Unit -I
Biosynthetic pathways introduction

Metabolic pathways in higher plants and their determination

The living plant may be considered as a biosynthetic laboratory not only for primary metabolites like sugars, Amino acids and fatty acids and also for a multitude of secondary products of pharmaceutical significance such as glycosides, alkaloids, flavonoids, volatile oils etc.

Biosynthesis is also known as anabolism since simple compounds are joined together to form macromolecules by enzymes. Biosynthesis is a process of forming larger organic compounds from small subunits within a living organism. Biosynthesis is mainly done by enzymes.

Biosynthesis is biological and catalyzed by enzymes. Occurrence Synthesis occurs outside living organisms. Biosynthesis occurs within a living organism.

Primary metabolites:

Substances that are widely distributed in nature either in the one form or the other, in virtually all organisms are needed for general growth and physiological development because of their basic cell metabolism.

Secondary metabolites:

Biosynthetically derived from primary metabolites but are more limited in distribution, usually being restricted to a taxonomic group.

They represent chemical adaptations to environmental stresses/ they serve as defensive, protective/offensive chemicals against microorganisms, higher animals etc.

In terms of cellular economy, secondary products are in general expensive to produce and accumulate.

Biosynthesis:

Biosynthesis is defined as building up of complex chemical compounds from simpler ones by a series of reactions catalysed by enzymes in cells, during the physiological processes of a living organism.

Other than enzymes, the process requires precursors like ATP, NADH, NADPH, FADPH, and FADH. Generally compounds generated via biosynthesis are carbohydrates, proteins, vitamins, antibiotics, fats, alkaloids, gums etc

Biogenesis:

Development of life from the pre-existing life

There are different biosynthetic pathways in plants like

Metabolic pathways in higher plants and their determination

Metabolism is integral to health and proper functioning.

Metabolites are the intermediate products of metabolic reactions catalyzed by various enzymes that naturally occur within cells.

An integrated network of enzyme-mediated and carefully regulated chemical reactions is used for this purpose, collectively referred to as 'intermediary metabolism', and the pathways involved are termed 'metabolic pathways'.

Some of the crucially important molecules of life are carbohydrates, proteins, fats, and nucleic acids.

By far the most important building blocks employed in the biosynthesis of secondary metabolites are derived from the intermediates acetyl coenzyme A (acetyl-CoA), shikimic acid, mevalonic acid, and 1-deoxyxylulose 5-phosphate.

These are utilized respectively in the acetate, shikimate, mevalonate, and deoxyxylulose phosphate pathways.

Primary metabolites:

Primary metabolites are involved in growth, development, and reproduction of the organism.

The primary metabolite is typically a key component in maintaining normal physiological processes; thus, it is often referred to as a central metabolite.

Primary metabolites are typically formed during the growth phase as a result of energy metabolism, and are deemed essential for proper growth.

Examples of primary metabolites include alcohols such as ethanol, lactic acid, and certain amino acids.

Within the field of industrial microbiology, alcohol is one of the most common primary metabolites used for large-scale production.

Specifically, alcohol is used for processes involving fermentation which produce products like beer and wine.

Additionally, primary metabolites such as amino acids including L-glutamate and L-lysine, which are commonly used as supplement which are isolated via the mass production of a specific bacterial species, *Corynebacteria glutamicum*.

Another example of a primary metabolite commonly used in industrial microbiology includes citric acid.

Citric acid, produced by *Aspergillus niger*, is one of the most widely used ingredients in food production.

It is commonly used in pharmaceutical and cosmetic industries as well.

Secondary Metabolites

Secondary metabolites are typically organic compounds produced through the modification of primary metabolite synthases.

Secondary metabolites do not play a role in growth, development, and reproduction like primary metabolites do, and are typically formed during the end or near the stationary phase of growth.

Many of the identified secondary metabolites have a role in ecological function, including defense mechanism, by serving as antibiotics and by producing pigments.

Examples of secondary metabolites with importance in industrial microbiology include atropine and antibiotics such as erythromycin and bacitracin. Atropine, derived from various plants, is a secondary metabolite with important use in the clinic.

Atropine is a competitive antagonist for acetycholine receptors, specifically those of the muscarinic type, which can be used in the treatment of bradycardia.

Antibiotics such as erythromcyin and bacitracin are also considered to be secondary metabolites. Erythromycin, derived from *Saccharopolyspora erythraea*, is a commonly used antibiotic with a wide antimicrobial spectrum. It is mass produced and commonly administered orally.

Lastly, another example of an antibiotic which is classified as a secondary metabolite is bacitracin. Bacitracin, derived from organisms classified under *Bacillus subtilis*, is an antibiotic commonly used a topical drug.

Bacitracin is synthesized in nature as a nonribosomal peptide synthetase that can synthesize peptides; however, it is used in the clinic as an antibiotic.

Difference between primary metabolite and secondary metabolites

S. No	Characteristics	Primary Metabolites	Secondary Metabolites
1	Definition	These are the products produced during the growth phase in the metabolism of organisms to carry out physiological functionalities thereby enabling the overall cell development of cells	Upon completion of the growth phase, end products of primary metabolism are synthesized. These are necessary for the ecological and other cell activities
2	Phase at which it occurs	Growth phase	Stationary phase
3	Other terms used to refer	Trophophase	Idiophase
4	Quantity of production	Large quantities	Small quantities
5	Extraction process	Easy	Difficult
6	Occurrence	They produce the same products in every species	They produce different products in every species
7	Applications	Widely used in industries for different purposes Play a role in cell growth, development and reproduction	Supports the cell indirectly, thereby helps in sustaining life for longer Economical importance
8	Some examples	Carbohydrates, proteins, lipids, vitamins	Steroids, essential oils, phenolics, alkaloids

Secondary metabolites are biosynthetically derived from primary metabolites but are more limited in distribution, usually being restricted to a taxonomic group.

They may represent chemical adaptations to environmental stresses, or they may serve as defensive, protective or offensive chemicals against microorganisms, insects and higher herbivorous predators.

They are sometimes considered to be waste or secretary product of plant metabolism.

The various biosynthetic reactions occurring in plant cells are enzyme dependent.

Wherein enzymes act as catalysts of metabolism and it is through the control of enzymatic activity that plant metabolism is directed into specific biosynthetic pathways.

The enzymatic reactions are reversible and in plants, many a time, the secondary metabolites are synthesized and hydrolysed under the influence of more or less specific enzymes.

The elucidation of biosynthetic pathways in plants for the production of various plant metabolites has been extensively examined by means of isotopically labelled precursors.

With the advancement of tracer technology, it is possible to incorporate isotopes into presumed precursors of plant metabolites and use as markers in biogenetic experiments.

With the use of radioactive carbon ^{14}C and hydrogen ^{3}H and to a lesser extent

The basic carbon reduction cycle by which carbon dioxide is converted to sugar phosphate is of primary importance, both as an energy yielding process and also as a source of various metabolic intermediates.

Two biosynthetic pathways of special importance in breakdown of sugar are pentose phosphate cycle (direct pathway) and glycolysis.

The hexose phosphate formed due to biosynthetic degradation may then be utilized as such or otherwise converted by a series of metabolic reactions into triose phosphate or by a reversal of glycolysis, into a hexose.

In glycolysis hexose phosphate is split hydrolytically to yield hexose phosphate is split hydrolytically to yield triosephosphate, which can then be oxidized.

The total number of natural products for which biosynthetic investigations have been carried out are quite limited compared with the diversity and number of natural products, our accumulation of knowledge in biogenetic field makes it possible to predict the gross biogenetic origin of practically all plant products.

Photosynthesis has three basic steps:

1. Energy is captured from the sunlight.
2. Light energy is converted into chemical energy in the form of ATP and NADPH.

3. Chemical energy is used to power the synthesis of organic molecules (e.g. carbohydrates) from carbon dioxide (CO_2).

Photosynthesis may be the most important process in ecosystems, both brings in energy needed within the ecosystem, and produce oxygen (O_2) needed for cellular respiration, and the production of more ATP.
Photosynthesis is the process where plants convert sunlight into energy, then store it as carbohydrates, sugars, such as glucose.

Importance of photosynthesis in formation of primary metabolites

H_2O + light + ADP + P ---

Photosynthesis > e- + NADP+ + H ---

After the above steps occur in photosystem II, the electron is finally sent to photosystem I, where the following happens.

O_2 + ATP + e- > CO_2 + ATP + NADPH ---

Now there are two high energy molecules, fully charged and ready to be used. Plants make more energy that it needs immediately, so the NADPH and ATP are used to make glucose as follows:

NADPH > This happens in Calvin cycle.

$C_6H_{12}O_6$ The remaining $5C_3$ continues moving through the Calvin cycle, being turned back into the starter C5 organic molecule.

Next, 6ATP and 6NADPH energizes the binding of a C_3 to make a 6- carbon molecule (C_6), glucose.

The first step in the Calvin cycle is for the $3C_5$ to bind with $3CO_2$, producing a six 3-carbon organic molecules ($6C_3$).

Where C_5 is a five carbon molecule, such as pyruvate, when is recycled as glucose is synthesized.

$C_5 + CO_2 + ATP + NADPH \rightarrow C_6H_{12}O_6$

The purpose of the Calvin Cycle is to take the energy from photosystem I and fix carbon. Carbon fixation means building organic molecules by adding carbon onto a chain. The following formula summarizes the Calvin cycle.

The Calvin cycle is the last step in photosynthesis.

Calvin Cycle

This pathway produces energy in the form of ATP. The starting product glucose is completely oxydized to water and carbon dioxide.

Pyruvate may be regarded as the preliminary final product of the degradation. Pyruvate is fed into the citric acid cycle via an intermediate product.

Sugars and polysaccharides are transformed into glucose or one of its phosphorylated derivatives before being processed any further. In the course of degradation, ATP is produced.

Glycolysis represents an anabolic pathway common in both aerobic and anaerobic organisms.

Glycolysis (Embden-meyerhoff pathway)

Each mole of NADH leads to 3moles of ATP and each mole of FADH2 leads to 2moles of ATP. Therefore, for each mole of pyruvate which enters the TCA cycle, 12moles of ATP can be generated.

The 3 moles of NADH and 1 mole of FADH2 generated during each round of the cycle feed into the oxidative phosphorylation pathway.

The GTP generated during the succinate thiokinase (succinyl- CoA synthetase) reaction is equivalent to a mole of ATP by virtue of the presence of nucleoside diphosphokinase.

It accounts for the major portion of carbohydrate, fatty acid and amino acid oxidation and produces at the same time a number of biosynthetic precursors.

The citric acid cycle, is the common mode of oxidative degradation in eukaryotes and prokaryotes.

Topic: Shikimic acid pathway

Citric Acid Cycle (Kreb's cycle)

The overall reaction of glucose in terms of ADP and ATP is

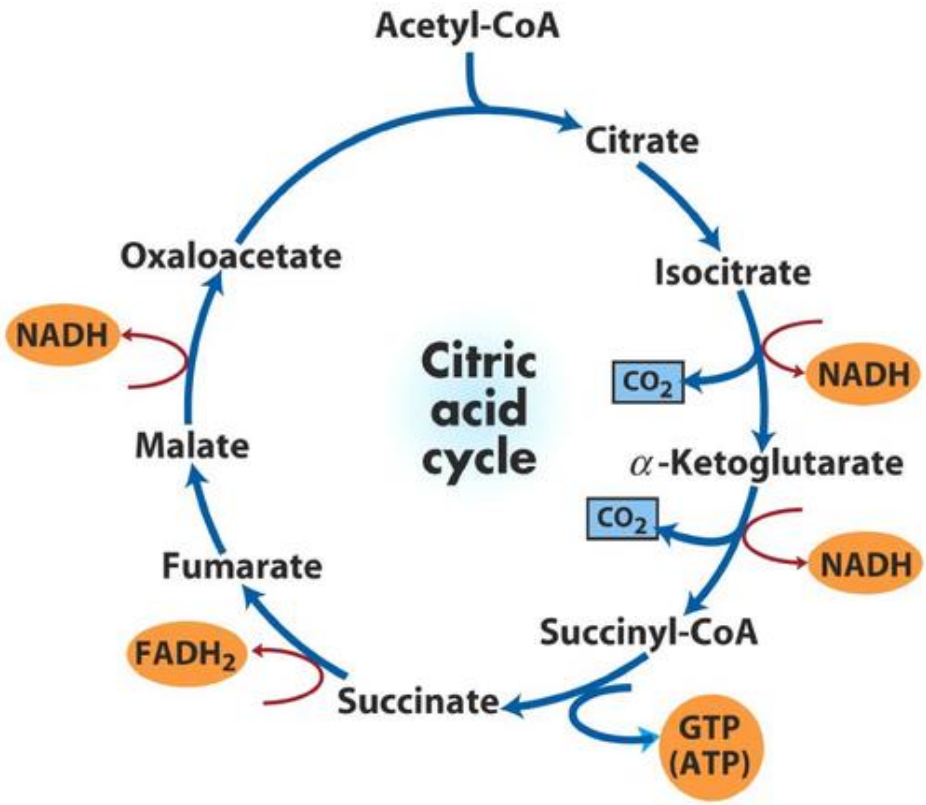
$C_6H_{12}O_6 + 6CO_2 + 38\ ADP + 38P\ (inorganic) \rightarrow 6H_2O + 6CO_2 + 38\ ATP$

Eventually the hydrogen combines with oxygen to form water.

During the process, the hydrogen atoms liberated are carried by coenzymes into the cytochrome system, in which energy is released in stages, with the possible formation of ATP and ADP and inorganic phosphate.

As a result of this, the energy-rich carbohydrate is eventually oxidized to $CO_2$and H_2O.

Storage carbohydrate such as the starch of plants or the glycogen of animals is made available for energy production by a process.

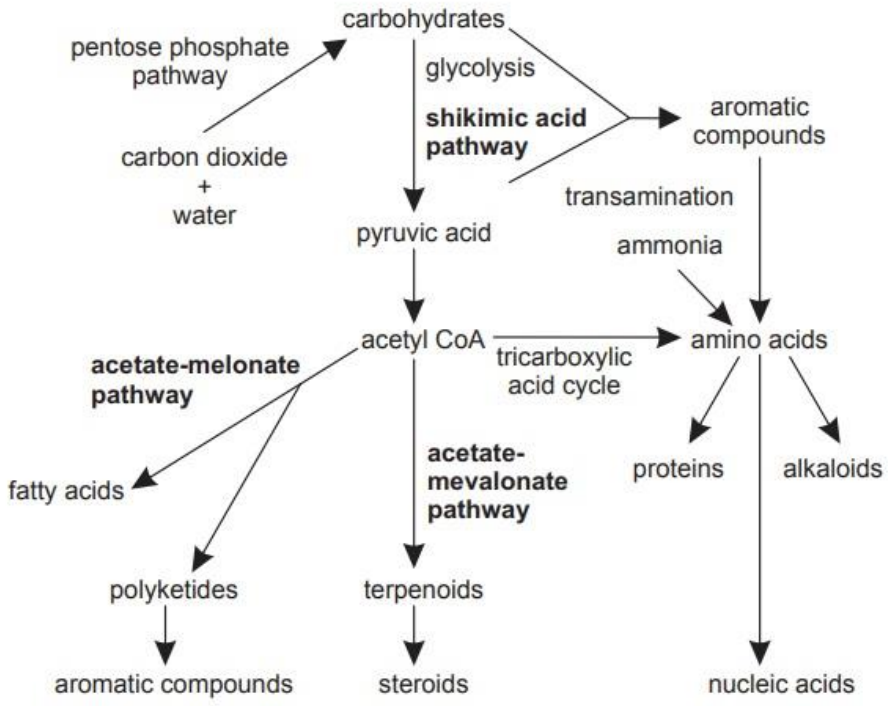


Carbohydrate Utilization

The primary and secondary metabolites derived from carbon metabolism difference between Primary and secondary metabolites

It involves 3 basic mechanism:

1. Acetate malonate pathway
2. Acetate-mevalonate pathway
3. Shikimic acid Pathway



Biosynthetic Pathway of Secondary Metabolites

1. Shikimic Acid Pathway

Shikimic acid is also the glycoside part of some hydrolysable

The elucidation of its structure was made nearly 50 years later.

Its name comes from the Japanese flower shikimi the Japanese star anise, Illiciumanisatum), from which it was first isolated in 1885 by Johan Fredrik Eykman.

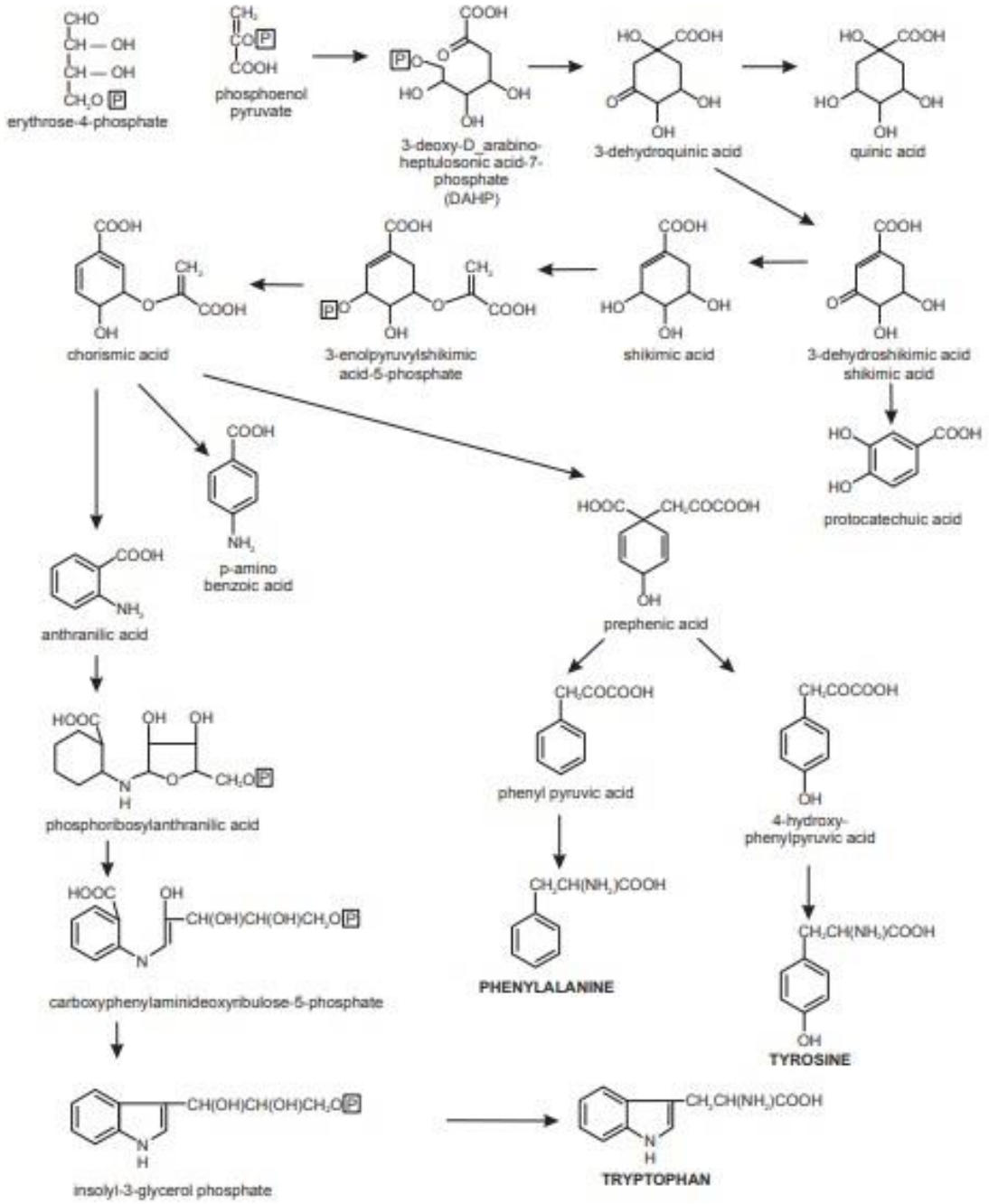
Animals can synthesize tyrosine from phenylalanine, and therefore is not an essential amino acid except for individuals unable to hydroxylate phenylalanine to tyrosine).

This pathway is not found in animals; therefore, phenylalanine and tryptophan represent essential amino acids that must be obtained from the animal's diet.

The shikimate pathway is a 7 step metabolic route used by bacteria, fungi, Algae, parasites, and plants for the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan).

The Shikimic acid pathway converts simple carbohydrate precursors derived from glycolysis and the pentose phosphate pathway to the aromatic amino acids.

The Shikimic acid pathway is a key intermediate from carbohydrate for the biosynthesis of C6-C3units (phenyl propane derivative).



Production of amino acids by shikimic acid pathway

Shikimic Acid Pathway

Although this reaction requires nicotinamide adenine dinucleotide (NAD) as a cofactor, the enzymic mechanism regenerates it, resulting in the net use of no NAD.

2-keto-3-deoxy-7-phosphoglucoheptonic acid is then transformed to 3- dehydroquinate (DHQ), in a reaction catalyzed by DHQ synthase.

Phosphoenolpyruvate and erythrose-4-phosphate react to form 2-keto3-deoxy-7-phosphoglucoheptonic acid, in a reaction catalyzed by the enzyme DAHP synthase.

Then 5-enolpyruvylshikimate-3-phosphate is transformed into chorismate by a chorismate synthase.

The next enzyme involved is shikimate kinase, an enzyme that catalyzes the ATP dependent phosphorylation of shikimate to form shikimate 3-phosphate.

Shikimate-3-phosphate is then coupled with phosphoenol pyruvate to give 5- enolpyruvylshikimate-3-phosphate via the enzyme 5-enolpyruvylshikimate-3- phosphate (EPSP) synthase.

DHQ is dehydrated to 3-dehydroshikimic acid by the enzyme 3-dehydroquinate dehydratase, which is reduced to shikimic acid by the enzyme shikimate dehydrogenase, which uses nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor.

Prephenic acid is then synthesized by a Claisen rearrangement of chorismate by Chorismate mutase. Prephenate is oxidatively decarboxylated with retention of the hydroxyl group by Prephenate dehydrogenase to give phydroxyphenylpyruvate, which is transaminated using glutamate as the nitrogen source to give tyrosine and α- ketoglutarate.

Shikimic acid is a precursor for: Other compounds

The latter compound spontaneously rearranges to gallic acid.

Gallic acid biosynthesis Gallic acid is formed from 3-dehydroshikimate by the action of the enzyme shikimate dehydrogenase to produce 3, 5- didehydroshikimate.

The phenylpropanoids are then used to produce the flavonoids, coumarins, tannins and lignin.

Starting Point in The Biosynthesis of Some Phenolics Phenyl alanine and tyrosine are the precursors used in the biosynthesis of phenylpropanoids.

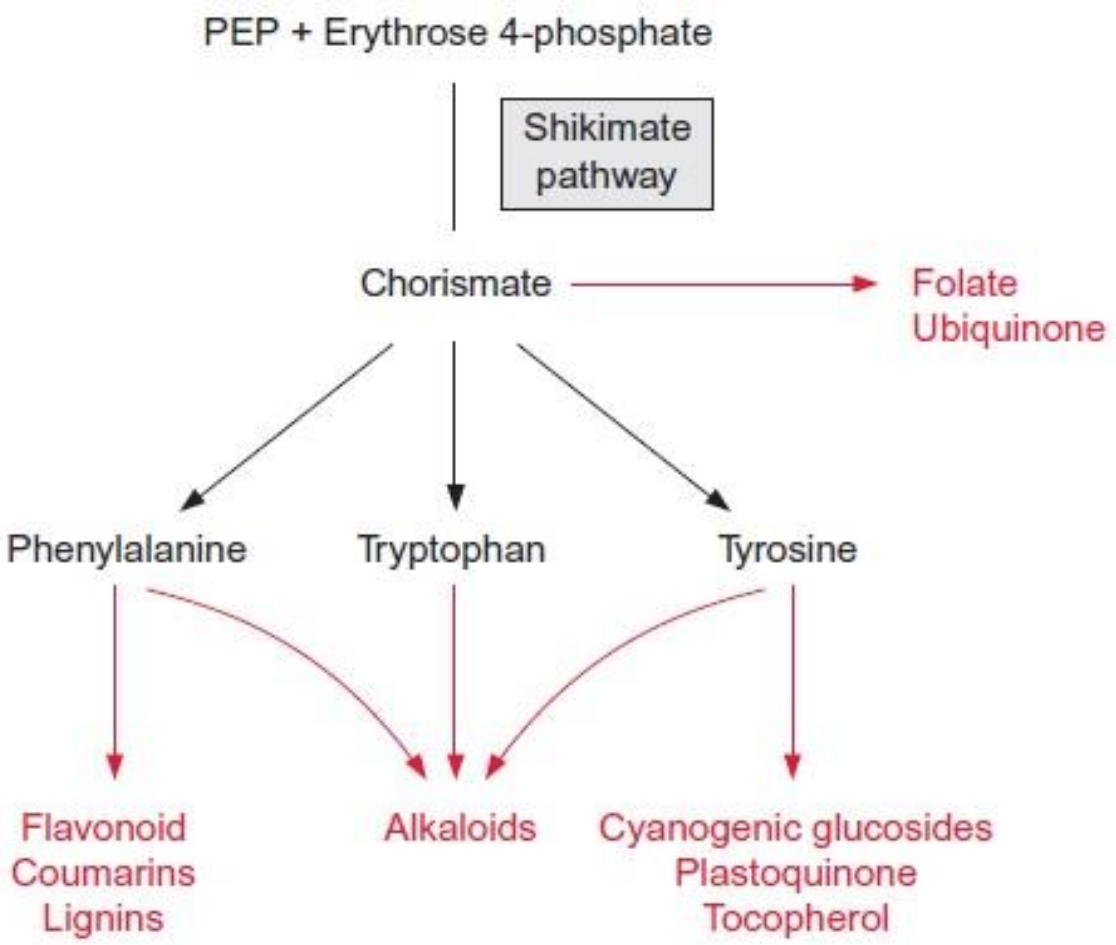


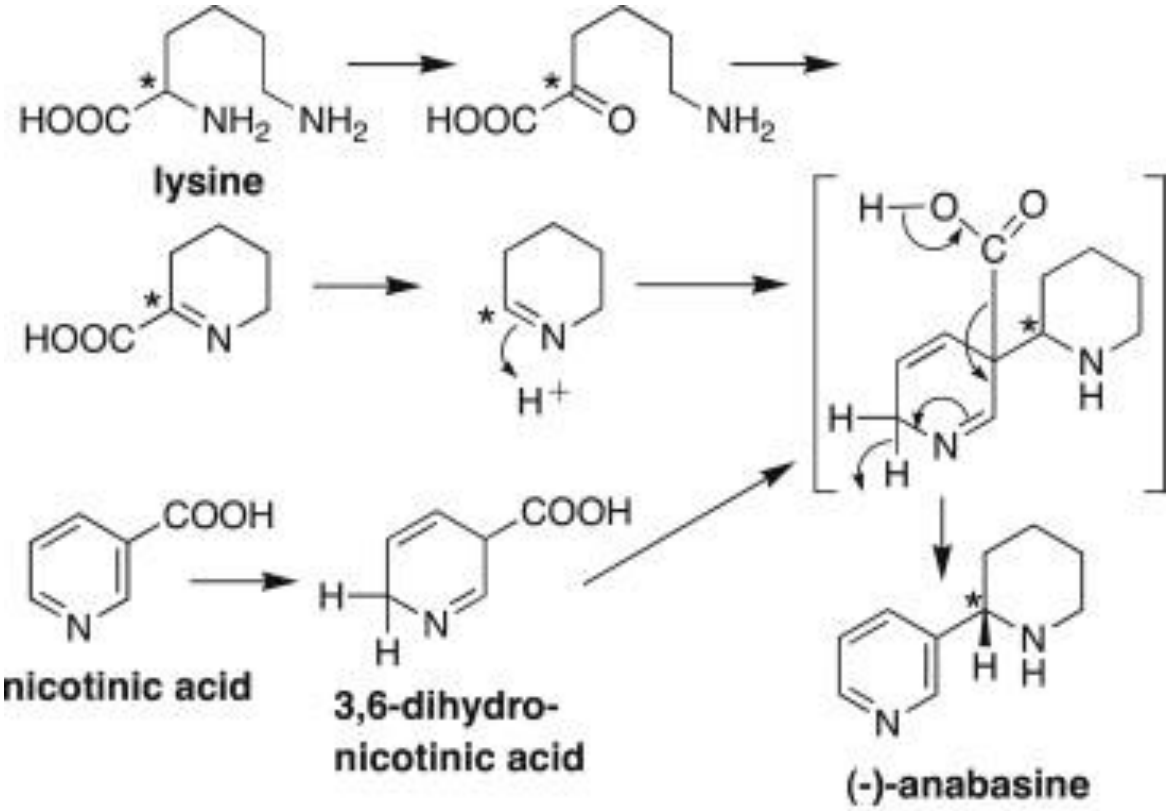
Figure 10.21 Several secondary metabolites are synthesized via the shikimate pathway.

Biosynthesis of alkaloids:

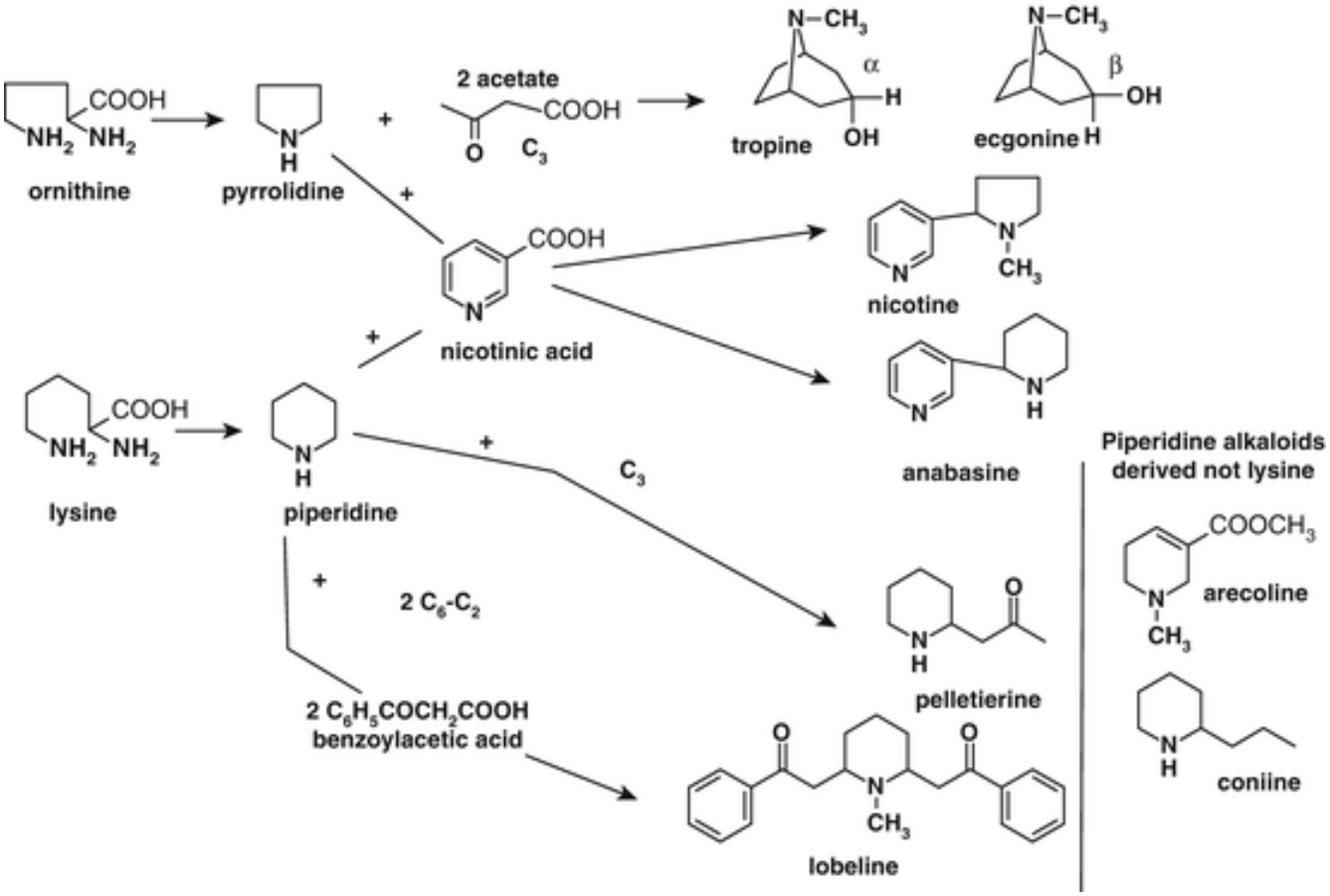
Most **alkaloids** derive from an amino acid as a precursor, such as ornithine, arginine, lysine, phenylalanine, tyrosine or tryptophan.

Alkaloids derived from lysine:

Lysine and its associated compounds are responsible for the biogenesis of anabasine, lupinine, isopelletierine and other related alkaloids



Biogenesis of (-)-Anabasine



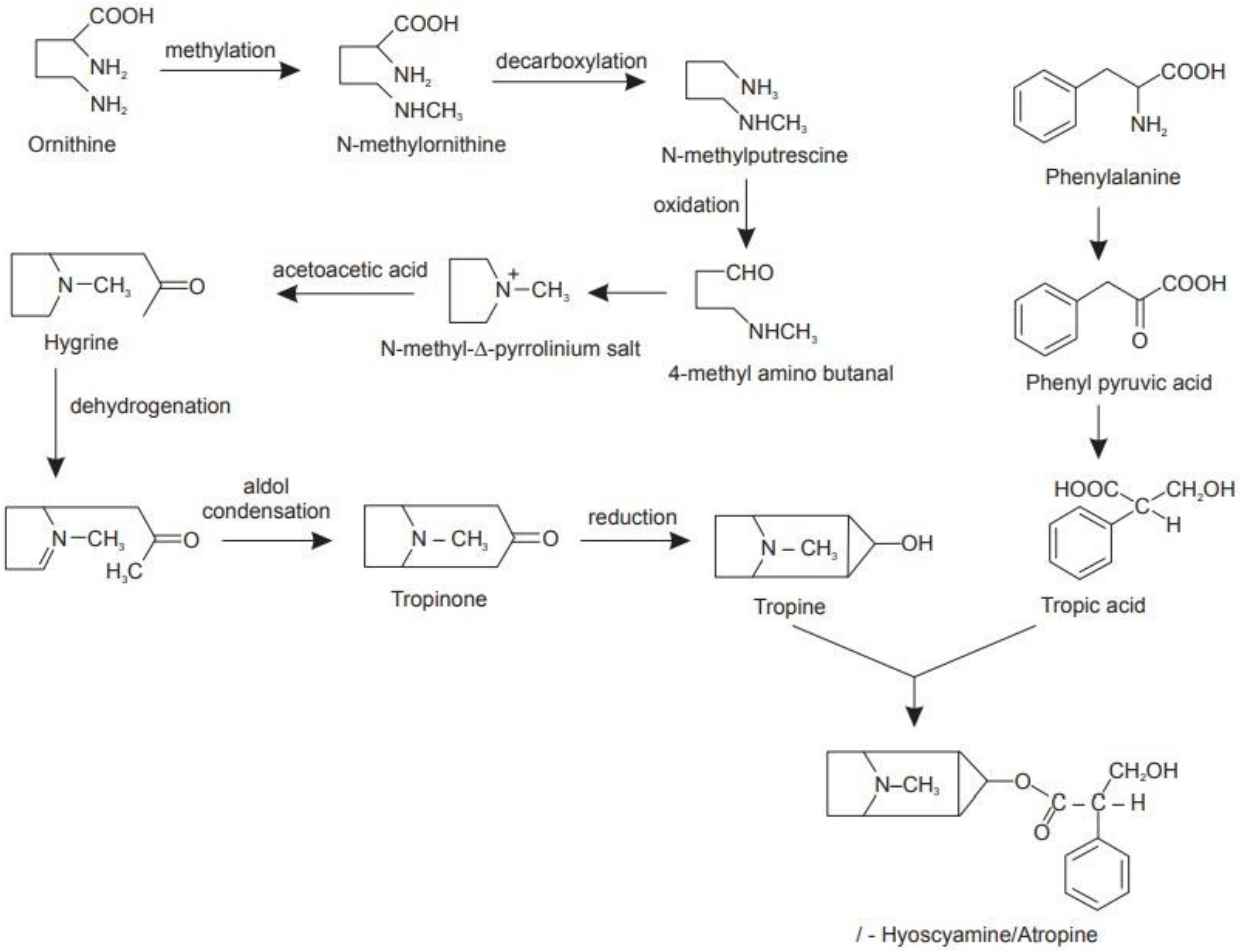
Alkaloids derived from ornithine:

The studies have related that ornithine is incorporated into both pyrrolidine and tropane alkaloids.

Ornithine is incorporated stereospecifically and unsymmetrically into pyrrolidine ring of tropane nucleus, the α – carbon of ornithine becoming the C1 of tropane nucleus.

The remaining three carbon atoms derived from acetate, thus completing peptidine moiety.

Methionine serves as the methyl group donor, whereas phenylalanine is the precursor of tropic acid. The different alkaloids derived from ornithine are grouped together.



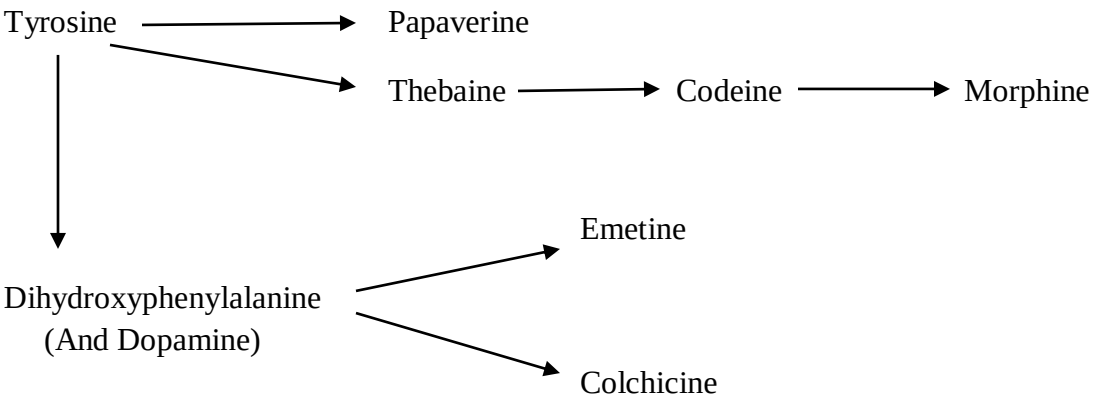
Alkaloids derived from phenylalanine, tyrosine and related aminoacids

These amino acids and their corresponding decarboxylation products serve as the precursor for a large number of alakloids including ephedrine, colchicine and opium alkaloids.

Earlier it was shown that tyrosine and dopamine could serve as precursor of morphine.

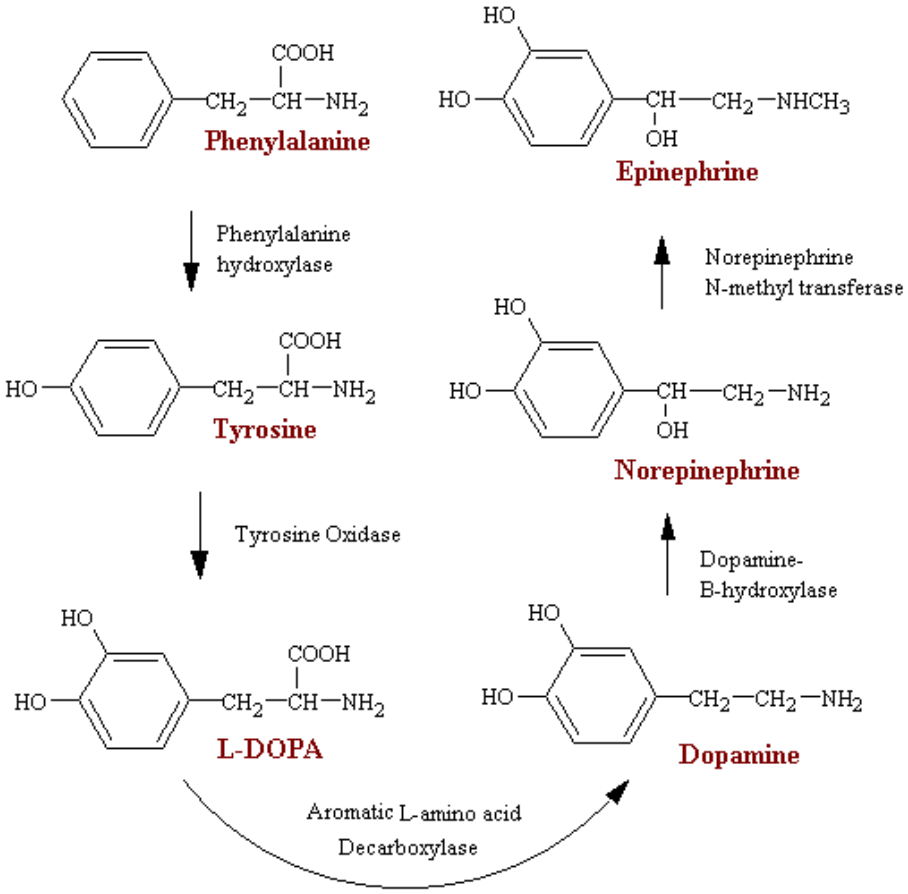
It was also proved that the first of the opium alkaloids synthesized is thebaine, followed by codeine and morphine.

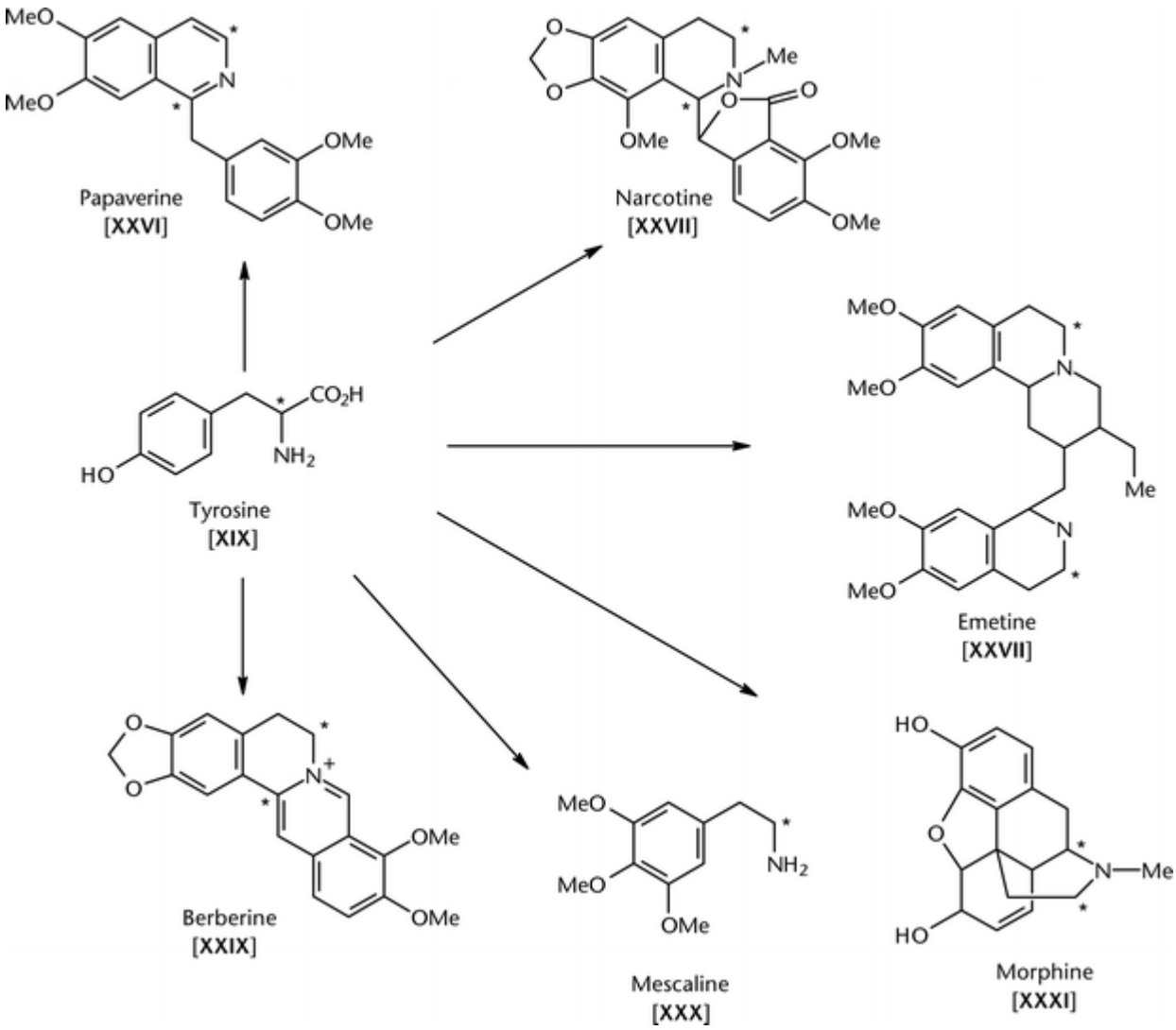
Phenylalaninine → Ephedrine
Phenylalaninine → Mescaline



Topic: Acetate mevalonate pathway

Some alkaloids derived from phenylalanine, tyrosine and their derivatives





2. Acetete- Mevalonate pathway

Acetate-Mevalonate Pathway Since a long time biochemists were aware of the involvement of acetic acid in the synthesis of cholesterol, squalene and rubber-like compounds. The discovery of acetyl coenzyme A called as ‘active acetate’ in 1950, further supported the role of acetic acid in biogenetic pathways. Later, mevalonic acid was found to be associated with the acetate.

Mevalonic acid further produced isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP). These two main intermediates IPP and DMAPP set the ‘active isoprene’ unit as a basic building block of isoprenoid compounds.

Both of these units yield geranyl pyrophosphate (C10-monoterpenes) which further association with IPP produces farnesyl pyrophosphate (C15-sesquiterpenes).

Farnesyl pyrophosphate with one more unit of IPP develops into geranyl-eranyl pyrophosphate (C20-diterpenes). The farnesyl pyrophosphate multiplies with its own unit to produce squalene, and its subsequent cyclization gives rise to cyclopentanoperhydrophen antherene skeleton containing steroidal compounds like cholesterols and other groups like triterpenoids.

The acetate mevalonate pathway thus works through IPP and DMAPP via squalene to produce two different skeleton containing compounds, that is, steroids and triterpenoids.

It also produces vast array of monoterpenoids, sesquiterpenoids, diterpenoids, carotenoids, polyprenols, and also the compounds like glycosides and alkaloids in association with other pathways

The **mevalonate pathway**, also known as the **isoprenoid pathway** or **HMG-CoA reductase pathway** is an essential metabolic pathway present in eukaryotes, archaea, and some bacteria.

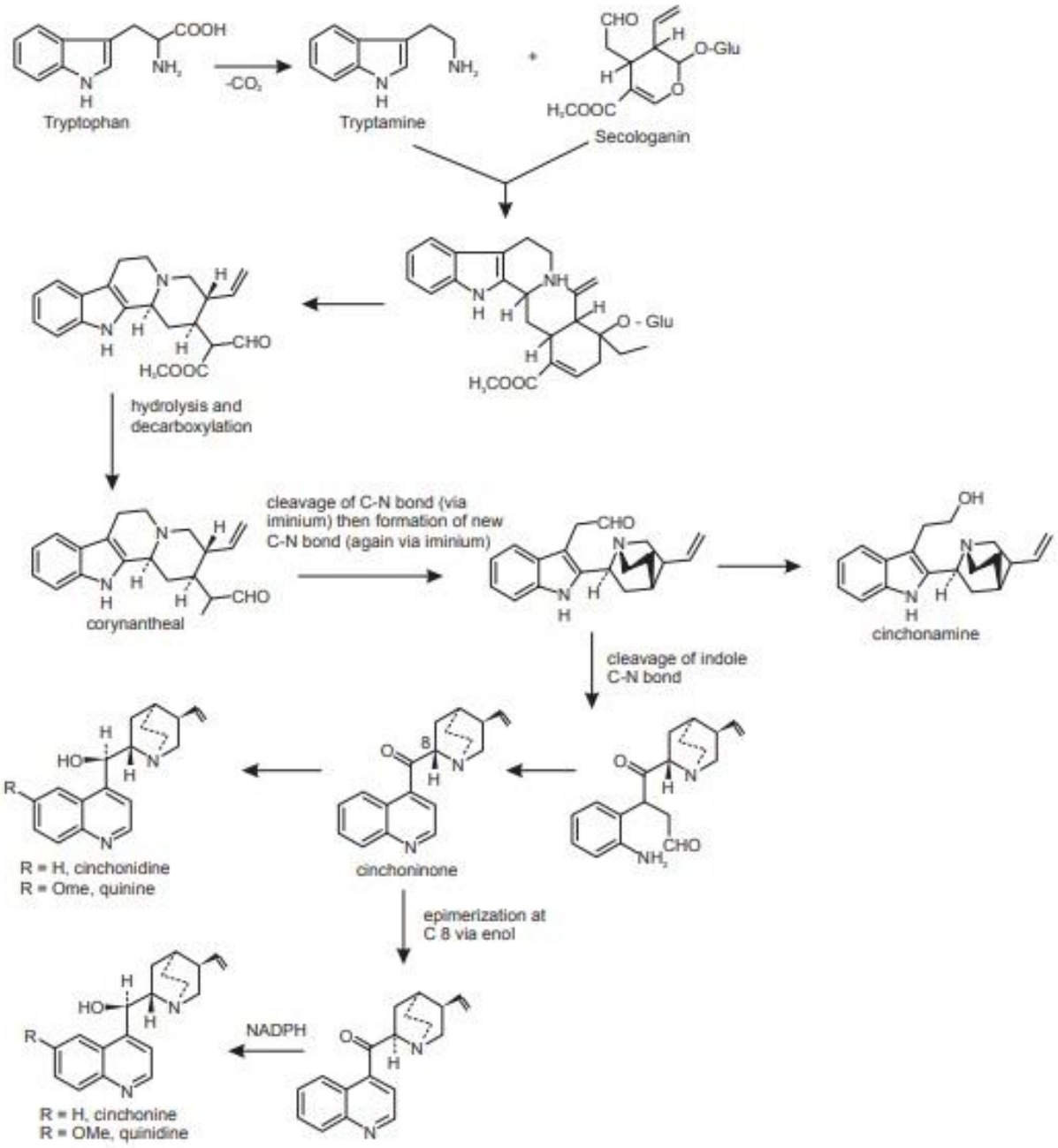
The pathway produces two five-carbon building blocks called isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which are used to make isoprenoids, a diverse class of over 30,000 biomolecules such as cholesterol, vitamin K, coenzyme Q10, and all steroid hormones.

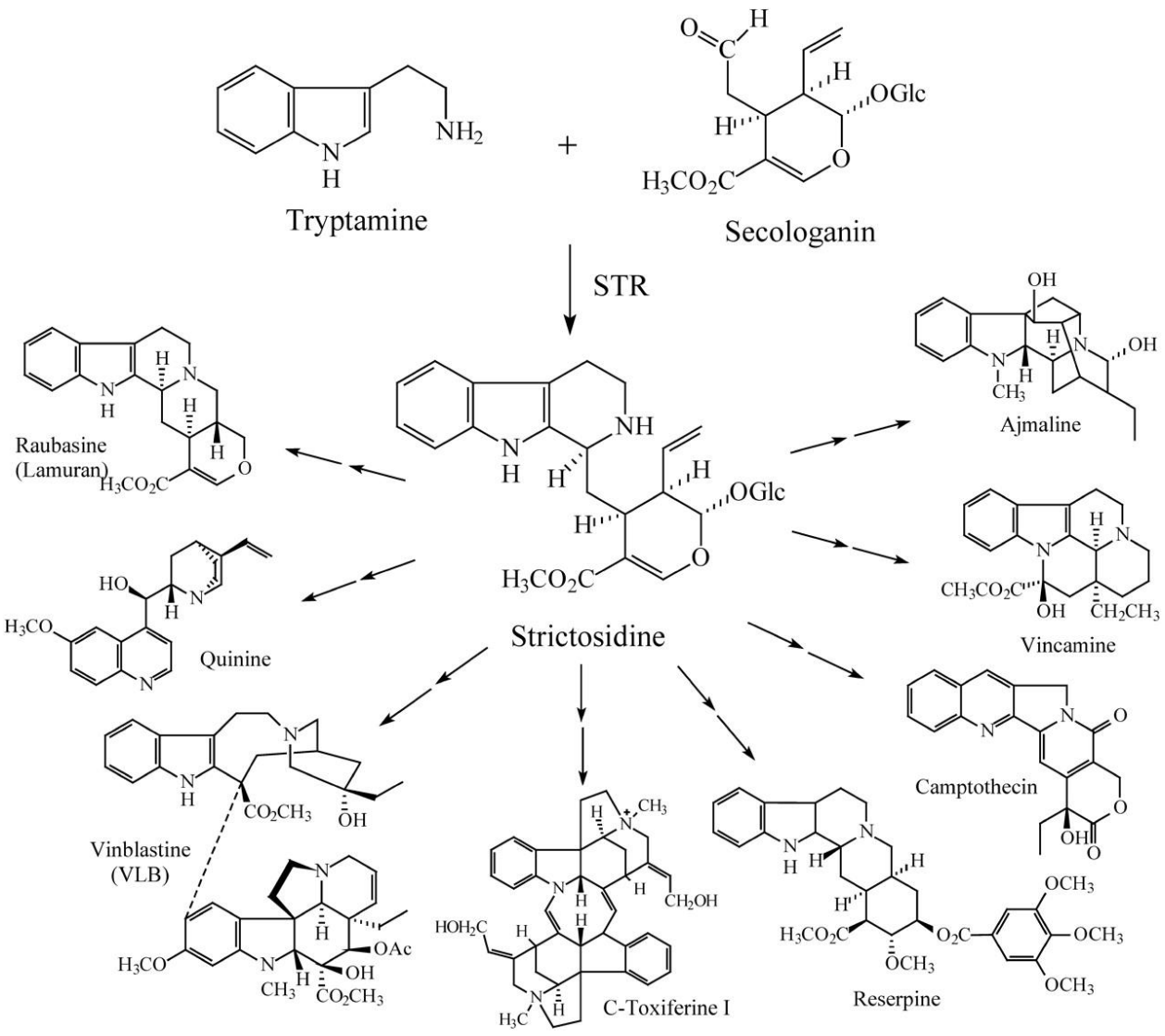
The mevalonate pathway begins with acetyl-CoA and ends with the production of IPP and DMAPP. It is best known as the target of statins, a class of cholesterol lowering drugs. Statins inhibit HMG-CoA reductase within the mevalonate pathway.

Alkaloids derived from tryptophan

- ✓ The biosynthesis of quinine and related alkaloids of cinchona proceeds through a route indicating transformation of indole to quinoline.
- ✓ In regard with the source of non-tryptamine derived portion, however, participation of a monoterpene glucoside, secologanin, from Mevalonate-pathway has been demonstrated.
- ✓ The most significsnt feature of quinine biosynthesis is cleavage of benzylpyrrole ring of tryptophan moiety and rearrangement to form the quinoline nucleus.
- ✓ Tryptophan and its decarboxylation product tryptamine, serve as the precursor for biosynthesis of a large class of indole alkaloids.

- ✓ The non-tryptophan portions of alkaloids are, however derived from monoterpenoid precursors which are designated as the Coryanthe, Iboga and Aspidosperma types.
- ✓ The reactive form of terpene involves an aldehyde group. The loss of one carbon atom during biogenesis is to give C_9 unit appears to be largely common.
- ✓ It is believed that Coryanthe type monoterpenoids moiety is metabolically most primitive.
- ✓ Condensation of tryptamine or tryptophan wth secologenin, a monoterpene glucoside, gives rise to nitrogenous glucoside, vincoside, from which a great variety of indole alklaoids, including monomeric alkaloids in Catharanthus roseus, are formed.


Tryptophan
Tryptamine
Secologanin
hydrolysis and decarboxylation
corynantheal
cleavage of C-N bond (via iminium) then formation of new C-N bond (again via iminium)
cinchonamine
cleavage of indole C-N bond
cinchoninone
R = H, cinchonidine
R = Ome, quinine
epimerization at C 8 via enol
NADPH
R = H, cinchonine
R = OMe, quinidine



The rauwolfia alkaloids such as reserpine, recinnamine, serpentine, ajmalicine, etc. are derived from a Coryanthe type monoterpenoid precursor.

The ergot alkaloids are also derived from a combination of acetate metabolism and tryptophan. The condensation of dimethylallylpyrophosphate at 4- position of tryptophan takes place as the first step inagroclavine.

The latter undergoes stepwise oxidation to elymoclavine and eventually lysergic acid. The carboxylic group of lysergic acid forms a peptide linkage with an amino group of a variety of amino acids to yield the medicinally important ergot alklaoids

Topic: Acetate- mevalonate pathway

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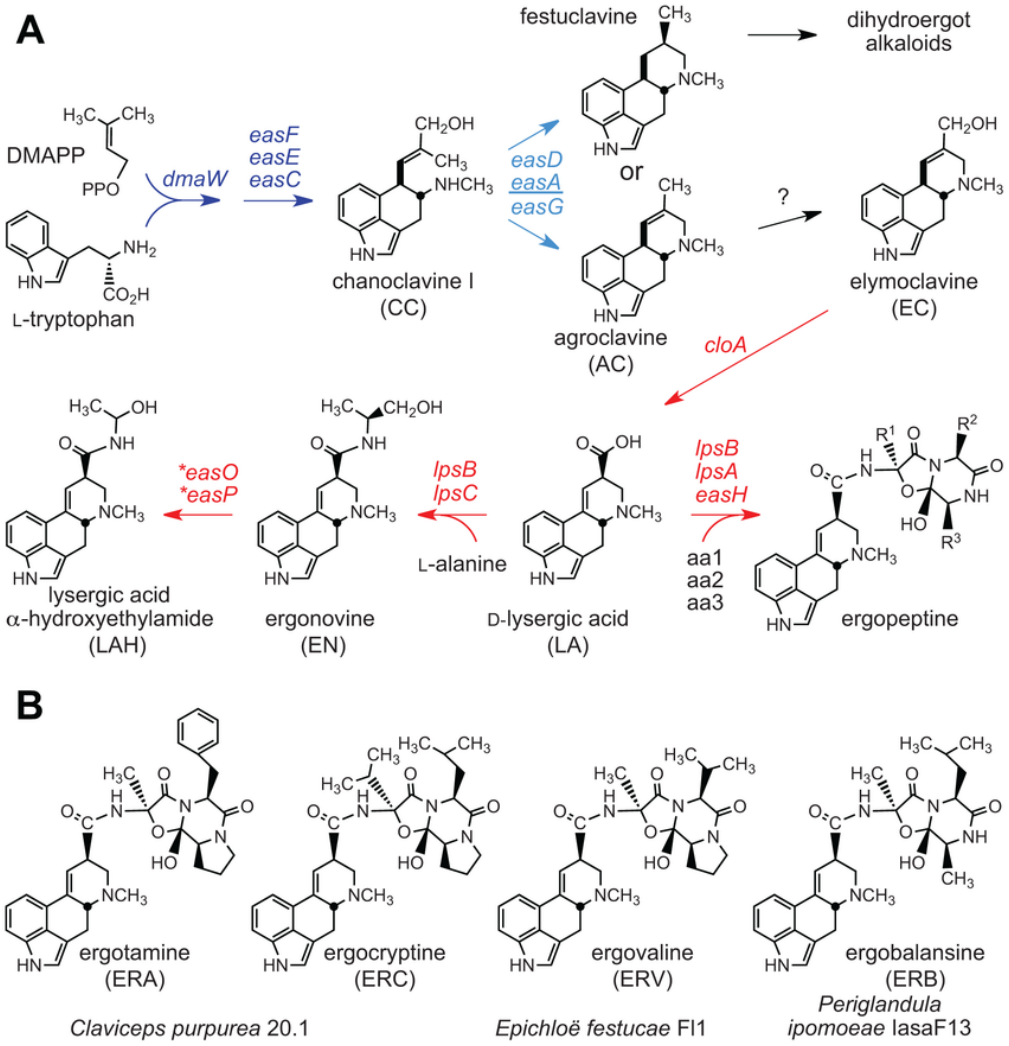
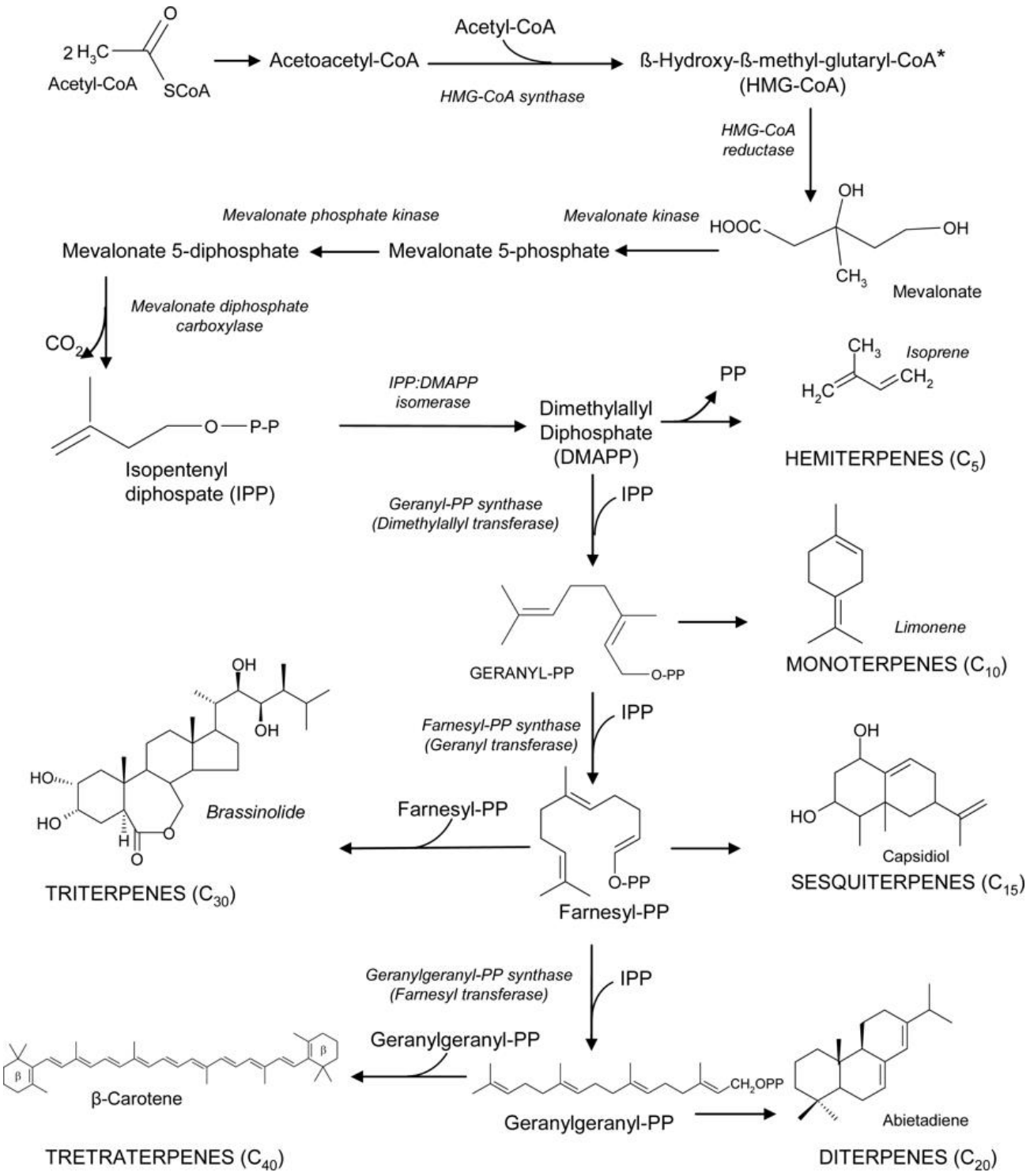


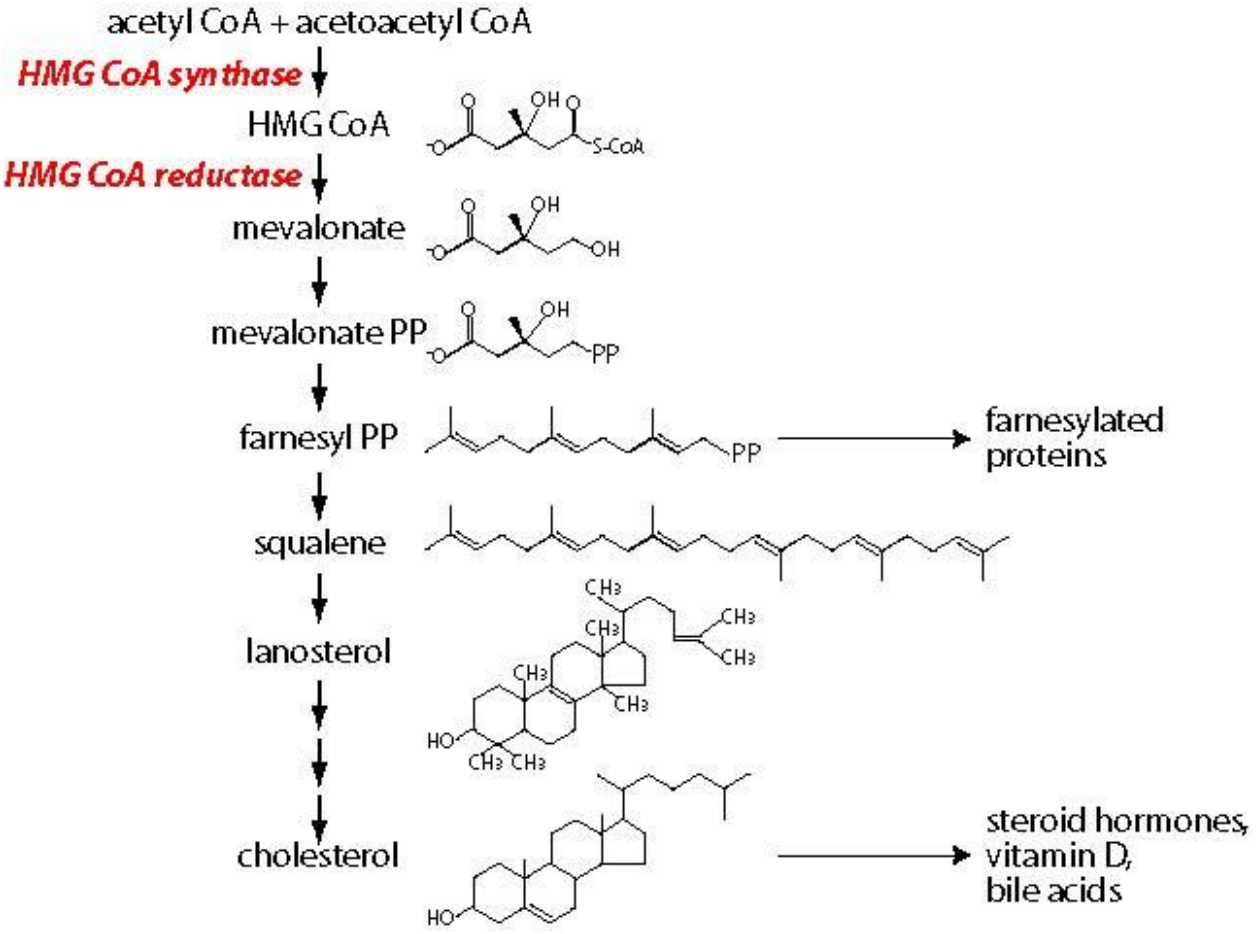
Biosynthesis of Isoprenoid compound



Acetate-mevalonate pathway

The discovery of mevalonic acid (3, 5-dihydroxy-3-methylavaleric acid) in 1956 and demonstration of its incorporation by living issues into these compound to which the isoprene rule is applied, were milestones in understanding biogenesis of terpene derivatives. It is established by research involving tracer technique, inhibitor studies and ionophoresis that C_5 compound – isopentynyl pyrophosphate is derived from mevalonic acid pyrophosphate by decarboxylation and dehydration.

The C10 compound geranyl pyrophosphate is formed by condensation of isopentenyl pyrophosphate with isomeric dimethylallyl pyrophosphate. Further, C6 units are added by participation of more isopentenyl pyrophosphate units. From geranyl and farnesyl pyrophosphate various isoprenoid structures are synthesized.



Topic: Acetate-melonate pathway **Book Reference: T2**

Acetate-Malonate Pathway

Acetate pathway operates functionally with the involvement of acyl carrier protein (ACP) to yield fatty acylthioesters of ACP. These acyl thioesters forms the important intermediates in fatty acid synthesis. These C2 acetylCoA units at the later stage produces even number of fatty acids from n-tetranoic (butyric) to n-ecosanoic (arachidic acid). Unsaturated fatty acids are produced by subsequent direct dehydrogenation of saturated fatty acids. Enzymes play important role in governing the position of newly introduced double bonds in the fatty acids.

Biosynthesis of triglycerides:

It occurs in 2 stages.

First stage is biosynthesis of fatty acids molecule while second stage is associated with formation of a triglyceride structure. For the formation of a fatty acid molecule, acetate is a precursor and reaction proceeds with presence of NADPH2, Mn++, ATP and two enzyme complexes (CoASH and Acetyl CoA) and Carbon dioxide.

Coenzyme A (CoA) comprises of adenosine 3, 5-diphosphate, Pantothenic acid-4-phosphate and thioethanolamine. Acetyl CoA is the activated form of acetate moiety. The total reaction pathway is represented here.

This reaction occurs only in the formation of saturated fatty acids. In case of unsaturated fattyacids, branched chain or other type of fatty acid, the biosynthetic pathways are not clearly known.

Second stage is the formation of triglyceride structure where required glycerol enters the reaction in the form of L-α-glycerophosphate, while fatty acids come as fatty acyl CoA.

Tracer techniques:

There are different biosynthetic pathways in plants like

1. Shikmic acid pathway
2. Mevalonic acid pathway
3. Acetate pathway

Various steps and intermediates are involved in biosynthetic pathways. These can be investigated by means of following techniques.

- Tracer techniques
- Use of mutant strain
- Use of isolated organs
- Grafting method

Tracer techniques It can be defined as a technique which utilizes a labeled compound to find out / to trace different intermediates and various steps in biosynthetic pathways in plants at a rate and time. The labeled compound can be prepared by use of two types of isotopes.

a. Radioactive isotopes

b. Stable isotopes

a. Radioactive isotopes:

Examples: C^{14}, P^{32}, H1, Na^{24}, S^{32}, P^{35}

- For biological investigations –carbon and hydrogen
- For metabolic studies – Sulphur, phosphorus, alkali, alkaline earth metals
- For studies on proteins and aminoacids – labeled nitrogen

b. Stable isotopes:

Examples: H^{22}, C^{13}, N^{15}, O^{18}

- These isotopes are scarcely available in nature.
- They are used for labeling compounds as possible intermediates in biosynthetic pathways.
- They are detected by mass spectroscopy and NMR spectroscopy.

Significance of tracer techniques:

1. Tracing of biosynthetic pathway: By incorporation of radioactive isotope into the precursors / starting material, the whole biosynthetic pathway can be traced Ex: By incorporation of radioactive isotope of carbon C^{14} in to phenylalanine, the biosynthesis of cyanogenetic glycoside prunasine can be traced.

2. Location and quantity of compound containing tracer: If location and quantity of glucose is determined in a biological system C^{14} labeled glucose may be used. The labeled glucose being

chemically indistinguishable from native glucose will mix completely with available glucose polls in the body of organism studied. Both location and quantity of glucose present in tissues can then be determined by radioactive assay.

3. Different tracers for different studies: For studies on proteins, alkaloids, amino acids, labeled N2 atom give more specific information than labeled carbon.

Criteria for tracer techniques:

- The starting concentration must be sufficient so as to withstand resistance with dilution in course of metabolism.
- The labeled compound must be involved in synthesis reaction.
- The labeled compound must be harmless to system to which it is used.
- Proper labeling is required. For proper labeling physical and chemical nature of compound must be known.
- The tracer should be highly pure. The radioactive isotope with greater half life period is preferred¬ Ex: ^{10}C - ^{11}C -8.8 Sec to 20mins
- ^{14}C –about 5000 years so it is preferred.

Steps for tracer techniques:

1. Preparation of labeled compound.

2. Introduction of labeled into biological system.

3. Separation and determination of labeled compound in various biochemical fractions at a later time.

Preparation of labeled compound:

Many compounds which are most conveniently prepared from natural sources

Ex: By growing chlorella in an atmosphere containing $^{14}CO_2$, all the carbon compounds of the organisms become labeled as ^{14}C.

The 3H labeled compound are commercially available Tritium labeling is effected by catalytic exchange in aqueous media by halogenation of unsaturated compound with tritium gas. ^{3}H is pure beta emitter of low intensity and its radiation energy is lower than ^{14}C.

By use of organic synthesis Grignard reagent

(CH_3MgBr). $CH_3MgBr + 14CO_2 \rightarrow CH_3 14COOMgBr + H_2O$

$CH_3 14COOMgBr + H_2O \rightarrow CH_3\ 14COOH + Mg\ (OH)\ Br$

Topic: Tracer technique **Book Reference: T2**

Introduction of labeled compound into biological system:

- **Root feeding and stem feeding:**

It is the most common method. Selection of the plant part depends upon the site of biosynthesis of desired metabolites.

Root biosynthesis – Tobacco alkaloids

Stem biosynthesis - Latex (Euphorbiaceae)

- **Direct injection method:**

Hollow stems (Umbelliferae)

Capsules (Opium poppy)

- **Wick feeding:**

To carry out feeding on plants rooted in soil/other support without disturbance to roots wick feeding is possible.

In this method cotton strands are passed through the plant stem. The terminal ends of these cotton strands are immersed in the reagent labeled with radioisotope.

- **Floating method:**

When the small amount of material is available leaf discs chopped leaves are made to float on the substrate solution.

- **Spray method:**

This method is used for those reagents which are readily absorbed from the leaf surface.

Eg : Steroids

The plant is exposed to the organic compound labeled with the radioisotope for a short period of time using one of the above techniques. The biosynthesis occurs sequentially and at each step radioactive products are formed. These products are isolated and identified.

Separation and determination of labeled compound:

Separation of compound depends upon the type of plant material.

- Soft and fresh tissue - Maceration, Infusion
- Hard tissue - Decoction hot percolation
- Unorganized drugs – Maceration

Different solvents are used depending upon the type of plant material.

- Fat, oils, alkaloids, glycosides - nonpolar solvent
- Flavonoids - slightly polar solvents
- Phenols - polar solvents

Determination oflabeled compound by various methods like Geiger muller counter

- Scintillation counter
- Auto radiography
- Gas ionization chamber
- Bernstein ballentine counter
- Mass spectrometer
- NMR spectrometer

Methods of tracer techniques:

I. Competitive feeding

II. Precursor product sequence method

III. Sequential analysis method

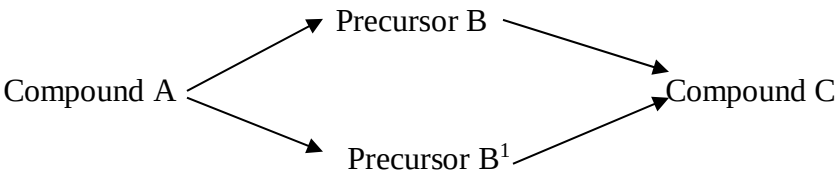
IV. Isotope incorporation method

1. Competitive Feeding:

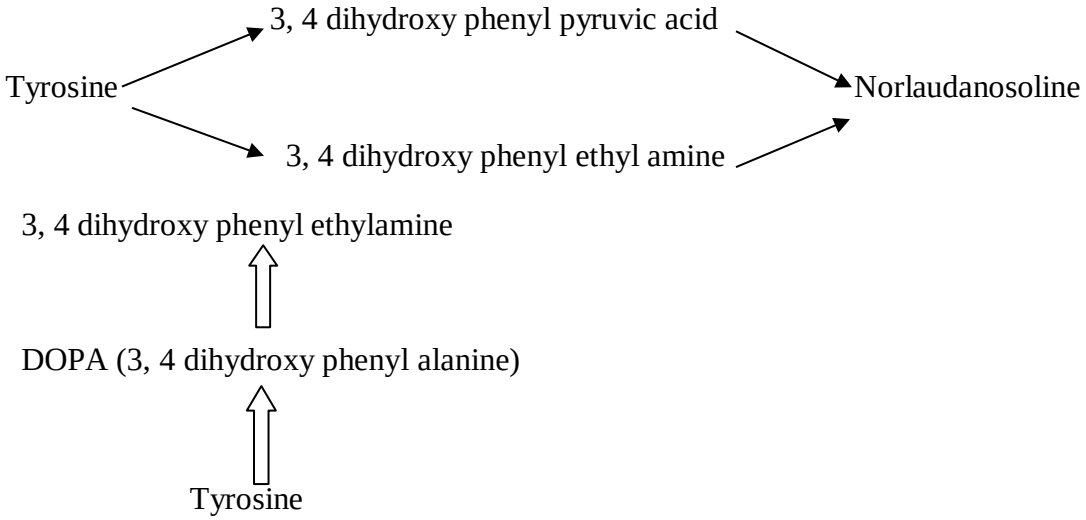
By this method, one can accurately determine the actual precursor involved in the biosynthesis of a particular metabolite.

Two precursors are then introduced into two separate groups of plants. If the radioactivity is observed in the group receiving precursor B and not in B1 receiving group, then the biosynthetic pathway for particular metabolite follows order.

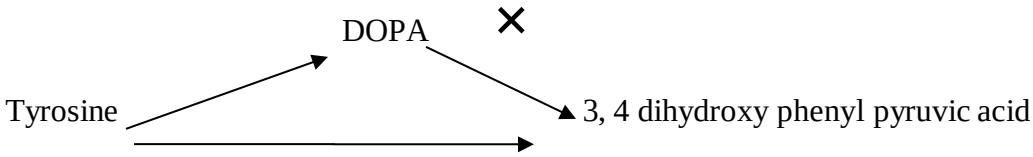
A → B → C but not A → B1 → C



E.g :



Similarly it was considered that 3, 4 dihydroxy phenyl pyruvic acid would also be synthesized through DOPA but by labeling experiments and competitive feeding it is confirmed that tyrosine directly gives 3, 4 dihydroxy phenyl pyruvic acid.



Applications:

Used for elucidation of biogenesis of propane alkaloids, biosynthesis of alkaloids like connine, conhydrine (hemlock) can be studied.

2. Precursor product sequence method:

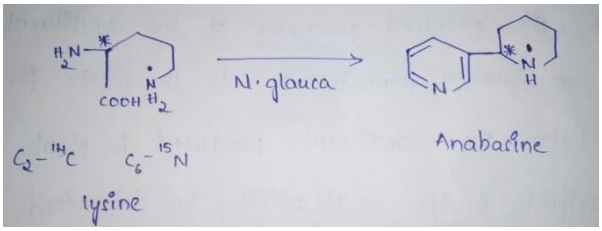
In this method, the presumed precursor of the constituent under investigation on a labeled form is fed into the plants for suitable time period. Later the constituents produced in plant are isolated and purified and its radioactivity is determined.

Disadvantages:

Sometimes radioactivity of isolated compound alone is not usually sufficient evident that the precursor fed during the studies is a direct precursor. It is due to the fact that the compounds may enter the general biogenetic pathways and get distributed randomly through the array of phytochemical constituents.

In such cases further proof can be obtained from studies by incorporating precursors from double and triple labeling experiments.

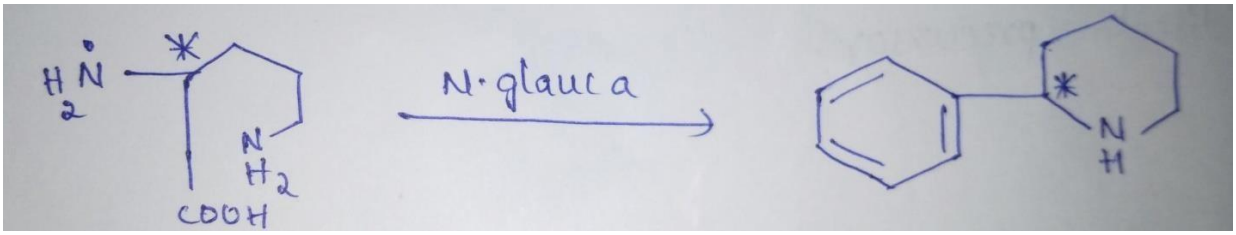
Eg : Anabasine



It is an alkaloid obtained from Nicotiana glauca.

Precursor - Lysine (which forms piperidine ring)

Radioactive labeled lysine (labeled with $_6C^{14}$ and $_7N^{15}$) at positions 2, 6 respectively was fed to Nicotiana



glauca.

Biogenesis resulted in formation of anabasine alkaloid. With the help of radioactivity determination study, it was proved that radioactive N_2 and C at 6th and 2nd positions respectively.

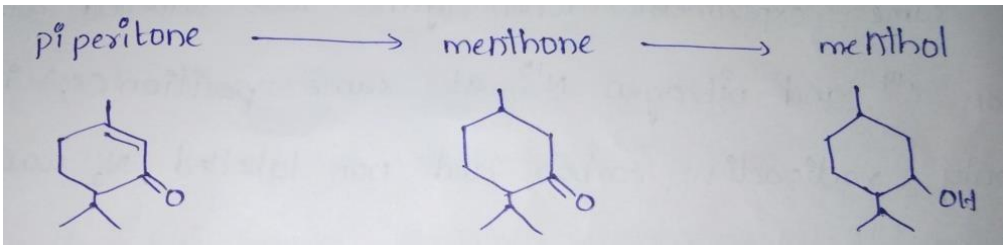
Suppose in the same experiment when lysine was labeled with radioactive carbon C^{14} and nitrogen N^{15} at same position it was proved that only radioactive carbon and non-labeled N_2 was involved in the formation of piperidine ring.

Applications:

1. Stopping of hordenine production in barley seedlings after 15- 20 days of germination.
2. Applied in study of biosynthesis of morphine and erogot alkaloids.

3. Sequential analysis method:

- The principle of this method of investigation is to grow plant in atmosphere of $^{14}CO_2$ and then analyze the plant metabolites at a given time intervals to obtain the sequence in which various related compounds become labeled.
  Example:
  Menthapiperita$^{14}CO_2$ for about 5 mins provided the evidence of probable biosynthetic sequences.



Applications:

1. $^{14}CO_2$ and sequential analysis has been very successfully used in elucidation of carbon in photosynthesis.
2. Determination of sequential formation of opium and tobacco alkaloids.

4. Isotope incorporation:

- This method provides information about the position of the bond cleavage and their formation during reaction.
- Example: the cleavage of glucose -1- phosphotase is catalysed by alkaline phosphatase.
- Reaction occurs with cleavage of either C-O bond/P-O bond. If the reaction is carried in presence of H_2O 18 enriched H_2O, the cleavage C-O cleavage path yields glucose containing one atom of ^{18}O.
- The P-O cleavage is characterized by phosphate containing one atom ^{18}O. During experimentation, the label invariably appears in inorganic phosphate identifying the P-O bond as the cleavage.

General applications of tracer techniques:

1. Study of sequence cyclisation by use of ^{14}C, ^{3}H labeled mevalonic acid.
2. Inter relationship among 4-methyl sterol and 4, 4 dimethyl sterols by use of ^{14}C acetate.
3. Terpenoid biosynthesis by chloroplasts, isolated in organic solvent by use of two ^{14}C mevalonate.
4. Study of formation of scopoletin by use of labeled phenyl alanine.
5. Study of formation of cinnamic acid in pathway of coumarin from labeled coumarin.
6. Origin of carbon and nitrogen atoms of purine ring system by use of ^{14}C or ^{15}N labeled precursor.
7. By using ^{45}Ca as a tracer it has been found that the uptake of calcium by plants from the soil is nearly the same both for CaO and $CaCO_3$ in acidic soils.
8. By adding ammonium phosphate labeled with ^{32}P of known specific activity thus uptake of phosphorous is followed by measuring the radioactivity as label reaches first the lower parts of the plant then the upper parts, branches, leaves etc
9.

II. Isolated organs, tissues and cells:

Cultures of the organs, tissues and cells growing under controlled aseptic conditions can be used for feeding experiments.

The radioactive tracers can be introduced by this process to the parenchymatous tissue of shoots, leaves, roots or other plant structures and the further analysis of such plant material can provide important information's about the incorporation of the labeled compounds for the determination of the sites of synthesis of particular compounds.

Isolated roots are also extensively used for the circulation of biogenetic pathways for tropane alkaloids in the roots of the solanaceous plants.

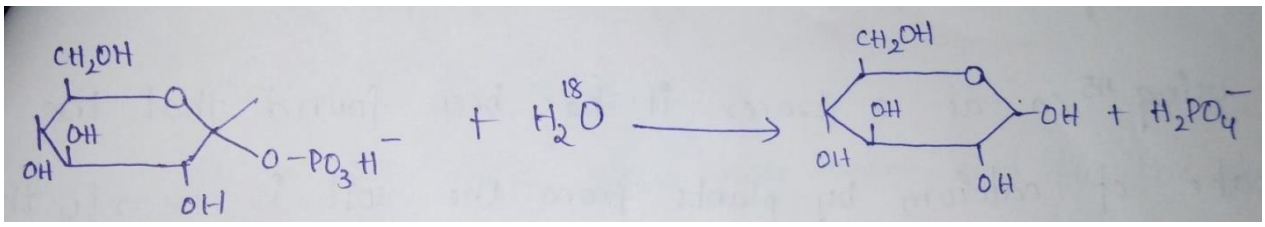
The studies on the petal discs have been used for the elucidation of pathways for essential oil components such as rose oil.

Isolated shoots and leaves can be maintained in a suitable sterile medium for the studies on Nicotiana and Datura spp. In such types of studies on rooted leaves to get large organization of roots facilitates the study of the tobacco alkaloid, for their biogenetic sites which is generally considered to be roots.

III. Grafting:

Grafting is an operation in which two cut surfaces of different but closely related plants placed so as to unite and further grow together.

- The major part of plant which is used for grafting is a stock.
- The portion that cut off from another plant is called as scion.



- In cases of plant propagation grafting has important place and plants like Cinchona, citrus, myristicaetc have been successfully grafted for the production of better quality drug.

- Grafting has also considerable utility in the biosynthetic studies for elucidation of the¬ pathways used for the biogenesis is of secondary metabolites. Solanaceous plants like Nicotiana, Datura have been intensively studied for the tobacco alkaloids and tropane alkaloids.

Eg: The scions of tomato grafted onto the stock of Datura, shows the accumulation of tropane alkaloid. On the contrary when Datura scions are grafted on Lycopersicon, tomato stock, the production of tropane alkaloids does not occur as usual and shows only traces of these alkaloids.

The above experiment suggests the possibilities that the major site for the biogenesis of tropane alkaloid is roots and no other organ of Datura.

IV. Mutant strains:

Mutant strains of lower plants like fungi and microorganisms are produced in nature which lacks one or other enzyme because of which the normal metabolic pathways are gently affected. In such mutant strains metabolites are found at the intermediate stage and needs artificial supply of another intermediate. Such mutant strains can be used in the biosynthetic studies of the natural products.

The biogenetic pathways of the gibberellins are mostly similar in both higher plants and Gibberella fugikuroi.

The mutant strains Gibberella can be used to obtain variety of novel C_{20}isoprenoid compounds which are produced at level of geranyl pyrophosphate in mevalonic acid pathway.

Very interesting results have been obtained by the studies on the ultraviolet induced strains of Claviceps purpurea.

These mutant strains can produce amino acids of diverseonessine contents nature. When the rye plant is introduced with the spore culture of these mutant strains, the sclerotia produced demonstrate the blockages of biogenetic pathway of certain intermediates and there by the accumulation of specific alkaloids (ergot alkaloid) is blocked.

The blockage occurs after the formation of ChanoclavineI in mutant strains to such strains if agroclavine and other intermediates had been supplied artificially it indicated the reinstallation of normal pathway to produce final or specific alkaloid compounds completely.

ALKALOIDS:

The term Alkaloid (Alk=Alkali; Oid=Like) was proposed by W. Meissner in 1819 for basic nitrogen containing compounds of plant origin. It may be defined as physiologically active basic compound of plant origin in which at least one nitrogen atom forms part of a cyclic system.

Properties of alkaloids:

Physical Properties

1. Most alkaloids are crystalline solids. Few alkaloids are amorphous solids e.g. emetine.
2. Some are liquids that are either:
3. Volatile e.g. nicotine and coniine, or Non-volatile e.g. pilocarpine and hyoscine.II
4. Color: The majority of alkaloids are colorless but some are colored e.g.: Colchicine and berberine are yellow, Canadine is orange,
5. The salts of sanguinarine are copper-red-III-
6. Solubility: Both alkaloidal bases and their salts are soluble in alcohol.Generally, the bases are soluble in organic solvents and insoluble in water
7. Exceptions: Bases soluble in water: caffeine, ephedrine, codeine, colchicine, pilocarpine and quaternary ammonium bases.
8. Bases insoluble or sparingly soluble in certain organic solvents: morphine in ether, theobromine and theophylline in benzene.
9. Salts are usually soluble in water and, insoluble or sparingly soluble in organic solvents.Salts insoluble in water: quinine monosulphate.
10. Salts soluble in organic solvents: lobeline and apoatropine hydrochlorides are soluble in chloroform.IV-
11. Isomerization: Optically active isomers may show different physiological activities. l-ephedrine is 3.5 times more active than d-ephedrine.l-ergotamine is 3-4 times more active than d-ergotamine.d-Tubocurarine is more active than the corresponding l- form. Quinine (l-form) is antimalarial and its d- isomer quinidine is antiarrythmic.
12. The racemic (optically inactive) dl-atropine is physiologically active.

13. **Oxygen: Most alkaloids contain Oxygen and are solid in nature e.g. Atropine.**
    Some alkaloids are free from Oxygen and are mostly liquids e.g. Nicotine, Coniine. Most alkaloids contain Oxygen and are solid in nature e.g. Atropine. IV

14. Stability: Effect of heat

15. Alkaloids are decomposed by heat, except Strychnine and caffeine (sublimable).Reaction with acids:1- Salt formation.2- Dil acids hydrolyze Ester Alkaloids e.g. Atropine

Chemical Properties:

I- Nitrogen:

Primary amines R-NH e.g. Norephedrine
Secondary amines R2-NH e.g. Ephedrine
Tertiary amines R3-N e.g. Atropine
Quaternary ammonium salts R4-N e.g d-TubocurarineII-

2. Basicity:
R2-NH > R-NH2 > R3-NSaturated hexacyclic amines are more basic than aromatic amines.

3. According to basicity Alkaloids are classified into:

1. Weak bases e.g. Caffeine

2. Strong bases e.g. Atropine

3. Amphoteric:

a. Phenolic Alkaloids e.g. Morphine

b. Alkaloids with Carboxylic groups e.g. Narceine

4. Neutral alkaloids e.g. Colchicine

Forms of Alkaloids:
Free bases Salts with Organic acids e.g. Oxalic, acetic acids Salts with inorganic acids e.g. HCl, H_2SO_4.
Salts with special acids: e.g. Meconic acid in Opium Quinic acid in Cinchona
Glycosidal form e.g. Solanine in Solanum.

Chemical tests:

Most Alkaloids are precipitated from neutral or slightly acid solution by various reagent. Following colour tests are used to detect the presence of alkaloid Test for alkaloids

1. Dragendorff's test Reagent: potassium bismuth iodide solution Test: 1 ml of extract + 1 ml of Dragendorff's reagent an orange-red precipitate indicate the presence of alkaloids.

2. Mayer's test Reagent: potassium mercuric iodide solution Test: 1 ml of extract + 1 ml of Mayer's reagent Whitish or cream colored precipitate indicate the presence of alkaloids.

3. Hager's test Reagent: saturated aqueous solution of picric acid Test: 1 ml of extract + 3 ml of Hager's reagent Yellow colored precipitate indicates the presence of alkaloids

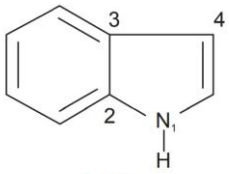
4. Wagner's test Reagent: iodine in potassium iodide
Test: 1 ml of extract + 2 ml of Wagner's reagent Reddish brown colored precipitate indicate the presence of alkaloids.

5. Tannic acid test: Reagent: Freshly prepared 5% saturated solution of tannic acid. Test: Alkaloids give precipitate with tannic acid solution which is soluble in dilute acid or ammonia solution. Test: 1 ml of extract + 1 ml tannic acid reagent PPT Dilute acid Soluble

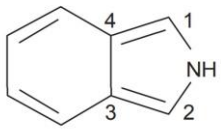
Indole Alkaloids

Indole (1-H-indole) is a benzopyrrole in which the benzene and pyrrole rings are through the 2, 3-positions of the pyrrole. The indole nucleus is found in a large number of naturally occurring compounds.

It is of commercial importance as a component of perfumes. Isoindole (I-H-isoindole), the isomer in which the benzene and pyrrole rings are fused through the 3- and 4-positions of the pyrrole, is not stable. A few of its derivatives are known, the simplest being N-methylisoindole.



Indole



Isoindole

Indole was first obtained (and its structure elucidated) in 1866 by Adolf von Baeyer. Interest in indole chemistry revived about 1930 when it was discovered that the essential amino acid, tryptophan, the plant growth hormone, heteroauxin, and several groups of important alkaloids are indole derivatives.

It was shown that 3-methylindole (skatole) is produced with indole during pancreatic digestion or putrefactive decomposition of proteins and, hence, both are found in the intestines and feces. Interest has centered on medicinal and biochemical aspects of indole chemistry.

Serotonin, which has been identified as a metabolite in brain chemistry; the psychotomimetic indoles, psilocin and psilocybin from mushrooms; the tranquilizer reserpine; and the melanin pigments are a few of the compounds that have been studied.

Indole is a colourless crystalline solid (mp 52–54°C, bp 254°C). The heat of combustion at constant volume is 4,268 MJ/mol (10–20 kcal/mol). The molecule is planar and has only moderate polarity.

Indole has good solubility in a wide range of solvents including petroleum ether, benzene, chloroform and hot water. The solubility in cold water is only 1:540 at 25°C; thus, water is a good solvent for purification by recrystallization. Indole forms salts with high concentrations of both strong bases and strong acids.

The various plants containing indole alkaloids are vinca, ergot, Rauwolfia, nux vomica, physostigma, etc.

VINCA

Synonyms

Vinca rosea, Catharanthus, Madagascar periwinkle.

Barmasi.

Biological Source

Vinca is the dried entire plant of *Catharanthus roseus* Linn., belonging to family Apocynaceae.

Geographical Source

The plant is a native of Madagascar and is found in many tropical and subtropical countries especially in India, Australia, South Africa and North and South America. The plant is cultivated as garden plant in Europe and India.

Cultivation and Collection

The plant is perennial and retains its glossy leaves through-out the winter. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and can grow in heavy clay soil. The plant prefers acid, neutral and basic (alkaline) soils. It can grow in full shade (deep woodland) semishade (light woodland) or no shade. It requires dry or moist soil and can tolerate drought. It is cultivated either by directly sowing the seeds or sowing the seeds in nursery. Nursery sowing method is found to be economical and

the fresh seeds are sown in nursery in the month of February or March. The seedlings attain a height of 5–8 cm after two months and then they are transplanted in to the field at a distance of 45 cm × 30 cm. Proper fertilization and weeding is done timely and leaves are stripped after nine months. In order to collect the whole plant, the stems are first cut about 10 cm above the grounds and the leaves, seeds, stems are separated and dried. The roots are collected by plugging which are later washed and dried under shade and packed.

Macroscopic Characteristics

The leaves are green in colour, flowers are either violet, pinkish white or carmine red and roots are pale grey in colour. It has characteristic odour and bitter taste. The flowers are hermaphrodite (have both male and female organs) and are pollinated by bees. Leaves are petiolate, entire margin, ovate or oblong, glossy appearance and with acute apex. Fruit is follicles with numerous black seeds.

Microscopy

Vinca has dorsiventral leaf structure. Epidermis is a single layer of rectangular cells covered with thick cuticle.

It consists of unicellular covering trichome and cruciferous stomata.

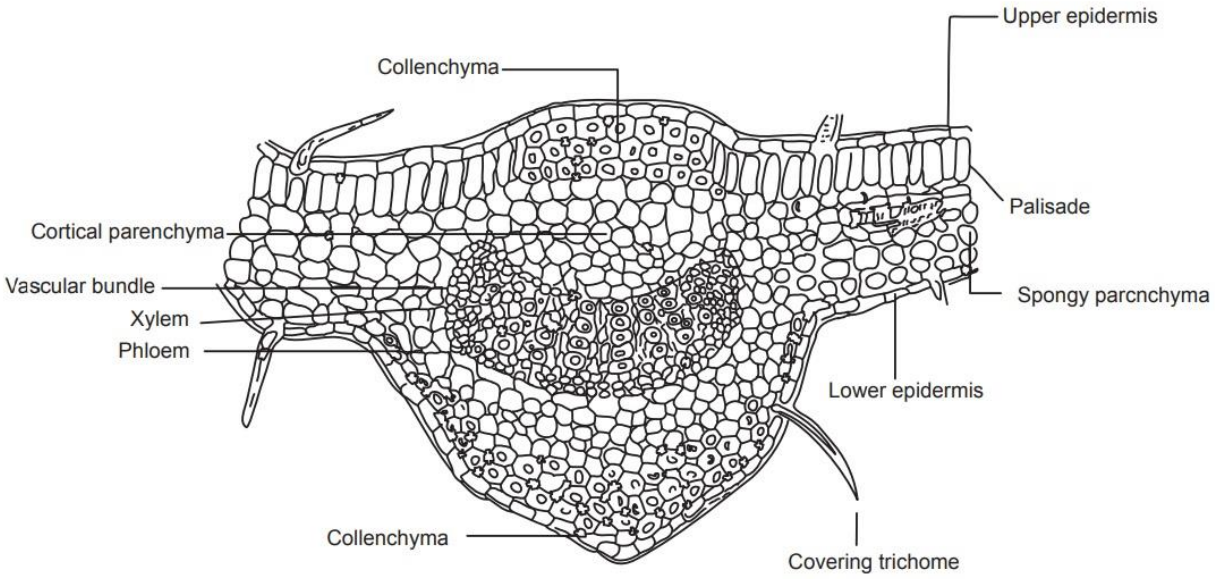
In the mesophyll region single layer of elongated and closely packed palisade parenchyma cells are present just below the upper epidermis.

In the midrib region two to three layers of collenchyma is present, both below the upper epidermis and above the lower epidermis.

Vascular bundle consisting of xylem and phloem is present in the middle of midrib region and rest of the intercellular space is covered by five to eight layers of spongy parenchyma.

Calcium oxalate crystals are absent.

Catharanthus roseus



Transverse section of Vinca leaf

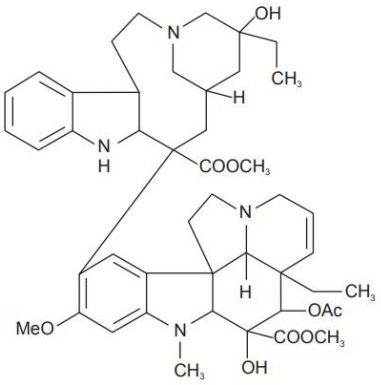
Chemical Constituents

Alkaloids are present in entire shrub but leaves and roots contain more alkaloids.

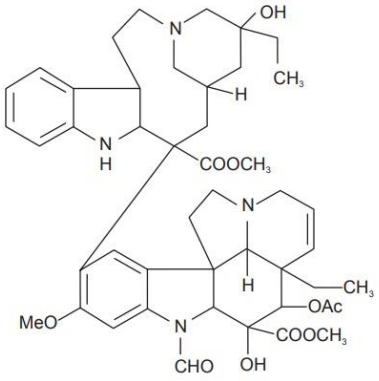
About 90 alkaloids have been isolated from Vinca from which some like Ajmalicine, Serpentine and Tetrahydroalstonine are known and are present in other species of Apocynaceae.

The important alkaloids in Catharanthus are the dimer indole indoline alkaloids Vinblastine and Vincristine and they possess definite anticancer activity.

Vindoline and Catharanthine are indole monomeric alkaloids. It also contains monoterpenes, sesquiterpene, indole and indoline glycoside.



Vinblastine



Vincristine

Uses

Vinblastin is an antitumour alkaloid used in the treatment of Hodgkin's disease.

Vincristine is a cytotoxic compound and used to treat leukaemia in children.

Vinca is used in herbal practice for its astringent and tonic properties in menorrhagia and in haemorrhages generally.

In cases of scurvy and for relaxed sore throat and inflamed tonsils, it may also be used as a gargle.

For bleeding piles, it may be applied externally, as well as taken internally. It is also used in the treatment of diabetes.

The flowers of the Periwinkle are gently purgative, but lose their effect on drying. If gathered in the spring and made into syrup, they impart all their virtues, and this, it is stated, is excellent as a gentle laxative for children and also for overcoming chronic constipation in grown persons.

Marketed Products

It is one of the ingredients of the preparation known as Cytocristin (Cipla).

RAUWOLFIA

Synonyms

Sarpagandha, Chandrika; Chootachand; Indian snake root.

Biological Source

Rauwolfia consists of dried roots of *Rauwolfia serpentina* Benth., belonging to family Apocynaceae.

Geographical Source

It is an erect, evergreen, small shrub native to the Orient and occurs from India to Sumatra.

It is also found in Burma, Thailand, Philippines, Vietnam, Indonesia, Malaysia, Paki-stan and Java. In India it occurs in the sub-Himalayan tracts from Sirhind eastwards to Assam, especially in Dehradun, Siwalik range, Rohelkhand, Gorakhpur ascending to 1,300 m, east and west ghats of Tamil Nadu, in Bihar (Patna and Bhagalpur), Konkan, Karnataka and Bengal.

Cultivation and Collection

Rauwolfia grows in tropical forests at an altitude of 1,200– 1,300 m at temperature 10–40°C. There should be enough rain or irrigation for its cultivation.

The soil should be acidic (pH 4–6), clayey and manure is applied for better crop. Propagation is done by planting seeds, root cuttings or stem cuttings. Better drug is obtained when the propaga-tion is carried out with fresh seeds. The plants should be protected from nematodes, fungus and Mosaic virus.

The drug is collected mainly from wild plants. Roots and rhizomes are dug out in October–November when the plant roots are two to four years old.

The aerial parts and roots are separated. The roots are washed and dried in air. The roots containing moisture up to 12% should be protected from light.

Seasonal variation, genetic differences, geographic location, improper handling and drying, and other factors account for percentage differences in alkaloid amount.

Rauwolfia should be packaged and stored in well-closed containers in a cool, dry place that is secure against insect attack.

Characteristics

The roots and rhizomes are almost identical in external characters. The drug occurs in cylindrical or slightly tapering, tortuous pieces, 2–10 cm long, 5–22 mm in diameter. The roots are rarely branched. Rootlets, 0.5–1 mm in diameter, are rare. The outer surface is greyish-yellow, light-brown or brown. Young pieces contain slight wrinkles while old pieces have longitudinal ridges. Circular scars of root-lets are present. Bark exfoliation is present in old samples leaving behind patches of exposed wood. The fracture is short. A narrow, yellowish-brown bark and a dense pale yellow wood are present on the smooth transverse surface at both the ends. Pieces of rhizome closely resemble the root but may be

identified by a small central pith. They are attached to them with small pieces of aerial stem. Slight odour is felt in recently dried drug which decreases with age; taste is bitter.

Root and twig of *Rauwolfia serpentina*

Microscopy

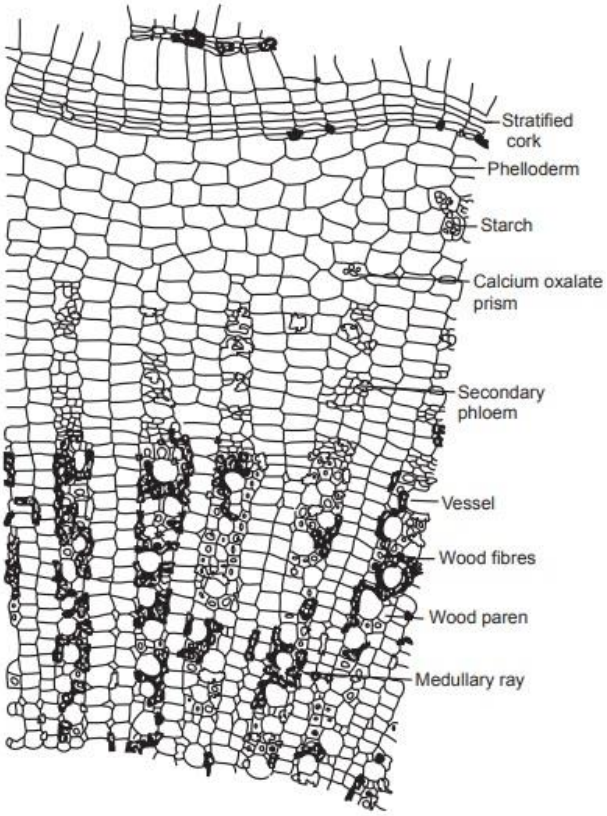
Transverse section of the root shows a stratified cork, which is divided, into two to eight alternating zones.

It consists of one to seven layers of smaller and radially narrower, suberised, nonlignified cells alternating with one to three layers of larger radially broader, lignified cells.

The phelloderm is composed of about ten to twelve layers of tangentially elongated to isodiametric, cellulosic parenchymatous cells. Cells of secondary cortex are parenchymatous and contain starch grains, simple and compound (two to four components), spherical with a distinct hilum in the form of a split.

Phloem is narrow and consists of parenchyma with scattered sieve tissue; parenchyma alternate with broader medullary rays composed of large cells and usually two to four cells wide.

Xylem is wide, entirely lignified and usually shows two to five annual rings. Medullary rays, one to five cells wide, contain starch grains and alternate with secondary xylem consisting of vessels, tracheids, fibres and parenchyma. Xylem vessels have pitted thickening.



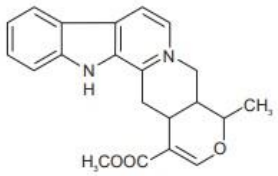
Transverse section of Rauwolfia root

Chemical Constituents

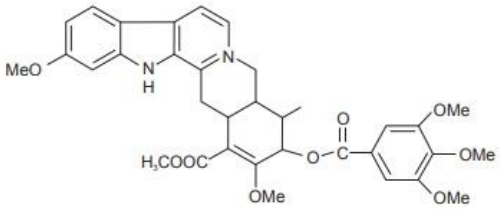
Rauwolfia contains about 0.7–2.4% total alkaloidal bases from which more than 80 alkaloids have been isolated.

The prominent alkaloids isolated from the drug are reserpine, rescinnamine, ψ-reserpine, rescidine, raubescine and deserpidine.

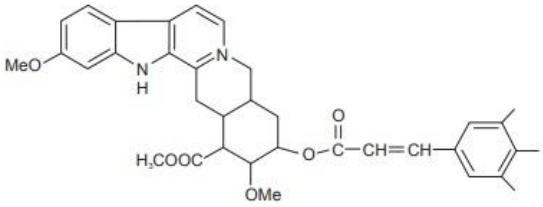
The other alkaloidal components are ajmalinine, ajmaline, ajmalicine (8-yohimbine), serpentine, serpentinine, tetrahydroreserpine, raubasine, reserpinine, isoajamaline and yohambinine.



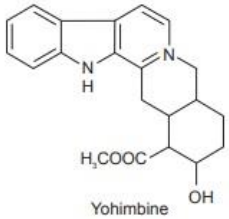
Serpentine



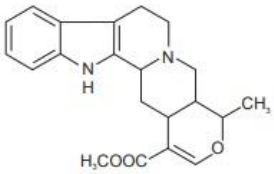
Reserpine



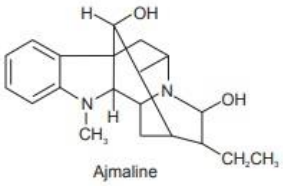
Rescinnamine



Yohimbine



Ajmalicine



Ajmaline

The other substances present are phytosterols, fatty acids, unsaturated alcohols and sugars.

Uses

Rauwolfia in used as hypnotic, sedative and antihypertensive. It is specific for insanity, reduces blood pressure and cures pain due to affections of the bowels.

It is given in labours to increase uterine contractions and in certain neuropsychiatric disorders. Ajmaline, which has pharmacological properties similar to those of quinidine, is marketed in Japan for the treatment of cardiac arrhythmias.

Reserpine is a white or pale buff to slightly yellow, odourless, crystalline powder that darkens slowly when exposed to light and rapidly when in solution.

Reserpine is an antihypertensive and tranquilizer. Rescinnamine is the methyl reserpate ester of 3,4,5-trimethoxy cinnamic acid. The usual antihypertensive dose of rescinnamine is 500 µg, two times a day. Higher doses may cause serious mental depression.

Deserpidine is 11-des-methoxyreserpine. It is a wide-range tranquilizer and antihypertensive and is free from the side effects.

Marketed Products

It is one of the ingredients of the preparations known as Confido, Lukol, Serpina (Himalaya Drug Company) and Sarpagandhan bati (Baidyanath).

TROPANE ALKALOIDS

The tropane alkaloids, which have the 8-azabicyclo octane nucleus, are commonly found in plants of three families, the Solanaceae, Erythroxylaceae and Convolvulaceae families. Tropane alkoloids are tropene derivatives.

Tropane ring is composed of pyrrolidine and piperidine rings. Tropane is 3-hydroxy tropene. There are two stereoisomers of tropene, tropine and pseudotropine.

They are esters combined with acids. These esters of tropic acid could be detected by vitali– morin reaction.

The acids present are tropic acid in atropine and atropic acid formed by the loss of water from tropic acid in apoatropine.

Other organic acids like tiglic acid, acetic acid, isobutyric acid and isovaleric acid are also present.

The alkaloids isolated from plants of these families, while having several legitimate medicinal uses, are probably best known for their toxic properties.

This can be a major problem since the plants produce very attractive berries which are tempting to small children. As few as three berries of henbane (Hyoscyamus niger) or deadly nightshade (Atropa belladonna) can cause death in infants.

Many of the plants in the Solanaceae family contain tropane alkaloids, which are responsible for the toxic effects of the plants.

Cleopatra is reputed to have tested the effects of henbane and deadly nightshade on her slaves to investigate the possibility of using these extracts to commit suicide (she found the toxic effects too painful).

The wives of the Roman emperors, Augustus and Claudius, used deadly nightshade to murder large numbers of Romans. The mandrake (Mandragora officinarum) was reputed to possess aphrodisiac properties and was prized for these properties.

However, the roots also contain large quantities of the tropane alkaloid hyoscine (scopolamine), making the plant highly toxic.

BELLADONNA

Synonyms

Belladonna herb; Belladonna leaf; Deadly night shade leaves; Banewort; Death's herb, Dwale; Poison black cherry; Folia belladonnae.

Biological Source

Belladonna consists of dried leaves and flowering tops of Atropa belladonna Linn. (European Belladonna), belonging to family Solanaceae. It contains about 0.35% of total alkaloids calculated as hyoscyamine.

Geographical Source

A . belladonna is cultivated in United States, Canada, UK, Germany and India.

Cultivation and Collection

- ✓ Plants are cultivated by sowing seeds in nurseries and seed-lings are transplanted in April to moist, calcareous and loamy soil.
- ✓ Weeds are removed and manure is applied for proper growth of the crop.
- ✓ During flowering session leaves and flowering tops are cut at least three times in a year at an interval of two months from one to three years old plants.
- ✓ When the plant is four years old, roots are dug out. The collected drug is dried at 40–50°C. Un-dried leaves deterio-rate and give off ammonia.
- ✓ Belladonna plant infected with the fungus Phytophthora belladonnae should be destroyed to prevent further infection. Sometimes the leaves are damaged by flea-bettle insect and the roots by a fungus.

Characteristics

- ✓ The drug contains leaves, smaller stems of about 5 mm diameter, flowers and fruits.
- ✓ Leaves are stalked, brittle, thin, entire, long-pointed, 5–25 cm long, 2.5–12 cm wide, ovate lanceolate, slightly decurrent lamina, margine-entire, apex acuminate, colour dull-green or yellowish-green, surface glabrous, lateral veins join the midrib at an angle of 60°C, curving upwards and are anastomose.
- ✓ The upper side is darker than the lower. Each has a petiole about 0.5–4 cm long and a broadly ovate, slightly decurrent lamina about 5–25 cm long and 2.5–12 cm wide. The margin is entire and the apex acuminate.
- ✓ A few flowers and fruits may be present. If the leaves are broken, they are characterized by the venation and roughness of the surface due to the presence of calcium oxalate in some mesophyll cells which causes minute points on the surface of the leaf on drying.
- ✓ The flowers blooming in June are solitary, shortly stalked, drooping and about 2.5 cm long. The corolla is campanulate, five-lobed and of a dull purplish colour.
- ✓ The five-lobed calyx is persistent, remaining attached to the purplish-black berry.
- ✓ The fruit is bilocular, contains numerous seeds and is about the size of a cherry.
- ✓ A yellow variety of the plant lacks the anthocyanin pigmentation.

Microscopy

- ✓ A transverse section of the leaf of *A. belladonna* has a bifacial structure.
- ✓ The epidermal cells have-wavy walls and a striated cuticle.
- ✓ Anisocytic type and some of the anomocytic type stomata arc present on both surfaces but are most common on the lower.
- ✓ Hairs are most numerous on young leaves, uni-seriate, two- to four-celled clothing hairs; or with a uni-cellular glandular head.
- ✓ Some hair has a short: pedicel and a multicellular glandular head. Certain of the cells of the spongy mesophyll are filled with micro-sphenoidal (sandy) crystals of calcium oxalate.
- ✓ The midrib is convex above and shows the usual bicollateral vascular bundle. A zone of collenchyma is present in epidermis near midrib.

Chemical Constituents

Belladonna contains 0.3–1.0% total alkaloids, the prominent base is l-hyoscyamine and other components are atropine, apoatropine, as choline, belladonnine, cuscohygrine, chrysa-tropic acid, volatile bases, such as atroscine, leucatropic acid; phytosterol, N-methylpyrroline, homatropine, hyoscyamine N-oxide, rutin, kaempferol-3-rhamnogalactoside and 7-glu-coside, quercetin-7-glucoside, scopoletin, calcium oxalate, 14% acid soluble ash and 4% acid-insoluble ash.

Addition of ammonia to the alcoholic solution of scopoletin shows blue florescence. This test is useful to detect Belladonna poisoning. Atropine is formed by racemization during the extraction process.

Uses

- ✓ The drug is used as adjunctive therapy in the treatment of peptic ulcer; functional digestive disorders, including spastic, mucous and ulcerative colitis; diarrhoea, diverticulitis and pancreatitis.
- ✓ Due to anticholinergic property, it is used to control excess motor activity of the gastrointestinal tract and spasm of the urinary tract.
- ✓ Belladonna is anticholinergic, narcotic, sedative, diuretic mydriatic and used as anodyne and to check secretion.
- ✓ Other uses are similar to Hyoscyamus. It relieves spasm of gut or respiratory tract.

- ✓ Consumption of Belladonna checks excessive perspiration of patients suffering from tuberculosis. Belladonna acts as a parasympathetic depressant.

Marketed Products

It is one of the ingredients of the preparation known as Belladona plaster (Surgi Pharma) for backache, stiffness of muscles and boil, swollen joints.

OPIUM

Synonyms

Crude Opium, Raw Opium, Gum Opium;

Biological Source

Opium is the air dried milky latex obtained by incision from the unripe capsules of *Papaver somniferum* Linn, or its variety *P. album* Decand, belonging to family Papaveraceae.

Opium is required to contain not less than 10% of morphine and not less than 2.0% of codeine. The thebaine content is limited to 3%.

Geographical Source

It is mainly found in Turkey, Russia, Yugoslavia, Tasmania, India, Pakistan, Iran, Afghanistan, China, Burma, Thailand and Laos. In India, Opium is cultivated in M.P. (Neemuch) and U.P. for alkaloidal extraction and seed production.

History

- ✓ The cultivation of opium dates back to 3400 B.C. in Mesopotamia and by 1300 B.C. Egyptians began the cultivation of opium thebaicum.
- ✓ Hippocrates 'the father of medicine', (460–357 B.C.) prescribed drinking the juice of the white poppy mixed with the seed of nettle and also acknowledged its use as narcotic and styptic in internal diseases.

- ✓ It was Alexander the Great, who introduced opium to India and Persia. During the 17th century tobacco smoking was introduced in China, which resulted in its extensive.
- ✓ In 1800 control on opium supply and prices was brought and in 1805 Friedrich W. Seiturner (German pharmacist) isolated and identified the chief chemical constituent of opium.
- ✓ The compound isolated was named morphium (morphine) after Morpheus, the god of dreams. Eventually many other constituents like codeine (1832) and papaverine (1848) were also isolated and identified.
- ✓ Due to the uncontrolled use of opium in china (late 18th century) the imperial court had to ban its use. The United States in 19th century made easy availability of the opium preparations and the 'patent medicines'. Later on during the war, the Union Army were provided with enough amount of opium pills, laudanum, morphine sulphate, etc., which made opium addiction known as the 'army disease or the 'soldier's disease'.
- ✓ By 1870s, substitute for morphine by acetylating morphine were prepared and in 1898 a German company manufactured 3, 6-diacetylmorphine (Heroin) in bulk quantity.
- ✓ In December 1914, Harrison Narcotics Act which called for control of each phase of the preparation and distribution of medicinal opium, morphine, heroin, cocaine, and any new derivative with similar properties, was enforced by the United States Congress.
- ✓ The Federal Controlled Substances Act of 1970 is the redefined act of the Har-rison Act. In 1999, opium was declared as the Bumper crop of Afghanistan by producing 75% of world's heroin. In December 2002 the U.K. government under the health plan, will make heroin available free on National Health Service to all those with a clinical need for it.

Cultivation and Collection

- ✓ Opium is cultivated under license from the government. Its seeds are sown in October or March in alluvial soil. After germination of seeds snow falls. In spring the thin plant attains the height of 15 cm. Fertilizers are used for better crop.
- ✓ The poppy of first crop blossoms in April or May and the capsule mature in June or July. When the capsules are about 4 cm in diameter, the colour changes from green to yellow; they are incised with a knife about 1 mm deep around the circumference between midday and evening.
- ✓ The knife, known as a 'nushtur' bears narrow iron spikes which are drawn down the capsule to produce several longitudinal cuts. The incision must not penetrate into the interior of the capsule otherwise latex will be lost.
- ✓ The latex tube opens into one another. The latex, which is white in the beginning, immediately coagulates and turns brown. Next morning it is removed by scrapping with a knife and transferred to a poppy leaf.

- ✓ Each capsule is cut several times at intervals of two or three days. After collection the latex is placed in a tilted vessel so that the dark fluid which is not required may drain off. By exposure to air the opium acquires a suitable consistency for packing.
- ✓ The dried latex is kneaded into balls, wrapped in poppy leaves and dried in shade. The principal commercial varieties of Opium are Turkish Opium, Indian Opium, Chinese Opium, Yugoslavian Opium and Persian Opium.

Characteristics

Opium occurs in rounded or flattened mass which is 8–15 cm in diameter and weighing from 300 g to 2 kg each.

The external surface is pale or chocolate-brown, texture is uniform and slightly granular.

It is plastic like when fresh and turns hard and brittle after sometime. Fragment of poppy leaves are present on the upper surface.

Internal surface is coarsely granular, reddish-brown, lustrous; odour is characteristic; taste is bitter and distinct.

Opium is intended only as a starting material for the manufacture of galenical preparations and is not dispensed as such.


Papaver somniferum capsules

Chemical Constituents

Opium contains about 35 alkaloids among which morphine (10–16%) is the most important base. The alkaloids are combined with meconic acid. The other alkaloids isolated from the drug are codeine (0.8–2.5%), narcotine, the-baine (0.5–2%). noscapine (4–8%), narceine and papaverine (0.5–2.5%). Morphine contains a phenanthrene nucleus. The different types of alkaloids isolated are:

1. *Morphine Type:* Morphine, codeine, neopine, pseudo or oxymorphine, thebaine and porphyroxine. Morphine consists of alkaloids which has phenanthrene nucleus whereas those of the papaverine group has benzyliso-quinoline structure.

Protopine and hydrocotamine are of different structural types. The morphine molecule has both a phenolic and an alcoholic hydroxyl group and acetylated form is diacetyl morphine or heroin.

Codeine is ether of morphine (methyl-morphine). Other morphine ethers which are used medicinally are ethylmorphine and pholcodine.

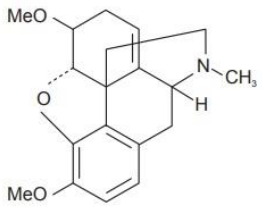
2. *Phthalide Isoquinoline Type:* Hydrocotarnme, narcotoline, 1-narcotine, noscapine, oxynarcotine, narceine, and 5'-O-demethyl-narcotine.

3. *Benzyl Isoquinoline Type:* Papaverine, dl-laudanine, lau-danidine, codamine and laudanosine.

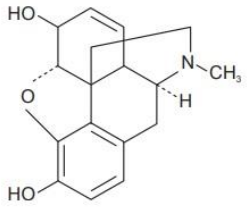
4. *Cryptopine Type:* Protopine, cryptopine.

5. *Unknown Constituents*: Aporeine, diodeadine, meconidine, papaveramine and lanthopine.

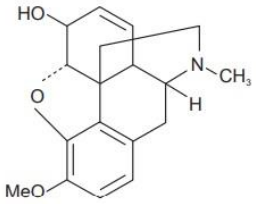
The drug also contains sugars, sulphates, albuminous compounds, colouring matter and moisture. In addition to these anisaldehyde, vanillin, vanillic acid, β-hydroxystyrene, fumaric acid, lactic acid, benzyl alcohol, 2-hydroxycinchonic acid, phthalic acid, hemipinic acid, meconin and an odorous compound have also been reported.



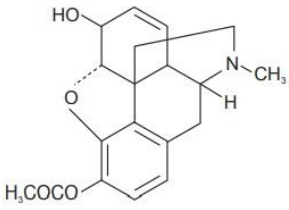
Thebaine



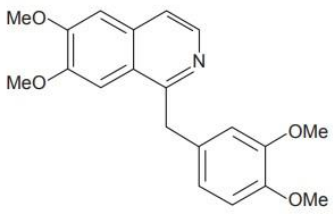
Morphine



Codeine



Heroin



Papaverine

Chemical Tests

Aqueous extract of Opium with $FeCl_3$ solution gives deep reddish purple colour which persists on addition of HCl. It indicates the presence of meconic acid.

Morphine gives dark violet colour with conc. H_2SO_4 and formaldehyde.

Uses

- ✓ Opium and morphine have narcotic, analgesic and sedative action and used to relieve pain, diarrhoea dysentery and cough.
- ✓ Poppy capsules are astringent, somniferous, soporific, sedative and narcotic and used as anodyne and emollient.
- ✓ Codeine is mild sedative and is employed in cough mixtures.
- ✓ Noscapine is not narcotic and has cough suppressant action acting as a central antitussive drug.
- ✓ Papaverine has smooth muscle relaxant action and is used to cure muscle spasms.
- ✓ Opium, morphine and the diacetyl derivative heroin, causes drug addiction.

Lignan

Introduction Lignans The term "Lignan" was first introduced by Haworth (1948) to describe a group of dimeric phenylpropanoids where two phenylpropanoid molecules are attached by its central carbon (C8). Lignans are a subgroup of non-flavonoid polyphenols. They are widely distributed in the plant kingdom, being present in more than 55 plant families, where they act as antioxidants and defence molecules against pathogenic fungi and bacteria.

a) Phenylpropanoid unit;

b) Lignan structure

Chemical structure of lignans their basic chemical structure consists of two phenylpropane unitslinked by a C-C bond between the central atoms of the respective side chains (position 8 or β), also called β-β' bond; in these cases the dimers are called neolignans.

Hence, their chemical structure is referred to as (C6-C3)2, and they are included in the phenylpropanoids group, as well as their precursors: the hydroxycinnamic acids Fig. 1 – Phenylpropane unit

Lignans can be found in more than 60 families of vascular plants and have been isolated from different plant parts, exudates and resins. Biological activity of Lignans are Antiviral ,Anticancer ,Cancer prevention, Anti-inflammatory, antimicrobial ,antioxidant , immunosuppressive, Hepatoprotective, Osteoporosis prevention.

Based on their carbon skeleton, cyclization pattern, and the way in which oxygen is incorporated in the molecule skeleton, they can be divided into 8 subgroups:

Main subclasses of Lignans-

1. Furofuran,

2. Furan,

3. Dibenzylbutane,

4. Dibenzylbutyrolactol,

5. Dibenzylbutyrolactones,

6. Aryltetralin,

7. Arylnaphtalene,

8. Dibenzocyclooctadienes.

Among these subgroups, the furan, dibenzylbutane and dibenzocyclooctadiene lignans can be further classified in “lignans with C9 (9′)-oxygen” and “lignans without C9 (9′)-oxygen”

In the last step, a NADPH-dependent cinnamyl alcohol dehydrogenase, also called monolignol dehydrogenase , catalyzes the reduction of the aldehyde group to an alcohol group, with the formation of the aforementioned monolignols.

In the second step, a NADPH-dependent cinnamoyl-CoA: oxidoreductase, also called cinnamoyl-CoA reductase catalyzes the formation of the corresponding aldehydes, and the release of coenzyme A.

The first step, which leads to the activation of the hydroxycinnamic acids, is catalysed by hydroxycinnamate:CoA ligases, commonly called p-coumarate:CoA ligases , with formation of the corresponding hydroxycinnamate-CoAs, namely, feruloil-CoA, p- coumaroyl-CoA and sinapil-CoA.

Biosynthesis of lignans, the pathway starts from 3 of the 4 most common dietary hydroxycinnamic acids: p-coumaric acid, sinapic acid, and ferulic acid (caffeic acid is not a precursor of this subgroup of poyphenols).

Therefore, they arise from the shikimic acid pathway, via phenylalanine.

The first three reactions reduce the carboxylic group of the hydroxycinnamates to alcohol group, with formation of the corresponding alcohols, called monolignols, that is, p-coumaric alcohol, sinapyl alcohol and coniferyl alcohol.

These molecules also enter the pathway of lignin biosynthesis.

TEA

Biological Source

It contains the prepared leaves and leaf buds of *Thea sinensis* (Linne) kuntz., belonging to family Theaceae.

Geographical Source

It is mainly cultivated in India (Assam), Ceylon, Japan and Java.

Cultivation and Collection

It is an evergreen shrub growing to 4 m by 2.5 m at a slow rate.

The plant prefers light (sandy) and medium (loamy) soils and requires well-drained soil.

The plant prefers acid and neutral soils and can grow in very acid soil.

It can grow in semishade (light woodland). It requires moist soil and prefers a pH between 5 and 7.

Prefers the partial shade of light woodland or a woodland clearing.

It is reported to tolerate an annual rainfall of 70–310 cm, an average annual temperature range of 14–27°C and a pH in the range of 4.5–7.3.

It prefers a wet summer and a cool but not very frosty dry winter.

Seed can be sown as soon as it is ripe in a green house.

Stored seed should be presoaked for 24 h in warm water and the hard covering around the micropyle should be filed down to leave a thin covering.

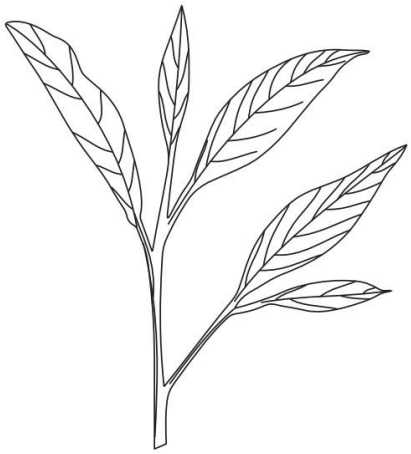
It usually germinates in one to three months.

Prick out the seedlings into individual pots when they are large enough to handle and grow them on in light shade in the green house for at least their first winter.

Plant them out into their permanent positions when they are more than 15 cm tall and give them some protection from winter cold for their first year or three outdoors.

Seedlings take 4–12 years before they start to produce seed.

Characteristics



Twig of tea plant

Leaves are dark green in colour, lanceolate or elliptical, on short stalks, blunt at apex, base tapering, margins shortly serrate, young leaves hairy, older leaves glabrous.

Microscopy

- ✓ The epidermal cells are made of polygonal cells which are slightly wavy walls. It consist on itself stomata and trichomes.
- ✓ The trichomes are thick walled, unicellular, conical (covering) which arise on the lower surface and in large number in young leaves.
- ✓ The mesophyll region consist of two rows of palisade parenchyma cells and large lignified sclereids which arise at some intervals and are extended across the mesophyll from one epidermis to the other.
- ✓ Cluster crystals of calcium oxalate are scattered in phloem and in parenchyma.
- ✓ In the midrib area a prominent ridge is present both above and below.

- ✓ Vascular bundle consisting of xylem and phloem are present; the entire region being covered by slightly lignified band of pericyclic fibres.
- ✓ The pericyclic fibres are up to four fibres in width at the widest region.
- ✓ The remaining portion is covered with spongy parenchyma with scattered lignified sclereids.

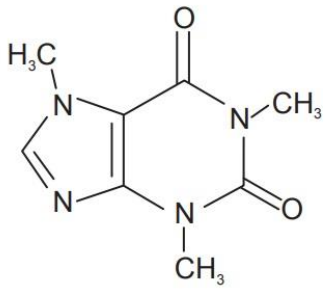
Chemical Constituents

The leaves are a rich source of caffeine (1–5%). It also contains theobromine and theophylline in minor quantities.

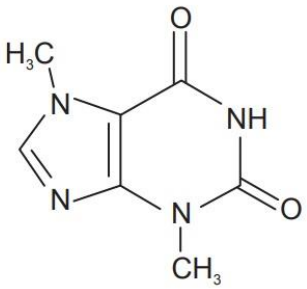
The colour of tea leaves is due to tannin (10–20% gallotannic acid).

The agreeable odour is due to presence of a yellow volatile oil.

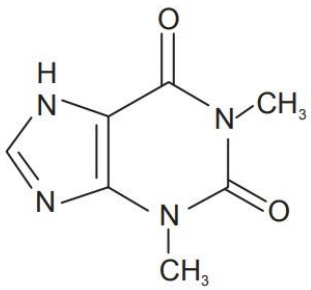
Tea leaves also contain protein, wax, resin and ash.



Caffeine



Theobromine



Theophyline

Chemical Tests

1. Caffeine and other purine alkaloids, gives murexide colour reaction. Caffeine is taken in a petridish to which hydrochloric acid and potassium chlorate are added and heated to dryness. A purple colour is obtained by exposing the residue to vapours of dilute ammonia. In addition of fixed alkali the purple colour disappears.

2. Caffeine also produces white precipitate with tannic acid solution.

Uses

It is used as stimulant, astringent and also as diuretic.

LIQUORICE

Synonyms

Radix Glycyrrhizae, Sweet liquorice

Biological Source

Liquorice consists of subterranean peeled and unpeeled stolons, roots and subterranean stems of *Glycyrrhiza glabra* Linn, and other species of *Glycytrhiza*, belonging to family *Leguminosae*.

Geographical Source

It is mainly found in China, Europe, India, Iraq, Japan, Kurdistan, Spain, Turkey, and the United States.

Cultivation and Collection

Liquorice is often cultivated for its edible root which is widely used in medicine and as flavouring. The plant requires a deep well cultivated fertile moisture-retentive soil for good root production.

Prefers a sandy soil with abundant moisture and does not flourish in clay. Slightly alkaline conditions produce the best plants. The plant thrives in a maritime climate.

It is propagated using seeds and roots. The seeds are presoaked for 24 h in warm water and then sown in spring or autumn in a greenhouse.

The seedlings are individually potted when they are large enough to handle, and grown them for their first winter in a green house. They are transplanted in late spring or early summer when in active growth. Plants are rather slow to grow from seed.

The plant parts are procured from old plantations, being waste from the harvesting process, consisting of those side roots or runners which have eyes or buds, cut into sections about 6 inches long.

They are dibbled in rows 3 or 4 feet apart, about 4 inches underneath the surface and about 18 inches apart in the rows. In the autumn, the ground is dressed with farmyard manure, about 40 tons to the acre.

Plants are slow to settle in and do not produce much growth in their first two years after being moved. The young growth is also very susceptible to damage by slugs and so the plant will require some protection for its first few years.

This species has a symbiotic relationship with certain soil bacteria; these bacteria form nodules on the roots and fix atmospheric nitrogen. Some of this nitrogen is utilized by the growing plant but some can also be used by other plants growing nearby.

Harvesting generally occurs in the autumn of the fourth year. The soil is carefully removed from the space between the rows to a depth of 2 or 3 feet as required, thus exposing the roots and rhizomes at the side, the whole being then removed bodily.

The earth from the next space is then removed and thrown into the trench thus formed and these operations are repeated continuously. Every portion of the subterranean part of the plant is carefully saved; the drug consists of both runners and roots, the former constituting the major part.

The roots are properly washed, trimmed and sorted, and either sold in their entire state or cut into shorter lengths and dried, in the latter case the cortical layer being sometimes removed by scraping.

The older or 'hard' runners are sorted out and sold separately; the young, called 'soft,' are reserved for propagation.

Characteristics

Liquorice root is in long, straight, nearly cylindrical, unpeeled pieces, several feet in length, varying in thickness from 1/4 inch to about 1 inch, longitudinally wrinkled, externally greyish brown to dark brown, warty; internally tawny yellow; pliable, tough; texture coarsely fibrous; bark rather thick; wood porous, but dense, in narrow wedges; taste sweet, very slightly acrid. The underground stem which is often present has a similar appearance, but contains thin pith. When peeled, the pieces of root (including runners) are shorter, a pale yellow, slightly fibrous externally, and exhibit no trace of the small dark buds seen on the unpeeled runners here and there. Otherwise it resembles the unpeeled.

Root and twig of *Glycyrrhiza glabra*

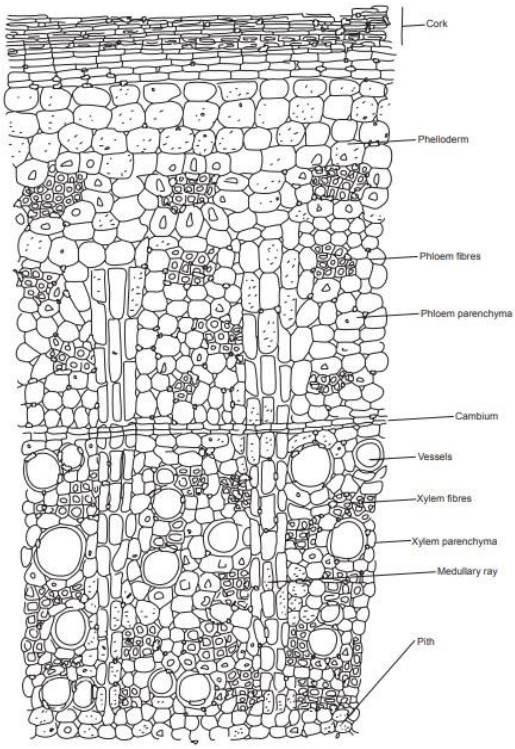
Microscopy

Cork consists of several rows of radially arranged thin walled tubular cells. Phelloderm is composed of parenchymatous and sometimes collenchymatous cells.

Starch grains and calcium oxalate crystals are seen in phelloderm. Pericyclic fibres are found in groups.

Phloem consists of sieve tissue alternating with thick walled, lignified fibres surrounded by a sheath of parenchymatous cells containing prisms of calcium oxalate.

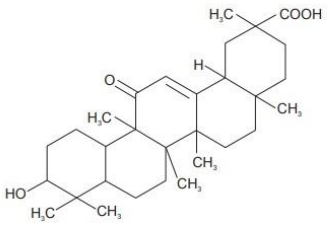
Xylem vessels and xylem parenchyma are present. Medullary rays are radially elongated. Pith is present in rhizomes and absent in root.



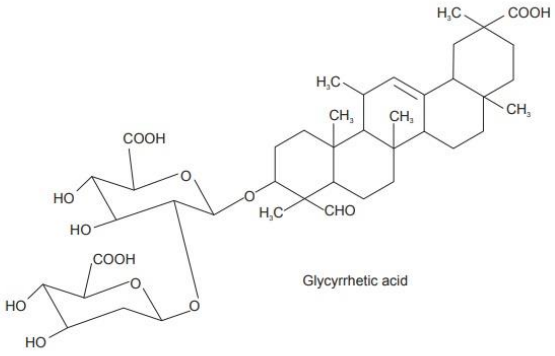
Transverse section of Liquorice stolon

Chemical Constituents

The chief constituent of liquorice root is Glycyrrhizin (6–8%), obtainable in the form of a sweet, which is 50 times sweeter than sucrose, white crystalline powder, con-sisting of the calcium and potassium salts of glycynhizic acid. Glycyrrhizic acid on hydrolysis yields glycyrrhetic or glycyrrhetinic acid. Glycyrrhizinic acid is a triterpenoid saponin having α-amyrine structure. It shows especially in alkaline solu-tion frothing but it has very weak haemolytic property. The yellow colour of the drug is due to chalcone glycoside isoliquiritin. The drug also contains sugar, starch (29%), gum, protein, fat (0.8%), resin, asparagin (2–4%), a trace of tannin in the outer bark of the root, yellow colouring matter, and 0.03% of volatile oil.



Glycyrrhetinic acid



Glycyrrhetic acid

Chemical Test

When 80% sulphuric acid is added to a section or powder of the drug orange yellow colour is produced due to transformation of flavone glycoside liquiritin to chalcone glycoside isoliquiritin.

Uses

1. Glycyrrhiza is widely used as a sweetening agent and in bronchial problems such as catarrah, bronchitis, cold, flu and coughs.

2. It reduces irritation of the throat and yet has an expectorant action. It produces its demulcent and expectorant effects. It is used in relieving stress.

3. It is a potent healing agent for tuberculosis, where its effects have been compared to hydrocortisone. Glycyrrhiza is also effective in helping to reduce fevers (glycyrretinic acid has an effect like aspirin), and it may have an antibacterial action as well.

4. It is used in the treatment of chronic inflammations such as arthritis and rheumatic diseases, chronic skin conditions, and autoimmune diseases in general.

5. It should be used in moderation and should not be prescribed for pregnant women or people with high blood pressure, kidney disease or taking digoxin-based medication.

6. Prolonged usage raises the blood pressure and causes water retention. Externally, the root is used in the treatment of herpes, eczema and shingles.

Marketed Products

It is one of the ingredients of the preparations known as Herbolex, Koflet, Regurin (Himalaya Drug Company), Jeevani malt (Chirayu Pharma), Eladi Bati, Madhume-hari (Baidyanath), J.P. Nikhar oil, J.P. Kasantak (Jamuna Pharma), Respinova (Lupin Herbal Laboratory) and Yasti madhu (Zandu Pharmaceuticals Works

DIOSCOREA

Synonym

Yam.

Biological Source

Dioscorea is the dried rhizome of several species of *Dioscorea* like *D. villosa, D. prazeri* Prain and Burk; *D. composite; D. spiculiflora; D. deltoidea* and *D. floribunda*, belonging to family Dioscoreaceae.

Geographical Source

It is mainly found in North America, Mexico, India (Himalayas from Kashmir and Punjab up to an altitude of 3,000 m), Nepal and China.

Cultivation and Collection

- ✓ It is a perennial climber growing to 3 m. The plant prefers sandy, loamy and clay soils and requires well-drained soil.
- ✓ The plant prefers acid, neutral and basic (alkaline) soils. It can grow in semishade or no shade. It requires moist soil.
- ✓ It can be cultivated in three methods, by sowing seeds or stem cuttings or by tubercles. Seeds are sown in the month of March to April in a sunny position in a warm green house and only just covered. It germinates in one to three weeks at 20°C.
- ✓ The seedlings are taken out as soon as they are large enough to handle and grown on in a green house for their first year.
- ✓ They are transplanted in late spring as the plant comes into new growth. Basal stem cuttings are done in the summer. Division is done in the dormant season, never when in growth.

- ✓ The plant will often produce a number of shoots, the top 5–10 cm of the root below each shoot can be potted up to form a new plant whilst the lower part of the root can possibly be eaten.
- ✓ Tubercles (baby tubers) are formed in the leaf axils. These are harvested in late summer and early autumn when about the size of a pea and coming away easily from the plant.
- ✓ They should be potted up immediately in individual pots in a greenhouse or cold frame and transplanted out in early summer when in active growth.

Characteristics

Colour : slightly brown,
Odour: odourless
Taste : bitter taste and vary in size.

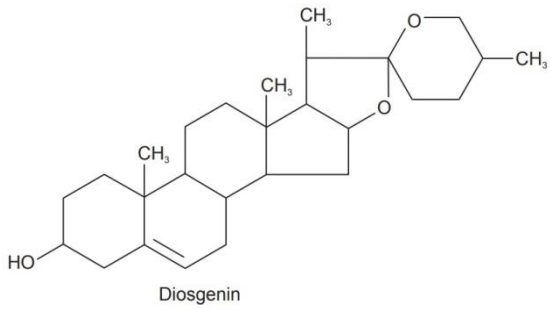
Microscopy

The transverse section of the drug when observed under the microscope shows the absence of epidermis, the cork is made up of few layers and next to cork it has corical parenchymatous tissue with thin wall.

The major part of the drug is occupied by stele and consists of collateral type of fibrovascular bundles. The drug has indistinguishable endodermis and pericycle.

Chemical Constituents

The roots contain diosgenin (4–6%) a steroidal sapogenin and its glycoside smilagenin, epismilagenin and beta isomer yammogenin. It also contains sapogenase (enzyme), phenolic compounds and starch (75%).



Diosgenin

Uses

1. It is a main source of diosgenin.
2. This is widely used in modern medicine in order to manufacture progesterone and other steroid drugs.
3. These are used as contraceptives and in the treatment of various disorders of the genitary organs as well as in a host of other diseases such as asthma and arthritis.

Marketed Products
It is one of the ingredients of the preparations known as Explode (Herbotech Pharmaceuticals).

DIGITALIS LEAVES

Synonyms

Digitalis, purple foxglove, finger flower, lady's glove, Fox-glove Leaves, Folia Digitalis

Biological Sources

Digitalis consists of dried leaves of *Digitalis purpurea* Linn., belonging to family Scrophulariaceae.

Geographical Sources

It is mainly found in England, Germany, France, North America, India, Iraq, Japan, Kurdistan, Mexico, Nepal, Spain, Turkey.

Cultivation and Collection

Digitalis is a biennial herb growing wild but good quality of the drug is obtained especially from cultivated plant. The plant will flourish best in well drained loose soil, prefer-ably of siliceous origin, with some slight shade. The plants growing in sunny situations possess the active qualities of the herb in a much greater degree than those shaded by trees, and it has been proved that those grown on a hot, sunny bank, protected by a wood, give the best results.

It grows best when allowed to seed itself, if it is desired to raise it by sown seed, 2 lb of seed to the acre are required. For cultivation special strains of the seeds are selected which would produce disease-resistant plants with maximum activity. Attention is specially paid to the structure of the soil in seed beds. As the seeds are so small and light, they should be mixed with fine sand in order to ensure even distribution. Before sowing, soil is sterilized. They should be thinly covered with soil. The seeds are uncertain in germination, but the seedlings may be readily and safely transplanted in damp weather, and should be pricked out to 6–9 inches apart. Sown in spring, the plant will not blossom till the following year. Seeds must be gathered as soon as ripe. In dry season sufficient water is supplied to the plant. In

the first year, a long stalk with rosette of leaves is produced. The flowers of the true medicinal type must be pure, dull pink or magenta, not pale-coloured, white or spotted externally.

Collection of these leaves is carried out from Septeber to November by hand and thus other organic matter and discoloured leaves are avoided. After collection the leaves should be dried as soon as possible at 60°C. By quick drying characteristic green colour of the leaves is maintained. Drying is carried out till moisture is not more than 5%. Leaves are packed under pressure in airtight containers.

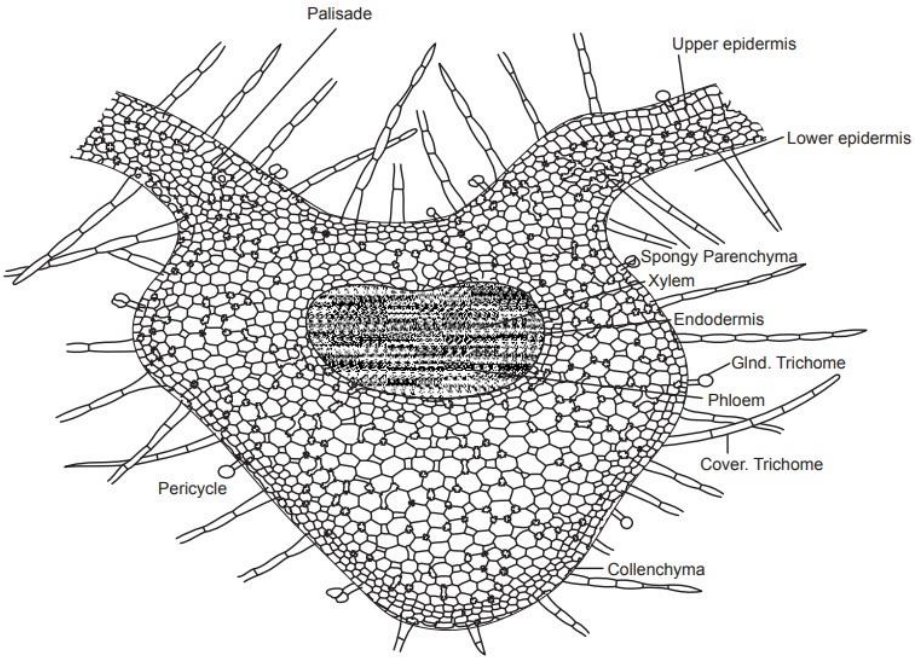
Morphology

Colour	Dark greyish green in colour
Odour	Odourless
Taste	Bitter
Shape	Ovatelanceolate to broadly ovate. Leaves have a subacute apex, decurrent base and crenate or dentate margin. The upper surface of leaf is hairy, slightly pubescent, dark green and little wrinkled. The lower surface of leaf is hairy, greyish-green and very pubescent.
Size	10–30 cm long and 4–10 cm wide

Digitalis purpurea

Microscopy

Digitalis has dorsiventral leaf structure. It has plenty of simple covering and glandular trichomes on both the surfaces. The covering trichomes are uniseriate, usually three to four cells long, having collapsed cells, acute apex and finely warty cuticle. The glandular trichomes have a short, unicellular stalk and bicellular or rarely unicellular head. It has anomocytic or ranunculaceous type of stomata. Trichomes and stomata are more in lower surface. The pericycle is parenchymatous above and collenchymatous below. Calcium oxalate crystals are absent.

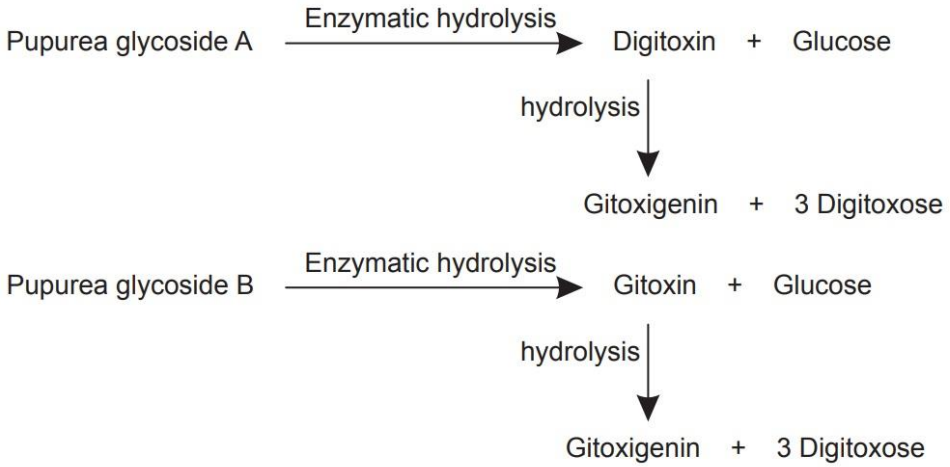


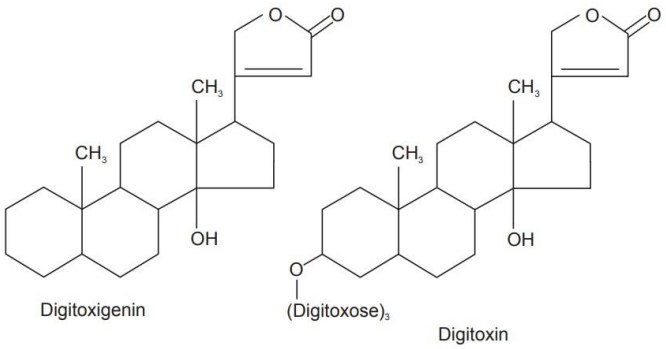
Transverse section of Digitalis leaf

Chemical Constituents

Digitalis leaves contains 0.2–0.45% of both primary and secondary glycosides. Purpurea glycosides A and B and glucogitoloxin are primary glycosides. Because of greater stability of secondary glycosides, and lesser absorption of primary glycosides a higher content of primary glycosides are not considered ideal and secondary glycosides are used. Purpurea glycosides A and B are present in fresh leaves and by their hydrolysis digitoxin and glucose or gitoxin and glucose are obtained respectively. Hydrolysis of purpurea glycosides can take place by digipuridase (enzyme) present in the leaves. Digitoxin yields on hydrolysis digitoxigenin and three digitoxose. By hydrolysis of verodoxin, gitaloxi-genin and digitalose are obtained. Digitalis leaves also con-tains glycosides like odoroside-H, gitaloxin, verodoxin and glucoverodoxin.

Verodoxin was found to potentiate the activity of digi-toxin by synergism. Digitoxose and digitalose are desoxy sugars found only in cardiac glycosides and answers Keller– Killiani test. The important saponins include digitonin, tigonin and gitonin, and luteolin, a flavone responsible for the colour of the drug are also present in the leaves.





Chemical Tests

Digitalis glycosides having five membered lactone ring answers positive for the following tests which are due to the intact lactone.

1. *Baljet Test:* To a thick section of the leaf sodium picrate reagent is added. Yellow to orange colour indicates the presence of glycoside.

2. *Legal Test:* Glycoside is dissolved in pyridine and sodium nitroprusside solution is added to it and made alkaline. Pink to red colour is produced.

3. *Keller–Killiani Test:* The isolated glycoside is dissolved in glacial acetic acid and a drop of ferric chloride solu-tion is added followed by the addition of sulphuric acid which forms the lower layer. A reddish-brown colour is seen in between two liquids and the upper layer becomes bluish green.

If the powdered leaves are used, 1 gm of the powdered leaves is extracted with 10 ml of 70% alcohol for couple of minutes, filtered and to 5 ml of filtrate 10 ml of water and 0.5 ml of strong solution of lead acetate is added and filtered and the filtrate is shaken with 5 ml of chloroform. Chloroform layer is separated in a porcelain dish and the test is carried out as mentioned above.

Uses

1. The foxglove is a widely used herbal medicine with a recognized stimulatory effect upon the heart.
2. It is also used in allopathic medicine in the treatment of heart complaints.
3. It has a profound tonic effect upon a diseased heart, enabling the heart to beat more slowly, powerfully and regularly without requiring more oxygen.
4. At the same time it stimulates the flow of urine which lowers the volume of the blood and lessens the load on the heart.
5. It has also been employed in the treatment of internal haemorrhage, in inflammatory diseases, in delirium tremens, in epilepsy, in acute mania and various other diseases.
6. Digitalis has a cumulative effect in the body, so the dose has to be decided very carefully.

Adulterants

Verbascum thapsus also known as Mullelin leaves. These leaves are covered with large woolly branched candelabra trichomes.

Primula vulgaris (Primrose leaves) can be detected by the presence of long eight- to nine-celled covering trichomes in them.

Symphytum officinale (Comfrey leaves), this leaves contains multicellular trichomes forming hook at the top.

Inula conyza (Ploughman's Spikenard), may be distinguished by their greater roughness, the less-divided margins, the teeth of which have horny points and odour when rubbed.

Marketed Products

It is one of the ingredients of the preparation known as Lanoxin tablets (Glaxo Smith Kline).

DIGITALIS LANATA

Synonym

Grecian Foxglove

Biological Source

It consists of the dried leaves of *Digitalis lanata* J. F. Ehrh., belonging to family Scrophulariaceae.

Geographical Source

It is mainly found in Central and Southern Europe, England, California and India.

Cultivation and Collection

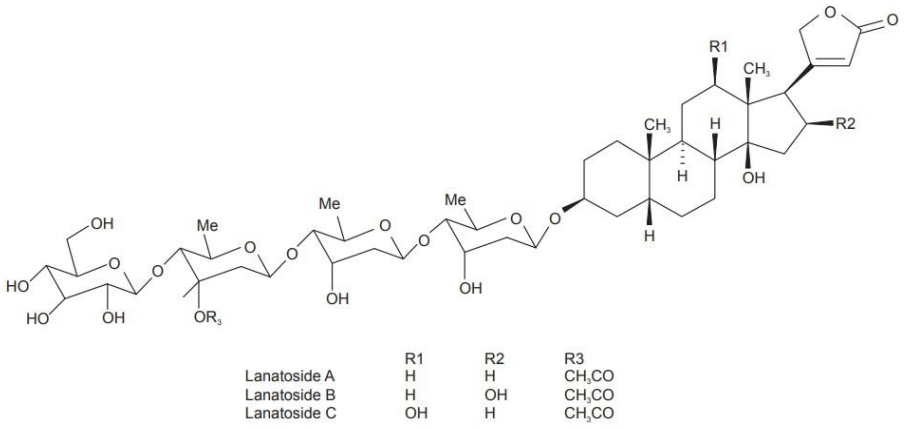
It is an evergreen biennial/Perennial growing to 0.6 m by 0.3 m. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils. The plant prefers acid, neutral and alkaline soils. It can grow in semishade or no shade. It requires dry or moist soil. It grows well even in ordinary garden soil, especially if it is rich in organic matter. It is propagated by seeds. Seed are sown on early spring in a cold frame. The seed usually germinates in 2–4 weeks at 20°C. When they are large enough to handle, seedlings are transplanted into individual pots and planted them out in the summer.

Characteristics

The leaves are sessile, linear-lanceolate, about 30 cm long and 4 cm broad with entire margin and apex is acuminate. The veins leave the midrib at an acute-angle. The epidermal cells are beaded with anticlinal walls, has 10–14 celled nonglandular trichomes, and the glandular one.

Chemical Constituents

Digitalis lanata contains cardiac glycosides like lanatoside A, B, C and E. Lanatosides A and B are acetyl derivatives of purpurea glycosides A and B respectively. Hydrolysis of Lanatoside C yields digoxin, a crystalline active glycoside.



	R1	R2	R3
Lanatoside A	H	H	CH_3CO
Lanatoside B	H	OH	CH_3CO
Lanatoside C	OH	H	CH_3CO

Uses

It has gained much importance in recent years because of the less cumulative effect and three to four times' greater activity than *D. purpurea.*

They have the same actions as that of the *D. purpurea*. It is the commercial source of digoxin. It is employed in the treatment of auricular fibrillation and congestive heart failure.

Their use should always be supervised by a qualified practitioner since in excess they cause nausea, vomiting, slow pulse, visual disturbance, anorexia and fainting.

Volatile oils:

Volatile oils are odorous volatile principles of plant and animal source, evaporate when exposed to air at ordinary temperature, and hence known as volatile or etheral oils. These represent essence of active constituents of the plant and hence also known as essential oils. In most instances the volatile oil preexists in the plant and is usually contained in some special secretory tissues, for example, the oil ducts of umbelliferous fruits, the oil cells, or oil glands occurring in the sub-epidermal tissue of the lemon and orange, mesophyll of eucalyptus leaves, trichomes of several plants, etc.

CLASSIFICATION OF VOLATILE OILS

Volatile oils are classified on the basis of functional groups present as given in Table below.

Table : Classification of volatile oil

Groups	Drugs
Hydrocarbons	Turpentine oil
Alcohols	Peppermint oil, Pudina, Sandalwood oil, etc.
Aldehydes	Cymbopogon sp., Lemongrass oil, Cinnamon, Cassia, and Saffron
Ketones	Camphor, Caraway and Dill, Jatamansi, Fennel, etc.
Phenols	Clove, Ajowan, Tulsi, etc.
Phenolic ethers	Nutmeg, Calamus, etc.
Oxides	Eucalyptus, Cardamom, and Chenopodium oil
Esters	Valerian, Rosemary oil, Garlic, Gaultheria oil, etc.

EXTRACTION OF VOLATILE OILS

Volatile oils are prepared by means of several techniques and those techniques are discussed below:

Extraction by Distillation

The distillation is carried out either by water or steam. The volatile oils from fresh materials are separated by hydrodistillation, and volatile oils from air dried parts are separated by steam distillation. However it is better to use fresh materials in either case.

Extraction by Scarification

This method is used for the preparation of oil of lemon, oil of orange, and oil of bergamot. These oils are found in large oil glands just below the surface in the peel of the fruit. The two principal methods of scarification are the sponge and the ecuelle method.

a. Sponge Process: In this process the contents of the fruit are removed after making longitudinal or transverse cut, and the peel is been immersed in water for a short period of time. Then it is ready for expression. The operator takes a sponge in one hand and with the other presses the softener peel against the sponge, so that the oil glands burst open and the sponge absorbs the exuded oil, which is transferred to a collecting vessel. The turbid liquid consisting of oil and water is allowed to stand for a short time, whereupon the oil separates from water and is collected. The whole of the above process is carried out in cool, darkened rooms to minimize the harmful effects of heat and light on the oil.

b. Ecuelle Process: In this process, the rinds are ruptured mechanically using numerous pointed projections with a rotary movement and the oil is collected.

Extraction by Volatile Solvent

In this the flowers are extracted by using the solvent light petroleum and the latter is distilled off at a low temperature, leaving behind the volatile oil.

TERPENOIDS

There are many different classes of naturally occurring compounds. Terpenoids also form a group of naturally occurring compounds majority of which occur in plants, a few of them have also been obtained from other sources. Terpenoids are volatile substances which give plants and flowers their fragrance. They occur widely in the leaves and fruits of higher plants, conifers, citrus and eucalyptus.

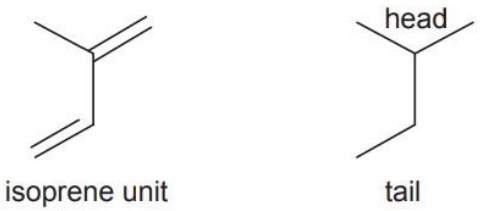
The term 'terpene' was given to the compounds isolated from terpentine, a volatile liquid isolated from pine trees. The simpler mono and sesquiterpenes is the chief con-stituent of the essential oils obtained from sap and tissues of certain plant and trees. The di and triterpenoids are not steam volatile. They are obtained from plant and tree gums and resins. Tertraterpenoids form a separate group of compounds called 'Carotenoids'.

The term 'terpene' was originally employed to describe a mixture of isomeric hydrocarbons of the molecular formula $C_{10}H_{16}$ occurring in the essential oils obtained from sap and tissue of plants and trees. But there is a tendency to use more general term 'terpenoids', which includes hydrocarbons and their oxygenated derivatives. However, the term terpene is being used these days by some authors to represent terpenoids.

According to modern definition, 'Terpenoids are the hydrocarbons of plant origin of the general formula $(C_5H_8)_n$ as well as their oxygenated, hydrogenated, and dehydrogenated derivatives.'

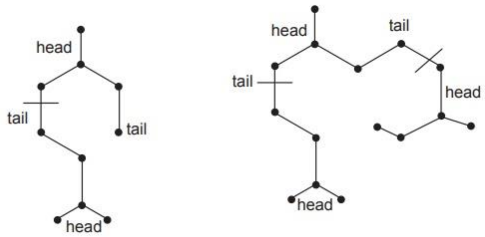
Isoprene Rule

Thermal decomposition of terpenoids gives isoprene as one of the product. Otto Wallach pointed out that terpenoids can be built up of isoprene unit. Isoprene rule states that the terpenoid molecules are constructed from two or more isoprene unit.

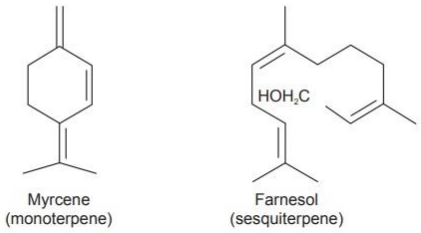


Special Isoprene Rule

It states that the terpenoid molecules are constructed of two or more isoprene units joined in a 'head to tail' fashion.



Examples



But this rule can only be used as guiding principle and not as a fixed rule. For example carotenoids are joined tail to tail at their central, and there are also some terpenoids whose carbon content is not a multiple of five.

CLASSIFICATION OF TERPENOIDS

Most natural terpenoid hydrocarbons have the general formula $(C_5H_8)_n$. They can be classified on the basis of number of carbon atoms present in the structure.

Table : Classification of Terpenoids

S. No.	Number of carbon atoms	Value of *n*	Class
1.	10	2	Monoterpenoids ($C_{10}H_{16}$)
2.	15	3	Sesquiterpenoinds ($C_{15}H_{24}$)
3.	20	4	Diterpenoids ($C_{20}H_{32}$)
4.	25	5	Sesterpenoids ($C_{25}H_{40}$)
5.	30	6	Triterpenoids ($C_{30}H_{48}$)
6.	40	8	Tetraterpenoids ($C_{40}H_{64}$)
7.	>40	>8	Polyterpenoids (C_5H_8)n

Each class can be further subdivided into subclasses according to the number of rings present in the structure.

1. Acyclic Terpenoids: They contain open structure.
2. Monocyclic Terpenoids: They contain one ring in the structure.
3. Bicyclic Terpenoids: They contain two rings in the structure.
4. Tricyclic Terpenoids: They contain three rings in the structure.
5. Tetracyclic Terpenoids: They contain four rings in the structure.

EVALUATION OF VOLATILE OILS

- Product from different manufacturers varies considerably, since it is inherently difficult to control all the factors that affect a plants chemical composition.
- Environmental conditions such as sunlight and rainfall, as well as manufacturing process can create substantial variability in essential oil quality. Various procedures are given for the evaluation of essential oils.
- Preliminary examinations like odour, taste, and colour.
- Physical measurements, which includes optical rotation, relative density, and refractive index.
- Chromatographic techniques are used to determine the proportions of individual components of certain oils.
- The ketone and aldehyde content of oils are determined by reaction with hydroxylamine hydrochloride (oxime formation) and titration of the liberated acid.

- The oil, which passes the above examinations, would be having good quality and therapeutic value.

Chemical Tests for Volatile Oils

Natural drugs containing volatile oils can be tested by following chemical tests:

1. Thin section of drug on treatment with alcoholic solution of Sudan III develops red colour in the presence of volatile oils.

2. Thin section of drug is treated with tincture of alkana, which produces red colour that indicates the presence of volatile oils in natural drugs.

PHARMACEUTICAL APPLICATIONS

Volatile oils are used as flavouring agent, perfuming agent in pharmaceutical formulations, foods, beverages, and in cosmetic industries. These are also used as important medicinal agent for therapeutic purposes like:

1. Carminative (e.g. Umbilliferous fruits)
2. Anthelminitic (e.g. Chenopodium oil)
3. Diuretics (e.g. Juniper)
4. Antiseptic (e.g. Eucalyptus)
5. Counter irritant (e.g. Oil of winter green)
6. Local anesthetic (e.g. Clove)
7. Sedative (e.g. Jatamansi)
8. Local irritant (e.g. Turpentine)
9. Insect repellent (e.g. Citronella)
10. Source of vitamin A (e.g. Lemongrass)

VOLATILE OILS CONTAINING HYDROCARBONS

- Hydrocarbons are present in all volatile oils. Limonene is the most widely distributed of the monocyclic terpenes.

- It occurs in Citrus, Mint, Myristica, Caraway, Thyme, Cardamom, Coriander, and many other oils.

- Another mono-cyclic hydrocarbon monoterpene is p-cymene, present in Coriander, Thyme, Cinnamon, and Myristica oils. Pinene, a dicyclic monoterpene, is also widely distributed.

- It occurs in many conifer oils and in Lemon, Anise, Eucalyptus, Thyme, Fennel, Coriander, Orange flower, and Myristica oils. Sabinene, a dicyclic monoterpene of the thujane class, present in cardamom and lemon oils.
- Acyclic monoterpene myrcene occurs in Myricia, Lemon, and Myristica. Cadinene occurring in juniper tar, is a sesquiterpene hydrocarbon.
- β-Caryophyllene is a sesquiterpene found in Wormwood, Peppermint, Cinnamon, and Clove oils.
- A volatile oil drug composed mainly of hydrocarbons is turpentine oil.

1. PEPPERMINT

Synonym

Brandy Mint.

Botanical Source

It is the oil obtained by the distillation of *Mentha piperita*, belonging to family *Labiatae.*

Geographical Source

It is mainly found in Europe, United States, and also in damp places of England.

Cultivation and Collection

Peppermint thrives best in a fairly warm, preferably moist climate, with well-drained, deep soils rich in humus. Peppermint will grow successfully, if once started into growth and carefully cultivated. The usual method of cultivation is to dig runners in the early spring and lay them in shallow trenches, 3 feet apart in well-prepared soil. The growing crop is kept well-cultivated and absolutely free from weeds and in the summer when the plant is in full bloom, the mint is cut by hand and distilled in straw. A part of the exhausted herb is dried and used for cattle food.

Characteristics

The leaves are shortly and distinctly stalked, 2 inches long and 3/4 to 1.5 inches broad. The margins are finely toothed, with smooth upper and lower surfaces.

The stems are 2 to 4 feet high, frequently purplish in colour. The flowers are reddish-violet in colour, present in the axils of the upper leaves, forming loose, interrupted spikes.

The plant has a characteristic odour and if applied to the tongue has a hot, aromatic taste at first and afterwards produces a sensation of cold in the mouth caused by menthol present in it.

Oil is colourless, yellowish or greenish liquid, with penetrating odour and a burning, camphorescent taste. On storage it becomes thick and reddish but increases the mellowness even if it is stored for 14 years.

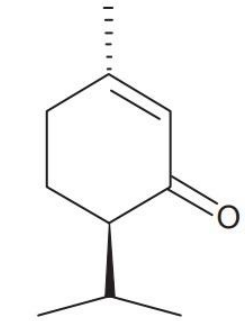
Mentha piperita

Chemical Constituents

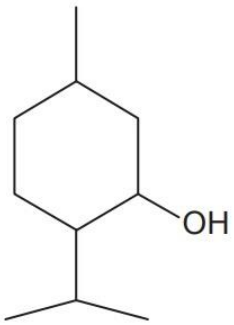
The chief constituent of Peppermint oil is Menthol, along with other constituents like menthyl acetate, isovalerate, menthone, cineol, inactive pinene, limonene, and other less important bodies.

Menthol separates on cooling it to a low temperature (–22°C). The flavouring properties of the oil are due to both the ester and alcoholic constituents, whereas the medicinal value is attributed only due to the alcoholic components.

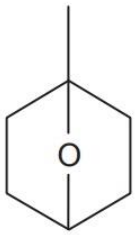
The English oil contains 60 to 70% of Menthol, the Japanese oil containing 85%, and the American has only about 50%.



(+)-Menthone



Menthol



Cineole

Uses

It is stimulant, stomachic, carminative, inflatulence, and colic; in some dyspepsia, sudden pains, for cramp in the abdomen and also in cholera and diarrhoea.

Oil of peppermint allays sickness and nausea, as infants cordial.

Peppermint is good to aid in raising internal heat and inducing perspiration.

It is also used in cases of hysteria and nervous disorders.

Adulterants

Camphor oil, Cedarwood oil, and oil of African Copaiba are occasionally used as an adulterant of Peppermint oil, the oil is also adulterated with one-third part of rectified spirit.

If adulterated with rectified spirit it can be identified by agitating it with water which produces milkiness.

Rosemary oil and Turpentine oil are also sometimes used as adulterants.

Marketed Products

It is one of the ingredients of the preparation known as Dabur lal tooth powder (Dabur).

2. CINNAMON

Synonyms

Cortex cinnamoni, Ceylon cinnamon, Saigon cinnamon, Chinese cassia, *Cinnamomum aromaticum*, *Cinnamomum laurus*

Biological Source

Cinnamon is the dried inner bark of the coppiced shoots of *Cinnamomum zeylanicum* Nees., belonging to family *Lauraceae.*

Geographical Sources

Cinnamomum zeylanicum is widely cultivated in Ceylon, Java, Sumatra, West Indies, Brazil, Mauritius, Jamaica, and India.

Cultivation and Collection

Cinnamon is cultivated by seed propagation method; about four to five seeds are placed in each hole at 2 m distance between the plants. The tree grows best in almost pure requiring only 1% of vegetable substance. It prefers shelter and constant rain of 75" to rainfall.

Cinnamon is an ever-green tree grows from 20 to 30 feet high, has thick scabrous bark, strong branches. The field is kept away from weeds and the plant is coppiced few inches above the ground, leaving five to six straight shoots on them.

The bark is loosened and the longitudinal incisions are made using copper or brass knife. The barks arc stripped off and made into bundles and wrapped in Coir. The bundles are kept aside for about 2 hours to facilitate fermentation due to enzymatic action.

The fermentation helps in the loosening of the outer layer up to pericycle. Each strip is taken and then they are scraped using a knife to separate the cork. The pieces are dried and they are categorized and packed one inside the other.

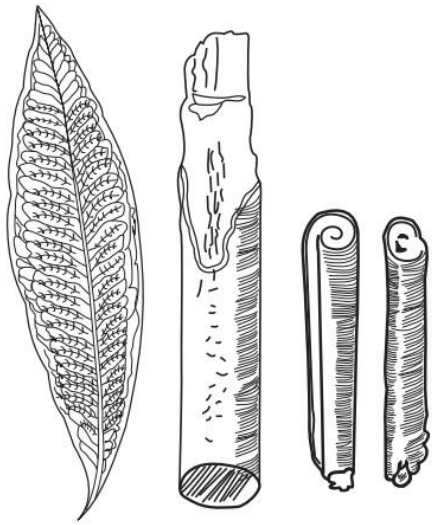
Then compound quills are made by packing the small, quills into larger ones. They are cut into pieces of 1 m length and dried first under shade and later under sun. During drying, the original pale colour changes to brown due to the presence of some pholobatannins in the bark

Characteristics

Cinnamon is either in single- or double-compound quills, with a size of 1 m length, 0.5 mm thickness, and 6 to 10 mm diameter.

The outer surface has yellowish brown colour having longitudinal lines of pericyclic fibre and scars and holes representing the position of leaves or the lateral shoots.

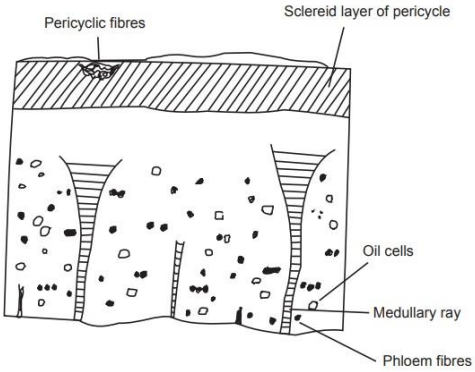
The inner surface is darker than the outer. Cinnamon has a fragrant perfume; taste aromatic and sweet.



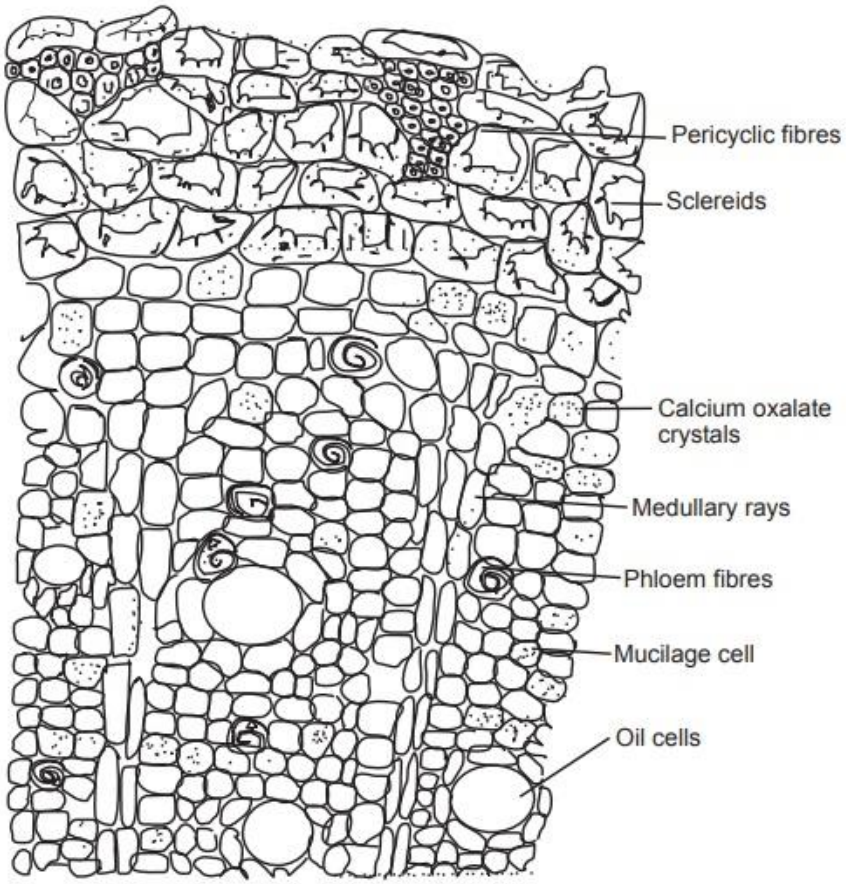
Leaf and bark of *Cinnamomum zeylanicum*

Microscopy

The transverse section shows the presence of three to four layers of sclereids which are horse shoe shaped consisting of starch grains. The pericyclic fibres (6 to 15) are present on the outer margin. It consists of sieve tubes which are completely collapsed and are arranged tangentially; lignified phloem fibres, arranged as tangential rows of four to five cells; biseriate medullary rays with needle-shaped calcium oxalate crystals; longitudinally elongated idioblast consisting of volatile oil; sub-rectangular parenchyma cells with starch grains and calcium oxalate crystals.



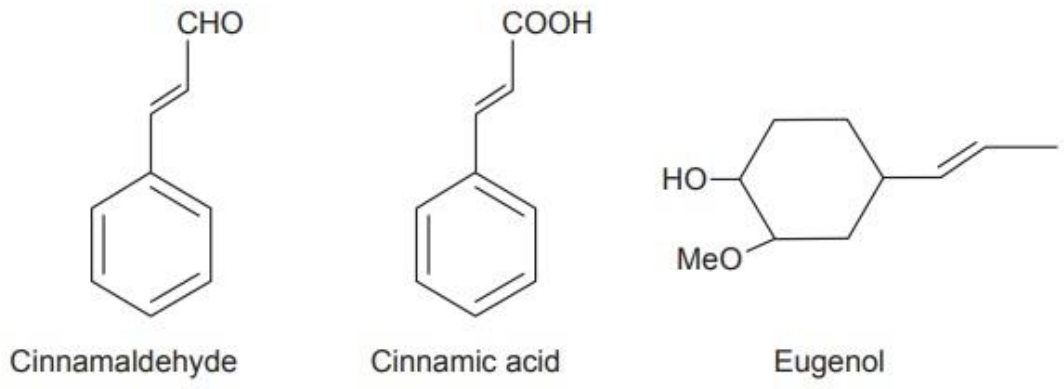
T.S. (schematic) Cinnamon bark



Transverse section of Cinnamon bark

Chemical Constituents

Cinnamon contains about 10% of volatile oil, tannin, mucilage, calcium oxalate and sugar. Volatile oil contains 50 to 65% cinnamic aldehyde, along with 5 to 10% eugenol, terpene hydrocarbons and small quantities of ketones and alcohols.



Cinnamaldehyde Cinnamic acid Eugenol

Chemical Tests

1. A drop of volatile oil is dissolved in 5 ml of alcohol and to it a drop of ferric chloride is added, A pale green colour is produced. Cinnamic aldehyde gives brown colour with ferric chloride, whereas eugenol gives blue colour.

2. The alcoholic extract is treated with phenylhydrazine hydrochloride, it produces red colour due to the formation of phenylhydrazone of cinnamic aldehyde.

Uses

1. It is used as an altenrative, aromatic, carminative, flavouring agent, analgesic, antiseptic, antirheumatic, antispasmodic, demulcent, digestive, expectorant, stomachic, diaphoretic, antibacterial, antifungal, etc.

2. It stops vomiting, relieves flatulence and is given with chalk and as astringents for diarrhoea and haemorrhage of the womb.

3. It is also used in the treatment of bronchitis, colds, palpitations, nausea, congestion, and liver problems.

Other Species

Cinnamon cassia is often used as a substituent. *C. culiawan* is native of Amboyna and the bark has the flavour of clove, *C. iners, Cassia burmarin, Saigon cinnamon*, and *C. nitidum* are also used.

Marketed Products

It is one of the ingredients of the preparations known as Rumalaya gel, Koflet lozenges, Chyavanprash (Himalaya Drug Company), Garbhapal ras, Sutsekhar ras (Dabur), and Sage Staminex capsules (Sage Herbals).

3. CLOVE

Synonyms

Clove buds, Clove flowers.

Biological Source

Clove consists of the dried flower buds of *Eugenia caryophyllus* Thumb., belonging to family *Myrtaceae.*

Geographical Source

Clove tree is a native of Indonesia. It is cultivated mainly in Islands of Zanzibar, Pemba, Brazil, Amboiana, and Sumatra. It is also found in Madagascar, Penang, Mauritius, West Indies, India, and Ceylon.

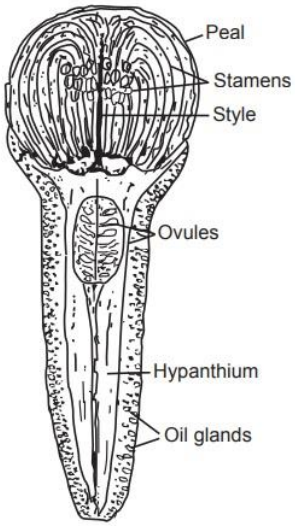
Cultivation and Collection

- ✓ Clove tree is evergreen and 10 to 20 m in height. The plant requires moist, warm and equable climate with well-distributed rainfall. It is propagated by means of seeds.
- ✓ The seeds are sown in well-drained suitable soil at a distance of about 25 cm. The plants should be protected against pests and plant diseases.
- ✓ Initially it has to be protected from sunlight by growing inside a green house or by con-structing frames about 1 m high and covering them with banana leaves.
- ✓ As the banana leaves decay gradually more and more sunlight falls on the young seedlings and the seeds are able to bear full sunlight when they are about 9 months old.

- ✓ The seedlings when become 1 m high, they are transplanted into open spaces at a distance of 6 m just before the rainy season.
- ✓ The young clove trees are protected from sun even for a longer period by planting banana trees in between. The drug can be collected every year starting from 6 years old till they are 70 years old.
- ✓ Clove buds change the colour as they mature.
- ✓ At the start of the rainy season long greenish buds appear which change to a lovely rosy peach colour and as the corolla fades the calyx turns yellow and then red.
- ✓ The buds are collected during dry weather in the month of August to December. The collection is done either by climbing on the tree or by using some ladders or with the help of mobile platforms.
- ✓ In some places the trees are even beaten using bamboo sticks for the collection of the bud.
- ✓ The drugs which are collected are then separated from the stalks and then placed on coconut mats for drying under sun.
- ✓ The buds loose about 70% of its weight, whereas drying and change their colour to dark reddish-brown. The dried clove is graded and packed.

Characterisitics

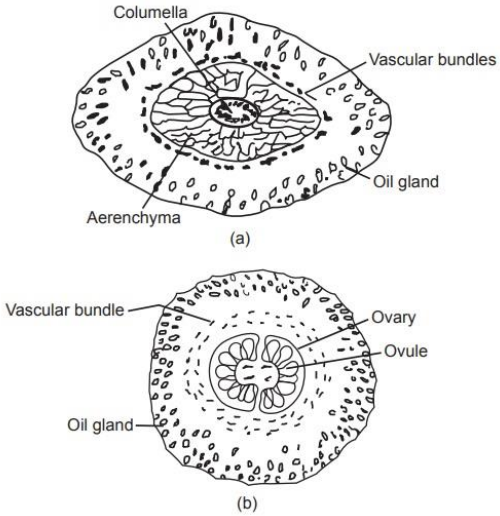
- ✓ Clove is reddish-brown in colour, with an upper crown and a hypanthium.
- ✓ The hypanthium is sub-cylindrical and tapering at the end.
- ✓ The hypanthium is 10 to 13 mm long, 4 mm wide, and 2 mm thick and has schizolysigenous oil glands and an ovary which is bilocular.
- ✓ The Crown region consists of the calyx, corolla, style and stamens.
- ✓ Calyx has four thick sepals. Corolla is also known as head, crown or cap; it is doineshaped and has four pale yellow coloured petals which are imbricate, immature, and membranous.
- ✓ The ovary consists of abundant ovules. Clove has strong spicy, aromatic odour, and pungent and aromatic taste.



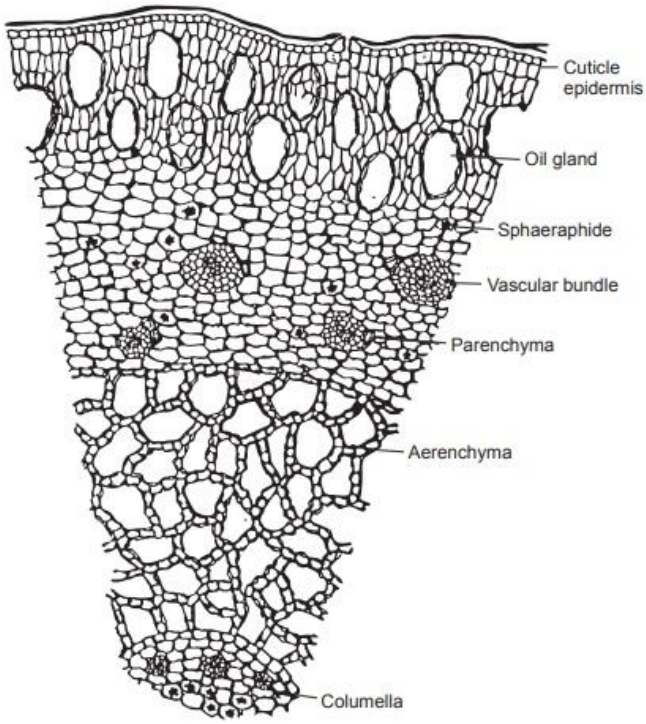
Clove bud

Microscopy

- ✓ The transverse section should be taken through the short upper portion which has the bilocular ovary and also through the hypanthium region.
- ✓ The transverse section through the hypanthium shows the following characters. It has a single layer of epidermis covered with thick cuticle.
- ✓ The epidermis has ranunculaceous stomata. The cortex has three distinct region: the peripheral region with two to three layers of schizolysigenous oil glands, embedded in parenchymatous cells.
- ✓ The middle layer has few layers of bicollateral vascular bundle. In the inner portion it has loosely arranged aerenchyma cells.
- ✓ The central cylinder contains thick-walled parenchyma with a ring of bicollateral vascular bundles and abundant sphaeraphides.
- ✓ The T.S. through ovary region shows the presence of an ovary with numerous ovules in it.



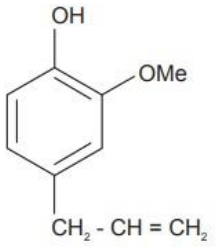
(a) T.S. passing through hypanthium. (b) T.S. passing through ovary



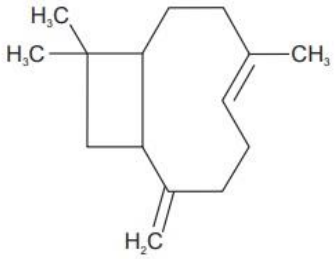
Transverse section of clove flower bud

Chemical Constituents

Clove contains 14–21% of volatile oil. The other constituents present are the eugenol, acetyl eugenol, gallotannic acid, and two crystalline principles; α- and β- caryophyllenes, methyl furfural, gum, resin, and fibre. Caryophyllin is odourless component and appears to be a phytosterol, whereas eugenol is a colourless liquid. Clove oil has 60–90% eugenol, which is the cause of its anesthetic and antiseptic properties.



Eugenol



Caryophyllene

Chemical Tests

1. To a thick section through hypanthium of clove add 50% potassium hydroxide solution; it produces needle-shaped crystals of potassium eugenate.

2. A drop of clove oil is dissolved in 5 ml alcohol and a drop of ferric chloride solution is added; due to the phenolic OH group of eugenol, a blue colour is seen.

3. To a drop of chloroform extract of clove add a drop of 30% aqueous solution of sodium hydroxide saturated with sodium bromide; Needle and pear shaped crystals of sodium eugenate arranged in rosette are produced immediately.

Uses

1. Clove is used as an antiseptic, stimulant, carminative, aromatic, and as a flavouring agent.

2. It is also used as anodyne, antiemetic.

3. Dentists use clove oil as an oral anesthetic and to disinfect the root canals.

4. Clove kills intestinal parasites and exhibits broad antimicrobial properties against fungi and bacteria and so it is used in the treatment of diarrhea, intestinal worms, and other digestive ailments.

5. Clove oil can stop toothache.

6. A few drops of the oil in water will stop vomiting, eating cloves is said to be aphrodisiac.
7. Eugenol is also used as local anaesthetic in small doses.
8. The oil stimulates peristalsis; it is a strong germicide, also a stimulating expectorant in bronchial problems.
9. The infusion and Clove water are good vehicles for alkalies and aromatics.

Adulterants

- ✓ The clove is generally adulterated by exhausted clove, clove fruits, blown cloves and clove stalks. The exhausted cloves are those from which volatile oil is either partially or completely removed by distillation.
- ✓ Exhausted cloves are darker in colour and can be identified as they float on freshly boiled and cooled water. Clove fruits are dark brown in colour and have less volatile oil content.
- ✓ These can be identified by the presence of starch present in the seed of the fruit. Blown Cloves are entirely developed clove flowers from which corolla and stamens get separated.
- ✓ While sepa-ration, sometimes the stalks are incompletely removed and the percentage of volatile oil in clove stalk is only 5%.
- ✓ As clove stalks contain prism type of calcium oxalate crystals and thick-walled stone cells which are absent in clove the clove stalk can also be detected.

Marketed Products

- ✓ It is one of the ingredients of the preparation known as Himsagar tail (Dabur).

4. FENNEL

Synonyms

Fructus foeniculli, Fennel fruit, Fenkel, Florence fennel, Sweet fennel, Wild fennel, Large fennel.

Biological Source

Fennel consists of the dried ripe fruits of *Foeniculum vulgare* Miller., belonging to family *Umbelliferae.*

Geographical Source

Fennel is indigenous to Mediterranean countries and Asia; it is largely cultivated in France, Saxony, Japan, Galicia, Russia, India, and Persia.

History

- ✓ Fennel was well-known to the Ancients, and it was also cultivated by the ancient Romans for its aromatic fruits and edible shoots.
- ✓ It is reported that during third-century B.C. Hippocrates prescribed fennel for the treatment of infant colic, and later on after 400 years Dioscorides called fennel as an appetite suppressant and recommended the seeds for nursing mothers to increase milk secretion.
- ✓ Pliny suggested that fennel cured eye problems and jaundice.
- ✓ Fennel seeds are commonly taken after meals to prevent gas and stomach upset.
- ✓ The use of fennel shoots and seeds are mentioned in ancient record of Spanish agriculture dating A.D. 961.

Cultivation and Collection

- ✓ Fennel, a hardy, beautiful plant, perennial, umbelliferous herb, with yellow flowers and feathery leaves, grows wild in many parts of the world.
- ✓ Fennel is propagated by seeds during April in ordinary soil.
- ✓ Fennel requires abundance sun light and is adapted to dry in sunny situations, it does not call for heavily manured ground but it will yield more on well-drained calcareous soil.
- ✓ About 4 1/2 to 5 lb of seed are sown per acre, either in drills or 15 inches apart, evenly covered with soil. The plants grow to a height of 2 m, erect and cylindrical and take enough space in branching.
- ✓ Most of the branches bearing leaves cut into the very finest of segments.

- ✓ The plant bears fruits in the second year and the bright golden flowers, flat terminal umbels bloom in July and August.
- ✓ The fruits are collected by cutting the stems in September, when the fruits are ripe.
- ✓ The stems are dried on sheaves under sun and later beaten to separate the fruits.

Characteristics

The fruit is an entire cremocarps with pedicels, oval-oblong and 5 to 10 mm long, 2 to 4 mm broad.

It has greenish-brown to yellowish brown colour with five prominent primary ridges and a bifid stylopod at the apex.

Foeniculum vulgare

Microscopy

The transverse section of mericarp region of fennel shows two prominent surfaces, the dorsal and the commissural surface.

The commisural surface has a carpophore and two vittae, and the dorsal surface has a total of five ridges.

The mericarp is divided into pericarp, consisting of the epicarp and mesocarp; the testa and the endocarp.

Epicarp consists of polygonal cells of epidermis which are tangentially elon-gated and covered by the cuticle.

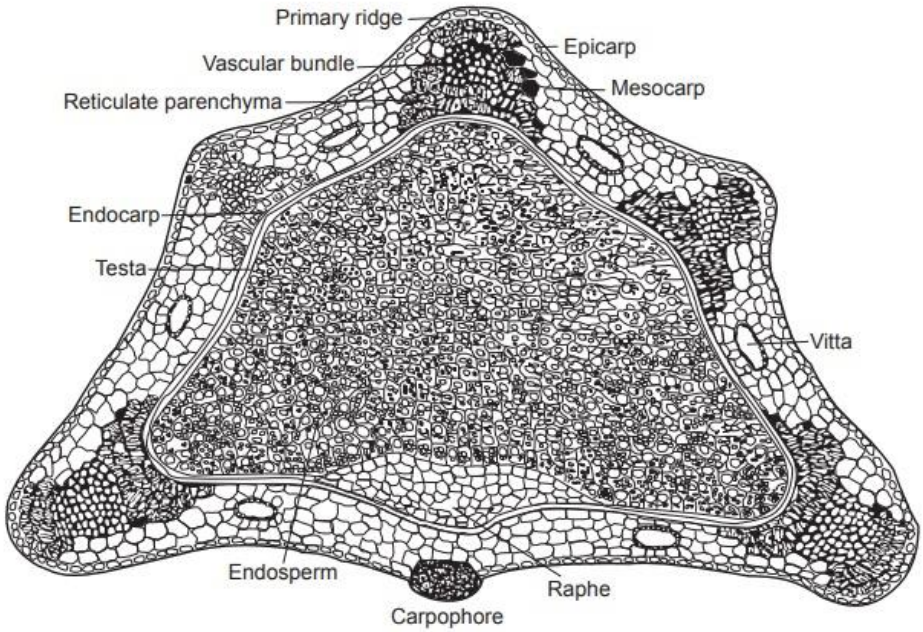
Mesocarp has parenchyma cells with five bicollateral vascular bundles; below each primary ridge a lignified reticulate parenchyma surrounds the vascular bundles.

There are four vittae on dorsal surface and two vittae on commisural or the ventral surface.

Inner Epidermis or Endocarp shows parquetry arrangement (a group of four to five cells arranged parallelly at acute angles with groups of similar cells in different direction).

Testa is a single-layered tangentially elongated cell with yellowish colour.

Endosperm consists of thick-walled, wide polyhedral, colourless cells. Cells contain fixed oil, aleurone grains, and rosette crystals of calcium oxalate.



Transverse section of Fennel fruit (Mericarp)

Chemical Constituents

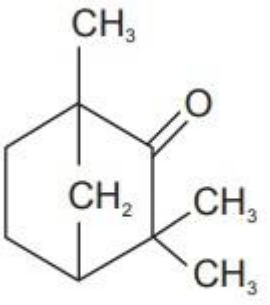
The best varieties of Fennel contain 4 to 5% of volatile oil.

The primary constituents of volatile oil are 50 to 60% of anethole, a phenolic ester; and 18 to 22% of fenchone, a ketone.

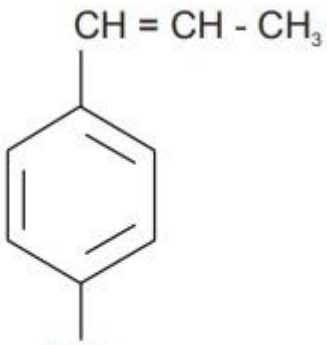
Fenchone is chemically a bicyclic monoterpene which is a colourless liquid and the odour and taste is pungent and camphoraceous.

The oil of Fennel has β-pinene, anisic acid, phellandrine, and anisic aldehyde.

Fennel also contains about 20% fixed oil and 20% proteins.



Fenchone



Anethole

Uses

1. Fennel is used as stomachic, aromatic, diuretic, carminative, diaphoretic, as a digestive, pectoral, and flavouring agent.

2. Anethole may have estrogen-like activity and inhibit spasms in smooth muscles.

3. Fennel can increase production of bile, used in the treatment of infant colic, to promote menstruation in women, can increase lactation, act as antipyretic, antimicrobial and antiinflammatory.

Adulterants

- ✓ Fennel is generally adulterated with exhausted fennel and due to improper caring during harvesting they are also adulterated with sand, dirt, stem, weed seeds, etc in which part of volatile oil is removed either by extraction with alcohol or steam distillation.

- ✓ Fruits exhausted by water or steam are darker in colour, contain less essential oil and sink in water, but those exhausted by alcohol still hold 1 to 2% of oil in them.

Marketed Products

- ✓ It is one of the ingredients of the preparations known as Abana, Shahicool, Anxocare (Himalaya Drug Company), Aptikid (Lubin Herbal Laboratory), Jalifaladi bati (Baidyanath), and Hajmola, Janum Gunti (Dabur).

5. CORIANDER

Synonyms

Fructus coriandri, Coriander fruits, Cilantro, Chinese parsley.

Biological Source

Coriander consists of dried ripe fruits of *Coriandrum sativum* Linn., belonging to family *Umbelliferae.*

Geographical Sources

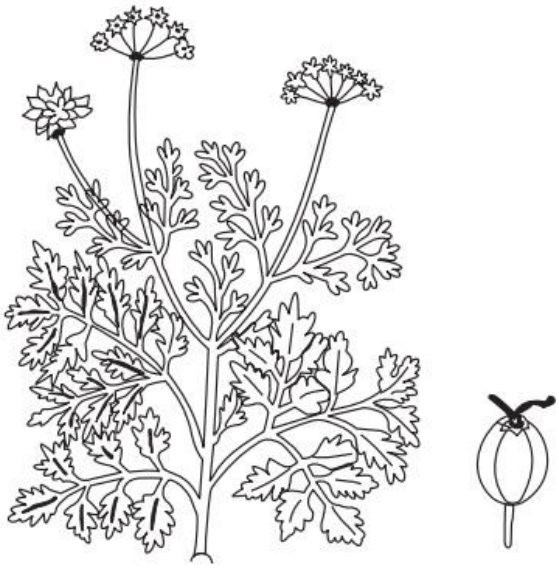
Cultivated in Central and Eastern Europe, particularly in Russia, Hungary, in Africa and India
In India it is cultivated in Maharashtra, U.P., Rajasthan, Jammu, and Kashmir.
It is also found in antiwild state in the east of England.

Cultivation and Collection

- ✓ The coriander seeds are sown in dry weather either in March or in early autumn.
- ✓ Shallow drills, about 1/2 inch deep and 8 inches apart are made and the seeds are sown in it, the rate of germination is slow.
- ✓ The plants are annual herb, which grow to a height of 1 to 3 feet high, slender, and branched. The flowers are in shortly stalked umbels with five to ten rays.
- ✓ The seeds fall as soon as ripe and when the seeds are ripe (about August), the disagreeable odour is produced. Plant is then cut down with sickles; the fruits are collected and dried.
- ✓ During drying fruits develop aromatic smell and the unpleasant odour disappears.

Characteristics

The fruit is a cremocarp, subspherical in shape, Yellowish-brown in colour. The size of the fruit is 3 to 4 mm in diameter, with aromatic odour, and spicy, aromatic taste.

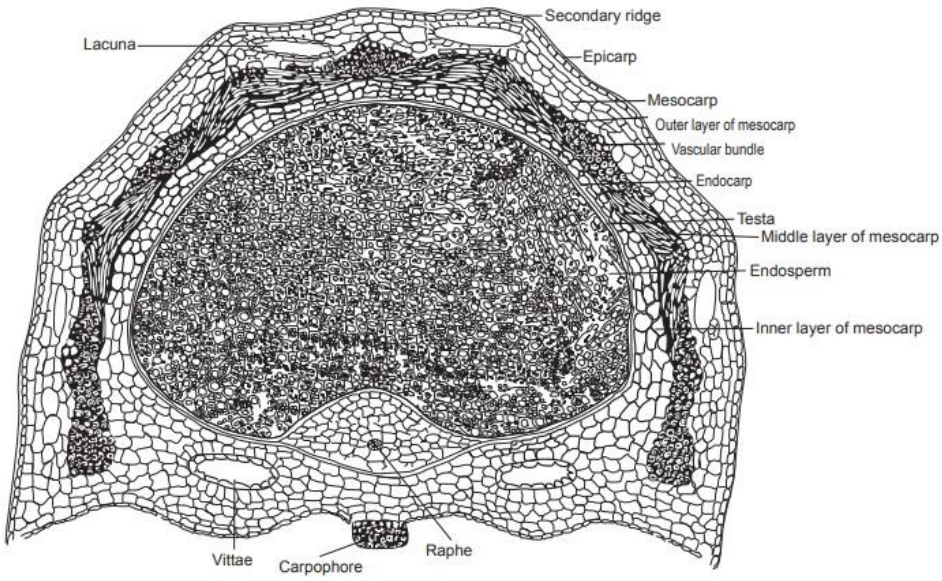


Coriandrum sativum

Microscopy

- ✓ The transverse section of coriander shows the presence of a dorsal surface and a commissural surface. The dorsal surface consists of two vittae and a carpophore.
- ✓ The dorsal surface has five primary ridges and four secondary ridges.
- ✓ The epicarp consists of a single row of small thick-walled cells with calcium oxalate crystals.
- ✓ The mesocarp has an outer loosely arranged tangentially elongated parenchyma cells and the middle layer consisting of sclerenchyma.
- ✓ The middle layer is again divided into; the outer region of sclerenchyma is represented by longitudinally running fibres, whereas the inner region has tangentionally running fibres.
- ✓ The vascular bundles are present below the primary ridges.
- ✓ The inner layer has polygonal, irregularly arranged parenchyma cells.

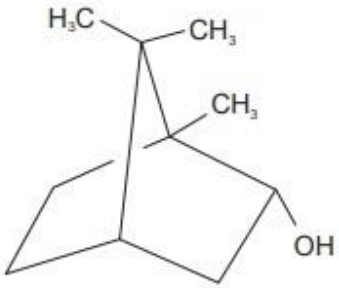
- ✓ The endocarp has the parquetry arrangement.
- ✓ In the testa it has single-layered, yellowish cells, and the endosperm is thick, polygonal, colourless parenchyma with fixed oil and aleurone grains.



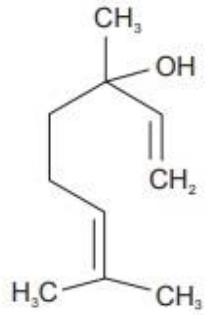
Transverse section of coriander fruit (mericarp)

Chemical Constituents

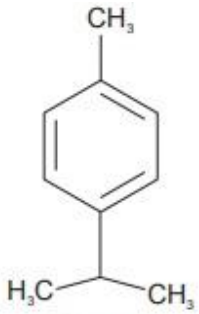
- ✓ Coriander consist of about 1% of volatile oil the chief volatile components are D-(+)-linalool (coriandrol), along with other constituents like, borneol, p-cymene, camphor, geraniol, limonene, and alpha-pinenes.
- ✓ The fruits also contain fatty oil and hydroxycoumarins.
- ✓ The fatty oils include acids of petroselic acid, oleic acid, linolenic acid, whereas the hydroxycoumarins include the umbelliferone and scopoletine.



Borneol



Linalool



p-Cymene

Uses

- ✓ Aromatic, carminative, stimulant, alterative, antispasmodic, diaphoretic and flavouring agent
- ✓ It is also used as refrigerant, tonic, apetizer, diuretic, aphrodisiac, and stomachic.
- ✓ Coriander can be applied externally for rheumatism and painful joints.
- ✓ The infusion of decoction of dried fruit of cardamom is useful for the treatment of sore-throat, indigestion, vomiting, flatulence, and other intestinal disorders.

Marketed Products

- ✓ It is one of the ingredients of the preparations known as Cystone (Himalaya Drug Company), Bilwadi churna (Baidyanath), and Sage massage oil (Sage Herbals).

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Dundigal, Quthbullapur Mandal, Hyderabad 500043, R.R. Dist.

Subject: Pharmacognosy and phytochemistry-II | **Unit No: III**
Faculty: Mrs. S. Jyothsna | **Lecture No: 1**
Topic: Tannins introduction | **Book Reference: T2**

TANNINS:

INTRODUCTION

The name 'tannin' is derived from the French 'tanin' (tanning substance) and is used for a range of natural polyphenols. Tannins are complex organic, non-nitrogenous plant products, which generally have astringent properties. These compounds comprise a large group of compounds that are widely distributed in the plant kingdom.

The term 'tannin' was first used by Seguin in 1796 to denote substances which have the ability to combine with animal hides to convert them into leather which is known as tanning of the hide. According to this, tannins are substances which are detected by a tanning test due to its absorption on standard hide powder. The test is known as Goldbeater's skin test.

CLASSIFICATION

The tannin compounds can be divided into two major groups on the basis of Goldbeater's skin test. A group of tannins showing the positive tanning test may be regarded as true tannins, whereas those, which are partly retained by the hide powder and fail to give the test, are called as pseudotannins.

Most of the true tannins are high molecular weight compounds. These compounds are complex polyphenolics, which are produced by polymerization of simple polyphenols.

They may form complex glycosides or remains as such which may be observed by their typical hydrolytic reaction with the mineral acids and enzymes.

Two major chemical classes of tannins are usually recognized based on this hydrolytic reaction and nature of phenolic nuclei involved in the tannins structure.

The first class is referred to as hydrolysable tannins, whereas the other class is termed as condensed tannins.

Hydrolysable Tannins

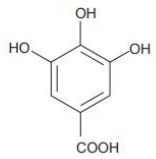
As the name implies, these tannins are hydrolysable by mineral acids or enzymes such as tannase.

Their structures involve several molecules of polyphenolic acids such as gallic, hexahydrodiphenic, or ellagic acids, bounded through ester linkages to a central glucose molecule.

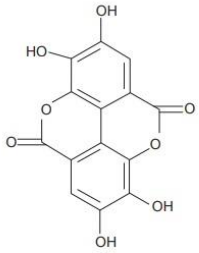
On the basis of the phenolic acids produced after the hydrolysis, they are further categorized under gallotannins composed of gallic acid or ellagitannins which contains hexahydrodiphenic acid which after intraesterification produces ellagic acid.

Hydrolysable tannins are sometimes referred to as pyrogallol tannins as the components of phenolic acids on dry distillation are converted to pyrogallol derivatives.

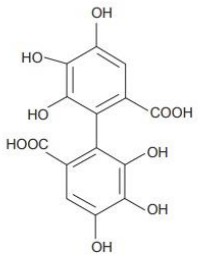
The hydrolysable tannins are soluble in water, and their solution produces blue colour with ferric chloride.



Gallic acid



Ellagic acid



Hexahydroxydiphenic acid

Nonhydrolysable or Condensed Tannins

Condensed tannins, unlike the previously explained group are not readily hydrolysable to simpler molecules with mineral acids and enzymes, thus they are also referred to as nonhydrolysable tannins. The term proanthocyanidins is sometimes alternatively used for these tannins.

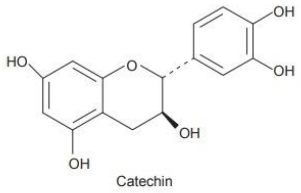
The compounds containing condensed tannins contain only phenolic nuclei which are biosynthetically related to flavonoids. Catechin which is found in tannins is flavan-3-o1, whereas leucoanthocyanidins are flavan-3,4-diol structures.

These phenolics are frequently linked to carbohydrates or protein molecules to produce more complex tannin com-pounds. When treated with acids or enzymes, they tend to polymerize yielding insoluble red coloured products known as phlobaphens. The phlobaphens give characteristic red colour to many drugs such as cinchona and wild cherry bark.

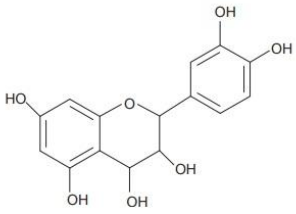
On dry distillation, they yield catechol derivatives. Condensed tannins are also soluble in water and produces green colour with ferric chloride.

The families of the plants rich in both of the above groups of tannins include Rosaceae, Geraniaceae, Leguminosae, Combretaceae, Rubiaceae, Polygonaceae, Theaceae, etc. The members of families Cruciferae and Papaveraceae on the other hand are totally devoid of tannins.

In the plants in which tannins are present, they exert an inhibitory effect on many enzymes due to their nature of protein precipitation and therefore contribute a protective function in barks and heartwood.



Catechin



Leucoanthocyanidin

Pseudotannins

Pseudotannins are simple phenolic compounds of lower molecular weight. They do not respond to the tanning reaction of Goldbeater's skin test. Gallic acid, Chlorogenic acid, or the simple phenolics such as catechin are pseudotannins which are abundantly found in plants, especially in dead tissues and dying cells.

CHARACTERISTICS OF TANNINS

1) Tannins are colloidal solutions with water.

2) Non crystalline substance.

3) Soluble in water (exception of some high molecular weight structures), alcohol, dilute alkali, and glycerin.

4) Sparingly soluble in ethyl acetate.

5) Insoluble in organic solvents, except acetone.

6) Molecular weight ranging from 500 to >20,000.

7) Oligomeric compounds with multiple structure units with free phenolic groups.

8) Can bind with proteins and form insoluble or soluble tannin—protein complexes.

BIOSYNTHESIS OF TANNINS

Tannins belong to the phenolics class of secondary metabolites. All phenolic compounds; either primary or secondary are in one way or another formed through shikirnic acid pathway (phenylpropanoid pathway). Other phenolics such as isoflavones, coumarins, lignins, and aromatic amino acids (tryptophan, phenylalanine, and tyrosine) are also formed by the same pathway.

Hydrolysable tannins (Hts) and condensed tannins (proanthocyanidins) are the two main categories of tannins that impact animal nutrition.

Common tannins are formed as follows:

1. Gallic acid is derived from quinic acid.
2. Ellagotannins are formed from hexahydroxydiphenic acid esters by the oxidative coupling of neighbouring gallic acid units attached to a D-glucose core.
3. Further oxidative coupling forms the hydrolysable tannin polymers.
4. Proanthocyanidin (PA) biosynthetic precursors are the leucocyanidins (flavan-3,4-diol and flavan-4-ol) which on autoxidation, in the absence of heat, form antho-cyanidin and 3-deoxyanthocianidin, which, in turn, polymerize to form PAs.

Isolation

Both hydrolysable and condensed tannins are highly soluble in water and alcohol but insoluble in organic solvents such as solvent ether, chloroform, and benzene.

Tannin compounds can be easily extracted by water or alcohol. The general method for the extraction of tannic acid from various galls is either with water-saturated ether, or with mixture of water, alcohol, and ether.

In such cases, free acids such as Gallic and ellagic acid go along with ether, whereas true tannin gets extracted in water. If the drug consists of chlorophyll or pigment, it may be removed by ether.

After extraction, the aqueous and ethereal layers are separately concentrated, dried, and subjected to further isolation and purification using various separation techniques of chromatography.

CHEMICAL TESTS

1. ***Goldbeater's skin test***: Goldbeater's skin is a membrane produced from the intestine of Ox. It behaves just like untanned animal hide. A piece of goldbeaters skin previously soaked in 2% hydrochloric acid and washed with distilled water is placed in a solution of tannin for 5 minutes.

It is then washed with distilled water and transferred to 1% ferrous sulphate solution. A change of the colour of the goldbeater's skin to brown or black indicates the presence of tannin.

2. Hydrolysable and condensed tannins both give the positive goldbeater's test, whereas pseudotannins show very little colour or negative test.

3. ***Phenazone Test:*** To 5 ml of aqueous solution of tannin containing drug, add 0.5 g of sodium acid phosphate. Warm the solution, cool, and filter. Add 2% phenazone solution to the filtrate. All tannins are precipitated as bulky, coloured precipitate.

4. ***Gelatin Test:*** To a 1% gelatine solution, add little 10% sodium chloride. If a 1% solution of tannin is added to the gelatine solution, tannins cause precipitation of gelatine from solution.

5. ***Test for Catechin* (*Matchstick Test*)**: Catechin test is the modification of the well-known phloroglucinol test for lignin. Matchstick contains lignin. Dip a matchstick in the dilute extract of the drug, dry, moisten it with concentrated hydrochloric acid, and warm it near a flame. Catechin in the presence of acid produces phloroglucinol which stains the lignified wood pink or red.

6. ***Test for chlorogenic acid***: A dilute solution of chlorogenic acid containing extract, if treated with aqueous ammonia and exposed to air, slowly turns green indicating the presence of chlorogenic acid.

7. ***Vanillin-hydrochloric acid test:*** Drug shows pink or red colour with a mixture of vanillin: alcohol : dilute HCl in the ratio 1:10:10. The reaction produces phloroglucinol which along with vanillin gives pink or red colour.

MEDICINAL PROPERTIES AND USES

1. Tannins are medicinally significant due to their astringent properties.

2. They promote rapid healing and the formation of new tissues on wounds and inflamed mucosa.

3. Tannins are used in the treatment of varicose ulcers, haemorrhoids, minor burns, frostbite, as well as inflammation of gums.

4. Internally tannins are administered in cases of diarrhoea, intestinal catarrh, and in cases of heavy metal poisoning as an antidote.

5. In recent years, these compounds have demonstrated their antiviral activities for treatment of viral diseases including AIDS.

6. Tannins are used as mordant in dyeing, manufacture of ink, sizing paper and silk, and for printing fabrics. It is used along with gelatine and albumin for manufacture of imitation horn and tortoise shell.

7. They are widely used in the leather industry for conversion of hide into leather, the process being known as tanning.

8. Tannins are also used for clarifying beer or wine, in photography or as a coagulant in rubber manufacture.

9. Tannins are used for the manufacture of gallic acid and pyrogallol, and sometimes as a reagent in analytical chemistry.

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Subject: Pharmacognosy and phytochemistry-II | **Unit No: III**
Faculty: Mrs. S. Jyothsna | **Lecture No: 2**
Topic: Catechu | **Book Reference: T2**

1. PALE CATECHU

Synonyms

Gambier, pale catechu, catechu

Biological Source

Gambier or pale catechu is a dried aqueous extract produced from the leaves and young twigs of *Uncaria gambier* Roxburgh., belonging to family *Rubiaceae*.

Geographical Source

U. gambier is a native of erstwhile Malaya. It is cultivated in Indonesia, Malaysia, Sumatra, Bornea, and Singapore at eleva-tion up to 150 m. The plant is used mostly for the production of the drug, which is marketed through Singapore.

Cultivation, Collection, and Preparation

Propagation of *U. gambier* is done by seeds. Seeds are sown in the nursery to raise the seedlings, which after about 9 months are planted out in the clearing about 3 meters apart. Leaves and young shoots are collected as a first crop during second year's growth.

Later the crop is taken every year. The plant continues to give sufficient leaves and twigs up to 20 years, but the maximum yield is obtained during eighth year of growth.

The collected leaves and twigs are transported to the factory as loose material. The material is put into large drums with about three quarters of boiling water.

It is boiled for about three hours with intermittent stirring. The marc is subsequently removed by large wooden forks and lodged on surface to drain the liquor back to the vessels. It is pressed and washed.

The washing is added to the extract. The combined total aqueous extract is then concentrated for one and half-hour till it becomes thick, yellowish-green paste. It is transferred from the vessels to wooden tubs, stirred while it is hot, and cooling in a stream of water to crystallize tannins.

Semicrystallized paste is again transferred to wooden trays in which it sets. They are cut into cubes by wooden knife and dried in sum. The drug is also made into large blocks in kerosene tins.

Morphology

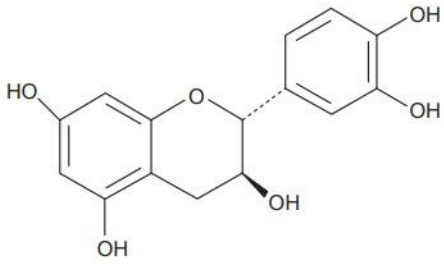
Colour	Dull reddish brown colour externally and pale brown to buff colour internally.
Odour	Odourless
Taste	At first it is bitter and astringent but later it is sweet.
Shape	Strips, flakes or coarse powder
Size	Pale catechu comes in the form of cubes or rectangular blocks of 2 to 4 cm length
Shape	Regular cubes or as rectangular blocks.

Microscopy

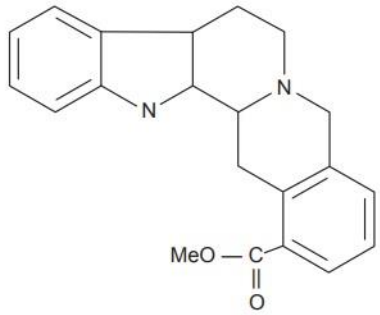
The powdered drug, if mounted in the solution of lactophenol or water, shows the small circular crystals of catechu under microscope. The water insoluble part of the pale catechu under the microscope exhibits epidermal pieces, unicellular hairs, cork tissues, lignified fibres, etc. Alcohol insoluble part shows the absence of starch. The pale catechu from Indonesia is reported to have minute starch grains.

Chemical Constituents

Pale catechu contain from about 7 to 30% of pseudotannin catechin and 22 to 55% of a phlobatannin catechutannic acid. Both of the about component constitute over 60% of the drug. It also contains catechu red, gambier fluorescin and quercetin. It contains indole alkaloid up to 0.05%, which includes gambirtannin and its derivatives. Gambirtannin gives a strong fluorescence under UV light. Catechin forms white, needle like crystals, which dissolves in alcohol and hot water. Catechutannic acid gives green colour with ferric chloride.



Catechin



Gambirtannin

Chemical Tests

1. ***Gambier fluorescin test***: Gambier fluorescin present in pale catechu gives the fluorescence. If to its alcohol extract, a little sodium hydroxide is added and shaken with petroleum ether. The petroleum ether layer shows green fluorescence. Black catechu gives negative test.

2. ***Vanillin-hydrochloric acid test:*** Drug shows pink or red colour with a mixture of vanillin:alcohol:dilute HCl in the ratio 1:10:10. The reaction produces phloroglucinol which along with vanillin gives pink or red colour.

3. A matchstick dipped in decoction of Pale catechu is air dried and again dipped into concentrated HCl and warmed near the burner. Pink or purple colour is produced.

4. Small quantity of powder is heated on water bath with 5 ml chloroform and filtered. The filtrate is evaporated in white porcelain dish on a water bath. A greenish-yellow residue is produced due to the presence of chlorophyll in the drug. Black catechu gives this test negative due to the absence of chlorophyll.

Uses

Pale catechu is medicinally used as local astringent. In diarrhoea, it is used as general astringent. It is largely used in various countries of east for chewing with betel leaf. Large proportion of gambier is used in dyeing and tanning industries. It is used for tanning of animal hides to convert it to leather.

2. BLACK CATECHU

Synonym
Cutch, black catechu, kattha

Biological Source
Black catechu is the dried aqueous extract prepared from the heartwood of *Acacia catechu* Willdenow, belonging to family, *Leguminosae*.

Geographical Source
A. catechu is common throughout the tract from Punjab to Assam ascending to an altitude of 300 m. It is also quite common in drier regions of peninsula such as Madhya Pradesh, Maharashtra, Gujarat, Rajasthan, Bihar, and Tamil Nadu.

History

Possibly, the use of black catechu could be traced back in history from the time of chewing betel leaf, in which it has been used as adjuvant. In old days, it was used by women as a colouring agent for the feet. Since 15th century, this natural material has been exported to Europe. The old information about catechu is by a Portuguese writer Garcia de Orta in 1574. Dr. Wrath first used the scientific process to extract catechu, and showed that catechu consists of two parts, such as, kattha and cutch.

Collection and Preparation

catechu is a medium-sized tree with thorns. For preparation of the drug the tree is cut off from the ground. The main trunk and branches are cleared of foliage and thorns.

The bark is stripped off, and the heartwood is made into chips. Heartwood is boiled in water in large earthen pots. The decoction is then strained and boiled in an iron pot with continuous stirring till it forms the syrupy mass.

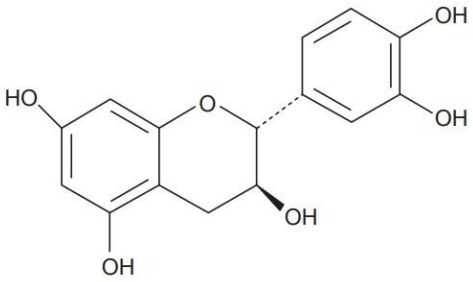
When the extract is cool enough, it is spread in the shallow wooden trays and kept for overnight. When sufficiently dry, it is cut into pieces. Since the decoction is concentrated in iron vessels, the colour of the catechu becomes darker due to its reaction with iron salts.

If the syrupy extract is stirred during cooling, it develops the shining crystals of catechin and produces translucent black catechu. Nowadays stainless steel vessels are used for the manufacture of catechu that produces a lighter coloured product.

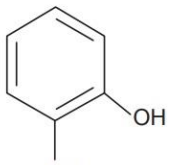
Colour	Black or brownish black mass
Odour	Odourless
Taste	Astringent and subsequently sweet taste
Size	Irregular mass
Extra features	Outer surface is firm and brittle. When broken the fractured surface appears glassy with small cavities

Chemical Constituents

Cutch or black catechu resembles pale catechu or gambier in its composition. It contains about 2–12% of catechin and about 25 to 33% of phlobatannin catechutannic acid. The principle fraction of cutch has been identified as a mixture of catechin isomers which includes (-) epicatechin, acatechin, DL-acacatechin, L-acacatechin and D-isoacacatechin. It also contains 20–30% gummy matter, catechin red, quercetin and querecitin. It yields 2–3% of ash.



Catechin



Catechol

Chemical Tests

1. Because of the presence of catechin, black catechu gives pink or red colour with vanillin and HCl.
2. Catechin when treated with HCl produces phlorogucinol, which burns along with lignin to give purple or magenta colour. For this purpose, tannin extract is taken on match stick dipped in HCl and heated near the flame.

3. Lime water when added to aqueous extract of black catechu gives brown colour, which turns to red precipitate on standing for some time.
4. Green colour is produced when ferric ammonium sulphate is added to dilute solution of black catechu. By the addition of sodium hydroxide, the green colour turns to purple.

Uses

1. Cutch is used in medicine as astringent.
2. It cures troubles of mouth, diseases of the throat and diarrhoea.
3. It also increases appetite.
4. In India and eastern countries, it is used in betel leaves for chewing.
5. In dyeing industries, cutch is used for dyeing fabrics brown or black. It is also used in calico printing.

Marketed Products

It is one of the ingredients of the preparation known as Koflet lozenge (Himalaya Drug Company) as cough expectorant, and Gum tone (Charak Pharma Pvt. Ltd.).

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Subject: Pharmacognosy and phytochemistry-II | **Unit No: III**
Faculty: Mrs. S. Jyothsna | **Lecture No: 3**
Topic: Pterocarpus | **Book Reference: T2**

3. PTEROCARPUS

Synonyms
Bijasal, Indian kino tree, Malbar kino

Biological Source
It consists of dried juice obtained by making vertical incisions to the stem bark of the plant *Pterocarpus marsupium* Linn., belonging to family *Leguminosae*.

Geographical Distribution

It is found in hilly regions of Gujarat, Madhya Pradesh, Uttar Pradesh, Bihar, and Orissa. It is also found in forests of Karnal, Kerala, West Bengal, and Assam.

Morphology

Colour	Ruby-red
Odour	Odourless
Taste	Astringent
Shape	Angular grains
Size	3 to 5 to 10 mm granules
Solubility	It is partly soluble in water (about 80—90%), completely soluble in alcohol (90%).
Extra features	The pieces of kino are angular, glistening, transparent, breaking with vitreous fracture.

Chemical Constituents

Kino contains about 70–80% of kinotannic acid, kino-red, k-pyrocatechin (catechol), resin and gallic acid. Kinotannic acid is glucosidal tannin, whereas kino-red is anhydride of kinoin. Kinoin is an insoluble phlobaphene and is produced by the action of oxydase enzyme. It is darker in colour than kinotannic acid.

Chemical Tests

1. When the solution of drug is treated with ferrous sulphate, green colour is produced.
2. With alkali (like potassium hydroxide) violet colour is produced.
3. With mineral acid, a precipitate is obtained.

Uses

1. Kino is used as powerful astringent and also in the treatment of diarrhoea and dysentery, passive haemorrhage, toothache, and in diabetes.

2. It is used in dyeing, tanning, and printing. The aqueous infusion of the wood is considered to be of much use in diabetes.

3. The alcoholic, as well as, aqueous extracts of heartwood are known to possess hypoglycaemic action.

4. The cups made of wood are available with Khadi and Gramodyog commission for treatment of diabetes.

Marketed Products

It is the one of the components of the preparation known as Gludibit (Lupin Herbal Laboratory) and Diabecon (Himalaya Drug Company) for diabetes mellitus.

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Dundigal, Quthbullapur Mandal, Hyderabad 500043, R.R. Dist.

Subject: Pharmacognosy and phytochemistry-II **Unit No: III**
Faculty: Mrs. S. Jyothsna **Lecture No: 4**

Topic: Resins introduction **Book Reference: T2**

RESINS

DEFINITION

Resin can be defined as the complex amorphous product of more or less solid characteristics which on heating first sets softened and then melt. Resins are produced and stored in the schizogenous or schizolysigenous glands or cavities of the plants. Isolated resin products which come as an unorganized crude drug in the market are more or less solid, hard, transparent, or translucent materials. Resins are insoluble in most polar and nonpolar solvents like water and petroleum ether, respectively, but dissolve completely in alcohol, solvent ether, benzene, or chloroform.

CLASSIFICATION

Resins are classified mostly on the basis of two important features, that is, on the basis of their chemical nature and secondly as per their association with the other group of compounds like essential oils and gums.

Chemical classification of resins categorizes these products according to their active functional groups as given below:

Resin Acids
Resin acids are the carboxylic acid group containing resinous substances which may or may not have association with phenolic compounds. These compounds are found in free states or as the esters derivatives. Being acidic compounds they are soluble in aqueous solution of alkalies producing frothy solution. Resin acids can be derivatized to their metallic salts known as resinates, which finds their use in soap, paints and varnish industries. The abietic acid and commiphoric acid present in colophony and myrrh respectively are the examples of resin acids.

Resin Esters
Resin esters are the esters of the resin acids or the other aromatic acids like benzoic, cinnamic, salicylic acids, etc.
They are sometimes converted to their free acids by the treatment with caustic alkali. Dragon's blood and benzoin are the common resin ester containing drugs.

Resin Alcohols
Resin alcohols or resinols are the complex alcoholic com-pounds of high molecular weight. Like resin acids they are found as free alcohols or as esters of benzoic, salicylic, and cinnamic acids. They are insoluble in aqueous alkali solution but are soluble in alcohol and ether. Resinols are present in benzoin as benzoresinol and in storax as storesinol.

Resin Phenols

Resin phenols or resinotannols are also high molecular weight compounds which occur in free states or as esters. Due to phenolic group they form phenoxoids and become soluble in aqueous alkali solution. However they are insoluble in water but dissolve in alcohol and ether. Resinotannols gives a positive reaction with ferric chloride. The resinotannol are found in balsam of Peru as peruresinotannol, in Tolu balsam as toluresinotannol and in benzoin as siaresinotannols.

Glucoresins

Resins sometimes get combined with sugars by glycosylation and produce glucoresins. Glycoresins can be hydrolysed by acidic hydrolysis to the glycone and aglycone.

Resenes

Chemically inert resin products are generally termed as resenes. They are generally found in free state and never form esters or other derivatives. Resenes are soluble in benzene, chloroform and to some extent in petroleum ether. Resenes are insoluble in water. Asafoetida is an example of resene-containing drug, which contains drug about 50% of asaresene B.

Accordingly, other simple classification based on the association of resin with gums and/or volatile oils is given below.

Oleoresins

Oleoresins are the homogenous mixture of resin with vola-tile oils. The oleoresins posses an essence due to volatile oils. A trace amount of gummy material may sometimes be found in oleoresins. Turpentine, ginger, copaiba, Canada resin are few important examples of oleoresins.

Gum Resins

Gum resins are the naturally occurring mixture of resins with gums. Due to solubility in water, gums can be easily separated out from resin by dissolving the gum in water. Ammoniacum is an example of natural gum resin.

Oleogum Resins

Oleogum resins are the naturally occurring mixtures of resin, volatile oil, and gum. The example includes gum myrrh, asafoetida, gamboage, etc. Oleogum resins oozes out from the incisions made in the bark and hardens.

Balsams

Balsams are the naturally occurring resinous mixtures which contain a high proportion of aromatic balsamic acids such as benzoic acid, cinnamic acid, and their esters. Balsams containing free acids are partially soluble in hot water. Some important balsams containing drugs are balsam of Peru, balsam of Tolu, benzoin, and storax. The oleogum resin containing drugs like copaiba and Canada are sometimes wrongly referred to as balsams.

CHEMICAL COMPOSITION

The chemical composition of the resin is generally quite complex and diverse in its nature. It can be a complex mixture of acids, alcohols, phenols, esters, glycosides, or hydrocarbons. When the resins are associated with volatile oils, contains the components like monoterpenoids, sesquiterpenonoids, and diterpenoids. The gums which are associated with resins are similar to acacia gum which sometimes possesses smaller quantities of oxidase enzymes. Resins can be of the physiological origin such as the secretions of the ducts. They can also be pathological products which are exuded through the incisions made on the plant.

ISOLATION

The process of the isolation of resin from crude drug can be a difficult task due to the presence of various combinations. However the most generalized technique can be the extraction of the drug with alcoholic solvents and then subsequent precipitation of resin by adding concentrated alcoholic extract to a large proportion of water. The method of distillation or hydrodistillation can be used for the separation of volatile oils from resin. This process is used largely for the separation of resin from turpentine.

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Subject: Pharmacognosy and phytochemistry-II **Unit No: III**
Faculty: Mrs. S. Jyothsna **Lecture No: 5**
Topic: Asafoetida and Colophony **Book Reference: T2**

4. ASAFOETIDA

Synonyms
Devil's dung; food of the gods; asafoda; asant; hing (Hindi)

Biological Source
Asafoetida is an oleo-gum resin obtained as an exudation by incision of the decapitated rhizome and roots of *Ferula asafoetida* L, *F. foetida*, Royel, *F. rubricaulis* Boiss, and some other species of Ferula, belonging to family Apiaceae.

Geographical Source
The plant grows in Iran, Turkestan and Afghanistan (Karam and Chagai districts).

Collection

The plant is a perennial branching, 3 m high herb possessing large schizogeneous ducts and lysigenous cavities containing milky liquid. Upon exudation and drying of the liquid, Asafoetida is obtained. For the collection of the drug the upper part of the root is laid bare and the stem cut off close to the crown in March–April. The exposed surface is covered by a dome-shaped structure made of twigs and earth. After separating each slice, exudation of oleo-gum-resin, present as whitish gummy resinous emulsion

in the schizogenous ducts of the cortex of the stem, takes place. It hardens on the cut surface which is collected, packed in tin-line cases and exported. Removal of the exudation and exposure of fresh surface proceeds until the root is exhausted. The yield is usually soft enough to agglomerate into masses when packed.

Characteristics

Asafoetida occurs as a soft solid mass or irregular lumps or 'tears', sometimes almost semi-liquid. Tears are rounded or flattened and about 5–30 mm in diameter, grayish-white or dull yellow or reddish brown in colour.

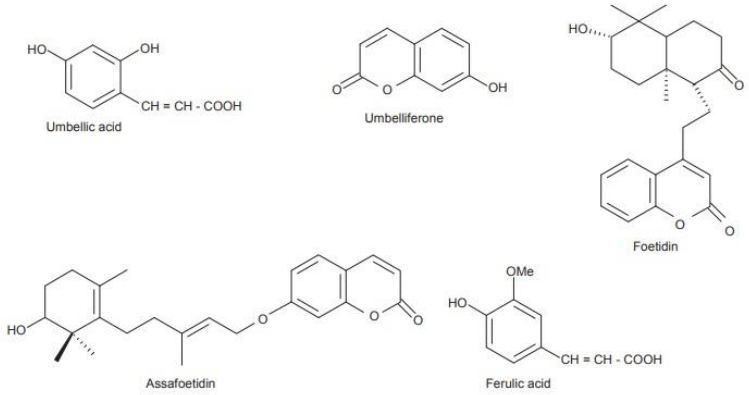
Asafoetida mass is mixed with fruits, fragments of root, sand and other impurities. Asafoetida has a strong garlic-like (alliaceous) odour and a bitter, acrid and alliaceous taste. When triturated with water, it makes a milky emulsion.

It should not have more than 50% of matter insoluble in alcohol (90%) and not more than 15% of ash.

Chemical Constituents

Asafoetida contains volatile oil (4–20%), resin (40–65%), and gum (25%). The garlic-like odour of the oil is due to the presence of sulphur compounds. The main constituent of the oil is isobutyl propanyl disulphide ($C_6H_{16}S_2$). The three sulphur compounds, such as, 1-methylpropyl-1-propenyl disulphide, l-(methylthio)-propyl-1-pro-penyl disulphide, and l-methyl-propyl 3-(methylthio)-2-propenyl disulphide have also been isolated from the resin; the latter two have pesticidal properties. The flavour is largely due to R-2-butyl-l-propenyl disulphide and 2-butyl-3-methylthioallyl disulphide (both as mixtures of diastereoisomers).

The drug also contains a complex mixture of sesquiterpene umbelliferyl ethers mostly with a monocyclic or bicyclic terpenoid moiety. Resin consists of ester of asaresinotannol and ferulic acid, pinene, vanillin and free ferulic acid. On treatment of ferulic acid with hydrochloric acid, it is converted into umbelliferone (a coumarin) which gives blue fluorescence with ammonia.



Asafoetida also contains phellandrene, sec-butylpropenyl disulphide, geranyl acetate, bornyl acetate, α-terpineol, myristic acid, camphene, myrcene, limonene, fenchone, eugenol, linalool, geraniol, isoborneol, borneol, guaiacol, cadinol, farnesol, assafoetidin, foetidin, etc.

Chemical Tests

1. On trituration with water it produces a milky emulsion.

2. The drug (0.5 g) is boiled with hydrochloric acid (5 ml) for sometime. It is filtered and ammonia is added to the filtrate. A blue fluorescence is obtained.

3. To the fractured surface add 50% nitric acid. Green colour is produced.

4. To the fractured surface of the drug, add sulphuric acid (1 drop). A red colour is obtained which changes to violet on washing with water.

Uses

Asafoetida is used as carminative, expectorant, antispas-modic, and laxative as well as externally to prevent bandage chewing by dogs; for flavouring curries, sauces, and pickles; as an enema for intestinal flatulence, in hysterical and epileptic affections, in cholera, asthma, whooping cough, and chronic bronchitis.

Adulteration

Asafoetida is adulterated with gum Arabic, other gum-resins, rosin, gypsum, red clay, chalk, barley or wheat flour, and slices of potatoes.

Allied Drugs

Galbanum and ammoniacum are oleo-gum-resins obtained, respectively, from *Ferula galbaniflua* and *Dorema ammoniacum.* Galbanum contains umbelliferone and umbelliferone ethers, up to 30% of volatile oil containing numerous mono- and sesquiterpenes, azulenes, and sulphur-containing

esters. Ammoniacum contains free salicylic acid but no umbeiliferone. The major phenolic constituent is ammoresinol. An epimeric mixture of prenylated chromandiones termed ammodoremin is also present. The volatile oil (0.5%) contains various terpenoids with ferulene as the major component.

Marketed Products

It is one of the ingredients of the preparation known as Madhudoshantak (Jamuna Pharma).

5. COLOPHONY

Synonyms

Rosin, yellow resin; Abietic anhydride; colophony resin; amber resin; resin; coloponium

Biological Source

Colophony is a solid residue left after distilling off the volatile oil from the oleoresin obtained from *Pinus palustris* (long leaf pine) and other species of *Pinus* such as *P. pinaster, P. halepensis, P. massoniana, P. tabuliformis, P. carribacea* var., belonging to family Pinaceae.

Geographical Source

The genus *Pinus* is widely found in United States, France, Italy, Portugal, Spain, Greece, New Zealand, China, India (Himalayan region), and Pakistan. Colophony is chiefly produced in the United States contributing about 80% of world supply. Other countries producing the resin are China, France, Spain, India, Greece, Morocco, Honduras, Poland, and Russia.

Collection

The collection of the oleoresin is very laborous procedure. Although Colophony is a normal (Physiological) resin of *Pinus* species, its amount is increased by injuring the plant. For its collection a few-feet long groove or blaze is made in the bark with the help of knife or some other instru-ment. A metal or earthenware cup is attached below the groove by nails. The cup is adjusted accordingly when the size of groove increases. The resin is taken out at different intervals and sent for further processing.

Cup and Gutter Method

This method is used in America, European countries, India, and Pakistan. The 60–100 cm long blaze or longitudinal groove is cut with a suitable instrument. It is enlarged at intervals and in about four years is about 4 m long. The metal or earthenware cups are attached to the trunk by nails and one or two strips of galvanized iron are placed above each to direct the flow of oleoresin. As the grooves are lengthened the cups are moved higher up the tree and new grooves are started when the old ones

become exhausted or collection is difficult. The cups are emptied at intervals and the oleoresin sent to the distillery. Trees can be tapped by this method for about 40 years.

Preparation

The crude oleoresin arrives at the distillery in barrels. It is mixed with about 20% by weight of turpentine in a heated stainless steel vessel and allowed to stand to separate water and other impurities. The diluted oleoresin is then transferred to copper or stainless steel stills and the turpentine is removed by steam distillation. When distillation is complete the molten resin is run through wire strainers into barrels, in which it cools and is exported.

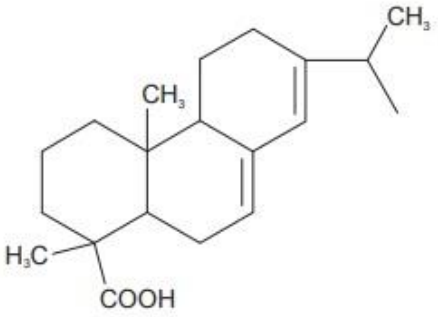
The resin obtained from trees during their first year of tapping is of a lighter colour than that obtained later on. The following grades of American rosin are recognized: B, FF (for wood rosin only), D, E, F, G, H, I, K, L, M, N, WG (window-glass), WW (water-white), and the extra-white X grades and American and Portuguese qualities (XA, XB. XC). A great deal of the American tall oil rosin is now paler than grade X. Grade B is almost black.

Characters

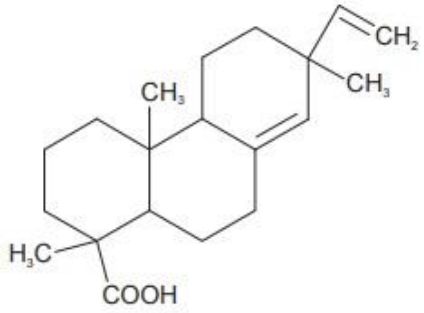
Colophony occurs as translucent, hard, shiny, sharp, pale yellow to amber fragments, fracture brittle at ordinary temperature, burns with smoky flame, slight turpentine-like odour and taste, melts readily on heating, density 1.07–1.09. Acid number is not less than 150. It is insoluble in water but freely soluble in alcohol, benzene, ether, glacial acetic acid, oils, carbon disulphide, and alkali solutions.

Chemical Constituents

Colophony contains resin acids (about 90%), resenes, and fatty acid esters. Of the resin acids about 90% are isomeric α-, β-, and γ-abietic acids; the other 10% is a mixture of dihydroabietic acid and dehydroabietic acid. Before distillation, the resin contains excess amounts of (+) and (-) pimaric acids. During distillation the (-) pimaric acid is converted into abietic acid while (+) pimaric acid is stable. The other constituents of Colophony are sipinic acid and a hydrocarbon.



Abietic acid



Pimaric acid

Chemical Tests

1. To a solution of powdered resin (0.1 g) in acetic acid (10 ml) one drop of conc. Sulphuric acid is added in a dry test tube. A purple colour, readily changing to violet, is formed.

2. To a petroleum ether solution of powdered Colophony twice its volume of dilute solution of copper acetate is shaken. The colour of the petroleum ether layer changes to emerald-green due to formation of copper salt of abietic acid.

3. To alcoholic solution of Colophony sufficient water is added. It becomes milky white due to precipitation of chemical compounds.

4. Alcoholic solution of Colophony turns blue litmus to red due to the presence of diterpenic acids.

Uses

- ✓ Colophony is used as stiffening agent in ointments, adhesives, plasters and cerates and as a diuretic in veterinary medicine.
- ✓ Commercially it is used to manufacture varnishes, printing inks, cements, soap, sealing wax, wood polishes, floor coverings, paper, plastics, fireworks, tree wax, rosin oil, and for water proofing cardboard.
- ✓ The abietic acids show antimicrobial, antiulcer and cardiovascular activity; some have filmogenic, surfactant, and antifeedant properties.

6. MYRRH

Synonyms
Gum-resin Myrrh; Gum Myrrh; Arabian or Somali Myrrh; Myrrha

Biological Source

Myrrh is an oleo gum-resin obtained from the stem of *Commiphora molmol* Eng. or *C. abyssinica* or other species of Commiphora, belonging to family *Burscraceae.*

Geographical Source

It grows in Arabian pennisula, Ethiopia, Nubia, and Somal-iland.

Collection

Myrrh plants are small trees up to 10 meters in height. They have the phloem parenchyma and closely associated ducts containing a yellowish granular liquid. The tissues between these ducts often collapse, thereby producing large cavities similarly filled, that is, schizogenous ducts become lysigenous cavities. The gum-resin exudes spontaneously or by incising the bark. The yellowish-white, viscous fluid is solidified readily to produce reddish-brown masses which are collected by the natives.

Characteristics

- Myrrh occurs as irregular masses or tears weighing up to 250 g.
- The outer surface is powdery and reddish-brown in colour.
- The drug breaks and is powdered readily.
- Fractured surface is rich brown and oily.
- Odour is aromatic and taste is aromatic, bitter, and acrid.

Chemical Constituents
Myrrh contains resin (25–40%), gum (57–61%), and volatile oil (7–17%). Large portion of the resin is ether-soluble containing α-, β-, and γ-commiphoric acids, resenes, the esters of another resin acid and two phenolic compounds.

The volatile oil is a mixture of cuminic aldehyde, eugenol, cresol, pinene, limonene, dipentene, and two sesquiterpenes. The disagreeable odour of the oil is due to mainly the disulphide.

The gum contains proteins (18%) and carbohydrate (64%) which is a mixture of galactose, arabinose, glucuronic acid, and an oxidase enzyme.

Chemical Tests

1. A yellow brown emulsion is produced on trituration with water
2. Ethereal solution of Myrrh turns red on treatment with bromine vapours. The solution becomes purple with nitric acid.

Uses

1. Myrrh is used as carminative and in incense and perfumes.
2. It has local stimulant and antiseptic properties and is utilized in tooth powder and as mouth wash. Topically it is astringent to mucous membranes.
3. It is used in a tincture, paint, gargle and rinse due to its disinfecting, deodourizing, and in inflammatory conditions of the mouth and throat.
4. Alcoholic extracts are used as fixatives in the perfumery industry.

Allied Drugs

Four different varieties of' bdellium are present. Of these, perfumed or scented bdellium or bissabol is obtained from *C. erythaea* var. *glabrescens.* It resembles soft myrrh in appearance but more aromatic odour and does not give a violet colour.

Marketed Products

It has been marketed as Guggulipid by CDRI, Lucknow, India. In ayurveda, it is sold as Yograj guggulu (Baidyanath) for antiinflammatory and antihyperlipidemic activity, and it is also a constituent of Madhumehari (Baidyanath).

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Subject: Pharmacognosy and phytochemistry-II | **Unit No: III**
Faculty: Mrs. S. Jyothsna | **Lecture No: 6**
Topic: Ginger and Siam Benzoin | **Book Reference: T2**

7. GINGER

Synonyms

Rhizoma zingiberis, Zingibere.

Biological Source
Ginger consists of the dried rhizomes of the *Zingiber officinale* Roscoe, belonging to family *Zingiberaceae*.

Geographical Source
It is mainly cultivated in West Indies, Nigeria, Jamaica, India, Japan, and Africa.

Cultivation
Ginger plant is a perennial herb that grows to 1 m. It is cultivated at an altitude of 600 to 1,500 m above sea level.

The herb grows well in well-drained rich, loamy soil, and in abundant rain fall. The rhizome is cut into pieces called fingers, and each finger consisting of a bud is placed in a hole filled with rotten manure in March or April.

The rhizomes get matured in December or January. By January the plants wither after flowering and then the flowers are forked up, buds and the roots removed and washed to remove the mould and clay or dirt attached to them.

The rhizomes are socked in water overnight and the next morning they are scraped with a knife to remove the outer cork and little of parenchyma. They are washed again and then dried under sun for a week.

The rhizomes are turned by the sides at regular intervals to facilitate proper drying. This is the ‘unbleached Jamaica’ or the uncoated ginger.

The coated or the unpeeled variety is prepared by dropping the rhizome for few minutes in boiling water, and then skin is removed such that the layer on the flat surface is removed but not in the grooves between the branches.

The ‘bleached’ or ‘limed’ is prepared by treating it with sulphuric acid or chlorine or dusting it with calcium sulphate or calcium carbonate.

Characteristics

The rhizomes are 5 to 15 cm long, 3 to 6 cm wide, and about 1.5 cm thick. The Jamaica ginger occurs as branches.

It has a sympodial branching and the outer surface has buff yellow colour with longitudinally striated fibres.

Small circular depressions at the portion of the buds are seen and fractured surface shows narrow bark, a well-developed endodermis, and a wide stele, with scattered small yellowish points of secretion cells and grayish points of fibrovascular bundles.

The ginger has agreeable and aromatic odour and pungent and agreeable taste.

Microscopy

The cork is the outermost layer with irregular parenchymatous cells and dark brown colour. The inner cork is few layered, colourless parenchymatous cells arranged in radial rows.

Cork is absent in Jamaica ginger. Phellogen is indistinct and the cortex consists of thin-walled rounded parenchyma with intercellular spaces consisting of abundant starch grains.

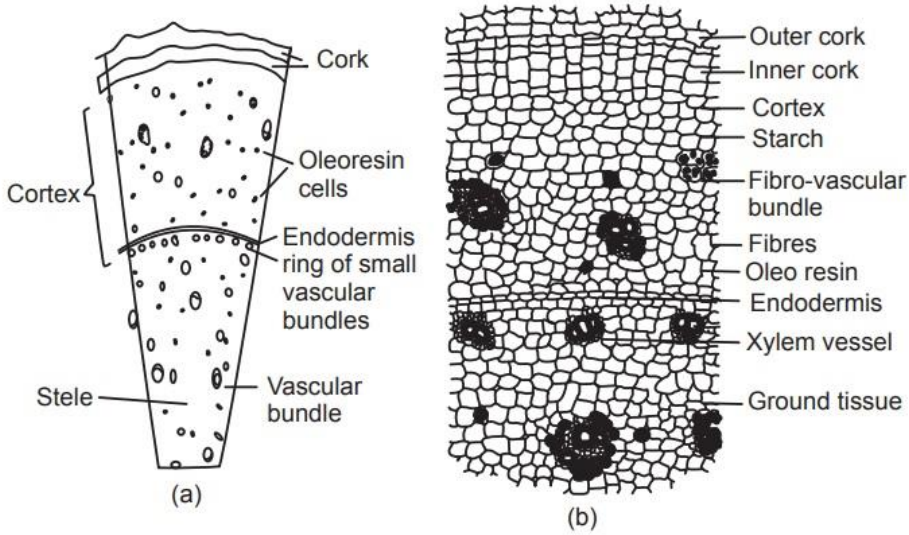
The starch grains are simple, ovate, or sac shaped. Numerous yellowish brown oleo-resin are also present along with the collateral fibro vascular bundles.

The endodermis is distinct without starch and consists of single layer of tangentially elongated cells containing suberin.

Just below the endodermis it has the ground tissue, a ring of narrow zone of vascular bundle which is not covered with sclerenchymatous fibres.

The ground tissues contain the large parenchymatous cells rich in starch, oleoresin, fibrovascular bundles.

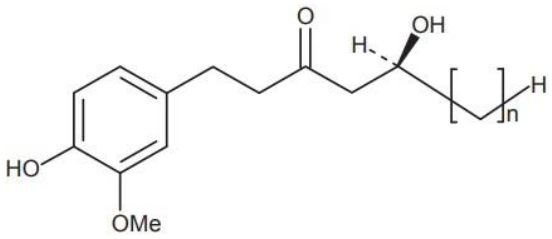
The phloem has well-developed sieve elements, and the xylem consist of vessels, tracheids either annual or spiral, or reticular in nature without lignin. The fibres are unlignified, pitted, and separate.



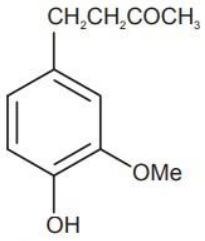
(a) Schematic diagram (T.S.) and, (b) Transverse section of Ginger rhizome

Chemical Constituents

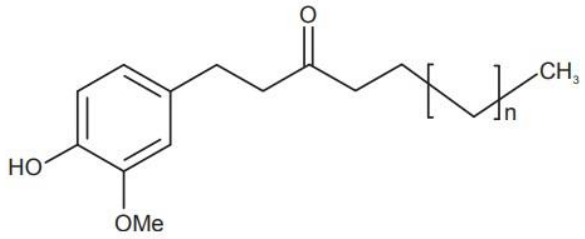
Ginger contains 1 to 2% volatile oil, 5 to 8% pungent resinous mass and starch. The volatile oil is responsible for the aromatic odour and the pungency of the drug is due to the yellowish oily body called gingerol which is odourless. Volatile oil is composed of sesquiterpene hydrocarbon like α-zingiberol; α-sesquiterpene alcohol α-bisabolene, α-farnesene, α-sesquiphellandrene. Less pungent components like gingerone and shogaol are also present. Shogal is formed by the dehydration of gingerol and is not present in fresh rhizome.



Gingerols (n = 0,2,3,4,5,7,9)



Zingerone



Shogaols (n = 4,5,7,9,10)

Uses

1. Ginger is used as an antiemetic, positive inotropic, spasmolytic, aromatic stimulant, carminative, condiment, and flavouring agent.
2. It is prescribed in dyspepsia, flatulent colic, vomiting spasms, as an adjunct to many tonic and stimulating remedies, for painful affections of the stomach, cold, cough, and asthma.
3. Sore throat, hoarseness, and loss of voice are benefited by chewing a piece of ginger.

Adulteration

- Ginger may be adulterated by addition of 'wormy' drug or 'spent ginger' which has been exhausted in the extraction of resins and volatile oil.
- This adulteration may be detected by the official standards, for alcohol-soluble portion, water-soluble portion, total ash and water-soluble ash.
- Sometimes pungency of exhausted ginger is increased by the addition of capsicum.

Marketed Products

It is one of the ingredients of the preparations known as Pain kill oil, J.P. Liver syrup (Jamuna Pharma), Abana, Gasex (Himalaya Drug Company), Hajmola (Dabur), Strepsils (Boots Piramal Healthcare), and Sage Massaj oil (Sage Herbals).

8. SIAM BENZOIN

Biological Source

Siam Benzoin is a balsamic resin derived from stem of *Styrax tonkinensis* Craib., belonging to family Styraceae.

Geographical Source

The trees are present in North Laos, North Vietnam, Annam, and Thailand.

Collection

Siam Benzoin is also a pathological resin produced by incising the bark and by fungus attack. The stem of 6–8 years old plant is incised when balsam exudates. The resin is obtained in the form of liquid which is solidified.

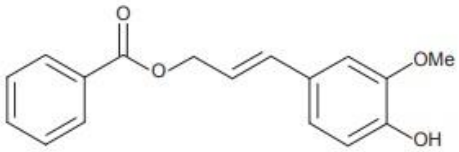
Characteristics

Siam Benzoin occurs as tears or in blocks of variable sizes and reddish brown externally, but milky-white or opaque internally.

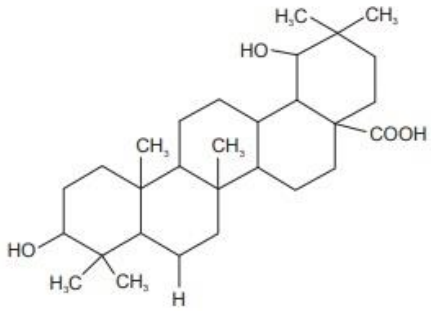
Matrix is glassy, reddish-brown, resinous, brittle but softening on chewing and become plastic-like on chewing. It has vanilla-like odour and a balsamic taste.

Chemical Constituents

The principal constituent of Siam Benzoin is coniferyl benzoate (60–80%) (3 – methoxy – 4 - hydroxycinnamyl alcohol). Other constituents are free benzoic acid (10%), triterpene siaresinolic acid (6%), vanillin, and benzyl cinnamate.



Coniferyl benzoate



Siaresinolic acid

Chemical Tests

1. Heat Sumatra Benzoin (5 g) with 10% aqueous potassium permanganate solution. A bitter almond-like odour is produced due to oxidation of cinnamic acid present in Sumatra Benzoin. This test is negative in case of Siam Benzoin.

2. To a petroleum ether solution of Benzoin (0.2 g), two to three drops of sulphuric acid are added in a China dish. Sumatra Benzoin produces reddish-brown colour, whereas Siam Benzoin shows purple-red colour on rotating the dish.

3. To alcoholic solution of Benzoin ferric chloride solution is added. A green colour is produced in Siam Benzoin due to the presence of phenolic compound coniferyl benzoate. This test is negative in case of Sumatra Benzoin which does not contain sufficient amount of phenolic constituents.

Uses

1. Siam Benzoin acts as antiseptic, culinary and expectorant;

2. It is used to prepare benzoinated lard, cosmetics, fixatives, and in perfumery.

3. It is superior to the Sumatra Benzoin with respect to antioxidative effect in lard and other fats.

Marketed Products

It is one of the ingredients of the preparation known as Friar's Balsam.

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Dundigal, Quthbullapur Mandal, Hyderabad 500043, R.R. Dist.

Subject: Pharmacognosy and phytochemistry-II **Unit No: III**
Faculty: Mrs. S. Jyothsna **Lecture No: 7**
Topic: Guggul and Sumatra Benzoin **Book Reference: T2**

9. GUGGUL

Synonyms
Gumgugul, Salai-gogil

Biological Source
Guggal is a gumresin obtained by incision of the bark of *Commiphora mukul* (H. and S.) Engl., belonging to family *Burseraceae.*

Geographical Source
The tree is a small, thorny plant distributed throughout India.

Collection

Guggul tree is a small thorny tree 4 to 6 feet tall branches slightly ascending.

It is sometimes planted in hedges. The tree remains without any foliage for most of the year.

It has ash-coloured bark, and comes off in rough flakes, exposing the innerbark, which also peels off.

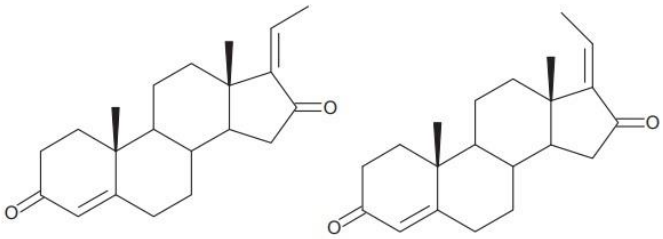
The tree exudes a yellowish resin called gum guggul or guggulu that has a balsamic odor.

Each plant yields about one kilogram of the product, which is collected in cold season.

Characteristics
Guggal occurs as viscid, brown tears; or in fragment pieces, mixed with stem, piece of bark; golden yellow to brown in colour. With water it forms a milk emulsion. It has a balsamic odour and taste is bitter, aromatic.

Chemical Constituents

Guggal contains gum (32%), essential oil (1.45%), sterols (guggulsterols I to VI, β-sitosterol, cholesterol, Z- and E-guggulsterone), sugars (sucrose, fructose), amino acids, α-camphorene, cembrene, allylcembrol, flavonoids (quercetin and its glycosides), ellagic acid, myricyl alcohol, aliphatic tetrols, etc.



Z-guggulsterone E-guggulsterone

Uses

1. Guggal significantly lowers serum triglycerides and cholesterol as well as LDL and VLDL cholesterols (the bad cholesterols).

2. At the same time, it raises levels of HDL cholesterol (the good cholesterol), inhibits platelet aggregation, and may increase thermogenesis through stimulation of the thyroid, potentially resulting in weight loss.

3. Also gum is astringent, aritirheumatic, antiseptic, expectorant, aphrodisiac, demulcent, and emmenagogue.

4. The resin is used in the form of a lotion for indolent ulcers and as a gargle in teeth disorders, tonsillitis, pharyngitis, and ulcerated throat.

Marketed Products

It is one of the ingredients of the preparations known as Arogyavardhini Gutika (Dabur) and Abana, Diabecon, Diakof (Himalaya Drug Company).

10. SUMATRA BENZOIN

Synonyms
Gum Benjamin; Benzoinum; Benzoin; Luban (Hindi)

Biological Source

Sumatra Benzoin is obtained from the incised stem of *Styrax benzoin* Dryander and *Styrax parallelo-neurus* Perkins., belonging to family *Styraceae*. It contains about 25% of total balsamic acids, calculated as cinnamic acid

Geographical Source
The trees are found in Sumatra, Malacca, Malaya, Java, and Borneo.

Collection
The plants are medium-sized trees. Sumatra Benzoin is a pathological resin which is formed by making incision and by attack of fungi. In Sumatra the seeds are sown in rice fields.

The rice plants provide protection to benzoin plants during first year. After harvesting of the rice crop the trees are allowed to grow.

When they are 7 years old, three triangular wounds are made in a vertical row. Tapping consists of making in each trunk three lines of incisions which are gradually lengthened. The first triangular wounds are made in a vertical row about 40 cm apart, the bark between the wounds being then scraped smooth.

The first secretion is very sticky and is rejected. After making further cuts, each about 4 cm above the preceding ones, a harder secretion is obtained. Further incisions are made at three-monthly intervals, and the secretion becomes crystalline.

About 6 weeks after each fresh tapping the product is scraped off, the outer layer (finest quality) being kept separate from the next layer (intermediate quality). About 2 weeks later the strip is scraped again, giving a lower quality darker in colour and containing fragments of bark.

Fresh incisions are then made, and the above process is repeated. Second exudation is milky white and is used for medicinal purpose.

The stem is incised four times during one year. AH types of exudations are sent to industry for further processing.

A single tree yields about 10 kg of resin per year and is completely exhausted by the 19th year of its life.

Characteristics

Sumatra benzoin occurs in brittle masses consisting of opaque, whitish, or reddish tears embedded in a translucent, reddish-brown or greyish-brown, resinous matrix.

Odour : agreeable and balsamic
Taste : slightly acrid. Siamese benzoin occurs in tears or in blocks.

The tears are of variable size and flattened; they are yellowish-brown or reddish-brown externally, but milky-white and opaque internally.

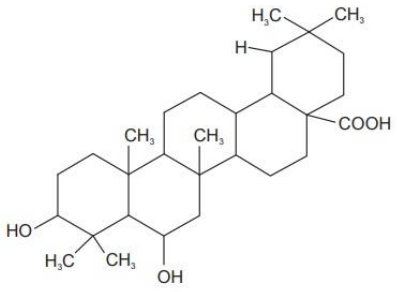
The block form consists of small tears embedded in a glassy, reddish-brown, resinous matrix. It has a vanilla-like odour and a balsamic taste.

When heated, benzoin evolves white fumes of cinnamic and benzoic acids which readily condense on a cool surface as a crystalline sublimate.

Chemical Constituents

Sumatra Benzoin consists of free balsamic acid (cinnamic and benzoic acids) (25%) and their esters. The amount of cinnamic acid is usually double that of benzoic acid.

It also contains triterpenic acids like siaresinolic acid (19-hydroxy-oleanolic acid) and sumaresinolic acid (6-hydroxy-oleanolic acid); traces of vanillin, phenylpropyl cinnamate, cinnamyl cinnamate, and phenylethylene.



Siaresinolic acid

Uses

1. Sumatra Benzoin possesses expectorant, antiseptic, carminative, stimulant, and diuretic properties.

2. It is used in cosmetic lotions, perfumery and to prepare Compound Benzoin.

3. It forms an ingredient of inhalations in the treatment of catarrh of upper respiratory tract in the form of Compound Benzoin Tincture.

4. Benzoin is used as an external antiseptic and protective, and is one of the main ingredients of Friar's Balsam.

5. It is also used to fix the odour of incenses, skin-soaps, perfumes and other cosmetics and for fixing the taste of certain pharmaceutical preparations.

6. Benzoin retards rancification of fats and is used for this purpose in the official benzoinated lard, also used in food, drinks and in incense.

Allied Drug

Palembang benzoin, an interior variety produced in Sumatra is collected from isolated trees from which the resin has not been stripped for some time.

It is very light in weight and breaking with an irregular porous fracture.

It consists of reddish-brown resin, with only a few very small tears embedded in it. *Palembang benzoin* is used as a source of natural benzoic acid.

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Subject: Pharmacognosy and phytochemistry-II | **Unit No: III**
Faculty: Mrs. S. Jyothsna | **Lecture No: 8**
Topic: Senna and Aloe | **Book Reference: T2**

11. SENNA LEAF

Synonyms

Alexandrian senna, Tinnevelly senna, Folia senna

Biological Source

Senna leaf consists of the dried leaflets of *Cassia acutifolia* Delile (*C. senna* L.) known as Alexandrian senna and of *C. angustifolia* Vahl., which is commercially known as Tin-nevelly senna. It belong family *Leguminosae*.

Geographical Source

Alexandrian senna is indigenous to South Africa.

It widely grows and sometimes is cultivated in Egypt and in the middle upper territories of Nile river.

It is also cultivated in Kordofan and Sennar regions of Sudan. Indian or Tinnevelly senna is indigenous to southern Arabia and cultivated largely in Tinnevelly and Ramnathpuram districts of Tamilnadu.

It also grows in Somaliland, Sindh and Punjab region.

Characteristics

Senna leaflets are 3–5 cm long, 2 cm wide and about 0.5 mm thick.

It shows acute apex, entire margin and asymmetric base.

Outline is lanceolate to ovate lanceolate. Pubescent lamina is found on both the surfaces.

Leaves show greyish green colour for Alexandrian senna and yellowish green for Tinnevelly senna. Leaves of Tinnevelly senna are somewhat larger, less broken and firmer in texture than that of Alexandrian senna.

Odour of leaves is slight but characteristic and the taste is bitter, mucilagenous.

Both the types of leaflets show impression or transverse markings due to the pressing of midrib. Distingushing characters of Alexandrian and Indian senna are given in Table below.

Table: Distinguishing characters of Alexandrian and Indian senna

Character	Indian Senna	Alexandrian senna
Appearance	Generally entire and less broken in good condition	Broken and brittle in nature
Size	2.5–5.0 cm long and 7–9 mm wide	2.4 cm long and 6–12 mm wide.
Shape	Lanceolate	Ovate lanceolate
Apex	Less acute with a sharp spine	Acute with a sharp spine
Margin	Entire, flat	Entire curled
Base	Less asymmetrical	Conspicuously asymmetrical
Veins	Pinnate, distinct towards the under surface and anastomosing towards margin	Pinnate, distinct towards the under surface and anastomosing towards margin
Surface	Transverse and oblique impressions, less pubescent (hairy)	Without transverse and oblique impressions and more pubescent
Texture	Flexible and less brittle	Thin more brittle
Odour	Faint	Faint
Colour	Light green	Light greyish green
Test	Bitter mucilaginous	Bitter mucilaginous
Vein Islet Number	19–22.5	25–29.5
Stomatal index	14–20	10–15
Palisade ratio	4–12	4.5–18

Leaflets and legumes of *Cassia angustifolia*

Microscopy

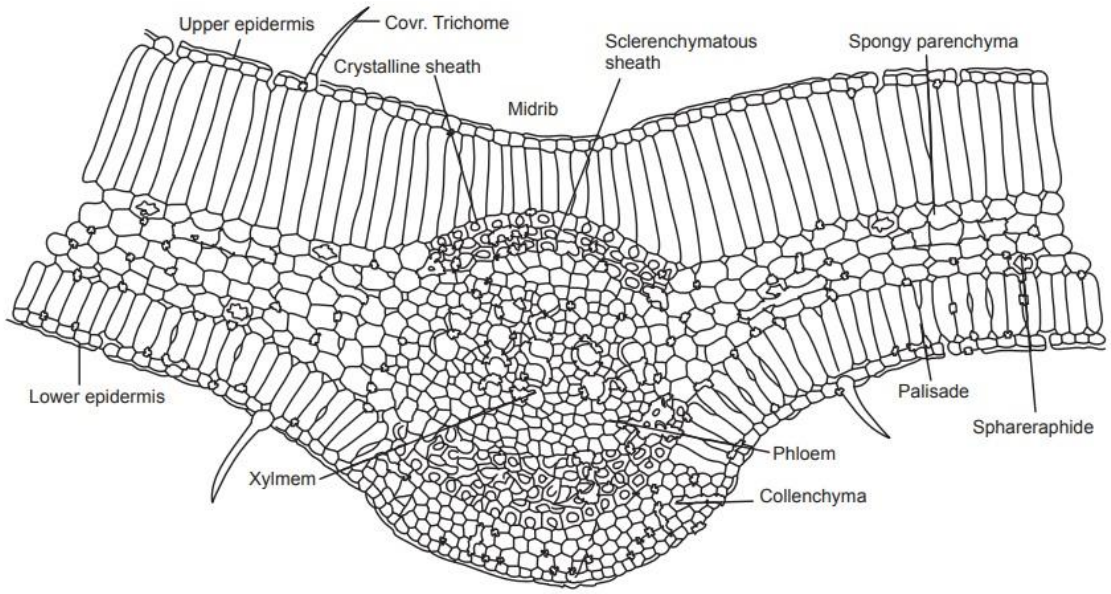
Being isobilateral leaf, senna shows more or less similar features at both the surfaces of leaf with few differences.

Transverse section of leaf shows upper and lower epidermis with straight wall cells, few of which contain mucilage.

Paracytic stomata and non-lignified unicellular trichomes are found on both the surfaces. A single layer of palisade parenchyma is observed at both the sides but it is discontinued in the midrib region of lower epidermis due to the zone of collenchymatous tissues.

Palisade is followed by spongy mesophyll which contains cluster crystals of calcium oxalate and vascular strands.

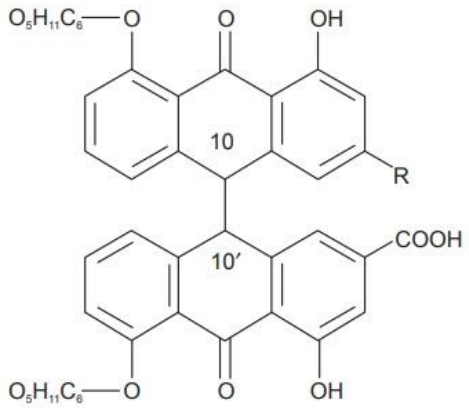
Midrib shows the vascular bundle containing xylem and phloem, almost surrounded by lignified pericyclic fibres and a sheath of parenchyma which contains prismatic crystals of calcium oxalate.



Transverse section of senna leaflet

Chemical Constituents

Senna contains sennosides A and B (2.5%) based on the aglycones sennidin A and B, sennosides C and D which are glycosides of heterodianthrones of aloe-emodin and rhein are present. Others include palmidin A, rhein anthrone and aloe-emodin glycosides. Senna also contains free chryso phanol, emodin and their glycosides and free aloe-emodin, rhein, their monoanthrones, dianthrones and their glycosides. Mucilage is present in the epidermis of the leaf and gives red colour with ruthenium red.



Glycoside	10 - 10′	R
Sennoside A	trans	COOH
Sennoside B	meso	COOH
Sennoside C	trans	CH_2OH
Sennoside D	meso	CH_2OH

Chemical Test

Borntrager test for anthraquinones: The leaves are boiled with dilute sulphuric acid and filtered. To the filtrate organic solvent like benzene, ether or chloroform is added and shaken.

The organic layer is separated, and to it ammonia solution was added. The ammoniacal layer produces pink to red colour indicating the presence of anthraquinone glycoside.

Uses

Senna leaves are used as laxative. It causes irritation of large intestine and have some griping effect. Thus they are prescribed along with carminatives. Senna is stimulant cathartic and exerts its action by increasing the tone of the smooth muscles in large intestine.

Adulterants

Cassia obovata (Dog Senna): They occur as small pieces with Alexandrian senna but can be easily identified by its obovata shape and obtuse and tapering apex.

It has only 1% anthraquinone derivatives. The presence of *Cassia auriculata* (Palthe senna) can be identified by treating it with 80% sulphuric acid. It gives red colour.

Cassia angustifolia (Bombay or Mecca or Arabian senna) a mild variety of Indian senna have the morphology similar to that of Tinnevelly senna but the leaflets are narrow, more elongated and brownish green in colour. *C. marilandica* or American Senna, Wild Senna, *Poinciana pulcherima*, formerly *Maryland Senna*, is a common perennial from New England to Northern Carolina.

Its leaves are compressed into oblong cakes like other herbal preparations of the Shakers. It acts like Senna, but is weaker, and should be combined with aromatics.

These leaves are also found mixed with or substituted for Alexandrian Senna. *Coriaria myrtifolia* is a Mediterranean shrub and highly poisonous, so that it should be recognized when present.

The leaves are green, very thin and soft, three veined, ovatelanceolate, and equal at the base. It is also used to adulterate sweet marjoram. *Cassia montana* yields a false Senna from Madras, partly resembling the Tinnevelly Senna, though the colour of the upper surface of the leaves is browner.

Marketed Products

It is one of the ingredients of the preparations known as Constivac, Softovac (Lupin Herbal Laboratory) and Isova powder, Kultab tablet (Vasu Healthcare).

12. ALOE

Biological Source

Aloe is the dried juice collected by incision, from the bases of the leaves of various species of Aloe.

Aloe perryi, *Aloe vera* or *Aloe barbadensis* and *Aloe ferox*, belonging to family *Liliaceae.*

Aloe perryi Baker is found in Socotra and Zanzibar islands and in their neighbouring areas and so the aloes obtained from this species is known as Socotrine or Zanzibar aloe.

Geographical Source

Aloes are indigenous to East and South Africa, but have been introduced into the West Indies and into tropical countries, and will even flourish in the countries bordering on the Mediterranean.

Aloe vera is also known as *Aloe vulgairis* Lamarek, or *Aloe barbadensis* Mil.or *Aloe officinalis* Forskal.

It was formerly produced on the island of Barbados, where it was largely cultivated, having been introduced at the beginning of the sixteenth century.

It is now almost entirely made on the Dutch islands of Curacoa, Aruba and Bonaire. The aloes obtained from this species is known as Curacao or Barbados aloe.

Aloe ferox Miller and hybrids of this species with *Aloe africana* and *Aloe spicata, A. platylepia* and other species of Aloe grows in Cape Colony and so is known as Cape aloe.

Preparation of Aloe

Curacao or barbados aloe

In West Indies the cut leaves are arranged with their cut surface on the inner side, on the sides of V shaped vessel of about 1–2 m long and the flowing juice is collected in a tin vessel that is placed below the V-shaped vessel This juice thus collected is concentrated either by spontaneous evaporation, or more generally by boiling until it becomes of the consistency of thick honey. These conditions favours the crystallization of barbaloin and this aloe contains crystals of barbaloin because of the presence of which it becomes opaque and so also known as hepatic or livery aloe. On cooling, it is then poured into gourds, boxes, or other convenient receptacles and solidifies.

Socotrine aloe

When it is prepared, it is commonly poured into goat skins, and spontaneous evaporation is allowed for about a month when it becomes viscous pasty mass which are then packed into cases. In European countries it is dried in wooden pans with hot air till moisture is about 10%.

Zanzibar aloe

This aloe is prepared similar to Socotrine aloe. It is packed in skins, of carnivorous animals. This aloe is also known as monkey skin aloe.

Cape aloe

The leaves of the plants from which Cape aloe is obtained are cut off near the stem and arranged around a hole in the ground, in which a sheep skin is spread, with smooth side upwards. When a sufficient quantity of juice has drained from the leaves it is concentrated by heat in iron cauldrons and

subsequently poured into boxes or skins in which it solidifies on cooling. Large quantities of the drug are .exported from Cape Town and Mossel Bay.

Characteristics

Curacao aloe

It is usually opaque and varies in colour from bright yellow-ish or rich reddish brown to black. Sometimes it is vitreous and small fragments are then of a deep garnet-red colour and transparent. It is then known as 'Capey Barbados' and is less valuable, but may become opaque and more valuable by keeping. Curacoa Aloes possesses the nauseous and bitter taste that is characteristic of all Aloes and a disagreeable, penetrating odour. It is almost entirely soluble in 60% alcohol and contains not more than 30% of substances insoluble in water and 12% of moisture. It should not yield more than 3% of ash. The fracture is waxy.

Aloe vera

Socotrine aloes

It may be distinguished principally from Curacoa Aloes by its different odour.

Much of the dry drug is characterized by the presence of small cavities in the fractured surface;

It is yellow-brown to dark-brown in colour and opaque.

Fracture is irregular and porous and taste is bitter.

Zanziber aloes

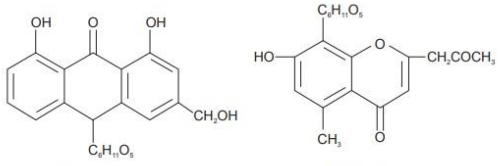
Zanzibar Aloes often very closely resembles Curacoa in appearance and is usually imported in liver-brown masses which break with a dull, waxy fracture, differing from that of Socotrine Aloes in being nearly smooth and even. It has a pleasant odour and bitter taste.

Cape aloes

It forms dark coloured masses which break with a clean glassy fracture and exhibit in their splinters a yellowish, reddish-brown or greenish tinge. Its translucent and glossy appearance is very characteristic and red-currant like odour sufficiently distinguishes it from all other varieties of Aloes.

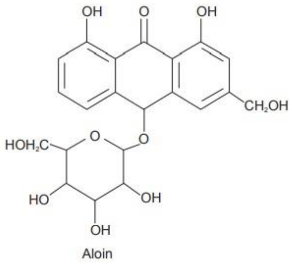
Chemical Constituents

The most important constituents of Aloes are the three isomers of Aloins, Barbaloin, β-barboloin and Isobarbaloin, which constitute the so-called 'crystalline' Aloin, present in the drug at from 10 to 30%. Other constituents are amor-phous Aloin, resin, emodin and Aloe-emodin. Barbaloin is present in all the varieties; it is slightly yellow coloured, bitter, water soluble, crystalline glycoside. Isobarbaloin is a crystalline substance, present in Curacao aloe and in trace amount in Cape aloe and absent in Socotrine and Zanzibar aloe. The chief constituents of Socotrine and Zanzibar aloe are Barbaloin and β-Barbaloin.



Barbaloin

Aloesin



Aloin

Chemical Tests

Boil 1 gm of drug with 100 ml water, allow it to cool; add 1 gm kieselguhr, stir it well and filter through filter paper

1. *Borax Test:* Take 10 ml of aloe solution and to it add 0.5 gm of borax and heat; a green coloured fluorescence is produced indicating the presence of aloe-emodin anthranol.

2. *Modified Anthraquinone Test:* To 0.1 gm of drug, 5 ml of 5% solution of ferric chloride is added followed by the addition of 5 ml dilute hydrochloric acid. The mixture is heated on water bath for 5–6 min and cooled. An organic solvent (benzene or chloroform) is added and shaken. Separate the organic solvent layer and add an equal volume of dilute ammonia. The ammoniacal layer produces pinkish red colour.

3. *Bromine Test:* To 5 ml of aloe solution, add equal volume of bromine solution; bulky yellow precipitate is formed due to the presence of tetrabromaloin.

4. *Nitrous Acid Test:* To 5 ml of aloe solution, add little of sodium nitrite and few drops of dilute acetic acid; it produces Pink or purplish colour. Zanzibar and Socotrine aloes give negative test.

5. *Nitric Acid Test:* 2 ml of concentrated nitric acid is added to 5 ml of aloe solution; Curacao aloe gives deep reddish-brown colour, Socotrine aloe gives pale yellowish-brown colour, Zanzibar aloe gives yellowish-brown colour and Cape aloe first produces brown colour which on standing changes to green.

6. *Cupraloin Test:* 1 ml of the aloe solution is diluted to 5 ml with water and to it 1 drop of copper sulphate solution is added. Bright yellow colour is produced which on addition of 10 drops of saturated solution of sodium chloride changes to purple and the colour persist if 15–20 drops of 90% alcohol is added. This test is positive for Curocao aloe, faint for Cape aloe and negative for Zanzibar and Socotrine aloes.

Uses

1. The drug Aloes is one of the safest and stimulating purgatives, in higher doses may act as abortifacient.

2. Its action is exerted mainly on the large intestine; also it is useful as a vermifuge.

3. The plant is emmenagogue, emollient, stimulant, stomachic, tonic and vulnerary.

4. Extracts of the plant have antibacterial activity.

5. The clear gel of the leaf makes an excellent treatment for wounds, burns and other skin disorders, placing a protective coat over the affected area, speeding up the rate of healing and reducing the risk of infection.

6. To obtain this gel, the leaves can be cut in half along their length and the inner pulp rubbed over the affected area of skin.

7. This has an immediate soothing effect on all sorts of burns and other skin problems.

Substituents and Adulterants

A. candelsbmm (Natal aloes) is dull greenish black to dull brown in colour, opaque. When scraped it gives a pale greyish green or a yellow powder.

It can be distinguished as it gives negative test to borax test and produces a deep blue colour. Jafferabad aloes and the Mocha aloes are the other two type of aloe which is used as adulterant.

Marketed Products

It is one of the ingredients of the preparations known as Diabecon, Evecare (Himalaya Drug Company), Mensonorm (Chirayu Pharma) and Kumari Asava (Baidyanath).

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Subject: Pharmacognosy and phytochemistry-II **Unit No: III**
Faculty: Mrs. S. Jyothsna **Lecture No: 9**
Topic: Bitter almond and Gentian **Book Reference: T2**

13. BITTER ALMOND OIL

Biological Source

Almond oil is a fixed oil obtained by expression from the seeds of *Prunus amygdalus* (Rosaceae) var. dulcis (sweet almonds) or *P. amygdalus var. amara* (bitter almonds).

Geographical Source

The oil is mainly produced from almonds grown in the countries bordering the Mediterranean (Italy, France, Syria, Spain, and North Africa) and Iran.

Macroscopic Characteristics

Almond trees are about 5 m in height. The young fruits have a soft, felt-like pericarp, the inner part of which gradually becomes sclerenchymatous as the fruit ripens to form a pitted endocarp or shell.

The shells, consisting mainly of sclerenchymatous cells, are sometimes ground and used to adulterate powdered drugs.

The sweet almond is 2–3 cm in length, rounded at one end, and pointed at the other.

The bitter almond is 1.5–2 cm in length but of similar breadth to the sweet almond.

Both varieties have a thin, cinnamon-brown testa which is easily removed after soaking in warm water.

The oily kernel consists of two large, oily planoconvex cotyledons, and a small plumule and radicle, the latter lying at the pointed end of the seed.

Some almonds have cotyledons of unequal sizes and are irregularly folded.

Bitter almonds are found in samples of sweet almonds; their presence may be detected by the sodium picrate test for cyanogenetic glycosides.

(a) Bitter almond and (b) Sweet almond

Chemical Constituents

Both varieties of almond contain 40–55% of fixed oil, about 20% of proteins, mucilage and emulsin. The bitter almonds contain in addition 2.5–4.0% of the colourless, crystalline, cyanogenelic glycoside amygdalin.

Almond oil is obtained by grinding the seeds and expressing, them in canvas bags between slightly heated iron plates.

The oil is clarified by subsidence and filtration. It is a pale yellow liquid with a slight odour and bland nutty taste.

It contains olein, with smaller quantities of the glycosides of linoleic and other acids. Bitter almonds, after maceration on hydrolysis of amygdalin yield a volatile oil that is used as a flavouring agent.

Sweet almonds are extensively used as a food, but bitter almonds are not suitable for this purpose.

Essential or volatile oil of almonds is obtained from the cake left after expressing bitter almonds.

This is macerated with water for some hours to allow hydrolysis of the amygdalin to take place.

The benzaldehyde and hydrocyanic acid are then separated by stem distillation.

Almond oil consists of a mixture of glycerides of oleic (62–86%), linoleic (17%), palmitic (5%), myristic (1%), palmitoleic, margaric, stearic, linolenic, arachidic, gadoleic, behenic, and erucic acid. Bitter almond oil contains benzaldehyde and 2–4% of hydrocyanic acid.

Purified volatile oil of bitter almonds has all its hydrocyanic acid removed and, therefore, consists mainly of benzaldehyde.

The unsaponifiable matter contains β-sitosterol, Δ^5-avenasterol, cholesterol, brassicasterol and tocopherols.

Uses

1. Expressed almond oil is an emollient and an ingredient in cosmetics.
2. Almond oil is used as a laxative, emollient, in the preparation of toilet articles and as a vehicle for oily injections.
3. The volatile almond oils are used as flavouring agents.

Marketed Products

It is one of the ingredients of the preparations known as Baidyanath lal tail (Baidyanath Company), Himcolin gel, Mentat, Tentex Royal (Himalaya Drug Company), and Sage badam roghan (Sage Herbals).

14. GENTIAN

Synonyms
Gentian Root, Yellow Gentian Root.

Biological Source
Gentian consists of dried unfermented rhizomes and roots of *Gentiana lutea* Linn., belonging to family *Gentianaceae*.

Geographical Source
Mountanious regions of Central and south Europe, of France and Switzerland, of Spain and Portugal, the Pyrenees, Sardinia and Corsica, the Apennines, the Mountains of Auvergne, the Jura, the lower slopes of the Vosges, the Black Forest and throughout the chain of the Alps as far as Bosnia and the Balkan States.

Cultivation and Collection

- It is a perennial plant growing to 1.2 m by 0.6 m. For cultivation, a strong loamy soil is most suitable, the deeper the better, as the stout roots descend a long way down into the soil.

- Plenty of moisture is also desirable and a position where there is shelter from cold winds and exposure to sunshine.
- Old plants have large crowns, which may be divided for the purpose of propagation, but growing it on a large scale, seeds would be the best method.
- It is advantageous to keep the seed at about 10°C for a few days after sowing, to enable the seed to imbibe moisture.
- Following this with a period of at least 5–6 weeks with temperatures falling between 0 and –5°C will usually produce reasonable germination.
- They could be sown in a frame, or in a nursery bed in a sheltered part of the garden and the young seedlings transplanted.
- They take about three years to grow to flowering size. It is, however, likely that the roots are richest in medicinal properties before the plants have flowered.
- Collection is done from two to five years old plants in spring.
- The rhizome and roots collected and dried. When fresh, they are yellowish-white externally, but gradually become darker by slow drying.
- Slow drying is employed to prevent deterioration in colour and to improve the aroma.
- Occasionally the roots are longitudinally sliced and quickly dried, the drug being then pale in colour and unusually bitter in taste.

Macroscopic Characteristics

- When fresh, they are yellowish-white externally, but gradually become darker by slow drying.
- Slow drying is employed to prevent deterioration in colour and to improve the aroma.
- Occasionally the roots are longitudinally sliced and quickly dried;
- The drug being then pale in colour and unusually bitter in taste, but this variety is not official.
- The dried root as it occurs in commerce is brown and cylindrical, 1 foot or more in length, or broken up into shorter pieces, usually 1/2 inch to 1 inch in diameter, rather soft and spongy, with a thick reddish bark, tough and flexible, and of an orange-brown colour internally.

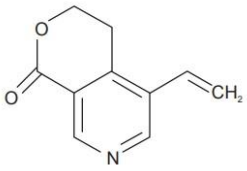
- The upper portion is marked with numerous rings, the lower longitudinally wrinkled.
- The root has a strong, disagreeable odour, and the taste is slightly sweet at first, but afterwards very bitter.

Microscopy

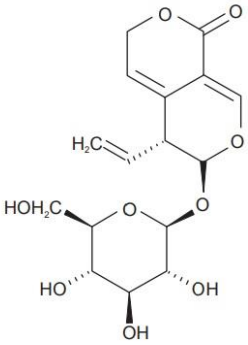
- The transverse section of root shows triarch primary xylem at the centre.
- Where each primary bundle, is represented by one to three very small vessels.
- The secondary xylem is very wide with parenchymatous and medullary rays not clearly marked.
- The drug also shows reticulately thickened xylem vessels very few being annular or spiral, scattered through-out the parenchyma of the xylem.
- Secondary phloem is wide and composed chiefly of parenchyma, with groups of sieve-tissue.
- The phloems are surrounded by a narrow parenchymatous phelloderm and externally are several rows of polygonal tabular, thin walled cork cells.
- Parenchyma cells in all regions of the root contain scattered needles of calcium oxalate crystals, about 3–6 μ long and 0.5–1.1 μ wide, also small prismatic crystals.

Chemical Constituents

- Gentian contains bitter glycosides. The dried gentian root contains Gentinin and Gentiamarin, bitter glucosides, together with Gentianic acid (gentisin), the latter being physiologically inactive.
- Gentiopicrin, another bitter glucoside, a pale yellow crystalline substance, occurs in the fresh root, and may be isolated from it by treatment with boiling alcohol.
- Gentinin, crystalline glycoside is not a pure chemical substance, but a mixture of gentiopicrin and a colouring substance gentisin (gentianine) or gentlanic acid.
- Gentian contains a bitter trisaccharide, gentianose which on hydrolysis yields two molecules of glucose and one molecule of fructose.
- The saccharine constituents of gentian are dextrose, laevulose, sucrose and gentianose, a crystallizable, fermentable sugar. It is free from starch and yields from 3 to 4% ash.



Gentianine



Gentiopicrin

Uses

1. Gentian root has a long history of use as an herbal bitter in the treatment of digestive disorders.
2. It contains some of the most bitter compounds known and is used as a scientific basis for measuring bitterness.
3. It is useful in states of exhaustion from chronic disease and in all cases of debility, weakness of the digestive system and lack of appetite.
4. It is one of the best strengthened of the human system, stimulating the liver, gall bladder and digestive system, and is an excellent tonic to combine with a purgative in order to prevent its debilitating effects.
5. It is also used as anthelmintic, anti-inflammatory, antiseptic, bitter tonic, cholagogue, emmenagogue, and febrifuge, refrigerant and stomachic.
6. It is taken internally in the treatment of liver complaints, indigestion, gastric infections and anorexia.
7. It should not be prescribed for patients with gastric or duodenal ulcers.

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Dundigal, Quthbullapur Mandal, Hyderabad 500043, R.R. Dist.

Subject: Pharmacognosy and phytochemistry-II | **Unit No: III**
Faculty: Mrs. S. Jyothsna | **Lecture No: 10**
Topic: Artemisia, Taxus and Carotenoid | **Book Reference: T2**

15. ARTEMISIA

Synonyms:

Sweet worm wood, Sweet sage work

For hundreads of years the Chinese have used the herb known as **Qing Hao** for the treatment of fevers including malaria.

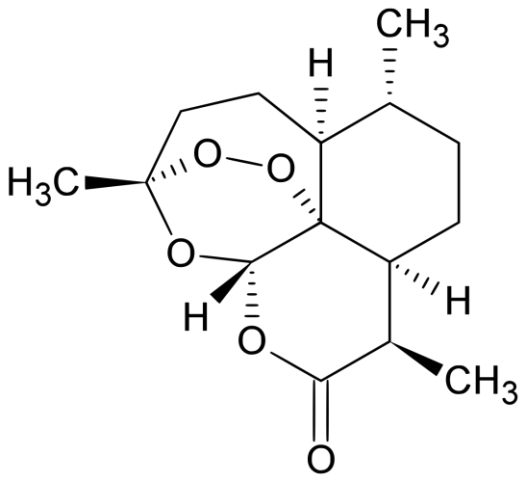
Biological source:

This consists of chinese traditional herb *Artemisia annua*, belongs to the family *Asteraceae*

Properties:

It is a sesquiterpene lactone with an endoperoxide moiety, an unaual functiona group in natural products.

In 1972, the drug was discovered with antimalarial activity



- Atimisinin is whitish crystalline compound.
- It is solube in most organic solvents.
- Slightly soluble in oil
- M.P is 156-157^{o}C

Artemisininn isolated from Artemisia annua is polymorphic in form and crystals posses higher dissolution rate.

Callus culture of Artemisia annua have been reported to produce Scopoletin and a triglyceride but no artemisinin.

Isolation:

Dried powdered leaves of Artemisia annua extract with petroleum ether, concentrate the extract and dry it.

Redissolved in chloroform and acetonitrile.

This precipitates inert plant constituents such as sugars and waxes.

Collect the extract separate by chromatographic fractionation of the concentrate on silica gel by eluting chloroform- ethylacetate.

It seperates polymorphic form of Artemisinin.

Crystallize it using Cyclohexane or 50% ethanol.

The chinese plants has been reported to yield as much as 0.9% of artemisinin.

The highest content of artemisinin is found in the leaves of the top 50cm of the plant.

The highest yeild is obtained from the leaves just before flowering.

Identification:

Separate the constituent (Artemisinin) using TLC on Silica gel-G plates.

Uses:

It has been successful in treating cases of Chloroquine-resistant Plasmodium falciparum and particularly celebral malaria.

Artemisinin doesnot react with the Iron in haemoglobin so that uninfected cells are unaffected.

Artemisinin derivatives are remarkably non-toxic

Artemisinin may, however be embryotoxic so that the use of these drugs are not recommended in early pregnancy.

Artemisinin is a complex molecule, much effort has been put into synthesizing compounds based on the 1,2,4-trioxane ring of artenmisinin.

A synthetic derivative known as artellene was evaluated in clinicaal trials but was abandoned due to high rate in recrudescence.

Taxol (or) Paclitaxel:

Synonyms:

Western yew, American yew, Pacific yew

Biological source:

Taxol is complex diterpenoid alkaloid obtained from the bark of *Taxus brevifolia* and *Taxus baccata*

Family: *Taxaceae*

The overall yield is only 0.01-0.02% w/w

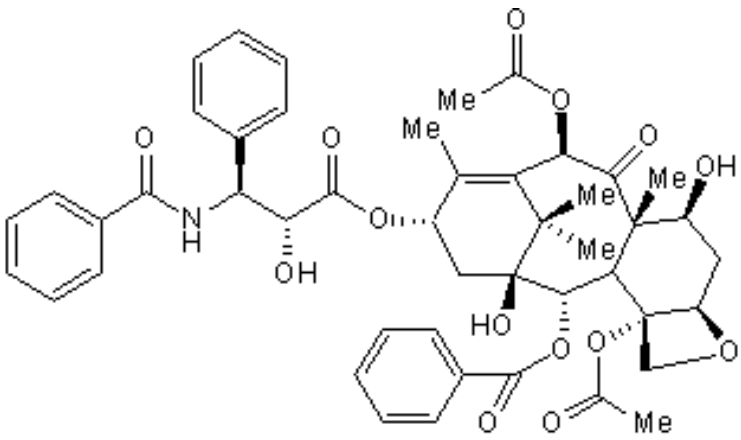
The major obstacle in the preparation of Taxol has been the limited supply of the drug.

It is not econoically feasible to synthesise taxol due to its complex chemistry.

It is not econnomically feasible to synthesise taxol

It requires the bark from about 3 mature 100yrs old trees to provide 1gm of taxol.

Properties:



It is hydrohobic in nature.

M.P 213-216^{o}C.

An ester side chain at the 13th position of the taxane ring is essential for its cyotoxic activity.

The presence of 2^1 hydroxy group of the side chain enhances the activity.

It is used in the treatment of metastatic carcinoma of the ovary afte failure of the 1st line treatment or subsequent chemotheraphy.

It is also used in the treatment of breast cancer.

Isolation:

The bark is removed from mature trees in the period May-August (1st isolated in 1971).

Extract dried and powdered bark of Taxus brevifolia with alcohol concentreate the total alcoholic extract.

Dry and subject it to sequential chromatographyon silica gel and florisil.

It yields taxol and cephalomannine.

Separate taxol by chromatography on silica gel using dichloromethane/ l-propanol as eluent.

Uses:

it is used for the treatment of ovarian cancer, breast cancer, lung cancer, cervical cancer and pancreatic cancer.

Taxol:

It is used chemically in the treatment of ovarian cancer , breast cancer and Non-small cell lung cancer

It may also have potential value against other cancers.

Taxotere:

It is a side chain analogue of taxol, which has also been produced by semi-synthesis from 10-desacetylbaccatin-III

Used in the treament of breast cancer.

16. CAROTENOIDS:

Carotenoids they are various colors usually yellow to red pigments

Carotenoids are composed of eight isoprene units (C40).

Carotenoids, also called tetraterpenoids, are organic pigments that are produced by plants and algae, as well as several bacteria and fungi.

The only animals known to produce carotenoids are aphids and spider mites, which acquired the ability and genes from fungi.

Hydroxylated, oxidized, hydrogenated or ring-containing derivatives exist.

Carotenoids contain a conjugated backbone composed of isoprene units, which are usually inverted at the center of the molecule, imparting symmetry.

Changes in geometrical configuration about the double bonds result in the existence of many cis and trans isomers.

Carotenoids absorb light in the 400-500 nm region of the visible spectrum. This physical property imparts the characteristic red/yellow color of the pigments.

Carotenoids are red, yellow and orange pigments that are widely distributed in nature. They are especially abundant in yellow-orange fruits and vegetables and dark green, leafy vegetables.

More than 700 naturally occurring carotenoids identified.

Hydrocarbon carotenoids are classified as carotenes while those containing oxygen are known as xanthophylls.

Classification:

There are two general classes of carotenoids:

1. Carotenes and

2. Xanthophylls.

Carotenes consist only of carbon and hydrogen atoms; beta-carotene is the most common carotene. Xanthophylls have one or more oxygen atoms; lutein is one of the most common xanthophylls.

General structure of the carotenoid is a polyene chain consisting of 9-11 double bonds and possibly terminating in rings general structure of carotenoids

Chemistry

Carotenoids belong to the category of tetraterpenoids (i.e. they contain 40 carbon atoms, being built from four terpene units each containing 10 carbon atoms).

Structurally, carotenoids take the form of a polyene hydrocarbon chain which is sometimes terminated by rings, and may or may not have additional oxygen atoms attached.

Carotenoids that contain unsubstituted beta-ionone rings (including beta- carotene, alpha-carotene, beta-cryptoxanthin and gamma-carotene) have vitamin A activity (meaning that they can be converted to retinol), and these and other carotenoids can also act as antioxidants.

Properties-

Carotenoids are usually lipophilic due to the presence of long unsaturated aliphatic chains as in some fatty acids.

The physiological absorption of these fat-soluble vitamins in humans and other organisms depends directly on the presence of fats and bile salts.

Foods beta carotene found in carrots and apricots, is responsible for their orange-yellow colors.

Dried carrots have the highest amount of carotene of any food per 100 gram serving, measured in retinol activity equivalents (provitamin A equivalents).

Vietnamese gac fruit contains the highest known concentration of the carotenoid lycopene.

The diet of flamingos is rich in carotenoids, imparting the orange-colored feathers of these birds

Carotenoids serve two key roles in plants and algae: they absorb light energy for use in photosynthesis, and they protect chlorophyll from photodamage.

Vietnamese gac fruit carrots flamingos

α- and β-carotene and lycopene appear predominantly in red, orange, and yellow fruit and vegetables, whereas lutein and zeaxanthin occur mainly in green-leaved vegetables.

Thereby, the plasma β-carotene level is a marker for fruit and vegetable uptake⎫ β-carotene is the most known carotenoid and the most often naturally occurring carotene.

β-carotene, also known as provitamin A, can be metabolized to vitamin A in different tissues (eg, small intestine, liver).

In turn, vitamin A (retinol) can be transformed to retinal, which is essential for vision, or retinoate, which is involved in cell proliferation and cell differentiation .

β-carotene itself is an antioxidant as are nearly all carotenoids.

About 600–700 different carotenoids are known of which α- and β-carotene, lycopene, lutein, and zeaxanthin are the most prominent ones. Alpha, Beta Carotenoids, vit A

Beta-carotene is an antioxidant. Antioxidants protect cells from damage caused by substances called free radicals.

Free radicals are believed to contribute to certain chronic diseases and play a role in the aging processes.

Dietary Sources The richest sources of beta-carotene are yellow, orange, and green leafy fruits and vegetables (such as carrots, spinach, lettuce, tomatoes, sweet potatoes, broccoli, cantaloupe, and winter squash).

In general, the more intense the color of the fruit or vegetable, the more beta-carotene it has. Alpha-Carotene foods include orange vegetables like pumpkin, carrots, and winter squash.

Other alpha-carotene food sources include tomatoes, napa cabbage, swiss chard, collards, green beans, tangerines, sweet bell peppers, Concentrations of preformed vitamin A are highest in liver and fish oils.

Other sources of preformed vitamin A are milk and eggs, which also include some provitamin A.

Most dietary provitamin A comes from leafy green vegetables, orange and yellow vegetables, tomato products, fruits, and some vegetable oils

Carotenoid pigments attach themselves to proteins or fats and can produce blue, green, purple, or brown pigments in addition to yellow, orange, and red.

If an animal's skin or feather color comes from carotenoids and it is not available in food, some or all of the color fades.

For example, many birds develop bright red, orange, or yellow carotenoid pigmentation that they use presumably to attract mates.

Because animals often obtain several different carotenoids from plant and animal food sources, it is possible that these pigments are accumulated at different levels, which results in the ultimate color expression of individual animals.

Beta carotene is composed of two retinyl groups, and is broken down in the mucosa of the human small intestine to retinol, form of vitamin A.

β -Carotene can be stored in the liver and body fat and converted to retinal as needed, thus making it a form of vitamin A.

Alpha carotene and gamma carotene, due to their single retinyl group (β- ionone ring), also have some vitamin A activity (though less than β-carotene).

All other carotenoids, including lycopene, have no beta-ring and thus no vitamin A activity (although they may have antioxidant activity and thus biological activity in other ways).

Molecular structure Chemically, carotenes are polyunsaturated hydrocarbons containing 40 carbon atoms per molecule, variable numbers of hydrogen atoms, and no other elements.

Some carotenes are terminated by hydrocarbon rings, on one or both ends of the molecule.

All are coloured to the human eye, due to extensive systems of conjugated double bonds. Structurally carotenes are tetraterpenes, meaning that they are synthesized biochemically from four 10-carbon terpene units, which in turn are formed from eight 5-carbon isoprene units.

α-Carotene is a form of carotene with a β-ionone ring at one end and an α- ionone ring at the opposite end. It is the second most common form of carotene.

Vitamin A (retinol)

α-carotene is one of the most abundant carotenoids in the diet and can be converted to vitamin A, but with only one-half the activity as β-carotene (contains only one β-ionone ring in contrast to two for β-carotene).

Another carotenoid, lycopene, is a red pigment found in fruits and vegetables.

Lutein and zeaxanthin are carotenoids found in green, leafy vegetables and algae and have been considered recently for potential benefits to sight and vision, particularly a decrease in the risk of cataracts.

Provitamin A carotenoids, those carotenoids possessing a β-inone ring, have vitamin A activity, and can be converted into vitamin A by the body.

β- Carotene has the highest provitamin A activity, but over 60 carotenoids have some provitamin A activity.

Vitamin A is toxic when taken in excess, but these carotenoids are safe sources because they are only converted to vitamin A as and when the body needs it.

The other biological roles of carotenoids, including their antioxidant functions, are completely independent of their provitamin A activity.

VITAMIN A Vitamin A is a fat-soluble vitamin that is stored in the liver.

There are two types of vitamin A that are found in the diet.

Preformed vitamin A is found in animal products such as meat, fish, poultry, and dairy foods. Provitamin A is found in plant-based foods such as fruits and vegetables.

The most common type of pro-vitamin A is beta-carotene.

Vitamin A is an essential nutrient for epithelial cell maintenance and repair.

Dietary vitamin A (retinol or retinol esters) is found in eggs, fish, and dairy products. Both beta- and alpha- carotenecan act as precursors for the synthesis of vitamin A and are postulated to function as antioxidants, inhibiting the oxidation of membrane lipids during infection.

Deficiency of vitamin A may cause night blindness, conjunctiva and corneal xeroses, dry thickened skin, and abnormalities of bronchial mucosal epithelialization.

Function Vitamin A helps form and maintains healthy teeth, skeletal and soft tissue, mucus membranes, and skin.

It is also known as retinol because it produces the pigments in the retina of the eye. Vitamin A promotes good vision, especially in low light.

It may also be needed for reproduction and breastfeeding. Retinol is an active form of vitamin A.

It is found in animal liver, whole milk, and some fortified foods

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Subject: Pharmacognosy and phytochemistry-II | **Unit No: IV**
Faculty: Mrs. S. Jyothsna | **Lecture No: 1**
Topic: Isolation of phytoconstituents, Menthol | **Book Reference: T2**

INTRODUCTION

The crude drug contains the active constituents, which can be isolated from these drugs by various methods of extraction and separation.

Extraction is defined as the process of isolation of soluble material from an insoluble residue, which may be liquid or solid, by treatment with a solvent on the basis of the physical nature of crude drug to be extracted, i.e. liquid or solid, the extraction process may be liquid-liquid or solid-liquid extraction.

The process of extraction is controlled by mass transfer. Mass transfer is a unit operation, which involves the transfer of mass of soluble material from a solid to a fluid.

If a crude drug panicle is immersesd in a solvent to be used for extraction, the particle is first surrounded by a boundary layer of the solute; the solvent starts penetrating inside the particle and subsequently forms solution of the constituents within the cells.

Escape of these dissolved constituents through the cell wall and through the boundary layer takes place.

The process is continued till equilibrium is set up between the solution in the cells and the free solution.

Few important factors, which affect the mass transfer, are agitation and temperature that increase the concentration gradient to bring about an efficient extraction.

Size reduction of the crude drug increases the area over which diffusion can occur. Overall extraction is also dependent on the selection of the method of extraction and the solvent selected for extraction.

There is a revival of interest in the use of plants in pharmacy both from pharmaceutical industry as a source of new lead molecules find from the general public who are using plant extracts in many ways in conventional and complementary therapies.

About one-quarter of all prescription drugs are of plant origin despite the fact that less than 5% of plant species have been investigated.

Many of the synthetic medicine currently in clinical use have been from natural sources.
It is a widely accepted fact that natural product chemistry surpasses the kind of chemistry that synthetic

chemist can ever accomplish in the laboratory

Phytochemical diversity in terms of structural novelty is unprecedented in laboratory synthesis. Indeed the plant kingdom provides enormous chemical diversity.

Advances in bioassay screening, isolation techniques and structural elucidation they have greatly shortened and accelerated the process of drug discovery from medicinal plants. Nowadays it is a common practice among natural product chemists to use some type of bioassay to direct progress of phytochemical investigation towards the discovery of new pure bioactive markers.

The increase use of herbal preparations has highlightened need for adequate standards to ensure quality, safety and efficacy of such drugs and preparations.

Many developing countries are becoming aware of the potential of their flora as a source of medicinally useful products.

Some of the most important alkaloids, glycosides, aglycones, resins and essential oil components of commercial use have been presented here in respect to their isolation and identification.

1. MENTHOL

Source:
Menthol is a monoterpene alcohol obtained from diverse types of mint oils or peppermint.

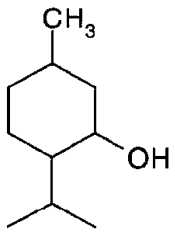
The sources of mint oil include black peppermint. *Mentha piperita* Va r. *vulgaris*; white peppermint, *M. piperita* Var. *officinalis*; *M. arvensis*; *M. canadensis* Va r. *piperascens* etc.

Peppermint contains about 1–3% of volatile oil. First two species contains not less than 45% of menthol while the later species contains menthol up to about 70–90%.

Along with menthol the oil contains (+) neomenthol, (+) isomenthol, menthone, menthofuran, menthyl acetate and cineol.

The menthol obtained from the natural sources is, Levorotatory (l-menthol) or racemic (dl-menthol). Menthol can be synthetically prepared by hydrogenation of thymol.

Menthol



Isolation of Menthol

- Mentha oil is obtained from the hydro-distillation or steam distillation of fresh above-ground parts just before flowering.
- For (-) menthol isolation from peppermint oil the oil is subjected to cooling.
- The crystals of menthol crystallize out from the oil which is separated by centrifugation.
- Cornmint oil obtained from the steam distillation of the flowering herb
- *Mentha arvensis* contains about 70–80% of free (-) menthol.
- Cornmint oil is cooled and the crystals of menthol produced are separated by centrifugation.
- Since the crystalline product contains traces of cornmint oil, this menthol has a slightly herbaceous minty note.
- Pure (-) menthol is obtained by re-crystallization from solvents with low boiling points.
- Dementholized corn mint oil from which (-) menthol is removed by crystallization and which still contains 40–50% free menthol can also be reused for producing (-) menthol.

Melting point: 41–44°C

Thin Layer Chromatography of Menthol

- Dissolve about 1 mg of menthol in about 1 ml of methanol.
- Apply the spot on silica gel-G plate and elute it in pure chloroform.
- Spray the dried plates with 1% vanillin-sulphuric acid reagent and heat the plate at 110°C for 10min.
- Menthol gives R_f value 0.48–0.62 in case of normal chamber saturation at 24°C.

2. CITRAL:

Citral, or 3,7-dimethyl-2,6-octadienal or lemonal, is either a pair, or a mixture of terpenoids with the molecular formula $C_{10}H_{16}O$. The two compounds are geometric isomers. The *E*-isomer is known as geranial or citral A. The *Z*-isomer is known as neral or citral B.

Due to cis–trans isomerism at the C=C bond nearly the aldehyde group obtained from essentials oils of plant sources.

Occurance:

Lemon grass:

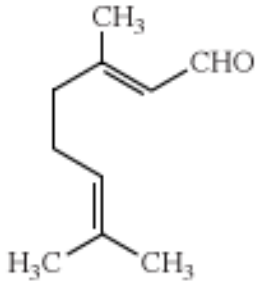
- It is cultivated in India, South East Asia and Africa.
- Lemon grass is a good source of lemon grass oil which is a good source of natural citral. Lemon grass oil contains 85-65%.

Lemon tea-tree:

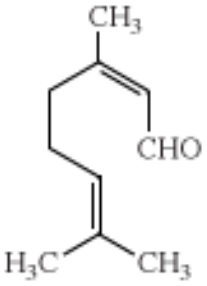
- It is grown in plantations in Kenya, South Africa, and Australia.
- The essential oil of tea tree contains 70-80% citral.

Lemon balm:

- The lemon balm contains 11% citral.
- It is cultivated in to south-central Europe, North Africa, the Mediterranean region, and Central Asia.



geranial
(citral a)



neral
(citral b)

Properties:

- The isomer neral has lemon odour is less strong and sweeter.
- The isomer geranal has strong lemon odour.
- Melting point of citral is less dense than water and insoluble in water.
- Citral is a clear yellow colored liquid with a lemon-like odor.
- When heated to decomposition it emits acrid smoke and irritating fumes.
- Citral is not stable to alkanes and strong acid.
- Density of citral is 0.9 g/cm^3.
- Melting point is < -10°C.
- Citral as an isolate in steam distilled lemon myrtle oil is typically 90–98%
- It is the highest natural source of citral, cultivated in Australia for flavouring and essential oil.
- Lemon myrtle Citral is present in the oils of several plants.

Isolation of Citral:

Lemon grass (chopped or unchopped) is filled in the distillation flask and fitted tightly so that the vapours and oils will not leak out.

The steam is injected in to it so that the upcoming steam carries away the essential oil from the plant material then the lemon grass oil as well as the vapours are passed through the condenser where they condensed as the oil is lighter then water so it will float through the surface of water and it is then easily separated now the thus obtained is the lemon grass oil which contain 85% Citral is isolated from lemon grass oil which is obtained from lemon grass by steam distillation.

Uses:

- Citral is an aroma compound used in perfumery for its citrus effect
- Citral is used as a flavor and for fortifying lemon oil
- Citral has strong antimicrobial qualities
- Citral is used in the synthesis of Vitamin A. ionone and methylionone,
- Mask the smell of smoke

3. ARTEMISININ:

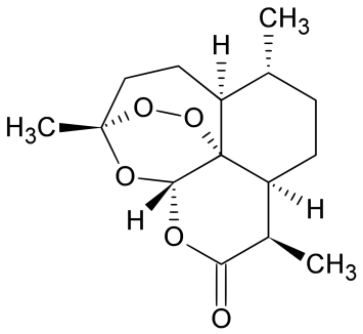
Synonyms:

Sweet worm wood, sweet sage work

For hundreads of years the Chinese have used the herb known as Qing Hao for the treatment of fevers including malaria.

Biological source:

This consists of Chinese traditional herb *Artemisis annua* belongs to the family *Asteraceae*.



Artemisinin

Properties:

- It is a sesquiterpene lactone with an endoperoxide moiety, an unusual functional group in natural products.
- In 1972, the drug was discovered with antimalarial activity.
- It is whitish crystalline powder.
- It is soluble in most organic solvents.
- It is slightly soluble in oil.
- M.P is 156-157^{o}C
- Artemisinin isolated from Artemisia annua is polymorphic in form and crystals posses higher dissolution rate.
- Callus culture of Artemisis annua have been reported to produce scopoletin and a triglyceride but no artemisinin.

Isolation:

- Dried powdered leaves of *Artemisia annua*, extract with petroleum ether, concentrate the extract and dry it.
- Redissolve it in chloroform and acetonitrile.
- This precipitates inert plant constituents such as sugars and waxes.
- Collect the extract, separate by chromatographic fractionation of the concentrate on silica gel by eluting chloroform- ethylacetate, It seperates polymorphic form of artemisinin.
- Crystallize it using cyclohexane or 50% ethanol
- The Chinese plant has been yield as much as 0.9% of artemisinin.
- The highest content of artemisinin is found in the leaves of the top 50cm of the plant.
- The highest yield is obtained from the leaves just before flowering.

Identification:

Separate the constituent (Atemesinin) using TLC on silica gel-G Plates

Uses:

- It is used to treat chloroquine resistant *Plasmodium falciparum.*
- Also used for the treatment of cerebral malaria.
- It has been successful in treating cases of chloroquine- resistant *Plasmodium falciparum* and particularly cerebral malaria.
- Artemisinin does not react with the iron in haemoglobin so that uninfected cells are unaffected.
- Artemisinin derivatives are remarkably non-toxic.
- Artemisinin may; however be embryotoxic so that the use of these drugs is not recommended in early pregnancy.
- Artemisinin is a complex molecule, much effort has been put into synthesiszing compounds based on the 1, 2, 4-trioxane ring of artemisinin.
- A synthetic derivative known as artellene was evaluated in clinical trials but was abandoned due to high rate in recrudescence.

4. RUTIN

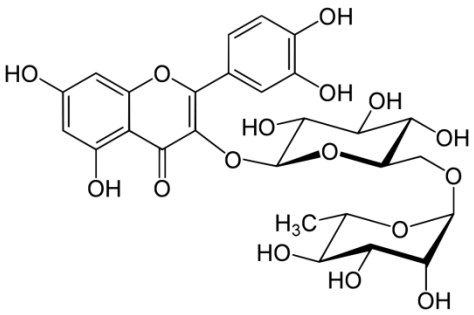
Rutin also called rutoside, quercetin-3-O-rutinoside and sophorin, is the glycoside between the flavonol Quercetin and the disaccharide rutinose.

Source:

- Rutin is the phenolic compound found in the invasive plant species *Carpobrotus edulis* and contributes to the antibacterial and antioxidant properties of the plant.
- Rutin is extracted from *Ruta graveolens L.* (garden rue), a member of *Rutaceae* family.
- Buckwheat-species: Herb of *Fagopyrum esculentum* Family: *Polygonaceae* upto 5% and *Fagopyrum tataricum* (*Polygonaceae*) (upto 3%).
- The flower buds of which contain 15-23% rutin.

Extraction and isolation:

- Take 20gm powder soxhlet with 250ml 80% ethanol.
- Filter it, mix it with 25ml water and extracted with petroleum ether and chloroform.
- Take aqueous layer keep in cold for 72hrs.
- Yellow precipitate gets separated.
- Wash it with chloroform: ethyl acetate: ethanol (50:25:25).
- Dissolve precipitate in hot methanol and fiter it.
- The filtrate is evaporate to dryness, yields yellow powder (Rutin).



Rutin

Properties:

- Rutin is a citrus flavonoid glycoside found in many plants including buckwheat, the leaves and petioles of Rheum species and asparagus.
- Rutin is one of the primary flavonols found in clingstone peaches.
- Rutin occurs as a yellow crystalline powder, soluble in alkali but only slightly soluble in water.
- Rutin on hydrolysis yields quercetin, rhamnose and glucose, while hesperidin yields hesperetin (or methyleriodictyol), rhamnose and glucose.

Estimation:

TLC chromatography:

Run the TLC using aluminium sheet with Silica gel G, using Ethyl acetate: butanone: formic acid: water (50:30:10:10) as mobile phase or Ethyl acetate: formic acid; acetic acid: water (100:10:11:27)

Uses:

- Rutin alone or in combination with other substances is used in the treatment of hemorrhages linked to diabetes and hypertension and to treat the functional symptoms of the acute attack of piles.
- It is used in many countries as medication for blood vessel protection.
- As an ingredient of numerous multivitamin preparations and herbal remedies.
- It is used as ligand.
- It is also used as antioxidant.
- It inhibits aldose reductase activity.
- It posses anti-inflammatory activity.
- Rutin inhibits platelet aggregation, decreases capillary permeability, making the blood thinner

improving circulation.

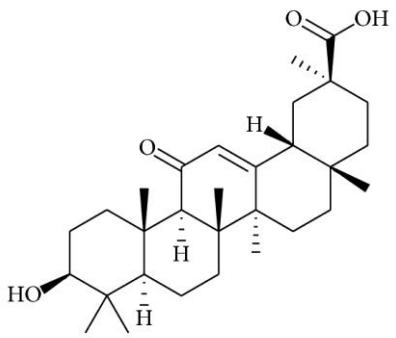
5. GLYCYRRHETINIC ACID

Source:

Glycyrrhetinic acid is a pentacyclic triterpenic acid obtained from the roots and stolones of *Glycyrrhiza glabra*; family *Leguminoseae* commonly known as liquorice.

A major component of liquorice root is a sweet triterpenic saponin glycoside glycyrrhizin, which is a potassium and calcium salt of glycyrrhizic acid about 6–14%.

After hydrolysis, it affords two molecules of gluconic acid and an aglycone glycyrrhetinic acid.



Glycyrrhetinic acid $C_{30}H_{46}O_4$

Isolation of glycyrrhetinic acid:

- The crude drug is first extracted with chloroform. Chloroform extract is distracted this time with 0.5 M sulphuric acid.
- The acid extract is cooled and shaken with chloroform.
- The combined chloroform extract is concentrated and dried to yield glycyrrhetinic acid during extraction with sulphuric acid.
- In another method of extraction, liquorice powder is extracted with boiling water to isolate glycyrrhizin.
- The aqueous extract is concentrated, dried and used as liquorice extract.
- The liquorice extract can be dissolved in water and acidified with hydrochloric acid to pH 3-3.4 to precipitate glycyrrhetinic acid.
- The precipitate is filtered, washed with water till neutral pH and then dried to yield glycyrrhetinic acid.

- Ammoniated glycyrrhizin, used in pharmaceutical trades is prepared by precipitating glycyrrhizic acid from liquorice extract, dissolving it in ammonia and drying the solution after spreading in a thin film on a glass plate to give shining dark brown flakes.

Melting point: 300°C

Thin Layer Chromatography of Glycyrrhetinic Acid

- Dissolve 1 mg of glycyrrhetinic acid in about 1 ml of methanol-chloroform (1:1) mixture.
- Apply the spots over silica gel-G plates and elute in the solvent system Toluene– ethyl acetate–glacial acetic acid (12.5:7.5:0.5).
- Spray the dried plates with 1% vanillin-sulphuric acid or anisalde hydrosulphuric acid and heat for 10 min at 110°C.
- Glycyrrhetinic acid gives purplish spot corresponding to the R_f value 0.41.

6. ATROPINE

Source:

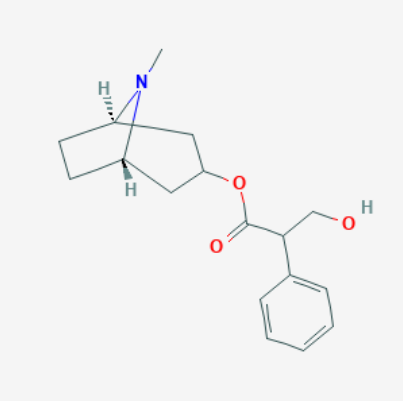
Atropine is a tropane alkaloid from the members of the *Solanaceae* family. It is present in *Atropa belladona* (Deadly Night shade), *Datura stramonium* (Thorn apple), and *Hyoscyamus niger* (Henbane), Other important solanaceous alkaloids are hyoscyamine, hyoscine (scopolamine), apoatropine,

Atropine is isolated from the juice or the powdered drug. *Hyoscyamus muticus* is the preferred source for the manufacture of atropine because of its high alkaloidal content, with *D. stramonium* next in order.

Isolation of atropine

- The powdered drug material is thoroughly moistened with an aqueous solution of sodium and then carbonate extracted with ether or benzene.
- The alkaloidal free bases are extracted from the solvent with water acidified with acetic acid.
- The acid solution is then shaken with solvent ether to remove colouring matter.
- The alkaloids are precipitated with sodium carbonate, filtered off, washed and dried.
- The dried mass is dissolved in solvent ether or acetone and dehydrated with anhydrous sodium sulphate before filtration.

- The filtrate after concentration and cooling yields crude crystals of hyoscyamine and atropine from the solution. The crude crystalline mass is separated from the solution.
- The crude crystalline mass obtained after filtration is dissolved in alcohol, and sodium hydroxide solution is added and the mixture is allowed to stand until hyoscyamine is completely racemized to atropine which is indicated by the absence of optical activity



Atropine

Properties:

The crude atropine is purified by crystallization from acetone.

Atropine sulphate is the most important salt of atropine.

It occurs in the form of colourless crystalline powder.

It is soluble in water and alcohol but insoluble in ether and chloroform.

Melting point: 115–116°C

Identification

Dilute solution of atropine, when treated with concentrated nitric acid and the mixture evaporated to dryness on the steam bath, produces a pale yellow residue. The residue gives a violet colouration when a drop of freshly prepared solution of potassium hydroxide is added. This is known as Vitali–Morin reaction.

Thin Layer Chromatography of Atropine

One percentage solution of atropine dissolved in 2 N acetic acid is spotted over silica gel-G plate and eluted in the solvent system of strong ammonia solution; methanol (1.5:100). TLC plate is spread with an acidified iodoplatinate solution. Atropine gives the R_f value 0.18. Likewise atropine sulphate shows the

R_f value 0.70, in the solvent system acetone : 0.5 M sodium chloride and spraying with Dragendorff's reagent.

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(Approved by AICTE & PCI, New Delhi and Affiliated to JNTUH, Hyderabad)
Dundigal, Quthbullapur Mandal, Hyderabad 500043, R.R. Dist.

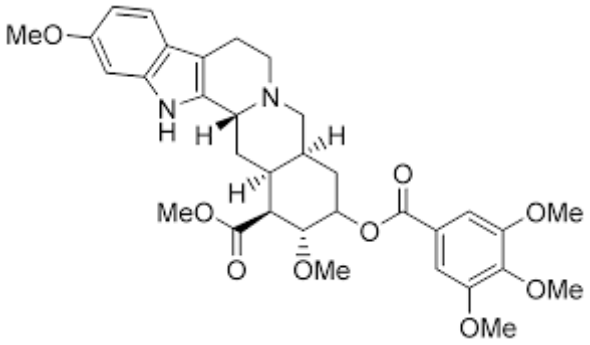
Subject: Pharmacognosy and phytochemistry-II | **Unit No: IV**
Faculty: Mrs. S. Jyothsna | **Lecture No: 6**
Topic: Isolation of Reserpine | **Book Reference: T2**

7. RESERPINE

Reserpine is an indole alkaloid obtained from the roots of *Rauwolfia serpentina,* family *Apocyanaeae* and also from other different species of *Rauwolfia*, such as *R. micrantha, R. vomiforia* and *R. tetraphylla.*

The material obtained from natural sources may contain closely related alkaloids, which includes ajmaline, ajmalicine, ajmalinine, rescinnamine, reserpinine, serpentine and yohimbine.

In *R. serpentina,* reserpine and rescinnamine both respond to the extraction procedures and extracted as a mixture of both while in *R. tetraphylla,* reserpine and deserpidine are extracted together.



Reserpine

Thin layer chromatography:
Dissolve 1 mg of rauwolfia alkaloidalextract or pure reserpine in methanol. Apply the spots over the TIC plate. In case of Silica gel-G plates elute the plate in solvent system chloroform–acetone–diethylamine (50:40:30).

In case of Alumina-G plate, elute in the solvent system cyclohexane–chloroform (30:70). Dry the eluted plates and spray with Dragendorff's reagent.

In both the cases orange spot is given by the alkaloidal components of rauwolfia and by reference standard. In cases of silica gel-G plate, reserpine gives Rf value 0.72 while in case of alumina G plate, it gives Rf value 0.35.

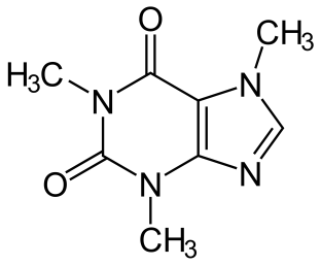
8. CAFFEINE

Source:

Caffeine or 1,3,7-trimethylxanthin is a purine base present along with other related bases like theophyline and theobromine in coffee, tea, cocoa, guarana, kola and mate.

Although caffeine is largely produced synthetically, it is usually isolated from tea leaves or recovered from coffee seeds during decaffeination process.

Tea leaves contain 1–4% of caffeine while coffee seeds contains 1–2% of caffeine. Caffeine was first discovered by Robiquet in coffee in 1821, and mid later in 1827, Oudry found it in tea leaves.



Caffeine

Isolation

- Variety of methods is in use for the isolation of caffeine from different sources. Some important processes are described below.
- The coarse powder of tea leaves is extracted with boiling water and the aqueous extract is filtered while hot.
- The warm extract is treated with lead acetate to precipitate tannins and filtered. The excess of lead acetate present in the solution is precipitated as lead sulphate with dilute sulphuric acid. The filtered solution is boiled with charcoal to remove colouring matter if any, and filtered to remove charcoal. The filtered decolourized solution is extracted with chloroform.
- The combined chloroform extract after evaporation affords caffeine as a white material. It is re-crystallized with alcohol.
- Finely or coarsely powdered tea leaves are extracted with ethanol in soxhlet extractor. The caffeine so extracted in ethanol is then adsorbed on magnesium oxide.
- Caffeine is then disorbed after treatment with 10% H2SO4. It is then extracted with chloroform and re-crystallized.
- Caffeine is extracted from coffee beans by the process of leaching with water. The highest yield up to about 90% was obtained when the coarse coffee powder is extracted with water at 75°C. The extraction takes about half an hour with water/coffee ratio of 9:1.

Decaffeination of coffee using super-critical fluid extraction:

- Super-critical fluid extraction has been efficiently used for the decaffeination of coffee.
- The process was first developed by K. Zosel using liquefied carbon dioxide the super critical liquid medium in a pressure vessel is circulated through moist coffee where it becomes charged with caffeine.
- It is then passed through second pressurized vessel containing an adsorbing medium such as activated carbon, resin or water which retains caffeine.
- Adsorbed caffeine is then separated by extraction with chloroform.

Melting point: 235–237°C

Thin layer chromatography of caffeine:

Dissolve 1mg of caffeine in 1ml of chloroform or methanol. Spot the sample on TLC plate and elute it in ethyl acetate- methanol- acetic acid (80:10:10). Visualize the dried TLC plate by exposure to iodine vapour. Caffeine develops a spot at R_f value 0.41.

9. CURCUMIN

Source:

Curcumins or curcuminoids are the diaryl heptanoid compounds obtained from the dried rhizomes of Turmeric, *Curcuma longa* family, *Zingiberaceae.*

It is a major colouring principle present up to 5% in the rhizomes, which constitutes about 50–60% of the mixture of three main curcuminoids namely

Curcumin,

Desmethoxycucumin and

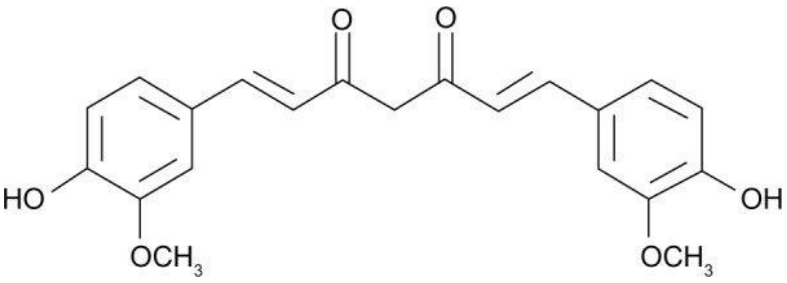
Bisdesmethoxycurcumin.

Isolation of Curcumin

The standardized extract of turmeric contains major proportion of the above curcuminoids

- Commercial curcumin isolated from turmeric rhizome contains up to 97% pure product.
- Curcumin can be obtained by different processes. Turmeric powder is extracted with alcohol in soxhlet extractor.
- The alcoholic extract is concentrated under reduced pressure and dried.
- In another procedure, turmeric powder is first extracted with hexane followed by acetone. The acetone extract is concentrated and dried to yield curcumin.
- The most efficient way of isolating curcumin was found to be to extract with hot ethanol, concentrate the filtrate, and throw the concentrate into superior grade kerosene, when a solid mass separates.
- The mass is stripped off kerosene with petroleum ether and re-crystallized from ethanol. The final product obtained is re-crystallized from hot ethanol to yield orange-red needles.

Melting point: Curcumin 183°C, desmethoxycucumin 168°C, and bisdesmethoxycurcumin 224°C



Curcumin

Thin Layer Chromatography of Curcumin

Dissolve 1 mg of curcumin in 1 ml methanol. Apply the spots on silica gel-G plate and elute the plate in the solvent system chloroform–ethanol–glacial acetic acid (94:5:1).

Dry the eluted plate and visualize under 366 nm light.

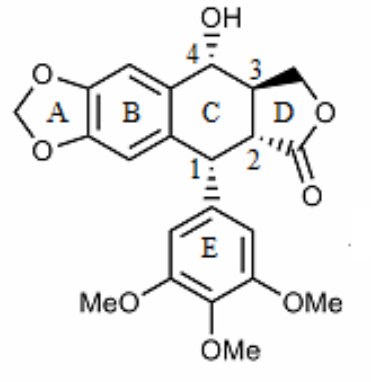
Curcumin exhibits a bright yellow fluorescent spot at R_f value 0.79.

The other spots appearing at R_f values 0.60 and 0.43 correspond to desmethoxycurcumin and bisdes-methoxycurcumin.

10. PODOPHYLLOTOXIN:

Source:

Indian podophyllum is the root and rhizome of *Podophyllum hexandrum Royle belongs to the family: Berberidaceae.*



Podophyllotoxin

ISOLATION OF PODOPHYLLOTOXIN

- Commercial podophyllum is obtained by extraction of powdered rhizome / roots of P. emodii with methanol. Then it is reduced under vaccum. Semi-solid mass is put into acidulated water (10 ml HCl in 100ml water).
- The precipitates are allowed to settle. Filtrate is decanted and then washed with cold water. Resin obtained is dried, and upon drying it gives dark brown amorphous powder called podophyllin.
- The obtained powder is extracted with chloroform and further purification is done by re-crystallization from benzene alone or alcohol benzene mixture followed by washing with petroleum ether / hexane yield podophyllotoxin.

- Another method of extraction to obtain pure podophyllotoxin is by dissolving the CHCl3 soluble fraction in alcohol.
- Then it is refluxed with neutral aluminium oxide so that solution becomes light yellow. To alcoholic solution benzene is added which yield podophyllotoxin of 95-98%.
- Another method of isolating podophyllotoxin from crude (*P. emodii* roots/rhizomes) podophyllin / crude podophyllotoxin involves extraction over a bed of neutral alumina with solvents like benzene, toluene, xylene, etc, for about 1.5-4hrs.
- Re-crystallization from organic solvents such as hot benzene, toluene and xylene yields pure podophyllotoxin (95–97%).
- Podophyllotoxin is a tetrahydronapthalin derivative with OH and lactone groups. The attachment at cis-position is responsible for the purgative property and the attachment trans-position corresponds for anticancer property of the drug.

Melting point: 114–118°C

Thin Layer Chromatography of Podophyllotoxin

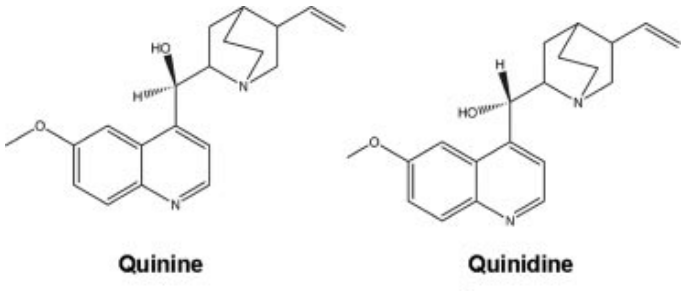
Podophyllotoxin is dissolved in methanol and is spotted on the TLC plate; the solvent used is Toluene:ethyl acetate (5:7) and detecting agent is sulphuric acid. Spot of Podophyllotoxin under day light has violet colour (R_f-0.39).

11. QUININE AND QUINIDINE

Source:

Cinchona is the dried bark of the stem or of the root of Cinchona calisaya Wedd, Cinchona *ledgeriana* Moens, *Cinchona officinalis* Linn and *Cinchona sucirubra* Pavon or hybrids of any of the first two species with any of the last two species *(Rubiaceae*).

Quinine is laevorotatory while quinidine is dextrorotatory stereoisomer.



Quinine Quinidine

Isolation of quinine and quinidine:

- The powdered chinchona bark is mixed with about 30%, of its weight of calcium hydroxide or calcium oxide and sufficient quantity of 5% sodium hydroxide solution.
- Make it into a paste and allow it to stand for few hours. The moistened mass is then transferred into soxhlet and extracted with benzene.
- To the benzene extract, add 5% sulphuric acid and mix well.
- The benzene layer is separated from that of the aqueous layer, the benzene layer is discarded and to the aqueous layer sodium hydroxide is added to adjust the pH to 6.5. Cool and on cooling precipitates of quinine sulphate is formed.
- The precipitate is filtered and separated. The separated precipitate is then recrystalized from hot water to free the salts from cinchonine and cinchonidine.
- The colouring matter is removed by treating it with activated charcoal. The quinine sulphate obtained is dissolved in dilute sulphuric acid, and it is later made alkaline with ammonia.
- Quinine precipitates become crystalline, which are washed and dried at 45–55°C.
- The mother liquor consisting of quinidine, cinchonine and cinchonidine are slightly made alkaline with ammonia, and the precipitate formed is again subjected to extraction with ether.
- Two portions are obtained: the first is ether insoluble fraction consisting of cinchonine crystals and the other is the ether extract with quinidine and cinchonidine.
- The ether soluble fraction consisting of quinine and cinchonidine is first stirred with dilute

hydrochloric acid followed by the addition of 25% of solution of sodium potassium tartarate.

- The resulting solution is allowed to stand for some time, and on standing, cinchonidine tartarate is precipitated. The cinchonidine is re-crystalized from alcohol.
- To the liquor obtained after the separation of cinchonidine tartarate add potassium iodide solution.
- Addition of potassium iodide results in the precipitation of quinidine hydroiodide.
- This on treatment with an alkali (ammonia) liberates a base, which is dissolved in acetic acid.
- The colouring matter is removed by the treatment with activated charcoal. The quinidine obtained is finally re-crystallized from alcohol.

Melting point: Quinine 177°C, Quinidine 174–l75°C

Identification tests:

Thalleoquin test:

- To the sample add one drop of dilute sulphuric acid 1ml of water.
- Add bromine water dropwise till the solution acquires permanent yellow colour and add 1ml of dilute ammonia solution, emerald green colour is produced.
- Thin layer chromatography of quinine and quinidine
- Alkaloids are dissolved in methanol and spotted in silica gel-G plate.
- The solvent system used are chloroform: acetone (9:1) and chloroform: acetone: diethylamine (5:4:1).
- Dried plates are sprayed with dragendroff's reagent the R_f value of quinine and quinidine in the first solvent system are 0.17 and 0.28 respectively, and in solvent system second 0.17 and 0.26

1. ARTEMISININ:

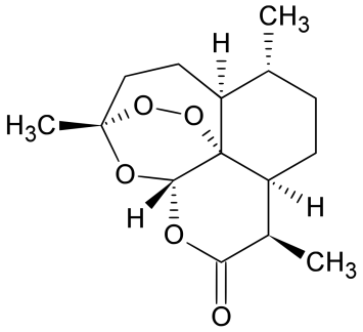
Synonyms:

Sweet worm wood, sweet sage work

For hundreads of years the Chinese have used the herb known as Qing Hao for the treatment of fevers including malaria.

Biological source:

This consists of Chinese traditional herb *Artemisis annua* belongs to the family *Asteraceae*.



Artemisinin

Properties:

- It is a sesquiterpene lactone with an endoperoxide moiety, an unusual functional group in natural products.
- In 1972, the drug was discovered with antimalarial activity.
- It is whitish crystalline powder.
- It is soluble in most organic solvents.
- It is slightly soluble in oil.
- M.P is 156-157^{o}C
- Artemisinin isolated from Artemisia annua is polymorphic in form and crystals posses higher dissolution rate.
- Callus culture of Artemisis annua have been reported to produce scopoletin and a triglyceride but no artemisinin.

Isolation:

- Dried powdered leaves of *Artemisia annua*, extract with petroleum ether, concentrate the extract and dry it.
- Redissolve it in chloroform and acetonitrile.
- This precipitates inert plant constituents such as sugars and waxes.
- Collect the extract, separate by chromatographic fractionation of the concentrate on silica gel by eluting chloroform- ethylacetate, It seperates polymorphic form of artemisinin.
- Crystallize it using cyclohexane or 50% ethanol
- The Chinese plant has been yield as much as 0.9% of artemisinin.
- The highest content of artemisinin is found in the leaves of the top 50cm of the plant.
- The highest yield is obtained from the leaves just before flowering.

Identification:

Separate the constituent (Atemesinin) using TLC on silica gel-G Plates

Uses:

- It is used to treat chloroquine resistant *Plasmodium falciparum.*
- Also used for the treatment of cerebral malaria.
- It has been successful in treating cases of chloroquine- resistant *Plasmodium falciparum* and particularly cerebral malaria.
- Artemisinin does not react with the iron in haemoglobin so that uninfected cells are unaffected.
- Artemisinin derivatives are remarkably non-toxic.
- Artemisinin may; however be embryotoxic so that the use of these drugs is not recommended in early pregnancy.
- Artemisinin is a complex molecule, much effort has been put into synthesiszing compounds based on the 1, 2, 4-trioxane ring of artemisinin.
- A synthetic derivative known as artellene was evaluated in clinical trials but was abandoned due to high rate in recrudescence.

2. ATROPINE

Source:

Atropine is a tropane alkaloid from the members of the *Solanaceae* family. It is present in *Atropa belladona* (Deadly Night shade), *Datura stramonium* (Thorn apple), and *Hyoscyamus niger* (Henbane), Other important solanaceous alkaloids are hyoscyamine, hyoscine (scopolamine), apoatropine,

Atropine is isolated from the juice or the powdered drug. *Hyoscyamus muticus* is the preferred source for the manufacture of atropine because of its high alkaloidal content, with *D. stramonium* next in order.

Isolation of atropine

- The powdered drug material is thoroughly moistened with an aqueous solution of sodium and then carbonate extracted with ether or benzene.
- The alkaloidal free bases are extracted from the solvent with water acidified with acetic acid.
- The acid solution is then shaken with solvent ether to remove colouring matter.
- The alkaloids are precipitated with sodium carbonate, filtered off, washed and dried.
- The dried mass is dissolved in solvent ether or acetone and dehydrated with anhydrous sodium sulphate before filtration.
- The filtrate after concentration and cooling yields crude crystals of hyoscyamine and atropine from the solution. The crude crystalline mass is separated from the solution.
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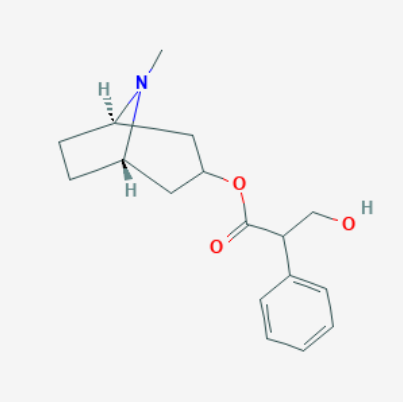

Atropine

Properties:

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It occurs in the form of colourless crystalline powder.

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Melting point: 115–116°C

Identification

Dilute solution of atropine, when treated with concentrated nitric acid and the mixture evaporated to dryness on the steam bath, produces a pale yellow residue. The residue gives a violet colouration when a drop of freshly prepared solution of potassium hydroxide is added. This is known as Vitali–Morin reaction.

Thin Layer Chromatography of Atropine

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CAFFEINE

Source:

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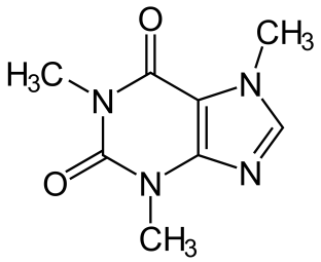
Although caffeine is largely produced synthetically, it is usually isolated from tea leaves or recovered from coffee seeds during decaffeination process.

Tea leaves contain 1–4% of caffeine while coffee seeds contains 1–2% of caffeine. Caffeine was first discovered by Robiquet in coffee in 1821, and mid later in 1827, Oudry found it in tea leaves.



Caffeine

Isolation

- Variety of methods is in use for the isolation of caffeine from different sources. Some important processes are described below.
- The coarse powder of tea leaves is extracted with boiling water and the aqueous extract is filtered while hot.
- The warm extract is treated with lead acetate to precipitate tannins and filtered. The excess of lead acetate present in the solution is precipitated as lead sulphate with dilute sulphuric acid. The filtered solution is boiled with charcoal to remove colouring matter if any, and filtered to remove charcoal. The filtered decolourized solution is extracted with chloroform.
- The combined chloroform extract after evaporation affords caffeine as a white material. It is re-

crystallized with alcohol.

- Finely or coarsely powdered tea leaves are extracted with ethanol in soxhlet extractor. The caffeine so extracted in ethanol is then adsorbed on magnesium oxide.
- Caffeine is then disorbed after treatment with 10% H2SO4. It is then extracted with chloroform and re-crystallized.
- Caffeine is extracted from coffee beans by the process of leaching with water. The highest yield up to about 90% was obtained when the coarse coffee powder is extracted with water at 75°C. The extraction takes about half an hour with water/coffee ratio of 9:1.

Decaffeination of coffee using super-critical fluid extraction:

- Super-critical fluid extraction has been efficiently used for the decaffeination of coffee.
- The process was first developed by K. Zosel using liquefied carbon dioxide the super critical liquid medium in a pressure vessel is circulated through moist coffee where it becomes charged with caffeine.
- It is then passed through second pressurized vessel containing an adsorbing medium such as activated carbon, resin or water which retains caffeine.
- Adsorbed caffeine is then separated by extraction with chloroform.

Melting point: 235–237°C

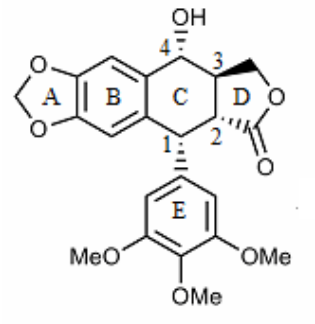
Thin layer chromatography of caffeine:

Dissolve 1mg of caffeine in 1ml of chloroform or methanol. Spot the sample on TLC plate and elute it in ethyl acetate- methanol- acetic acid (80:10:10). Visualize the dried TLC plate by exposure to iodine vapour. Caffeine develops a spot at R_f value 0.41.

PODOPHYLLOTOXIN:

Source:

Indian podophyllum is the root and rhizome of *Podophyllum hexandrum Royle belongs to the family: Berberidaceae.*



Podophyllotoxin

ISOLATION OF PODOPHYLLOTOXIN

- Commercial podophyllum is obtained by extraction of powdered rhizome / roots of P. emodii with methanol. Then it is reduced under vaccum. Semi-solid mass is put into acidulated water (10 ml HCl in 100ml water).
- The precipitates are allowed to settle. Filtrate is decanted and then washed with cold water. Resin obtained is dried, and upon drying it gives dark brown amorphous powder called podophyllin.
- The obtained powder is extracted with chloroform and further purification is done by re-crystallization from benzene alone or alcohol benzene mixture followed by washing with petroleum ether / hexane yield podophyllotoxin.
- Another method of extraction to obtain pure podophyllotoxin is by dissolving the CHCl3 soluble fraction in alcohol.
- Then it is refluxed with neutral aluminium oxide so that solution becomes light yellow. To alcoholic solution benzene is added which yield podophyllotoxin of 95-98%.
- Another method of isolating podophyllotoxin from crude (*P. emodii* roots/rhizomes) podophyllin /

crude podophyllotoxin involves extraction over a bed of neutral alumina with solvents like benzene, toluene, xylene, etc, for about 1.5-4hrs.

- Re-crystallization from organic solvents such as hot benzene, toluene and xylene yields pure podophyllotoxin (95–97%).
- Podophyllotoxin is a tetrahydronapthalin derivative with OH and lactone groups. The attachment at cis-position is responsible for the purgative property and the attachment trans-position corresponds for anticancer property of the drug.

Melting point: 114–118°C

Thin Layer Chromatography of Podophyllotoxin

Podophyllotoxin is dissolved in methanol and is spotted on the TLC plate; the solvent used is Toluene:ethyl acetate (5:7) and detecting agent is sulphuric acid. Spot of Podophyllotoxin under day light has violet colour (R_f-0.39).

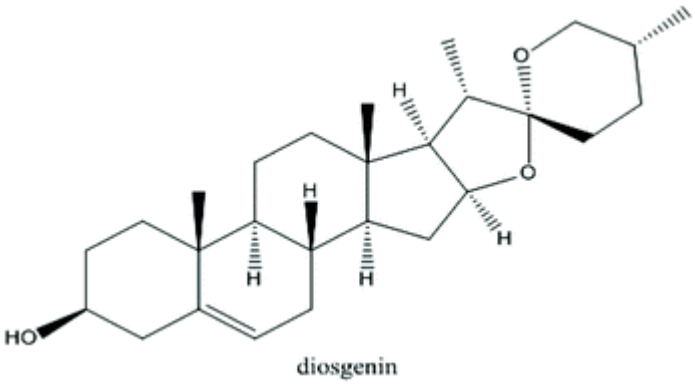
SGENIN

Diosgenin, a precursor for partial synthesis of oral contraceptives, sex hormones, and other steroids, is widely used in pharmaceutical industry.

Nowadays, many researchers found that diosgenin has antiproliferative and proapoptotic effects on cancer cells or on rheumatoid arthritis synoviocytes.

It also shows pharmacological activities such as antilipoperoxidative and antiskin aging effects.

The main raw material used in industry is *Dioscorea zingiberens* is C. H. Wright (DZW) because of the high content of diosgenin in its tubers.



diosgenin

Plant material:

Seeds were surface sterilized with a sodium hypochlorite solution (1.5% w/v available chlorine) supplemented with two drops Triton X- 100 for 6 min and rinsed 3 times with sterile distilled water.

They are left for germination on agar plates containing 0.5% sucrose at 25°C in the light with a daily 16-h photoperiod.

Plantlets are transferred onto solid half-strength McCown's woody plant (WP) medium (Lloyd and McCown 1980) +3% sucrose (1/2 WP 3) and maintained at 25°C in the light.

Establishment of hairy root cultures:

Hairy roots are induced by direct infection of the stems of 2- weekold sterile plantlets with A. rhizogenes A4. Approximately 4 weeks after infection, hairy roots were transferred onto solid 1/2 WP 3 medium containing 0.25 g/l cefotaxime and 1 g/l ampicillin.

After elimination of the bacteria, the hairy roots are inoculated into 1/2 WP 3 liquid medium (50 ml in 250-ml conical flasks), cultured in darkness at 25°C on a gyratory shaker (80 rpm) and subcultured at 4-week intervals.

To prove transformation, theopines are extracted and identified by paper electrophoresis.

Extraction of diosgenin:

The contents of flasks are harvested and fresh and dry weight, after lyophilization, will be determined individually.

The hairy roots are powdered, extracted and hydrolyzed by refluxing for 5 h with 1 M sulfuric acid in 70% isopropanol.

The extract is adsorbed on an Extrelut column and diosgenin was eluted with n-hexane.

The organic solvent is evaporated to dryness. Root tissues of normal 8-month-old plants grown in the field are extracted.

After filtering off cell debris, the liquid media are lyophilized and extracted using the same procedure.

Cellulase

Pectinase and

Glucoamylase

- Diosgenin was identified by high-performance liquid chromatography coupled with mass spectrometry and comparison with reference material.
- 2 ml of dried pyridine and 0.1 ml of benzoyl chloride were added to the dried extract.
- The sample was heated at 80°C for 30 min then, after addition of 2 ml of methanol, heated again at 100°C for 30 min.
- After cooling to room temperature, 10 ml of dichloromethane was added, followed by 20 ml of water and 2 ml of concentrated hydrochloric acid.
- The organic phase was washed successively with water, with a saturated sodium carbonate solution and finally twice with water.
- The dichloromethane layer was evaporated to dryness and the residue was dissolved in chloroform (HPLC grade) before injection into the HPLC column.
- Vitamin K1 was used as internal standard. Twenty microliters of filtered extract-internal standard mixture was injected. Diosgenin benzoate concentrations were determined by comparison with an external standard curve over the range 25–250 µg/ml.
- HPLC analyses were performed using a Waters 600E apparatus on a Nucleosil 100-5 C-18 column fitted with a Nucleosil 120-5 C-18 guard column.
- The isocratic solvent system was acetonitrile-water 92:8 (v/v) for 60 min with UV detection at 230 nm. The flow rate was set at 2 ml/min and the column temperature was maintained at room temperature.

Cell culture conditions:

Stock suspension cultures of the *Digitalis lanata* cell line W.1.4 were grown in Erlenmeyer flasks kept in the dark at 24°C on gyratory shakers (120 rpm).

Cells were sub-cultured every 10.5 d by inoculating 20 g cells (wet weight) into 300 ml of fresh GM1

Growth media:

The maintenance medium (GM1) was based on MS medium with twice the MS phosphate and glycine and no caseine hydrolysate added.

Phytohormones were omitted and the glucose concentration was 3%. Growth medium 2 (GM2), with increased concentrations of sulfate, phosphate, ammonium, magnesium, potassium and glucose, was used to supply fresh medium when the cells were grown in the semi-continuous mode.

Production media:

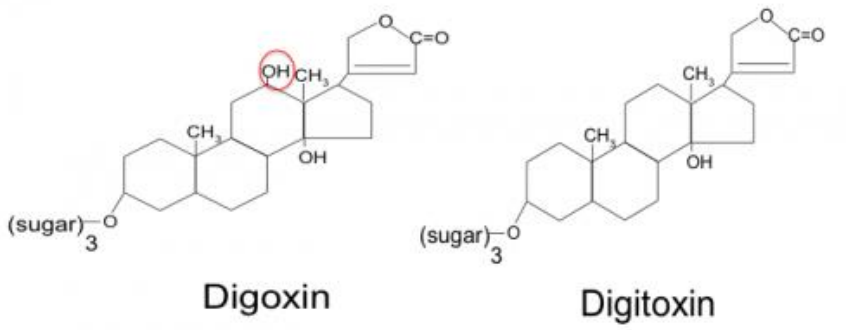
Production medium 1 (PM1), i.e., an 8% glucose solution with the pH adjusted to 5.5, was used as the production medium for all experiments run in the batch mode.

For the production of digoxin under semi-continuous culture conditions a medium termed PM 3 (16% glucose solution, pH 5.5) was used to replace part of the GM 2 at the beginning of the pre- incubation phase.

Growth of cell supension cultures in bioreactors. The contents of 24 stock culture flasks (for a total of 7.7 l suspension) were added to 28 1 GM 2 in a 40-1 air-lift bioreactor, which was used to produce the inoculum for a 300-1 bioreactor.

2. DIGOXIN

Digoxin or Lanoxin is the most widely used cardiac glycoside obtained from the leaves of *Digitalis lanata; family Scrophulariaceae.* It is a secondary glycoside which is produced from a primary glycoside Lanatoside C. Its hydrolysis yields three molecules of digitoxose sugar and digoxigenin. It is a highly potent drug and should be handled with exceptional care.



Production of digoxin in bioreactors:

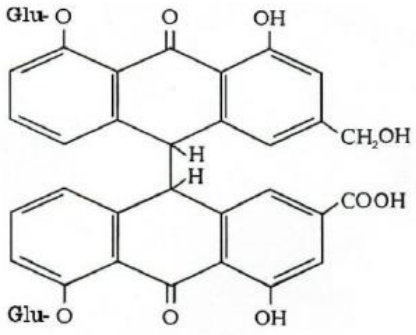
- In preliminary runs digoxin production was achieved in a 1-1 exsiccator vessel fitted with an aeration line ending in a ring-shaped sparger fixed to the bottom of the jar.
- The vessel was filled with 300-400 ml of cell suspensions pre-incubated for 48 h in an 8% glucose solution. The suspension was agitated by sparged air at an aeration rate of 1 1 min-1.
- These portable glass jars were sterilized in an autoclave and then each filled with 18-19 L of cell suspension withdrawn from the 300-L bioreactor.
- During incubation the glass vessels were shaded.
- The suspensions were aerated at 4.5-12.0 1 min with sterile air and the incubation temperature was maintained at 21°C.
- The production cycle was started by the addition of 0.65 mmol 1-1 digitoxin.
- Plants are capable of synthesizing a variety of low-molecular-weight organic compounds, called secondary metabolites, usually with unique and complex structures.

- Plant secondary metabolites are of tremendous importance, both for the plant itself (for plant–environment interactions) and to humans, for their biological activities that can have therapeutic value.
- Compared to the main molecules found in the plants, these secondary metabolites were soon defined by their low abundance, often less than 1% of the total carbon, or storage, usually occurring in dedicated cells or organs.
- India's booming export trade in medicinal plants has risen almost three-fold during the last decade.
- This boom in local use and export trade is depleting many species from the wild, bringing some to the edge of extinction.
- The major problem in conventional procurement of MAPs is that only a few are cultivated; over 95% of the medicinal plants used in India are collected from the wild.
- Some species are becoming difficult to obtain in sufficient amounts to meet increasing demands.
- Destruction of natural habitats and technical difficulties in cultivation are also driving the drastic reductions in plant availability.
- For the production of desirable medicinal compounds from plants, biotechnological approaches, specifically plant tissue culture, has the potential to supplement traditional agriculture in the industrial production of bioactive plant metabolites.
- Large-scale plant tissue culture is found to be an attractive alternative approach to traditional methods of plantation, as it offers a controlled supply of biochemicals independent of plant availability.
- In vitro propagation and complete plant regeneration can be a viable method of ex situ conservation of these important species.
- Biotechnological production of important phytopharmaceuticals (secondary metabolites) can indirectly help in the conservation of important plants, by reducing the demand for raw materials from the wild.

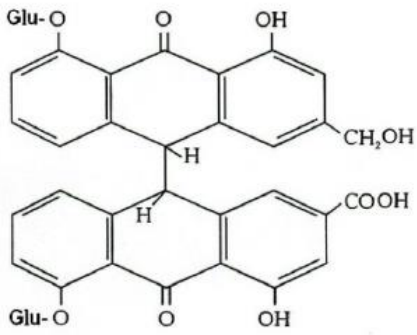
3. SENNOSIDES

Source:

Dianthrone glycosides, leaflets of *Cassia angustifolia* (Indian senna) and *C. acutifolia* (Alexandrian senna). Family- *Leguminosae.*



Sennoside A



Sennoside B

Method-I

- The leaves are dried and powdered. The powdered drug is shaken with benzene for 2 h on an electronic shaker.
- Filter and distill off the solvent and marc is dried at room temperature and extracted with 70% methanol for 4–6 hours.
- The extract is filtered under vacuum and it is re- extracted with 70% of methanol for 2 h, and filtered.
- The methanolic extract is combined and concentrated to l/8th portion of its original volume. The concentrated solution is acidified with hydrochloric acid to a pH of 3.2.
- The acidified solution is kept aside for 2 h at a temperature of 5°C.
- The this solution is filtered and to the filtrate add anhydrous calcium chloride dissolved in 25 ml of denatured spirit with constant and vigorous stirring.
- The pH is again adjusted to 8 by ammonia and it is set aside for 2 h. The solution is filtered; the precipitate obtained is dried over P O in a dessicator.

Method-II

- Powdered drug is extracted by shaking with ethanolic chloroform (93 parts of chloroform and 7 parts of ethanol) for 30 min.
- Filtered and the leaves are again extracted with acidic methanol (1.2 g of oxalic acid per liter of methanol).
- Both the extracts are combined and concentrated. It was kept for whole night in

room temperature.

- Sennoside A precipitates out, Sennoside B remains in solution. Sen- noside A is re-crystalized using triethylamine.
- Sennoside B solutions are precipitated by 10% methanolic solution of $CaCl_2$. Further seperated by methanolic ammonia solution (40 ml ammonia + 60 ml methanol).
- Dried washed with water and kept for one day. It is then re-crystylized using glycolmonoethylether.

Melting point: Sennoside A 200–240°C, Sennoside B 180–186°C

Identification tests: To the crude extract, organic solvent like benzene, ether or chloroform is added and shaken. The organic layer is separated and to it ammonia solution is added, the ammoniacal layer produces pink to red colour indicating the presence of anthraquinone glycoside.

Thin Layer Chromatography of Sennosides

Sennosides are spotted in Silica gel-G plates and devel- oped using ethyl acetate: methanol: water (100:16.5:13.5) as solvent system. Red coloured spots will appear when the spots are sprayed with 25% nitric acid and turns to yellow when sprayed after drying with alcoholic potassium hydroxide solution.

Industrial production:

- Dried senna leaves powder extracted with benzene for 2-3 hrs.
- Marc is dried and extracted with methanol for 4-6 hrs.
- Mix both the extracts and concentrated.
- pH of extract adjusted to 3.2 by HCl.
- Extract is mixed with hydrous calcium chloride in 25 ml denatured spirit.
- pH adjusted to 8 using ammonia & set aside for 2hrs, results into precipitate of sennosides.

Estimation:

Column- C18 Mobile phase- 1% acetic acid in water: Acetonitrile (82:18) Flow rate- 1ml/min
Detection- 350 nm

Utilization:

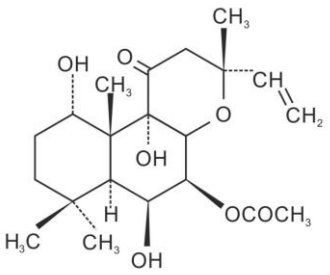
1. Treatment of constipation
2. Used in the skin diseases
3. Used as an anthelmintic

4. Useful in loss of appetite, dysentry, indigestion, malaria, jaundice, gout, rheumatism & anaemia.

4. FORSKOLIN

Biological Source:

Labdane diterpenoid extracted from roots of *Coleus forskohlii*, family- *Lamiaceae*.



Chemical Structure of Forskolin

Industrial Production:

Roots& bark powder extracted with toluene at 60˚C for 2 hours.
Filtrate collected & concentrated at temperature not exceeding 40˚C.
Concentrated extract mixed with n- hexane, yields crude forskolin in the form of brown ppt.
It is purified using column chromatography.

Estimation:

TLC & HPTLC Mobile phase – Toluene: ethyl acetate (8.5: 1.5 v/v) Stationary phase- Silica gel F254
Visualizing agent- 5% vanillin in glacial acetic acid and 10% sulphuric acid in water.

Utilization:

1. Antidepressant
2. Vasodilating
3. Antiobesity
4. In glaucoma
5. Antiasthmatic

Forskolin, an important secondary metabolite in Coleus forskohlii (Lamiaceae), has several biological and pharmacological activities.
Central Drug Research Institute (CDRI), Lucknow, India, in 1974, revealed the presence of a hypotensive and spasmolytic component of C. forskohlii that was named coleonol.

Further investigation (Saksena et al., 1985) determined the exact chemical structure of this labdane diterpene and its name was changed to forskolin (7b-acetoxy- 8, 13-epoxy-la, 6b, 9a-trihydroxy-labd-14-en-11-one).

- It shows positive inotropic, positive chronotropic and hypotensive activity, inhibits thrombocyte aggregation, and decreases intraocular pressure.
- Forskolin Micropropagation and in vitro culture for production of forskolin
- Forskolin synthesis in transformed cultures: gall calluses, cell suspension cultures, rhizogenic calluses, and rooty and shooty teratomas
- Transformed cell suspension culture
- Transformed hairy root cultures
- Molecular cloning

- ✓ Transformed hairy root cultures Establishment of C. forskohlii hairy root culture
- ✓ The shoots (2 cm in length) were surface-sterilized with 75% ethanol for 30 s and NaClO2 (available chlorine concentration of 2%) for 10 min.
- ✓ Shoot buds (ca. 1 cm) excised from the sterilized shoots were incubated on hormone-free 1/2 Murashige and Skoog (1962) (MS) solid medium containing 3% sucrose, and cultured at 25°C in 16 h light/day.
- ✓ The axenic plantlets of C. forskohlii were used as explants for the induction of hairy roots. Agrobacterium rhizogenes strain MAFF 03-01724 subcultured on YEB liquid medium, was inoculated by needle on a newly cut surface of the plantlets.
- ✓ After 2–3 weeks, several hairy roots appeared at the inoculated sites.
- ✓ Tips of the hairy roots were cut off and cultured on woody plant (WP) solid medium containing 3% sucrose and 0.5 g/l Claforan for the elimination of bacteria.

5. VINCRISTINE & VINBLASTINE

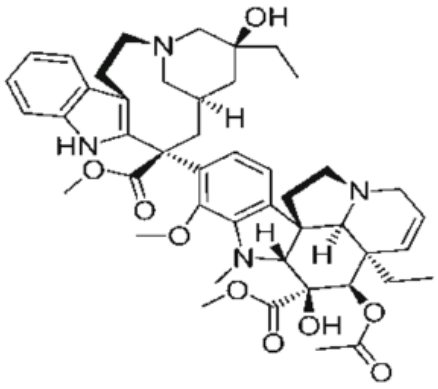
Source:

Indole alkaloid, It consists of whole plant of *Catharanthus roseus*, family- *Apocynaceae.*

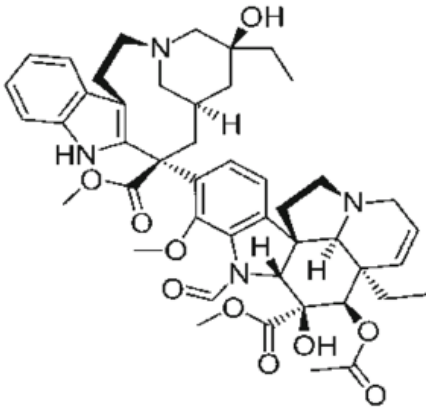
Extraction and Isolation

The dried leaf material is taken and is extracted with a solution of hot ethanol–water–acetic acid in a ratio of 9:1:1. The solvent is removed and to the residue hot hydrochloric acid solution of 2% is added. The pH of the acidic extract is adjusted to 4, for the precipitation of the non-alkaloidal components, which can be separated by filtration. The pH of the aqueous acidic solution is now adjusted to 7 and then extracted with benzene. The benzene layer is evaporated to obtain vinblastine and other alkaloids.

Fig. 1 Chemical structure of vinblastine and vincristine



Vinblastine



Vincristine

Isolation of Vinblastine and Vincristine

- ✓ The phenolic materials are removed by the washing the extract with dilute alkali.
- ✓ The washed extract is subjected to chromatography on alumina and elution is carried out in 18 fractions starting with benzene–methylene chloride (65:35) mixture to pure methylene chloride.
- ✓ Vinblastine recovered in the ninth fraction. Further elution of the column results in separating the fractions of vincristine.

Melting point: Vinblastine: 284–285°C, Vincristine: 273– 281°C

Thin Layer Chromatography of Vincristine

Vincristine dissolved in 25% water in methanol solution, spotted in Silica gel-G plate and developed using the solvent, acetonitrile: benzene (30:70). The dried plates are sprayed with 1% solution of ceric ammonium sulphate in 85% phosphoric acid. The R_f value of the appeared spot would be 0.39

Utilization:

1. Used in the chemotherapy regimens
2. Used to treat leukemia in children
3. Immunosuppressant

Estimation:

HPLC method Mobile phase- acetonitrile: 0.1 M phosphate buffer.

Wavelength: 254nm.
Production: Plant tissue culture technique.

6. TAXOL (OR) PACLITAXEL:

Synonyms:

Western yew, American yew, Pacific yew

Biological source:

Taxol is complex diterpenoid alkaloid obtained from the bark of *Taxus brevifolia* and *Taxus baccata*

Family: *Taxaceae*

The overall yield is only 0.01-0.02% w/w

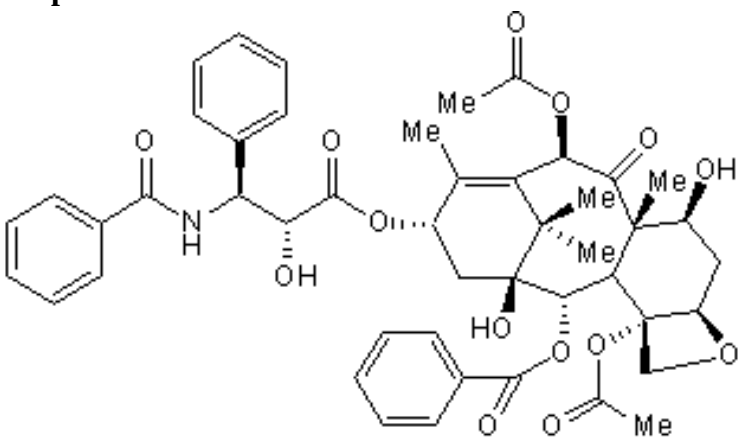
The major obstacle in the preparation of Taxol has been the limited supply of the drug.

It is not econoically feasible to synthesise taxol due to its complex chemistry.

It is not econnomically feasible to synthesise taxol

It requires the bark from about 3 mature 100yrs old trees to provide 1gm of taxol.

Properties:



- ✓ It is hydrohobic in nature.
- ✓ M.P 213-216^{o}C.
- ✓ An ester side chain at the 13th position of the taxane ring is essential for its cyotoxic activity.
- ✓ The presence of 2^{1} hydroxy group of the side chain enhances the activity.
- ✓ It is used in the treatment of metastatic carcinoma of the ovary afte failure of the 1st line treatment or subsequent chemotheraphy.

- ✓ It is also used in the treatment of breast cancer.

Isolation:

- The bark is removed from mature trees in the period May-August (1st isolated in 1971).
- Extract dried and powdered bark of *Taxus brevifolia* with alcohol concentreate the total alcoholic extract.
- Dry and subject it to sequential chromatographyon silica gel and florisil.
- It yields taxol and cephalomannine.
- Separate taxol by chromatography on silica gel using dichloromethane/ l-propanol as eluent.

Uses:

It is used for the treatment of ovarian cancer, breast cancer, lung cancer, cervical cancer and pancreatic cancer.

Taxol:

- It is used chemically in the treatment of ovarian cancer , breast cancer and Non-small cell lung cancer
- It may also have potential value against other cancers.

Taxotere:

- It is a side chain analogue of taxol, which has also been produced by semi-synthesis from 10-desacetylbaccatin-III
- Used in the treament of breast cancer.

Extraction:

- The crude drug contains the active constituents, which can be isolated from these drugs by various methods of extraction and separation.
- Extraction is defined as the process of isolation of soluble material from an insoluble residue, which may be liquid or solid, by treatment with a solvent on the basis of the physical nature of crude drug to be extracted, i.e. liquid or solid, the extraction process may be liquid-liquid or solid-liquid extraction.
- The process of extraction is controlled by mass transfer. Mass transfer is a unit operation, which involves the transfer of mass of soluble material from a solid to a fluid.
- If a crude drug panicle is immersed in a solvent to be used for extraction, the particle is first surrounded by a boundary layer of the solute; the solvent starts penetrating inside the particle and subsequently forms solution of the constituents within the cells.

- Escape of these dissolved constituents through the cell wall and through the boundary layer takes place. The process is continued till equilibrium is set up between the solution in the cells and the free solution.

- Few important factors, which affect the mass transfer, are agitation and temperature that increase the concentration gradient to bring about an efficient extraction.

- Size reduction of the crude drug increases the area over which diffusion can occur. Overall extraction is also dependent on the selection of the method of extraction and the solvent selected for extraction.

EXTRACTION METHODS

Majority of the small-scale extraction processes of maceration and percolation are generally slow and time consuming and also give the inefficient extraction of the crude drugs. These processes are generally modified for more efficient and faster extraction at the laboratory scale. Large-scale industrial batch operations demand some more modifications of extraction process where the small-scale directions are inappropriate.

MACERATION

- Maceration process involves the separation of medicinally active portions of the crude drugs. It is based on the immersion of the crude drugs in a bulk of the solvent or menstruum.
- Solid drug material is taken in stoppered container as shown in below figure, with about 750 ml. of the menstrum and allowed to stand for at least three to seven days in a warm place with frequent shaking.
- The mixture of crude drug containing solvent is filtered until most of the liquid drains off. The filtrate and the washing are combined to produce 1,000 ml of the solution.

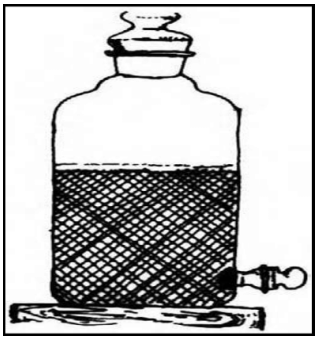


Fig. Macerating bottle

- Maceration method is modified to multiple stage extraction to increase the yield of the active ingredients in the extracts.

- The crude drug material is charged in the extractor, which is connected with a circulatory pump and spray distributor, along with number of connected tanks to receive the extraction solution.

- This is known as multiple stage extraction because the solvent added and circulated in the extractor containing drug is removed as extracted solution and is stored in the receiver tanks.

- This operation is repeated thrice. When the crude drug material is charged in the extractions, the stored solution is once again circulated through fresh drug and then removed as an extract. Likewise, after three extractions, the drug is removed from the extractor, again recharged with fresh drug and the whole cycle is repeated.

PERCOLATION

- As the term indicates, percolation is a continuous flow of the solvent through the bed of the crude drug material to get the extract.
- In this process, first the powdered drug is treated with sufficient menstruum to make it uniformly wet. Damp material is allowed to stand for about 15 min, and then transferred to a percolator winch is generally a V- shaped vessel open at both the ends.
- To it, sufficient menstruum is added to saturate the drug. The lid is placed on the top. When the liquid starts dripping out from the outlet of the percolator, the lower opening is closed.
- The drug material is allowed to macerate in the vessel for 24 h and then the percolation is continued

gradually using sufficient menstruum to produce 1,000 ml of solution.

- The percolation process is dependent on the flow of solvent through the powered drug, and it yields the products of greater concentration than the maceration process.

Fig. Precolator

MODIFIED PERCOLATION

- The conventional percolation process is modified especially when the solvent is dilute alcohol. In cases when the strength of alcohol needs to be unaffected by concentration of the extract, percolation is continued and the first quantity of the percolate is collected and set aside.

- The subsequent quantities of the percolates are collected, concentrated and lastly, the first volume of the percolate is added in the final product. In this way it maintains the required alcohol strength and also produces the higher concentration of the products. The process is known as reserve percolate method.
- In modified process of percolation techniques, continuous or semi-continuous extraction devices are used in some industries for handling the batches of varying size.

- The extraction batteries which consists of a number of vessels in series are inter-connected through pipelines and are arranged in such a way that the solvent can be added and the product removed from any vessel.

- Such type of extraction battery gives maximum efficiency of extraction with minimum use of solvent. The product obtained is more concentrated and less losses of solvent take place due to evaporation.

CONTINUOUS EXTRACTION

SOXHLET EXTRACTION

- Soxhlet extraction is the process of continuous extraction in which the same solvent can be circulated through the extractor for several times.
- This process involves extraction followed by evaporation of the solvent. The vapours of the solvent are taken to a condenser and the condensed liquid is returned to the drug for continuous extraction

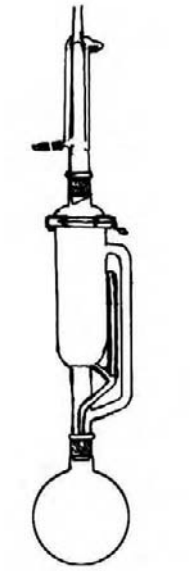


Fig. 24.3 Soxhlet extractor

- Soxhlet apparatus, designed for such continuous extraction, consists of a body of extractor attached with a side tube and siphon tube as shown in the above figure.
- The extractor from the lower side can be attached to distillation flask and the mouth of the extractor is fixed to a condenser by the standard joints.
- The crude drug powder is packed in the soxhlet apparatus directly or in a thimble of filter paper or fine muslin.
- The diameter of the thimble corresponds to the internal diameter of the soxhlet extractor. Extraction assembly is set up by fixing condenser and a distillation flask.
- Initially for the setting of the powder, solvent is allowed to siphon once before heating. Fresh activated porcelain pieces are added to the flask to avoid bumping of the solvent.
- The vapours pass through the side tube and the condensed liquid gradually increases the level of liquid in the extractor and in the siphon tube.
- A siphon is set up as the liquid reaches the point of return and the contents of the extraction chamber are transferred to the flask.
- The cycle of solvent evaporation and siphoning back can be continued as many times as possible without changing the solvent so as to get efficient extraction. This method, although a continuous extraction process, is nothing but a series of short macerations.
- Similar methodology can be adopted in large-scale production in which the operation principles

may resemble the laboratory equipment. Soxhlet extraction is advantageous in a way that less solvent is needed for yielding more concentrated products.

- The extraction can be continued until complete exhaustion of the drug. The main disadvantage is that this process is restricted to pure boiling solvents or to azeotropes.

Large-scale extraction

- As the large-scale extraction is meant for the extra large batches of drug material, the various assemblies which are generally in attachment with the body of soxhlet extractor are modified.
- The pilot plant extractor generally has a separate extractor and condenser unit. Separate inlet for loading the drug and an outlet for drug discharge are provided. The extractor body is divided into two parts: the upper one for drug material and the lower one as a distillation chamber.
- The distillation chamber is electrically heated. The vapours of the solvent are passed to condenser and the condensed liquid is sprayed on the bed of crude drug with the help of solvent distribution nozzle.
- Solution return to the distillation chamber via solution return pipe. Such large-scale extractors are provided with the outlet from the lower side of the extractor, for removing extract.

Supercritical fluid extraction:

- The supercritical fluid extraction is a comparatively recent method of extraction of crude drugs. Certain gases behave like a free flowing liquids or supercritical fluids at the critical point of temperature and pressure.
- Such super critical have a very high penetration powers and extraction efficiency. This principle was first used in the food packing industries for the deodarization of the packed food products.
- The gases like carbon dioxide are held as supercritical fluid at the critical point of 73.83 barpressure and 31.06^{o}C temperature.
- At this critical point CO_2 behaves as a liquefied gas or free-flowing liquid and assist the extraction of the phytochemical constituents from the crude drugs.
- The below phase diagram of CO_2 indicates the characteristic areas for the deodorization, extraction and fractionation.

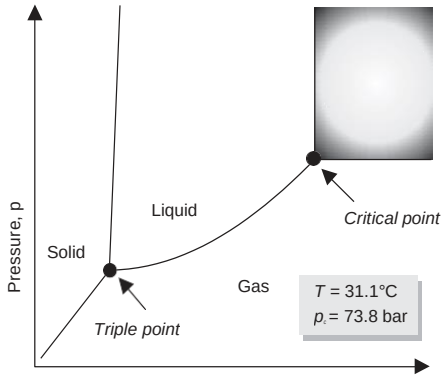
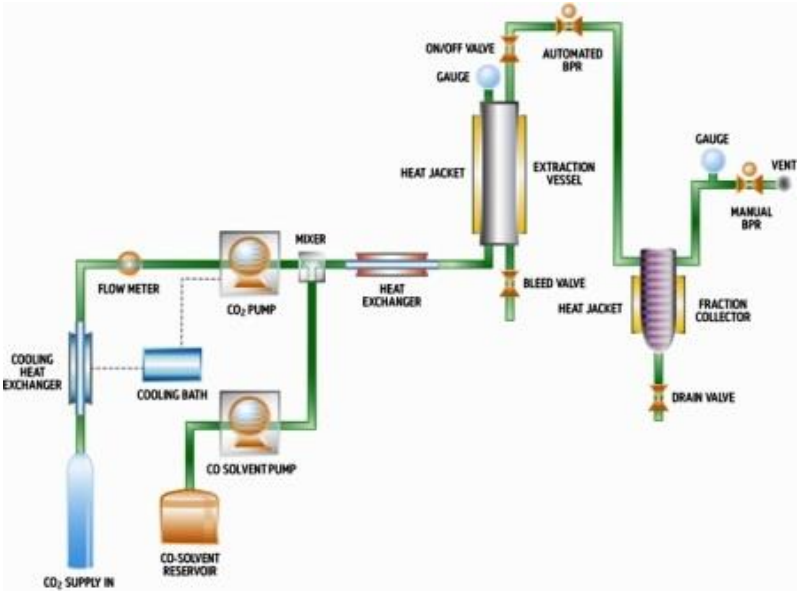


Fig. Phase diagram of CO_2

- The advantages of CO_2 in supercritical fluid extraction are that it is sterile and bacteriostatic. It is noncombustible and nonexplosive.
- CO_2 is harmless to environment and no waste products are generated during the process, and it is available in large amount under favourable condition.
- The mixture to be fractionated is passed in the extraction column along the length of which the heater is located.
- CO_2 is purged through the column. Once the extraction column is pressurized, drug material gets saturated in the supercritical fluid which moves along the length of the column.
- The operating conditions, i.e. pressure and temperature, are selected. In the pressure controlled type of extraction, the solution is just expanded in the separation stage to precipitate the extract and then again the gas is recompressed for recycle.
- In temperature control type operation, the extract is precipitated by heating the solution which lowers the solvent density. The density is then increased by isobaric cooling for recycling.
- Operation of supercritical fluid extraction system is controlled from a PC. PC is used to set the operating conditions like pressure, temperature and flow rate. PC is programmed to safely shut down the unit in case of overpressure or over temperature situations.



Supercritical fluid extractor

- Supercritical fluid extraction has many applications in pharmacognosy especially in the extraction and isolation of the active constituents. The process is successfully used in the decaffeination of coffee, extraction of pyrethrins and for the production of terpeneless oils.
- It has been successful in selectively extracting larger proportions of active ingredients than conventional methods of hydrodistillation or extraction, i.e. in cases of acorone from Acorus calamus, matricin and bisabolol from chamomile flowers, heat labile sesquiterpene hydrocarbons of Valerian and Nardostachys species.
- It is useful for removing the off odours or 'still notes' from the freshly distilled essential oil. The temperature pressure conditions for the extraction of certain constituents from crude drugs are given in Table below.

Table Temperature and pressure conditions for supercritical fluid extraction

Drugs	Temp (°C)	Pressure (bar)	Degree of Extraction
Caffeine from Coffee	40–80°	200–300	Decaffeination
Pyrethrins	50°	250	98%
Chamomile principles	40°	160	1.4%
Acorone from Calamus	40°	90	8.3%

There are two essential steps to SFE, transport (by diffusion or otherwise) from with the solid particles to the surface, and dissolution in the supercritical fluid. Other factors, such as diffusion into the particle by the SF and reversible release such as desorption from an active site are sometimes significant, but not dealt with in detail here.

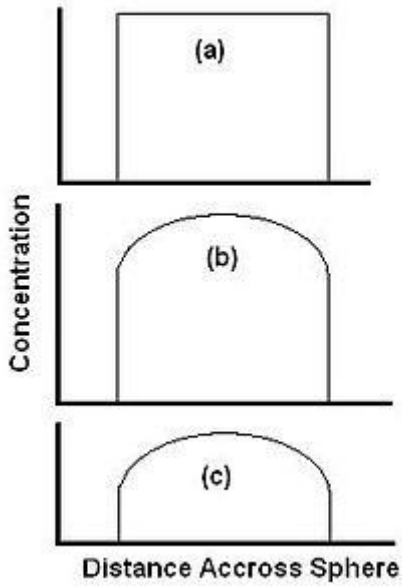


Figure 2. Concentration profiles during a typical SFE extraction

- Figure 2 shows the stages during extraction from a spherical particle where at the start of the extraction the level of extractant is equal across the whole sphere (Fig. 2a).
- As extraction commences, the material is initially extracted from the edge of the sphere. The concentration in the center is unchanged (Fig 2b).
- As the extraction progresses, the concentration in the center of the sphere drops as the extractant diffuses towards the edge of the sphere (Figure 2c).

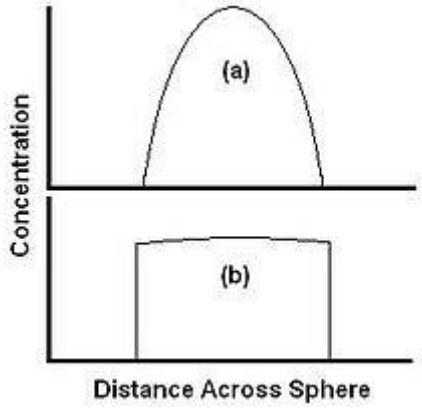


Figure 3: Concentration profiles for

a. Diffusion limited and
b. Solubility limited extraction

- The relative rates of diffusion and dissolution are illustrated by two extreme cases in Figure 3. Figure 3a shows a case where dissolution is fast relative to diffusion.
- The material is carried away from the edge faster than it can diffuse from the center, so the concentration at the edge drops to zero.
- The material is carried away as fast as it arrives at the surface, and the extraction is completely diffusion limited.
- Here the rate of extraction can be increased by increasing diffusion rate, for example raising the temperature, but not by increasing the flow rate of the solvent. Figure 3b shows a case where solubility is low relative to diffusion.
- The extractant is able to diffuse to the edge faster than it can be carried away by the solvent, and the concentration profile is flat. In this case, the extraction rate can be increased by increasing the rate of dissolution, for example by increasing flow rate of the solvent.

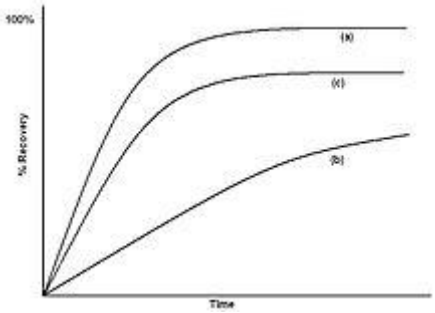


Figure 4: Extraction Profile for Different Types of Extraction

The extraction curve of % recovery against time can be used to elucidate the type of extraction occurring.

Figure 4(a) shows a typical diffusion controlled curve. The extraction is initially rapid, until the concentration at the surface drops to zero, and the rate then becomes much slower. The % extracted eventually approaches 100%.

Figure 4(b) shows a curve for a solubility limited extraction. The extraction rate is almost constant, and only flattens off towards the end of the extraction.

Figure 4(c) shows a curve where there are significant matrix effects, where there is some sort of reversible interaction with the matrix, such as desorption from an active site. The recovery flattens off, and if the 100% value is not known, then it is hard to tell that extraction is less than complete.

Advantages of SFE:

1. Higher diffusion rates than liquid solvents
2. Lower viscosities than liquid solvents
3. Higher vapour pressure than liquid solvents
4. Higher densities compared to gases, higher solvating power
5. Solubility and (to some extent) selectivity can be controlled by modification of parameters
6. Low polarity of carbon dioxide can be modified with cosolvents
7. Suitable for heat-sensitive compounds

Disadvantages of SFE:

1. Carbon dioxide, which is the most commonly used solvent, has low polarity and hence cannot extract polar compounds
2. Presence of water may cause problems
3. Unpredictability of matrix effect
4. Need for specialized/expensive equipment

MICROWAVE ASSISTED EXTRACTION

- Microwaves heat up the molecules by dual mechanism of ionic conduction and dipole rotation.
- Non-ionizing electromagnetic waves positioned between the X-ray and infrared rays in the electromagnetic spectrum with frequency between 300 MHz to 300 GHz are called microwaves.
- The two types of oscillating perpendicular fields that generate microwaves are the electric field and magnetic field. Both The ionic conduction and dipole rotation are responsible for heating of substances.
- When the microwaves interact with polar solvents, heating of substance is caused due to any one of the above mentioned phenomena, individually or simultaneously.
- The electrophoretic migration of ions under the influence of the changing electric field is called Ionic conduction. If the solution offers a resistance to this migration of ions, a friction is generated and the solution is heated.

Principle:

- The target for heating in dried plant material is the minute microscopic traces of moisture that occurs in plant cells. The heating up of this moisture inside the plant cell due to microwave effect, results in evaporation and generates tremendous pressure on the cell wall.
- The cell wall is pushed from inside due to the pressure and the cell wall ruptures. Thus the exudation of active constituents from the ruptured cells occurs, hence increasing the yield of phytoconstituents. The yields from plant matrices can also be enhanced if the plant matrix is soaked with solvents with higher tan δ value.
- The ether linkages of cellulose are hydrolyzed and are converted into soluble fractions within a few minutes. Higher yields can be obtained also by increasing the temperature, which facilitates faster penetration of solvent into the cell walls.
- It is observed that when one compares the Scanning Electron Micrographs (SEM) of untreated samples, heat-reflux extraction sample and MAE samples, there may be no structural difference between heat-reflux extraction and those of untreated samples, whereas there is a complete rupture of cell wall in the MAE treated samples.

- In extraction by heat-reflux, extraction takes place by a series of permeation and solubilization processes to bring the analytes out of the matrix and in the case of MAE the process is based on exposing the analytes to the solvent through cell rupture. Desorption of components from plant matrix occurs in MAE.

- The free water molecules present in the gland and vascular systems of plant matrices are heated and this causes a localized heating and expansion of their walls, thus resulting into the flow of constituents outside the cells.

- The dielectric susceptibility of the solvent and matrix is the factor that affects utilization of microwave energy in the process. In order to prevent the degradation of thermolabile components the sample matrix is immersed in a microwave transparent solvent like hexane.

Microwave systems for extraction and laboratory use are available in two forms:

- Closed extraction vessels/Multi-mode microwave ovens and
- Focused microwave ovens

- The extraction in a closed extraction vessel/ Multi-mode microwave oven is brought about by controlled pressure and temperature.
- Whereas in focused microwave assisted Soxhlet or solvent extraction (FMASE), as the name indicates, only the part of the extraction vessel containing the sample is focused for irradiation with microwave.
- Both the closed vessel type and the focused type are available commercially as multimode and single- mode or focused systems.
- A multimode system allows random dispersion of microwave radiation within the microwave cavity, so every zone in the cavity and sample is irradiated evenly

Principle components of a microwave device:

- The microwave extraction assembly comprises of four major components. A Microwave generator also called as the magnetron is responsible for generation of microwaves; a Wave guide is used to direct the propagation of microwave from the source to the microwave cavity.

- The third component is the applicator, where the sample holder along with the sample is placed. The next component is the Circulator which regulates the movement of microwaves only in the forward direction.

- In case of multi- mode systems the applicator is a closed cavity inside which a random dispersion of microwaves is brought about.

- Beam reflectors or turntables help in bringing about a uniform distribution of microwave energy inside the cavity, irrespective of the position of placement of sample.

- In case of focused microwave systems, the microwave waveguide acts as the applicator and the extraction vessel is placed directly in the cavity.

- Only a few inches of the bottom of the vessel is exposed to the microwaves and as glass is transparent to microwaves the upper region of the vessel remains cool. Thus an effective condensing mechanism is a result of such an inbuilt design of the microwave.

Advantages of closed-vessel systems:

- In a closed vessel system higher temperatures can be reached due to the increased pressure inside the vessel that raises the boiling point of the solvents used.
- There is considerably no loss of volatile substances in a closed system vessel and very less volume of solvent is required.
- There is no need of addition of solvent/s repeatedly and hence risk of air-borne contamination is lowered.

Drawbacks or limitations of closed-vessel systems:

- The disadvantage of a closed vessel system include the risk involved in use of high pressures and the limited amount of sample that can be processed.
- The material like PTFE (polytetraflouroethylene) that is used for vessel construction does not allow use of **high temperature**s and while using volatile compounds the vessel must be opened only after a cooling step to avoid loss of extracted volatile constituents.

Advantages of open-vessel and over pressurized/closed-vessel:

- Open vessels have an increased safety as they can be operated at atmospheric pressure and the reagents can be added at any time during the treatment.
- The material to be used for vessels of the oven may be PTFE, glass or quartz and excess of solvents can be removed easily.
- The major advantage of the instrument is the ability to process large samples without the requirement of a cooling process.
- The equipment can be obtained at a low cost and a complete automation with Open-vessel operation can be done.

Short comings of open-vessel systems:

- In open vessel systems the methods used are usually less precise than the ones used in closed-vessel systems.
- The open vessel system cannot process many samples simultaneously, while closed-vessel systems can handle about 10-14 samples at a time.
- To obtain extraction efficiencies similar to those of closed-vessel systems, the open vessel systems require longer extraction times.