PHARMACOGNOSY

Introduction of Plant Constituents and their Tests

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Plant Constituents

Plant constituents, as the word implies are the individual chemicals from which plants are made. These constituents are organic in nature and synthesized in plants by the activity of individual cells. The process by which these complex organic chemical constituents are formed, utilizing simple substances and enzymes are known as biosynthesis.

As historically, plants and their products form the basis of medicines and also in present days. Several compounds, which are pharmaceutically and medicinally important, derived from plant sources. However, the medicinal value of plant depends on the nature of plant constituents present in it, which is known as active principal or active constituent. Active constituents are those chemical substances, which are solely responsible for therapeutic activity of plant. A large number of theories have been proposed as to why these compounds are formed in plants, it is likely that many of them are produced as part of chemical defense system to protect the producing organism.

The chemical constituents present in plants that do not possess any definite therapeutic value are known as inactive constituents. As the formation of different active and inactive constituents of plants involves various metabolic pathways, hence in general the inactive plant constituents are termed as primary plant metabolites whereas active plant constituents are termed as secondary plant metabolites.

Primary plant metabolites are simple molecules or polymers of simple molecules synthesized by plants, generally do not possess therapeutic as such but essential for the life of plants and contains high-energy bonds. These are usedup for the biosynthesis of secondary metabolites. e.g. Carbohydrates, proteins, lipids and nucleic acids.

Secondary metabolites are complex organic molecules biosynthesized from primary plant metabolites in plant cells (Fig.1). Unique to plants or group of plants, generally possess therapeutic activity, neither essential for plants life nor contains high energy bonds. These are usually stored in vacules. Secondary metabolites are classified as: alkaloids, glycosides, tannins, phenolic compounds, volatile oils, terpenoids, saponins, steroids, resins and bitter principles. These are used as medicine, food, flavors, colours, dyes, poisons and perfumes etc. It is estimated that $1/4^{th}$ of prescription drugs contains at least one chemical originally identified from plants.

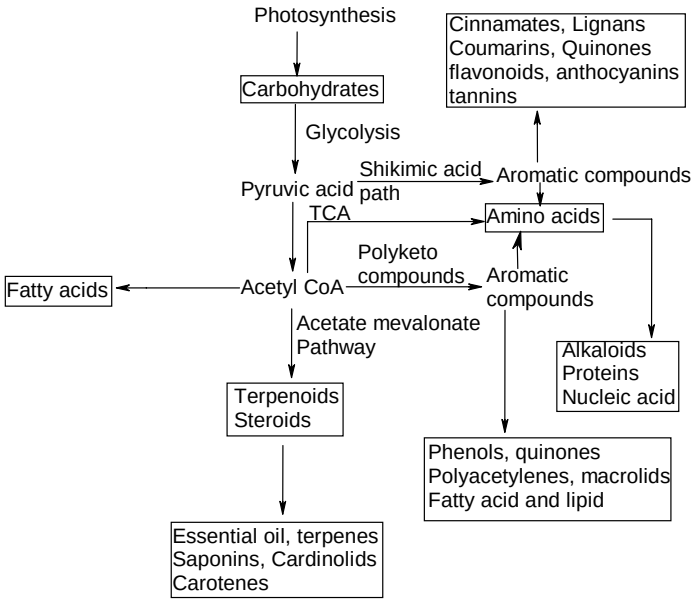


Fig.1. Relationship between primary and secondary metabolites

Carbohydrates

Carbohydrates are organic polyhydroxy carbonyl compounds with universal occurrence in living organisms. These are made up of C, H and O and are the first complex organic compounds formed in the plants as a result of photosynthesis. These provide means of storage and transport of energy and also building blocks of cell wall. These are the constituents of various metabolites as glycosides and required precursor for biosynthesis of all other metabolites as well as basis of all organic compounds of living world.

These are classified in three main categories on the basis of their molecular size:

1. Monosacchrides (True sugars)
2. Oligosacchrides (True sugars)
3. Polysacchrides (Non sugars)

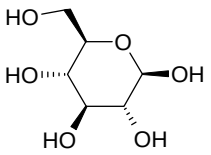
1. Monosacchrides: Monosacchrides are characterized by general formula $Cn(H_2O)n$, presence of carbonyl group and (n-1) hydroxyl group. The number of carbon atoms most often five or six. These are simplest sugar molecules and hence cannot be hydrolyzed into simpler form. These are crystalline substances, soluble in water, practically insoluble in organic solvents like ether, chloroform and absolute alcohols. These are optically active and exist in more than one isomeric form. Further, these are sub-classified on the basis of no. of carbon atoms present.

a. Trioses – 3 carbon atom e.g. glyceraldehydes
b. Tetroses – 4 carbon atom e.g. threose, erythrose
c. Pentoses – 5 carbon atom e.g. xylose, arabinose, rhamnose, ribose and ribulose.
d. Hexoses – 6 carbon atom e.g. glucose, fructose, mannoseand galactose.
e. Heptoses – 7 carbon atom e.g. cymarose

These are also classified on the basis of presence of carbonyl group:

1. Aldoses- (containing aldehydic group) e.g. glucose, arabinose and galactose
2. Ketoses- (containing ketone group) e.g. fructose.

Carbohydrates are found in plants in more than one isomeric form i.e. D or L, but most of the natural monosachharides belongs to D series (except L-rhamnose, L-arabinose and L-fucose). Monosachharides can exist in both cyclic structures i.e. either pyrano (six membered ring) or furano (five membered ring) depending on nature of bridge (1-4 or1-5). Although, aldohexoses generally forms pyranose whereas ketohexoses forms furanose ring, e.g. glucose occurs in pyranose configuration (Fig.2a) while fructose occurs in furanose configuration (Fig.2b).



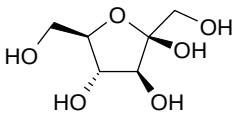


Fig.2a. β-D Glucose Fig.2b. β-D Fructose

The carbon atoms in ring are of sp^3 hybridization and may not be in the planner form; hence it adopts various conformations like chair, boat, half chair etc. Preffered conformation for aldohexo-pyranose is always chair conformation (most stable) (Fig.3), which has minimal interaction and lowest energy.

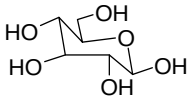


Fig.3. Aldohexo-pyranose in most stable Chair confirmation

2. Oligosaccharides: These are formed by condensation of 2-10 monosacchride molecules, which involves *in vivo* formation of glycosidic linkage. The glycosidic linkage can easily be cleaved by chemical hydrolysis and specifically by enzymatic hydrolysis. On the basis of type of glycosidic linkage present the disaccharides are of two types:

1. Non reducing disaccharides (linkage involving reducing function of both sugar) e.g. sucrose
2. Reducing disaccharides (linkage involving reducing function of only one sugar) e.g. Lactose, maltose, cellobiose etc.

Sucrose is commonly obtained from sugar cane and hence known as cane sugar. It yields glucose and fructose on hydrolysis. **Lactose** is obtained from milk and known as milk sugar, it yields glucose and galactose on hydrolysis. **Maltose** is obtained by hydrolysis of starch during germination of grains, it yields two molecules of glucose on hydrolysis. **Lactulose** is a synthetic disaccharide, acts as osmotic laxative, yields galactose and fructose on hydrolysis.

Cyclodextrins are cyclic oligosaccharides produced by enzymatic degradation of starch using different Bacillus species. The α, β and χ cyclodextrins are made-up of 6, 7 and 8 glucose units,

respectively. These have ability to form non-covalent inclusion compounds which permits molecular encapsulation to increase: stability and solubility to improve bioavailability of drug molecules.

3. Polysacchrides (Glycans): These are high molecular weight polymers of larger number of monosaccharide molecules. The sugar molecules are attached to each other by glycosidic linkage between the hemi-acetal hydroxyl group on C-1 of one sugar and any of the hydroxyl group on other sugar molecules. These serve as skeletal material (cellulose) or reserve food material (starch, glycogen and inulin). These are non-sugar carbohydrates; do not have sweet taste, either insoluble in water (cellulose) or forms colloidal solution (starch). Glycans are further sub-divided in two categories:

i. Homoglycans (homogenous polysaccharides): formed by the condensation of larger number of same sugar molecules, e.g. starch, cellulose, glycogen etc.
ii. Heteroglycans (heterogeneous polysaccharides) e.g. gums, mucilages and pectins.

Starch is main reserve energy source, universal constituent of plant and derived from glucose. It composed of two components:

a. Amylose (20-30%) is a water soluble, linear chain molecule, composed of 250- 300 α-D – glucose having 1-4 linkage. It forms blue color with iodine.

b. Amylopectin (70-90%), it is insoluble in cold-water, branched chain molecule, composed of about 1000 α-D –glucose with 1-4 and 1-6 linkage. It forms red to violet colour with iodine. Pharmaceutical products like dextrins and cyclo-dextrins are derivatives of starch.

Cellulose is the most universal biological polymer. It is fibrous substance of cell wall and responsible for structural rigidity of plants in combination with lignin. Cellulose is a linear polymer made up of β (1-4) linked D-glucose units, its molecular weight ranges from 50,000 – 25,00,000. Cotton fibers are the purest form of cellulose (98%), whereas oxidized cellulose and methylcellulose are cellulose derivatives used in pharmacy.

Gums are complex heterogeneous, branched and uronic acid containing polysaccharide macromolecules. These are translucent, amorphous substances, exudates of plants and produced as the result of trauma. Gums are insoluble in organic solvents but most of them are soluble in water and forms colloidal viscous solutions. These are optically active and dilute solutions (<1%) precipitates upon addition of ethanol or lead sub acetate. Gums on hydrolysis yields sugars (arabinose, galactose, glucose, mannose and xylose) with sugar acids (glucoronic acid and galacto-uronic acid).

Mucilages are normal cell constituents of high molecular weight compounds, composed mainly of sulphuric acid esters of sugar. These neither dissolves in water to form clear colloidal solutions but swells nor precipitates by addition of alcohol. Mucilages on hydrolysis yields sugar (galactose and arabinose) and sugar acids (uronic acids). Seaweed agar and carrageenan contains mucilage composed of salts of sulphate esters of polysaccharides.

Pectins are defined as the group of polymers made-up of partially methylated 1-4 linked α galacturonic acid residues associated with arabinan and galactan units. These are localized in middle lamella of vegetable cell wall but for industrial purpose it is obtained from inner portion

of rind of citrus fruit or from apple. It absorbs water and swells up because of its hydrophilic property forming stiff jelly. Hence, it is used in pharmaceutical and food technology.

Chemical Test for Carbohydrates: Mono-saccharides are the building blocks of carbohydrates. Di, oligo and polysaccharides on hydrolysis in presence of mineral acid yield monosaccharide units. Monosaccharides are soluble in water and practically insoluble in organic solvents like chloroform, ether and in absolute alcohols. These are optically active compounds and respond to various color reactions and identification tests.

i. Charring test
ii. Molish test
iii. Iodine test
iv. Barford test
v. Seliwanoff''s test
vi. Fehling solution test
vii. Benedict test
viii. Tollens test
ix. Bials test
x. Osazone test

1. Charring test: Carbohydrates on heating in testube or in presence of Conc. H_2SO_4, produces charring with smell like burning sugar.

2. Molish test: Aqueous solution of drug/carbohydrate mixed with few drops of Molish reagent (alpha naphthol) and Conc. H_2SO_4 was added from sidewall of testube. Formation of purple coloured ring at junction indicates presence of carbohydrates.

3. Iodine test: It is specific for polysacchrides. Few drops of Iodine solution was added to aqueous solution of drug/polysaccharide. Formation of blue colour, which disappears on heating and reappears on cooling, indicates the presence of starch.

4. Barford test: This test is used to distinguish between monosacchride and disacchrides. Two ml of Barford reagent (Cupric acetate, acetic acid and water) was added to 1 ml aqueous solution of drug and boil. Formation of brick red precipitate in 5 minutes indicates presence of monosacchride while in 7 minutes indicates disaccharide.

5. Seliwanoff's test: This test is used for identification of keto-hexoses or to distinguish between ketoses and aldoses. To 1 ml aqueous solution of drug, 5 ml of Seliwanoff's reagent (resorcinol in 6M HCl) was added and boiled. Formation of cherry red colour in presence of ketose (Fructose) due to formation of hydroxyl methyl furfural, which condensed with resorcinol to produce cherry red colour.

6. Fehling solution test: It is generally used for reducing sugars and composed of two solutions, which are mixed in situ. Fehling solution A composed of 0.5% of copper sulphate whereas Fehling solution B composed of Sodium Potassium Tartarate.

Equal volumes of Fehling A and Fehling B solutions were mixed (1 ml each) and 2 ml of aqueous solution of drug was added followed by boiling for 5-10 minutes on water bath.

Formation of reddish brown coloured precipitate due to formation of Cuprous oxide indicates presence of reducing sugar.

Di-, oligo and poly-sacchrides having reducing sugars can be tested by first boiling in dilute acid solution followed by neutralization with ammonia. This neutralized aqueous is used for testing.

7. Benedict's test: It is used for reducing sugars and composed of mainly Copper sulphate and sodium hydroxide. To the 4 ml of aqueous solution of drug, 1 ml of Benedicts solution was added and heated almost to boiling. Formation of green, yellow, orange, red or brown colour in order of increasing concentration of simple sugar in the test solution, due to formation of cuprous oxide.

8. Tollens test: Tollens reagent (Silver Nitrate, NaOH and Ammonia) is Ammonical Silver Nitrate (diaminesilver (I) complex), an oxidizing agent, which is itself reduced to silver metal in a clean glass reaction vessel and forms a "silver mirror", when raects with aldehydes to form carboxylic acids. Add few drops of freshly prepared Tollens reagent to 2 ml of aqueous solution of drug in clean testube and heat gently. Formation of black mirror on the sidewall of testube indicates the presence of aldehydic group.

9. Bials test: It is used to distinguish between pentoses and hexoses. Pentoses reacts with Bial's reagent (Orcinol in Conc. HCl and traces of $FeCl_3$ as catalyst) to form furfural, which condenses with orcinol to produce blue-green product. Aqueous solution of drug (2 ml) was mixed with 4 ml of Bial's reagent and heat to boiling, it produces blue-green colour in presence of pentose sugar.

10. Osazone test: The osazone test was developed by Emil Fischer to identify aldose sugars differing in configuration only at the alpha-carbon. These sugars react with 2, 4-dinitro-phenyl hydrazine effecting only alpha-carbon with formation of pink-red coloured bis-phenylhydrazone, known as an osazone. Application of the osazone reaction to D-glucose and D-mannose demonstrates that these compounds differ in configuration only at C-2.

Chemical test for Starch: It is soluble in hot water, gives positive test for Molish reagent and some specific tests like Jelly test and Lugol's iodine test.

Jelly test: To 0.5 gm of starch in a testube add 5 ml of distilled water and boil on water bath. Formation of translucent jelly indicates presence of starch.

Lugol's iodine test: It is also known as iodine – KI reagent and composed of aqueous Iodine solution in presence of KI. Few drops of iodine – KI reagent was added to the aqueous solution of starch, which produces deep blue to bluish black colour due to presence of amylase. The colour developed disappears on warming and reappears on cooling. Starch amylopectin, disacchrides and cellulose do not produce any colour.

Lipids

These are a large and diverse group of naturally occurring organic compounds, esters of fatty acids and alcohols and polyols. These are soluble in nonpolar organic solvents (e.g. ether, chloroform, acetone & benzene) and generally insoluble in water.

These can be classified as:

1. Simple lipids (esters of fatty acids with alcohols) e.g. triglycerides like fats and oils, waxes.
2. Compound lipids e.g. phospholipids and glycolipids.

They show great structural variety and can be studied in following sections:

a. Fatty Acids
b. Fats and Oils
c. Waxes
d. Soaps and Detergents
e. Phospholipids

a. Fatty Acids: Lipids on hydrolysis by acids or bases yield the component fatty acid. These long-chain carboxylic acids are generally referred by their common names, which in most cases reflect their sources. Natural fatty acids may be saturated or unsaturated, and the saturated acids have higher melting points than unsaturated acids of corresponding size.

Saturated

Lauric acid:	$CH_3(CH_2)_{10}CO_2H$
Myristic acid:	$CH_3(CH_2)_{12}CO_2H$
Palmitic acid:	$CH_3(CH_2)_{14}CO_2H$
Stearic acid:	$CH_3(CH_2)_{16}CO_2H$
Arachidic acid:	$CH_3(CH_2)_{18}CO_2H$

Unsaturated

Arachidonic acid:	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2CO_2H$
Palmitoleic acid:	$CH_3(CH_2)_5CH{=}CH(CH_2)_7CO_2H$
Oleic acid:	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2H$-
Linoleic acid:	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7CO_2H$
Linolenic acid:	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7CO_2H$

The higher melting points of the saturated fatty acids are due to the uniform rod-like shape of their molecules. The presence of cis-double bond in the unsaturated fatty acids introduces a twist in their shape, which makes it more difficult to pack their molecules together in a stable repeating array or crystalline lattice. The trans-double bond isomer of oleic acid, known as elaidic acid, has a linear shape and a melting point higher than its cis isomer.

Two polyunsaturated fatty acids, linoleic and linolenic, are designated "essential" because their absence in the human diet has been associated with health problems, like scaly skin, stunted growth and increased dehydration. These acids are also precursors to the prostaglandins, a family of physiologically potent lipids present in minute amounts in most body tissues.

b. Fats and Oils: Theses are phospholipids of fatty acids with glycerol commonly known as triglycerides, found in both plants and animals, and compose one of the major food groups of our diet. Triglycerides that are solid or semisolid at room temperature are classified as fats, and found predominantly in animals. The liquid triglycerides are called as oils and originate mainly from plants; triglycerides obtained from fish are also oils.

Fats composed of mainly saturated fatty acids while oils are composed of unsaturated fatty acids. Saturated and trans-fatty acid glycerides in the diet have been associated with atherosclerosis. Triglycerides having three identical acyl chains, like tristearin and triolein are called as "simple", while those composed of different acyl chains are called "mixed".

c. Waxes: Waxes are esters of fatty acids with long chain monohydric alcohols and may also contain hydrocarbons. These are widely distributed in nature like leaves and fruits of many plants have waxy coatings, which protect them from dehydration. E.g. of some common waxes are: Spermaceti wax- $CH_3(CH_2)_{14}COO\text{-}(CH_2)_{15}CH_3$, Beeswax-$CH_3(CH_2)_{24}COO\text{-}(CH_2)_{29}CH_3$ and Carnuaba wax- $CH_3(CH_2)_{30}CO_2\text{-}(CH_2)_{33}CH_3$.

d. Soaps and Detergents: Carboxylic acids and salts having alkyl chains longer than eight carbons exhibit unusual behavior in water due to the presence of both hydrophilic (COO^-) and hydrophobic (alkyl) regions in the same molecule. Such molecules are known as amphiphilic or amphipathic. Fatty acids made up of ten or more carbon atoms are nearly insoluble in water, and float on the surface when mixed with water because of their lower density. These fatty acids spread evenly over water surface and form a monomolecular layer in which the polar carboxyl groups are hydrogen bonded at the water interface, and the hydrocarbon chains are aligned together away from water surface. These substances accumulate at water surface and change the surface properties called as surfactants.

Alkali metal salts of fatty acids are more soluble in water than the acids themselves, and the amphiphilic character of these substances also make them strong surfactants. The most common examples of such compounds are soaps and detergents, each of these molecules has a nonpolar hydrocarbon chain, the "tail", and a polar (ionic) "head group". The use of such compounds as cleaning agents is facilitated by their surfactant character, which lowers the surface tension of water, allowing it to penetrate and wet a variety of materials.

e. Phospholipids: These are main constituents of cell membranes; resemble the triglycerides in being ester or amide derivatives of glycerol or sphingosine with fatty acids and phosphoric acid. The phosphate moiety of the resulting phosphatidic acid is further esterified with ethanolamine, choline or serine in the phospholipids itself. The fatty acid components may be saturated or unsaturated.

Liposomes are microscopic vesicles consisting of an aqueous core enclosed in one or more phospholipids layers. They are formed when phospholipids are vigorously mixed with water.

The bilayer membrane that separates the interior of a cell from the surrounding fluids is largely composed of phospholipids, but it incorporates many other components, such as cholesterol, that contribute to its structural integrity. Protein channels that permit the transport of various kinds of chemical species in and out of the cell are also important components of cell membranes.

The sphingomyelins are also membrane lipids. They are the major component of the myelin sheath surrounding nerve fibers. Multiple Sclerosis is a devastating disease in which the myelin sheath is lost, causing paralysis

Several vegetable oils have been included in different pharmacopoeias like: almond oil, castor oil, olive oil, sesame oil, peanut oil, sunflower oil and γ-linolenic acid containing evening primrose oil and borage oil.

There are a number of quality control parameters for the assessment of quality of lipid drugs mentioned in various pharmacopoeias like: refractive index, specific gravity, iodine value, saponification value, determination of unsaponifiable matter, acid value, peroxide value, anisidine value, GLC profile, fatty acid composition as well as TLC and HPTLC fingerprints.

Chemical tests for lipids:

1. Solubility in Polar and Nonpolar Solvents: Lipids are insoluble in polar solvents like water and soluble in nonpolar solvents like petroleum ether, benzene and mineral oil.

2. Sudan IV test: Lipids stain red when Sudan IV (a common stain) is added. Sudan IV is a lipid soluble dye. When Sudan IV is added to a mixture of lipids and water, the dye will move into the lipid layer and makes it red:

3. Grease Spot Test: A simplest test for lipid is based on the ability of lipids to produce a translucent spot on paper

4. Emulsification test: If emulsifiers like bile salts, tween or soap solution is mixed with lipids and water; the lipids broken down into smaller fragments, which remained suspended for long periods of time in water.

Proteins and Amino Acids

Proteins are class of organic compounds, which are present in and vital to every living cell. It is present in the form of skin, hair, callus, cartilage, muscles, tendons and ligaments; it protects and provides structure to the body of a multicellular organism. The enzymes, hormones and antibodies are made up of proteins, which catalyze and regulate the physiological activities of plant and human being. Hemoglobins and various lipoproteins transport oxygen and other substances within an organism. Protein deficiency may cause deficiency disease like kwasharkor. These are also toxic in nature like botulinum toxin, venom toxins, ricin and toxins produced by

microorganisms like tetanus and diphtheria. Proteins and peptides are composed of 15 to 25% nitrogen and about an equal amount of oxygen. Proteins and peptides can be distinguished by their size; peptides are small proteins having molecular weights less than 10,000.

Classification of Proteins: Proteins are classified mainly in two groups on the basis of their chemical nature and solubility.

a. Simple proteins: Composed of amino acids and soluble in water, dilute acid solutions, dilute alkali solutions, dilute salt solutions or in 70% alcohol. e.g. Albumins, globulins, glutelins, protamines and histones.
b. Conjugate proteins: Composed of other structural elements besides aminoacids. e.g. chromoproteins, lipoproteins, metalloproteins and glycoproteins.

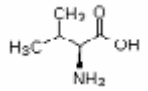
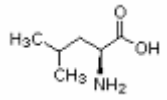
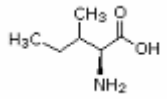
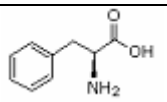
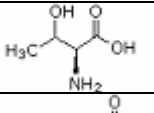
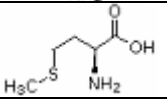
In general plant proteins contain less content of sulphur containing amino acids whereas plant cell wall is rich in hydroxyl praline. Plants do not vary in their protein make up

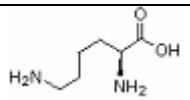
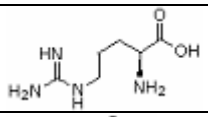
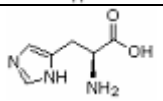
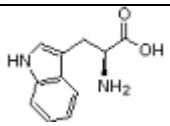
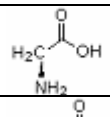
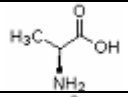
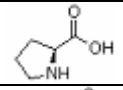
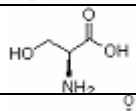
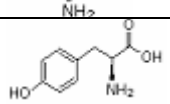
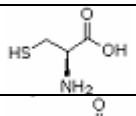
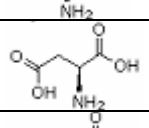
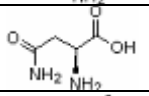
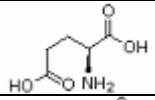
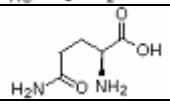
Amino Acids

Hydrolysis of proteins by boiling aqueous acid or base yields an assortment of small molecules identified as α-aminocarboxylic acids. More than twenty such components have been isolated. The amino acids are classified generally in two categories on the basis of their synthesis in human body (Table 1):

1. **Essential amino acids**: Essential component of diet since they are not synthesized by human body.
2. **Non-essential amino acids**: Synthesized in human body.

Table 1: Some common Amino acids

	S. No.	Name	Structure	Properties
Essential amino acids	1	Valine		-
	2	Leucine		-
	3	Iso-leucine		-
	4	Phenyl alanine		-
	5	Threonine		Hydroxyl group
	6	Metheonine		Sulphide group

	7	Lysine		NH_2 group in side chain
	8	Argentine		NH_2 group in side chain
	9	Histidine		N-containing heterocyclic ring
	10	Tryptophan		N-containing heterocyclic ring
Non essential amino acids	1	Glycine		Achiral
	2	Alanine		-
	3	Prioline		2° amine,
	4	Serine		Hydroxyl group
	5	Tyrosine		Hydroxyl group
	6	Cysteine		R-configuration Thiol group
	7	Aspartic acid		-COOH side chain
	8	Asparagine		-Aspartic acid amide
	9	Glutamic acid		-COOH side chain
	10	Glutamine		-Glutamic acid amide

Isoelectric Point of Amino acids: The isoelectric point (pI) is the pH of an aqueous solution of an amino acid (or peptide) at which the molecules on average have no net charge. In other words, the negatively charged groups exactly balance the positively charged groups. The pI simple amino acids like alanine, is the average of pK_a's of carboxyl (2.34) and ammonium (9.69) groups. Hence, the pI of alanine can be calculated as: (2.34 + 9.69)/2 = 6.02.

Chemical Test for Proteins and Amino Acids: Proteins are high molecular weight polymers of amino acids. Amino acids are colourless ionic compounds, more or less soluble in water and present in acid hydrolysates of plant and animal proteins.

The presence of proteins and amino acids can be detected by following chemical tests:

1. Millons test
2. Biurate test
3. Xanthoprotic test
4. Ninhydrin test
5. Lead sulphide test

1. Biuret test: To the aqueous solution of protein in hot water, few drops of Biuret reagent (KOH, $CuSO_4$ and sodium potassium tartarate) is added, which turns blue reagent to violet. In laboratory, it is usually done by adding few drops of 0.5% $CuSO_4$ solution to the alkaline aqueous protein solution. At least one peptide linkage is necessary for this test; individual amino acids do not produce violet colour.

2. Millons test: Any compound containing a phenolic hydroxyl group gives Millon's test positive. Consequently, any protein containing phenolic hydroxyl group (like tyrosine and phenyl alanine etc) will give a positive test of a pink to dark-red colour due to formation of a mercury salt of nitrated amino acid. The Millon reagent is a solution of mercuric and mercurous ions in nitric and nitrous acids. Take 1 ml of protein solution in a test tube and add few drops of Millons reagent. White precipitate is produced, which turns red after heating for 5 minutes on water bath.

3. Ninhydrin test: The Ninhydrin test is used to detect the presence of alpha-amino acids and proteins containing free amino groups. Protein solution when heated with ninhydrin molecules, it gives characteristic deep blue or pale yellow colour due to formation of complex between two ninhydrin molecule and nitrogen of free amino acid.

4. Lead sulphid test: Sulfur-containing amino acids like cysteine can be determined by converting the sulfur of amino acid to inorganic sulfide by using base. The resulting sulfide when reacts with lead acetate, a black precipitate of lead sulfide is formed.

Take 1 ml of protein solution in a testube and add 2 ml of 10% NaOH solution followed by few drops of lead acetate solution. Shake the solution and boil on water bath for few minutes, it produces black precipitate in presence of sulfur containing amino acids.

5. Xanthoprotic test: It is used for detection of presence of aromatic ring in amino acids or aromatic ring containing amino acid in proteins. To the aqueous solution of amino acid/protein (2 ml) in a testube add 2 ml of 65% HNO_3. Formation of yellow precipitate due to nitration of aromatic ring indicates presence of aromatic ring containing amino acid like tyrosine, tryptophane etc.

Chemical Test for Gelatin: It can be identified using following chemical tests:

1. Solubility test: Soluble in hot water whereas insoluble in cold water.

2. Soda lime test: It produces ammonia gas on heating with soda lime solution.

3. Precipitation test: Aqueous solution of gelatin produces crystalline yellow precipitate on addition of saturated solution of picric acid whereas buff precipitate on addition of saturated solution of tannic acid.

Nucleic Acids

Nucleic acids are the major components of chromosomes present in the nuclei of cells. Elemental analysis of nucleic acids revealed the presence of phosphorus, in addition to the C, H, N & O. Complete hydrolysis of chromosomal nucleic acids gave inorganic phosphate, 2-deoxyribose and four different heterocyclic bases, chromosomal nucleic acids are called deoxyribonucleic acids (DNA). Analogous nucleic acids in which the sugar component is ribose are termed ribonucleic acids (RNA). The acidic character of the nucleic acids was attributed due to the phosphoric acid moiety.

The heterocyclic bases present in nucleic acid are of two types:

1. **Pyrimidines** (monocyclic bases): e.g. Adenine and guanine
2. **Purines** (bicyclic bases): e.g. Thymine, cytosine and uracil

N-glycosides of the common sugar ribose with heterocyclic bases (nucleoside) are the building blocks of RNA, and are named adenosine, cytidine, guanosine and uridine (a thymidine analog without the methyl group). Nucleic acids are the alternating copolymers of phosphoric acid (**P**) and nucleosides (**N**) (Table 2 and 3).

Table 2: Nucleic acid sugars and phosphate

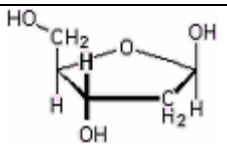
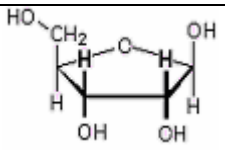
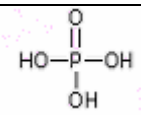
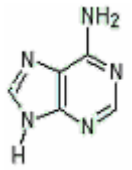
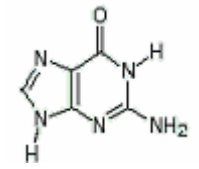
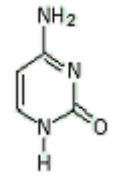
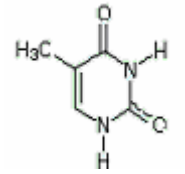
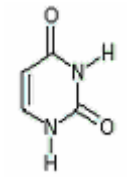
	DNA	RNA
Sugars	 2-Deoxyribose sugar	 Ribose sugar
Phosphate		

Table 3: Nucleic acid bases and their derivatives

Base	Structure	Nucleoside	5'-Nucleotide
Adenine[1 & 2]		2'-Deoxyadenosine[1] Adenosine[2]	2'-Deoxyadenosine-5'-monophosphate[1] Adenosine-5'-monophosphate[2]
Guanine[1 & 2]		2'-Deoxyguanosine[1] Guanosine[2]	2'-Deoxyguanosine-5'-monophosphate[1] Guanosine-5'-monophosphate[2]
Cytosine[1 & 2]		2'-Deoxycytidine[1] Cytidine[2]	2'-Deoxycytidine-5'-monophosphate[1] Cytidine-5'-monophosphate[2]
Thymine[1]		2'-Deoxythymidine[1]	2'-Deoxythymidine-5'-monophosphate[1]
Uracil[2]		Uridine[2]	Uridine-5'-monophosphate[2]

[1]present in DNA, [2]present in RNA

The analysis of DNA by Erwin Chargaff revealed its composition to be species specific and the amount of adenine (A) always equaled the amount of thymine (T) whereas the amount of guanine (G) always equaled the amount of cytosine (C), regardless of the DNA source.

Information is stored or encoded in the DNA polymer by the pattern in which the four nucleotides are arranged. To access this information the pattern must be "read" in a linear fashion, just as a bar code is read at a supermarket checkout. Because living organisms are extremely complex, a correspondingly large amount of information related to this complexity must be stored in the DNA. The nuclei of multicellular organisms incorporate chromosomes, which are composed of DNA combined with nuclear proteins called histones.

In contrast to DNA, a lower molecular weight, but much more abundant nucleic acid, RNA is distributed throughout the cell, most commonly in small organelles like ribosomes. Three kinds of RNA are identified:

a. Ribosomal RNA (rRNA, 85 to 90%) the major component of ribosomes with proteins. The size of rRNA molecules varies, but is generally less than a thousandth the size of DNA.

b. Messenger RNA (mRNA)

c. Transfer RNA (tRNA). Both of them are shorter than rRNA and present in smaller quantities.
Careful examination of the purine and pyrimidine base components of the nucleotides reveals that three of them could exist as hydroxy pyrimidine or purine tautomers, having an aromatic heterocyclic ring.

Watson and Crick have proposed a complementary pairing, via hydrogen bonding, of guanosine (G) with cytidine (C) and adenosine (A) with thymidine and suggested a double helix structure for DNA.

The G#C association involves three hydrogen bonds, and is therefore stronger than the two-hydrogen bond association of A#T. The RNA base uracil corresponds to thymine and hence A#U in RNA.

Two strands of DNA were aligned anti-parallel to each other, i.e. with opposite 3' and 5' ends. Complementary primary nucleotide structures for each strand allowed intra-strand hydrogen bonding between each pair of bases. Coiling of coupled strands leads to a double helix structure as a cross-linked ribbons. Each helix has ten base pairs per turn, and of 3.4 Å height in each turn. This right-handed helix is the favored conformation in aqueous systems, and has been termed the B-helix. Two alternating grooves are evident, a wide and deep major groove, and a shallow and narrow minor groove. Other helical structures of DNA have also been observed, and are designated by letters A and Z.

Dische diphenylamine test for DNA: In acidic conditions it converts deoxyribose to a molecule (-CHO) that binds with diphylamine and forms a blue complex. The intensity of the blue color is proportional to the concentration of DNA. The reaction depends on the conversion of the pentose to □-hydroxylaevulinic aldehyde, which then reacts with diphenylamine to gives a blue colored complex.

Alkaloids

Alkaloids are organic compounds of basic nature hence named alkali like or alkaloids and have one or two nitrogen atom within nucleus or outside the nucleus. Alkaloids are usually derived from amino acids and show prominent pharmacological action in small doses. Most alkaloids are alkaline solids like quinidine, emetine and atropine. Some alkaloids are found in liquid form like coniine, nicotine etc. These are colorless but some of them are colored like berberine (yellow), betain (red). Alkaloids contain carbon, hydrogen, one or more than one nitrogen, usually oxygen and some time sulphur. These are optically active laevorotatory form is pharmacologically more active than dextrorotatory. Alkaloids are bitter in taste, available in the form of salt, less toxic

and don't show addiction property like morphine. Free bases of alkaloids are insoluble in water and soluble in organic solvent like chloroform, ether etc. (caffeine and colchicine are soluble in water) whereas alkaloidal salts and quaternary alkaloids are highly soluble in water but insoluble in organic solvents (lobelline HCl soluble in $CHCl_3$, quinine sulphate is sparingly soluble in water). Alkaloids, taken in their broadest sense, may have nitrogen atom which is primary e.g. mascaline, secondary e.g. ephedrine, tertiary e.g. atropine and quaternary e.g. tubocurarine.

Most alkaloid skeletons are derived from amino acids whereas some derived from other groups of molecules also such as the steroidal alkaloids with the nitrogen from glutamine or another N donor being added in later biosynthetic steps.

Alkaloids nomenclature ends by adding suffix ine, prefix is usually the name of genus (cocain from coca sp.), species (vasicine from *Adhatoda vasica*), common name (ergotamine from ergot sp.), discoverer (Pelleterine and Ajmalicine) and physiological activity (emetine).

Like many secondary metabolites, plants apparently synthesize alkaloids for defensive purposes. Nicotine and derivatives are among the earliest known and most potent insecticides. Major source of alkaloids are flowering plants (angiosperm) and distributed unevenly in families. The plant families with the highest alkaloid levels are the Papaveraceae, Berberidaceae, Leguminosae, Boraginaceae, Apocynaceae, Asclepiadaceae, Liliaceae, Gnetaceae, Ranunculaceae, Rubiaceae, Solanaceae and Rutaceae. Some families are characterized by presence of specific alkaloidal nucleus like morphinan in papaveraceae. Some animals also contain alkaloids like butterflies contain pyrrolizidine alkaloids because they feed upon the plants containing these alkaloids. Salamanders, amphibians and arthropods also contains small amount of alkaloids. Insects, marine organisms and lower plants have also been reported to contain small amount of alkaloids like ergot alkaloids from fungus.

Alkaloids are distributed in different parts of plants like in seed (nux-vomica, colchicum); fruits (black pepper, opium); leaves (datura, belladonna, hyoscyamus, vinca, tobacco); barks (cinchona, cinnamon, kurchi); roots and rhizomes (rauwolfia, ipecac, aconite) and also in whole plant (ephedra, lobelia).

Classification of alkaloids on the basis of origin:

(a) **True Alkaloids:** These are basic in nature, derivatives of amino acids having nitrogen in heterocyclic ring, occurs in plants as salts of organic acids. e.g.: Quinine, Morphine, Atropine.

(b) **Proto/amino-alkaloids:** These are simple biological amines, basic in nature, derivatives of amino acids, don't have heterocyclic nitrogen atom in ring system (but in side chain). e.g. ephedrine, colchicine and mescaline

(c) **Pseudo alkaloids:** These are weakly basic nitrogenous compounds, do not derived from amino acid but have heterocyclic nitrogen atom. e.g. purine bases like caffeine and steroidal alkaloids like solasodine.

1. True and proto alkaloids (Amino acid derived alkaloids)
    a. Ornithine and Lysine derived alkaloids
        i. Pyrolidine alkaloids e.x. Nicotine

ii. Tropane alkaloids e.x. Atropine, Hyoscine
iii. Pyrolizidine alkaloids e.x. Alkaloids of Borage and Symphytum
iv. Quinolizidine alkaloids e.x. Lupanine and sparteine
v. Indolizidine alkaloids e.x. Castanospermine (Anti-HIV)
vi. Piperidine alkaloids e.x. Lobeline, Piperine, pelleterine and coniine

b. Phenylalanine and tyrosine derived alkaloids

i. Phenyl ethyl amine alkaloids e.x. Ephedrine
ii. Isoquinoline alkaloids e.x. Papaverine
iii. Aporphine alkaloids e.x. Apomorphine
iv. Morphinans e.x. Morphine, codine, thebain

c. Tryptophan derived alkaloids

i. Tryptamine and carbolines e.x. Muscarine, serotonine, harmine and harmaline
ii. Indolines e.x. Neostigmine and physiostigmine
iii. Ergolines e.x. Lysergic acid derivative ergot alkaloids
iv. Monoterpenoid indole e.x. vinca, cinchona, nux-vomica, rauwolfia and camptotheca alkaloids.

d. Histidine derived alkaloids e.x. Pilocarpine

2. Pseudo alkaloids (non amino acid derived alkaloids)

a. Alkaloids derived from terpene metabolism

i. Mono and sesque terpene alkaloids e.x. alkaloids of Nymphea sp.
ii. Diterpene alkaloids e.x. alkaloids of Aconite sp.
iii. Steroidal alkaloids e.x. alkaloids of Solanum and Veratrum sp. and kurchi alkaloids.

b. Purine bases e.x. caffeine and theine

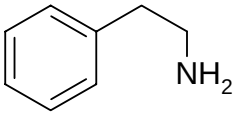
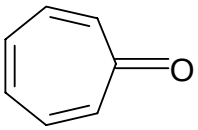
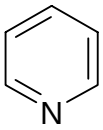
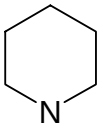
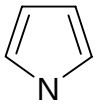
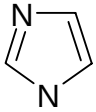
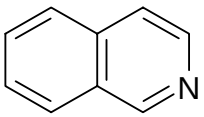
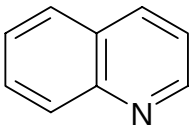
c. Miscellaneous alkaloids like peptide alkaloids of Ziziphus sp. and maytansinoids.

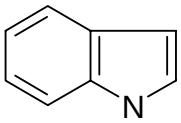
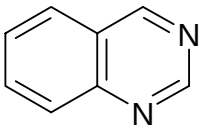
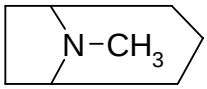
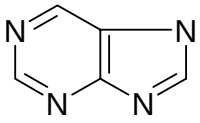
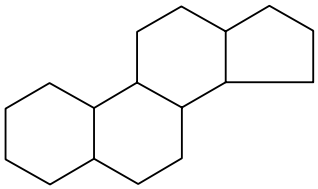
Chemical classification of alkaloids on the basis of heterocyclic ring:

Alkaloids are mainly divided into two categories on the basis of their chemical structure i.e. heterocyclic rings Table 4.

1. Atypical alkaloids: These are also known as non-heterocyclic alkaloids and contain nitrogen in aliphatic chain.
2. Typical alkaloids: These are also known as heterocyclic alkaloids and contain nitrogen in heterocyclic ring system.

Table 4: Classification of Alkaloids

Groups	Example	Source	Chemical structure	Uses
1. Non-Heterocyclic Alkaloids				
Phenyl ethyl amine alkaloid	Ephedrine, Mescaline Hordenine	Ephedra sp. *Lophophora wiliamsoii*		Asthama
Tropolone alkaloids	Colchicine	Colchicum sp.		Gout
Modified diterpene	Taxol	Taxus sp.		Anti cancer
2. Heterocyclic Alkaloids				
a. Mono-nuclear Heterocyclic Alkaloids				
Pyridine	Lobeline	Lobelia sp.		Asthama
Piperidine	Piperine	Piper sp		Gonorrhea Anti oxidant
Pyrrole	Hygrine	Coca sp.		CNS Stimulant
Pyrrolidine	Nicotine	Tobbaco sp.		CNS Stimulant
Imidazole	Pilocarpine	Pilocarpus sp.		Contraction of pupil
b. Poly-nuclear Hetero-cyclic alkaloids				
Isoquinoline	Morphine, papaverine	Opium		Narcotic analgesic
Quinoline	Quinidine, quinidine	Cinchona		Anti-malarial

Indole	Ergotamine, reserpine, vincrystine Strychnine	Ergot Rauwolfia Vinca Nux-vomica		Oxytocic, Anti-HT, Anti_cancer CNS stimulant
Quinazoline	Vasicine	Vasaka		Anti-tussive
Tropane	Atropine, hyoscine	Datura, belladona		Parasympatholytic
Purine	Caffeine Theine	Coffee, tea		CNS stimulant
Steroid	Solasodine	Solanum sp.		Steroidal precursor
Terpenoid	Aconitine	Aconite sp.		CNS

Extraction and isolation of alkaloids: Alkaloids can be extracted by following two methods:

1. Direct extraction using non polar organic solvent
2. Extraction using polar solvent

1. Direct extraction using non polar organic solvent:

a. The powered material is moistened with limewater, which combines with acids, tannins and other phenolic substances and set free the alkaloids (exists in the plant as salts).

b. The extraction is then carried out with organic solvent such as ether or chloroform, filtered and concentrates the filtrate.

c. The concentrated organic extract is then shaken with aqueous acid solution to form alkaloidal salts, which are soluble in aqueous acidic layer. The impurities present in the extract remain in the organic liquid.

d. The aqueous acidic phase is separated and alkaloidal salts present are precipitated using strong ammonia solution (make alkaline).

e. Precipitated alkaoidal bases are back extracted with chloroform or ether and evaporated to dryness.

f. The dried residue is weighed, which gives total alkaloidal content of drug.

2. Extraction using polar solvent:

a. Powered drug is extracted with aqueous or alcoholic dilute acid solution, filtered and concentrated on water bath till complete evaporation of alcohol.

b. The concentrated extract is shaked with organic solvent like chloroform or ether to remove pigments and other unwanted materials.

c. Excess of ammonia solution is then added to concentrated extract containing alkaloidal salts to precipitate alkaloidal bases.

d. Alkaline extract is extracted with chloroform or ether and evaporated to dryness.

e. The dried residue is weighed, which gives total alkaloidal content of drug.

Chemical Test for Alkaloids: The chemical test are performed from neutral or slightly acidic solution of drug following type of chemical test given by alkaloids are-

1. **Dragendorff's Test:** Drug solution + Dragen droff's reagent (Potassium Bismuth Iodide), formation of Orangish red colour.

2. **Mayer's Test:** Drug solution + few drops of Mayer's reagent (K_2HgI_4), formation of creamy-white precipitant.

3. **Hager's Test:** Drug solution + few drops of Hagers reagent (Saturated aq. Solution of Picric acid), formation of crystalline yellow precipitate.

4. **Wagner's Test:** Drug solution + few drops of Wagner's reagent (dilute Iodine solution), formulation of reddish-brown precipitate.

5. **Tannic Acid Test:** Drug solution + few drops of tannic acid solution, formation of buff coloured precipitate.

Glycosides

In nature glycosides are formed by interaction of nucleotide glycosides like uridine phosphate glucose with alcohol, phenol, steroid, triterpenoid and flavonoids etc. and joined by glycosidic linkage.

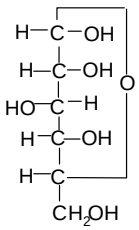
These are non-reducing (do not reduce Fehling solution) organic substances which on hydrolysis yields one or more sugar molecules along with non-sugar molecules. The sugar molecule known as glycon and non-sugar molecule termed as aglycon part. Sugars are hemiacetal and occur as oxide rings.

Glycosides can be defined as the condensation product of hydroxyl group of aglycon and hemiacetal hydroxyl group of sugar. The aglycon may be any compound containing at least one hydroxyl group to which glycosidal hydroxyl group of sugar joints.

Glycosides are colorless, crystalline or amorphous solid substances (flavonoids are yellow colored whereas anthracene glycosides are red to orange) generally, poisonous in nature. These

are soluble in water and alcohol but insoluble in ether and chloroform, optically active, usually levorotatory.

Glycosidic hydroxyl group reacts with large number of organic compounds and acid liable i.e. the organic moiety attached at glycosidic hydroxyl group is hydrolyzed with acids whereas others are not. The sugars present in glycoside are of two isomeric form i.e. α form and β form (Fig. 4), but all the natural glycosides contain β-type of sugar.



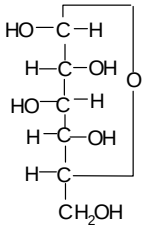


Fig. 4a. α- Glucose Fig. 4b. β-Glucose

The simplest glycosides are alpha methyl glycoside and beta methyl glycoside. Both type of glycosides can be hydrolyzed using mineral acids whereas these can specifically be hydrolysed using enzymes like maltase (α glycoside) and emulsine (β glycoside). This reaction is used for determination of type of linkage and nature of aglycon present in glycoside.

Glycoside (α or β) —Acid→ Aglycon + glycon

α-Glycoside —Maltase→ Aglycon + glycon

β-Glycoside —Emulsin→ Aglycon + glycon

Classification of Glycosides: Glycosides are generally classified in following two ways:

A. **On the basis of glycosidic linkage**

1. O - glycosides: Sugar molecule is combined with phenol or OH group of aglycon. e.g Amygdaline, Indesine, Arbutin, Salicin, cardiac glycosides, anthraxquinone glycosides like sennosides etc.

2. N – glycosides: Sugar molecule is combined with N of the –NH (amino group) of aglycon. e.g. Neucleosides

3. S – glycosides: Sugar molecule is combined with the S or SH (thiol group) of aglycon. e.g. Sinigrine

4. C – glycosides: Sugar molecule is directly attached with C – atom of aglycon. e.g. Anthraquinone glycosides like Aloin, Barbaloin, cascaroside and flavan glycosides etc.

B. On the basis of chemical nature of aglycon and pharmacological activity

1. **Anthraquinone glycosides:** These are derivative of anthraquinones (Fig. 5a), possess purgative property, may be dihydroxy phenol (chrysophanol), trihydroxyphenol (emodine), or tetra hydroxy phenols. Anthraquinone derivatives are often orange red coloured compounds, soluble in hot water and alcohols. e.g Sennosides A, B, C and D.

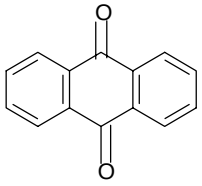
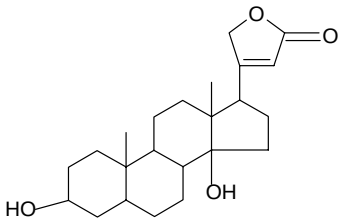


Fig. 5a. Anthraquinone

2. Cardiac glycosides: Cardiac glycosides are composed of steroidal aglycone with a 5 membered lacton ring and acts on cardiac muscles. These are also known as cardiotonic glycosides and the cardiac activity dependent on both the aglycone and sugar molecule. Activity resides in the aglycone but the sugar renders the compounds more soluble, which increases the power of fixation of the glycoside to the heart muscles. These are divided into two sub-groups:

a. Cardenolides: Cardenolides have 5 membered lactone ring at C-17 position (Fig. 5b). e.g. Digoxine, Dixitoxine, Oliandrine.

b. Bufadenolides: Bufadenolides have 6- member lactone ring at C-17 position of steroidal rings (Fig. 5c). e.g. Scillarenin A, obtain from Squill.



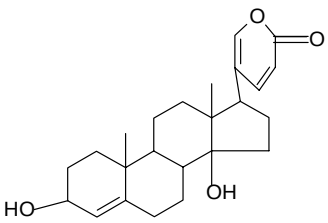


Fig. 5b. Cardenolide (Dixitoxigenine) Fig. 5c. Bufadenolide (Scillarenin A)

3. Saponin glycosides: These forms honey comb like foam when shaken with water and causes haemolysis of blood. Saponine complex organic compounds distributed in higher plants and are toxic to cold-blooded animals and lower organisms like earthworm and fishes. These are soluble in alcohol but insoluble in ether and light petroleum; on hydrolysis it gives aglycone known as sapogenin (generally steroids) and sugars. Depending on the aglycone moiety, saponins are of two types:

a. Steroidal saponin (tetracyclic triterpenoids): These contain cyclopenteno-perhydro-phenanthrene ring e.g. Diosgenin- obtained from Dioscorea species.

b. Pentacyclic triterpene saponin: These are polymer of isoprenes and mainly derivatives of β-amyrine (Fig. 5d). e.g Glycyrrhetinic acid (Fig. 5e)– obtained from *Glycyrrhiza glabra*.

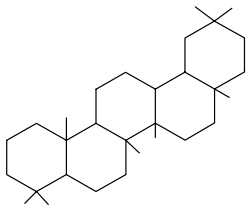


Fig. 5d. β-amyrine

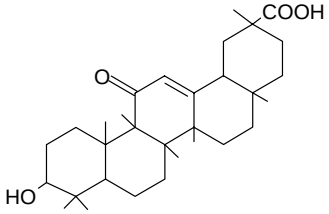


Fig. 5e. Glycyrrhetinic acid

4. Cynophoric or Cynogenetic Glycosides: The glycosides which on hydrolysis yields hydrocynic acid (HCN), benzaldehyde and sugars. The medicinal activity of cyanogenetic glycosides is due to presence of hydrocyanic acid and these are the characteristics of family rosaceae. e.g. Amygdalin:-obtained from bitter almond (*Prunus amygdalus*), Prunasin: obtained from wild cherry bark.

5. Isothiocyanate glycoside: These are sulphur-containing compounds rich in family cruciferae, also known as glucosinolates and on hydrolysis yields isothiocyanate (-NCS) group. These glycosides are generally irritant and hence used externally as counter irritant. e.g. Sinigrin from black mustard, sinalbin from white mustard and gluconapin from rapeseed.

6. Bitter glycosides: These are complex organic compounds containing lactone ring, soluble in water and very bitter in taste even in much diluted solutions.These increase the secretions of GIT by reflex action and are used as stomachic, febrifuge and bitter tonic. e.g. gentiopicrin (Fig. 5f) and amarogentin from Gentian root and Chirata, picroside and kutkoside from Picrorrhiza, andrographolides from kalmegh and quassin from Quassia wood.

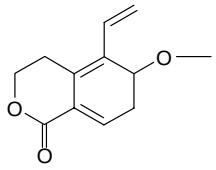


Fig. 5f. Gentiopicrin

7. Coumarin glycosides: These are aromatic compounds containing benzo-α-pyrone ring system (Fig. 5g). The alcoholic solution of coumarins shows blue-green fluorescence on addition of alkali. Some coumarins are containing furan ring attached at 6-7 or 7-8 position in coumarin ring and are called as furano coumarin. These are generally used externally, in skin disorders and in sun tan preparations because they have property to absorb UV radiation of sunlight. e. g. Aesculin from plants of family rosaceae and scopolin; furano coumarins are generally present in family rutaceae, umbelliferae and leguminosae like psoralen, xanthotoxin, bergapten and imperatorin.

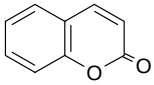


Fig. 5g. Benzo-α-pyrone

8. Flavone glycosides: These are complex organic compounds containing phenyl-benzo-χ-pyrone ring system (Fig. 5h and i). Flavones are present in plants in free state or in glycosidal state (O-glycoside or C-glycoside) with its different derivatives like flavane, flavonol, flavonone, isoflavone and chalcones. e.g. Rutin, quercitrin, hyperoside, diosmin (buchu leaf), hesperidin (lemon and orange peel) and vitexin (Caratégus).

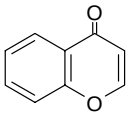


Fig. 5h. Benzo-χ-pyrone

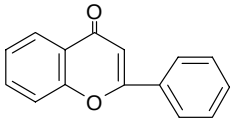


Fig. 5i. 2-phenyl-benzo-χ-pyrone

Extraction and isolation of glycosides:

1. Powdered drug was extracted with alcohol in soxhlet extractor.

2. Alcoholic extract was then treated with lead acetate solution to precipitate tannins, proteins, coloring matter and other non-glycosidal part

3. The precipitate formed was filtered and to the filtrate H_2S gas was pass to precipitate excess lead as lead sulphide and removed by filtration.

4. Filtrate was evaporated to dryness on water bath and dried residue was collected and weighed to get total glycoside content.

Specific classes of glycosides and their aglycones are extracted with specific methods.

Distribution of glycosides: Glycosides are the class of compounds abundant in nature, some plant families containing important glycosides are listed bellow:

1. Scrophulareacea: e.g. *Digitalis purpurea* and *Digitalis lanata*, *Picrorhiza kurroa.*
2. Apocyanacea: e.g. *Nerium oliander* and *Thevetia peruviana*.
3. Liliacea: e.g. *Urgenea indica* and *U.maritima* , *Aloe vera*
4. Leguminocae: e.g. *Cassia acutefolia* and *C. angustefolia*, *Glycyrrhiza glabra, Psoralea corylifolia*
5. Dioscoreaceae:-e.g. *Dioscorea floribunda*
6. Rosaceae: e.g. *Prunus amygdalus, Carategus oxycantha*
7. Cruciferae: *Brassica sp.*
8. Gentianaceae: Gentian and Chirata.
9. Acanthaceae: Kalmegh
10. Simarubaceae: Quassia
11. Umbelliferae: *Ammi majus, Ammi visnaga*
12. Rutaceae: Citrus sp., *Ruta graveolens*
13. Polygonaceae: Fagopyrum sp.
14. Myrtaceae: Eucalyptus sp.

Chemical Tests of Glycosides: Glycosides are the compounds with organic molecules having attached glucose or any mono-oligo sacchrid unit. Usually, these are crystalline or amorphous solids; optically active, soluble in water and alcohol but insoluble in organic solvents like ether, chloroform and benzene etc. Generally, aqueous or alcoholic extracts of crude drugs are tested with specific reagents for presence of various types of glycosides.

1. Chemical tests for anthraquinone glycosides
   - Borntrager's test
   - Modified Borntrager's test

2. Chemical tests for saponin glycosides
   - Heamolysis test
   - Foam test

3. Chemical tests for steroid and triterpenoid glycosides
   - Libermann Bruchard test
   - Salkovaski test

- Antimony trichloride test
- Trichloro acetic acid test
- Tetranitro methane test
- Zimmermann test

4. Chemical tests for cardiac glycosides
   - Keller Killiani test
   - Legal test
   - Baljet test
   - 3,5-dinitro benzoic acid test

5. Chemical tests for Coumarin glycosides
   - $FeCl_3$ test
   - Fluorescence test

6. Chemical tests for Cynophoric glycoside
   - Sodium picrate test

7. Chemical tests for flavonoid glycosides
   - Ammonia test
   - Shinoda test
   - Vanillin HCl test

1. Chemical tests for anthraquinone glycosides

a. **Borntragor's Test:** To 1 gm of drug add 5-10 ml of dilute HCl boil on water bath for 10 minutes and filter. Filtrate was extracted with CCl_4/benzene and add equal amount of ammonia solution to filtrate and shake. Formation of pink or red colour in ammonical layer due to presence of anthraquinone moiety.

b. **Modified Borntragor's Test:** To 1 gm of drug add 5 ml dilute HCl followed by 5 ml ferric Chloride (5% w/v). Boil for 10 minutes on water bath, cool and filter, filtrate was extracted with carbon tetrachloride or benzene and add equal volume of ammonia solution, formation of pink to red colour due to presence of anthraquinone moiety. This is used C-type of anthraquinone glycosides.

2. Chemical tests for saponin glycosides

a. Haemolysis test: A drop blood on slide was mixed with few drops of aq. Saponin solution, RBC's becomes ruptured in presence of saponins.

b. Foam test: To 1 gm of drug add 10-20 ml of water, shake for few minutes, formation frothing which persists for 60-120 seconds in presence of saponins.

3. Chemical tests for steroid and triterpenoid glycosides

a. Libermann Bruchard test: Alcoholic extract of drug was evaporated to dryness and extracted with $CHCl_3$, add few drops of acetic anhydride followed by conc. H_2SO_4 from side wall of test tube to the $CHCl_3$ extract. Formation of violet to blue coloured ring at the junction of two liquid, indicate the presence of steroid moiety.

b. Salkovaski test: Alcoholic extract of drug was evaporated to dryness and extracted with $CHCl_3$, add conc. H_2SO_4 from sidewall of test tube to the $CHCl_3$ extract. Formation of yellow coloured ring at the junction of two liquid, which turns red after 2 minutes, indicate the presence of steroid moiety.

c. Antimony trichloride test: Alcoholic extract of drug was evaporated to dryness and extracted with $CHCl_3$, add saturated solution of $SbCl_3$ in $CHCl_3$ containing 20% acetic anhydride. Formation of pink color on heating indicates presence of steroids and triterpenoids. .

d. Trichloro acetic acid test: Triterpenes on addition of saturated solution of trichloro acetic acid forms colored precipitate.

e. Tetranitro methane test: It forms yellow colour with unsaturated steroids and triterpenes.

f. Zimmermann test: Meta dinitro benzene solution was added to the alcoholic solution of drug containing alkali, on heating it forms violet colour in presence of keto-steroid.

4. Chemical tests for cardiac glycosides

a. Keller Killiani test: To the alcoholic extract of drug equal volume of water and 0.5 ml of strong lead acetate solution was added, shaked and filtered. Filtrate was extracted with equal volume of chloroform. Chloroform extract was evaporated to dryness and residue was dissolved in 3 ml of glacial acetic acid followed by addition of few drops of $FeCl_3$ solution. The resultant solution was transferred to a testube containing 2 ml of conc. H_2SO_4. Reddish brown layer is formed, which turns bluish green after standing due to presence of digitoxose.

b. Legal test: To the alcoholic extract of drug equal volume of water and 0.5 ml of strong lead acetate solution was added, shaked and filtered. Filtrate was extracted with equal volume of chloroform and the chloroform extract was evaporated to dryness. The residue was dissolved in 2 ml of pyridine and sodium nitropruside 2 ml was added followed by addition of NaOH solution to make alkaline. Formation of pink colour in presence of glycosides or aglycon moiety.

c. Baljet test: Thick section of leaf of digitalis or the part of drug containing cardiac glycoside, when dipped in sodium picrate solution, it forms yellow to orange colour in presence of aglycones or glycosides.

d. 3,5-dinitro benzoic acid test: To the alcoholic solution of drug few drops of NaOH followed by 2% solution of 3,5-dinitro benzoic acid was added. Formation of pink colour indicates presence of cardiac glycosides.

5. Chemical tests for Coumarin glycosides

a. $FeCl_3$ test: To the concentrated alcoholic extract of drug few drops of alcoholic $FeCl_3$ solution was added. Formation of deep green colour, which turned yellow on addition of conc. HNO_3, indicates presence of coumarins.

b. Fluorescence test: The alcoholic extract of drug was mixed with 1N NaOH solution (one ml each). Development of blue-green fluorescence indicates presence of coumarins.

6. Chemical tests for Cynophoric glycoside

a. Sodium picrate test: Powdered drug moistened with water in a conical flask and few drops of conc. Sulphuric acid was added. Filter paper impregnated with sodium picrate solution followed

by sodium carbonate solution was trapped on the neck of flask using cork. Formation of brick red colour due to volatile HCN in presence of cynophoric glycosides.

7. Chemical tests for flavonoid glycosides

a. Ammonia test: Filter paper dipped in alcoholic solution of drug was exposed to ammonia vapor. Formation of yellow spot on filter paper.

b. Shinoda test:

i. To the alcoholic extract of drug Magnesium turning and dil. HCl was added, formation of red colour indicates the presence of flavonoids.

ii. To the alcoholic extract of drug Zinc turning and dil. HCl was added, formation of deep red to magenta colour indicates the presence of dihydro flavonoids.

c. Vanillin HCl test: Vanillin HCl was added to the alcoholic solution of drug, formation of pink colour due to presence of flavonoids.

Pharmaceutical application of Glycosides: Glycosides are widely used in pharmaceutical industries including production of steroidal hormones, vitamins, active pharmaceutical ingredients and also for therapeutic purposes. Some important applications are listed below:

1. Digitalis: Used in the treatment of conjustive heart failure .
2. Strophanthus: As a cardiotonic and diuretic
3. Squill: as rodenticide
4. Oliandrin and adynerin: As anti cancer
5. Senna and rhubarb: Used as laxative and purgative.
6. Aloe: Purgative
7. Diosgenin: Used for production of steroidal hormone like estrogen and progesterone
8. Glycyrrhetinic acid: Used as anti inflammatory
9. Quillia: Used as emulsifier.
10. Almond (Bitter): Used as expectorant.
11. Picrorrhiza and Kalmegh: Bitter and in liver disorders
12. Rutin: Venoactive and as a source of Vit. P.

Terpenoids

These are hydrocarbons of plant origin and their oxygenated, hydrogenated and dehydrogenated derivatives having general formula $(C_5H_8)_n$. These are building blocks of isoprene (2-methyl-buta 1, 3 diene, C_5H_8) units (Fig. 6), joined together in head to tail fashion. Terpenoids are colorless compounds, lighter than water with boiling point 150-180 °C. These are optically active liquids (few terpenoids are solid), insoluble in water but soluble in organic solvents. The term terpene originates from the mixture of isomeric hydrocarbons of molecular formula $C_{10}H_{16}$ present in turpentine oil. Now, terpenes are only limited to a class of compounds of terpenoids that is monoterpene hydrocarbon of molecular formula $(C_5H_8)_2$, whereas terpenoids represents hydrocarbon and their oxygenated derivatives, hence it can be stated that all the terpenes are terpenoids but not vice-versa.

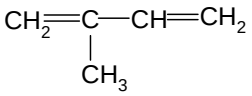


Fig. 6. Isoprene (2-methyl-buta 1, 3 diene)

The pleasant smelling parts of the plants like flowers, leaves, stem, bark, wood and fruits, are due to the presence of some steam volatile oils known as essential oils. The chief constituents of essential oils are terpenoids having atoms up to C_{15} i.e. mono and sesque terpenoids and their oxygenated derivatives. Terpenoids are located in cytoplasm of plant cell. Essential oils are some times present in special glandular cells on leaf surface whereas carotenoids are mainly associated with chloroplast in leaf and chromoplast in petals. Di and triterpenoids are found mainly in gums and resins. Some triterpenoids are also present in animal kingdom.

Classification of terpenoids

Terpenoids are classified on the basis of number of isoprene units present (Table 5):

Table 5: Classification of Terpenoids

Class	**No's of isoprene**	**Molecular formula**	**General formula**	**Remarks**
Hemiterpene	1	C_5H_8	C_5H_8	Isoprene, rare, detected in *Hamamelis japonica*
Monoterpenoids	2	$C_{10}H_{16}$	$(C_5H_8)_2$	Essential oils e.g. Menthol from mint Monoterpene lactone e.g. Nepeta lacone from Nepeta Tropolone from gymnospermous woods
Sequiterpenoids	3	$C_{15}H_{24}$	$(C_5H_8)_3$	Essential oils e.g. farnesol, neroledol and caryophyllene Lactones e.g. santonine, xanthinine and in family compositae Abscisins e.g. abscisic acid
Diterpenoids	4	$C_{20}H_{32}$	$(C_5H_8)_4$	Diterpene acids in plant resins Gibberllins e.g. gibberellic acid
Sesterpenoids	5	$C_{25}H_{40}$	$(C_5H_8)_5$	
Triterpenoids	6	$C_{30}H_{48}$	$(C_5H_8)_6$	Sterols e.g. sitosterol Triterpene e.g. amyrins Saponins e.g. yamogenin Cardiac glycosides
Tetraterpenoids	8	$C_{40}H_{64}$	$(C_5H_8)_8$	Carotenoids e.g. carotenes
Polyterpene	n	Rubber	$(C_5H_8)_n$	Polyisoprene e.g. rubber from *Hevea brasiliensis*

These are further subdivided into the separate classes on the basis of cyclic ring:

1. **Monoterpenoids:**

a. Acyclic e.g. geraniol, linalool and myrecene
b. Monocyclic e.g. limonene, menthol, menthone, carvone etc.
c. Bicyclic e.g. camphor, fenchone, α-pinene, thujone etc.
d. Irregular e.g. Loganin, nepetalactone etc.

2. **Sesquiterpenoids:**

a. Acyclic e.g. farnesol, nerolidol etc.
b. Monocyclic e.g. abscisic acid, bisabolene etc.
c. Bicyclic e.g. caryophyllene, cadinene etc.
d. Sesqueterpene lactone e.g. xanthinin, santonin etc.

3**. Diterpenoids**

a. Acyclic e.g. Phytol
b. Bicyclic e.g. Agathic acid
c. Tricyclic e.g. Abietic acid
d. Gibberllins e.g. gibberellic acid

4. **Triterpenoids**

a. Triterpenes
b. Steroids
c. Saponins
d. Cardiac glycosides
e. Steroidal alkaloids

5. **Tetraterpenoids (carotenoids)**: These are lipid soluble pigments widely distributed in all plants including bacteria. Carotenoids are present either in simple unsaturated hydrocarbon form or their oxygenated derivatives known as xanthophylls. Crocin obtained from *Crocus sativus* is only water soluble carotenoid present in plants, which on hydrolysis with acid yields crocetin dicarboxylic acid and glucose.

a. Hydrocarbons: e.g. Carotenes and Lycopene
b. Xanthophylls: e.g. Zeaxanthin, Capsanthin, Violaxanthin, Fucoxanthin, Lutein etc.

Extraction and Isolation

1) Ecuelle/Crushing/Expression
2) Hydrodistilation/Steam distillation
3) Solvent extraction
4) Enfleurage method
5) Supercritical fluid method
6) Steam distillation by microwave
7) Rectification or selective extraction

Extraction of Essential Oil

1. Expression method: By this method generally oil of fruit are separated by crushing like citrus and lemon.

2. Steam distillation/hydro distillation: Usually the volatile oil, which is less affected by heat, is isolated by this method. In this method plant material is either macerated with H_2O or directly passed with steam and the essential oil obtained in distillate is extracted with organic volatile solvent like petroleum ether.

3. Solvent extraction: This is very widely used extraction method in perfume industry for the production of volatile oil using lipid solvent like ether or benzene. This is very economical as there are very less deterioration of volatile oil and solvent is distilled off.

4. Enflurage method/adsorption on purified fat: This is very old method and still used now days generally flowers petals are strain on the surface of a glass plate having a layer a purified fat and maintained at temperature of 50 ^{o}C. The petals are kept in contact with fat for few days their wax is separated, digested with ethanol and cooled at 20 ^{o}C to remove fat. The alcohol is then removed by distillation under reduced pressure.

5. Supercritical fluid method: At certain critical pressure and temperature the gasses behaves like a liquid, which diffuse well through solids and acts as good solvent. The gasses like CO_2 are chemically inert, non-inflammable, nontoxic, easy to eliminate, selective, readily available and do not cause any hydrolysis or rearrangement of component to be extracted. Hence, in spite of high cost this method is enormously spreading for supercritical fluid to liquid-liquid extraction. Now days this method is used for production of decaffeinated coffea, nicotinless tobacco products and terpenless oils.

Separation and isolation of terpenoids from volatile oil

Terpenoids are present in volatile oils in the form of mixture. These terpenoids are present either in the form of hydrocarbon or their oxygenated derivative (alcohol, aldehyde, ketone etc.). These are separated usually by two methods: Physical method, and Chemical method.

1. Physical method: In physical method different chromatographic methods and fractional distillation is applied for separation of constituent terpenoids.

2. Chemical method:

a. **Separation of terpenoid hydrocarbon:** These are separated by using Tilden reagent composed of solution of Nitrosyl chloride (NOCl) in chloroform. The terpenoid hydrocarbons on treatment with Tilden reagent forms crystalline adduct having sharp m.p., which is separated from volatile oil followed by hydrolysis or decomposed to get back the terpenoid hydrocarbon.

b. **Separation of terpenoid alcohol:** Terpenoid alcohols on reaction with thallic anhydride forms di-ester, which precipitate out from volatile oil. These di-esters on treatment with $NaHCO_3$ in presence KOH, yields back terpene alcohol and thallic acid (Fig. 7).

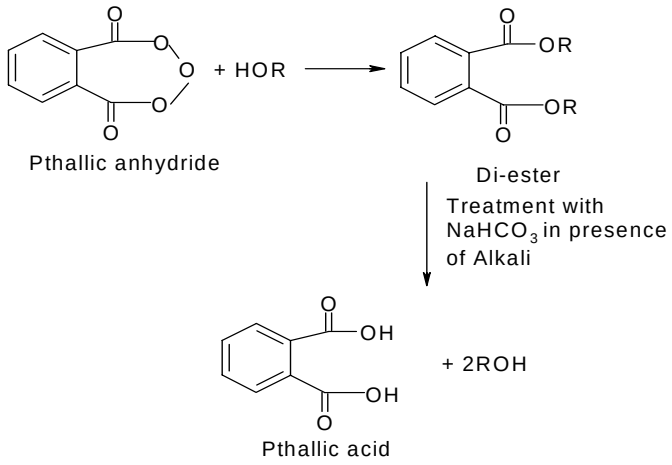


Fig.7. Separation of terpenoid alcohol

c. **Separation of terpenoid aldehyde and ketone:** Terpenoid aldehydes and ketones forms crystalline adduct on reaction with $NaHSO_3$ and phenyl hydrazines etc. These crystalline adducts can be hydrolyzed to get back carbonyl compounds.

Chemical test for terpenoids

1) Un-saturation test due to presence of double bond.
2) Addition reaction with H_2 + HX and forms characteristic addition product with NaCl and NaBr.
3) Undergoes polymerization.
4) Thermal decomposition yields isoprene.

Pharmaceutical Importance: It has great importance in perfumery, cosmetic, soap, food and pharmaceutical industry. Therapeutically these are used as antiseptic, stimulant, carminative diuretic, antihelminitic, analgesics, anti-rheumatic, aromatic and counter irritant. Some times these are also used as repellant and deodorant.

Volatile Oil/ Etheral Oil /Essential Oil

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

These are chemically derived from terpenes (mainly mono and sesqui terpenes) and their oxygenated derivatives. These are soluble in alcohol and other organic solvents, practically insoluble in water, lighter than water (Clove oil heavier), possess characteristic odour, have high refraction index and most of them are optically active. Volatile oils are colourless liquids but when exposed to air and direct sunlight these become darker due to oxidation. Unlike fixed oils, volatile oils neither leave permanent grease spot on filter paper nor saponified with alkalis.

Volatile oils are secreted in special structures like duct cells, schizogenous or lysogenous glands, trichomes and vittae etc. These are commonly found in the families like:

- Labiatae: e.g. *Oscimum sanctum,* Mentha
- Zingiberaceae: e.g. Cardamom, Ginger
- Umbelliferae: e.g. Coriander, Fennel, Caraway, Cumin
- Myrtaceae: e.g. Clove, Eucalyptus
- Lauraceae: e.g. Cinnamon
- Graminae: e.g. Lemon grass
- Compositeae: Flowers

Isolation of Essential oils: Perfume industry mainly utilize solvent extraction technique for volatile oils production using non polar or lipid solvent like ether, benzene etc. The solvent extraction techniques are advantageous over other methods like it is economical, cause less deterioration of volatile oils, solvent can be reused after distillation. Terpeneless volatile oils have more prices in perfumery industries because of their specificity and stability. They are processed by removing hydrocarbon and undesired products by the fractional distillation. These are obtained from the plant parts by distillation, by mechanical methods like ecuelle or sponge, by extraction with non-volatile or volatile solvents.

Classification of volatile oils: Volatile oils are classified on the basis of functional groups present as given in Table 6.

Table 6: Classification of volatile oil

Groups	**Examples**	**Drugs**
Hydrocarbons	Acyclic monoterpene: e.g. Myrecene Monocyclic monoterpene: e.g. Limonene, α and β phellandrene, α-terpinene etc. Bicyclic monoterpene: e.g. α and β pinene Sesquiterpenes: e.g. Zingiberene and Caryophyllene	Turpentine oil
Alcohols	Acyclic terpenes: e.g. Geraniol, Coriandrol etc. Cyclic terpenes: e.g. Menthol and borneol Sesquiterpenes: e.g. Santalol and Gingerol	Coriander Mentha and camphor Chandan and Ginger
Aldehydes	Acyclic: e.g. Citral, Geranial, Citronellal etc Cyclic: Cinnamicaldehyde, Cumin aldehyde, Safranal	Cymbopogon sp., Lemon Cinnamon, Cumin and Safron
Ketones	Monoterpene ketone (Monocyclic): e.g. Menthone, Carvone, Peperitone and Pulegone etc Diterpene ketone (Bicyclic): e.g. Camphor, Thujone and Jatamansone	Mentha, Caraway Spearmint and Dill Camphor, Jatamanshi
Oxides	Cineol and Ascaridol	Eucalyptus, Cardamom and Chenopodium oil
Phenolics	Eugenol, Thymol and Carvacrol	Clove and Thyme
Phenolic ethers	Anethol, Safrol, Myresticin and Dillapiol	Fennel,Anise,Nutmeg,Dill
Esters	Borneol acetate, Terpeneol acetate and Geraniol acetate Methyl salicylate Vatrates Pyrethrines and Jasmolines	Common Oil of wintergreen Valerian Pyrethrum
Lactones	Costus lactone Santonin Cantheridine (Lactone and anhydride)	Costus or Saussurea Santonica Cantharides

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

Storage of volatile oils: Volatile oils are liable to oxidation on storage in presence of air, moisture and light. The oxidation is followed by the change in color, increase in viscosity and change in ordour. Hence, volatile oils must be stored in well-closed completely filled containers and away from light in cool places.

Pharmaceutical applications of Volatile Oils: Volatiles are used as flavoring 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 repellant: e.g. Citronella
10. Source of vitamin A: e.g. Lemon grass

Resins

Resins are amorphous mixture of essential oils, oxygenated products of terpenes and carboxylic acids, obtained as exudates from plants and considered as end product of metabolism. These are solid or semi solid amorphous products of complex chemical nature usually insoluble in water but soluble in organic solvent like alcohol, volatile oils, fixed oils, benzene and ether.

Resins are poorly defined chemically but more related to each other in their physical properties and their appearance. Generally these are complex mixtures of several compounds however isoprene (C_5H_8) units are the fundamental building blocks of all true resins. These are non-crystalizable translucent masses, soften and melt on heating, burns with smoky flames on ignition, heavier than water and contain large number of carbon atoms. The thin film of resin on drying becomes hard and transparent which is unaffected by moisture and air. These are usually found in homogenous combination with other plant metabolites and hence, collectively known as resin combinations.

The exact mechanism of formation in unknown, but it seems to be the product of oxidation of volatile oils followed by polymerization. They usually formed in shizogenous and shizolysogenous cavities or ducts.

Distribution of resins: Resins are widely distributed in the animal and plant kingdom, occurs in different secretory structures like resin cells (Ginger), Schizo or Schizolysogenous ducts or cavities (*Pinus sp.*) and in glandular hairs (Cannabis). These are present in plant kingdom mainly in seed bearing plants whereas absent in thallophytes.

Pteridophytes (negligible): It present in family polypodiaceae i.e. in male Fern (*Dryopteris flix-mass*) composed of oleoresins.
Gymnosperms: Present in family pinaceae (Pinus sp.), commonly known as Colophony and Tar, which mainly composed of hydrocarbons.
Angiosperms-
Monocots- Cucurbitaceae: Colocynth (*Citrulus colosynthis*).
Dicots: Leguminosae: Tolu Balsam; Dipterocarpaceae: Gurjan Balsam; Burseraceae: Myrrh; **Umbelliferae**: Asafoetida; Zingiberaceae: Ginger and Turmeric; Cannabinaceae: Cannabis; **Convovulaceae**: Jalap (*Ipomea sp*.).
Animals: Lac (*Lacifer lacca*)

Classification of resins: These are classified on the basis of their occurrence, formation and chemical nature as given below:
1. **On the basis of their formation**
a. Physiological resins- These are formed as a normal product of metabolism without making injury to the plants and mainly present in specialized cells like:
Schizogenous glands: e.g. Copaiba
Secretion cells: e.g. Ginger
Oil glands: e.g. Clove
Oil ducts: e.g. Umbelliferous fruits

b. Pathological Resins- These are formed as a result of wound, injury or abnormal circumstances the plant gets shock and produces number of resin ducts. e.g. Benzoin, Colophony, Balsams, Aloe resin etc.

2. On the basis of chemical nature
A. Resin acids: These are high molecular weight acids, also called resinolic acids. On combination with alkali these forms their metallic salts known as resonates. Resin acids are generally found in free state or in combination with resin alcohols, as esters. e.g. Colophony: Abietic acid, Copaiba: Copavic acid, Myrrh: Commiphoric acid, and Shellac: Alleurhetic acid

B. Resin alcohols: These are high molecular weight compounds also known as resinols and occur either in free form or as esters in combination with balsamic acids or resin acids. These are tetra or penta cyclic alcohols and usually derivatives of amyrines.
e.g. Gurjan balsam- (gurjuresinols), Gauecum Resins- (gaucoresinols), Storax- (storesinols).

C. Resin phenols (Resinotannols): These are also high molecular weight compounds with phenolic group, occurs either in free form or as esters. Resinotannols are soluble in aqueous

alkali solution due to formation of phenoxide. However these are insoluble in water and soluble in alcohol and ether. These gives color reaction with $FeCl_3$. e.g Peru balsam-(peruresinotannols), Tolu balsam- (toluresinotannols), Benzoin- (siaresinotannols).

D. Ester Resins: These are esters of resin alcohol or resinotannols with resin acids or balsamic acids and on treatment with alkali mostly converted to free acids. e.g. Benzoin- Conyferyl benzoate, Storax- cinnamyl cinnamate

E. Resenes: These are complex neutral chemically inert high molecular weight hydrocarbons generally found in free state; neither shows any characteristic property nor effected by moisture and light. Resenes are soluble in chloroform and benzene but insoluble in water. e.g. Asafoetida (assaresene), Gum copal, Colophony, Gutta parcha and Dammar etc.

3. On the basis of occurrence with other secondary metabolites

Resins are generally found in homogenous combinations with other plant constituents and hence collectively known as resin combinations. These can be simply classified on the basis of its association with other constituents.

1. Oleoresins: These are homogenous mixtures of resins and volatile oils and depending on the amount of volatile oil these may exists as liquid, solid or semisolid. e.g. Copaiba, Canada balsam, Capsicum, Ginger and Turpentine.

2. Gum resin: These are naturally occurring mixtures of resin with gum. e.g. Gamboage and Ammoniacum.

3. Oleogum resins: These are naturally occurring combinations of volatile oil, gum and resin. e.g. Myrrh, Guggal, Asafoitida, Olibanum and Gamboage.

4. Glycoresins: Resins some times occur in combination with sugars as glycosides and also called as glucoresins, if glucose is the sugar part. These can be hydrolyzed with acids to get glycone and aglycone part. e.g. Jalap, Ipomoea and Podophyllum.

5. Balsams: These are naturally occurring resinous mixtures containing large amount of cinnamic acid, benzoic acids and or their esters. Their solubility in hot water varies with the content of free balsamic acids. Balsams containing higher amount of free acids are partially soluble in hot water. e.g. Benzoin, Balsam of tolu, Balasam of peru, Storax etc.

Isolation of resins: The isolation of resins is a tedious job because of its complexity in chemical composition and presence of different combination. However, it can be isolated from plants or animal by one of the following methods:

a. By extracting the drug with alcohol and precipitating resins present in concentrated extract by addition of large proportion of water. e.g. Jalap, Ipomea and Podophyllum.
b. By distillation for separation of oils. e.g. Copaiba, Colophony etc.
c. By heating the plant parts. e.g. Guacum
d. As plant exudates by making incision. e.g. Myrrh, Asafoetida, and Balsams
e. By collecting fossil resins. e.g. Copal and Kaury
f. By processing the incrustations. e.g. Shellac

Chemical tests of resins:

a. Solubility test: Resins are insoluble in water, rarely soluble in light petroleum (except Colophony and Dammar) soluble in alcohol, ether, acetone, chloroform, fixed oils and volatile oils etc.

b. Turbidity test: Resinous drug was extracted with alcohol and water is added in excess to form turbidity, because these are insoluble in aqueous solutions.

c. HCl test: One gram of drug was extracted with few ml of acetone and 3ml of dilute HCl was added. Formation of pink colour after heating the solution on water bath for 30 minutes indicates presence of resins.

d. $FeCl_3$ test: Few drops of $FeCl_3$ solution was added to the alcoholic extract of drug. Formation of greenish blue color indicates presence of resins.

Pharmaceutical application of resins: Generally resins are local irritant and hence act as local cathartics (e.g. Jalap and Ipomea), as anti cancer (Podophyllum), in bronchial asthma (e.g. Cannabis) also used externally as mild antiseptic in the form of tinctures (Benzoin) ointment and plasters (Turpentine and Colophony). Resins are also used in the preparation of emulsion and sustained released formulations.

Tannins

Tannins are mixture of complex organic, non-nitrogenous substances derivatives of polyhydroxy benzoic acid (polyphenols) with ability to precipitate proteins and having high molecular weight (500 to > 20000). These are non-crystalizable, astringent substances, soluble in water (forming colloidal solution), dilute alkali, alcohol, glycerol and acetone while sparingly soluble in ethyl acetate, chloroform and other organic solvents. Aqueous solutions of tannins are acidic in nature due to presence of free phenolic and carboxylic groups. It precipitates heavy metals, alkaloids, glycosides and gelatin (proteins) from solutions. Tannins combined with proteins by cross-links making raw animal skin to leather and on application to living surface it renders proteins resistant to proteolytic enzymes (astringent property), which forms the basis of therapeutic application of tannins.

Historically, tannin containing drugs are related to their tanning property i.e. their ability to transform fresh hides into imputrescible leather, which is due to the formation of hydrophobic bonds between collagen fibers that imparts resistance to water, heat and abrasion.

Tannins are widely distributed in plant kingdom; localized in specific plant parts such as leaves, fruits, barks or stem; often found in immature fruits and disappear during ripening. These are mainly present in higher plants in solution form in cell saps and vacuoles. The hydrolysable tannins are characteristic to dicot plants while condensed tannins are found in all plants including pteridophytes and Gymnosperms.

Classification of tannins: These are mainly classified in two categories on the basis of their chemical nature and biogenetic origin.

1. **Hydrolysable tannins:** These are ester of sugars and phenolic acid molecules (gallic acid and ellagic acid), hydrolyzed by mineral acids, alkalis and tannase enzyme yielding gallic acid or hexahydroxydiphenic acid with sugar (mainly glucose). Hydrolyzable tannins on dry distillation yield pyrogallol and on reaction with $FeCl_3$ produce bluish black precipitate. These are further sub divided into gallotannins and ellagitannins on the basis of their product of hydrolysis.

a**. Gallotannins:** These are the simplest one also known as galloylglucose, in which a glucose core is surrounded by five or more galloyl ester groups and on hydrolysis yields gallic acids (Fig. 8) and glucose molecule. The molecular weight of gallotannins ranges between 1000-1500. e.g. Nutgal, Rhubarb, Clove and Chest nut etc.

b**. Ellagitannins:** This is composed of phenolic acids as dimer of gallic acid i.e. hexahydroxydiphenic acids (HHDP) and attached glucose molecule, molecular weight ranging between 1000-3000. These on hydrolysis yield HHDP (readily lactonised to give ellagic acid, Fig. 8) with glucose molecule. e.g. Oak, Myrobalan, Pomegranut bark etc.

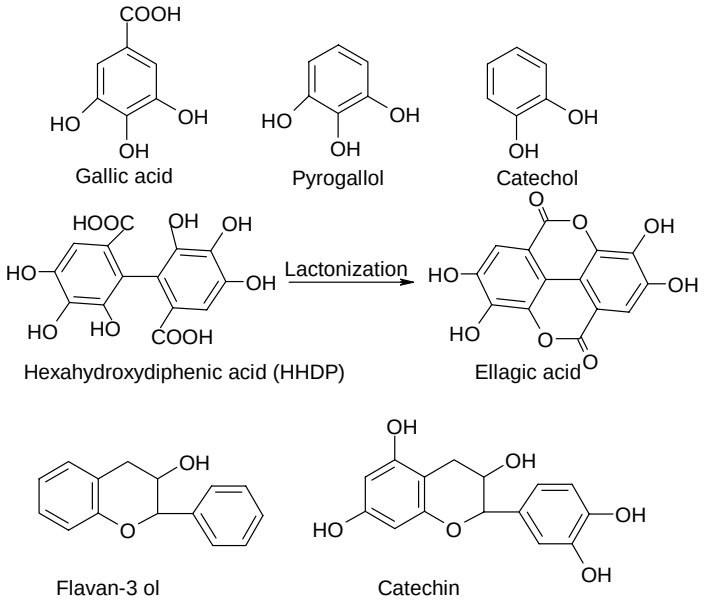


Fig.8. Precursors of tannins

2. Condensed tannins: These are also called flavolans, proanthocyanidins, nonhydrolyzable tannins, catechol tannins and phlobatannins, molecular weight ranging between 1000-3000. Condensed tannins are resistant to hydrolysis, does not contain sugar molecule, derivatives of flavones like catechin, flavan –3-ol (Fig. 8), flavan 3-4 diol etc. Condensed tannins on treatment with acids or enzyme converted to a red water insoluble compound called as phlobaphene which imparts typical brownish color to many of the plant materials mainly bark. Thses on dry distillation gives catechol; on treatment with ferric chloride give brownish green color, with dil. HCl yields phloroglucinol that gives red colour with matchstick on reaction with lignin, with

vanillin HCl gives red colour and precipitated with bromine water. e.g. Green tea, Cinnamon bark, Cinchona bark, Willd cherry bark, Oak, Coca, Catechu etc.

3. Complex tannins: These are formed by the combination of hydrolyzable and condensed tannins. The formation of complex tannins occurs by the union of C-C bond between C1 of glucose unit of ellagitannins and C8 or C6 of flavone –3-ol. These are present in dicot plants mainly in family Rosidae and Dilenidae etc.

4. Pseudo tannins: These are also known as prototannins or tannin precursors, high molecular weight compounds do not respond to protein precipitation test (Gold beaters skin test) with the molecular weight ranging between 200-600. e.g. Gallic acid, Chlorogenic acid, Nux-vomica seed etc.

Extraction and isolation of tannins: General methods for extraction of tannins are as follows:

1. Fresh or lyophilized drug containing tannins are extracted with water-acetone mixture and filtered.
2. Filtrate is distilled to remove acetone and aqueous extract remaining is then extracted with dichloromethane to remove lipids and fats.
3. Aqueous extract is further extracted with ethyl acetate to separate demeric proanthocyanidine and gallotannins.
4. The remaining aqueous phase contains polymeric proanthcyanidins and high molecular weight gallotannins.
5. Further, desired compounds are separated by appropriate chromatographic technique.

Chemical tests for tannins: Tannins show specific chemical reaction like solution of tannins precipitate gelatin, alkaloids, salt of Cupper, Lead and Tin etc. and shows color reaction with $K_2Cr_2O_7$, chromic acid and iron salts.

1. Test with Iron salts: It show color reaction with iron salt like $FeCl_3$ and potassium ferrrocyanide $K_4Fe(CN)_6$ in presence of ammonia. Addition of $FeCl_3$ solution to the solutions of hydrolysable tannins forms bluish black precipitate whereas with condensed tannins it forms greenish brown coloured precipitate.

2. Goldbeater's skin test: Goldbeater's skin is the membrane prepared from ox intestine and behaves like untanned hide. Small piece of ox-intestine is dipped in 2% dilute HCl, rinse with distilled water then soaked in test solution for few minutes again rinsed with distill water and transfer to 1% solution of Ferrous sulphate. Formation of brownish black color indicates the presence of tannin. This is positive for all true tannins but negative for pseudo tannins.

3. Gelatin test: To the aqueous solution of gelatin (1% w/v) solution of gelatin 0.5-1.0% solution of tannin was added, formation of buff coloured precipitate indicates presence of tannins. Pseudo tannins also show this test positive if tannin is present in sufficient amount.

4. Phenazone test: Aqueous extract of drug (5 ml) was mixed with 0.5 gm of solid sodium acid phosphate ($NaHPO_4$), heated the solution to boiling, cooled and filtered. Filtrate was treated with 2% solution of phenazone drop wise to form bulky precipitate of all tannins.

5. Test for catechin (Matchstick test): Catechins forms phlorogluecinol when heated in presence of acids and can be detected by reaction with lignin forming woody red to magenta colour. The paste of test drug (tannin) was applied on the rear end of matchstick and moistened with conc. HCl. Formation of woody pink to magenta colour on heating near the flame indicates presence condensed tannins.

6. Test for Chlorogenic acid: Extracts of drug containing chlorogenic acid on treatment with aqueous ammonia converted to green color after exposing with air.

7. Vanilline HCl test: Solution of test drug was mixed with few drops of vanilline HCl. Development of pink colour in presence of tannins due to conversion of phloroglucinol from catechin.

8. Bromine water test: Condensed tannins are precipitated in presence of bromine water.

Pharmaceutical applications of tannins: The basis of its use therapeutically is depends on its astringent property. Tannins are used in GIT problems, burns, leather industry and for the formation of inks. It precipitates proteins and traditionally used as styptic agent and internally for the protection of surface of mouth and throat. These also act as anti-diarrheal agent and antidotes in poisoning of heavy metals, alkaloids and glycosides. The use of tannins is reduced now days because tanninc acid causes necrosis of liver. Recently it has been demonstrated that tannins are useful as anti cancer and anti-HIV agent.

Suggested Readings:

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