COMPILED BY
Dr. NISHA SHARMA, ASSOCIATE PROFESSOR,
SUBJECT TEACHER, PHARMACOGNOSY & PHYTOCHEMISTRY-I ,
B.PHARM. IV SEM,
UNIT IV- INTRODUCTION TO SECONDARY METABOLITES

Syllabus: *Definition, classification, properties and test for identification of Alkaloids, Glycosides, Flavonoids, Tannins, Volatile oil and Resins*

PHYTOCONSTITUENTS: Plants are the natural bio-chemical factory to generate the various chemicals categorized as Primary and secondary metabolites.

Primary metabolites

- Distribution: These are widely distributed in plants and are simple molecules
- They are involved in various process in plants like reproduction, development & growth
- The isolation process of primary metabolites is quite easy and in-expensive, for example: starch isolation from potatoes
- Most of primary metabolites as such do not have therapeutic activity, but these are precursors for synthesis of important secondary metabolites
- Examples of Primary metabolite are: Carbohydrates, lipids, Proteins, amino acids, nucleic acid

Secondary metabolites

- Distribution: Their distribution is restricted to plant species or as per taxonomic classification. These are complex molecules generated in plants.
- They are involved in various processes like chemical adaptation, environmental stress, as protective chemicals against microbes, animals, insects.
- Their production and isolation is quite expensive as compared to primary metabolites.
- They have marked therapeutic activity ex: Antihypertensive: Rauwolfia, Anticancerous: Vinca etc.
- Various examples of secondary metabolites from plants include Alkaloids, Glycosides, Flavonoids, Terpenoids, Resins, Tanins etc.

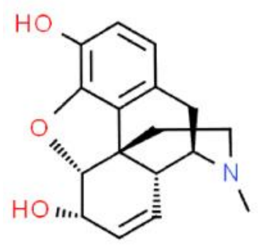
ALKALOIDS

- The term Alkaloid was coined by the German Scientist: **Carl F.W. Meissner** in **1815** Since they are basic or Alkali like, therefore the name coined was "**Alkal**oids"
- **Definition:** These are defined as group of naturally occurring organic compounds, basic in nature, containing 1 or more nitrogen atoms mostly present within heterocyclic ring, with specific physiological action.
- **Distribution**: They are found abundantly in angiosperms i.e. higher plants
- **Families rich in alkaloids** : They are abundantly found in various families like Apocynaceae (Rauwolfia) Rubiaceae (cinchona), Solanaceae (belladona), Papaveracea (opium), Berberidaceae (podoplyllum)
- **They are Present in various part of plants like**: Aerial parts: ex: Ephedra- ephedrine, Entire plant: Vinca- Vincristine, vinblastine, Leaves- Tea- Caffeine, Root- Rauwolfia- Reserpine, Bark- Cinchona- quinine, Seed: Nuxvomica- struchnine,Fruit- Black pepper- piperine, Latex: Opium- Morphine,codeine etc.
- **Uses:** Analgesic, Antimalarial, Antispasmodic, hypertension, mental disorders, anticancerous
- **Occurance**: They occur in plants as salts of organic acid (oxalic , citric, acetic, maleic, lactic, fumaric, acid etc)
- **Function in plants**: **Protective**: They are the protective agents of plants and protect plants against insects, herbivores (as of their bitterness, toxicity)
- Some of them are **Product of detoxification** (waste products)
- They are Reservoir for protein synthesis, **Source of N &** in case of deficiency are utilized in plants

A. CLASSIFICATION BASED ON ORIGIN

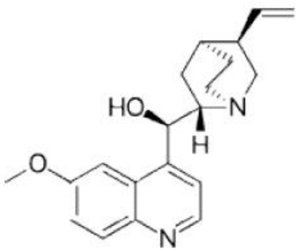
1. True alkaloid

- They contain Heterocyclic N atom within the ring
- They are obtained from various Amino acids directly (Tryptophan, tyrosine, PA, Lysine, ornithine, histidine etc.)
- They are Basic in nature and form water sol. Salts
- Various Examples include: Quinine, Atropine, Morphine



Morphine

Precursor is Tyrosine



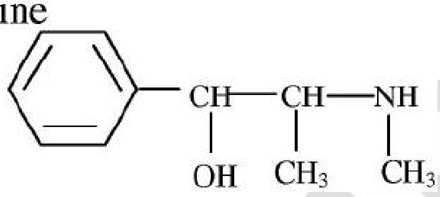
Quinine

Precursor is Tryptophan

2. **Proto alkaloid /Amino alkaloid**

- They contain N but not inside the heterocyclic ring. It is side chain.
- They are obtained from Amino acids, biological amine
- They are basic in nature
- Various Examples include: Colchicine, ephedrine

Ephedrine



Precursor is Phenyl alanine

3. **Pseudo Alkaloid**

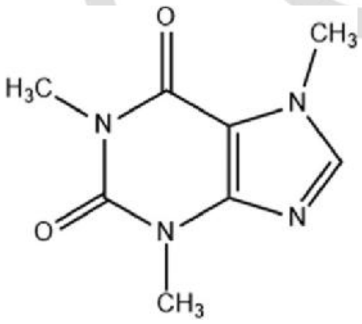
They contain Heterocyclic N atom

They are not derived from amino acid precusor

They are weakly basic nitrogenous compounds

Various Examples include: Purine alkaloids: example of purine alkaloid is caffeine obtained from Tea leaves

Steroidal alkaloids: Solanidine, conessine



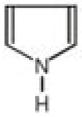
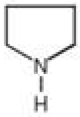
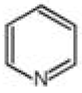
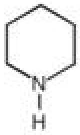
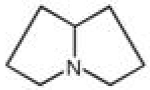
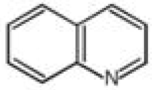
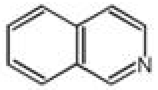
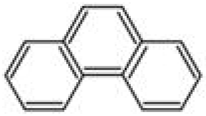
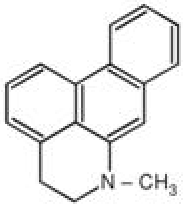
Caffeine

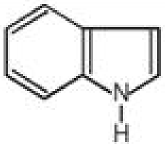
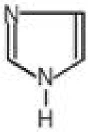
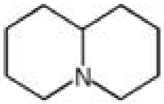
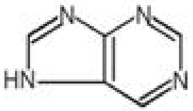
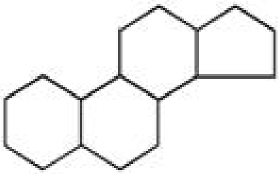
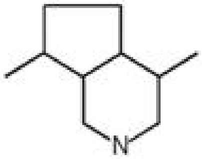
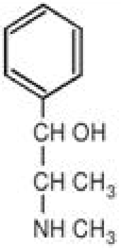
B. CLASSIFICATION OF ALKALOIDS

1. BIOSYNTHETIC	2. PHARMACOLOGICAL	3. TAXONOMICAL
• Based on types of precursor used for biosynthesis of alkaloid in plants • Same precursor same is group of alkaloids • Ex: ornithine: Pyrrolidine: Nicotine, Tropane: Atropine, cocaine • Lysine:Pipperidine & pyridine: Coniine, lobeline Quinolizidine: Lysine • Tryptophan:Indole: vincristine, reserpine, Quinoline: qunine • Histidine: Imidazole: pilocarpine • Phenylalanine: Amino alkaloid: ephedrine • Tyrosine: Isoquinoline: papaverine	• CNS: Caffeine, Strychnine • Anticancer: Taxol: Taxus, vincristine, VB • Bronchodialator Ephedrine (ephedra) vascisine (vasaka) • Narcotic analgesic Morphine: opium Codeine • Antihypertensive: Reserpine (rauwolfia)	Based on distribution in variuos plants Solanaceae: Tropane- Atropine, hyoscine Steroidal: Solanidine, withanolide Paplionaceae: Pyrolidine, Quinolizidine Grouped as per the genus ex. Ephedra, Cinchona

C. CLASSIFICATION OF ALKALOIDS BASED ON THE CHEMICAL RING

A. Heterocyclic Alkaloids (True Alkaloids)

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

Type	Basic ring structure	Examples
Indole (Benz pyrrole)		Ergometrine, ergotamine, reserpine, vincristine, vinblastine, strychnine, brucine, physostigmine
. Imidazole		Pilocarpine, Isopilocarpine, Pilosine
. Norlupinane		Cytisine, laburnine, lupanine, sparteine
. Purine (pyrimidine/imidazole)		Caffeine, theobromine, theophylline
. Steroidal (cyclopentano-perhydrophenanthrene ring)		Protoveratrine, solanidine, conessine, funtumine
	B. Non-heterocyclic alkaloids Pseudo alkaloids	
. Diterpene		Aconitine, Aconine, hypoaconitine
	Proto alkaloids (Amino – alkaloids)	
. Alkylamine (Amino alkaloid)		Ephedrine, pseudoephedrine. Mescaline, colchicine

PHYSICAL & CHEMICAL PROPERTIES OF ALKALOIDS

PHYSICAL PROPERTIES	CHEMICAL PROPERTIES
1. Crystalline solid, some are amorphous solid: emetine • Liquid: Nicotine, coniine (volatile) 2. Colorless, some are colored, Berberine, colchicine: yellow, Betanidine: orange 3. Solubility: Free bases soluble in organic solvents (ether, chloroform) • Alkaloidal salt sol. In water & alcohol • Exceptions: bases soluble in water (caffeine), ephedrine bases insoluble or sparingly soluble in organic solvent-Morphine in ether 4. Isomerisation: optically active isomers show physiological activities, l-ephedrine > d ephed 5. Bitter in taste, mainly poisonous	1. Basic in nature, lone pair of electrons in N atom 2. N exists as Primary (RNH2) Nor ephedrine Secondary (R2NH) Ephedrine Tertiary: (R3N) Atropine Quaternary ammonium: Tubocurarine 3. Basicity: R2NH> RNH2 >R3N Weak base: caffeine (unstable salt) Strong base: Atropine Amphoteric: Morphine Neutral alkaloids: Colchicine 4. Alkaloid + Acid → Akaloidal salt 5. Dilute alkali liberate most of alkaloid from its salt

IDENTIFICATION TEST OF ALKALOIDS

- General chemical Test
- Precipitation Reactions

1. **Dragendorff's test**: Potassium bismuth Iodide sol: Orange ppt
2. **Mayers Test**: Potassium mercuric iodide solution: Cream ppt
3. **Wagners test:** Iodine potassium iodide sol. : Reddish brown ppt
4. **Hagers Test**: Sat. picric acid sol. Yellow ppt

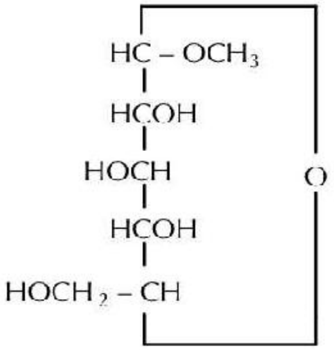
Specific Reagent test:

1. **Vitali morin test**: Tropane alkaloid+ fuming HNO_3 + alc. KOH sol. → violet color
2. **Van Urk's Test**: Ergot alk. (Indole)+ paradimethyl amino benzaldehyde (in dil. H_2SO_4+ traceof $FeCl_3$)→ Blue
3. Thalloquin test: Chinchona (quinine)+ Br_2 water+ dil. NH_3 sol. → Emrald green
4. Murexide Test : Caffeine (Purine) + $KClO_3$+ HCl → evaporate to dryness → dried residue exposed to NH_3 vapors → purple color

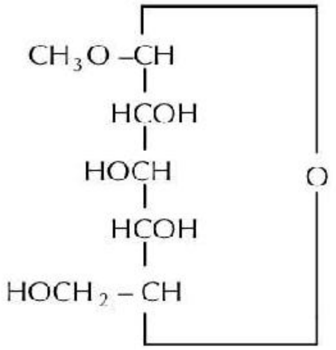
GLYCOSIDES

- Glycoside-It is a group of naturally occurring complex compd.

  sugar part (glycone)+ non sugar part (aglycone) = Glycoside
- Bond joining is glycosidic bond, formed b/w hemiacetal group of a saccharide and -OH group of an alcohol.
- The glycone and aglycone portions can be chemically separated by hydrolysis in the presence of acid. There are also numerous enzymes that can form and break glycosidic bonds.
- Chemically they are acetyl or sugar ethers formed by interaction of –OH of non sugar & sugar, with loss of water. –OH of aglycone- alcoholic/phenolic/ amines
- Sugars are of β D glucose, others- mannose, galactose, rhamnose, digitoxose, cymarose etc.



α - methyl glycoside



β - methyl glycoside

In plants β form exist

PROPERTIES OF GLYCOSIDES

Physical Properties	**Chemical Properties**
1. Crystalline/ amorphous, solid, non volatile 2. Solubility: sol. In polar solvents Exceptions: Resin glycoside Insoluble in other organic solvents CHCL3, Ether Aglycone solubility- Soluble in non polar organic solvent (C_6H_6, Ether) 3. Optically active, Laevo form active 4. Bitter taste except Glycyrrhizin, stevia 5. More the sugar units, more is polar solubility	1. Hydrolysis: Hydrolysed by water, mineral acids, & enzymes Glycosides → Aglycone + Glycone Exception: C glycosides are stable, undergo oxidative hydrolysis (Aloin) 2. Alkali Hydrolysis: Strong & mild alkali hydrolyses ether group. Opens lactone ring (cardiac glycosides) 3. Enzymatic hydrolysis: Emulsin hydrolyses β glycoside Maltase, invertase hydrolyse α glycoside

CLASSIFICATION

A. On the Basis of Glycone

Glycone group of a glycoside is glucose → glucoside;

If it is fructose → fructoside;

If glucuronic acid → glucuronide, etc.

B. On the Basis of Glycosidic Linkage

1. O-glycosides: Sugar molecule is combined with phenol or –OH group of aglycon, for example, higher plants: senna, rhubarb, Amygdaline, Salicin, cardiac glycosides, anthraquinone glycosides like sennosides etc.

Glycone –O H+HO − aglycone → Glycone −O− Aglycone + H_2O

2. **N-glycosides**: Sugar molecule is combined with N of the –NH (amino group) of aglycon, for example, nucleosides

Glycone –OH+H N − aglycone → Glycone− N − Aglycone + H_2O

3. **S-glycosides**: Sugar molecule is combined with the S or SH (thiol group) of aglycon, Isothiocyanate glycosides: for example, Sinigrin from black mustard

Glycone –OH+H S − aglycone → Glycone− S − Aglycone + H_2O

4. **C-glycosides**: Sugar molecule is directly attached with C—atom of aglycon, for example, Anthraquinone glycosides like Aloin, Barbaloin, Cascaroside and Flavone glycosides, etc.cochineal coloring matter- carminic acid

Glycone –OH+H C − aglycone → Glycone− C − Aglycone + H2O

C. On the Basis of Aglycone

Class	Example
Anthraquinone glycosides	Senna, Rhubarb, Aloe etc.
Sterol or cardiac Glycoside	Digitalis, Thevetia, Squill etc.
Saponin glycosides	Dioscorea, Liquorice, Ginseng etc.
Cyanogenetic & Cyanophoric Glycosides	Bitter Almond, Wild cherry bark etc.
Thiocyanate & Isothiocyanate Glycosides	Black mustard etc.
Flavone Glycosides	Ginko etc.
Aldehyde Glycosides	Vanilla etc.
Phenol Glycosides	Bearberry etc.
Steroidal Glycosides	Solanum etc.
Bitter & Misc. Glycosides	Gentian, Picrrohiza, Chirata etc.

IDENTIFICATION TEST

- Chemical Tests for Anthraquinone Glycosides
- ***Borntrager's test***

1 gm of drug + 5–10 ml dil. HCl, boil on water bath for 10 min, filter. extract filterate with CCl_4/ benzene. Add equal amount of NH_3, shake. Pink or red colour in ammonical layer due anthraquinone moiety.

- ***Modified borntrager's test***

1 gm of drug +5 ml dil. HCl + 5 ml $FeCl_3$ (5% w/v). Boil for 10 min on water bath, cool and filter, extract filterate with CCl_4/ benzene, add equal volume of NH_3 solution → formation of pink to red colour due to anthraquinone moiety. This is used C-type of anthraquinone glycosides.

CHEMICAL TESTS FOR SAPONIN GLYCOSIDES

Haemolysis test

A drop of blood on slide+ mix with few drops of aq. Saponin solution → RBC's rupture in presence of saponins.

Foam test

1 gm drug + 10–20 ml H_2O, shake for few minutes → frothing persists for 60–120 s in presence of saponins

IDENTIFICATION TEST FOR STEROID & TRITERPENOID GLYCOSIDE

- ***Libermann burchard test***

Evaporate alcoholic extract of drug to dryness, extract with $CHCl_3$ + few drops of acetic anhydride followed by conc. H_2SO_4 from sides of test tube → violet to blue coloured ring at the junction of two liquid, indicate the presence of steroid moiety.

- ***Salkowaski test***

Evaporate alcoholic extract of drug to dryness, extract with $CHCl_3$ + conc. H_2SO_4 from sidewall of test tube to extract → yellow coloured ring at the junction of two liquid, which turns red after 2 min, indicate the presence of steroid moiety.

- ***Antimony trichloride test***

Evaporate alcoholic extract of drug to dryness, extract with $CHCl_3$ + saturated solution of $SbCl_3$ in $CHCl_3$ containing 20% acetic anhydride → Formation of pink colour on heating indicates presence of steroids and triterpenoids.

- ***Zimmermann test***

Meta dinitrobenzene solution + alcoholic solution of drug containing alkali + heat → violet colour in presence of keto steroid

IDENTIFICATION TEST FOR CARDIAC GLYCOSIDE

- ***Keller-kiliani test***

Alcoholic extract of drug + equal vol. of H_2O + 0.5 ml of strong lead acetate solution shake, filter. Filtrate + eq. vol. $CHCl_3$. Evaporate $CHCl_3$ extract to dryness, Dissolve residue in 3 ml of gl. CH_3COOH +few drops of $FeCl_3$ solution. Transfer resultant solution to a TT containing 2 ml conc. H_2SO_4 → Reddish brown layer is formed, turns bluish green after standing due to presence of digitoxose.

- ***Legal test***

Alcoholic extract of drug + equal vol. of H_2O + 0.5 ml of strong lead acetate solution , shake, filter. Filtrate + eq. vol. $CHCl_3$. Evaporate $CHCl_3$ extract to dryness, Dissolve residue in 2 ml of pyridine and sodium nitropruside 2 ml + NaOH to make alkaline → pink colour in presence of glycosides or aglycon moiety.

- ***Baljet test***

Thick section of leaf of digitalis or the part of drug containing cardiac glycoside + sodium picrate solution → yellow to orange colour in presence of aglycones or glycosides.

- ***3,5-dinitro benzoic acid test***

Alcoholic sol. Drug + drops of NaOH+ 2% sol. of 3,5-dinitro benzoic acid → pink colour indicates presence of cardiac glycosides

TEST FOR COUMARIN GLYCOSIDES

- **$FeCl_3$ test**

Conc. alcoholic extract of drug + few drops of alcoholic $FeCl_3$ solution → deep green colour, turn to yellow on addition of conc. HNO_3 , indicates presence of coumarins.

- ***Fluorescence test***

Alcoholic extract of drug + mixed with 1N NaOH (one ml each) → blue-green fluorescence indicates presence of coumarins.

CHEMICAL TESTS FOR CYNOPHORIC GLYCOSIDE

- ***Sodium picrate test***

Powdered drug + moistened with H_2O + drops of conc. H_2SO_4 in a C Flask

Filter paper impregnated with sodium picrate solution followed by Na_2CO_3 sol. trapped on the neck of flask using cork → brick red colour due to volatile HCN in presence of cynophoric glycosides

CHEMICAL TESTS FOR FLAVONOID GLYCOSIDES:

- ***Ammonia test***

Dip Filter paper in alcoholic sol. of drug + expose to NH_3 → yellow spot filter paper

- ***Shinoda test***

alcoholic extract of drug + Mg turning + dil. HCl → red colour (Flavonoids)

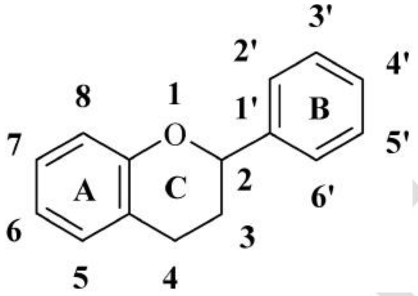
alcoholic extract of drug + Zn turning +dil. HCl → deep red to magenta colour, presence of dihydro flavonoids.

- ***Vanillin HCl test***

Vanillin HCl + alcoholic sol. of drug → pink colour due to presence of flavonoids.

FLAVONOIDS

- Class of plant secondary metabolites
- Word Flavonoids derived from latin word: “Flavus”= yellow
- Group of polyphenolic compounds which are found in fruits, flowers, seeds & vegetable
- Structure of flavonoids The flavonoids are possessing 15 carbon atoms; two benzene rings joined by a linear three carbon chain the skeleton can be represented as the C6 - C3 - C6 system.



PROPERTIES OF FLAVONOIDS

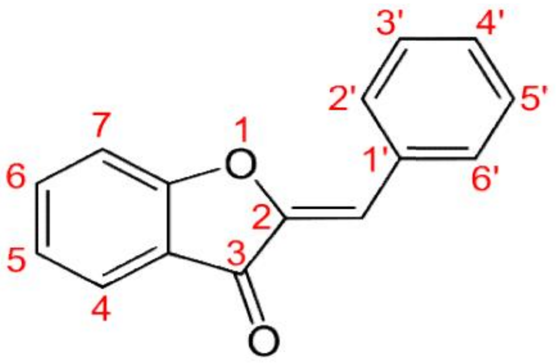
Physical Properties	Chemical properties
1. Crystalline solids, sharp MP 2. Solubility: in H2O & alcohol (Flavonoid glycoside) Non glycosidic flavonoid: Aglycon part-sol. in organic solvents 3. Color: Flavonones, Flavanol, Isoflavones- Colorless Flavonols, Flavones: Yellow, Chalchones, aurones: Y, Orange Anthocyanidine acid med.: Red In basic: Blue 4. Flavanols: optically active	1. 15 C skeleton, 2 benzene linked by heterocyclic pyran ring 2. Being phenolic, dissolves in alkalies → Yellow sol. +HCl → colorless 3. Glycosidic linkage located at 3 or 7 C 4. Flavanones, Flaonoes are unstable compound , on oxidation → Chalcones, leucocyanidines 5. Flavonoid + $FeCl_3$ → green/ purple/ red brown color

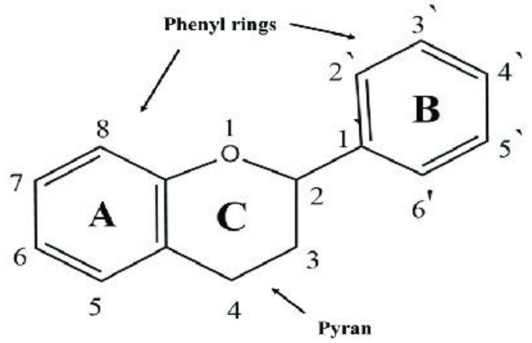
FLAVONOIDS

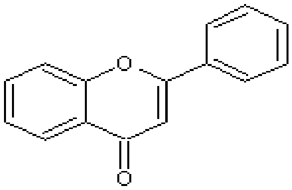
- The three-carbon (-C3-) may be included through an oxygen bond between the two phenyl rings into:

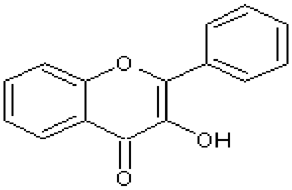
1- A five-membered heterocyclic ring (furan) as in aurones.

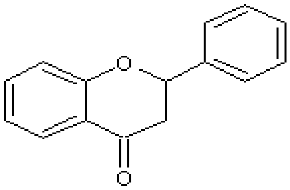
2- A six-membered heterocyclic ring (pyran) to give flavonoids which constitute the largest group. The flavonoid aglycone consists of a benzene ring (A) condensed with a six membered ring (C) pyran ring, which at 2nd position adds a phenyl ring (B) as a substituent. C6-C3-C6 structure

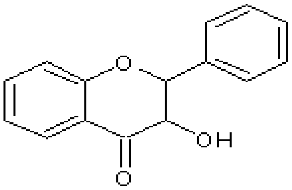


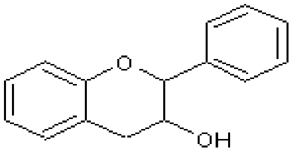


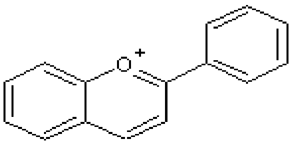

Flavones

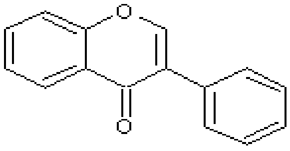

Flavonols

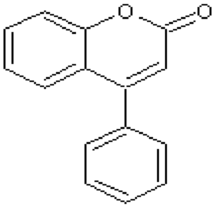

Flavanones

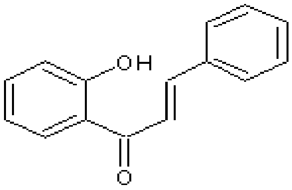

Flavanonols


Flavanols


Anthocyanidins


Isoflavones


Neoflavonoids


Chalcones

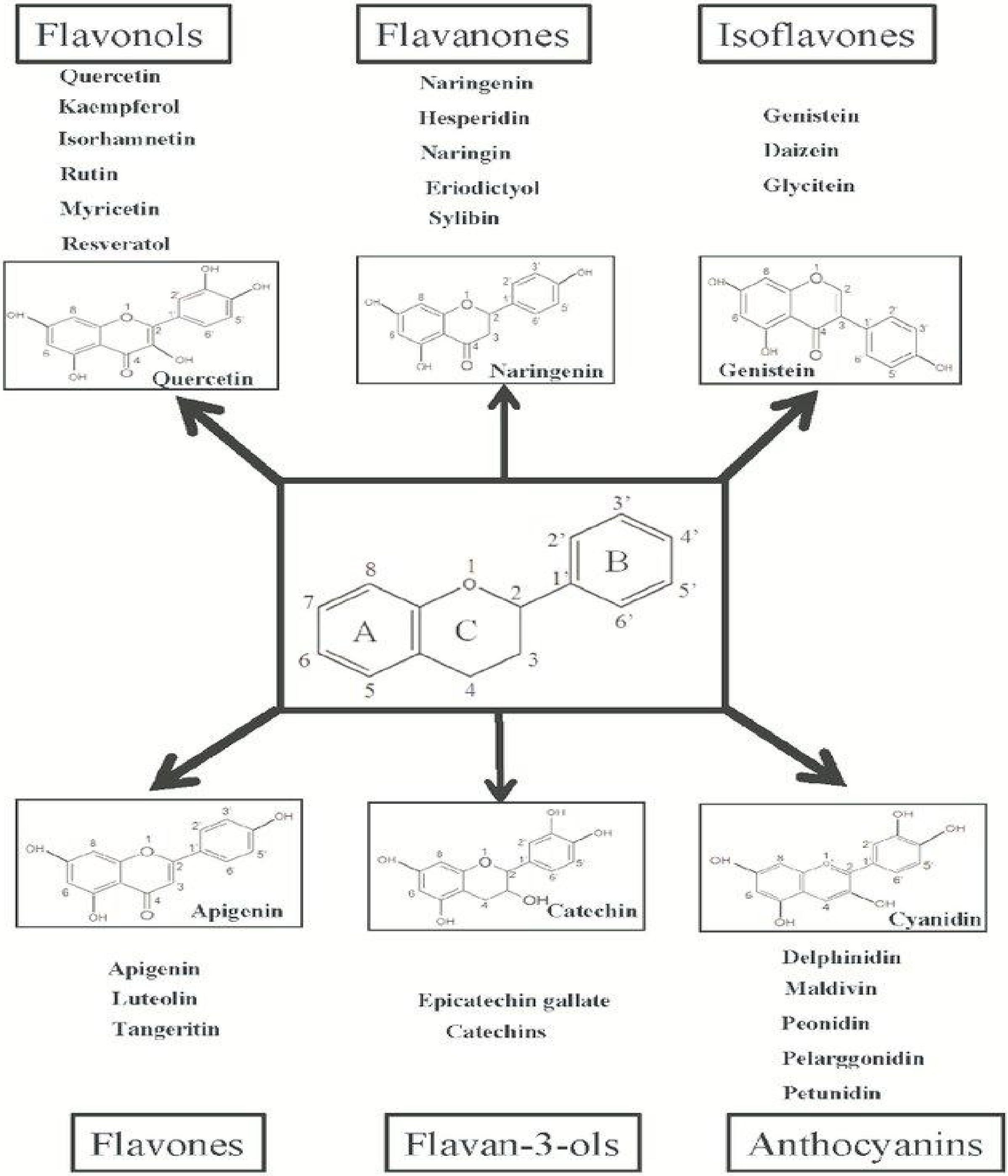
CLASSIFICATION OF FLAVONOIDS

- Depending on the carbon of the C ring on which B ring is attached, and the degree of unsaturation and oxidation of the C ring.
- B ring is linked in position 3 of the ring C are called isoavones;
- B ring is linked in position 4, neoavonoids,
- B ring is linked in position 2 further subdivided on the basis of the structural features of the C ring.
- These subgroup are: avones, avonols, avanones, avanonols, avanols or catechins and anthocyanins.
- Finally, avonoids with open C ring are called chalcones.

1. **Flavones** (2-phenylchromen-4-one)
   - Have a double bond between positions 2 and 3 and a ketone in position 4 of the C ring. ex:- Apigenin, Luteolin
2. **Flavonols** (3-hydroxy-2-phenylchromen-4-one)
   - Have a hydroxyl group in position 3 of the C ring, which may also be glycosylated. Ex: Kaempferol, Rutin, Myricetin, Quercetin.
3. **Flavanones**/ dihydroavones (2,3-dihydro-2-phenylchromen-4- one
   - Have C ring saturated; the double bond between positions 2 and 3 is saturated, ex: Hesperetin, Hespereidin, Naringenin

Subclassified: furanoavanones, prenylated avanones, pyranoavanones or benzylated avanones

4. **Flavanonols** (dihydroavonols)/ 3-hydroxy-2,3-dihydro-2-phenyl chromen-4-one
   - Are 3-hydroxy derivatives of avanones; ex:Taxifolin, Silymarin
5. **Isoflavones** / phytoestrogens.3-Phenylchromen-4-one skeleton
   - subgroup of avonoids in which the B ring is attached to position 3 of the C ring. have structural similarities to estrogens, such as estradiol
6. **Neoflavonoids ,** Isoflavonoids and the Neoflavonoids can be regarded as abnormal flavonoids.
   - They have the B ring attached to position 4 of the C ring.
7. **Flavanols** or avan-3-ols or catechins
   - Also referred to avan-3-ols as the hydroxyl group is bound to position 3 of C ring
   - Another important feature of avanols, particularly of catechin and epicatechin, is the ability to form polymers called as proanthocyanidins or condensed tannins. Ex: Catechin (β-OH) 2- Epicatechin (α-OH)
8. **Anthocyanidins** (Flavylium (2-Phenylchromenylium) ion skeleton
   - Sugar units are bound mostly to position 3 of the C ring and they are often conjugated with phenolic acids, such as ferulic acid.
   - Color of the anthocyanins depends on pH, methylation or acylation at the hydroxyl groups on the A and B rings. Ex: Cyanidin. 2- Delphenidin.
9. **Chalcones and dihydrochalcones** are avonoids with open structure



Flavonols

Quercetin
Kaempferol
Isorhamnetin
Rutin
Myricetin
Resveratol

Flavanones

Naringenin
Hesperidin
Naringin
Eriodictyol
Sylibin

Isoflavones

Genistein
Daizein
Glycitein

Flavones

Apigenin
Luteolin
Tangeritin

Flavan-3-ols

Epicatechin gallate
Catechins

Anthocyanins

Delphinidin
Maldivin
Peonidin
Pelarggonidin
Petunidin

IDENTIFICATION OF FLAVONOIDS

1. **Reaction with $AlCl_3$** : Give yellow color with $AlCl_3$ but fluoresce differently in UV as shown in the following table .

Light	Flavones	Flavonols	Flavanones
Visible	Yellow	Yellow	Yellow
UV	Green	Yellowish- Yell. Green	Pale brown

2. **Lead subacetate test**:- all flavonoids give yellowish precipitate with Pb subacetate.

3. **Shinoda test** for flavanones and flavonols:

alcoholic solution + Mg metal ⁄ HCl → orange, red or violet color

4. **Antimony pentachloride** test for chalcones:

alcoholic solution + SbCl5⁄CCl4 → red or violet color

TANNINS

- Name ‘tannin’ –derived French ‘tanin’ (tanning substance), denotes range of natural polyphenols.
- Tannins- complex organic, nonnitrogenous plant products, generally have astringent properties.
- Denotes large group of compounds, distributed in the plant kingdom.
- Term ‘tannin’- first used by Seguin , 1796 to denote substances having ability to combine with animal hides to convert them into leather which is known as tanning of the hide.
- According to this, tannins are substances which are detected by a tanning test due to its absorption on standard hide powder → test is Goldbeater’s skin test.

TANNINS CLASSIFICATION

Two major groups on the basis of Goldbeater’s skin test.

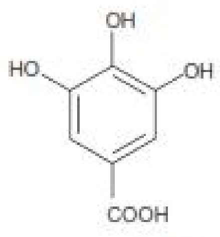
- **True tannins**- show positive tanning test, high molecular weight compounds, complex polyphenolics
- **Pseudotannins-** partly retained by the hide powder and fail to give the test

Based on hydrolytic reaction and nature of phenolic nuclei involved in the tannins structure, Two major chemical classes of tannins:

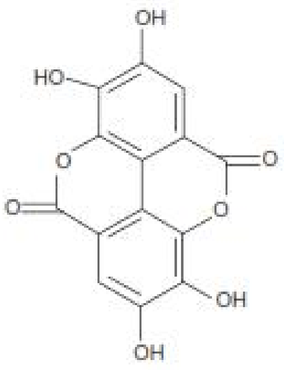
- **hydrolysable tannin * condensed tannins**
- **Hydrolysable Tannins**: hydrolysed by mineral acids or enzymes such as tannase. Invloves many molecules of polyphenolic acids such as gallic, hexahydrodiphenic, or ellagic acids, bounded through ester linkages to a central glucose molecule
- **On the basis of the phenolic acids** produced after the hydrolysis, they are further categorized under
- **Gallotannins** composed of gallic acid or

Ellagitannins -contains hexahydrodiphenic acid which after intraesterification produces ellagic acid.

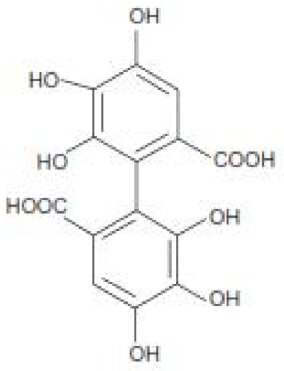
- **Hydrolysable tannins**- Known as pyrogallol tannins- as the components of phenolic acids on dry distillation are converted to pyrogallol derivatives.
- They are soluble in water, and their solution produces blue colour with ferric chloride.



Gallic acid



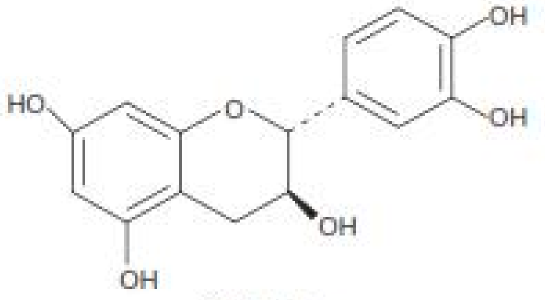
Ellagic acid



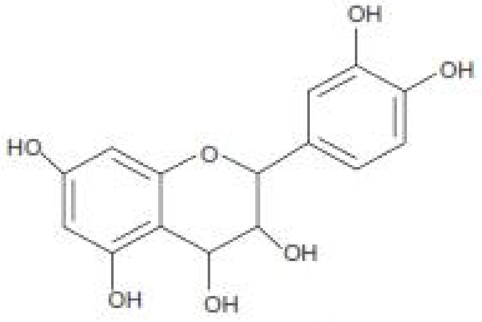
Hexahydroxydiphenic acid

NONHYDROLYSABLE OR CONDENSED TANNINS/ PROANTHOCYANIDINS

- not readily hydrolysable to simpler molecules with mineral acids and enzymes,
- contain only phenolic nuclei which are biosynthetically related to flavonoids.
- Catechin, found in tannins is flavan-3-o1, leucoanthocyanidins are flavan-3,4-diol structures. linked to carbohydrates or protein molecules to produce more complex tannin compounds.
- Treated with acids or enzymes, polymerize yielding insoluble red coloured products – phlobaphens
- Phlobaphens give red colour to many drugs such as cinchona and wild cherry bark. On dry distillation, they yield catechol derivatives.
- Condensed tannins are also soluble in water and produces green colour with ferric chloride.
- include Rosaceae, Geraniaceae, Leguminosae, Combretaceae, Rubiaceae, Polygonaceae, Theaceae, etc
- Cruciferae and Papaveraceae - totally devoid of tannins



Catechin



Leucoanthocyanidin

- **PSEUDOTANNINS**
- Pseudotannins are simple phenolic compounds, low molecular weight. do not give Goldbeater's skin test. Gallic acid, Chlorogenic or the simple phenolics such as catechin are pseudotannins found in plants, especially in dead tissues and dying cells.
- **PROPERTIES OF TANNINS**

1. Give colloidal solutions with water.
2. Non crystalline substance.
3. Soluble in water (exception of some high molecular weight structures), alcohol, dilute alkali, and glycerin.
4. Sparingly soluble in ethyl acetate.
5. Insoluble in organic solvents, except acetone.
6. Molecular weight ranging from 500 to >20,000.
7. Oligomeric compounds with multiple structure units with free phenolic groups.
8. Can bind with proteins and form insoluble or soluble tannin—*protein complexes.*

TANNINS IDENTIFICATION TEST

- ***Goldbeater's skin test:*** *Goldbeater's skin is a membrane* from the intestine of Ox. It behaves just like untanned animal hide.

A piece of goldbeaters skin previously soaked in 2% HCl + wash D H_2O + placed in a solution of tannin for 5 minutes + wash with D H_2O and transferred to 1% $FeSO_4$solution. A change in colour of the goldbeater's skin to brown or black indicates the presence of tannin.

- Hydrolysable and condensed tannins give +ve
- pseudotannins show very little colour or –ve test.
- ***Phenazone Test:***

5 ml of aq. Sol. of tannin containing drug+0.5 g of sodium acid phosphate. Warm, cool, and filter + 2% phenazone solution to the filtrate. All tannins are precipitated as bulky, coloured precipitate

- ***Gelatin Test:***

1% gelatine solution, + little 10% sodium chloride + 1% sol. of tannin → precipitation of gelatine from solution.

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

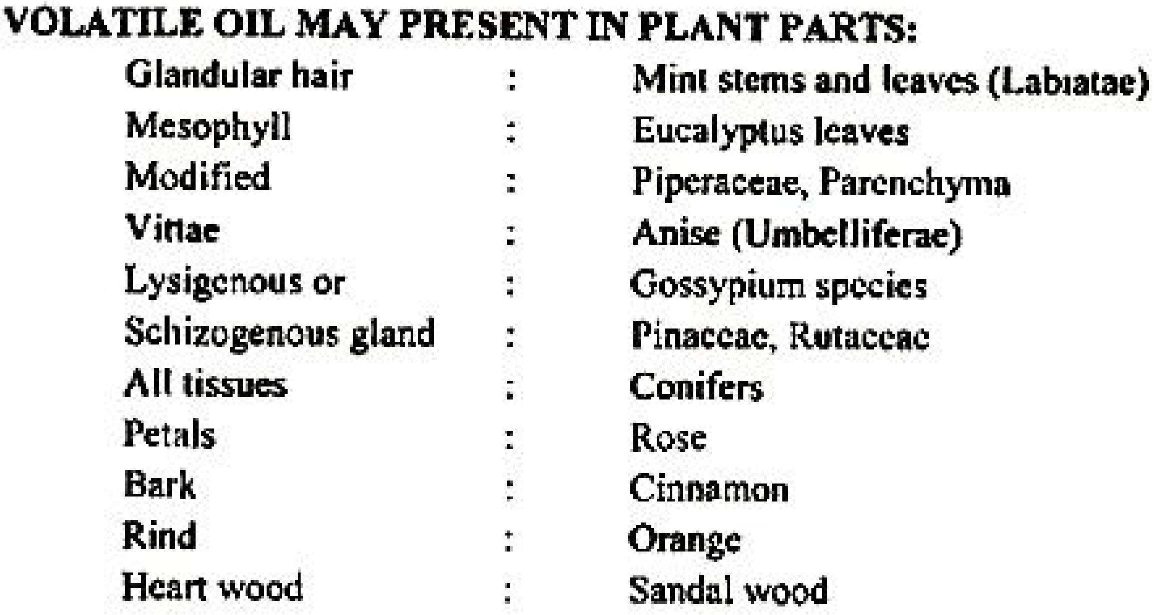
A dilute solution of chlorogenic acid + extract + aq. NH_3 , exposed to air, slowly turns green → presence of chlorogenic acid.

- Vanillin-hydrochloric acid test:

Drug + mixture of vanillin: alcohol : dilute HCl (1:10:10) The reaction produces phloroglucinol which along with vanillin gives pink or red colour.

VOLATILE OILS

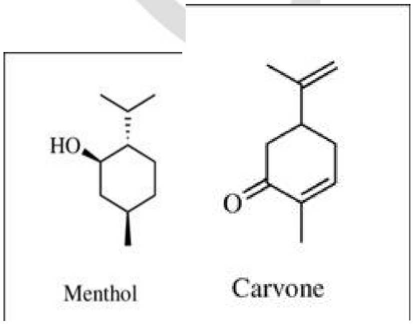
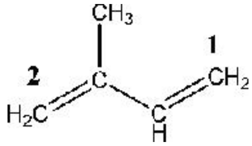
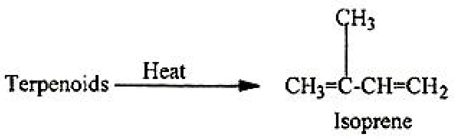
- These are Odorous volatile principles of plant & animal source, they evaporate if exposed to air at ordinary temperature, hence known as volatile or etheral oils.
- Essence of active constituents of the plant - known as essential oils.

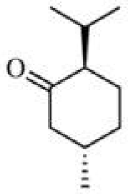
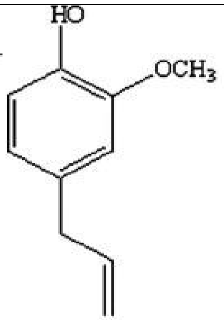


VOLATILE OIL MAY PRESENT IN PLANT PARTS:

Glandular hair	:	Mint stems and leaves (Labiatae)
Mesophyll	:	Eucalyptus leaves
Modified	:	Piperaceae, Parenchyma
Vittae	:	Anise (Umbelliferae)
Lysigenous or	:	Gossypium species
Schizogenous gland	:	Pinaceae, Rutaceae
All tissues	:	Conifers
Petals	:	Rose
Bark	:	Cinnamon
Rind	:	Orange
Heart wood	:	Sandal wood

PROPERTIES OF VOLATILE OILS

Physical Properties	Chemical Properties
1. Volatile in nature 2. Have characteristic odor & flavor 3. Solubility: Immiscible in water, fairly sol. in alcohol, freely sol. in ether, CHCl3 4. Lighter than water, exception: clove oil is heavier 5. Most of them are colorless liq. 6. Have ↑ Refractive index ex. Cinnamon oil: 1.562-1.582 7. Most of them are optically active Cumin oil: +4 to +8  Menthol Carvone	1. They are derived from terpene Mostly mono & sesquiterpenes & their oxygenated derivatives i.e. terpenoids 2. Terpenes are built of Isoprene unit (C_5H_8)  3. They are open chain or cyclic unsaturated compound, have 1 or more double bonds 4. Thermal decomposition: Isoprene as one of the product  $\text{Terpenoids} \xrightarrow{\text{Heat}} CH_3{=}C(CH_3){-}CH{=}CH_2$ Isoprene 5. Have olefinic bond, easily oxidised by all oxidising agents 6. Terpenoids undergo addition reaction with

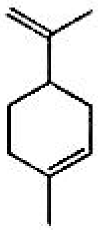
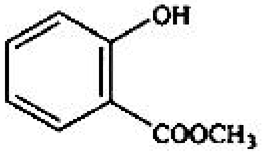
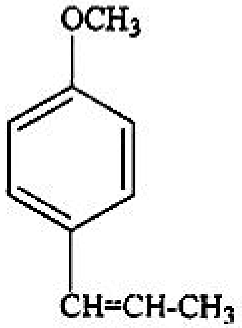
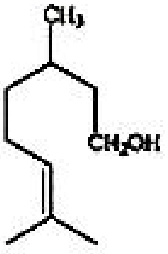
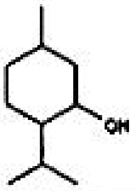
 *p*-Menthone  Eugenol	H_2 & halogen acids.

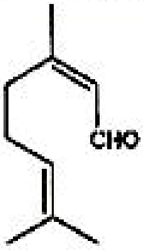
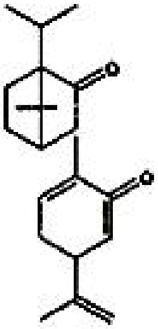
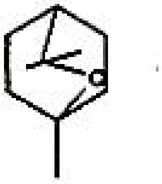
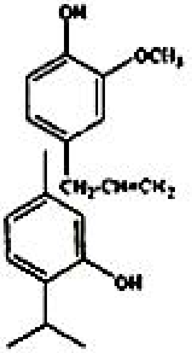
CLASSIFICATION

- Volatile oils are classified on the **basis of functional groups present**

• Groups	• Drugs
• Hydrocarbons	• Turpentine oil
• Alcohols	• Peppermint, sandalwood oil
• Aldehydes	• Lemongrass, cinnamon, cassia, saffron
• Ketones	• Camphor, caraway, dill, jatamansi, fennel
• Phenols	• Clove, ajowan, tulsi
• Phenolic ethers	• Nutmeg
• Oxides	• Eucalyptus, cardamom, chenopodium oil
• Esters	• Valerian, rosemary, garlic, gaultheria oil

CLASSIFICATION OF VOLATILE OIL

S.No	Class	Structure	Constituents	Name of Drugs
1	Hydrocarbon volatile oil			
a.	Monocyclic		Limonene	Cardamom
b.	Bicyclic		α-Pinine	Coriander oil
2	Esters volatile oil		Methyl salicylate	Winter green oil
3	Ether volatile oil		Anethole	Fennel, Anise
4	Alcohol volatile oils			
a.	Acyclic		Citronellol	Lemon grass
b.	Monocyclic		Menthol	Peppermint

5	Aldehydes Volatile oil		Citral	Lemon
6	Ketone volatile oil		Camphor Carvone	Camphor Caraway oil
7	Oxides volatile oils		Cineole	Eucalyptus oil
8	Phenol volatile oils		Eugenol Thymol	Clove Mint

TERPENOIDS

- Group of naturally occurring compounds
- Terpenes or Terpenoids are volatile substances which give plants and flowers their fragrance.
- Term 'terpene' was given to the compounds isolated from terpentine, a volatile liquid isolated from pine trees.
- Terpenoids are the hydrocarbons of plant origin of the general formula $(C_5H_8)_n$ as well as their oxygenated, hydrogenated, and dehydrogenated derivatives.
- The simpler mono and sesquiterpenes is the chief constituent of the essential oils obtained from sap and tissues of certain plant and trees.
- The di- and triterpenoids are not steam volatile. They are obtained from plant and tree gums and resins.
- Tertraterpenoids form a separate group of compounds called 'Carotenoids'.
- The term 'terpene' was originally used to describe a mixture of isomeric hydrocarbons with molecular formula $C_{10}H_{16}$ occurring in the essential oils. But more general term is 'terpenoids', which includes hydrocarbons and their oxygenated derivatives.

CLASSIFICATION TERPENOIDS

Classified on the basis of **number of carbon atoms** present in the structure

Number of carbon atoms	Value of n	Class	Formula
10	2	Monoterpenoids	$C_{10}H_{16}$
15	3	Sesquiterpenoids	$C_{15}H_{24}$
20	4	Diterpenoids	$C_{20}H_{32}$
25	5	Sesterpenoids	$C_{25}H_{40}$
30	6	Triterpenoids	$C_{30}H_{48}$
40	8	Tetraterpenoids	$C_{40}H_{64}$
>40	>8	Polyterpenoids	$(C_5H_8)_n$

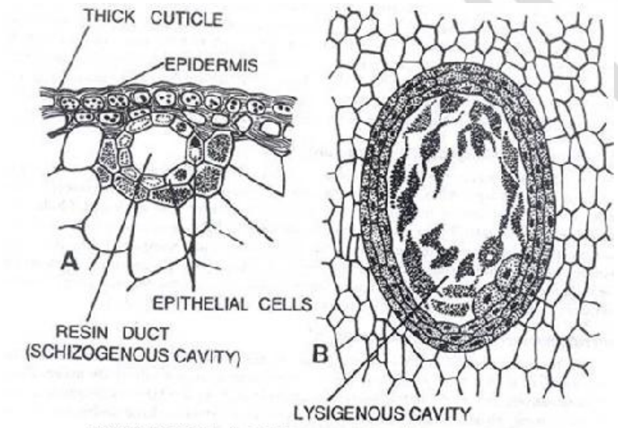
IDENTIFICATION TESTS

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

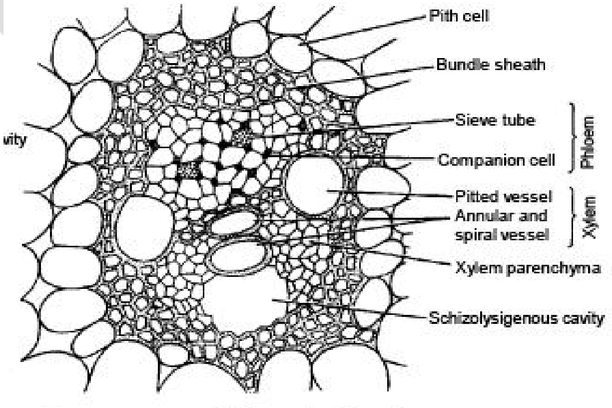
1. Thin section of drug + alcoholic solution of Sudan III → red colour → confirm volatile oils.
2. Thin section of drug + tincture of alkana → red colour → presence of volatile oils
3. Volatile oil smeared on thin paper → Temporary stain → disappears on exposure
4. Specific test for camphor: natural camphor +vanillin + H_2SO_4 (1:100) → Yellow → changes to red, violet and finally blue (series of color changes)

RESINS

- Resins: Solid/semisolid amorphous products of complex chemical nature containg large number of C atom.
- These are mixture of essential oil, oxygenated products of terpenes & carboxylic acids.
- Source: Natural- found in Most of the plant parts or excudate of plant because of injury or incision made
- Ex: In plants: Asafoetida (Hing), Benzoin (lobaan), Ginger, Podophyllum, Capsicum etc
- From animals: Shellac (Lac), From Fossils ex: Copal
- Distributes mainly in Spermatophyta i.e. seed plants, sometimes in ferns (Pteridophyta)
- In higher plants- they are end products of their metabolites
- Occurance: secreted in special form of structures (may be internally or surface)
- In Ginger: Resin cells, In cannabis- Glandular hair, In pine- Schizogenous or schizolysogenous ducts or cavities
- Schizogenous: developed by separation or splitting apart of cells, enclosing a cavity which then enlarges, lined by secretory epithelial cells, formed by the division of surrounding cells.
- Lysigenous: cavities in the bodies of plants form because of break down of certain cells. considered to originate from a single cell by the division in different directions forming a solid mass of secreting cells and then by the gradual disintegration or solution of the resulting cells starting from the center.
- Schizolysigenous: develops at first schizogenously but, then increases in size by the break down of the bounding cells producing secretion



intercellular spaces. A, a schizogenous intercellular space in *Bryophyllum* ster B, a schizogenous intercellular space in leaf of *Sequoia sempervirens*.



A Vascular Bundle

PROPERTIES OF RESINS

PHYSICAL	CHEMICAL
• Transparent/ Translucent in nature (solid/ semisolid) • Specific gravity greater than water i.e. heavier than water (0.9-1.25) • When heated- soft, melt as sticky or adhesive fluid	• Present as mixture of various chemical compd. • Complex mixture of resin acid, ester, alcohol, HC etc. • Enriched with Carbon, but no N, some have O

• Amorphous rarely crystalline • Solubility : insol. In H_2O Soluble in organic solvents Exception colophony insol. in ether Resin + Heat → Burn smoky flame	• Amorphous mixture of essential oil, oxygenated product of terpenes, carboxylic acid • Get darken on atmospheric oxidation • Acid resin + aklali → Resin Soap

CLASSIFICATION OF RESINS

1**. Based on process of formation**

- **Physiological resin:** Formed as normal product of metabolism Ex: cannabis, podophyllum, ginger
- **Pathological resins:** Formed as a result of wound, injury or abnormal circumstances: Ex: benzoin, asafoetida, guggul

2. **On the basis of occurance with other secondary metabolites** (Resin combination)

i) **Oleo resin :** Naturally occuring mixture of resin & volatile oil ex: Ginger, capsicum, copaiba

ii) **Gum Resin:** Resin associated with gum example: colophony, cannabis

iii) **Oleo Gum Resin**: Mixture of volatile oil, Gum & resin Ex: Guggul, Asafoetida, Myrrh

iv) **Balsam Resin:** Resinous mixture of benzoic acid/ cinnamic acid or esters of tehse acids in free or combined form

Ex: Benzoin, Tolu balsam, peru balsam

v) **Glycoresins:** occur in combination with sugar by Glycoside linkage Ex: Jalap, Podophyllum, Ipomoea

3) Depending upon the chemical nature of predominent chemical constituent

i) **Resin acid** (Resinolic acid) : Found in free or esterified form, Large proportion of oxy acids, soluble in alkali forming soap, derivatized as Metallic salt forming Resinates, Ex: Abietic acid-Colophony, commiphoric acid- Myrrh, Ferulic acid- Asafoetida

ii) **Resin alcohol** (Resinols): Complex alcohol of high mol. Weight, found in free or ester with balsamic acid or acid resin, gives –ve test with $FeCl_3$, Ex: Cannabinol- cannabis, Gingerol-Ginger, Nenzoresinol- Benzoin

iii) **Resin Phenol** (Resinotannols): Contain phenolic group, high Mol. Wt. , Give color reaction with $FeCl_3$, occur as free or ester Ex: Peruresinotannol- Balsam of peru

4**) Resin ester**: Ester is chief constituent- Ester of resinols/ resinoltannols with resin acid/ Balsamic acid Ex: Coniferyl benzoate, Benzyl benzoate- Benzoin, Cinnamyl cinnmate- Storax

5) **Resones:** Complex natural inert substances, don't undergo hydrolysis, don't form salt, no specific chemical properties Ex: Asaresenes- Asafoetida,

6) **Glucoresin**: combined with sugar by glycosylation & produce glucoresin. Can be hydrolysed to aglycon & glycon portions.

IDENTIFICATION TEST FOR RESIN

1. **Benzoin:** Benzoin + Ether → shake→ Extract +H_2SO_4→ sumatra benzoin deep brown color & siam benzoin (deep purple color)

2. **Colophony** : Drug + acetic anhydride + 1 drop of Conc. H_2SO_4

Purple color that rapidly changes to violet color

3. **Myrrh**: Triturate with solvent ether → Filter → Evaporate thin film → violet color on contact with Br_2 vapor

Triturate with water → Yellow emulsion

4. **Podophyllum**: Drug+ alcohol → Macerate → Filter → Filtrate + strong copper acetate solution → Brown ppt

5. **Tolu balsam**: Acidic to litmus

Drug + $FeCl_3$ → Green color (Resinotannol)