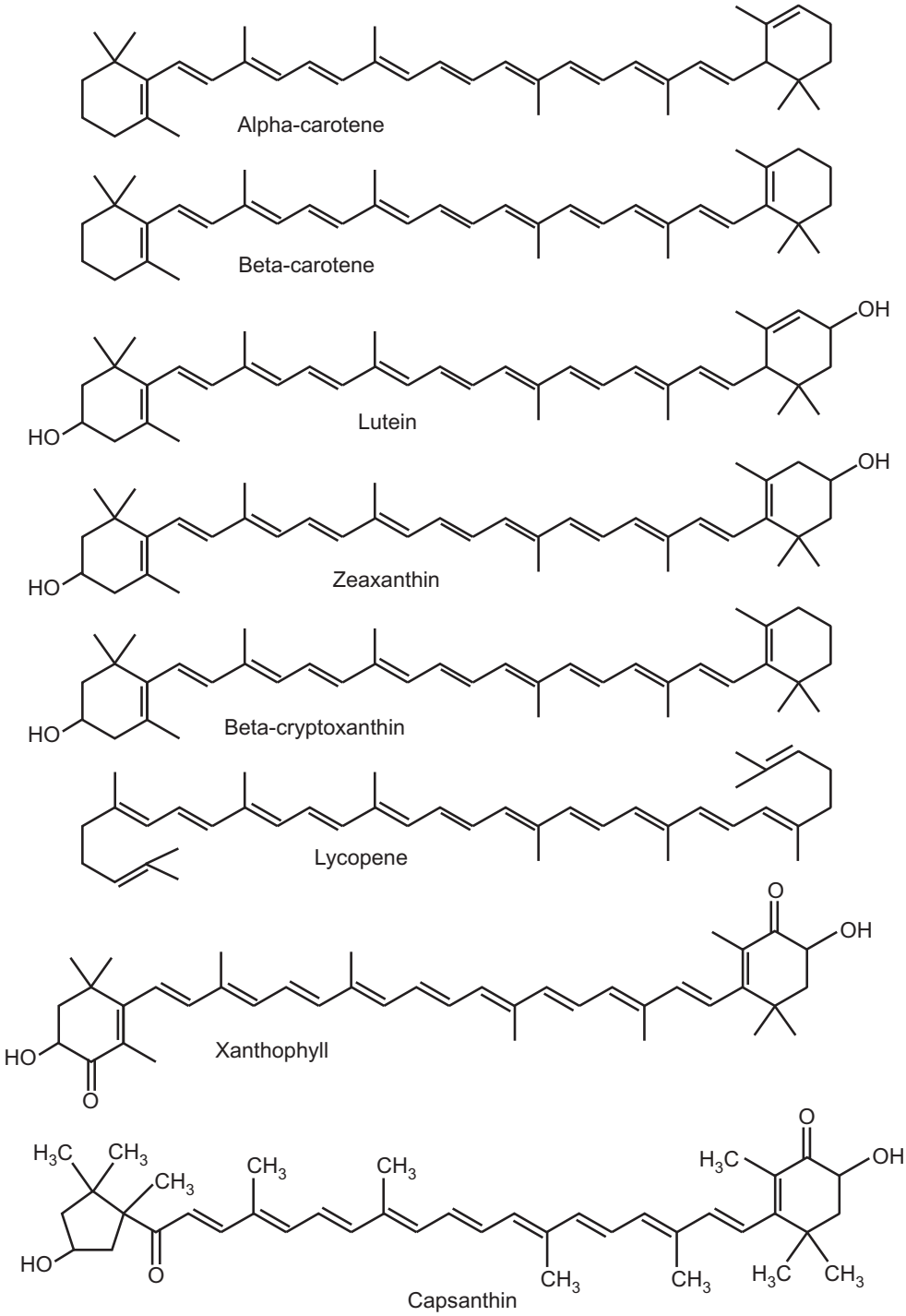


Fig. 10.1: Chemical structure of few examples of carotenes

10.3 CLASSIFICATION OF CAROTENOIDS

Chemically, carotenoids are fat soluble plant pigments which provide colour in nature. These are polyisoprenic compounds which consist of isoprene units nearly eight carbon atoms and forty carbon atoms.

There are nearly 600 compounds which exists in carotenoids group which can be classified as:

1. **Carotenes or carotenoids:** In these pigments only carbon and hydrogen atoms are present.
2. **Xanthophylls:** These pigments are oxygenated hydrocarbon compounds which contain at least one oxygen atom, hydroxyl, keto, epoxy, methoxy or carboxyl groups.
3. **Apocarotenoids:** These pigments typically formed by cleavage of carotenoids.

Properties of Carotenoids:

1. Carotenoids are found in chloroplast in green plants as part of photosynthesis but they are more visible and more coloured in roots, fruits and flowers.
2. Carotenoids are yellow, red or orange in colour which occurs in both plants and animals.
3. They are synthesized in plants but in animals they originate from foods of plant origin.
4. They occur in free state as well as in combination with holoproteides and carbohydrates (carotenoproteides, carotene glycosides).
5. Due to hydrocarbon structure these compounds are hydrophobic in nature and solubilizes only in organic solvents, oils and fats.
6. They are characterized by the presence of conjugated double bond structure.
7. They are unsaturated in nature and causes oxidation and autoxidation reactions in open air.
8. Carotenoid pigments plays an important role in plant photosynthesis and in protection of self photo destruction of chlorophyll molecule and other active substances like cytochromes, peroxidases, catalases, vitamin B 12, vitamin E and vitamin K.

10.4 NATURALLY OCCURRING CAROTENOIDS

- **Hydrocarbons:** Hexahydrolycopene, Lycopersene, Phytofluene, Torulene etc.
- **Alcohols:** Alloxanthin, Crustaxanthin, Cryptomonaxanthin, cynthiaxanthin, Lutein etc.
- **Glycosides:** Oscillaxanthin and Phleixanthophyll.
- **Ethers:** Rhodovibrin and Spheroidene.
- **Epoxides:** Citroxanthin, Diadinoxanthin, Luteoxanthin, Zeaxanthin etc.
- **Aldehydes:** Rhodopinal. Torularhodin methyl ester.
- **Acids and acid esters:** Torularhodin and torularhodin methyl ester.
- **Ketones:** Phoenicoxanthin, Astacene, Astaxanthin, Capsanthin, Flexixanthin etc.
- **Alcohol esters:** Astacein, Flucoxanthin, Isoflucoxanthin, Physalien, Siphonein etc.
- **Apocarotenoids:** Bixin, Paracentrone, Lycopenoate, Crocetin etc.

10.5 EXTRACTION OF CAROTENOIDS

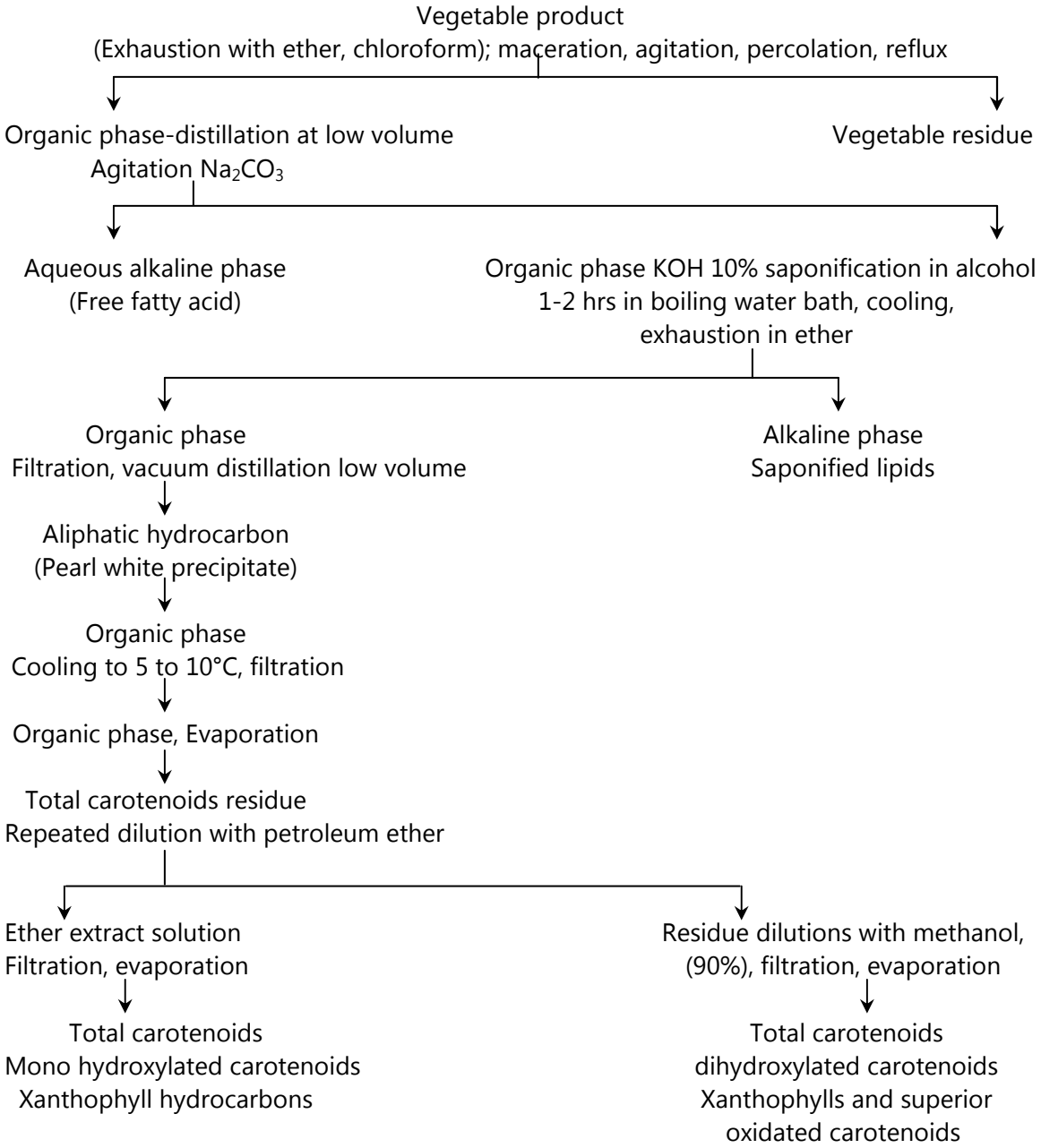


Fig. 10.2: Extraction of Carotenoids

10.6 METHODS OF ISOLATION

- Isolation methods depend on physical and chemical properties of the biosubstances to be separated. Commonly used methods of separation are:
  1. **Distillation:** It is a process of separation of constituents from a liquids mixture by selective evaporation and condensation.
  2. **Crystallisation:** It is the process of formation of solid crystals precipitating from a solution melts or deposited directly from a gas.
  3. **Electrophoresis:** It is a method of separation and analysis of macromolecules and their fragments, based on their size and charge/separates organic molecules based on their different interaction with a gel under an electric potential.
  4. **Column chromatography and thin layer chromatography:** This is a physical method of isolation which distributes components to separate between two phases, one stationery phase and other is mobile phase moving in a definite direction; subtle differences in a compounds partition coefficient which results in differential retention on the stationary phase and changed the separation.

QUESTIONS

1. Define Carotenoids. Classify them.
2. Draw chemical structure of some examples of carotenoids.
3. What are the properties of carotenoids?
4. How carotenoids are extracted?
5. What are the isolation methods of carotenoids?
6. Write examples of naturally occurring carotenoids.

■■■

Unit III

Chapter ... 11

Isolation, Identification and Analysis of Phytoconstituents

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *Isolation, Identification and Analysis of Terpenoids:*
  - *Menthol*
  - *Citral*
  - *Artemisinin*
- *Brief study about Isolation, Identification and Analysis of Alkaloids:*
  - *Atropine*
  - *Quinine*
  - *Reserpine*
  - *Caffeine*
- *Brief study about method of Isolation, Identification and Analysis of Glycosides:*
  - *Glycyrhetinic acid*
  - *Rutin*
- *Brief study about method of Isolation, Identification and Analysis of Resins:*
  - *Podophyllotoxin*
  - *Curcumin*

11.1 ISOLATION AND PHYTOCHEMICAL ANALYSIS OF TERPENOIDS

Terpenoids are the hydrocarbons of plant origin having general formula $(C_5H_8)_n$. They are also oxygenated, hydrogenated and dehydrogenated derivatives. Basically terpenoids are volatile substances which give fragrance or aroma to plants. They are also known as essential oils because they represent essence. They mainly found in leaves and fruits of plants like conifers, citrus and eucalyptus. The term 'terpene' was assigned to the compounds after isolation of volatile liquid turpentine from pine trees. They are colorless, fragrant liquids lighter than water and volatile in nature. A few of them are solids e.g. camphor. They are soluble in organic solvents, alcohol and fixed oils and usually insoluble in water. They are open chain or cyclic unsaturated compounds having one or more double bonds. They give addition reaction with hydrogen, halogen, acids, etc and addition products possess antiseptic properties. They undergo polymerization and dehydrogenation. They are easily oxidized

nearly by all the oxidizing agents. On thermal decomposition, most of the terpenoids yields isoprene as one of the product. They are optically active. It becomes darker in colour upon long standing in air or sunlight. Volatile oils should be stored in well closed air tight umber colour containers or bottles.

11.1.1 Isolation of Menthol

Occurrence: Menthol is a monoterpene alcohol found in mint oil or peppermint oil. The sources of mint oil are *Mentha piperita* Var. *vulgaris*, *Mentha piperita* Var. *officinalis*, *Mentha arvensis*, *Mentha canadensis* Var. *piperascence* belongs to the family Piperaceae. 1 to 3 percent volatile oil is present in peppermint. The two varieties *Mentha piperita* Var. *vulgaris* and *Mentha piperita* Var. *officinalis* contains not less than 45 percent of menthol whereas the varieties *Mentha arvensis* and *Mentha canadensis* Var. *piperascence* contains upto 70 to 90 percent of menthol. The other constituents are (+) neomenthol, (+) isomenthol, menthofuran, menthone, menthyl acetate and cineole. Menthol can be synthesized chemically by hydrogenation of thymol.

Isolation: Mentha oil is obtained by hydro distillation and steam distillation of fresh parts of mentha plant. For the isolation of (-) menthol from peppermint oil the oil is subjected for cooling. The menthol crystal crystallizes out and separated by centrifugation.

The corn mint oil is obtained from steam distillation of *Mentha arvensis* flowering tops which contains about 70 to 80 percent of free (-) menthol. The oil is cooled and menthol crystals are separated by centrifugation. Pure (-) menthol is obtained from recrystallisation from low boiling solvents. The Melting point of menthol is 41 to 44°C.

Thin layer chromatography of Menthol: 1 mg menthol is dissolved in 1 ml of methanol. Then apply a spot on silica gel-G plate and elute it in chloroform. The dried plates are sprayed with 1 percent vanillin-sulphuric acid reagent. Heat the plate at 110°C for 10 minutes. The Rf value of menthol is 0.48 to 0.62.

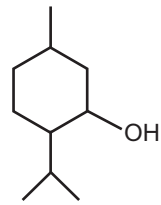


Fig. 11.1: Menthol

Identification test

1. 10 mg menthol crystals are dissolved in 4 drops of concentrated sulphuric acid and add few drops of vanillin sulphuric acid reagent. It shows orange yellow colour which changes to violet upon addition of few ml of water.
2. Crystals of menthol are dissolved in glacial acetic acid and add 3 drops of sulphuric acid and one drop of nitric acid. Menthol does not give green or blue green colour (thymol gives green colour).

11.1.2 Isolation of Citral

Occurrence: Citral occurs abundantly in lemongrass oil *Cymbopogan flexuosus* (Nees) stapf and *Cymbopogan citrates* (DC) stapf belongs to the family Graminae. Lemongrass contains about 75 to 85 percent of citral in their oil. It also occurs in verbena oil, lemon, lime, orange and ginger root. Citral is also present in other species like *Ocimum pilosum* (contains 35 percent), *Liptospermum citratum*, *Eucalyptus staigeriana* and other *citrus* species.

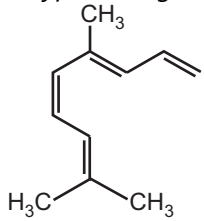


Fig. 11.2: Citral

Isolation: The lemongrass oil is shaken with 5 percent sodium bisulphate solution for 25 to 30 minutes. The resultant is first separated on Buchner funnel and washed with solvent ether or ethanol. The crude citral is regenerated by decomposing the sodium bisulphate adducts with dilute sodium hydroxide solution. The pure citral is obtained by distillation of crude citral under reduced pressure. Its boiling point is 92 to 93°C.

Separation of Geranial (Citral-a) and Neral (Citral-b):

It was observed by Tiemann that geranial (Citral-a) is obtained free from neral (Citral-b) during the regeneration process from bisulphate adduct. The crystalline sodium bisulphate adduct of Geranial or Citral-a is sparingly soluble in water whereas Neral or Citral-b adduct is readily soluble in water. Tiemann observed that neral is isolated from citral (mixture) by shaking it with alkaline cyanoacetic acid solution ($NC.CH_2.COOH$) for short period of time when geranial reacts at much faster rate with acid than neral. Citral-a have melting point 108-110°C and Citral-b melting point is 96°C.

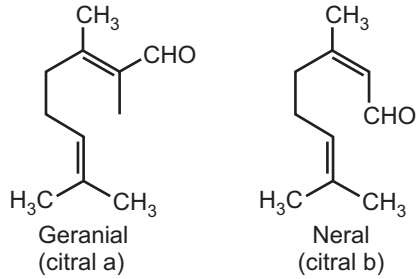


Fig. 11.3: Chemical structure of geranial and neral

Identification Test:

1. Citral is very sensitive to oxidizing agents (even exposure to air) and yields linalool which have intense yellow colour.
2. Geranial with Tollen's reagent (ammoniacal silver nitrate) gives geranic acid ($C_{19}H_{15}COOH$).
3. Geranial upon hydrogenation with sodium amalgum in acidic solution gives citronellal and citronellol.

4. Geranial gets converted into ρ-cymene on treatment with potassium bisulphate or dilute sulphuric acid.
5. When citral interacts with acetone, it forms pseudo-ionone or Ψ–ionone. The aliphatic ketone pseudo-ionone undergoes cyclisation with variety of reagents like sodium acetate, concentrated sulphuric acid, formic acid, dilute mineral acid etc and yields β-ionone and α-ionone.

11.1.3 Isolation of Artimisinin

Artemisnin is isolated from the leaves or aerial part of the plant *Artemisia annua* (Family-Compositae). It is a sesquiterpene lactone with prominent antimalarial activity with melting point 156-157°C. Artemisinin and their derivatives (Artemether and artesunate) can treat both chloroquine resistant and chloroquine sensitive *Plasmodium falciparum*. The plant is grown in US, Vietnam and China. It is not native of India but grow successfully in Kashmir. The yellow flower of the plant contains 2-4 times more artimisinin concentration than leaf. Cultivated varieties have 1.0 percent artemisinin content while wild varieties have 0.01 to 0.5 percent.

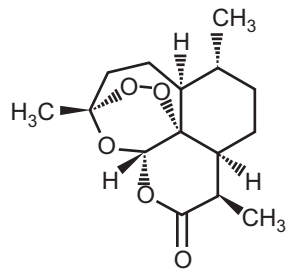


Fig. 11.4: Artemisinin

Aerial parts of the plant are extracted with petroleum ether (Boiling point 40-60°C) and concentrate the petroleum ether under vacuum. The concentrated extract is redissolved in chloroform and acetonitrile is added to separate out inert plant component like waxes and sugars. Column chromatography of this concentrated extract was performed using silica gel as stationary phase. High artemisnin content fraction will crystallize rapidly. Then recrystallised this fraction using cyclohexane or 50 percent ethanol. Artemisinin is less water soluble, colourless or white, crystalline compound having molecular weight 282.3 with odourless crystalline powder.

Melting point: 156-157°C.

Thin layer chromatography of Artemisinin:

Dissolve the standard and sample both in chloroform and perform the TLC using mobile phase petroleum ether: Ethyl acetate (1:2). Dry the plate and sprayed with either ρ-di methyl amino benzaldehyde (densitometric scan at 600 nm) or 2 percent solution of vanilline-sulphuric acid (densitometric scan 560 nm). Compare the spot of standard and sample. Sesquiterpene can also be estimated by HPLC with UV detection (220 nm). Sesquiterpene can or cannot be converted into their derivative which can absorb UV radiation. In alkaline medium open the oxygen containing heterocycles then converted into lactol in acidic medium. It can be also estimated using GC-MS technique by estimating the degradation product (artemisinin is thermal degradable).

Identification Test:

1. 1 gm finely powdered drug is boiled with 10 ml alcohol and filtered. Then add sodium hydroxide to the filtrate and heat again. The red colour develops in liquid.

11.2 ISOLATION AND PHYTOCHEMICAL ANALYSIS OF ALKALOIDS

Alkaloids are an organic compound of natural origin which contains more or less basic nitrogen atom with limited distribution and has marked pharmacological properties at low doses. Their nitrogen atom is a part of heterocyclic system and causes a significant pharmacological activity. The term alkaloid was introduced by W. Meissener at the beginning of the 19th century to designate natural substances reacting like base, in other words like alkalis (from the Arabic alkaly means soda and from the greek word eiods means appearance). For a long time alkaloids used to be considered products of metabolism of plant only. In fact alkaloids also occur in animals (Arthopods who secrete them in very small quantities in their exocrine glands). In the plant, alkaloid occurs as soluble salt (citrate, Malate, tartarate, melonates, benzoates, isobutyrates) or in combination with tannins.

The various functions of alkaloids are- Alkaloids are poisonous in nature but when used in small quantities exert useful physiological effects on animals and humans beings. They are reserve substances which can supply nitrogen. They might have defensive mechanism for plant growth in dry region to produce from grazing animals, herbivores and insect. They may be end product of detoxification mechanism in plant and by this way check formation of substance which may be prove to harmful to the plant. The possible role is growth regulatory factor in the plant. They are present normally in conjugation with plant acid like mercuric acid, cinchotannic acid etc. Therefore alkaloids could be acting as carrier within plant for transportation of such acids.

11.2.1 Isolation of Atropine

It is a tropane alkaloid obtained from plants like *Atropa belladonna*, *Datura strawmonium* and *Hyoscyamus niger* belongs to the family Solanaceae. The other important alkaloids are Hyoscine, hyoscyamine, belladonine, apoatropine and norhyoscyamine. Atropine is optically inactive laevorotatory isomer of hyoscyamine.

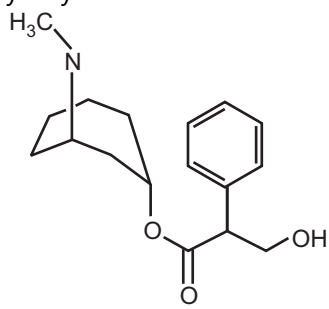


Fig. 11.5: Chemical structure of Atropine

Hyoscyamus muticus contains high alkaloidal content than other sources like *Datura strawmonium*. The powdered drug is moist with aqueous solution of sodium carbonate and extracted with ether or benzene. The free alkaloidal bases are extracted from the dilute

solvent and acidified with acetic acid. The acidic solution is shaken with ether solvent which separates colouring matter. The alkaloids get precipitated with sodium carbonate which is filtered, washed and dried. The dried residue is dissolved in ether/acetone and dehydrated with anhydrous sodium sulphate. This all treatment should be done before filtration. The filtrate is concentrated and cooled. This process will yield crystals of hyoscyamine and atropine. The crystalline mass is separated and dissolved in alcohol. Then sodium hydroxide solution is added and mixture is kept for some time. The hyoscyamine will completely be racemized into atropine. The purification of crude atropine should be done by crystallization with acetone.

Atropine sulphate is the most important salt of atropine which occurs in colourless crystalline powdered form. It has solubility in water and alcohol and insolubility in ether and chloroform.

Melting point: 115 to 116°C

Thin layer chromatography of Atropine: Atropine solution (1%) is dissolved in 2N acetic acid and it is spotted over silica gel-G plate and eluted into solvent system strong ammonia solution: methanol (1.5:100). The TLC plates are spreaded with acidified iodoplatinate solution. It gives Rf value 0.18. Atropine sulphate gives Rf value 0.70 in solvent system acetone: sodium chloride with Dragendorff reagent as spraying agent.

Identification Test:

Vitali-Morin reaction: Dilute solution of atropine is treated with concentrated nitric acid. The mixture is evaporated to dryness which produces pale yellow residue. A violet colour appears when a drop of potassium hydroxide solution is added in the residue.

11.2.2 Isolation of Quinine

Quinine is an alkaloid obtained from the dried stem bark and roots of *Cinchona calisaya* Wedd, *Cinchona ledgeriana* Moens, *Cinchona officinalis* Linn and *Cinchona succirubra* Pavon or hybrids of these species, belongs to the family Rubiaceae. Quinine is optically laevorotatory compound whereas quinidine is dextrorotatory isomer.

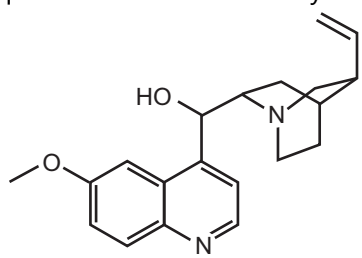


Fig. 11.6: Quinine

The powdered bark of Cinchona is mixed with calcium hydroxide or calcium oxide and enough quantity of sodium hydroxide (5%) solution. Then the pasty material is allowed to stand for few hours. The moistened material is packed in Soxhlet apparatus and extracted with benzene. 5 percent sulphuric acid is added in benzene extract and mixed well. The two layers are formed which are separated cautiously. The benzene layer is discarded and sodium

hydroxide is added in aqueous layer to maintain the pH 6.5. Then it is allowed to cool and quinine sulphate precipitate is formed. The precipitate is filtered, separated and recrystallized from hot water. This process will free the salts from cinchonine and cinchonidine. The activated charcoal treatment will remove the colouring matter. The obtained quinine sulphate is dissolved in dilute sulphuric acid and made alkaline with ammonia. This process will precipitate the quinine and formed crystals are washed and dried at 45-55°C.

Melting point: 177°C.

Thin layer chromatography of Quinine: The methanolic solution of alkaloid is spotted in Silica gel-G plates and eluted in solvent systems chloroform: diethylamine (9:1) and chloroform: acetone: diethylamine (5:4:1). The plates are dried and sprayed with Dragendorff reagent. The Rf value of quinine is 0.17 in both solvent systems.

Identification Test:

Thalleioquin test: Dilute sulphuric acid (one drop) and water (1 ml) is added in the sample solution. Then add bromine water drop by drop till the solution acquires permanent yellow colour and add 1 ml dilute ammonia solution till emerald green colour develops.

11.2.3 Isolation of Reserpine

Reserpine, an indole alkaloid, is obtained from roots of *Rauwolfia serpentina*, *Rauwolfia micrantha, R. vomiforia* and *R. tetraphylla* belongs to the family Apocynaceae. Rauwolfia also contains ajmaline, ajmalicine, ajmalinine, rescinnamine, reserpinine, serpentine and yohimbine.

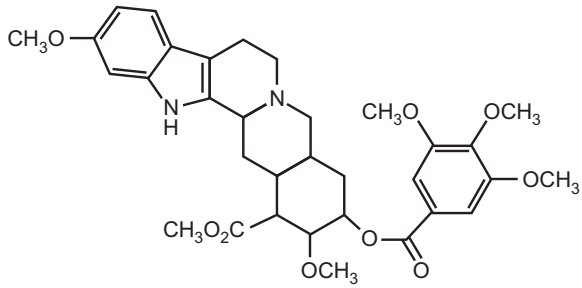


Fig. 11.7: Reserpine

Powdered root of drug is extracted with alcohol (90%). The obtained extract is concentrated and dried under reduced pressure (below 60°C). This process will yield dried extract of rauwolfia which contain total alkaloids (about 4%). This extract is again extracted with ether-chloroform-alcohol (90%) in a ratio of 20:8:2.5. Then add dilute ammonia in little quantity to the extract with continuous shaking. The alkaloids will convert into water insoluble base. Then add water and keep the drug to settle after shaking and filter it. The obtained residue is extracted with 4 volumes of 0.5N sulphuric acid in a separating funnel. The obtained total acidic extract is combined and filtered. To the filtrate dilute ammonia is added to make it alkaline. This will liberate the alkaloids. Finally it is extracted with chloroform and obtained extract is filtered. The solvent chloroform is separated by distillation and total extract is dried under vacuum. The alkaloids are isolated by column chromatography or fractionation method.

Melting point: 270°C.

Thin layer chromatography of Reserpine: 1 mg alkaloidal extract or purified Reserpine is dissolved in methanol. The silica gel-G TLC plates are spotted and eluted in solvent system chloroform-acetone-diethylamine (5:4:3). In case of alumina-G plates, elute the plates in solvent system cyclohexane-chloroform (3:7). Then eluted plates are dried and sprayed with Dragondorff's reagent. The alkaloidal component gives orange spot in both cases. The Rf value of Reserpine is 0.72 (in silica gel-G stationary phase) and 0.35 (in alumina-G stationary phase).

Identification Test:

1. A red coloration along the medullary rays is observed when the freshly fractured surface is treated with concentrated nitric acid.
2. Reserpine shows violet red colour when treated with solution of vanillin in acetic acid.

11.2.4 Isolation of Caffeine

Caffeine can be obtained from different sources like Tea (*Camellia sinensis*, Family- Theaceae), coffee (*Coffea arabica*, Family- Rubiacea), cacao (*Theobroma cacao* Family- Malvaceae), cola (*Cola acuminata* and *Cola nitida* Family- Malvaceae) and others. Caffeine can be extracted from tea sweepings, coffee roaster, tea dust or tea. Caffeine is white in colour or white powder. It is anhydrous or on hydration only one one molecule of water will be attached. It is bitter, needle shape, sublimined crystals. In the presence of salicylate, citric acid, benzoates its solubility in water increases very much.

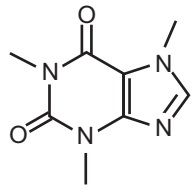


Fig. 11.8: Caffeine

Around 50 gram of tea powder are taken and extracted with alcohol for around 6 hours in Soxhlet extractor. The extract is transferred into porcelain dish which already contain magnesium oxide (30 gram in 200 ml water). Heat it on steam bath with continuous stirring and evaporate it. Boil the obtain residue three times with 100 ml water. Filter it in hot condition through Buchner funnel. Add the 10 percent sulphuric acid (30 ml) in combined filtrate. Concentrate the above filtrate to one third of the volume. Filter the solution when hot and extract it with 5 times with chloroform (25 ml). Add sodium hydroxide (1 percent, few ml) in chloroform extract so that the decolourisation takes place. Equal quantity of water is added in this and separate the chloroform layer. Evaporate the chloroform extract to obtain crude caffeine which can be recrystallized with hot water. Caffeine shows the positive murexide test in which caffeine is treated with few drop of nitric acid in porcelain dish and evaporates it to dryness. Add ammonium hydroxide (few drops) in this residue then purple colour obtained.

For the isolation of caffeine from coffee beans the leaching process with water is followed. When coarse coffee powder is extracted with water at 75°C, it will yield 90 percent of caffeine. The extraction procedure will take about 30 minutes with water/coffee ratio of 9:1.

Caffeine can be also extracted by super critical fluid extraction technique by using liquefied carbon dioxide. Liquefied carbon dioxide absorbs the caffeine through moist coffee. Then it is passed through another pressurized vessel containing absorbing media like resin or activated carbon which retain the caffeine. Separate this caffeine by extracting with chloroform.

Melting point: 235-237°C.

Thin layer chromatography of Caffeine: Dissolve caffeine (1 mg) in chloroform (1 ml) or methanol. Then spot the sample on TLC plates and eluted in solvent system ethyl acetate: methanol: acetic acid (8:1:1). Then visualize the spots on dried TLC plate by exposure to iodine vapour. The Rf value was 0.41.

Identification Test:

Murexide test: Caffeine or purine derivatives do not precipitate like other alkaloids. It is detected by mixing with a very small amount of potassium chlorate and a drop of HCl, evaporating to dryness and exposing the residue to ammonia vapour. A purple colour is produced with caffeine and other purine derivatives.

11.3 ISOLATION AND PHYTOCHEMICAL ANALYSIS OF GLYCOSIDES

Glycosides are the naturally occurring organic compounds found in plants and some animals, which upon hydrolysis (either acid or enzymatic) gives one or more sugar (glycone) moiety and non sugar (aglycone) moiety. The non sugar or aglycon moiety is called genin. The pharmacological activity of any glycoside is mainly due to the presence of genin part whereas glycone part facilitates the transportation of genin part to the site of action. Most frequently occurring sugar is β-D-glucose, although rhamnose, digitoxose, cymarose and other sugars are components of glycosides. When sugar part is glucose then it is known as glucoside. Other sugars may be developed during hydrolysis then term glycoside is applied. Glycosides have role in plant life include regulatory, protective and sanitary functions. They have many functions like cardioactive (digitalis, stropanthus, squill, convallaria, apocynum etc), laxative (senna, aloe, rhubarb, cascara, frangula etc.), analgesic (methyl salicylate from wintergreen), local irritant (allyl isothiocynate from black mustard). They are colorless, amorphous, solid, non-volatile compounds. They give positive test with Molisch and Fehling's solution test. They have solubility in water but insoluble in organic solvents. Most of them possess bitter taste but some exceptions are populin, glycyrrhizin, stevioside. They are odourless compounds except saponin (glycyrrhizin). Glycosides get hydrolyzed by mineral acids and temperature or by enzymes.

11.3.1 Isolation of Glycyrhetinic Acid

Glycyrrhetinic acid is a pentacyclic triterpenoidal glycoside (mainly aglycone) which is obtained from roots and stolons of *Glycyrrhiza glabra* belongs to the family Leguminosae. It is used as an antiulcer compound.

Coarsely powdered drug of Glycyrrhiza is extracted with chloroform. The chloroform extract is filtered and filtrate is discarded. Then obtained marc is extracted with 0.5M sulphuric acid for few hours. Filter it and filtrate is extracted with chloroform (3 parts). Separate the chloroform layer and mix them. Then distill off the chloroform extract which will yield the dry residue of glycyrrhetinic acid.

In another method of extraction powdered liquorice is extracted with boiling water. The obtained aqueous extract is concentrated and dried. Then this extract is dissolved in water and acidified with hydrochloric acid (pH 3 to 3.4) to precipitate glycyrhetinic acid. Then precipitate is filtered and washed with water upto neutral pH and dried. This will yield glycyrhhetinic acid.

Melting point: 300°C.

Thin layer chromatography of Glycyrhetinic acid: 1 mg of glycyrhhetinic acid is dissolved in 1 ml methanol chloroform (1:1) solvent. Then apply the spots on silica gel G plates and elute the plate in solvent system Toluene-ethyl acetate-glacial acetic acid (12:8:0.5). Then dried plates are sprayed with 1% vanillin-sulphuric acid or anisaldehyde-sulphuric acid and heat the plates for 10 minutes at 110°C. The purplish spot of isolated compound i.e. glycyrhetinic acid have Rf value 0.41.

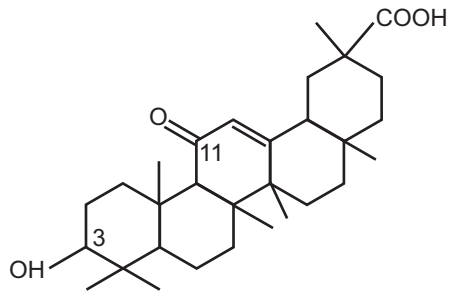


Fig. 11.9: Glycyrrhetinic acid

Identification Test:

1. Liebermann test- 2 ml test extract is mixed with 2 ml acetic anhydride. Boil the solution and add 0.5 ml of sulphuric acid which will develop blue colour.
2. Liebermann Burchard test- 2 ml test extract is mixed with 1 ml chloroform and 1 ml acetic anhydride. Then add one drop of concentrated sulphuric acid. Blue green to red orange colour develops.

11.3.2 Isolation of Rutin

Rutin is a flavonoidal compound which is obtained from citrus fruits. It is also extracted from the leaves of buck wheat *Fagopyrum esculentum* belongs to the family Polygonaceae. It is used as capillary fragility factor.

Coarsely powdered drug of buckwheat is first defatted with n-hexane. Then extract the marc with alcohol (78%) for 60 minutes. Filter the solution and evaporate the solvent. The obtained dried residue is dissolved in sufficient quantity of acetone (30%). Filter the solution and evaporate the filtrate to one fourth of its original weight. Then add sufficient quantity of borax solution (5%) until pH 7.5 with continuous shaking. Then add enough quantity of solid sodium chloride with stirring. Filter the solution and acidify it with phosphoric acid to lower the pH 5.5. Stir the solution for 15 minutes and filter it. Wash the residue with sodium chloride (20%) solution. Again filter the solution and evaporate the filtrate to 500°C to one fourth of its original volume. Then add hydrochloric acid in hot condition to lower the pH to 1.5. Cool the solution and kept in refrigerator for overnight. Rutin crystals will separate out, collected and dried.

Melting point: 242°C.

Thin layer chromatography of Rutin: 1% methanolic solution of rutin is spotted on silica gel-G plates. The plates were eluted in solvent system of n-butanol- glacial acetic acid and- water (3.6:0.5:0.5). The plates were dried and isolated compound detected under UV cabinet at 366nm.

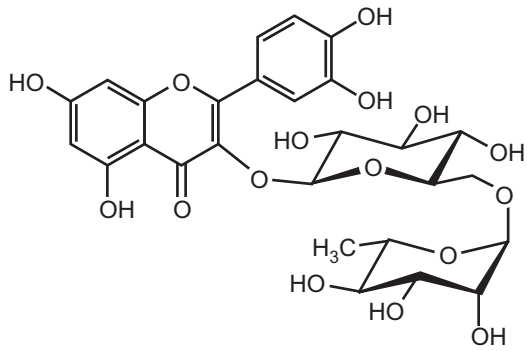


Fig. 11.10: Rutin

Identification Test:

1. **Shinoda test:** Add few drops of concentrated hydrochloric acid or sulphuric acid and magnesium powder in 2 ml of sample solution. An orange, pink, red or purple colour develops.
2. Add sulphuric acid in sample solution shows deep yellow colour or orange colour.
3. A yellow precipitate is formed when test solution is mixed with lead acetate.

11.4 ISOLATION AND PHYTOCHEMICAL ANALYSIS OF RESIN

Resins are amorphous mixtures of essential oils and oxygenated products of terpenes, transparent or translucent solids, semi solid or liquid substances. They have complex chemical nature and contains large no of carbon atoms. Resins are insoluble in water and heavier than water but they are soluble in non polar solvents like benzene or ether, volatile oils, fixed oils and alcohol. Resins are abundantly distributed in plants and rarely in insects

(**Example:** Shellac). Resins are present in ducts or cavities which are called schizolysigenous ducts. There are 2 types of resin which exist in nature. Normal or Physiological resins are preformed in the plants and their yield increases upon injury or incision. Example- Resin of Pinus and Abnormal or Pathological resin formed only upon injury or incision made to the plant. Example- Benzoin, Tolu Balsam.

Resins are hard, non conductor of electricity and combustible in nature. They soften and finally melt upon heating. They are usually formed in schizogenous glands, lysigenous glands or ducts as end product of metabolism. The pharmaceutical applications of resins are local irritant, local cathartic (e.g. Jalap, Ipomoea), as anticancer (podophyllum), in bronchial asthma (Cannabis), used externally as mild antiseptic in the form of tinctures (Benzoin), ointment and plasters (Turpentine and Colophony) and used in the preparation of emulsion and sustained release formulations. Majority of resins undergo slow atmospheric oxidation which darkens its colour and impaired solubility.

11.4.1 Isolation of Podophyllotoxin

Podophyllotoxin consists of dried roots and rhizomes of *Podophyllum emodi* or *Podophyllum hexandrum* belongs to the family Berberidaceae or Podophyllaceae. The content of resin in the podophyllum depends upon the season of collection, area, part of the plant (i.e root or rhizome.). The roots contain more resin than rhizomes. Podophyllin (resin) extracted from the podophyllum not only contain the podophyllotoxin and demethyl podophyllotoxin it also contain the podophyllotoxin-1-o-βD gluco pyranoside, quercitine, kaempferol and tannins.

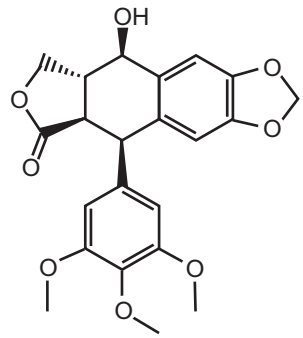


FIg. 11.11: Podophyllotoxin

Podophyllotoxin

Root and rhizome of podophyllum emodi is the commercial source of podophyllotoxin. Powder the root and rhizome and extract with methanol or ethanol and concentrate the extract under vaccum. Treat the semisolid mass with acidulated water (10 ml HCl in 1000 ml water) and cool to 10°C slowly. Decanted the settled precipitate and wash it with cold water. On drying this precipitate we get amorphous dark brown powder known as podophyllin.

Extract podophyllin with chloroform and purify by recrystallisation with benzene or benzene and ethyl alcohol mixture and then wash with hexane/ petroleum ether which gives the commercial podophyllotoxin.

Thin layer chromatography of Podophyllotoxin: Dissolve podophyllotoxin in methanol and spot the solution on Silica gel G TLC plates. Elute the TLC plates in solvent system containing toluene: ethyl acetate (5:7). Sulphuric acid should be used as detecting reagent. Compare with standard by scanning 280nm densitometric scan. Violet colour spot will be seen with approx Rf value 0.39.

Identification Test:

1. Podophyllotoxin shows violet blue colour when treated with 50 percent sulphuric acid solution.

11.4.2 Isolation of Curcumin

Curcumin or curcuminoids compounds are obtained from dried rhizomes of *Curcuma longa* belonging to the family Zingiberaceae. These compounds are diarylheptanoid compounds. Curcuminoids constitutes about 50 to 60 percent of the mixture of three main curcuminoids named as Curcumin, desmethoxycurcumin and bisdesmethoxycurcumin. Commercial Curcumin contains 97 percent pure product when it is isolated from curcuma rhizomes.

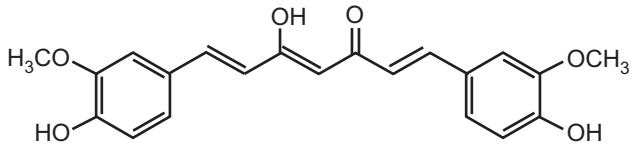
Powdered drug of turmeric is extracted with alcohol in Soxhlet apparatus. The obtained alcoholic extract is concentrated under reduced pressure and dried.

The other method also used for extraction of Curcumin form curcuma. In this method the powdered drug is extracted with hexane followed by acetone. The obtained acetone extract is concentrated and dried. This process will yield Curcumin. The most efficient method for Curcumin isolation is to extract the drug with hot ethanol. Then concentrate the filtrate and treat the concentrate with superior grade kerosene, a solid mass will separate out. The separated mass will treat with petroleum ether and recrystallised from alcohol which will yield orange red needle crystals.

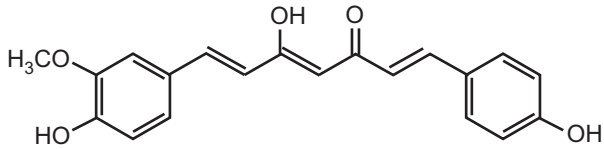
Melting point: Curcumin 183°C, Desmethoxycurcumin 168°C and Bisdesmethoxy-curcumin 224°C

Thin layer chromatography of Curcumin.

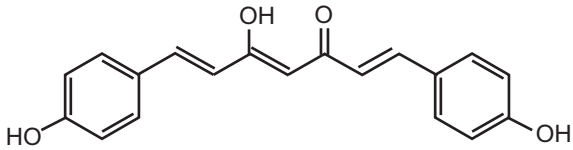
1 mg of Curcumin is dissolved in 1 ml methanol and applied spots of silica gel G plates. Then elute the silica gel G plates in the solvent system chloroform-ethanol-glacial acetic acid in a ratio of 94:5:1. The eluted plate should be dried and visualized under 366nm light. Curcumin shows bright yellow fluorescent spot at Rf value 0.79. The Rf value for desmethoxycurcumin is 0.60 and bisdesmethoxycurcumin is 0.43.



1 Curcumin



2 Demethoxycurcumin



3 Bis-demethoxycurcumin

Fig. 11.12: Chemical structure of Curcumin, Demethoxycurcumin, Bis-demethoxycurcumin

Identification Test:

1. Powdered drug of turmeric is treated with acetic anhydride and concentrated sulphuric acid (few drops) develops violet colour of solution. When this solution is observed under UV light shows intense red fluorescence which indicates the presence of Curcumin.

QUESTIONS

1. What do you understand by terpenoids? Write in brief.
2. Write method of isolation and analysis of Menthol.
3. What are the identification tests for Menthol?
4. Write method of isolation and analysis of Citral.
5. What are the identification tests for Citral? Write in short.
6. Write method of isolation and analysis of Artemisin.
7. Write identification test for Artemisin.
8. What do you understand by alkaloids? Explain in short.
9. Write method of isolation and analysis of Atropine.
10. Write identification tests for Atropine.
11. Write method of isolation and analysis of Quinine.
12. What are the identification tests for Quinine?
13. Write method of isolation and analysis of Reserpine.
14. Write identification tests for Reserpine.
15. Write method of isolation and analysis of Caffeine.
16. Write identification test for Caffeine.
17. What are Thalleioquin and Murexide test? Describe in short.
18. What do you understand by glycosides? Explain.

19. Write method of isolation and analysis of Glycyrhhetinic acid.
20. Write identification tests for Glycyrhhetinic acid.
21. What are Liebermann and Liebermann Burchard test?
22. Write method of isolation and analysis of Rutin.
23. What are the identification tests for Rutin.
24. What is shinoda test? Describe in short.
25. What do you understand by resins? Explain.
26. Write method of isolation and analysis of Podophyllotoxin.
27. Write identification tests for Podophyllotoxin.
28. Write method of isolation and analysis of Curcumin.
29. Write identification test for Curcumin.

■■■

Unit IV

Chapter ... 12

Industrial Production and Utilization of Phytoconstituents

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *Industrial production, estimation and utilization of the following phytoconstituents:*
  - *Forskolin*
  - *Taxol*
  - *Podophyllotoxin*
  - *Atropine*
  - *Digoxin*
  - *Diosgenin*
  - *Artemisinin*
  - *Sennoside*
  - *Vincristine and Vinblastine*
  - *Caffeine*

12.1 INTRODUCTION

Primary metabolites or central metabolites are that compounds which directly take part in normal growth, development and reproduction. They perform a physiological function in the plant or organism. This metabolite occurs in many organisms or cells and it is also referred as a central metabolite. Some common examples of these compounds are ethanol, acid and amino acids.

Secondary metabolites do not involve in growth pattern and development of any plant. They have an important ecological function, a relational function. Secondary metabolites are present in a taxonomically restricted set of plants. Few examples of secondary metabolites are alkaloids, antibiotics, nucleosides, phenazines, quinolines, terpenoids, peptides and growth hormones etc. Secondary metabolites are mainly produced by modified primary metabolite synthesis.

Phytoconstituents, also known as chemical constituents, are those compounds which are obtained after extraction (by any suitable method) and isolation of phytopharmaceuticals or phytoconstituents of the drugs. These are biosynthesized compounds in plants and have

potent therapeutic activity in humans and animals for example- Forskolin, Taxol, Podophyllotoxin, Atropine, Digoxin, Diosgenin, Artemisinin, Sennoside, Caffeine, Vincristine, Vinblastine etc.

12.2 FORSKOLIN

Forskolin is obtained from the plant coleus. Coleus is grown in warm or subtropical temperate weather in Burma, India and Thailand. The plant is peperennial in nature and the root is fleshy fibrous. In various state of India it is use as a condiment. The botanical source of the crude drug is root of *Coleus forskohlii* synonym *Plectranthus barbatus* belonging to Family Labiatae. The marker constituent of the drug is forskolin (labdone diterpenoids). Other important phytoconstituents are deoxy forskolin, 1,9dideoxy forskolin, volatile oil (β elemene, humulene, β bisabolene). The drug is used as a hypotensive, cardiotonic and spasmolytic.

Fig. 12.1: Structure of Forskolin, *Coleus forskohlii* plant and its roots (Image)

Extraction:

Method 1:

- Collect the tubers → washed and dried → pulverized into granules → extract the crude drug with methanol → concentrate the extract → add chloroform in concentrate and equal volume of water → shake well → allow the separate chloroform layer and concentrate the chloroform layer → Precipitate the forskolin by adding n-hexane (ice cold) into concentrate chloroform a reddish brown powder of forskolin will get.

Method 2 :

- Take root or tubers of the plant → dried to reduce the moisture 6% → charged the drug into extractor solvent → ethyl acetate or benzene poured into the extractor.
- Extract 2-3 hrs under 80^{o}C temperature with 8-10 lbs/sq inch pressure and agitate for maximum extraction (60-70% in first extraction).
- Repeat 2-3 more washing with same solvent.
- Distill off the solvent. A uniform consistency paste will be obtained.
- In this paste add pet ether 60-80^{o}C (temperature 6-8^{o}C with 2-3 times quantity of petroleum ether compare to paste) and agitate 200-250 rpm for half an hours.
- Filter the formed precipitate and wash again with chilled petroleum ether and dry the precipitate.
- Concentrate the residual pet ether and chill at temperature 2-4^{o}C for 10 hrs collect the precipitate and dry it. Combine both the precipitate which is forskolin.

Estimation:

The drug is estimated with HPLC at constant temperature 30^{o}C using photodiode detector 210 nm the retention time may be around 6.9 minutes. The column was RP18 (150 x 4.6mm particle size 5µm) and methanol use as mobile phase.

TLC-Mobile Phase - benzene: methanol (9:1)

Stationary Phase: Silica gel 60F 254

Detection: UV Cabinet (long range 365 nm)

Utilization:

Forskolin (a diterpenoid compound) shows the hypotensive effect, positive ionotropic action and spasmolytic action. Forskolin is also reported for antiplatelet aggregation, antiglaucoma, anti-inflammatory, bronchodilator, increase pepsinogen and acid secretion. It is also recommended in the congestive cardio myopathy treatment and bronchial asthama. Forskolin derivatives are more potent antiglaucoma agent.

12.3 TAXOL

Taxol is isolated from the plant *Taxus brevifolia* or *Taxus cuspidate* (family Taxaceae). In this genus there are eight different species present in northern in northern hemisphere. Taxol isolated from the trunk of *Taxus brevifolia* but the yield was very low (0.01%). That means a plant with 100 years old produce 3 kg bark (or 300 mg taxol). Generally 1 Kg taxol will be isolated from seven tons dried bark. The content of taxol in the leaves is comparatively more (as much as 0.1%). Drying process of the plant is very much related to the content of taxol. The drying should be done at 40-50°C.

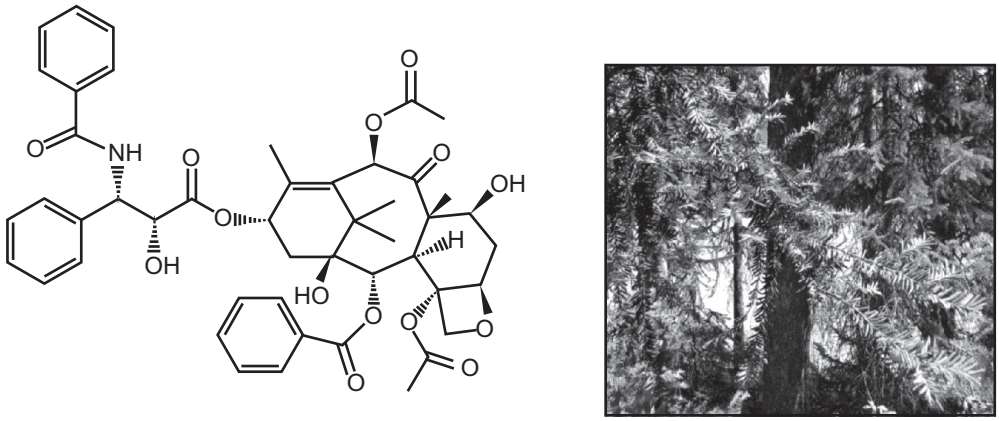


Fig. 12.2: Structure of Taxol, *Taxus brevifolia* plant (Image)

Production:

Put the dried powder of the leaves of plant in the rotary extractor. Extract the leaves with ethanol with gentle agitation and rotation at 40-45°C. Concentrate the extract and repeat the same process two more times so that the active constituents are completely extracted from the leaves. Separate all the extract and leave the residue. This extract is distributed with

four time methanol and then treats with hexane to remove the pigment and fatty materials. Extract this solvent with chloroform through liquid- liquid extraction. Concentrate the chloroform extract and perform the column chromatography of this residue using chloroform and methanol as mobile phase and column of silica gel G to segregate different taxanes. Last purification is done by HPLC using Hexane: ethyl acetate (1:1) as solvent system.

Estimation:

Weight around 0.125 gm of drug in conical flak and add 25 ml water add 50 ml of 0.83 M potassium permagnate and add 100 ml of sulphuric acid. Boil the solution and then cool it and transfer in volumetric flask. The solution is distilled with water to 250 ml. From this solution separate out the 50 mi solution and add 1 M of ferrous ammonium sulphate. Perform blank and make necessary correction.

Each ml of 0.1M $Fe(NH_4)_2SO_4$ ≡ 0.000675Gm of drugs.

Thermospray ionization HPLC-MS:

The analysis was performed using triple stage quadrupole mass spectrometer with TSP_2 interface

Source blank temperature	:	230°C
Vaporizer temperature	:	70°C
Repeller Voltage	:	30 V
Discharge on mode	:	1800 V

Instrument scanned from m/z 300 to m/z1000. The mobile phase use in HPLC is H_2O:MeCN:MeOH (85:5:10) using flow rate 1ml/min. Dissolve the sample in methanol at concentration 1mg/ml.

Utilization:

Taxol inhibits the polymerization of tubulin similar to podophylotoxin and vincristine. It was used to treat the ovarian tumors and other cancer. Taxol can be dispensing at the rate of 135-175mg/m^2 depending on the indication. Infuse the drug after every three week with proper premedication. The toxicity produce by the treatment of taxol is neutropenia, peripheral neuropathy, cardiovascular problems, vomiting, alopecia, nausea, hypersensitivity etc.

12.4 PODOPHYLLOTOXIN

The biological source of podophyllotoxin consists of dried roots and rhizome of *Podophyllum emodi* or *Podophyllum hexandrum* (family berberidaceae). Roots and rhizome of *Podophyllum peltatum* is the source of American podophyllum. The content of podophyllin (resin) in Indian podophyllum is about 7-15%.

The content of resin in the podophyllum depends upon the season of collection, area, part of the plant (i.e root or rhizome.). Currently the content of resin in the root is more than rhizome. Podophyllin (resin) extracted from the podophyllum not only contain the podophyllotoxin and demethyl podophyllotoxin it also contain the podophyllotoxin-1-o-βD gluco pyranoside, quercitine, kaempferol and tannins. Podophyllin is light brown to greenish yellow amorphous powder with bitter taste to characteristic odour. It is irritating to mucous membrane and eyes having melting point 114-118°C.

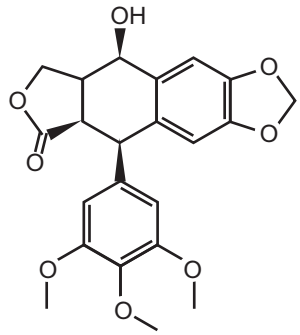


Fig. 12.3: Structure of Podophyllotoxin, *Podophyllum emodi* and *P. hexandrum* (Image)

Production:

- Root and rhizome of podophyllum emodi is the commercial source of podophyllotoxin. Powder the root and rhizome and extract with methanol or ethanol and concentrate the extract under vaccum.
- Treat the semisolid mass with acidulated water (10 ml HCl in 1000 ml water) at cool to 10°C slowly. Decanted the settled precipitate and wash it with cold water. On drying this precipitate we get amorphous dark brown powder known as podophyllin.
- Extract podophyllin with chloroform and purify by recrystallisation with benzene or benzene and ethyl alcohol mixture and then wash with hexane/ petroleum ether which gives the commercial podophyllotoxin.

Utilization:

The famous anticancer drug teniposide and etoposide are semi synthesized by the podophyllotoxin (natural teralin lignan). The production of podophyllotoxin in total synthetic way is still not economical and production of podophyllotoxin is based on either use of tissue culture technique or the systematic cultivation. Alone with anticancer activity it also show purgative, bitter tonic and emetic property. In veterinary medicine it is also used as cathartic for dog and cat but nowadays its major use is in anticancerous drug synthesis.

Estimation:

Weigh the sample and shake it with chloroform for half an hour. Transfer the filtrate in Erlenmeyer flask which contains 50 ml of petroleum ether (40-60°). Collect the precipitate. Rewash the precipitate and conical flask with the petroleum ether. Dry the precipitate at 70°C for one hour and weight the podophyllotoxin.

TLC study

Solvent - Toluene: Ethyl acetate (5:7)

Stationary phase: Silica gel G

Detecting reagent: Sulphuric acid

Sample: Dissolve in methanol

Compare with standard by scanning 280nm densitometric scan. Violet colour spot will be seen with approx Rf value 0.39.

12.5 ATROPINE

Atropine is an alkaloid isolated from the *Hyoscyamus niger* (leaf and flowering tops) or dried leaves of *Hyoscyamus muticus* or *Atropa belladonna* (aerial parts) or *Atropa acuminata* family Solanaceae. Atropine is a poisonous product and its production on synthetic way is costly compare to extraction from natural way. Atropine is needle like crystal, white colour or colourless, optically inactive usually present with laevorotary hyoscyamine. The melting point of atropine is 115-116°C.

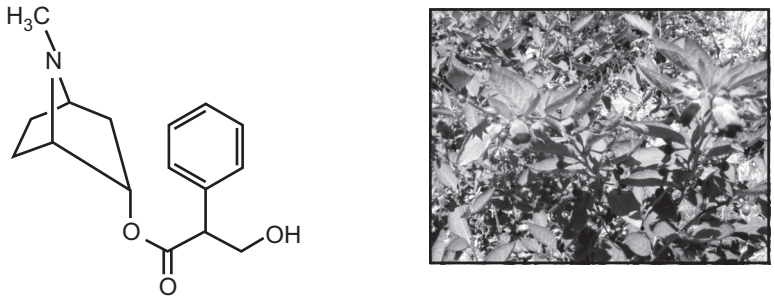


Fig. 12.4: Structure of Atropine, *Atropa belladonna* plant (Image)

Production:

- The powdered drug is moistened with sodium carbonate aqueous solution and then it is extracted with benzene or ether. The residue is then extracted with acidified water. Remove the coloring matter by treating the aqueous extract with solvent ether.
- From this solution add sodium carbonate to precipitate the alkaloid. Wash the precipitate and dry it. Dissolve the dried precipitate in ether or acetone and dehydrate it using anhydrous sodium sulphate.
- Concentrate the filtrate and make it cool. After cooling the crystal of hyoscyamine and atropine are separate out. Separate out the crystal and dissolve in alcohol. To this solution add the sodium hydroxide.
- Then allow standing this mixture. The crystal of hyoscyamine will be completely racemised in the atropine. For the purification the crude atropine, dissolve it into acetone and then recrystallised it.

Estimation:

Add alcohol in atropine sulphate and evaporate the alcohol. Dissolve the residue again in alcohol and add calculated quantity of 0.1N HCl in this alcohol and titrate excess of this acid with 0.1N NaOH using methyl red as indicator. Each ml of 0.1 N HCl is equivalent to 0.3384 gm of atropine sulphate.

In another method atropine sulphate (around 0.1 gm) was weighed and dissolved in glacial acetic acid (50 ml). Titrate this solution with 0.1N per chloric acid and determine end point by potentiometrically. Make the necessary correction by performing the blank determination.

Factor 1 ml of 0.1N $HClO_4$ ≡ 0.0670 gm of atropine sulphate.

Utilisation:

Atropine sulphate acts as anticholinergic drug. It is used in surgery as antisialogogue to reduce nasal, salivary, bronchial and pharyngeal secretion. It is dispensed through intra muscularly injection before the anesthesia. Atropine is used as an antidote for the poisoning of organophosphate insecticide and physostigmine. It also shows mydriatic and anti spasmodic property. In ophthalmology it is also used to measure the refractive error.

12.6 DIGOXIN

Digoxin is the one of the important cardiotonic glycosides obtained from the leaf of *Digitalis lanata* (Family- Scrophulariaceae). Digoxin produces three molecule of digitoxose sugar and one molecule of digoxygenin during hydrolysis. Digoxin hydrolyzed at acidic pH but stable at pH 7. It starts its action within 30 minutes to 2 hours, half life is 30-40 hours and major eliminated by kidney. The melting point of the digoxin is 230-265°C.

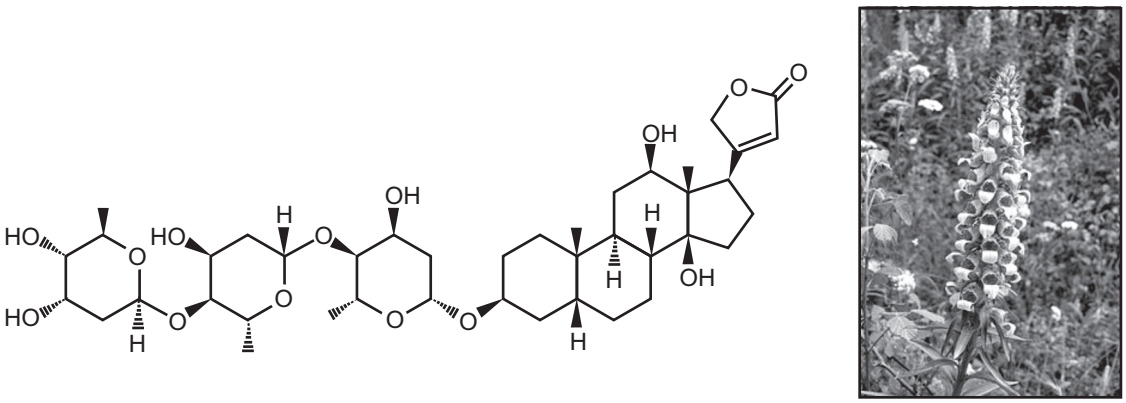


Fig. 12.5: Structure of Digoxin and *Digitalis lanata* plant

Production:

- The dried powdered leaf extracted with petroleum ether under reduced pressure. Discard the extract and digested the marc with water at 0-4°C so that the polysaccharide may remove. Again the filtrate is discarded and the marc is extracted with alcohol and water.
- Under reduced pressure alcoholic extract is concentrated at 50°C. This concentrate solution is treated with lead acetate to remove the impurities. In the water soluble glycoside portion (obtain after the treatment of lead acetate) maintain the pH around 6 and then wash with non polar solvent.
- Discard the organic layer and treat the aqueous layer with 0.5 percent sodium sulphate to remove the lead sulphate. The aqueous layer is then treated with chloroform and afterwards with ethanol.
- Chloroform portion contain less polar glycoside while ethanolic portion contain more polar glycoside. The ethanolic portion further treated and subjected to chromatography to separate the digoxin.

Estimation:

Dissolve digoxin (equivalent to 500 mg) in 5 ml solvent (mixture of chloroform: methanol 65:35) and add 20 ml of glacial acetic acid. Shake one hour continuously and filter the solution. Dilute 5 ml of this filtrate to 25 ml with glacial acetic acid which contain 0.005 percent W/V ferric chloride and 2 percent V/V sulphuric acid and allow to stand for 90 minutes. Measure the absorbance at 519 mμ and compare with this standard solution prepared in same way.

TLC study"

Stationary phase: Silica gel G.

Mobile phase - Cyclohexane: Acetone: Acetic acid (49:49:2).

Detecting reagent: 5 percent sulphuric acid.

View under UV far light digoxin appears as blue colour, compare with standard

Utilisation:

Digoxin is important glycoside from the plant of *Digitalis lanata*. It increases the myocardial contractility which empties the ventricles completely. The beginning dose of digoxin is 1 to 1.5 ml but due to its great accumulation tendency the maintenance dose maintains to 0.25 mg. Comparative to digitoxin, digoxin is short acting and rapidly eliminated. The use and precaution of the digoxin is same as digitalis.

12.7 DIOSGENIN

There are 15 specieces existed from which the diosgenin can be produce but the major varieties are *Dioscorea composita* and *Dioscorea floribunda*. In Indian region *Dioscorea deltoidea* is prominent (Family- Dioscoreaceae). Most varieties are rich in starch and widely used as edible products. Those varieties which have more than 2 percent of saponin concentration are industrially important. The solasodine are also extracted from the tubers of Dioscorea. Generally 3-5 year old plant produce 1-8 percent of total sapogenin.

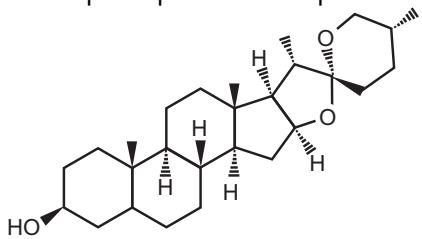


Fig. 12.6: Structure of Diosgenin

Fig. 12.7: Images of *Dioscorea floribunda* and *D. composita*

Production from Acid Hydrolysis Method:

- Dry the tubers of Dioscorea and reduce its size to 100-200 mesh. The powdered tuber is reflexed or autoclave with 2-4 N mineral acid for the duration of about 2-6 hours. Filter the acid and wash the residue with distill water till the solution become neutral.
- Dry the residue and re-extracted with hydrocarbon (Petroleum ether) solvent for about 6 hours.
- Concentrate the liquid and keep in refrigerator for 1 hour, the crystal of diosgenin will separate out, wash them with acetone.

Incubation and Acid Hydrolysis Method:

The fresh tuber is incubated for few days in water. The incubated drug material kept with acid for acid hydrolysis. Concentrate the hydrolysed liquid and further subjected to extraction with petroleum ether or hydrocarbon solvent to obtain the diosgenin.

Extraction Form Alcohol:

The tubers of dioscorea are cut into small pieces and sun dried. Powder the sun dried tubers and extracted with alcohol for 6-8 hours. Filter the extract and concentrate it to the syrupy mass. Then hydrolyze the concentrated syrupy liquid with acids like sulphuric acid and hydrochloric acid for 2-12 hours. Crude diosgenin will be precipitated. Filter the precipitate wash with water and further purify with alcohol.

Fermentation and Acid Hydrolysis Method:

The fresh tubers of the plant are collected and smash it with the help of hammer mill. This smashed product is allowed for fermentation for around two days. Reduce the moisture from the fermented product by sun drying up to 7-8 percent. Further the product is hydrolyzed with the help of mineral acid under reduced temperature. It is then subjected to extraction with hydrocarbon (9 heptanes) to collect the diosgenin. The melting point of diosgenin is 204-207°C.

Estimation:

By UV standard curve method.

Prepare the solution A (0.5 ml ρ-anisaldehyde in 99.5 ml ethyl acetate) and solution B (50 ml sulphuric acid with 50 ml ethyl acetate). The test samples is dissolved in 2 ml ethyl acetate and add 1 ml of reagent A and B. Stirred well and maintain the temperature 60° C for 10 minutes to develop the colour. Allow to cool at 25°C and measure the absorbance at 430 nm using ethyl acetate as blank. Similarly the calibration curve of standard diosgenin (2-70 µg) in ethyl acetate was made and determine the concentration of unknown sample.

TLC Method:

Stationary phase: Silica gel G.

Mobile phase - Toluene: Ethyl acetate (7:3).

Detecting reagent: Anisaldehyde sulphuric acid.

(Compare the standard and sample spot).

HPTLC Method:

Stationary phase: Silica gel G plate.

Mobile phase - n-Hexane: Ethyl acetate (4:2).

Detecting reagent: 3 gm of antimony trichloride in 100 ml concentrated HCl.

(Spot of green black colour is observed the standard and sample are stand by using densitometer).

Utilization:

Diosgenin is very important precursor for the synthesis of steroidal drug like sex hormone, corticosteroids and anti fertility compounds. The demand of corticosteroids is around 10000 kg but the production is around 4000 kg. The synthetic method for the production of diosgenin is very costly. Natural production of diosgenin is economically feasible. Along with the production of steroidal drugs diosgenin is also used for the treatment of natural hormone replacement therapy or rheumatic arthritis and digestive system improvement.

12.8 ARTEMISININ

Artemisnin is isolated from the leaves or aerial part of the plant *Artemisia annua* (Family-Compositae). It is a sesquiterpene lactone with prominent antimalarial activity with melting point 156-157°C. Artemisinin and their derivatives (Artemether and artesunate) can treat both chloroquine resistant and chloroquine sensitive *Plasmodium falciparum*. The plant is grown in US, Vietnam and China. It is not native of India but grow successfully in Kashmir. The yellow flower of the plant contains 2-4 times more artimisinin concentration than leaf. Cultivated varieties have 1.0 percent artemisinin content while wild varieties have 0.01 to 0.5 percent.

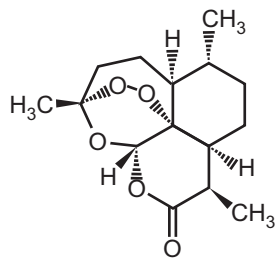


Fig. 12.8: Structure of Artemisinin and *Artemisia annua* plant

Production of Artemisinin:

- Aerial parts of the plant are extracted with petroleum ether (Boiling point 40-60°C) and concentrate the petroleum ether under vacuum.
- The concentrated extract is redissolve in chloroform and in the acetonitrile is added to separate out inert plant component like waxes and sugars.

- Column chromatography of this concentrated extract was performed using silica gel as stationary phase.
- High artemisnin content fraction will crystallize rapidly.
- Recrystallised this fraction using cyclohexane or 50 percent ethanol.
- Artemisnin is less water soluble, colourless or white, crystalline compound having molecular weight 282.3 with odourless crystalline powder.

Estimation:

It can be estimated by TLC densitometric scan. Dissolve the standard and sample both in chloroform and perform the TLC using mobile phase petroleum ether: Ethyl acetate (1:2). Dry the plate and sprayed with either ρ-di methyl amino benzaldehyde (densitometric scan at 600 nm) or 2 percent solution of vanilline-sulphuric acid (densitometric scan 560 nm). Compare the spot of standard and sample. Sesquiterpene can also be estimated by HPLC with UV detection (220 nm). Sesquiterpene can or cannot be converted into their derivative which can absorb UV radiation. In alkaline medium open the oxygen containing heterocycles then converted into lactol in acidic medium. It can be also estimated using GC-MS technique by estimating the degradation product (artemisnin is thermal degradable).

Utilization:

Artemisnin or their derivatives are very useful for their antimalarial activity. It can be dispense in oily suspension (IM) or in tablet or suppositories form. Orally administered dose is 50 mg/Kg for 3-5 days. The dose at IM or suppositories route is 2.8 gm/3 days but the drawback is that artemisnin has very low yield and remain expensive and less available globally.

12.9 SENNOSIDE

Senna, the popular drug uses for constipation was introduced by Arabian physician and they use their leaves and pods. The two varieties of Senna are the *Cassia acutifolia* Family Leguminosae (popularly known as Alexandrian or Khartoum Senna) and the other variety is *Cassia angustifolia* (Tinnevelly senna). The drug contains important phytoconstituents sennoside responsible for their purgative activity.

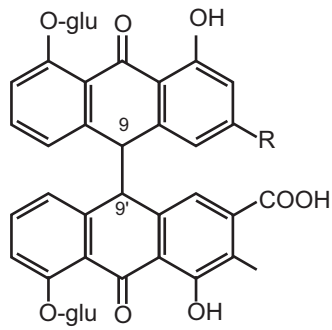


Fig. 12.9: Structure of Sennoside


Fig. 12.10: Images of *Cassia acutifolia* and *C. angustifolia* plant

Production of Sennoside:

Method A:

- The dried senna leaves extracted with 70 percent methanol.
- Shake the drug with methanol for around 4 hours at room temperature.
- Filter the extract and reduce the volume to 1/8 under vacuum.
- The extract is acidified to pH 3 with the help of Hydrochloric acid.
- After acidification filtration is done and removes any soluble aglycone with chloroform.
- The filtrate is neutralized with liquid ammonia and then centrifuge to isolate the sennoside.

Method B:

- The extraction of the crude drug was performed with the help of benzene for two to three hours with frequent shaking at room temperature.
- Dry the residue (marc) left after the extraction with benzene at oven or room temperature which should be not exceeded to 40°C. The dry drug or marc should be extracted with 70 percent methanol for 4-6 hour on shaker at room temperature. Repeat the same process with fresh methanol for 2 hours.
- Club both the methanol extract and concentrate to 1/8 volume under reduced pressure. The pH is adjusted to 3 with hydrochloric acid and kept on side for 3 hours afterwards filter it. Anhydrous calcium chloride dissolves in denatured spirit and added to the filtrate with vigorous shaking.
- Maintain the pH 8 of the solution by ammonia solution and kept aside for two hours. Filter the solution and dry the precipitate over dessicant like phosphorus pentaoxide in a desiccator.

Estimation of Sennoside:

Hot water is useful for the extraction of anthraquinone glycosides. Acidified the aqueous extract and treat with chloroform to make free the aglycone which is largely present in the solution.

First neutralize the solution and then centrifuge it afterwards add ferric chloride to the solution.

Reflux the solution and further acidified it to bring oxidation and hydrolysis. The aglycone present should be extracted with ether and re dispersed in the magnesium acetate solution. Measure the absorbance of the sennoside and express the concentration in comparison to sennoside B at 515 nm.

Utilization:

The main use of sennoside is cathartic. It may be used in habitual constipation or occasional constipation. The sennoside glycoside absorb in the gastro intestinal tract and aglycone portion released in the colon. The released aglycone stimulates and irritates the colon. Thus movement in the colon portion is increased due to local action. The peristalsis movement is increased which generate soft and bulky stool.

12.10 VINCRISTINE AND VINBLASTIN

Vincristine and vinblastin are important important alkaloidal phytoconstituents isolated from the plant of Vinca (*Catharanthus roseus* L, Family Apocynaceae) which is an erect herb or sub herb everblooming plant with 40-80 cm height. Flower may be white, violet or rose in colour. The alkaloids isolated from the vinca plant are generally referred as Vinca alkaloids. Root may contain more amounts of alkaloids (0.15-1.795 percent). More than 100 alkaloids of indole group are present in the plant in which 25 are dimeric in nature. Vincristine and vinblastine are also dimeric in nature and uses for the treatment of human neoplasm.

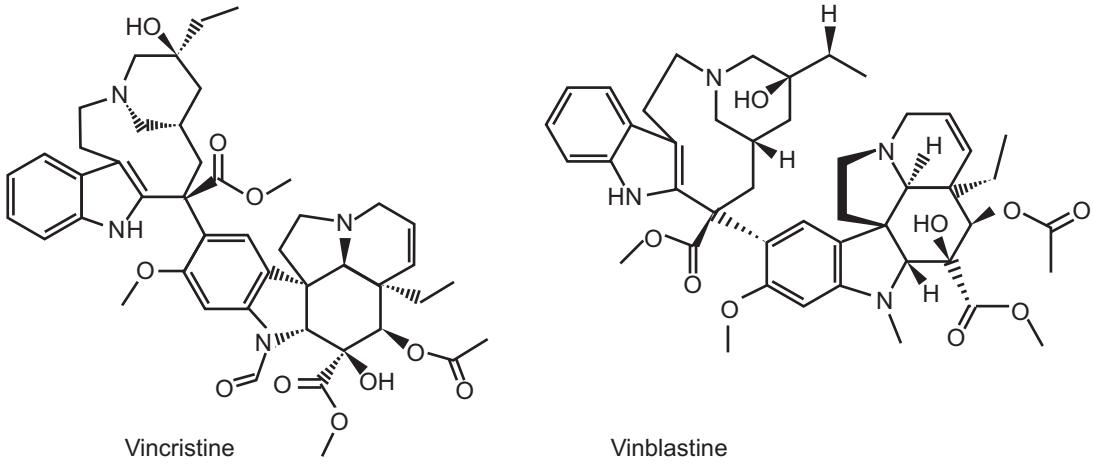


Fig. 12.11: Structure of Vinca alkaloids

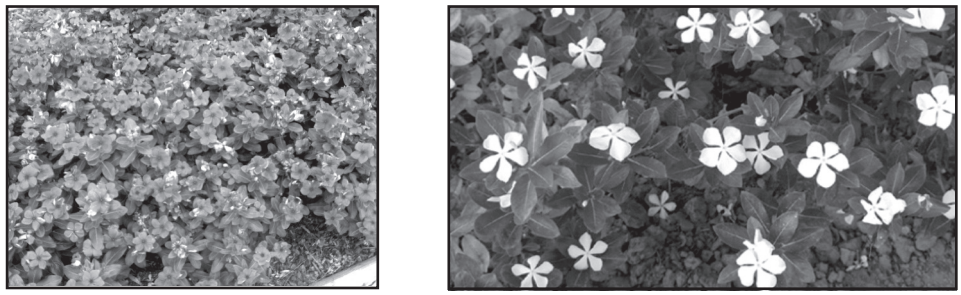


Fig. 12.12: Images of Catharanthus Roseus Plant

Extraction and Semisynthesis of Vinblastin:

Dry leaf powder of the Vinca was extracted with 0.1M HCl for half an hour in ultrasonic bath, centrifuge the mixture for 10 minutes at 2000 rpm. Obtained sediment is re-extracted with more quantity of HCl. Mix both supranatant and filter it. Treat this filterate with petroleum ether to remove lipophilic compound and chlorophyll. Separate the acidic fraction and treat it with alkaline solution (pH 10.5) of 10 percent in embonic acid add slowly for the precipitate of alkaloid. Increase the pH upto 5.0. separate the precipitate with decantation and this precipitate can be use for the semisynthesis of vinblastin.

Mix this precipitate with 0.1M HCl and 0.1 M Citric acid and cool the mixture from 0 to -5°C using dichloro methane and ice bath. Slowly add 30 percent aqueous hydrogen peroxide, 10 percent aqueous sodium hypochlorite and 1 percent solution of sodium borohydride in methanol for three to five hours. Increase the pH of mixture upto 9.5, collect the organic layer step wise and dry it.

Production of Vincristine Sulphate:

Dried homogenous ground material treated with dilute tartaric acid was extracted with benzene. Concentrate the benzene extract and perform the steam distillation. The benzene will be separated as distillate and the residue will be left out. Dissolve the residue in methanol and treat it with dilute tartaric acid solution. Perform the distillation. The methanol will separate as distillate and treat the bottom product with dilute solution of ammonia. Extract it and then evaporate the extract to dryness. Dissolve the dry powder and separate the vincristine sulphate by chromatographic method by using alumina column and eluted with benzene, benzene + chloroform, chloroform and chloroform + methanol. Vincristine will be isolated and treat it with sulphuric acid, vincristin sulphate will be obtained.

Estimation:

Vincristin and vinblastin sulphate are estimated with the help of HPLC. The following solution are prepared for the estimation:

Solution 1: 0.1 percent w/v of the substance being examine.

Solution 2: Contain 0.2 percent w/v each of vinblastine sulphate RS and vincristine sulphate RS solution.

Solution 3, 4 and 5: 0.1 percent w/v, 0.002 percent w/v and 0.0001 w/v respectively of vinblastin sulphate (if vinblastin estimated) or vincristin sulphate (if vincristin sulphate estimated).

Column: Packed with stationary phase LC2.

Flow rate: 1.0 ml/min.

Mobile phase: (For vinblastin) Mixture of 70 volume of methanol 30 volume of 1.5 percent w/v of diethylamine (pH adjusted 7.5 with phosphoric acid).

Detection wavelength: (For vinblastin sulphate) 297 nm.

Mobile phase: (For vincristin sulphate) Mixture of 50 volume of methanol 38 volume of 1.5 percent w/v of diethylamine (pH adjusted 7.5 with phosphoric acid) 12 volume of acetonitrile.

Detection wavelength: (For vincristin sulphate) 262 nm.

Utilization:

Vinblastin sulphate should be store in refrigerator otherwise it may be unstable. It is available in sealed ampoules. The vinblastin sulphate is highly used in the treatment of neoplasm, lymphocytic lymphoma, hodgkin's disease, testicular carcinoma. Vinblastin can show its potency in its individual form but generally it is dispensed in combination of other drugs to improve its therapeutic efficacy. It is dispensed through intravenous route by considering other factors like body surface, patient age, WBC count etc.

Vincristine sulphate is also available in ampoule and stored in refrigerator to improve its stability. It is also useful in lymphosarcoma, small cell lung cancer, neuroblastoma, hodgkin's disease, cervical and breast cancer. These alkaloids show antimitotic activity which inhibits cell growth. They disrupt the microtubules which causes the dissolution of cell mitotic spindle and the growth of cell arrest in metaphase.

12.11 CAFFEINE

Caffeine can be obtained from different sources like Tea (*Camellia sinensis*, Family- Theaceae), coffee (*Coffea arabica*, Family- Rubiacea), cacao (*Theobroma cacao* Family- Malvaceae), cola (*Cola acuminata* and *Cola nitida* Family- Malvaceae) and others. Caffeine can be extracted from tea sweepings, coffee roaster, tea dust or tea. Caffeine is white in colour or white powder. It is anhydrous or on hydration only one one molecule of water will be attach. It is bitter, needle shape, sublimined crystals. In the presence of salicylate, citric acid, benzoates its solubility in water increases very much.

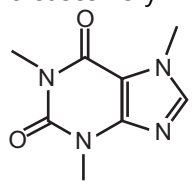


Fig. 12.13: Structure of Caffeine

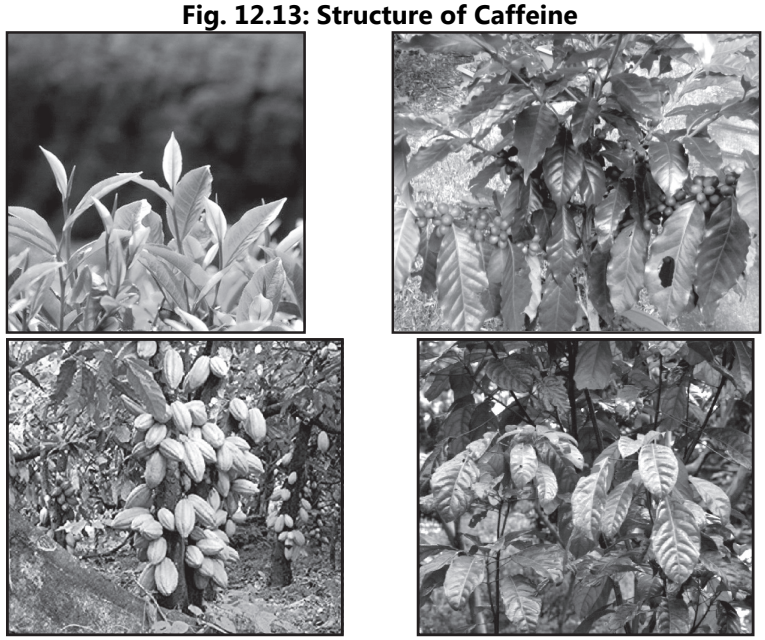


Fig. 12.14: Images of Tea, Coffee, Theobroma and cola plant

Production:

Caffeine can be extracted from the tea. Around 50 gram of tea powder are taken and extracted with alcohol for around 6 hours in Soxhlet extractor. The extract is transferred into porcelain dish which already contain magnesium oxide (30 gram in 200 ml water). Heat it on steam bath with continuous stirring and evaporate it. Boil the obtain residue three times with 100 ml water. Filter it in hot condition through Buchner funnel. Add the 10 percent sulphuric acid (30 ml) in combined filtrate. Concentrate the above filtrate to one third of the volume. Filter the solution during hot and extract it with 5 times with chloroform (25 ml). Add sodium hydroxide (1 percent, few ml) in chloroform extract so that the decolourisation takes place. Equal quantity of water add in this and separate chloroform layer. Evaporate the chloroform extract to obtain crude caffeine which can be re crystallized with hot water. Caffeine shows the positive murexide test in which caffeine is treated with few drop of nitric acid in porcelain dish and evaporates it to dryness. Add ammonium hydroxide (few drops) in this residue then purple colour obtained.

Caffeine can be also extracted by super critical fluid extraction technique by using liquefied carbon dioxide. Liquefied carbon dioxide absorbs the caffeine through moist coffee. Then it is passed through another pressurized vessel containing absorbing media like resin or activated carbon which retain the caffeine. Separate this caffeine by extracting with chloroform.

Estimation:

Dissolve the accurately weigh 0.18 gm powder in 5 ml of anhydrous glacial acetic acid with warming. If caffeine powder present in hydrated form dehydrate it by drying at 100 to 105°C. Cool the solution and then add 20 ml toluene and 10 ml acetic anhydride. Perform the non aqueous titration. Determine the end point by potentiometrically. Perform blank determination to make necessary correction.

Factor - each ml of 0.1 M perchloric acid ≡ 0.01942 gm of $C_8H_{10}N_4O_2$ (Caffeine)

Utilization:

Caffeine is CNS stimulant drug with the category of methyl xanthine. It is very popular psychoactive drug without any legal boundation. Caffeine citrate comes under essential medicines in WHO list. Caffeine can be used for broncho pulmonary dyspepsia. It also develops in mild form of drug dependence. It can be used as dietary supplements.

QUESTIONS

1. What are plant metabolites? Explain in short.
2. What are the differences between primary metabolite and secondary metabolite?
3. What do you understand by the term 'Phytoconstituents'?
4. What is the importance of 'Forskolin'? How it will be isolated from Coleus plant.
5. Write in short about the production, estimation and utilization of Taxol.
6. Write in short about the production, estimation and utilization of Podophyllotoxin.

7. Write in short about the production, estimation and utilization of any resin containing anticancerous drug.
8. How the atropine isolated from Atropa belladonna? Mention its estimation and utilization.
9. Write the importance of digoxin in the treatment of CVS diseases. How digoxin is isolated and estimated?
10. Mention the importance of diosgenin in the synthesis of steroidal drugs.
11. What is the procedure for the isolation and estimation of diosgenin?
12. Write the importance of artemisinin in the treatment of malaria.
13. How artemisinin is isolated and estimated?
14. What are the uses of sennosides?
15. Write the production and estimation of a drug which is used as cathartic.
16. How the vinca alkaloids are isolated and estimated?
17. What are the role of vincristine and vinblastine in the treatment of diseases?
18. Write the different sources for caffeine extraction.
19. What is the utilization of caffeine?
20. How caffeine is isolated and estimated? Explain.

■■■

Unit V

Chapter ... 13

Extraction Methods

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *General Introduction about Traditional and Modern methods of Extraction*
- *Methods for extraction process of Phytoconstituents:*
  - *Infusion*
  - *Decoction*
  - *Digestion*
  - *Maceration*
  - *Percolation*
  - *Soxhalation*
  - *Microwave assisted extraction*
  - *Ultrasonic assisted extraction*
  - *Steam distillation*
  - *Counter current extraction*
  - *Super critical fluid extraction*

13.1 INTRODUCTION

In the extraction process the animal / plant drug is treated with particular solvent. The solvent dissolves the medicinally active constituents in itself but animal or plant tissue and other component are not dissolved in the solvent.

OR

Extraction is the process in which the separation of the soluble constituents occurs from insoluble substance either solid or liquid by processing with a specific solvent.

The active constituents from the crude drug can be separated by different separation and extraction methods. In the extraction process there is a mass transfer process in which transfer of mass occur from soluble material like solid to a fluid. The different factor which effect the process of mass transfer are temperature, agitation, size reduction and others. The extraction may be solid extraction, solid-liquid extraction or liquid-liquid extraction. In the solid separation of the drug the active constituents of a solid drug are extracted from solid substances. In the solid liquid extraction the solid drug is extracted from a liquid solvent. In the case of liquid – liquid extraction liquid solvent is selected to extract the active constituents present in another liquid. Both liquid are immiscible. Some important term uses in extraction process are-

Menstrum: Solvent or mixture of solvent use for extraction.

Miscella: Solution with extracted substance.

Rinsing: Dissolution of extractive substance out of disintegrated cell.

Lixative or leaching: Water is use as solvent for extraction.

Marc: Residue left after extracting the desired constituents.

The various methods of extraction are:

1. Infusion
2. Decoction
3. Digestion
4. Maceration
5. Counter current extraction
6. Super critical fluid extraction
7. Hot continuous extraction (soxhalation)
8. Percolation
9. Ultra sound extraction
10. Steam distillation
11. Microwave assisted extraction

13.2 INFUSION

In the infusion process the crude drug is left with solvent to soak them properly at room temperature for a particular period of time with or without intermittent shaking. After the specific time period the filtration will follow which will separate the plants debris with the miscella. Various times the plant material has settled down which has separated by decantation and can replace with other solvent. The apparatus use in the infusion process is depending upon the type of the process. It can be a small test tube or flask to a very large industrial vessel. The infusion process is generally followed when active constituents are water soluble and they are soft in nature so that water can penetrate into the tissue. The final volume is not adjusted in infusion. Infusion can be fresh or concentrated. Examples are Infusion of quassia, senna.

Fig. 13.1: Infusion method of extraction

13.3 DECOCTION

The process is more suitable for the vegetables drugs which are heat stable and water soluble because in this method the drug is boiled for about 15 min.

Take drug in a vessel add 1000 ml water and cover it
↓
Boil for this for around 15 minutes
↓
Cool it to room temperature
↓
Strain the liquids and press the marc

Fig. 13.2: Flowchart of decoction method

The term decoction is taken from latin word decoquere (meaning to boil down) de means from and coquere means to cook. In the decoction process the plant drug is first mashing and then it is boiled with water which helps to extract the active constituents from the drugs. Herbal tea and coffee is good example of decoction.

13.4 DIGESTION

Digestion is modified form of maceration in which the temperature of the menstrum is kept heated at particular temperature at under pressure. This rise of temperature and pressure help the menstrum to penetrate the drug and extract it completely. The apparatus use for the digestion is the digestor which is generally made up of metal. The drug is placed inside of it with menstrum under specified temperature and pressure and then screw the cover with the help of nuts.

13.5 MACERATION

In the process of maceration the crude drug is immersed into the bulk of menstrum or solvent for at least 3 days (generally 3-7 days). During this period the menstrum is agitated frequently. The menstrum and container should be kept in the stoppered container. The mixture is then filtered or strained through net or sieves. Filter almost all the liquids and then press the marc and clarified liquid by decantation after standing or by filtration. The loss of solvent can be adjusted by prescribed extracted juices. Stoppered container are generally use for maceration so that the loss of solvent by evaporation should be avoided. The drug is allow to stand for 3-7 days with menstrum so that solvent penetrate the cell more perfectly and get the time for portioning of active constituents into the solvents. Frequent agitation help the distribution of active constituents in the entire solvent and prevent localization of active constituents around the tissue and cell.

The maceration may be of various types like:

1. Simple
2. Modified
3. Multiple
4. Kinetic maceration
5. Remaceration

Simple and modified Maceration

Both can be better understand by their differences. The differences between these are as following:

Table 13.1: Comparison of Simple Maceration and Modified Maceration Methods

Sr. No.	Simple Maceration	Modified Maceration
1.	Use for the organized drugs (bark, root).	Use for unorganized drugs (gum, resin).
2.	Drug and menstrum shaken occasionally during 3-7 days.	Drug and 80% menstrum shaken occasionally during 3-7 days.
3.	Strain the liquid and press the marc.	Decant the liquid and marc is not pressed.
4.	Mix the liquid obtained and clarify.	Filter one liquid and pass remaining menstrum.
5.	Filtrate is not adjusted to volume.	Filtrate is adjusted to the volume
6.	Here marc is pressed because contain considerable amount of macerate (organized drug).	Marc is gummy and compact and do not contain any macerate so pressing of marc is not necessary (unorganized drugs);

Multiple Maceration:

The maceration process is use for the concentrated preparation where the entire menstrum is segregated into two parts (for double maceration) or in three parts (in triple maceration). Each menstrum are use individually for the maceration process. In this process the drug menstrum ratio is low and extraction is performed with less amount of menstrum.

Kinetic Maceration:

Like the simple maceration kinetic maceration is also preceded into the room temperature but the single difference is that the material is under constant motion. The intensity and type of movement play important role in the maceration.

Re-Maceration:

In the re-maceration process part of the solvent is added to the drug. After filtration process the residue is treated with the remainder solvent.

Uses of Maceration:

BP permits the preparation of the tincture by maceration methods of following drugs Squill tincture, Catechu tinctures, Senna liquid extract compound Benzoin tincture and opium tincture.

Mother tinctures of homeopathic Pharmacopoeia are prepared by maceration methods. Maceration is most widely use methods of extraction.

Maceration can be performed easily with pharmacist lab with small amount of sample. There are number of drug which can extract by only maceration methods due to their high swelling property or high mucilage content.

Disadvantages:

Maceration is not exhaustive extraction process. This factor becomes more significant when the drug is expensive. It is a batch process.

13.6 PERCOLATION

The word percolation refers to the "First pass through". Percolation can be defined as 'Short successive maceration or extraction by the method of displacement'. Percolation can be performed into the conical percolator, cylindrical percolator or steam jacketed percolator. The various stages involved in the percolation are following:

(a) Size reduction: To assure the complete exhaustion of the crude drug, the drug should be suitably size reduced. Size reduction also increase the surface area of the crude drug and more surface area of the crude drug will be available to react with the menstrum. Size reduction also helpful of uniform packing of crude drug in percolator and reduce the moment of menstrum in the percolator.

(b) Imbibition: Imbibition is a process in which the powdered drug is kept along with menstrum for 4 hours in a well stoppered container. During this duration the menstrum penetrate into cell wall. This initial moistening of the crude drug powder is very important because it reduces the chances of choking of percolator though the dried drug swells when come into the contact of menstrum. The swelling of the drug reduces porosity of the powder and choke the percolator. Imbibitions replace the interstices air which can otherwise affect the flow of menstrum. Imbibitions also prevent the washing of fine particle of crude drug during percolation.

(c) Packing: After imbibition the lump of crude drug must be broken. The lower end of percolator should be plucked with cotton and then place the drug powder layer by layer. The packing should be perfect, neither too tight nor too loose which may affect the flow of menstrum. Two third of the percolator should be cover with the drug on which place the piece of filter paper and wash sand should be placed on the top of the filter paper. This prevents any type of disturbance of the crude drug by flow of menstrum.

(d) Maceration: Sufficient amount of menstrum should be added after packing the percolator. During the addition of menstrum the lower tap of percolator should be open so that air present in the percolator is displaced by the menstrum. When menstrum starts to come from lower tap close the tap and allowed to stand, the percolator for 24 hours as the primarily maceration take place. The menstrum level should be above the drug bed.

(e) Percolation: Open the lower tap after 24 hours of maceration and collect the menstrum from lower end until the three fourth portion of the final product is obtained. Meanwhile sufficient menstrum added over the powdered drug so that packed drug does not become dry. Completion of the percolation process should be check by various method like:

- By checking the specific gravity of end few ml of percolate and compare with the specific gravity of fresh menstrum.

- Evaporate the last few ml of percolate to dryness and see that any residue comes after evaporation or not.
- Perform the specific chemical test for last few ml of percolate like tannins, glycoside, alkaloids etc whether it is positive or negative.

(f) Pressing the marc: In the last, the marc should be pressed and obtain liquid is added into the collected menstrum. More menstrum should be added to obtain the desired volume. Allow the liquid to stand and separate the suspended particles by filtration or decantation. Example- Strong tincture of ginger, tincture of belladona etc.

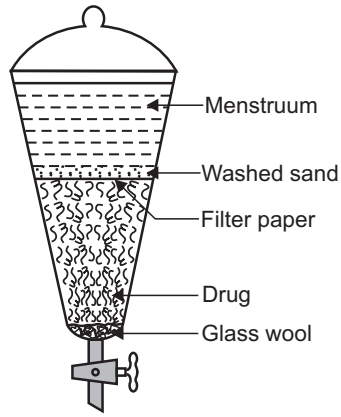


Fig. 13.3: Simple percolation method

Reserved Percolation:

In this type of percolation the initial portion (near about three fourth percentage of menstrum) which have the major quantity of active ingredient is kept separate just like the simple percolation process and the last one fourth amount of menstrum is collected in another vessel and concentrated it by the method of evaporation. This concentrated syrupy fluid should be added into the previous three fourth portion of menstrum. Adjust the final volume. The last one fourth portions contain very less amount of active ingredient so by the help of evaporation generally we concentrate the menstrum. Example- Liquid extract of liquorice.

The process has advantage that we obtain the concentrate menstrum without heating the major portion (three fourth portions) which have more active ingredient and just heat the last one fourth portions which also save the energy as well as handling of less amount of fluid for process.

Modified Percolation:

In the modified percolation process we reduce the drug / percolate ratio 1:3 which is generally 1:4 in common percolation process. By this we can save menstrum, time and heat. Percolation is type of displacement method. It has been observed that a stationary menstrum dissolve the more active constituents because stationary menstrum may remain in contact with more time to drug compare to the moving menstrum. Hence more quantity of menstrum is required to exhaust the drug in the case of simple percolation process. But if a percolation process is break into various step of maceration the drug/ product ratio will be reduced to 1:3.

Soxhlet Extraction/Continuous Hot Percolation Process:

This extraction process is more suitable where menstrum have less penetration into the cellular tissue. Active constituent are not readily extracted with solvent. The quantity of solvent for extraction of the active constituents is very less.

Soxhlet extraction is preferable in above mentioned conditions. Here the hot menstrum cycle is passed again and again over the crude drug and solubilize its active ingredient until the drug become exhausted. The apparatus consist of three important parts:

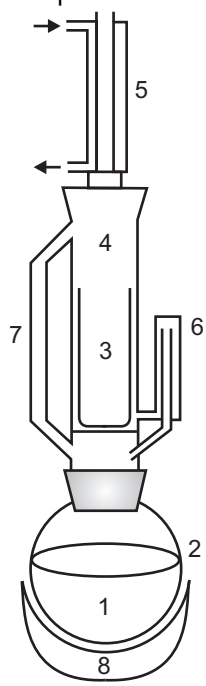
(a) A round bottom flask (RBF) which contain menstrum.
(b) Extraction chamber in which drug is kept in thimble.
(c) Condenser.

The size reduction of the crude drug was performed and packed the size reduced drug into the thimble (made of filter paper or cellulose) which is helpful to prevent choking of the extractor.

Place the solvent in the round bottom flask (RBF) and boiled it by heating mantle. The vapour of the solvent goes to the condenser via side tube. Condenser condenses the vapour of solvent and this solvent penetrates the packed drug. It percolates the crude drug and extracted the active ingredient. The level of menstrum is increased in the extractor until it reaches to maximum point from where it is siphoned into the round bottom flask.

This menstrum is again heated in RBF where active constituent remain in RBF and vaporized solvent again pass through the side tube and condenser which penetrates the drug present in the extractor. The process will continue till the drug become exhausted. Some drug like resin, opium, gum blocks the Soxhlet apparatus so this is not suitable method for these drugs.

Thermolabile drugs and constituents are not suitably extracted by this method because the active ingredient can be destroyed. Pure solvent or only those solvent mixtures which have constant boiling temperature can be used for extraction purpose.



1 - Solvent (ethanoi)
2 - Round bottom flask
3 - Soxhlet thimble
4 - Sozhlet extractor
5 - Condenser with running water
6 - Siphon
7 - Side arm (lagged with glass woll)
8 - Isomentle (heat source)

Fig. 13.4: Continuous Hot Percolation or Soxhlet Apparatus

13.7 MICROWAVE ASSISTED EXTRACTION

The electromagnetic radiation which have a frequency 0.3-300 GHz are called microwave. Industrial microwaves are generally operated as 2.45 GHz so that it avoid the interferences of domestic instruments like radio and other. Due to their electromagnetic property microwaves contain magnetic and electric field. They are perpendicular to one another. Electric field generates heat through two simultaneously method i.e. ionic conduction and dipolar rotation. Microwave transfer the energy to solid matrix and solvent homogenously and very efficiently. Substance (solid matrix and solvent) absorb the energy as per dielectric constant. The plant material which is present in the microwave transport solvent absorbs the heat of microwave which causes the heating of moisture present inside the drug. Evaporation occurs due to heating of moisture and this will produce high vapour pressure. This high vapour pressure crack the cell wall of plant drug and release the active constituent into the solvent. The extraction property of the solvent and their interaction with microwave can be altered by using solvent mixtures.

The benefit of microwave assisted extraction are that less solvent are required, time consumption is less, high extraction rate and good reproducibility but additional centrifugation or filtration are necessary to remove residue and the efficiency will be poor if the solvent or compound have non polar property or they are volatile. Example- Extract of glycyrrhizic acid from mulethi.

13.8 ULTRASONIC ASSISTED EXTRACTION

The frequencies above the 20,000 Hz are known as ultrasound. Ultrasonic waves are using in ultrasonic extraction. These waves cause cavitations effect on the dry cell and destruct the cell wall and release the active constituents. When ultrasonic waves are passed through the liquid media it compresses (produce high pressure) and rarefaction (low pressure) to the liquid media. Due to this process small voids or vacuum bubbles are formed in the solvent. After certain duration these bubbles are not able to absorb more energy produce by microwave and they bursted. At the high pressure cycle they bursted which is known as cavitation. Due to this cavitation cell wall destructed and active chemical constituent are extracted.

This process is efficient, inexpensive and simple compare to conventional extraction techniques. It has high yield, reduce operating temperature and easy to operate but sometime active constituent can convert into free radical which have undesirable side effect.

13.9 STEAM DISTILLATION

It is one of the most popular methods to separate the essential oil from the crude drugs. A still (large stainless steel container) contain the crude drug material. The steam is injected through inlet valve into the plant material which contains the essential oil. The inlet steel evaporates the volatile molecule. This volatile molecule collected into the condenser. The water molecule and volatile oil molecule can be easily separated because they are not mixable. Direct prolonged heating should be avoided which deteriorates the volatile components.

13.10 COUNTER CURRENT EXTRACTION

In counter current extraction the substance which is to be extracted is distributed between two different solvent depends upon their distribution coefficient. This can be best performed by Craig apparatus. There are number of glass tubes (r = 0, 1, 2, 3, ----) in the Craig apparatus and they are designed such that the solvent which are lighter in density can be transferred from first tube to second tube and then forward. The tubes are driven or shaken electromechanically. The lower phase which have higher density is called as stationary phase and the solvent which have the lighter density is called the mobile phase. The substance to be extracted is present in the stationary phase. Equal quantity of stationary phase is present in all the tubes.

The mobile phase (solvent have less density) is transferred into the first tube (tube =0) shake properly so that extraction take place and then phases are allowed to separate down. The mobile phase (or upper phase) then transferred to next tube no 1and repeat the same process and transfer it into further tube like tube no 2, 3, 4 and so on after every shaking at the fresh mobile phase to tube no 0.

The substance having higher distribution ratio separate more quickly compare to lower distribution ratio. Now a day's Craig apparatus is less frequently used because of the availability of more recent chromatographic techniques but it has more significant to clear the concept of extraction to the students. It is very difficult to construct and operate a Craig apparatus having more than 100 test tubes.

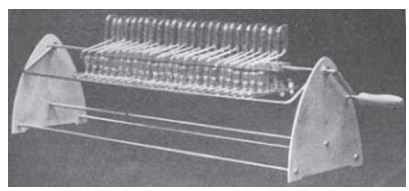


Fig. 13.5: Counter current extraction apparatus

13.11 SUPER CRITICAL FLUID EXTRACTION

A critical point is a particular temperature and pressure at which two phases (like liquid and vapour) can co-exist. The substance at above the critical temperature and pressure or above the critical point is known as supercritical fluid. The property of super critical fluid is in between gas and pure liquid so that they are also known as dense gases or compressible liquid. Super critical fluid has good solvation power, low viscosity, higher diffusibility and good penetrating power. By the help of super critical fluid extraction technique we can separate the constituents from the drug with the help of super critical fluid. Generally we can separate the constituents from the solid matrix but it may also be liquid. CO_2 is one of the most common super critical fluid. To make CO_2 super critical fluid the critical temperature is 31°C and critical pressure is 74 bars.

Instrumentation:

The different component in the super critical fluid extraction is the first one is fluid reservoir which contains the fluid like gas cylinder in the case of CO_2. Another one is the pump; the pump may be syringe pump or reciprocating pump. Another important is the extractor which may be made of stainless steel and can with stand to high pressure like 300 to 600 atmosphere. Another important component of the super critical fluid extractor is the restrictor. It is important for the controlled and systematic release of the pressure inside the extractor vesicle. The restrictor may be fixed type or variable type. The isolated constituent is collected in a collector where it can be detected with the help of detector. Sometimes modifiers are also added into the supercritical fluid to increase its versatility like one to ten percent of methanol is added in CO_2.

During the process the liquid is pumped into a heating zone and heated to supercritical temperature then the super critical fluid passes through the extraction vessel where it diffuses to the solid matrix and dissolves the active constituents. The extracted material with super critical fluid comes into the separator or collector unit where the pressure is low. The extracted material settle down here and the CO_2 can then be cooled, recycled or discharge to the environment. The isolated constituent can be detected with the help of detector.

Advantages and Limitations:

The super critical fluid extraction technique is very rapid and there is no need of organic solvent for the extraction purpose. The thermolabile substance can also be extracted with this technique. It is a versatile and efficient continuous method with complete separation and recovery of the solvent.

Sometimes its consistency and reproducibility may vary. It is expensive technique because it need high pressure which increases its cost. CO_2 cannot always be used as solvent as it is non polar and so limited dissolving power particularly in case of polar solvent.

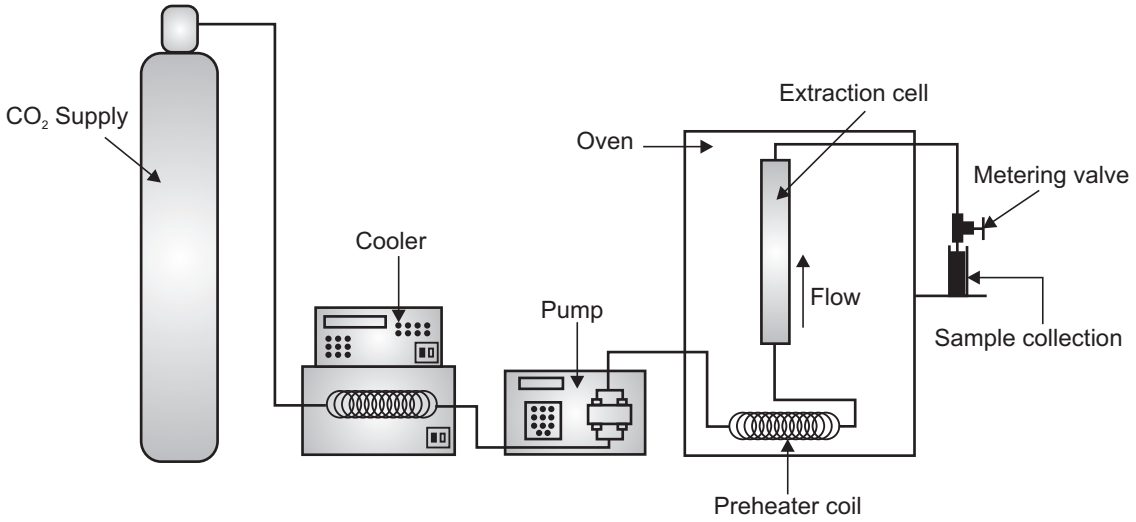


Fig. 13.6: Super critical fluid extraction apparatus

QUESTIONS

1. What do you understand by the term 'Extraction'?
2. What are the different techniques uses for extraction of crude drugs?
3. What is Infusion? Explain in short.
4. What are the examples of decoction method? Write in brief about this method.
5. What are digestors? Explain its role in the process of digestion.
6. Describe the maceration technique in short.
7. What are the differences between simple maceration and modified maceration technique?
8. Draw a diagram of percolator and explain the percolation technique.
9. What is reserved percolation process? Describe in brief.
10. What is continuous hot percolation process?
11. Draw a well labeled diagramme of Soxhlet apparatus. Explain its functioning.
12. Describe microwave assisted extraction technique.
13. Describe ultrasonic assisted extraction technique.
14. What are the differences between ultrasonic and microwave assisted extraction.
15. Explain briefly steam distillation method.
16. What is the counter current technique for the extraction of crude drugs?
17. What is supercritical fluid extraction?
18. Write in brief about instrumentation of super critical fluid extraction?
19. What are the merit and demerit of super critical fluid extraction?
20. Mention the different technique for the extraction of thermolabile drugs.

■■■

Chapter ... 14

Isolation and Characterization Techniques

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *Description about Isolation and Characterization techniques:*
  - *Chromatography*
    - *Column chromatography*
    - *Paper chromatography*
    - *Thin layer chromatography*
    - *High pressure liquid chromatography (HPLC)*
    - *Gas liquid chromatography (GLC)*
    - *High performance thin layer chromatography (HPTLC)*
  - *Spectroscopic studies*
    - *UV spectroscopy*
    - *Infrared spectroscopy*
    - *Mass spectroscopy*
    - *Nuclear Magnetic Resonance (NMR) spectroscopy*
  - *Electrophoresis*
    - *Paper electrophoresis*
    - *Gel electrophoresis*
    - *Capillary electrophoresis*

14.1 INTRODUCTION: CHROMATOGRAPHY

Chromatography is the separation technique used for the separation of complex mixture. For example in a tablet the active ingredient is mixed with various excipients. In this the chromatography will be helpful to separate the excipient with other ingredients. Similarly in plant extract there are various phytoconstituents are present. Chromatography will be helpful to separate this phytoconstituents into its individuals.

Chromatography word was derived from the two words chroma and graphy. Chroma the greek word indicate colour and graphy represent the meaning writing and recording. The person who invented the chromatography first was a Russian botanist Mikhail Tswett. He separated the chlorophyll and xanthophylls from the leaves of the plant by using a glass column packed with the fine particle of calcium carbonate. So the chromatography is very primitive technique used for the separation of the mixtures.

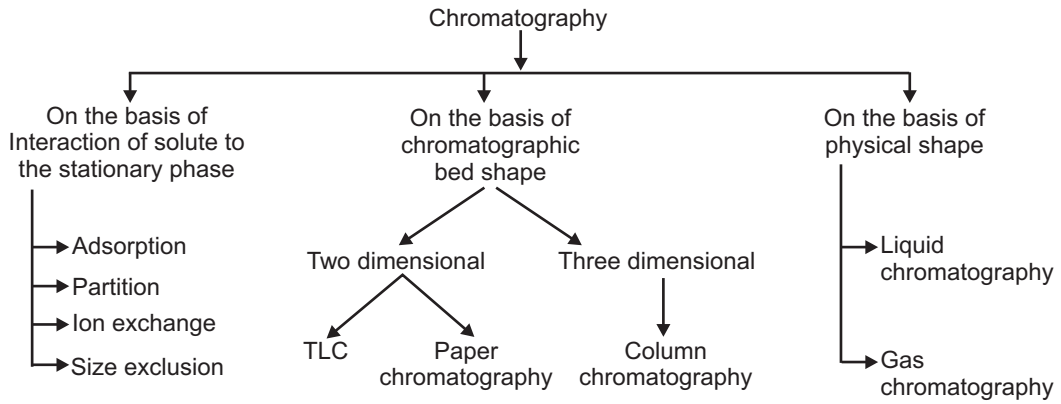


Fig. 14.1: Classification of chromatography

14.1.1 Column Chromatography

Generally in all the types of chromatography there is one stationery phase and one mobile phase. In a column chromatography we use packed column as a stationery phase. The column may be packed with solid or liquid. If the packing occur with the solid the principle of separation is adsorption while if packing will be done by liquid then the principle of separation will be partition. Generally in the column chromatography the most separation is based on the adsorption principle. The adsorption principle is used in the separation of the mixture from column chromatography. The extract which is to be analyzed should be dissolve in the mobile phase and add into the column. The individual constituent in the extract will move with different rates which depend upon their affinities towards the stationery phase or adsorbent. The compounds which have more affinity towards stationery phase will be separated in the last while those have less affinity towards stationery phase will move fast and separated first.

The stationary phase which is used to fill the column should have uniform spherical size with high mechanical stability, inert with solute or other components and mobile phase have free flow, freely available, inexpensive and able to separate the wide variety of compounds.

Example of weak adsorbent is sucrose, starch, inuline, talc while medium adsorbent is calcium carbonate, magnesium oxide, calcium hydroxide and magnesium carbonate and the example of strong adsorbent is activated alumina, activated charcoal, fuller's earth etc.

The mobile phase may be used in individual or can be used into the mixture depending upon their polarity and the type of constituent to be separated. The column should be made of neutral glass and not affected by acid, alkali, mobile phase or stationery phase. The length: diameter ratio may be 10:1 to 30:1. Sometimes it may be 100:1 ratio.

Packing of the Column:

The lower portion of the column should be pack with glass wool or cotton wool to above which the adsorbent should be placed. After packing with adsorbent, a paper disc can be placed above the adsorbent layer. So that on introduction of sample or mobile phase will not disturb the adsorbent layer. There are generally two types of column packing techniques

1. Dry packing technique.
2. Wet packing technique.

In the dry packing technique the column is pack with dry adsorbent and then flown the solvent through the column till equilibrium is obtained but the major drawback is that air bubbles are trapped between the solvent and adsorbent which produce trouble in the separation of compounds.

In the wet packing technique the mobile phase is mixed with adsorbent and poured into the column. So the air entrapment is comparatively less and uniform packing occur in the column. If the flow of mobile phase will be uniform there will be no development of crack and a good and uniform separation of the extract will occur.

Sample Introduction:

The sample should be dissolved in the least quantity of mobile phase. The complete sample should be place on the top portion of column and get them absorbed. Then the sample can be isolated by elution. Elution may be of two types:

1. In Isocratic elution the composition of mobile phase will be same for entire separation.
2. In gradient elution the composition of mobile phase can be changed slowly to improve the separation. The polarity of mobile phase can be changed by changing the mobile phase combination.

Detection of Components:

Colored constituents can be detected visually and colored band moving in the column can be collected separately. Colourless compound can be detected by UV technique, refractive index detector, monitoring by TLC, by flame ionization detector or such other technique. The component can be recovered by different technique some time even by cutting the column (column made of plastic) into several distinct zones but the best method is elution in which the component eluted by the mobile phase and the constituent is separated from the mobile phase.

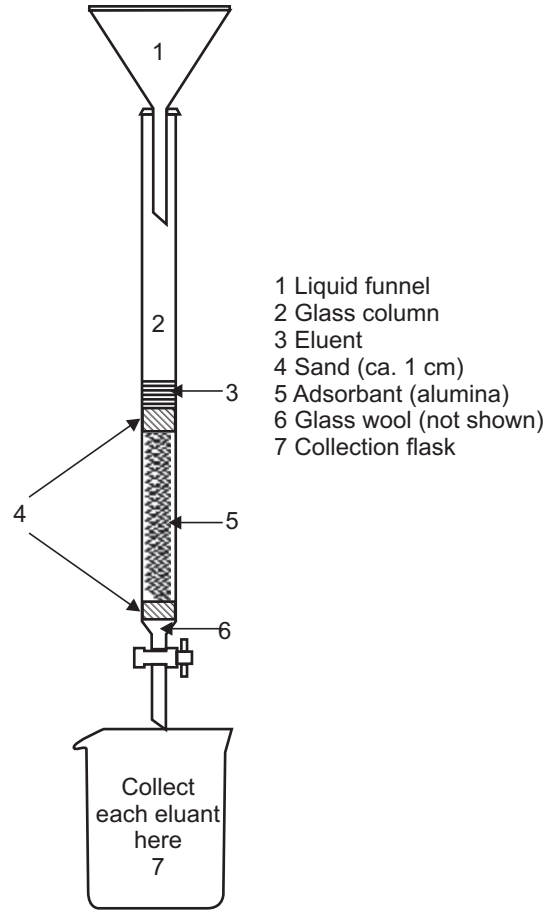


Fig. 14.2: Diagrammatic representation of Column Chromatography

14.1.2 Paper Chromatography

Paper chromatography is carried out by specially designed filter paper. The principle of separation may be here partition or adsorption. If the filter paper is impregnated with alumina or silica, the adsorption principle will be applied for separation whereas if moisture/water present in the pores of cellulose fibre it works as stationery phase and solvent as mobile phase then the principle of separation will be partition. In general paper chromatography refers to the partition principle.

Various types of filter paper are used for paper chromatography. It may be Whatmann paper of different grade, acid or base wash filter paper, paper modified with glycol, formamide, methanol, glass fibre type paper, hydrophobic paper (OH group can be acetylated) or paper can be impregnated with alumina, silica or ion exchange resin.

The size of the paper should be suitable for the size of the chamber and apply the sample by using capillary or micro pipette. The sample should be dissolved in the mobile phase and applied with low concentration with small zone. In the mobile phase pure solvent or mixture of solvent or buffer solution can be used.

There may be ascending, descending, circular, two dimensional and ascending-descending type different development techniques.

Ascending development technique is conventional technique in which the mobile phase moves against the gravity and spot the sample at bottom portion. While in descending development the mobile phase kept at the top and the solvent flow down the paper. Here the samples applied at the top and the development is fast due to gravity assisted solvent flow.

In circular or radiant development the sample applied at the centre of the paper and the mobile phase flown through a wick at the centre and spread uniformly in all direction. In two dimensional development techniques the samples is applied in one corner and develop the paper on one axis then dry the paper. After drying turn the paper on ninety degree angle and develop the paper on another axis. This technique is used for more complex sample. By ascending descending development the length of separation can be increase. First here ascending takes place later descending development follow.

After development of the chromatogram the isolated compound can be visualized by detecting agents. Detecting agents can be two types:

(a) Destructive type

(b) Non destructive type

In destructive type the sample cannot be recovered or it will be destroyed due to chemical reaction of spraying reagent with sample. While in non destructive method sample can be recovered. In non destructive method sample can be detected by UV chamber method, densitometric method or iodine chamber method.

Paper chromatography can be use for both qualitative and quantitative purpose. For qualitative purpose Rf value can be determined.

$$\text{Rf (Retardation factor)} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

For quantitative purpose the density of the spot can be measured or the spot can be eluted with the solvent and analysed by conventional techniques like spectrophotometric method or electrochemical methods.

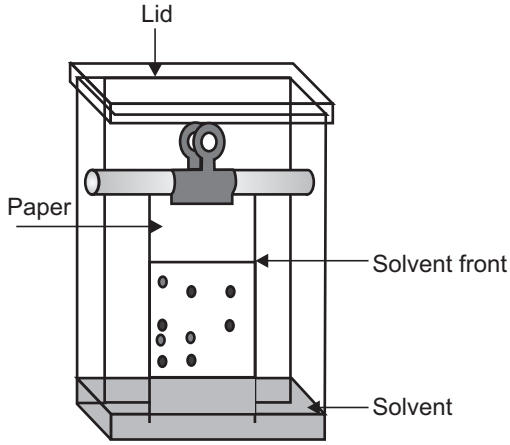


Fig. 14.3: Diagrammatic representation of paper chromatography

14.1.3 Thin Layer Chromatography (TLC)

Stahl in 1958 developed the standard equipment and technique for analysis by thin layer chromatography. Before that in 1938 Izmailou and Shraiber separate the phytoconstituent using 2 mm thick alumina on glass plate. TLC is comparatively better separation tool then paper chromatography. Though the adsorbent term use various times in TLC but principle of separation may be any one of four fundamental mechanism of separation:

1. Ion exchange
2. Partition
3. Adsorption
4. Molecular exclusion

Principle of separation totally depend upon the coating substance apply on the plate. If silica or alumina is apply as coating substance adsorption principle will take place. If coating substance is sephadex molecular exclusion will be principle of separation. Ion exchange will be the mechanism of separation if resin will be the coating substance and cellulose like coating substance adopt partition mechanism.

The extract of plant spotted on the plate and it moves along with mobile phase. The phytoconstituent of extract have different affinity towards the stationary phase and mobile phase. Those constituents have more affinity towards mobile phase separate faster compare to those who have more affinity with coating substances.

Silica gel G is one of the most common adsorbent uses for the coating of plate. Here G represents the gypsum ($CaSO_4$) which is around 15 percent of the silica gel and act as binder. The other adsorbent which are use in the stationary phase is alumina, cellulose, kiesselguhr, polyamide powder and others. Most often the adsorbent are applied on the glass plate. The dimension of the glass plate may be vary from 20X20 cm to 20X5 cm. sometime even microscopic plates are also used to examine the progress the chemical reaction by TLC method.

The glasses are coated with adsorbent with different techniques like: 1. Pouring, 2. Dipping, 3. Spraying, 4. Spreading techniques.

In pouring technique the prepared slurry is poured onto the glass plate and try to maintain an equal level surface. In dipping technique two plates are stick together and dip into the slurry and then remove from the slurry. Separate both plates, one surface will be coated and other one will be dried. In the spray technique adsorbent is sprayed on glass plate using a machine sprayer. The best applying technique is spreading technique in which the slurry is kept in the TLC spreader box. The glass plates of particular size are kept on base plate. The prepared slurry kept in the reservoir of spreader box. Adjust the thickness of slurry by rotating the knob present in the spreader box then spreader box is moved over the glass plate. Allow them for air drying and activate them at 100-120°C for one hour.

Sample can be applied by micro pipette or capillary tube. It should be spotted at least 2 cm above the base of or on such height that spotted area should not immersed in mobile phase. The area of the spotted sample should be minimum with sufficient concentration. Mobile phase and TLC plate should be kept in the development chamber. Chamber should be saturated with mobile phase otherwise **edge effect** may found in which the solvent front in the middle of plate move faster than the edge. The mobile phase selected on the basis of phytoconstituent which have to be separated and the nature of stationary phase. It may be single or the mixture of the solvent. The polarity of the solvent adjusted such that it easily separated the phytoconstituents.

The different development techniques used in the TLC are one dimensional, two dimensional, horizontal and multiple developmental techniques. In one dimensional or vertical technique, the mobile phase flows against the gravity due to the capillary action. Most separations are done by this technique in which one spot is applied at the corner of the plate and the plates are developed in one axis then dried it. Further turn the plate to 90 degree and developed the plate on another axis. After development of the chromatogram the isolated compound can be visualized by detecting agents. Detecting agents can be two types a) Destructive type b) Non destructive type.

In destructive type the sample cannot be recovered or it will be destroy due to chemical reaction of spraying reagent with sample. While in non destructive method sample can be recovered. In non destructive method sample can be detected by UV chamber method, densitometric method or iodine chamber method.

This chromatography can be use for both qualitative and quantitative purpose. For qualitative purpose R_f value can be determine.

$$R_f \text{ (Retardation factor)} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

For quantitative purpose the density of the spot can be measured or the spot can be eluted with the solvent and analysed by conventional techniques like spectrophotometric method or electrochemical methods.

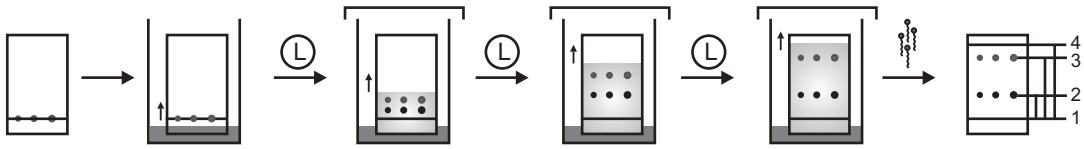


Fig. 14.4: Process involved in Thin layer chromatography

14.1.4 High Pressure Liquid Chromatography (HPLC)

Compare to the classical chromatography high pressure liquid chromatography or high performance liquid chromatography has much better performance. Compare to classical column chromatography it work under high pressure. HPLC have smaller particle size for adsorbent phase so that it provides more surface area which improves the separation.

In HPLC the particle size of stationary phase is less (3-20µm) with small metal column packed under high pressure which run under high plow rate and high pressure (>5000 psi) while in conventional column chromatography particle size is less (60-200µm) with glass column packed under gravity pressure, run at very low flow rate and work under low pressure (< 20psi).

HPLC run under normal phase mode or reverse phase mode which depends upon the polarity of stationary phase and mobile phase. In normal phase mode the mobile phase is non polar and stationary phase is polar while in the reverse phase mode mobile phase is polar and stationary phase is non polar.

The separation technique in HPLC may isocratic or gradient. In the isocratic separation technique the polarity of mobile phase is maintained same throughout the process while in gradient separation the polarity of mobile phase can be gradually change by changing the ratio of the different solvent present in the mobile phase.

The HPLC unit consists of different parts like pump, mixing unit, injector, guard column, analytical column, detectors and recorder. The solvent pushed into the column under very high pressure (1000 to 5000 psi) because the particle size of the stationary phase is very small (5-10 µm) so the resistance force by mobile phase is very high. To generate such pressure mechanical or pneumatic pumps are used. Mechanical pump works under constant flow rate and for generally analytical purpose. Pneumatic pumps work under constant pressure with highly compressed air. Flow rate and back pressure is controlled by the check valve. The pulse generate by the pump is dampen by pulse dampeners. In the mobile phase there may be single solvent or may be more than one solvent. To mix properly more than one solvent there is a mixing unit in HPLC apparatus. It may be two types a) low pressure mixing chamber b) high pressure mixing chamber. In low pressure mixing chamber helium is used for degassing the solvent. The quality of solvent should be very good or may be HPLC grade.

Various gases are soluble in the mobile phase. Mobile phase under high pressure when pumped in soluble gas becomes undissolved and forms the bubble which interferes in the

separation of constituent. To remove this hindrance the degassing of mobile phase is very important. For degassing the various techniques like vacuum filtration, helium purging, ultrasonication can be used. Vacuum filtration is not very much reliable, helium purging is efficient but helium is costly gas so that the ultrasonication is the better technique. Ultrasonicator removes the air bubble into the mobile phase by converting ultra high frequency into the mechanical vibration.

The sample can be introduced into the HPLC by manual method or auto injection method. It can be septum injection, stop flow (on line) injection or loop valve (Rheodyne injectors). In septum injection one rubber septum is use to inject the sample but the septum should withstand to high pressure and leaching (erosion of rubber) also exist. Sometime stop the flow of mobile phase and introduce the sample through a valve device but the most popular is loop valve (Rheodyne) injector type. In this first sample is loaded on the injector (20- 50 µl) which is the load position and then inject the sample without disturbing the flow of mobile through inject mode.

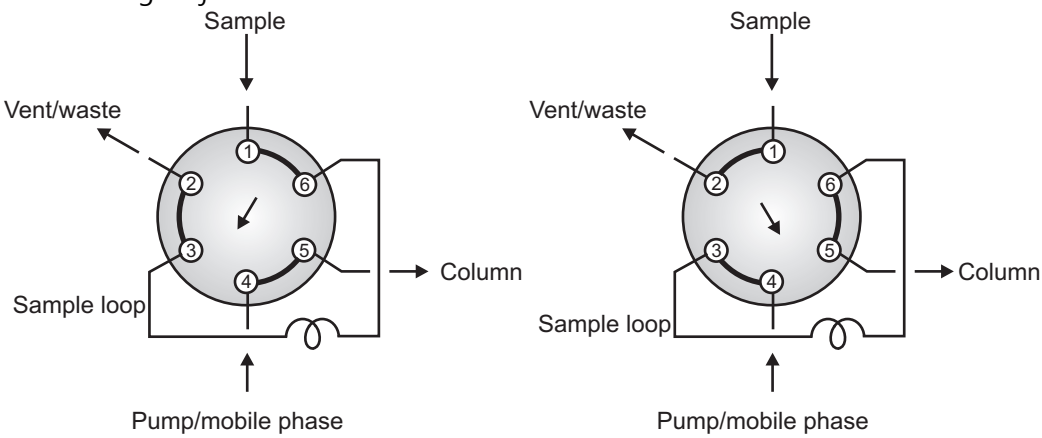


Fig. 14.5: Diagrammatic representation of Rheodyne injector of HPLC

A very important part of the HPLC is the analytical column but before the analytical column one guard column is also provided which is comparatively cheaper to the analytical column and helpful to increase the life of analytical column by removing the entry of unwanted material in the analytical column. The performance of the analytical column decides the efficiency of separation. Column can be made of polyether ketone, stainless steel, polyethylene or sometime glass. Stainless steel can be most widely used column because it can withstand the high pressure compare to PEER column. The column length can vary from 5-30 cm with diameter 2-50 mm using adsorbent particle size 1 µm to 20 µm with uniform spherical porous material. 1gm stationary phase may have average 400 sqm area.

The detector use in the HPLC can be divided into the two categories, the solute property detector and bulk property detector. Bulk property detector also known as universal detector, measure the characteristic to all analyte by analyzing mobile phase without or with sample. The good examples of these are conductivity detector or refractive index detector. Solute property detector corresponds to the particular unique property of the analyte like UV detector, Flourimetric detector, Photodiode array detector.

The response of the separated constituents can be recorded by the recorder. Recorder amplifies the response which is detected by the detector. It record the time at which the constituent are separated or retention time. Integrator measures the height and width of the peaks, peak area and percentage of the area.

HPLC can be use for the qualitative analysis by measuring the retention time of sample under standard condition but generally it is use for quantitative analysis by direct comparison method, calibration curve method and by internal standard method.

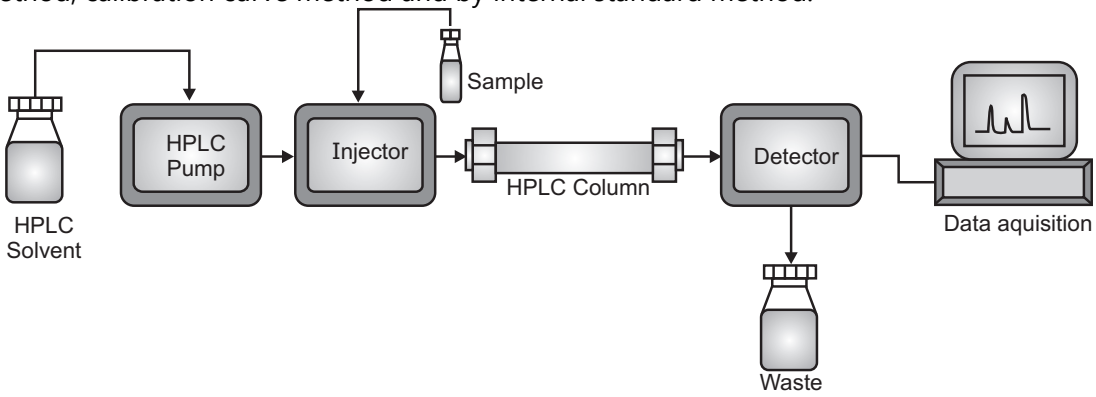


Fig. 14.6: Diagrammatic representation of High performance liquid chromatography (HPLC)

14.1.5 Gas Liquid Chromatography (GLC)

Gas chromatography generally divided into GSC (gas solid chromatography) and GLC (gas liquid chromatography). In either type the gas is a mobile phase but the stationary phase vary in GC (gas chromatography). In GLC stationary phase is liquid and in GSC it is solid. In GLC the principle of separation is partition while in GSC it is adsorption. Most of the time when named GC it is GLC.

Generally a liquid is coated on solid support used as stationary phase. The mixture which has to separate into the individual constituent has to be converted into the vapour and mix with mobile phase (gas). The constituent which have more affinity towards stationary phase travels slowly compare to those which have high affinity towards mobile phase. The constituents are separated out on the basis of their partition coefficient. GLC is a good technique to detect those compounds which are **volatile and thermostable**.

The volatile compound should be mixed with the carrier gas. The carrier gas may be hydrogen, helium, nitrogen. Hydrogen is a good option for carrier gas because of good thermal conductivity and low density but it reacts with unsaturated compounds. Helium is also good choice but it is expensive. Nitrogen is inexpensive but has reduced sensitivity. Gases are generally stored under high pressure. To blow the gases under uniform pressure and flow rate there is a need of flow meter. Generally rotameter and soap bubble meter are used to control the flow of gases.

The sample can be introduced into any form like solid, liquid or gaseous form. Valves are suitable device to introduce the gas sample. Solid samples are generally dissolved in appropriate solvent and then injected through septum. Liquid samples can be dispensed through either loop or septum devices. Septum should be made of high quality silicone rubber and can tolerate high temperature and suitable for repeated injection.

Another important part of GLC which effect the separation of the constituent is column. They are made of either glass or stainless steel. The stainless steel column have long life and easy to handle but sometime it may react the constituent which are not in the case of glass column but glass column are fragile and difficult to handle. The column may be analytical (length 1-1.5 mt, diameter 3-6 mm) or preparative column (length 3-6 mt, outer diameter 6-9 mm). Depending upon its nature it may be packed column, open tubular or golay or capillary column and support coated open tubular column (SCOT).

Though the sample should be converted into the vapour the pre-heaters are required in the GLC which convert the sample into vapour form and mix with the carrier gas. They are installed along with injecting device. Thermostatically controlled oven can be used for this purpose which can operate on isothermal programming base (same temperature during entire operation) and linear programming (oven is heated linearly over a period of time).

Different type of detector like kathrometer, FID (flame ionization detector), AID (argon ionization detector), ECD (electron captured detector) are used. The most sensitive of them are ECD (10^{-12}). Recorders record the response and amplify it. Recorder record the retention time, record base line and record all the peak. Heights, width, area of the individual peak, percentage of area are calculated by integrators.

The separation or detection of the sample can be improved in GLC by derivatisation techniques. It can be precolumn derivatisation or post column derivatisation. In precolumn derivatisation the sample is converted into more volatile and thermostable derivative (like carboxylic acid, phenols, sugars are converted into less polar by reagent BSA bis trimethyl silyl acetamide). Post column derivatisation is done generally to improve the detector response for isolated constituents.

GLC can be use for the qualitative analysis by measuring the retention time of sample under standard condition but generally it is used for quantitative analysis by direct comparison method, calibration curve method and by internal standard method.

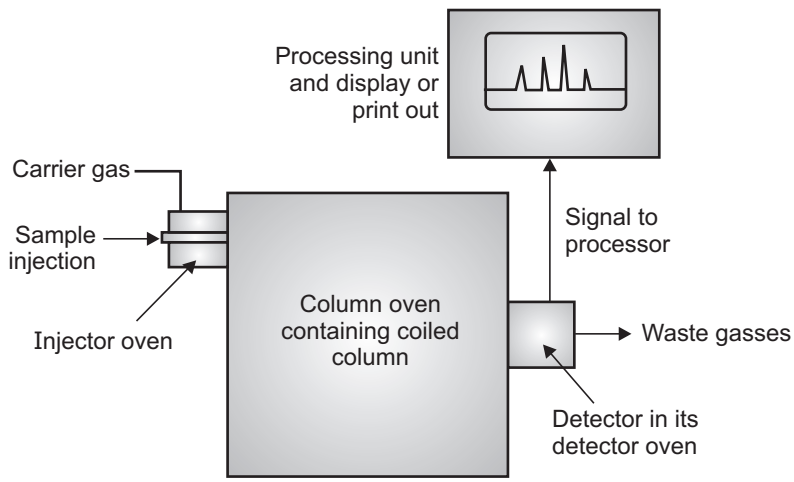


Fig. 14.7: Diagrammatic representation of Gas Liquid Chromatography (GLC)

14.1.6 High Performance Thin Layer Chromatography (HPTLC)

In contrast to the column chromatography (like GLC, HPLC) planar chromatography utilizes a planar (flat) stationary phase for the separation.

Sample Preparation:

The sample should be homogenous and representative to the entire batch. The sample should be prepared such as that analyte present in the traces quantity can be detected. If the sample is pure and concentrated can be directly apply to the HPTLC plate but if analyte is present at less concentration with impurities than purification, isolation of analyte from interfering reagent and concentration procedure should be taken.

Sample Application:

The physical dimension of the isolated sample should be very much compact. The optimum amount of the sample should be applied on the stationary phase. In case of manual application care should be taken that manual application not damages the surface of the layer. During sample application chemical fumes and vapours should be absent and temperature and humidity should be constant. Fully automatic sample application is better. It dispenses precise volume on precise position with precise delivery rate. Generally 0.5-5µl sample applied in case of spot or 2-10µl as narrow band. Sample can be applied as 1) Manual application 2) Instrumental techniques 3) Semiautomatic application. The adverse effect of overloading of samples shows unresolved components.

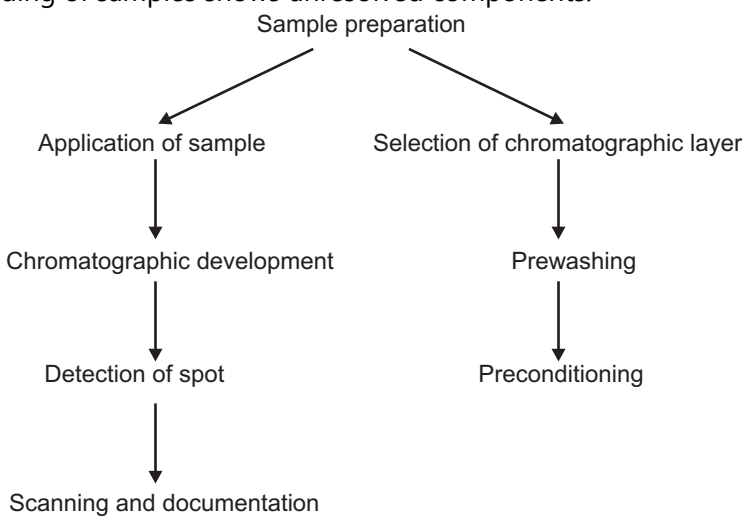


Fig. 14.8: Flowchart representation of High performance thin layer chromatography (HPTLC)

Stationary phase, TLC plates and solvents

Silica gel is a most popular solvent used in HPTLC afterwards cellulose. Homemade sheets/plates show bigger particle and not shows homogenous so it is not suitable for HPTLC analysis. Precoated HPTLC plates which are available in the market are more appropriate for HPTLC analysis. Plastic sheet or aluminium foil supported HPTLC plate are now more popular compare to the glass plate supported HPTLC plate because they can easily cut into desired

sizes as well as require less space to keep in the lab but glass is more resistant to heat and chemical reaction compare to the aluminium and plastic sheets. Mean particle size in HPTLC is 5-6µm compare to the 10-12µm in classical TLC. The layer thickness is 100-200µm in HPTLC but 250 µm in TLC. The relative humidity plays a crucial role to reproduce the result. The relative humidity is variable in the laboratory condition. To avoid such variation it is desirable to **precondition** (saturate the TLC chamber with the vapours of mobile phase) the TLC chamber. Preconditioning is more required with highly polar mobile phase.

The environment of the lab contains various dirt particles, vapour of various gases which can be deposited into the HPTLC plate. This impurity can be removed by **prewashing** of the plate. Run the methanol or such other solvent without apply the sample on HPTLC plate so that all dirt particle or impurities on the HPTLC plate will be collected on the upper edge which can be removed. After prewashing the plate are kept in the oven for 15-20 min at 120°C which is known as conditioning.

Mobile Phase:

The mobile phase use for HPTLC should be utmost pure. Presence of antioxidant and stabilizer altered the nature of chemical. They should be kept in proper storage condition. Polarity, viscosity, volatility are some points which effect chromatographic procedure. The solvent used for mobile phase are categorized into 8 different classes. The mobile phase should be kept as simple as possible.

Chromatographic Development:

Chromatographic development is another important aspect in HPTLC. Generally the glass development chambers are use for such purpose. The various development chambers which can be used for chromatographic development are:

1. Flat bottom chamber
2. Twin trough chamber
3. Sandwich chamber
4. Horizontal chamber
5. Automatic development chamber
6. Forced flow development chamber
7. Automatic multiple development chamber.

Twin trough chamber uses less solvent. The linear development of the chromatogram is the best method in which the HPTLC plate is placed vertically in an appropriate chamber. The solvent is run by capillary action.

Detection:

After the development of chromatogram HPTLC plates are dried and evaluated for following method:

Evaluation by non destructive method:

1. Direct visual method
2. Evaluation under UV light:

Reversible Reaction:

1. Iodine Vapour
2. Ammonia Vapour

Non Reversible Reaction:

1. Fluoroscent dye
2. pH indicator
3. Wetting/ Dipping
4. Spraying technique

All the above technique come under non reversible detection technique in which the isolated constituents cannot be recovered and destroyed after detection.

Scanning and Documentation:

After the development of spot the HPTLC plate are scanned at selected UV regions wavelength and the selected can be measured in the computer in the form of peak and can be compared with standard compound or other constituents.

The obtained band can be converted into the peak. The height and area peak of the peak correspond to the concentration of isolated constituents. This document can be stored in the computer for the further references.

14.2 UV SPECTROSCOPY

UV spectroscopy plays a very important role for the identification of plant constituents. This may be either in the screening of crude plant extract or observation of the eluents of chromatographic column during separation of plant products. Generally the UV spectroscopy of the phytoconstituents should be measured in dilute solution using an appropriate blank. The absorbance of colored compounds can be measured in between 200 to 700nm while the colorless compound can be measured in between 400-700nm. The particular wavelength at which the maximum and minimum absorption take place should be recorded. A pure phytoconstituent which show the characteristic UV spectra should be purified repeatedly from the mixture until the particular characteristic spectra will obtain with the help of UV visible spectra. Sometime we can also predict the structure of phytoconstituents like carotenoids have generally three small absorption peak in the region 400-500nm.

If single peak persist between 250-260nm the phytoconstituents may be purine aromatic amino acids phenol or pyrimidine polyacetylenes (unsaturated compounds). Hydroxyl cinnamic acid (aromatic compound) and ketones have also shows the characteristic UV absorption pattern. The BP give identification test of various phytoconstituents based on their UV absorption characteristics like lanatoside C, benzyl penicillin and various alkaloids (i.e. morphine, cocaine, reserpine, tubocuraine chloride and colchicine). It is also useful for the quantitative evaluation of the phytoconstituents. For this first the standard curve are prepared by using the standard substance and then by this curve the concentration of unknown compound are determined.

Table 14.1: Different phytoconstituents with their wavelength

Sr. No	Region of Spectrum	Constituents	Optimum wavelength
1.	Ultraviolet	Reserpine	268 nm
(a)		Vinblastin	267 nm
(b)		Vincristine	297nm
(c)		Morphine	286 nm
(d)		Glycyrrhizinic acid	250 nm
(e)		Capsaicin	248 and 296 nm
2.	Visible	Ergot (total alkaloids)	550 nm
(a)		Morphine	442 nm
(b)		Reserpine	390 nm
(c)		Anthraquinone	500 nm

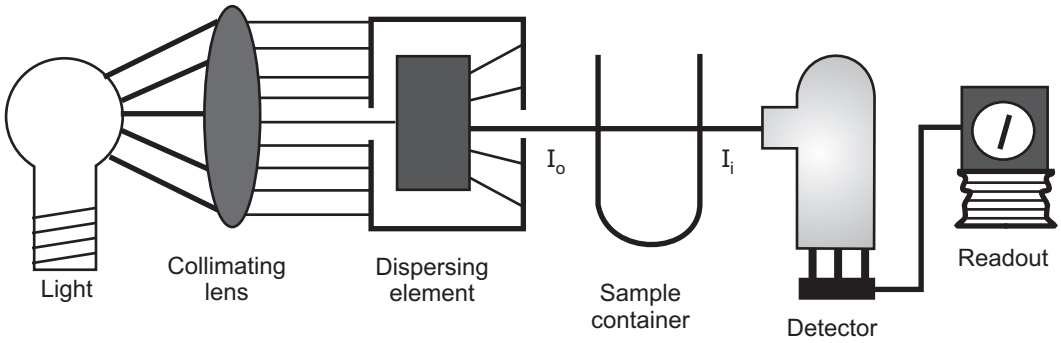


Fig. 14.9: Diagrammatic representation of UV –Visible spectrophotometer

14.2.1 Infrared Spectroscopy

In the infra red region the energy involve in the absorption is very small and the spectrum in this region is much more complex. The energy associated to absorption is so small that it will not produce electronic transition but enough to produce rotational and vibrational energy changes. The spectrum of infrared can be divided into two regions. First one is the Fingerprint region (7-11µm) and another one is the functional group region.

In fingerprint region it is difficult to assign the peak but can assign the peak in functional group region. The intensity of the band can be recorded as (S) strong (M) medium (W) or weak.

By the help of characteristic vibrational frequencies we can assign the various functional groups in the molecule (mentioned in the table). We can compare the natural phytoconstituents to the synthetic compounds by comparing the fingerprint region. IR spectra are most commonly use to identify known essential oil component which are separated by gas liquid chromatography (GLC).

IR spectroscopy is a great tool in the structure elucidation of a new phytoconstituent isolated from the natural origin. The interpretation of the spectrum of new compound is very complex and need very experiences. By the help of IR spectroscopy we can also perform the quantitative analysis of crude herbal sample or their formulation. In the quantitative analysis the area of the peak of the band are measured and the ratio of area of two sample are compare by the help of which we can assign the percentage of pure drug or the herbal drug present in the formulation.

Table 14.2: Characteristic infrared frequencies of natural products

Sr. No.	Compounds	Approx positions of characteristic bands above 1200 cm^{-1}
1.	Alkanes	2940 (S), 2860 (M), 1455 (S), 1380 (M)
2.	Alkenes	3050 (W-M), 1850 (W), 1650 (W-M), 1410 (W)
3.	Aromatics	3050 (W-M), 2100-1700 (W), 1600, 1580, 1500 (W-M)
4.	Acetylenes	3310 (M), 2225 (W), 2150 (W-M), 1300 (W)
5.	Alcohols and Phenols	3610 (W-M), 3600-2400 (broad), 1410 (M)
6.	Aldehydes and Ketones	2750 (W), 2680 (W), 1820-1650 (S), 1420 (W-M)
7.	Esters and Lactones	1820-1680 (S)
8.	Carboxylic acids	3520 (W), 3400-2500 (broad, M), 1760 (S), 1710 (S)
9.	Cyanides	2225 (W-S)
10.	Isocyanates	2270 (VS)

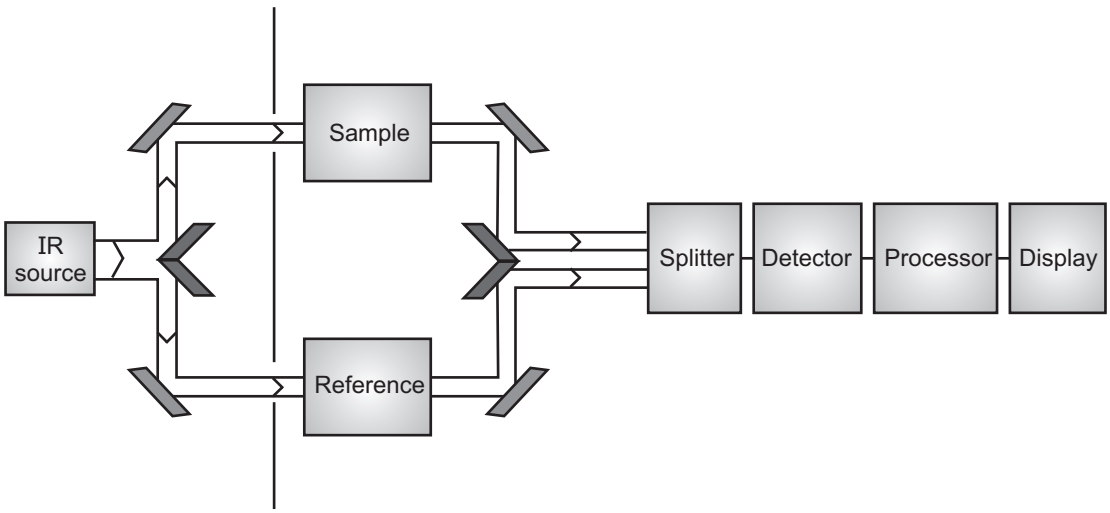


Fig. 14.10: Diagrammatic representation of Infra-red spectrophotometer apparatus

14.2.2 Mass Spectroscopy

By the help of mass spectroscopy we can get the accurate molecular weight of the phytoconstituent by using only microgram quantity of the sample. Mass spectroscopy produces the characteristic fragmentation pattern which is helpful to identify the phytoconstituents.

The microgram amount of phytoconstituents is introduced into the mass spectrometer where the compound become ionized and caused fragmentation of the chemical bonds.

Mass spectra are the graph in between the relative proportion of a fragmented ion versus the ratio of mass/charge of these ions. Mass spectroscopy nowadays is very useful to get the information of about structure of unknown phytoconstituent. Nowadays mass spectra is attached to the high performance liquid chromatography (HPLC) where isolated constituent direct enter into the mass spectra and help the identification of the unknown phytoconstituents.

Mass spectrometer is very complicated and sophisticated instrument and unlike the UV and IR where the instrument operated by individual phytochemist, it is operated by some technical expert person. The mass spectroscopy is also useful to very low molecular weight phytoconstituents. In generally the mass spectroscopy is used for the structure elucidation of phytoconstituents but sometime when two mass spectrometers are attached simultaneously it is also useful for the quantitative analysis.

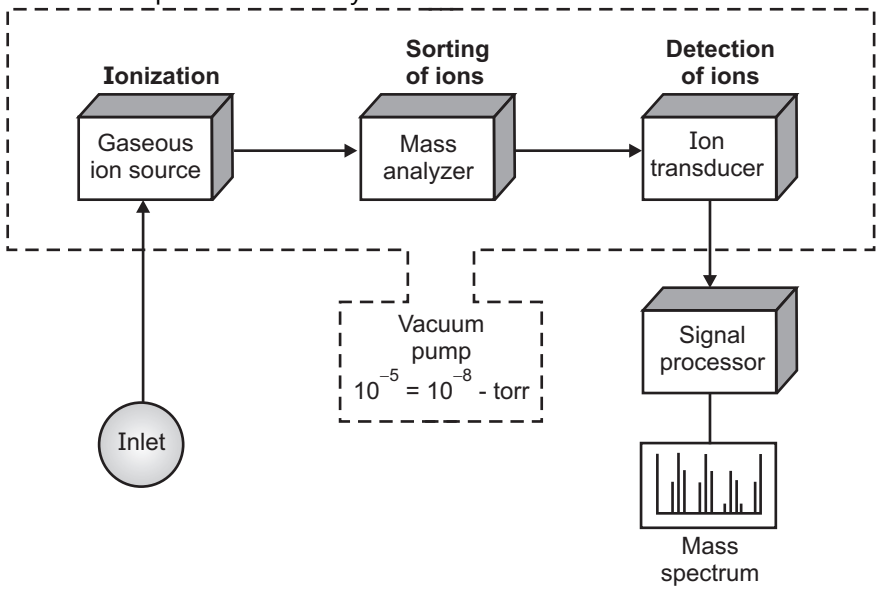


Fig. 14.11: Diagrammatic Representation of Mass Spectrophotometer Apparatus

14.2.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

In the nuclear magnetic resonance (NMR) spectroscopy when we apply radio frequency energy under the presence of magnetic field there is a change of spin at nuclear level. Generally we study hydrogen or proton and thus NMR is called as proton magnetic resonance (PMR). When ^{13}C, ^{35}Cl, ^{19}F and other nuclei are studied it is known as NMR. Nuclei with only odd mass number only give NMR spectra.

Generally the proton with uneven mass number spin on their own axis and when the energy applied on it in the form of radio frequency under magnetic field at particular precessional frequency it absorbs the energy and give the NMR signal. The nucleus after absorbing this energy moves from ground state to excited state and spin in reversal or anti parallel orientation. The nucleus comes back to ground state when the externally applied radio frequency is stopped. Generally the graph plot in the form of chemical shift and it is represented by δ–value which is from 0-10 δ for most compounds. Each proton or hydrogen atom in the organic molecule feel the different environment due to the nearness of heteroatom, double bond or triple bond, aromatic, alicyclic or benzene ring and each proton need different energy for its excitation. Therefore the each proton give the different peak of signal due to their different environmental surrounding there. The chemical shift is the difference between the absorption peak of sample and absorption peak of the reference compound. The reference compound is generally TMS (Tetra methyl silane). Generally the sample is kept into the solution with inert solvent in between the pole of powerful magnet. The major importance of Proton NMR comes under structure elucidation of phyto-constituents. The more different type of proton present in the molecule will produce more complex spectrum. So highly substituted alkaloids give fewer signal. Due to interaction between proton attached to adjacent carbon atom the spectral signal may be doublet or triplet compare to single peak. Thus more skill required for the interpretation of NMR spectra. Though the quantity requires in mg in NMR but it is recoverable compare to mass spectroscopy.

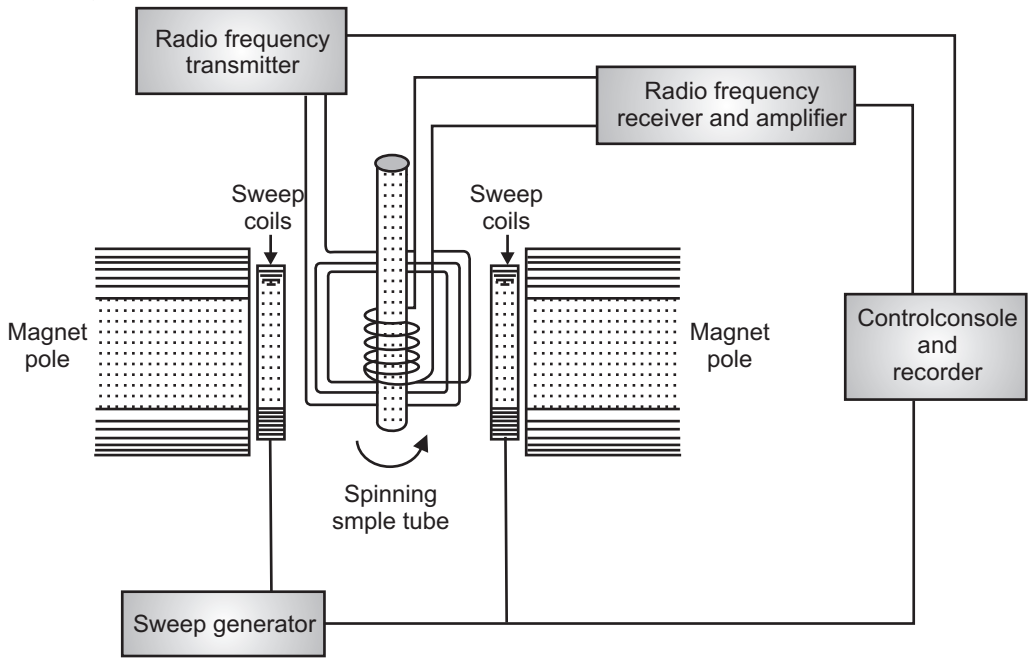


Fig. 14.12: Diagrammatic representation of Nuclear magnetic resonance apparatus

14.3 ELECTROPHORESIS

Like chromatography, electrophoresis is another very important technique which is used for the separation of constituents. It is also known as cataphoresis. The separation in electrophoresis is based on the different migration rate of charged ion in an electric field. The Swedish chemist in 1930 named Arne Tiselius performed the serum protein study by this technique and got noble prize in 1948. Electrophoresis use extensively for the separation of various types of constituents like Vitamins, Inorganic anions and cations, amine drugs, catechol, nucleic acid, polynucleotide, nucleotide, proteins, carbohydrates, drugs etc.

Electrophoresis is one of the most common tools for the separation of protein (antibodies, enzymes, hormones) and nucleic acid (RNA and DNA). In the electrophoresis separation process a small amount of sample is introduced (in the form of band) into aqueous buffer solution and applied high voltage, entire length of buffer by the help of a pair of electrodes (present at the end of buffer). Due to the field the charged sample will move towards the electrodes (depending upon their charges) and the separation will take place.

The movement of the sample ions depends upon the different factors like size of the ions, voltage applied, charge of ions, viscosity of the medium, ionic strength and pH of the buffer media etc.

(a) Size of the ions: Mobility of the sample is inversely proportional to their size. It means larger the size of the sample particle will give the lower the rate of separation.

(b) Voltage applied: If we have to obtain the sharp band we should apply higher voltage which also speed up the separation process. But due to high voltage the problem of evaporation of solvent or buffer may arise.

(c) Charge of ions: Higher the charge on the ions the mobility of the sample will be higher or rate of the separation will be faster.

(d) Viscosity of the medium: Mobility is proportional to viscosity of medium.

(e) Adsorption: Sample is retained over the supporting medium is known as adsorption which causes tailing of sample and this reduces the resolution and rate of separation.

(f) Temperature: The migration time decreases with increase in the temperature.

Type of Electrophoresis:

1. **Zone electrophoresis:** In zone electrophoresis the charged molecule or ion moves on the supporting media like gel, paper etc. Example of zone electrophoresis are:
   (a) Paper electrophoresis
   (b) Gel electrophoresis
   (c) Thin layer electrophoresis
   (d) Cellulose acetate electrophoresis
2. **Moving boundary electrophoresis:** In the moving boundary electrophoresis the charged molecule can move freely in a free moving solution. There is no supporting media like gel or paper required. Examples are:
   (a) Capillary electrophoresis
   (b) Isotacto electrophoresis
   (c) Isoelectric focusing
   (d) Immuno electrophoresis

14.3.1 Paper Electrophoresis

This technique can be useful for the separation of amino acids, small proteins or small charged molecules. The paper strips moist with buffer and the end of this paper strip dipped into buffer solution which contain electrode. The sample should be applied in the centre of the paper and the high voltage applied. The sample will migrate depending on their charges. After electrophoresis the isolated constituents can be identified by various techniques of staining depending upon their chemical structure.

14.3.2 Gel Electrophoresis

Just like the other electrophoresis techniques it also involves the electric field. Those phytoconstituents which have to be separated are introduced into the gel under electric field. The gel contains the pore through which phytoconstituents have to move. The small molecules of the phytoconstituents will travel the larger distance (because it can penetrate small pore of the gel) through the gel compare to the larger molecules. Generally the DNA and RNA (contain negative charge) can be easily separated by gel electrophoresis. If protein have to be separated by this technique they are first treated with sodium dodecyl sulphate which unfold the protein and makes them linear and coated with negative charge. Currently agarose gel or polyacrylamide gel are used for gel electrophoresis.

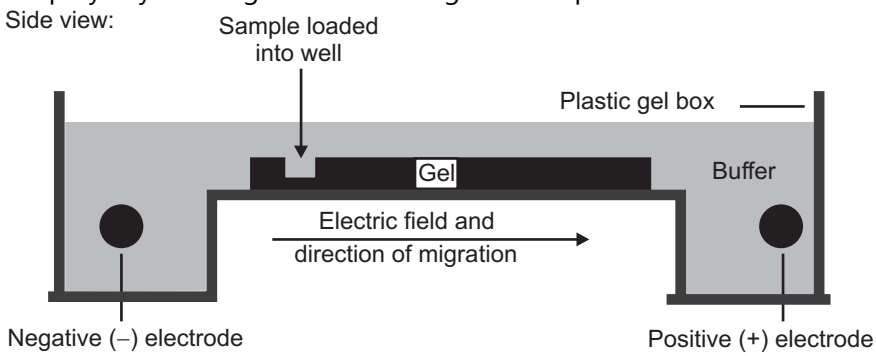


Fig. 14.13: Diagrammatic representation of Gel electrophoresis

14.3.3 Capillary Electrophoresis

In the capillary electrophoresis separation takes place inside the capillary having internal diameter 10 to 100 μm. The ends of fused silica capillary tube dip into the buffer solution. It contain platinum electrode (cathode and anode). Capillary tube contain optical window for detection which attached with UV detector. To introduce the sample the inlet buffer should be replaced with sample and sample can be injected by pressure, electro kinetically, capillary action and siphoning. On applying the voltage the migration of the sample starts which is known as electrophoretic migration but this electrophoretic migration is also effected by electron osmotic flow of the buffer solution. Generally the electro osmotic flow is towards the cathode (or negative charged). Though the electrophoretic mobility is lower than electro osmotic flow all the analytes are carried towards cathode with different speed. These can be detected by detector.

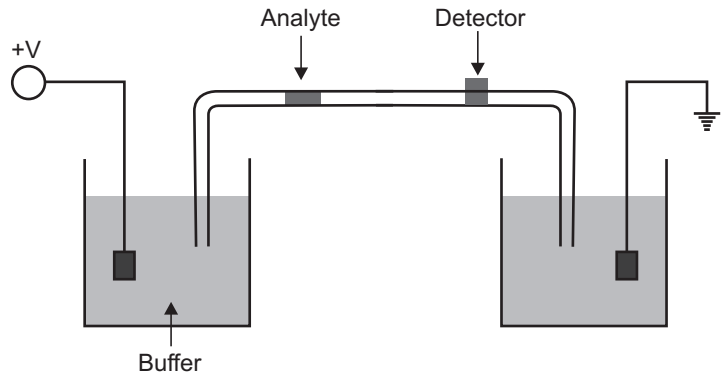


Fig. 14.14: Diagrammatic representation of Capillary electrophoresis

QUESTIONS

1. What do you understand by 'Chromatography'?
2. What are the techniques for the isolation of phytoconstituents?
3. Explain the column chromatography.
4. What is the principle involved in paper chromatography? Explain.
5. What is the role of TLC in isolation of compounds?
6. How TLC performed in lab scale? Write in short.
7. Write in short about rheodyne sampler.
8. How HPLC is differentiated from Column chromatography?
9. Write about the instrumentation and uses of HPLC.
10. What are the different detectors are used in GLC?
11. Which types of compounds are preferable to estimate by GLC?
12. Write about the instrumentation and uses of GLC.
13. How the HPTLC differs from TLC? Explain.
14. Explain the instrumentation and uses of HPTLC.
15. What is the importance of spectroscopy in characterization of phytoconstituents?
16. Write about the importance of UV spectroscopy.
17. Write a brief note on IR spectroscopy.
18. What is the importance of Mass spectroscopy in characterization of constituents?
19. 'Can the isolated constituent recovered in NMR spectroscopy'? Explain it.
20. Write the importance of NMR spectroscopy.
21. How the electrophoresis is different from chromatography?
22. Classify electrophoresis with examples.
23. Write a note on gel electrophoresis.
24. Describe in brief about paper electrophoresis.
25. Mention in short about capillary electrophoresis.

■■■