

## Unit II

### Pharmacognosy (DP-103)



**By**

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## Alkaloids

The term ‘**Alkaloid**’ or ‘**Pflanzenkalien**’ was coined by the German pharmacist **Carl F.W. Meissner** in **1819**. Since they are basic or Alkali like, therefore the name coined was “**Alkaloids**”. Alkaloids are naturally occurring organic substances, predominantly found in plant sources including marine algae and rarely in animals (e.g. in the toxic secretions of fire ants, ladybugs and toads). They occur mostly in seed-bearing plants mainly in berries, bark, fruits, roots and leaves.

**Definition:** These are the organic products of natural or synthetic origin which are basic in nature containing one or more nitrogen atoms, normally of heterocyclic nature, and possess specific with specific physiological actions on human or animal body, when used in small quantities.

They are Present in various part of plants like: Aerial parts: ex: Ephedra ephedrine, Entire plant: Vinca-Vincristine, vinblastine, Leaves- Tea- Caffeine, Root- Rauwolfia- Reserpine, Bark- Cinchona- quinine, Seed: Nuxvomica- struchnine, Fruit- Black pepper- piperine, Latex: Opium- Morphine, codeine etc.

**Uses:** Analgesic, Antimalarial, Antispasmodic, hypertension, mental disorders, anticancerous

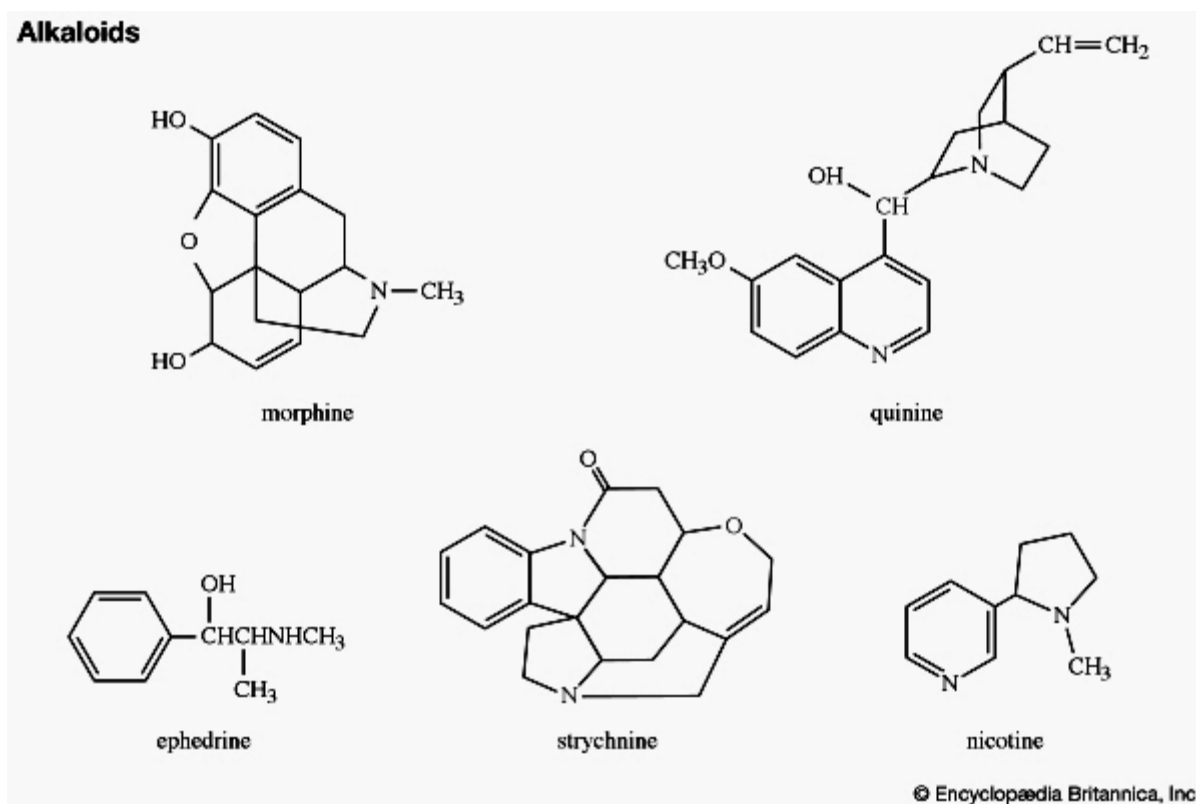
**Distribution:** They are found abundantly in angiosperms i.e. higher plants. Alkaloids are found primarily in plants and are especially common in certain families of flowering plants.

**Families rich in alkaloids :** They are abundantly found in various families like Apocynaceae (Rauwolfia) Rubiaceae (cinchona), Solanaceae (belladonna), Papaveraceae (opium), Berberidaceae (podophyllum)

In fact, as many as one-quarter of higher plants are estimated to contain alkaloids, of which several thousand different types have been identified. In general, a given species contains only a few kinds of alkaloids, though both the opium poppy (*Papaver somniferum*) and the ergot fungus (*Claviceps*) each contain about 30 different types. Certain plant families are particularly rich in alkaloids; all plants of the poppy family (*Papaveraceae*) are thought to contain them, for example. The *Ranunculaceae* (buttercups), *Solanaceae* (nightshades), and *Amaryllidaceae* (amaryllis) are other prominent alkaloid-containing families. A few alkaloids have been found in animal species, such as the New World beaver (*Castor canadensis*) and poison-dart frogs (*Phyllobates*). Ergot and a few other fungi also produce them.

**The function** of alkaloids in plants is not yet understood. It has been suggested that they are simply waste products of plants' metabolic processes, but evidence suggests that they may serve specific biological functions. In some plants, the concentration of alkaloids increases just prior to seed formation and then drops off when the seed is ripe, suggesting that alkaloids may play a role in this process. Alkaloids may also protect some plants from destruction by certain insect species.

The chemical structures of alkaloids are extremely variable. Generally, an alkaloid contains at least one nitrogen atom in an amine-type structure—i.e., one derived from ammonia by replacing hydrogen atoms with hydrogen-carbon groups called hydrocarbons. This or another nitrogen atom can be active as a base in acid-base reactions. The name alkaloid (“alkali-like”) was originally applied to the substances because, like the inorganic alkalis, they react with acids to form salts. Most alkaloids have one or more of their nitrogen atoms as part of a ring of atoms, frequently called a cyclic system. Alkaloid names generally end in the suffix -ine, a reference to their chemical classification as amines. In their pure form most alkaloids are colourless, nonvolatile, crystalline solids. They also tend to have a bitter taste.



Interest in the alkaloids stems from the wide variety of physiological effects (both wanted and unwanted) they produce in humans and other animals. Their use dates back to ancient civilizations, but scientific study of the chemicals had to await the growth of organic chemistry, for not until simple organic bases were understood could the intricate structure of the alkaloids be unraveled. The first alkaloid to be isolated and crystallized was the potent active constituent of the opium poppy, morphine, in about 1804.

Alkaloids are often classified on the basis of their chemical structure. For example, those alkaloids that contain a ring system called indole are known as indole alkaloids. On this basis, the principal classes of alkaloids are the pyrrolidines, pyridines, tropanes, pyrrolizidines, isoquinolines, indoles, quinolines, and the terpenoids and steroids. Alternatively, alkaloids can be classified according to the biological system in which they occur. For example, the opium alkaloids occur in the opium poppy (*Papaver somniferum*). This dual classification system actually produces little confusion because there is a rough correlation between the chemical types of alkaloids and their biological distribution.

The medicinal properties of alkaloids are quite diverse. Morphine is a powerful narcotic used for the relief of pain, though its addictive properties limit its usefulness. Codeine, the methyl ether derivative of morphine found in the opium poppy, is an excellent analgesic that is relatively nonaddictive. Certain alkaloids act as cardiac or respiratory stimulants. Quinidine, which is obtained from plants of the genus *Cinchona*, is used to treat arrhythmias, or irregular rhythms of the heartbeat. Many alkaloids affect respiration, but in a complicated manner such that severe respiratory depression may follow stimulation. The drug lobeline (from *Lobelia inflata*) is safer in this respect and is therefore clinically useful. Ergonovine (from the fungus *Claviceps purpurea*) and ephedrine (from *Ephedra* species) act as blood-vessel constrictors. Ergonovine is used to reduce uterine hemorrhage after childbirth, and ephedrine is used to relieve the discomfort of common colds, sinusitis, hay fever, and bronchial asthma.

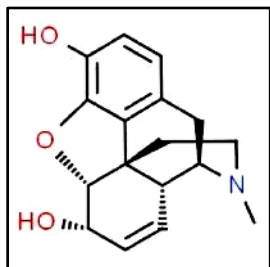
Many alkaloids possess local anesthetic properties, though clinically they are seldom used for this purpose. Cocaine (from *Erythroxylum coca*) is a very potent local anesthetic. Quinine (from *Cinchona* species) is a powerful antimalarial agent that was formerly the drug of choice for treating that disease, though it has been largely replaced by less toxic and more effective synthetic drugs. The alkaloid tubocurarine is the active ingredient in the South American arrow poison, curare (obtained from *Chondrodendron tomentosum*), and is used as a muscle relaxant in surgery. Two alkaloids, vincristine and vinblastine (from *Catharanthus roseus*, formerly *Vinca rosea*), are widely used as chemotherapeutic agents in the treatment of many types of cancer.

Nicotine obtained from the tobacco plant (*Nicotiana tabacum*) is the principal alkaloid and chief addictive ingredient of the tobacco smoked in cigarettes, cigars, and pipes. Some alkaloids are illicit drugs and poisons. These include the hallucinogenic drugs mescaline (from *Lophophora* species) and psilocybin (from *Psilocybe mexicana*). Synthetic derivatives of the alkaloids morphine and lysergic acid (from *Claviceps purpurea*) produce heroin and LSD, respectively. The alkaloid coniine is the active component of the poison hemlock (*Conium maculatum*). Strychnine (from *Strychnos* species) is another powerful poison.

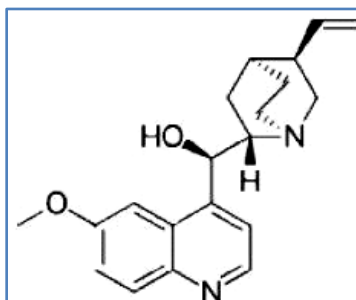
## CLASSIFICATION BASED ON ORIGIN

### I. True alkaloid

They contain one or more nitrogen atom within the ring. They are obtained from various Amino acids directly (Tryptophan, tyrosine, phenyl alanine, Lysine, ornithine, histidine etc.). They are Basic in nature and form water soluble salts. Examples: Quinine, Atropine, Morphine.



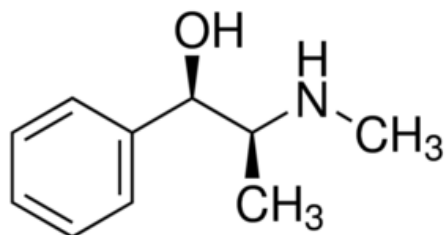
Morphine (Obtained from Tyrosine)



Quinine (Obtained from Tryptophan)

### II. Proto alkaloids /Amino alkaloids

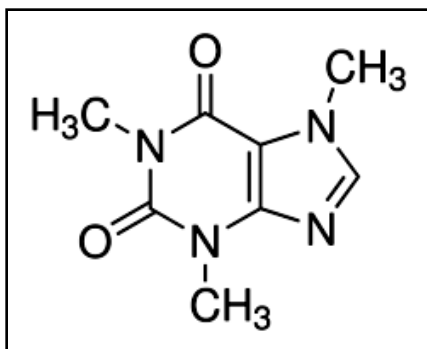
Proto-alkaloids or amino alkaloids are simple amines in which the nitrogen is not in a heterocyclic ring. Sometimes they are considered as biological amines. They are basic in nature and prepared in plants from amino acids. Examples: Colchicine, Ephedrine



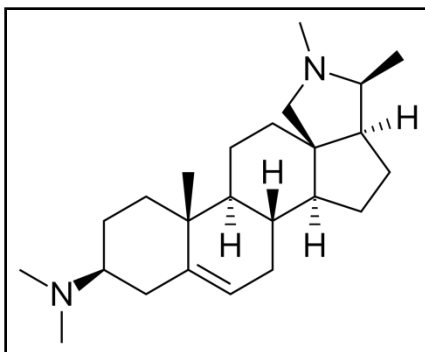
Ephedrine (Obtained from Phenyl alanine)

### III. Pseudo Alkaloid

Pseudo alkaloids includes mainly steroidal and terpenoidal alkaloids and purines. They are not derived from amino acids. They do not show many of the typical characters of the alkaloids, but give standard qualitative tests for alkaloids. Examples: Conessine, Caffeine.



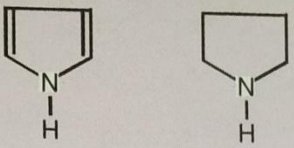
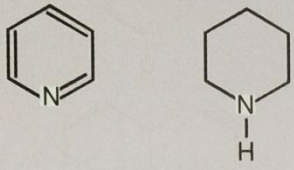
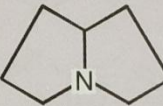
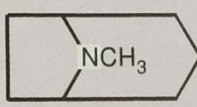
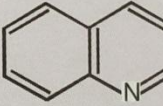
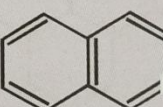
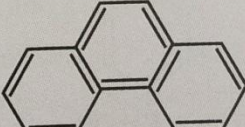
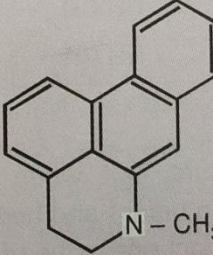
Caffeine



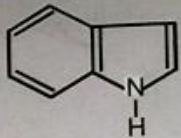
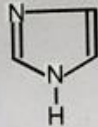
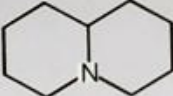
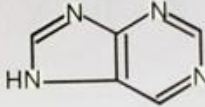

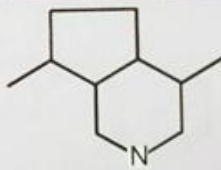
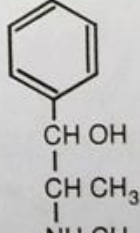
Conessine

## CLASSIFICATION OF ALKALOIDS

- I. Taxonomical classification:** This classification is based on the distribution of alkaloids in various plant families, like solanaceous or papilionaceous alkaloids. Sometimes they are grouped as per the name of grouped genus in which they occur, e.g. ephedra, cinchona, etc.
- II. Biosynthetic classification:** This method gives significance to the precursor from which the alkaloids are biosynthesized in the plant. Hence the variety of alkaloids with different taxonomic distribution and physiological activities can be brought under same group, if they are derived from same precursor, e.g. all indole alkaloids from tryptophan are grouped together. Alkaloids derived from amino acid precursor are grouped in same class such as ornithine, lysine, tyrosine, phenylalanine, tryptophan, etc.
- III. Pharmacological classification:** This classification is based on the physiological action or biological activity of alkaloids on animals like CNS stimulants or depressants, sympathomimetics, analgesics, purgatives, etc. This method does not take account of chemical nature of alkaloids. Within the same chemical structure the alkaloids can exhibit more than one physiological action e.g. morphine is narcotic-analgesic, while quinidine is cardiac depressant.
- IV. Chemical Classification of alkaloids**

Type	Basic ring structure	Examples
1. Pyrrole and Pyrrolidine		Hygrine, coca species
2. Pyridine and Piperidine		Arecoline, anabasine, coniine, lobeline, pelletierine, trigonelline
3. Pyrrolizidine		Echimidine, senecionine, seneciphylline, symphitine
4. Tropane (piperidine. N-methyl pyrrolidine)		Atropine, hyoscine, hyoscyamine, cocaine, pseudo- pelletierine, meteloidine
5. Quinoline		Quinine, quinidine, cinchonine, cinchonidine, cupreine, camptothecin.
6. Isoquinoline		d-tubocurarine, berberine, emetine, cephaeline, papaverine, narcotine, narceine
7. Phenanthrene		Morphine, codeine, hydrastine
8. Aporphine (reduced isoquinoline-naphthalene)		Boldine

Condt...

Type	Basic ring structure	Examples
9. Indole (Benz pyrrole)		Ergometrine, ergotamine, reserpine, vincristine, vinblastine, strychnine, brucine, physostigmine
10. Imidazole		Pilocarpine, Isopilocarpine, Pilocosine
11. Norlupinane		Cytisine, laburnine, lupanine, sparteine
12. Purine (pyrimidine/imidazole)		Caffeine, theobromine, theophylline
13. Steroidal (cyclopentano-perhydrophenanthrene ring)		Protoveratrine, solanidine, conessine, funtumine
<b>Table 15.1: B. Non-heterocyclic alkaloids Pseudo alkaloids</b>		
14. Diterpene		Aconitine, Aconine, hypoaconitine
<b>Proto alkaloids (Amino - alkaloids)</b>		
15. Alkylamine (Amino alkaloid)		Ephedrine, pseudoephedrine. Mescaline, colchicine

## CHEMICAL TESTS FOR ALKALOIDS

The chemical tests used for detection of alkaloids depend on their character to precipitate with organic acids in the form of their salts. These are also precipitated by the reaction of compounds of heavy metals like mercury, gold, platinum etc. Caffeine and some other alkaloids which are highly water soluble, do not give the tests with usual reagents. Some common reagents, used to the detection of alkaloids are summarized in Table 2.

**Table 2.** Reagents used for the detection of alkaloids

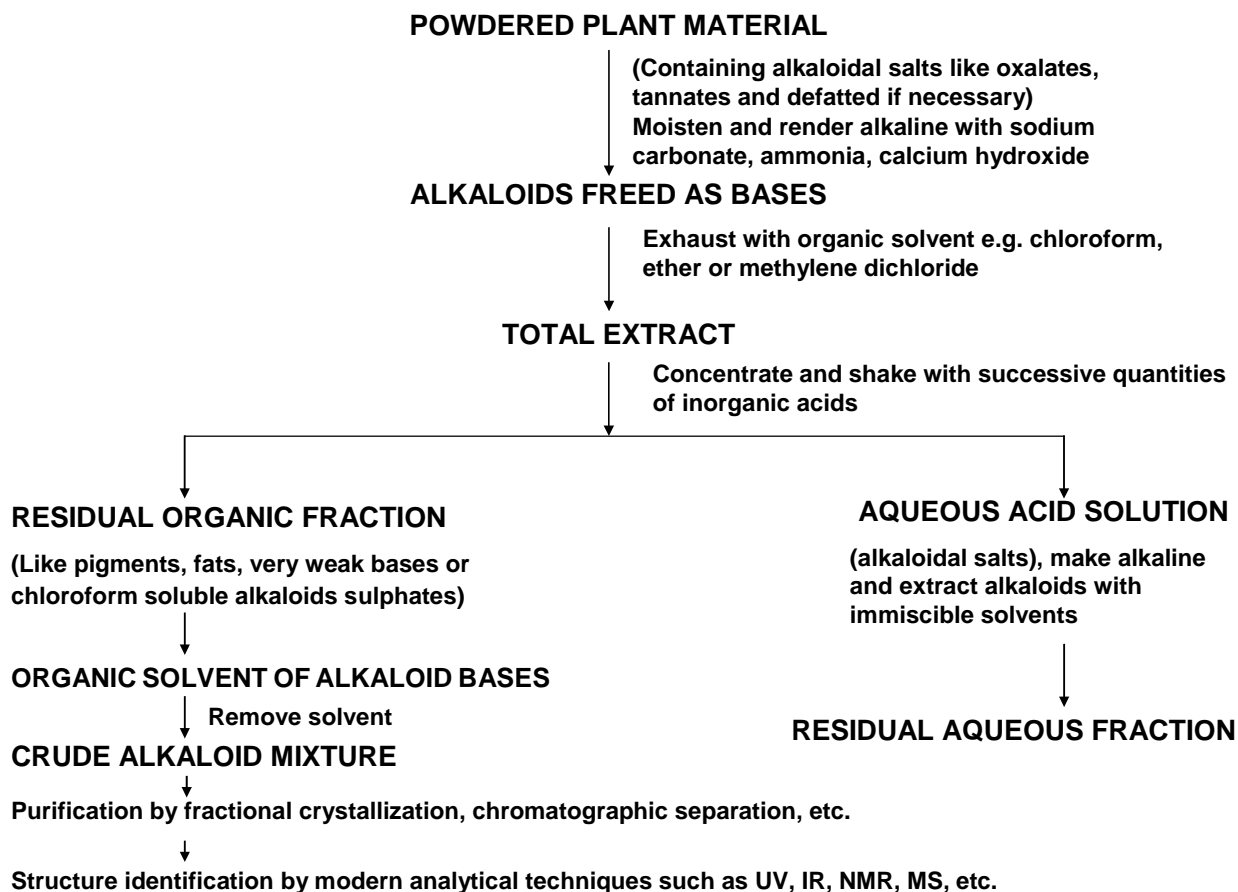


S.No	Name of reagent	Chemical composition	Colour obtained	Example
1	Mayer's reagent	Potassium mercuric iodide solution	Cream	Common
2	Wagner's reagent	Solution of iodine in potassium iodide	Reddish-brown	Common
3	Dragendorff's reagent	Potassium bismuth iodide solution	Reddish-brown	Common
4	Hager's reagent	Saturated solution of picric acid	Yellow	Common
5	Picrolonic acid	Solution of picrolonic acid	Yellow	Common
6	Tannic acid	Solution of tannic acid		Common
7	Murexide test	*Potassium chlorate+HCl+NH <sub>3</sub>	Purple	Caffeine
8	Mineral acids	Phosphotungstic acid, phosphomolybdic acid	Yellow	Colchicine
9	Acidic p-methyl-aminobenzaldehyde	p-Methyl-aminobenzaldehyde and sulphuric acid	Bluish-violet to red	Indole
10	Nitric acid	Dilute nitric acid	Orange-red	Morphine

## EXTRACTION AND ISOLATION

The extraction of alkaloids is based on their basic character and solubility pattern. The general scheme for extraction is shown in Fig. Extraction is usually served by one of the following general methods;

1. The plants are defatted with petroleum ether, especially in case of seeds and leaves to remove the fat soluble constituents and then with polar solvents. The extract is concentrated under reduced pressure and treated with alkali so that the free bases convert in their salts and separated with organic solvents. This process is known as Stas-Otto process. This method is frequently used in the extraction of ergotamine from ergot.
2. The powdered material is moistened with water and mixed with lime, which combines with acids, tannins and other phenolic substances and sets free the alkaloid salts. Extraction is then carried out with organic solvents such as ether or petroleum spirit. The concentrated organic liquid is then shaken with aqueous acid and allowed to separate. Alkaloid salts are now in aqueous liquid, while many impurities remain behind in the organic liquid.
3. The powdered material is extracted with polar solvents such as water or aqueous alcohol containing dilute acid. Pigments and other unwanted materials are removed by shaking with chloroform or other organic solvents. The free alkaloids are then precipitated by the addition of excess sodium bicarbonate or ammonia and then separated by filtration or by extraction with organic solvents.
4. The extract is treated with ammonia so as to convert the alkaloid salts into their free bases. Such liberated alkaloids in free base form are conveniently extracted with organic solvents like ether, benzene, chloroform etc. This method is not useful for the isolation of alkaloids of quaternary nitrogen.
5. The alkaloids present in the extract are converted into their reineckates by treating with  $\text{H}[\text{Cr}(\text{NH}_3)_2(\text{SCN})_4]$  (Reinecke's solution). The product is then dissolves in acetone and then passed this solution through an ion exchange column which afforded the alkaloids in a high state of purity.



**Fig. 6. General scheme for extraction of alkaloids**

Further purification of crude extract of alkaloids is done by following ways, which may, however vary for individual alkaloid.

1. **Direct crystallization from solvent:** It is a simple method of isolation in which the alkaloids crystallise directly by fractionation process and may not be useful in case of complex mixtures.
2. **Steam distillation:** This method is specially employed for volatile liquid alkaloids like coniine, sparteine and nicotine. However this method is not suitable for alkaloids of high molecular weights. In this method, the aqueous extract is made alkaline with caustic soda or sodium carbonate and then alkaloids are distilled off in steam.
3. **Gradient pH technique:** There is variation in the extent of basicity of various alkaloids of same plant. On the basis of this property, the crude alkaloid mixture is dissolved in 2% tartaric acid solution and extracted with benzene so that the first fraction contains neutral

and/or very weakly basic alkaloids. The pH value of the aqueous solution is increased gradually by 0.5 increments to pH 9 and extraction is carried out at each pH with organic solvents. By this way, the alkaloids of different basicity are extracted and strongly basic alkaloids extracted at the end.

**4. Chromatographic techniques:** Chromatography is an ideal method for separation of a vast number of alkaloids. The separation of alkaloids carried out by using stationary and mobile phase of different organic solvents. The different techniques of chromatography used for separation of individual alkaloid from complex mixture are as following;

- i. Paper chromatography (PC):** This technique is simplest and most widely used among other chromatographic techniques because of its applicability to isolation, identification and some times quantitative determination of all type of natural products. It is a partition as well as an absorption type technique, in which the mobile phase is either individual or mixture of organic solvents and the stationary phase is hydrophilic surface of paper. The choice of the solvent used to run a chromatogram depends upon the nature of alkaloid.
- ii. Thin layer chromatography (TLC):** TLC is an important tool extensively used for identification, separation and determination of the purity of isolated alkaloid. Although TLC method is used in qualitative analysis but it having a great importance in quantitative analysis also. The TLC separation of alkaloids can be performed on silica gel, alumina, cellulose powder or kieselguhr. Silica gel is the most active stationary phase and good separation is achieved even when several milligrams substance applied. This method is also used for preparative separation of alkaloids.

## GLYCOSIDES

Glycosides are certain molecules in which a sugar part is bound to some other part. Glycosides play numerous important roles in living organisms.

A glycoside is an organic compound, usually of plant origin, and comprising a **sugar portion** linked to a **non-sugar** moiety in a particular manner. The molecule from which the non-sugar moiety of a glycoside is derived is called the **aglycones or genin**. The linkage between the sugar and the aglycones is a hemiacetal linkage formed by the reducing group (usually aldehydes or keto group) of the sugar and an alcoholic or phenolic hydroxyl group of the aglycone.

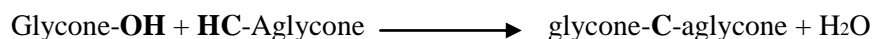


The glycone and aglycone portions can be chemically separated by hydrolysis in the presence of acid. There are also numerous enzymes that can form and break **glycosidic bonds**. The most important cleavage enzymes are the glycoside hydrolases, and the most important synthetic enzymes in nature are glycosyltransferases.

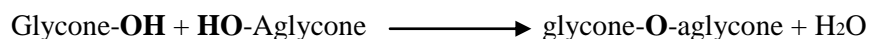
Mutant enzymes termed glycosynthases have been developed that can form glycosidic bonds. There are a great many ways to chemically synthesize glycosidic bonds.

### Classification based on Linkages

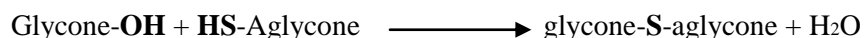
**i. C-glycoside:** Some of the anthraquinone glycoside such as cascarosides from cascara & aloin from aloe show the presence of C-glycoside.



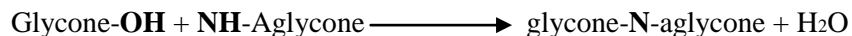
**ii. O-glycoside:** They are common in very higher plants like senna, rhubarb etc.



**iii. S-glycoside:** Their occurrence is restricted to isothiocyanate glycosides like sinigrin. They are formed by interaction of sulfhydryl group of aglycone & hydroxyl group of glycone.



**iv. N-glycoside:** The most typical representative example of N-glycoside is nucleosides, where the amino group react with OH-group of ribose/deoxyribose & ultimately gives N-glucosidic form.



### Classification of glycoside:

**1. By glycone:** If the glycone group of a glycoside is glucose, then the molecule is a glucoside; if it is fructose, then the molecule is a fructoside; if it is glucuronic acid, then the molecule is a glucuronide; etc. In the body, toxic substances are often bonded to glucuronic acid to increase their water solubility; the resulting glucuronides are then excreted.

**2. By type of glycosidic bond:** Depending on whether the glycosidic bond lies "above" or "below" the plane of the cyclic sugar molecule, glycosides are classified as  **$\alpha$ -glycosides** or  **$\beta$ -glycosides**. Some enzymes such as  $\alpha$ -amylase can only hydrolyzed  $\alpha$ -linkages; others, such as emulsin, can only affect  $\beta$ -linkages. Stereochemically these considered as a theoretical aspect, because the plants contain only  $\beta$ - glycosides.

**3. By aglycone:**

- i. Steroidal glycosides or cardiac glycosides:** Here the aglycone part is a steroidal nucleus. These glycosides are found in the plant genera *Digitalis*, *Scilla*, and *Strophanthus*. They are used in the treatment of heart diseases e.g. congestive heart failure and arrhythmia.
- ii. Alcoholic glycosides:** An example of an alcoholic glycoside is salicin which is found in the genus *salix*. Salicin is converted in the body into salicylic acid, which is closely related to aspirin and has analgesic, antipyretic and antiinflammatory effects. Example: **Salicin**
- iii. Anthraquinone glycosides:** These glycosides contain an aglycone group that is a derivative of anthraquinone. They are present in senna, rhubarb and aloes; they have a laxative effect. E.g. Barbaloin.
- iv. Coumarin glycosides:** Here the aglycone is coumarin. An example is apterin which is reported to dilate the coronary arteries as well as block channels those obtained from dried leaves of *Psoralea corylifolia* have Main glycosides psoralin and corylifolin. [Coumarin (benzopyrone)] [Apterin]
- v. Cyanogenic glycosides:** In this case, the aglycone contains a cyanide group and the glycoside can release the poisonous hydrogen cyanide if acted upon by some enzyme. An example of these is amygdalin from almonds.
- vi. Flavonoids glycosides:** Here the aglycone is a flavonoid. This is a large group of flavonoid glycosides. E.g. - Hesperidin (aglycone: Hesperetin, glycone: Rutinose). Here, the aglycone is a flavonoid. Examples of this large group of glycosides include:  
Hesperidin (aglycone: Hesperetin, glycone: Rutinose),

Naringin (aglycone: Naringenin, glycone: Rutinose)

Rutin (aglycone: Quercetin, glycone: Rutinose)

Quercitrin (aglycone: Quercetin, glycone: Rhamnose)

- vii. Saponins:** These compounds give a permanent froth when shaken with water. They also cause hemolysis of red blood cells. Saponin glycosides are found in liquorice (Glycyrrhizin). Their medicinal value is due to their expectorant effect. [Glycyrrhizin]
- viii. Thioglycosides:** As the name implies, these compounds contain sulfur. E.g.- sinigrin, found in black mustard, and sinalbin, found in white mustard. Example: Sinigrin
- ix. Phenolic glycosides:** Here the aglycone is a simple Phenolic structure. E.g. - arbutin found in the Common Bearberry *Arctostaphylos uva-ursi*. It has a urinary antiseptic effect.

### Chemical Tests For Cardiac Glycosides:

S.No	Test	Observation	Inferences
1	<b>Kellar-Killiani Test:</b> The glycosides dissolved in glacial acetic acid containing a traces of FeCl <sub>3</sub> , add the same amount of FeCl <sub>3</sub> dissolved in conc. H <sub>2</sub> SO <sub>4</sub> along the side of the test tube.	A reddish brown colour changing to bluish green colour appear at the junction of two reagents within 2-5 mins spreading slowly into the acetic acid layer.	For α-deoxy sugars (Digitoxose)
2	<b>Legal test:</b> Few mg of the glycosides (Scillaren) Is dissolved in a few drops of pyridine. To this add a drop of sodium nitroprusside solution (2%) and a drop of NaOH (20%)	Appearance of pink or deep red colour	Cardiac Glycosides present
3.	<b>Baljet test:</b> The aglycone portion of the glycosides is mixed with Baljet reagents. [Baljet reagents: Aqu. Solution of picric acid (1%) and NaOH (10%), both are mixed immediately before used]	Appearance of Yellow to orange colour	Cardiac Glycosides present
4	<b>Kedde test:</b> A solution of glycosides is treated with small amount of Kedde reagents [Kedde reagents: Mix equal volume of a 2%	Development of blue or violet colour, which fades out in 1-2 hours.	Cardenolides and Bufadienolides

	solution of 3, 5, dinitrobenzoic acid in methanol and 7.5% aqu. Solution of KOH]		
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## Isolation of glycosides

The dried plant material is rendered into a moderately coarse powder. The powder is then extracted in a Soxhlet apparatus with aqueous ethanol. The non-glycosidal impurities which get extracted along with glycosides are removed by precipitating them with lead acetate solution. The excess of lead acetate is then removed by passing hydrogen sulphide gas through the extract. Lead gets precipitated as lead sulphide, which is filtered out.

The filtrate contains the glycosides. The glycoside can be obtained by removal of the solvent under reduced pressure or any other suitable procedure. Further purification of the isolated glycosides is done by column chromatography.

## Flavonoids

These are mostly yellow pigments in plants. They are phenolic in nature. They are derivatives of 2-phenylbenzopyrones. A large number of physiological activities have been attributed to them. Some flavones may act as cardiac stimulants, some strengthen weak capillary blood vessels, some are good diuretics and of late some have proved extremely good against liver damage.

### *Chemical tests for flavonoid:*

1. Shinoda test to dry powder or extract, add 5 ml of 95% ethanol, few drops of conc. HCl and 0.5 g magnesium turnings. Pink colour observed.
2. To small quantity of residue, add lead acetate solution. Yellow coloured precipitate is formed. Addition of increasing amount of sodium hydroxide to the residue show yellow coloration, which decolouration after addition of acid.

## Classification of flavonoids

### 1. Flavone glycosides:

- (a) Parsley- Apin (b) Buchu- Diosmin.



## **2. Flavonol glycosides:**

(a) Buck wheat- Rutin. (b) Ring (Crataegus oxycantha)- Quercitrin.

## **3. Flavanone glycosides:**

(a) Lemon, sweet orange- Hesperidin (b) Bitter orange

## **4. Chalone glycosides:**

Safflor red- Carthamin

## **5. Isoflavonoid glycoside:**

(a) Sharapunkha- Tephrosin. (b) Gilas- Prunetrin

## **6. Anthocyanidin glycosides:**

(a) Pelargonium flower- Plargonidin (b) Petunia flower- Petunidin

## **General properties of flavones and flavonols:**

These are crystalline compounds, soluble in water, dilute mineral acids, alkalis, alcohol, etc. they are precipitated by means of lead acetate. They give a dull green or a red brown colour with ferric chloride. They are more highly coloured in the acidic medium than in the bases from which they are derived. In acidic medium they form oxonium salts colour.

## **Isolation of flavones and flavonols:**

The plant material containing flavones or flavonols is extracted with boiling water and the tannins are removed as lead salts by means of lead acetate the filtrate is diluted with water, acidified with HCl and boiled for some hours when the sugar free flavones or flavonols are precipitated. They are extracted with alcohol and may be purified by fractional crystallization of their acetates or by re-crystallization from some organic solvents like benzene, carbon disulphide, alcohol, etc.

## **Cardiac Glycosides**

The cardiac glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or

animal. Small amount would exhibit a much needed stimulation on a diseased heart, whereas an excessive dose may cause even death.

Lactones ring is very important for therapeutic activity. Removal of lactone ring or even a slight disturbance to the lactone ring means that the activity of the activity is lost. Cardiac glycosides contain a special sugar called digitoxose; through they do carry other sugar like glucose and rhamnose. Very often diuretic action is also associated with steroidal glycosides as they also promote improved circulation of blood through kidneys.

## **Classification of cardiac glycosides**

### **Cardenolides:**

They are C<sub>23</sub> steroids that have a 17-β side chain and α, unsaturated β-membered lactone ring.

### **Example:**

Digitalis, Quabain, Strophanthin, etc

### **Bufadienolide:**

The bufadienolides are C<sub>24</sub> homologues of the cardenolides and carry a doubly unsaturated 6-membered lactone ring at the 17-position. The bufadienolides derived their name from the genetic name for the toad, Bufo (the prototype compound bufalin was isolated from the skin of toads).

### **Example:**

Squill, etc

### ***Chemical tests for cardiac glycosides:***

#### **1. Raymond's test:**

To the drug, add a few ml of 50% ethanol and 0.1 ml of 1 % solution of m- dinitrobenzene in ethanol. To this solution, add 2-3 drops of 20% sodium hydroxide solution. Violet colors appears, this is due to presence of active methylene group.

## **2. Legal test:**

To the drug, add few ml of pyridine and 2 drops of nitroprusside and a drop of 20% sodium hydroxide solution. A deep red colour is produced.

## **3. Killer killiani test:**

Glycoside is dissolved in a mixture of 1 % ferric sulphate solution in (5%) glacial acetic acid. Add one or two drop of concentrated sulphuric acid. A blue colour develops due to the presence of deoxy sugar.

## **4. Xanthydrol test:**

The crude is heated with 0.1 to 5% solution of Xanthydrol in glacial acetic acid containing 1% hydrochloric acid. A red colour is produced due to the presence of 2-deoxysugar.

## **5. Baljet test:**

Take a piece of lamina or thick section of the leaf and add sodium picrate reagent. If glycoside is present yellow to orange colour will be seen.

## **Cyanogenic Glycosides**

Cyanogenic glycoside is hydrolysis to yielding hydrocyanic acid one of the products found in the plant. Some common cyanogenic glycosides are derivatives of mandelonitrile (benzaldehyde-cyanohydrin). The group is represented by amygdalin, which is found in large quantities in bitter almonds, in kernels of apricots and plum and also by prunasin, which is occurs in *Prunus serotina*.

### ***Chemical tests:***

#### **1. Ferriferrocyanide test:**

Macerate 1 g of the powdered drug with 5 ml of alcoholic KOH for 5 min. transfer it to an aqueous solution containing  $\text{FeSO}_4$  and  $\text{FeCl}_3$ , and maintain at 60-70°C for 10 minutes. Now transfer the contents to HCl (20%) when the appearance of a distinct Prussian blue colour confirms the presence of HCN.

#### **2. Precipitation of Hg from $\text{HgNO}_3$ :**

The reduction of aqueous mercurous nitrite solution to metallic Hg by HCN being observed by an instant formation of black metallic Hg in the cells

### CYANOGENETIC OR CYANOPHORIC GLYCOSIDE CONTAINING DRUGS

S. No.	Name of drug	Name of part	Biological source	Chemical constituents	Uses
1	Wild cherry	Dried bark	<i>Prunus serotina</i> (Rosaceae)	Prunasin, scopoletin	local anesthetic action, bronchitis
2	Bitter almond	dried ripe seeds	<i>Prunus amygdalus</i> (Rosaceae)	Amygdalin, fixed oil proteins	demulcent, mild laxative, stimulant and nervine tonic

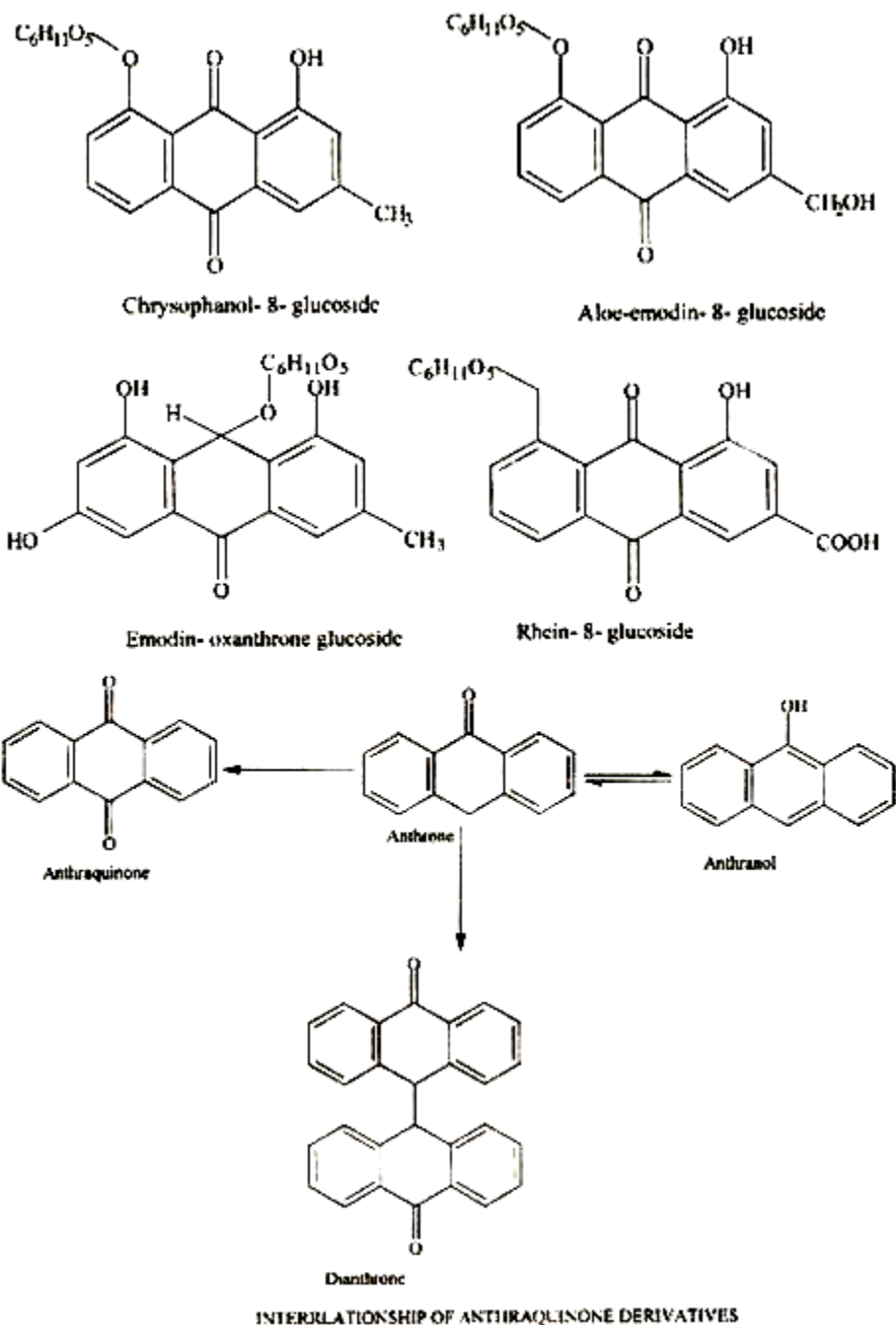
### Anthraquinones Glycosides

The anthraquinone glycosides are the ones whose aglycone component is a polyhydroxyanthraquinone derivative. The drugs having these glycosides possess cathartic activity. The polyhydroxyanthraquinone derivatives present in these drugs are chrysophanic acid (1, 8- dihydroxy- 3- methylanthraquinone), aloe emodin (1, 8- dihydroxy-3- methyl anthraquinone), Frangula emodin and rhein (1, 8- dihydroxy anthraquinone -3-carboxylic acid).

Glycosides of anthranol and anthrones, reduced derivatives of anthraquinones, also occurs in the plant materials, and they make significant contributions to the therapeutic action of these natural products. The free anthraquinone aglycones exhibit little therapeutic activity.

The sugar residue facilitates absorption and translocation of the aglycone to the site of action. The anthraquinone and related glycosides are stimulant cathartics and exert their action by increasing the tone of the smooth muscle in wall of the large intestine.

Glycosides of anthranol and anthrones elicit a more drastic action than the corresponding anthraquinone glycosides and a preponderance of the former constituents in the glycosidic mixture can cause discomforting griping action.



**Chemical Test:**

**1. Borntranger's test:**

Powdered leaves of Senna are boiled with dilute sulphuric acid. Filtered and cooled. The filtrate is extracted with chloroform or benzene and dilute ammonia is added to it. The ammonical layer becomes pink to red due to the presence of anthraquinones derivative.

## **2. Modified Anthraquinones test:**

Take 0.1 gm of drug and add 5ml of 5% solution of ferric chloride and 5ml dilute hydrochloric acid and heat on boiling water-bath for 5 minutes, cool the solution and shake gently with an organic solvent like benzene. Separate the organic solvent layer and add an equal volume of dilute ammonia. A pinkish red colour is formed in ammonical layer. This test is of C. glycoside.

## **3. Isolation of sennosides:**

Senna leaves are powdered to 20-40 mesh and loaded into vertical/ continuous extractors. Acetone at ambient temperature is circulated through the material to remove adherent impurities of pesticides, and other acetone soluble unwanted material of no therapeutic value. It is then made free of acetone and extracted with 70% V/V alcohol (ethyl or methyl) preadjusted to pH 3.9 with citric acid at temperature 45-50°C.

The extraction is continued till washing show a positive test for anthraquinones glycosides (colour reaction or TLC). After extraction, the marc is desolventised and discarded. The extracted liquid is filtered and transferred to a tank fitted with stirrer. The pH is adjusted to 6.0-6.2 with limewater.

It is then concentrated to a paste of 65-70% total solids in a multiple effect evaporator. The paste is dried in rotary vacuum drier at temperature 50-55°C. The flakes obtained are pulverized to a fine powder. It is then sifted to 80 mesh and packed preferably by vacuum sealing

## **4. Isolation of aloin from aloes:**

Aloes is dissolved in about 10 times its weight of boiling water. A small proportion of sulphuric acid is then added to precipitate the resin and after the elapse of sufficient time for complete sedimentation, the liquid is filtered to remove the resin and other insoluble matter.

The clear filtrate is neutralized and concentrated by evaporation under reduced pressure to form a liquid two parts of which represent approximately one part of aloes originally used.

This concentrated liquid is then allowed to cool, 'sown' with a few crystals of aloin and set aside for crystallization to take place, the crystals are filtered off, washed on the filter with a small proportion of dilute alcohol and then dissolved in the minimum amount of hot dilute alcohol (or methyl alcohol) and the solution set aside to crystallize. Aloes yields 10- 15% of aloin by this process.

## **Resins**

### **Definition:**

The term 'resin' is applied to more or less solid, amorphous products of complex chemical nature. These mixtures of essential oils, amorphous oxygenated products of terpenes and carboxylic acids. Resins and related resinous products are produced in plants during normal growth or secreted as a result of injury to the plants. They usually occur in schizogenous or schizolysigenous cavities or ducts.

### **General properties**

#### **Physical characters –**

All resins are heavier than water, they are usually amorphous, hard, and brittle solids. They are insoluble in water, insoluble in petroleum ether and usually but dissolve more or less completely in alcohol, chloroform and ether. Chemically, resins are complex

### **CHEMICAL PROPERTIES:**

- On heating, they soften and finally melt
- Insoluble in water and usually insoluble in petroleum spirit, but dissolve more or less completely in alcohol, chloroform and ether.
- Chemically, resins are complex mixtures of resin acids, resin alcohols (resinol), resin phenols (resinotannols), esters and chemically inert compounds known as resenes.

➤ **CLASSIFICATION:**

- Resin acid: carboxylic acid group containing resinous substances e.g. abietic acid (colophony) & commiphoric acid (myrrh)
- 2. Ester resins – This group contains esters as chief the constituents of the resins- Examples Benzoin and Storax, Benzoin contains benzyl benzoate, Storax contains cinnamyl cinnamate
- Resin alcohols – □ □ □ They occurs as in free state or as esters , examples – Balsam of peru with perru resino tannol Guaiaccum resin with guaic resinol
- Resin phenol (resino tannols): contains phenol group e.g. peru- resinotannol in peru balsam & tolu-resinotannol in tolu balsam & siaresinotannol in benzoin
- Gluco resins: resins when get combined with sugars by glycosylation e.g. Convolvulaceae family drugs.
- Resin: stable, neutral, unaffected by most chemical reagents or by exposure to moisture produced a hard film. e.g. asafetida
- Oleo resin: resin+ volatile oil e.g. turpentine, ginger, copaiba, Canada
- Gum resin: resin+ gum e.g. ammoniacum
- Oleogum resin: resin+volatile oil+gum e.g. myrrh, asafetida, gamboges
- Balsam: contains aromatic acids like benzoin & cinnamic e.g. Tolu balsam, peru balsam, storax

**CHEMICAL COMPOSTION AND ISOLATION:**

- Complex mixture of acids, alcohols, phenols, esters, glycosides or hydrocarbons.
- When associated with volatile oils, contains monoterpenoids, sesequiterpenoid and diterpenoids.
- Gums when associated with resin: acacia gum: oxidase enzymes.

• **ISOLATION:**

- Difficult task due to presence of various combinations.
- Extraction with alcoholic solvents and then the subsequent precipitation by adding concentrated alcoholic extract to a large proportion of water.



- Hydro distillation or distillation can be used for separation of volatile oils from resins.  
(separation of resin from turpentine)

## Uses

### Plant resins

Plant resins are valued for the production of varnishes, adhesives, and food glazing agents. They are also prized as raw materials for the synthesis of other organic compounds and provide constituents of incense and perfume. The oldest known use of plant resin comes from the human babies in late Middle Stone Age in Southern Africa where it was used as an adhesive for hafting stone tools. The hard transparent resins, such as the copals, dammars, mastic, and sandarac, are principally used for varnishes and adhesives, while the softer odoriferous oleo-resins (frankincense, elemi, turpentine, copaiba), and gum resins containing essential oils (ammoniacum, asafoetida, gamboge, myrrh, and scammony) are more used for therapeutic purposes, food and incense. The resin of the Aleppo Pine is used to flavour retsina, a Greek resinated wine.

### Synthetic resins

Many materials are produced via the conversion of synthetic resins to solids. Important examples are bisphenol A diglycidyl ether, which is a resin converted to epoxy glue upon the addition of a hardener. Silicones are often prepared from silicone resins via room temperature vulcanization.

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## **Tannins**

Tannins (or tannoids) are a class of astringent, polyphenolic biomolecules that bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids.

The term tannin (from Anglo-Norman tanner, from Medieval Latin tannāre, from tannum, oak bark) refers to the use of oak and other bark in tanning animal hides into leather. By extension, the term tannin is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with various macromolecules.

The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation (including as pesticides) and might help in regulating plant growth. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit, red wine or tea. Likewise, the destruction or modification of tannins with time plays an important role when determining harvesting times.

Tannins have molecular weights ranging from 500 to over 3,000 (gallic acid esters) and up to 20,000 (proanthocyanidins).

### **Classification of tannins**

1. Hydrolysable tannins
2. Condensed tannins
3. Pseudotannins.

#### ***1. Hydrolysable tannins:***

These tannins are hydrolyzed by acids, or enzyme and produce gallic acid and ellagic acid. Chemically, these are esters of phenolic acid like gallic acid and ellagic acid. The tannins derived from gallic acid are known as gallitannins and from that of ellagic acid are known as gallitannins. The gallic acid is found in rhubarb, clove and ellagic acid is found in eucalyptus

leave and myrobalans and pomegranate bark. These tannins treated with ferric chloride to produced blue or black colour.

### **2. Condensed tannins:**

These tannins are resistant to hydrolysis and they derived from the flavonols, catechins and flavan-3, 4-diols. On treatment with acids or enzymes they are decomposed into phlobaphenes. On dry distillation condensed tannin produce catechol. These tannins are called as catechol tannins. These tannins are found in cinchona bark, male fern, areca seeds, tea leaves and wild cherry bark, bahera fruits, Amla, etc. they produce green colour with ferric chlorides.

### **3. Pseudotannins:**

They are phenolic compounds of lower molecular weight and do not show the goldbeater's test. They are found in catechu and nux- vomica, etc.

(i) Catechins: Catechu, cocoa, etc.

(ii) Ipecacuanhic acid: Ipecacuanha

(iii) Chlorogenic acid: Coffee and nux-vomica

### **Properties of tannins:**

Tannins are freely soluble in water, alcohol, glycerol, and acetone and dilute alkalies. They are sparingly soluble in chloroform, ethyl acetate and other organic solvents. They have an astringent taste. They yield purple, violet or black precipitate with iron compounds. They are precipitated by number of metallic salts notably potassium dichromate, and lead acetate and sub acetate. They combine with skin and hide to form leather and with gelatin and isinglass to form an insoluble compound. They combine with alkaloids to form tannates, most of which are insoluble in water.

### **Chemical tests:**

#### **1. Gelatin test:**

To a solution of tannin, aqueous solution of gelatin and sodium chloride are added. A white buff coloured precipitate is formed.

### **2. Goldbeater's skin test:**

A small piece of goldbeater skin (membrane prepared from the intestine of an ox) is soaked in 20% hydrochloric acid, rinsed with distilled water and placed in a solution of tannin for 5 minutes. The skin piece is washed with distilled water and kept in a solution of ferrous sulphate. A brown or black colour is produced on the skin due presence of tannins.

### **3. Phenazone test:**

A mixture of aqueous extract of a drug and sodium acid phosphate is heated and cooled and filtered. A solution of phenazone is added to the filtrate. A bulky coloured precipitate is formed.

### **4. Match stick test (Catechin test):**

A match stick is dipped in aqueous plant extract, dried near burner and moistened with concentrated hydrochloric acid. On warming near flame, the matchstick wood turns pink or red due to formation of phloroglucinol.

### **5. Chlorogenic acid test:**

An extract of chlorogenic acid containing drug is treated with aqueous ammonia. A green colour is formed on exposure to air.

### **6. Vanillin-hydrochloric acid test:**

Sample solution and added vanillin- hydrochloric acid reagent (Vanillin 1 gm, alcohol 10 ml, concentrated hydrochloric acid 10 ml). A pink or red colour is formed due to formation of phloroglucinol.

### **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 while 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

### **Medicinal properties and uses**

- i.** Tannins occur in crude drugs either as major active constituent as in oak bark, or as a subsidiary component as in clove. In many cases, they synergistically increase the effectiveness of active principles.
- ii.** Tannins are medicinally significant due to their astringent properties. They promote rapid healing and the formation of new tissues on wounds and inflamed mucosa. They are used in the treatment of varicose ulcers, hemorrhoids, minor burns, frostbite as well as inflammation of gums.
- iii.** Internally tannins are administered in cases of diarrhoea, intestinal catarrh and in cases of heavy metal poisoning as an antidote.
- iv.** Recently, these compounds have demonstrated their antiviral activities for treatment of viral diseases including AIDS.
- v.** They are used as mordant in dyeing, manufacture of ink, sizing paper and silk, and for printing fabrics.
- vi.** They are used along with gelatin and albumin for manufacture of imitation horn and tortoise shell.
- vii.** They are widely used in the leather industry for conversion of hide into leather, the process being known as tanning.
- viii.** They are also used for clarifying beer or wine, in photography or as a coagulant in rubber manufacture.
- ix.** They are used for the manufacture of gallic acid and pyrogallol, and sometimes as a reagent in analytical chemistry.

## **Volatile oil (Essential oil)**

An essential oil is a concentrated hydrophobic liquid containing volatile (easily evaporated at normal temperatures) chemical compounds from plants. Essential oils are also known as volatile oils, ethereal oils, aetherolea, or simply as the oil of the plant from which they were extracted, such as oil of clove. An essential oil is "essential" in the sense that it contains the "essence of" the plant's fragrance—the characteristic fragrance of the plant from which it is derived. The term "essential" used here does not mean indispensable or usable by the human body, as with the terms essential amino acid or essential fatty acid, which are so called because they are nutritionally required by a given living organism.

### **Isolation**

Essential oils are generally extracted by distillation, often by using steam. Other processes include expression, solvent extraction, absolute oil extraction, resin tapping, wax embedding, and cold pressing. They are used in perfumes, cosmetics, soaps and other products, for flavoring food and drink, and for adding scents to incense and household cleaning products. Essential Oils should not be confused with Perfume, Fragrance, etc. as the latter usually include pure chemical components whereas essential oils are derived from plants.

### **Distillation**

Most common essential oils such as lavender, peppermint, tea tree oil, patchouli, and eucalyptus are distilled. Raw plant material, consisting of the flowers, leaves, wood, bark, roots, seeds, or peel, is put into an alembic (distillation apparatus) over water. As the water is heated, the steam passes through the plant material, vaporizing the volatile compounds. The vapors flow through a coil, where they condense back to liquid, which is then collected in the receiving vessel. Most oils are distilled in a single process. The recondensed water is referred to as a hydrosol, hydrolat, herbal distillate, or plant water essence, which may be sold as another fragrant product. Hydrosols include rose water, lavender water, lemon balm, clary sage, and orange blossom water. The use of herbal distillates in cosmetics is increasing.

## **Expression**

Most citrus peel oils are expressed mechanically or cold-pressed (similar to olive oil extraction). Due to the relatively large quantities of oil in citrus peel and low cost to grow and harvest the raw materials, citrus-fruit oils are cheaper than most other essential oils. Lemon or sweet orange oils are obtained as byproducts of the citrus industry. Before the discovery of distillation, all essential oils were extracted by pressing.

## **Solvent extraction**

Most flowers contain too little volatile oil to undergo expression, but their chemical components are too delicate and easily denatured by the high heat used in steam distillation. Instead, a solvent such as hexane or supercritical carbon dioxide is used to extract the oils. Extracts from hexane and other hydrophobic solvents are called concretes, which are a mixture of essential oil, waxes, resins, and other lipophilic (oil-soluble) plant material.

## **Uses**

Essential oils are often used for aromatherapy, a form of alternative medicine in which healing effects is ascribed to aromatic compounds. Aromatherapy may be useful to induce relaxation, but there is not sufficient evidence that essential oils can effectively treat any condition. Improper use of essential oils may cause harm including allergic reactions and skin irritation, and children may be particularly susceptible to the toxic effects of improper use.

## **Terpenes**

Terpene, any of a class of hydrocarbons occurring widely in plants and animals and empirically regarded as built up from isoprene, a hydrocarbon consisting of five carbon atoms attached to eight hydrogen atoms ( $C_5H_8$ ). The term is often extended to the terpenoids, which are oxygenated derivatives of these hydrocarbons.

The true terpenes are usually grouped according to the number of isoprene ( $C_5H_8$ ) units in the molecule: monoterpenes ( $C_{10}H_{16}$ ) contain two such units; sesquiterpenes ( $C_{15}H_{24}$ ), three; diterpenes ( $C_{20}H_{32}$ ), four; triterpenes ( $C_{30}H_{48}$ ), six; and tetraterpenes ( $C_{40}H_{64}$ ), eight.