UNIT-III

Isolation, Identification and Analysis of Phytoconstituents

- **Terpenoids** : Menthol, Citral, Artemisin
- **Glycosides** : Glycyrhetinic acid & Rutin
- **Alkaloids** : Atropine, Quinine, Reserpine, Caffeine
- **Resins** : Podophyllotoxin, Curcumin

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Terpenoids

- The terpenoids sometimes called isoprenoids, are a large and diverse class of naturally occurring organic chemicals derived from the 5-carbon compound isoprene.
- Terpenoids contribute to the scent of eucalyptus, the flavors of cinnamon, cloves, and ginger, the yellow color in sunflowers, and the red color in tomatoes.
- Well-known terpenoids are citral, menthol, camphor.
- The **terpenoids** usually have a number of such **isoprene units ($CH_2=C(CH_3)-CH=CH_2$ or (C_5H_8)** joined together in a head to tail manner.

Classification of Terpenoids

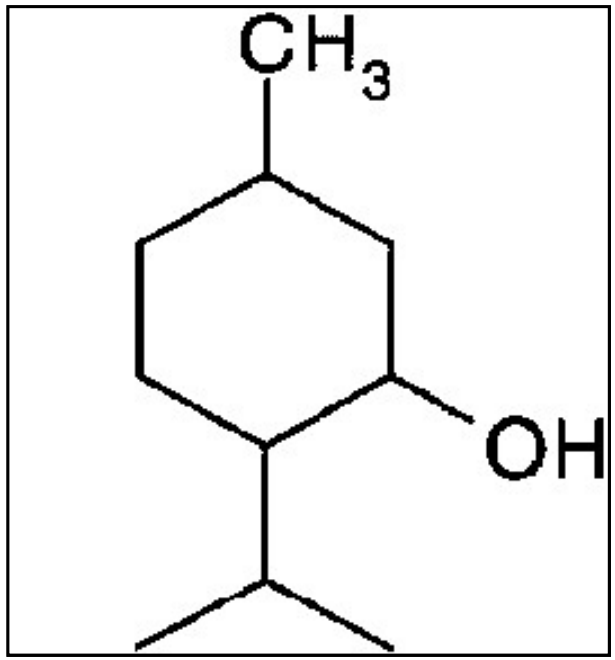
Class	No. of Isoprene unit (C_5H_8)	Formula
Isoprene	01	C_5H_8
Monoterpenes	02	$C_{10}H_{16}$
Sesquiterpenes	03	$C_{15}H_{24}$
Diterpenes	04	$C_{20}H_{32}$
Triterpenes	06	$C_{30}H_{48}$
Tetraterpenes	08	$C_{40}H_{64}$
Polyterpenes	N	$(C_{10}H_{16})n$

Menthol

- Mentha is a monoterpenoid obtained from ***Mentha piperita,*** family Lamiaceae.

Extraction and Isolation:

- Accurately weight quantity of coarse powder of ***Mentha piperita*** *parts just before flowering.*
- *Extract the peppermint oil by water distillation method*
- *Separate the oil and allow cooling.*
- *Crystals of menthol will separate out.*
- *Collect the crystals by centrifugation.*
- *Recrystallise from acetone.*



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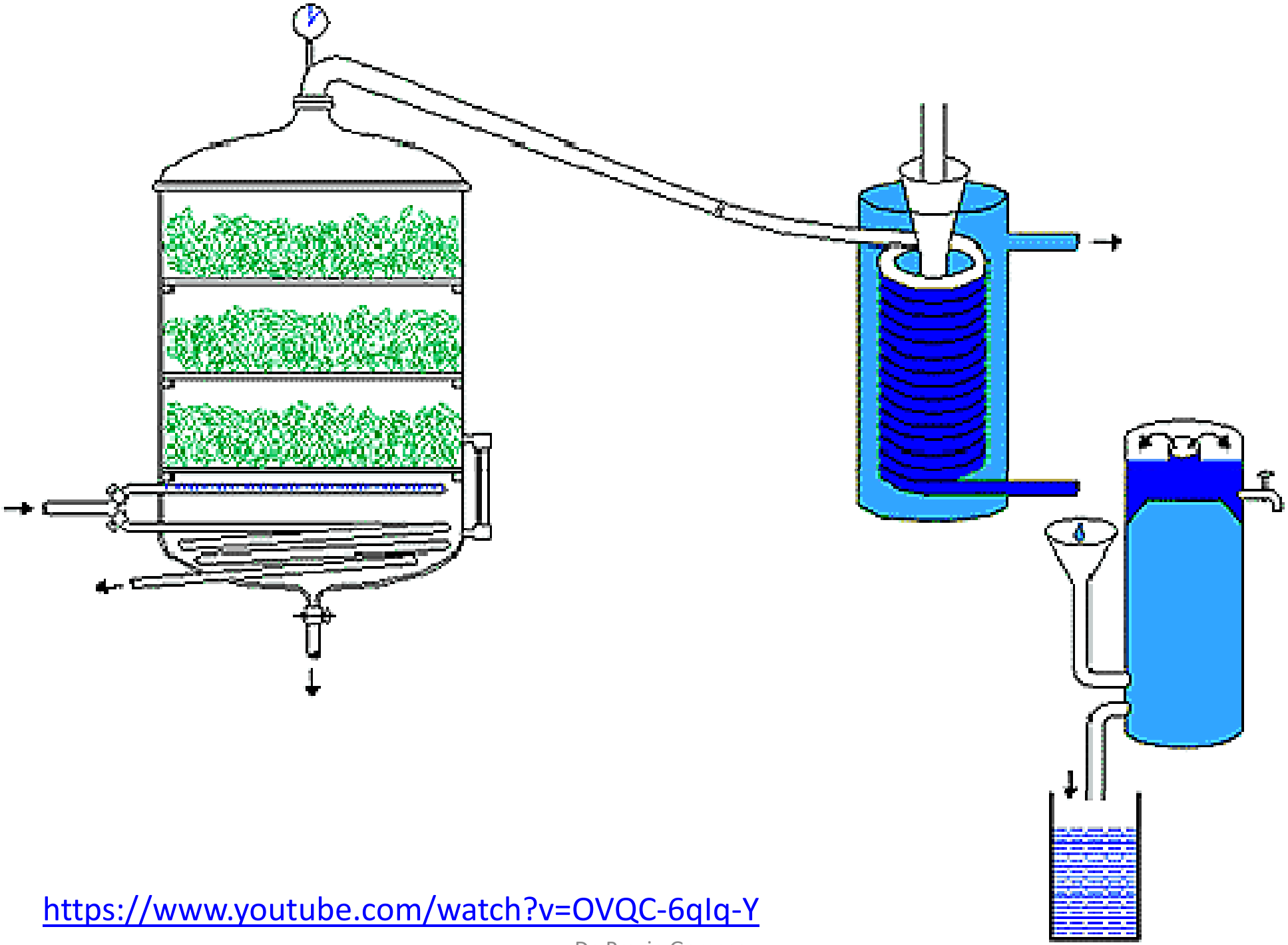

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Identification:

1. Few drops of sample + 5 ml nitric acid heat on water bath----- liq. Develops blue colour, after some time it becomes yellow.
2. 1 mg Menthol + 1 ml methanol

- Spot applied on silica gel G plate
- Run with plane chloroform (100 %)
- Dried plate sprayed with 1 % vanillin-sulphuric acid reagent and heated at 110 C for 10 min.
- Shows Rf value 0.48-0.62.

https://www.youtube.com/watch?v=ch8UkjdIAE8

Analysis:

- Take10gm sample + 10 ml acetic anhydride + 2gm Sodium acetate, shifted to reflux
- Cool and then add 30 ml water, and boil with continue stirring for 15 min.
- Transfer to separating funnel and separate oil layer.
- Wash oil layer with water up to neutral.
- Then add 2 gm Sodium sulphate and shake
- Allow for 30 min and filter
- To oil layer add 5 ml ethanol + 25 ml Alc.KOH + Phenapthaleine indicator.
- Reflux above mix. For 1 hr And titrate with HCL

- **Formula for calculation of menthol**

$$\% \text{ Methanol} = \frac{7.814 \times (c-a)\{0.021 \times 9c-b)/U\}}{A - 0.021 \times (c-a)}$$

Where,

a & b = ml of HCL for oil

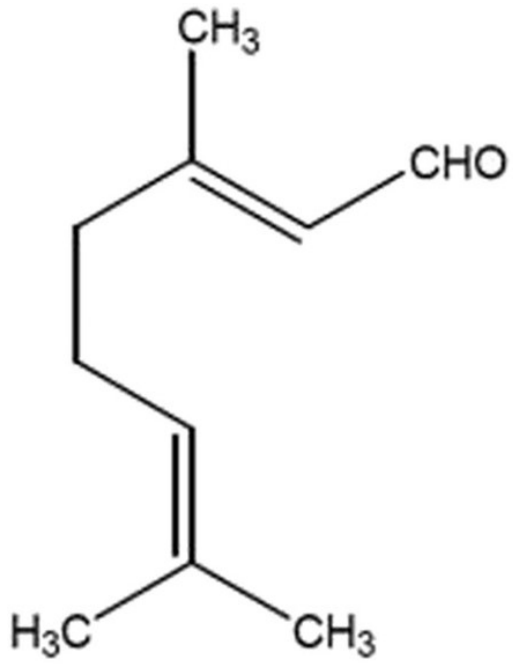
c = ml of HCL for blank

U = Unacetylated oil

A = Acetylated oil

Citral

- Citral is a monoterpens aldehyde found in variety of sources which include lemon grass, ***Cymbopogon flexuosus, C. martini*** family Graminae.

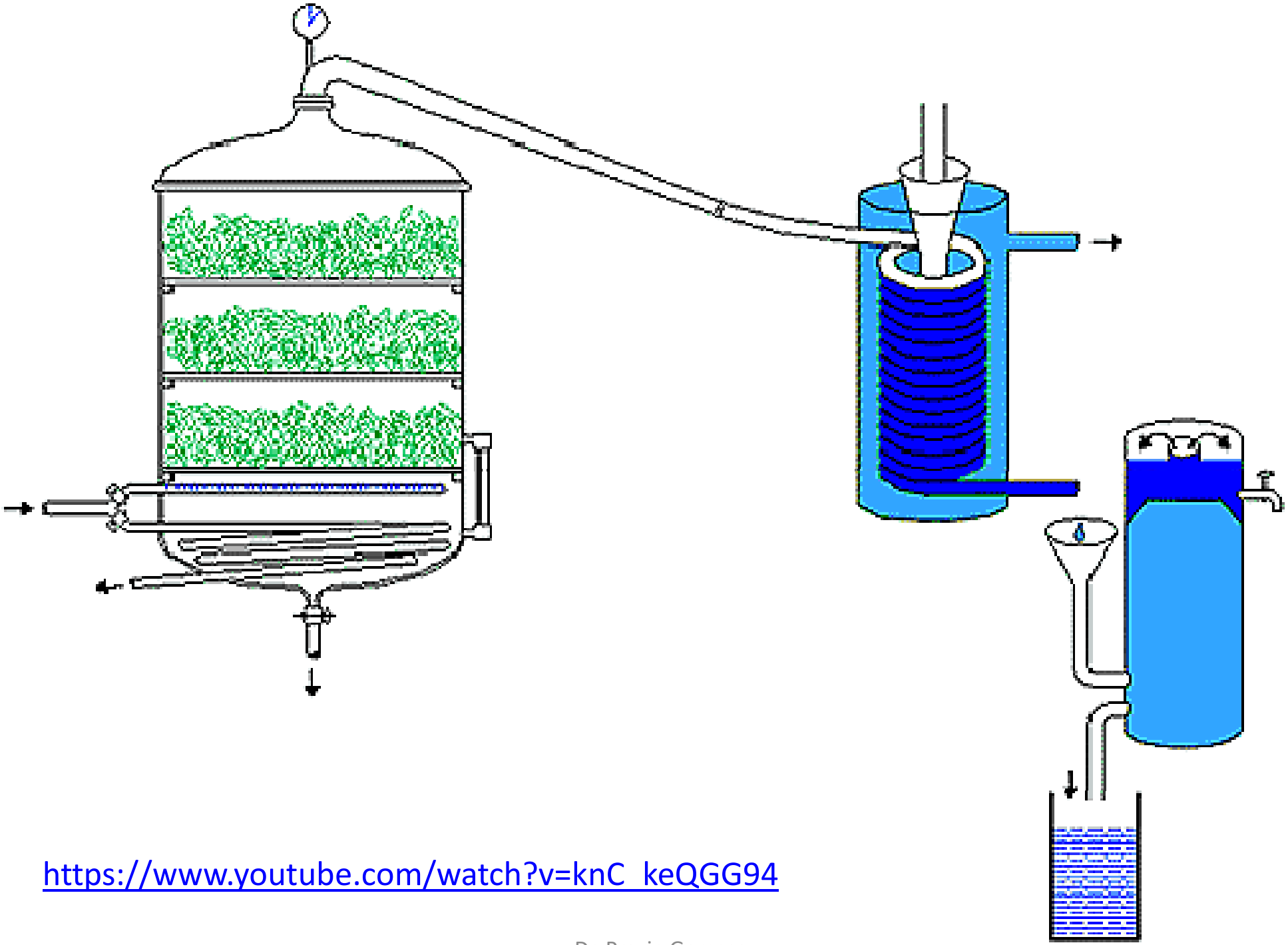


Properties:

- Citral is practically insoluble in water but miscible with alcohol, ether, benzyl benzoate.

Isolation:

- Fresh plant material shifted to hydrodistlation to obtained lemon grass oil
- Oil shifted for fractional crystallization for citral
- First sodium sulphite is added to the total oil
- Citral get converted to sulphite salt
- Crystals are filter and washed with ether or chloroform
- Product shifted to sodium carbonate to recover citral



https://www.youtube.com/watch?v=knC_keQGG94

Identification:

1. Sample + alc. Sudan red III shows red colour
2. Sample + tincture alkane shows red colour

Analysis-

- 1 mg citral + 1 ml methanol
- Spot applied on silica gel plate
- Run with plane chloroform (100 %)
- Dried plate sprayed with 2,4-dinitrophenyl hydrazine acid reagent and heated at 110 C for 10 min.
- Shows Rf value 0.51.

http://www.olabs.edu.in/?pg=topMenu&id=41

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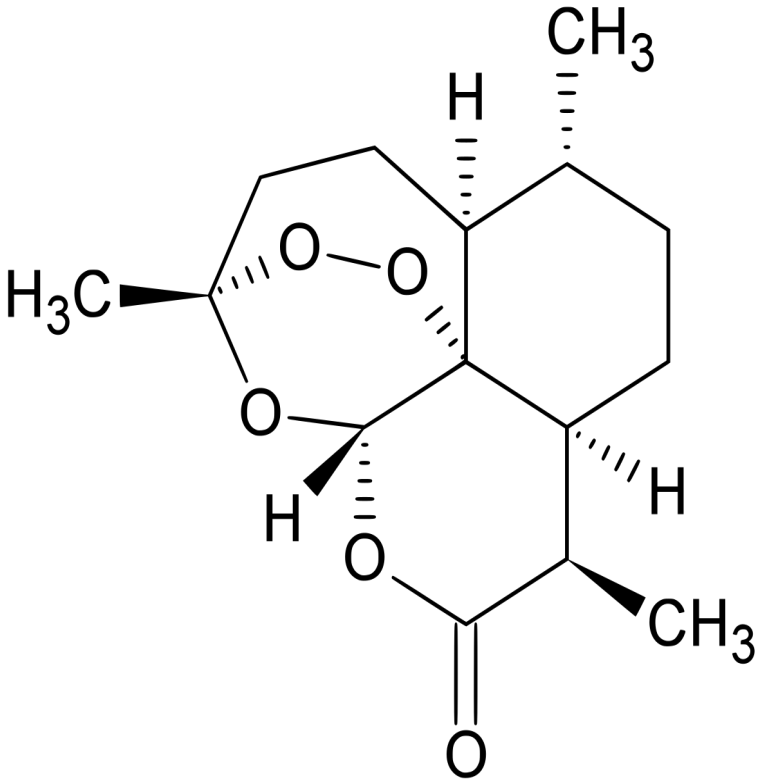
Artemisinin

- Artemisinin is an active antimalerial constituents of herb ***Artemisia annua*** family Compositae.



- **Properties**
- It is white crystalline powder, soluble in most organic solvent. It is slightly soluble in oil

Isolation

- 100 gm powder macerated with methanol for 1 hr.
- After complete maceration evaporate using vacuum at 40 C until volume reduce to 100 ml.
- Add 50 ml hexane in methanolic solution until the hexane layer shows colorless.
- Separate the layer by using separating funnel.

- Take methanolic extract and add 10 ml distilled water and 50 ml ethyl acetate
- Again separate the ethyl acetate and methanolic layer
- Each extract was concentrated and fractionated in Coolum chromatography using silica gel 60-120

https://www.youtube.com/watch?v=UmWMlKJAdSk

https://www.youtube.com/watch?v=a2wwgLV80U8

Identification:

- Extract + dissolve in 1 ml chloroform
- Spot applied on silica gel G plate
- Run with solvent system of petroleum ether: ethyl acetate (1:2)
- Dried plate sprayed with p-dimethyl-aminobenzaldehyde reagent and heated at 80 C for 10 min.
- Shows Rf value 0.28.

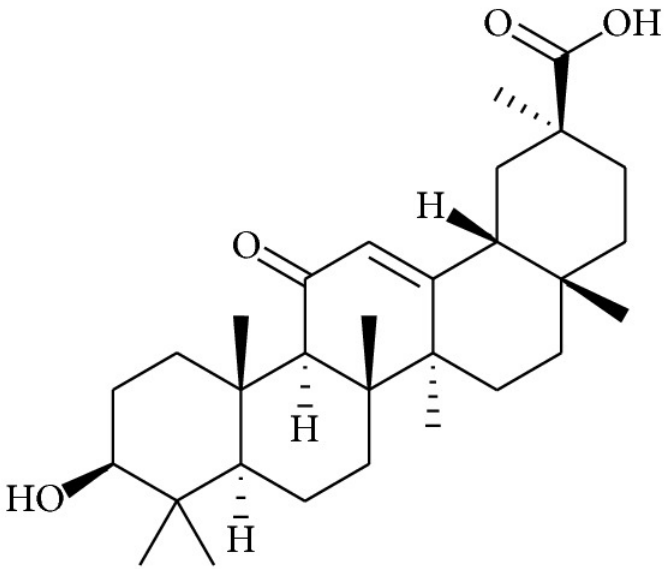
- **Analysis**
- UV: 1 mg sample mix with 10 ml methanol and analyse λ 200-400 nm
- TLC: Run with solvent system of petroleum ether: ethyl acetate (1:2) Shows Rf value 0.28.

Glycosides

Glycosides is defined as the organic compound from plants and animals sources which on enymatic or acid hydrolysis give one or more sugar moieties along with nonsugar moiety. The former is called as glycon and the latter as aglycones or genin

Glycyrhetinic acid

- It obtained from of subterranean peeled and unpeeled stolons, roots, and subterranean stems of ***Glycyrrhiza globra*** Linn. Family Leguminosae.
- **Properties:**
- Glycyrhetinic acid is a white crystalline powder
- Soluble in water but freely soluble in alcohol



Isolation:

- Glycyrhetinic acid extracted with solvent chloroform.
- Chloroform extract decant and mark again extracted with 0.5 M sulphuric acid
- Acid extract is extracted with three portion of chloroform.
- The combine chloroform extract is concentrated and dried to yield Glycyrhetinic acid

Identification & Analysis:

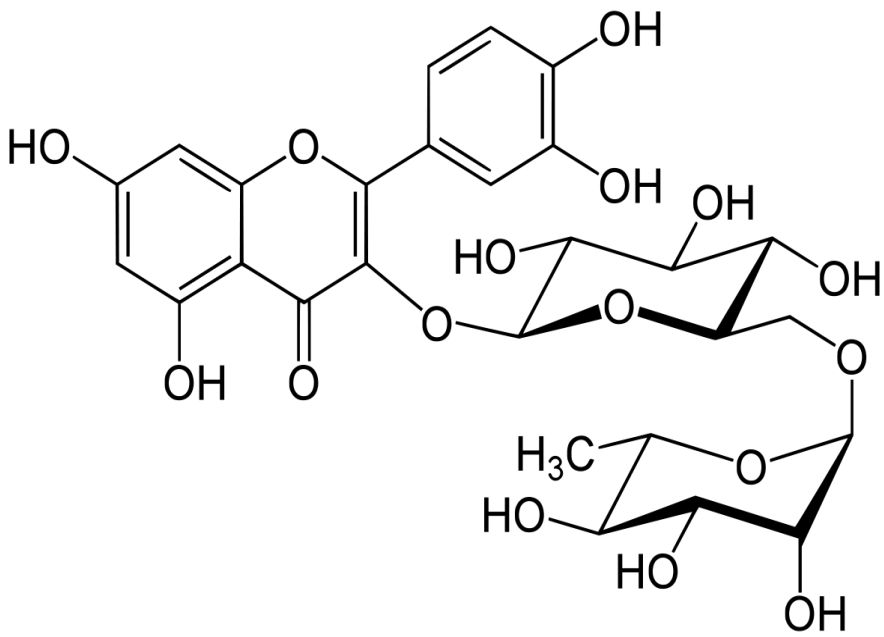
- 1 mg Glycyrhetinic acid + dissolve in 1 ml methanol-chloroform (1:1)
- Spot applied on silica gel G plate
- Run with solvent system of toluene: ethyl acetate: glacial acetic acid(12.5 :7.5: 0.5)
- Dried plate sprayed with 1 % vanillin sulphuric acid or anisaldehyde-sulphuric acid reagent and heated at 110 C for 10 min.
- Rf value shows 0.41.

Rutin

- Rutin is the most widespread of all quercetin glycosides obtained from various plant source.

Properties:

- Rutin is a pale or greenish yellow crystalline powder which gradually darkens on exposure to light.
- It is tasteless and odorless
- Sparingly soluble in water
- It is soluble in methanol.



- **Isolation:**
- Fresh leaves and flowers subjected to grinding extracted with boiling ethanol (80 %).
- Alcoholic extract is concentrated under vacuum and filter
- Concentrated extract treated with ether and ether extract is rejected
- The aqueous solution is again concentrated and allowed to crystalline to yield rutin.

- **Identification**

Shinoda test-

Sample + 3 ml ethanol + few drops of Sulphuric acid + 0.5 mg Magenesium turning----- pink colour develops.

Analysis

- 1 mg Rutin+ dissolve in 1 ml methanol
- Spot applied on silica gel G plate
- Run with solvent (mobile phase) system of

Ethyl acetate: butanol: formic acid: water (50:30:10:10)

Ethyl acetate: formic acid: acetic acid: water (100:10:11:27)

- Dried plate sprayed with anisaldehyde-sulphuric acid reagent and heated at 110 C for 10 min.
- Rf value shows 0.34.

Alkaloids

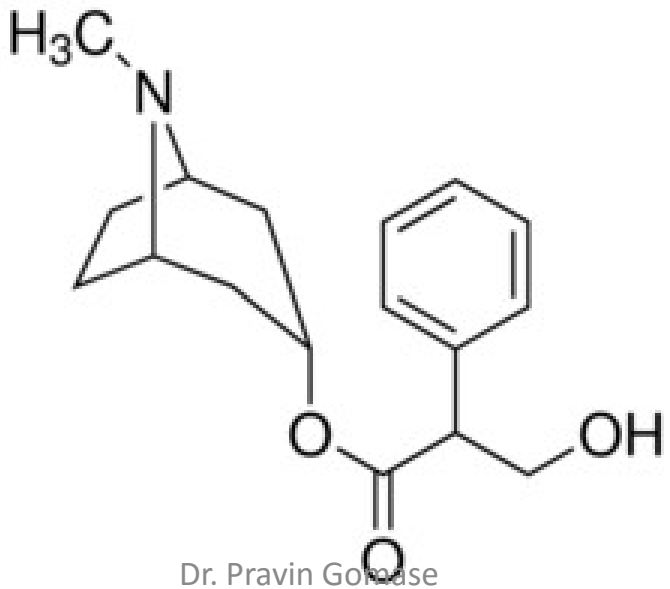
Definition: The term “alkaloid” (alkali-like) is commonly used to designate basic heterocyclic nitrogenous compounds of plant origin that are physiologically active and shows pharmacological action in small dose. Derived from amino acids.

OR

These are the organic products of natural or synthetic origin which are basic in nature and containing one or more nirogen atom normally of heterocyclic nature and posses pharmacological action in small dose on human and animals

Atropine

- Atropine, poisonous crystalline substance belonging to a class of compounds known as alkaloids and used in medicine.
- Atropine occurs naturally in belladonna (***Atropa belladonna***), from which the crystalline compound was first prepared in 1831.



Isolation:

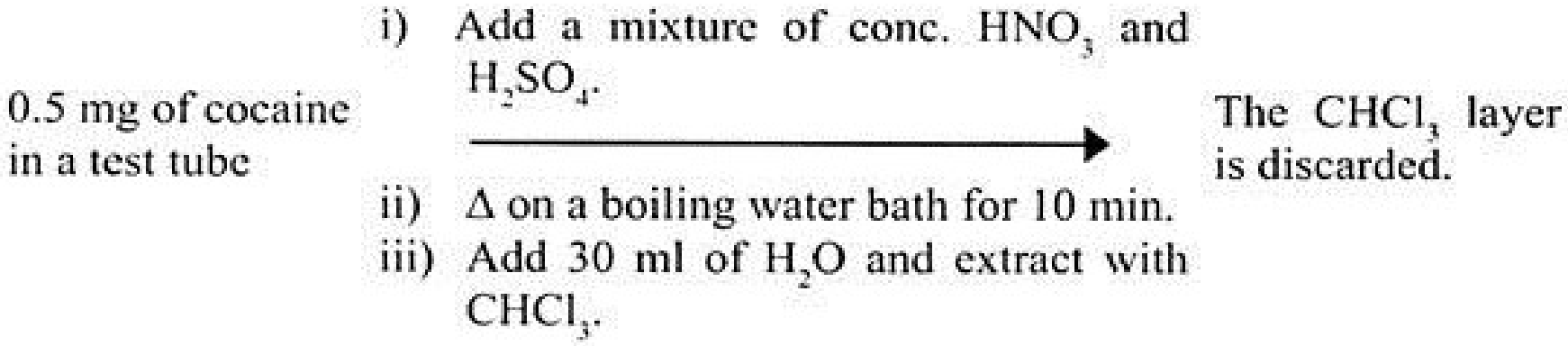
- Belladonna leaves extracted with 95 % alcohol in Soxhlet Apparatus
- Concentrated ethanolic extract in vacuum to remove alcohol
- Add dil HCL in solution and filter
- Again extracted with petroleum ether to remove impurities.
- Aqueous solution contain alkaloid and make it alkaline with ammonia solution.

Soxhlet Apparatus Parts, Function and Extraction Process (Full HD).mp4

https://www.youtube.com/watch?v=XBZkVEWxlnU

- Extracted this liquid with chloroform three times
- Combine chloroform extract and concentrated under vacuum to obtained crude alkaloid
- Treat again with dilute solution of oxalic acid to obtained alkaloid crystals
- Recrystalise to obtained pure crystals

- **Identification:**
- **Vitali-Morin reaction**
- Sample + drop of Sulphuric acid and evaporate + 0.3 ml 3 % Methanolic KOH----- Purple colour produce the indication of Atropine.
- **Rathenasinkam's Test:**



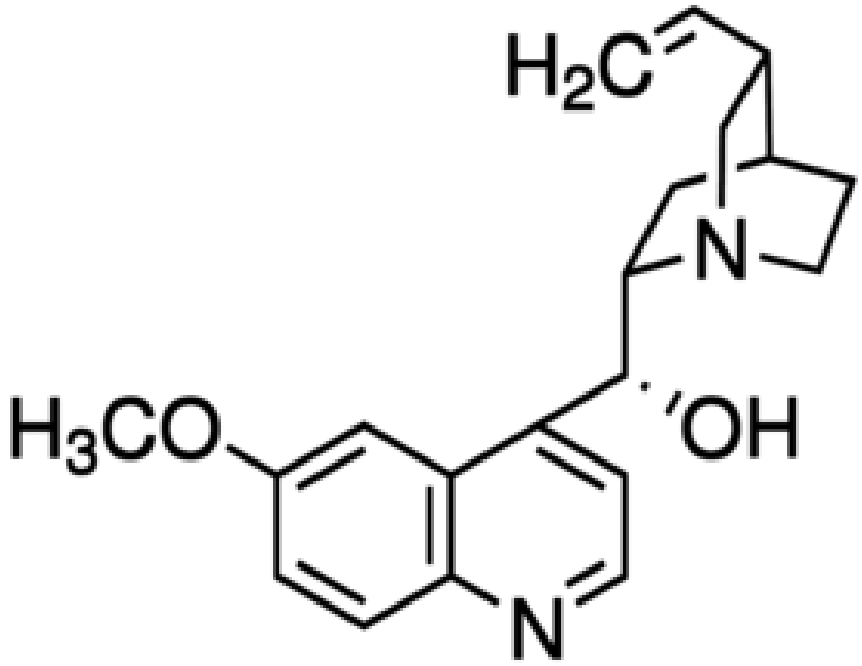
- To the residue, few drops of NH_3 is added and extracted with $CHCl_3$. The $CHCl_3$ is evaporated again and the residue is dissolved in acetone. A few drops of 10% NaOH are added. A bluish-purple colour is formed.

Identification & Analysis :

- 1 % solution of Atropine + dissolve in 2N acetic acid
- Spot applied on silica gel plate
- Run with solvent system of Strong ammonia solution: methanol (1.5 : 100)
- Dried plate sprayed with acidified iodoplatinate reagent and heated at 110 C for 10 min.
- Rf value shows 0.18.

Quinine

- Quinine, cinchonine are the popular qunoline alkaloid obtained from the dried bark of ***Cinchona officinalis, C. calisaya*** Family Rubiaceae.



Isolation

- Powder of cinchona bark weigh accurately
- Moisten with alcoholic KOH (20%). Keep the mixture aside to dry.
- Extract this mass with benzene for 6 hr in Soxhlet apparatus.
- After complete extraction filter the extract and shake with 5 % Sulphuric acid
- Filter the solution using filter paper coated with charcoal

- Basify to PH 8.5 by using ammonia solution. Extract the solution with 3 portion of chloroform
- Combine an evaporate chloroform extract to obtained dry residue of total cinchona.
- Dissolve residue in hot water to obtained white quinine sulphate crystals.

- **Identification:**
- **Thalloquin test:**

Take test sample to this add bromine water and ammonia solution it shows **emerald green** colour indicate presence of quinine.

Analysis:

- Extract + dissolve in methanol
- Spot applied on silica gel G plate
- Run with solvent system.
- Dried plate sprayed with Dragendorff reagent 110 C for 10 min.
- Chloroform: diethyl amine (9:1)
- Rf value shows-0.17
- Chloroform: acetone: diethyl amine (5:4:1)
- Rf value shows 0.17

Reserpine

- Reserpine is an Indole alkaloid obtained from the roots of ***Rauwolfia serpentina*** Family **Apocyanaceae** and also other species of Rauwolfia.
- **Isolation**
- Root powder extracted with 90 % alcohol by percolation.
- Alcoholic extract is concentrated and dried under vacuum to obtained dry extract.
- Dry extract is extracted further with proportion of ether-chloroform-alcohol (20:8:2.5)

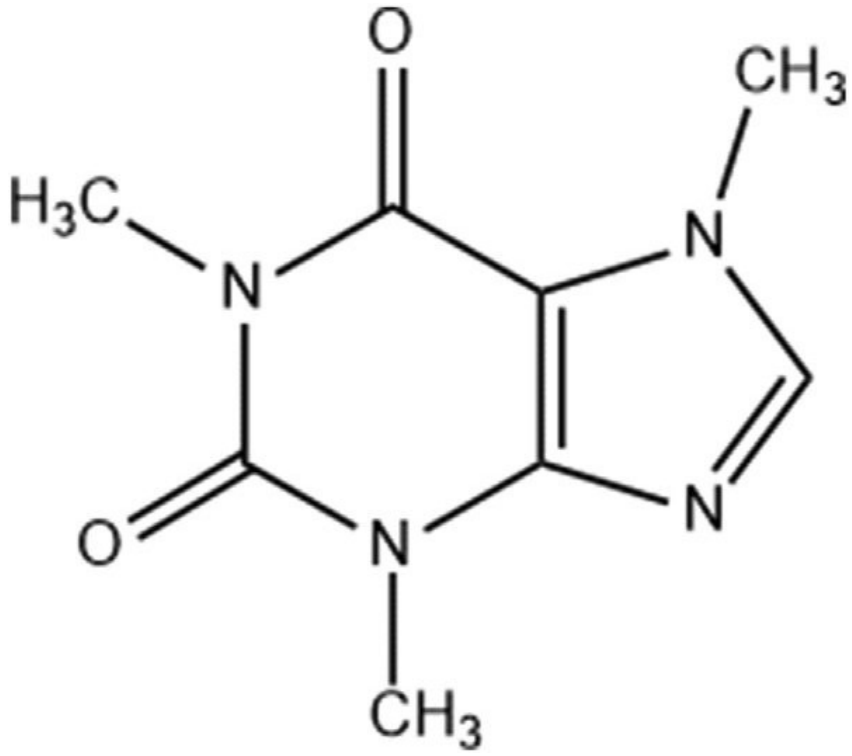
- To extract add little dil. ammonia with shaking.
- Alkaloid is converted to water insoluble base
- Water added and allow to settle
- Filter and residue extracted with 4 volume of 0.5 NH_2SO_4 in separating funnel.
- The extract filter made alkaline with dil ammonia to liberate alkaloid
- Finally extracted with chloroform and dried to yield alkaloid

Identification:

- 1 mg Extract + dissolve in methanol
- Spot applied on silica gel G plate
- Run with solvent system chloroform: acetone: diethyle amine (50:40:10)
- Dried plate sprayed with Dragendorff reagent 110 C for 10 min.
- Rf value shows 0.72

Caffeine

- Caffeine is xanthine alkaloid obtained from coffee, cocoa beans, cola nuts and tea leaves.
- It is obtained from prepared leaves of ***Thea sinensis.***



Method for isolation(Method 1)

- Weigh quantity of tea leaves transfer to 250 mi distil water
- Boil the water for 30 min with occasional stirring
- Allow to cool and filter the solution
- Take filtrate in separating funnel and to it add 100 ml chloroform. Shake vigorously so total caffeine will transfer to chloroform
- Separate chloroform layer and evaporate over water bath. To yield white caffeine crystals.

- **Method for isolation(Method 2)**
- Tea leaves powder boiled with water and filter the extract
- Treat it with lead acetate solution to ppt tanines
- Filter solution and add dil Sulhuric acid to remove excess lead acetate in the form lead sulphate.
- Filter and treat with charcoal to remove any coloring matter
- Take filtrate and add chloroform in separating funnel and shake
- Separate the chloroform layer and evaporate to yields white crystals of caffeine.

- **Method for isolation(Method 3)**
- 30 gm tea powder mix with 250 ml distilled water and 5 g sodium carbonate (removal of tannins) and boil for 10 min.
- Filter the content while hot
- Again take residue and add 100 ml water and boil
- Filter and combine all filtrate in 250 ml separating fennel and extract with 3 portion of dichloromethane without vigorous shaking.
- Drain the dichloromethane layer.
- Place in Petri dish for evaporation to yield needle shape caffeine.

https://www.youtube.com/watch?v=sPhJWBL17OQ&list=PLtEqsPSBZlXu4dJUFMnNmM5zQuKUIQjbu&index=43&t=0s

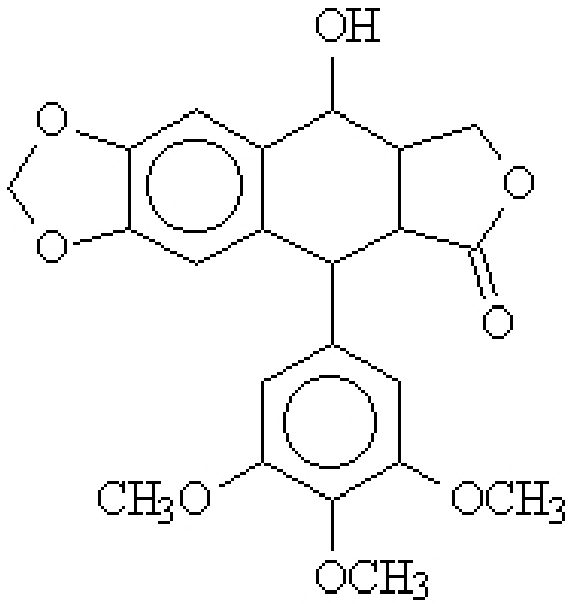
- **Identification and Analysis:**
- **Murexide test:**
- Take test sample add HCl and KCL. Heat it till it gets dry. Expose this powder to dil. Ammonia. Purple color indicates presence of caffeine.
- **TLC**
- Sta. phase: Silica gel G
- Mob. Phase: **Ethyl acetate: Methanol: acetic acid (8:1:1) or Chloroform : methanol (9:1)**
- Spraying reagent: Iodine chamber
- Rf 0.70

Resins

- Resins are typically viscous substances that convert into rigid polymers through a curing process.
- Resins are usually mixtures of organic compounds.
- Resins are naturally occurring but are now often made synthetically.
- Some synthetic resins have similar properties to natural plant resins, but many are very different.
- Plants secrete resins for their protective benefits in response to injury.
- The resin protects the plant from insects and pathogens

Podophyllotoxin

- Podophyllotoxin is the lactone resin obtained from root and rhizome of ***Podophyllum hexandrum*** or ***Podophyllum emodi*** Family Berberidaceae.
- It is used as anticancer agent.



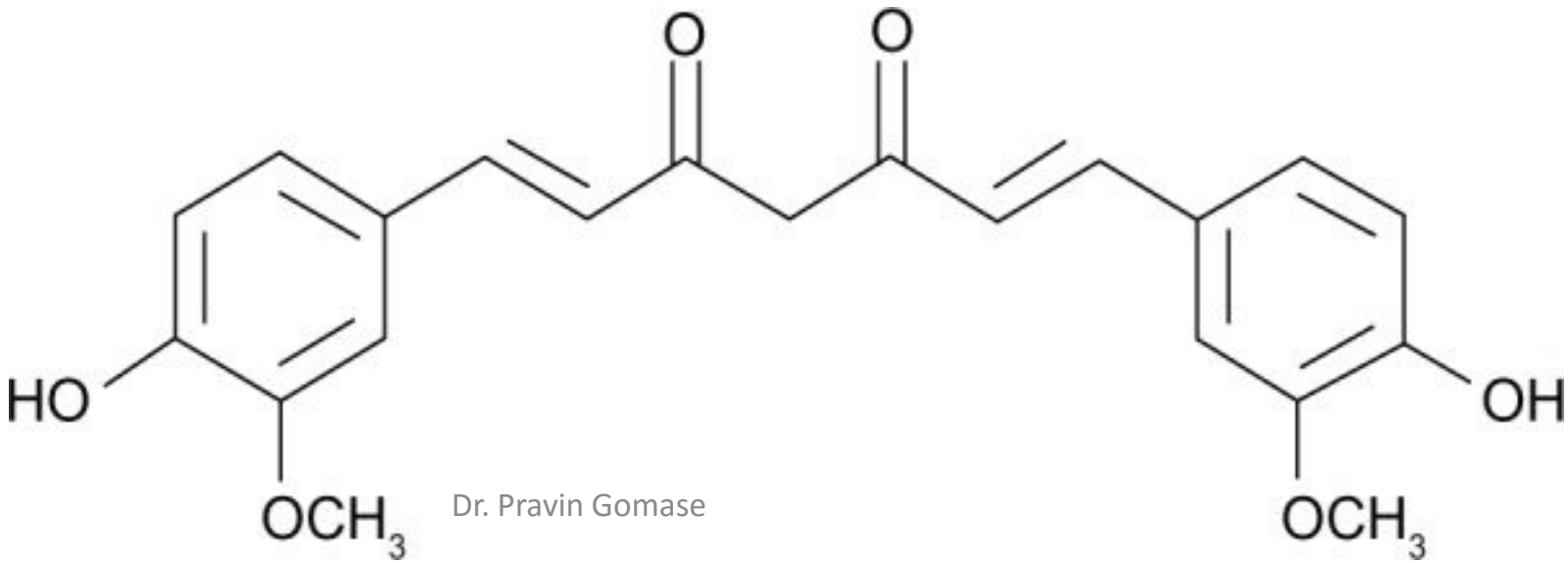
podophyllotoxin

- **Isolation method**
- Extract 120 gm of root powder with methanol in Soxhlet apparatus.
- Filter and concentrate to obtained semisolid mass.
- To this add 200 ml acidified water and cool it.
- Allow the mixture to stand for 2 hr at 5 c to form precipitation.
- Filter under vaccum.
- Dissolve this residue in sufficient quantity of 90 % ethanol, filter and evaporate to dryness.
- Recrystalise the residue in benzene to get podophyllotoxin.

- **Identification**
- Sample treated with 50 % Sulphuric acid shows violet blue colour.
- Macerate 0.5 gm of drug with 10 ml alcohol and filter. To filtrate add 0.5 ml copper acetate. It shows brown ppt.
- **Analysis:**
- Sample spotted on Silica gel G plate
- Mobile phase: acetonitrile : water (4:6)
- Rf value: 0.41

Curcumin

- Curcumin is a bright orange yellow colored needle chemical compound produced by the fresh as well as dried rhizomes of the plant ***Curcuma longa*** belong to family Zingiberaceae.
- It is commonly known as haldi in hindi.
- India is the largest producer of haldi in the world.



- **Isolation**
- Material is extracted in Soxhlet by using 95 % alcohol as a solvent until no colour solution observe in syphon tube.
- Concentrate the extract to yield semisolid mass
- Dissolve this semisolid mass in benzene and further extracted with 0.1 % sodium hydroxide in separating funnel.
- Alkalkine extract is acidified by addition of dil HCl which result the formation of yellow colored ppt.

- Concentrate the extract by boiling on water bath and at the same time dissolving the ppt. in boiling water.
- During this process the resinous material will separate out and will form lumpy mass.
- Filter the solution while hot and concentrate the filtrate to a very small volume and finally cool to get orange yellow colored needle shape curcumin.

https://www.youtube.com/watch?v=WK6qdFNIAho

- **Identification:**
- Curcumin gives yellow-red colour with conc. Sulphuric acid and with sodium hydroxide gives deep brown colour.
- **Analysis:**
- **TLC:** Sample spotted on Silica gel G plate
- Mobile Phase: Chloroform: Ethanol : Glacial acetic acid (9.4 : 5 : 0.1)
- 0.69 Rf

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Save Trees.
Save the World.

Thank you.