आईएफटीएम विश्वविद्यालय, मुरादाबाद, उत्तर प्रदेश

IFTM University, Moradabad, Uttar Pradesh

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IFTM University, Moradabad

Pharmacognosy and Phytochemistry-II (BP 504T)

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Subject: Pharmacognosy and Phytochemistry-II (BP 504T)

UNIT-III

Isolation, Identification and Analysis of Phytoconstituents

a) Terpenoids: Menthol, Citral, Artemisin

b) Glycosides: Glycyrhetinic acid & Rutin

c) Alkaloids: Atropine,Quinine,Reserpine,Caffeine

d) Resins: Podophyllotoxin, Curcumin

Terpenoids

Terpenoids are the natural products whose structures are divided into several isoprene (C_5H_8) units. Hence they are also termed as isoprenoids. Terpenoids include hydrocarbons and their oxygenated derivatives whereas terpenes include only hydrocarbons. Terpenoids are found in all volatile oils obtained from plants and animal sources.

Properties of Terpenoids:

Terpenoids are present in all volatile oils so they normally colorless liquid or solids. They possess a characteristic smell and most of them are optically active. They easily get oxidized by oxidizing agents. They are soluble in alcohol, chloroform, ether, acetone and carbon disulphide but are insoluble in water.

Classification of Terpenoids:

Terpenoids are broadly classified on the basis of number of isoprene units they contain are following as:

Terpeniods	**No of Isoprene units**	**Molecular formula**
Monoterpeniods	**2**	$C_{10}H_{16}$
Sesquiterpeniods	**3**	$C_{15}H_{24}$
Diterpeniods	**4**	$C_{20}H_{32}$
Triterpeniods	**6**	$C_{30}H_{48}$
Tetraterpeniods	**8**	$C_{40}H_{64}$

Uses:

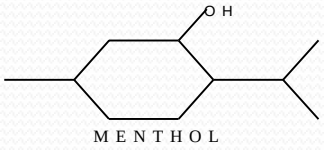
Therapeutically terpenoids exhibit activities like analgesic, carminative, anthelmintic, antiseptic, Counter-irritant, stimulant etc. They are also used in the preparation of soap cosmetic, incense sticks, perfumery and food articles. They are also employed in pesticide and insecticide industries.

Menthol

Menthol is a monoterpenes alcohol obtained from diverse types of mint oils or peppermint. It is found in the peppermint oil obtained from the fresh flowering tops of *Mentha piperita* Linn. or other allied species of Mentha i.e. *Mentha arvensis* and *Mentha Canadensis*, belonging to family Labiatae.

Isolation of Menthol: Menthol oil is obtained from the hydro distillation or steam distillation of fresh above ground parts just before flowering. For (-) menthol isolation from peppermint oil the oil is subjected to cooling. The crystals of menthol crystallize out from the oil which is separated by centrifugation. Cornmint oil is obtained from the steam distillation of the flowering herbs Mentha arvensis contains about 70-80 % of free (-) menthol. Cornmint oil is cooled and the crystals of menthol produced are separated by centrifugation. Since the crystalline product contains traces of cornmint oil. This menthol has a slightly herbaceous minty note. Pure (-) menthol is obtained by recrystallization from solvents with low boiling points. Dementholized corn mint oil from which (-) menthol is removed by crystallization and which still contains 40-50% free menthol can also be reused for producing (-) menthol.

Melting point: 41-44 ^{o}C



Menthol

Identification of Menthol:

- On heating the crystals of menthol in watch glass on water bath, the entire material evaporates without leaving any residue.
- A small quantity of menthol is taken in a test tube and adds equal quantity of camphor or thymol. Liquefactions of contents of test tube indicate the presence of menthol.

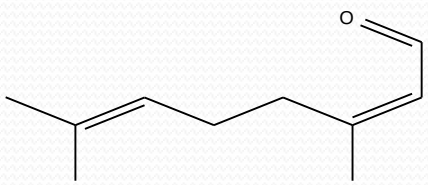
Analysis of Menthol: TLC analysis of menthol can be performed by using silica gel 60 as stationary phase. Sample prepared in menthol is applied to the TLC plates. The TLC plate is then subjected to linear ascending development in TLC chamber previously saturated for 30 min with mobile phase, hexane: ethyl acetate 8:2 (% v/v). The plates are dried at room temperature for 15 min before derivatization. The plate is then sprayed with anisaldehyde sulphuric acid spray reagent followed by heating at 105 ^{o}C for 10 min. Menthol shows the R_f value of about 0.34.

Citral

Citral is a monoterpenes aldehyde found in variety of sources which include lemon grass, *Cymbopogen flexuousus* belonging to family Graminae.

Isolation of Citral: In case of lemon grass, the fresh plant material is hydrodistillation to obtained lemon grass oil. Citral has a low boiling point, therefore it is not hydrodistilled due to the risk o degradation. The fractional distillation of oil gives a low boiling component containing higher citral content. Fractional crystallization is a suitable method for the purification of citral. First sodium sulphite is added to the total oil. Thus the citral gets converted to its sulphite salt. The salt crystallizes out of the solution. The crystals are filtered and washed with ether or chloroform. The product is then subjected to sodium carbonate treatment to recover citral.

Melting point: 91-92 ^{o}C

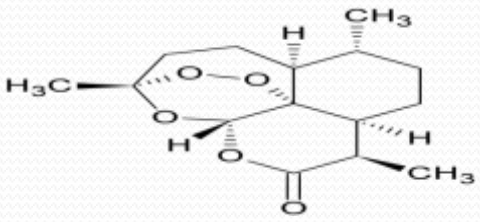


Citral

Identification and analysis of Citral: 1 mg of citral is dissolved in 1ml of methanol and the spots are applied over silica gel-G plate. The TLC plates are eluted in toluene: ethyl acetate (93:7). The dried plates are sprayed with 1 % vanillin sulpuric acid reagent. Citral gives yellow to orange spots with R_f value 0.51.

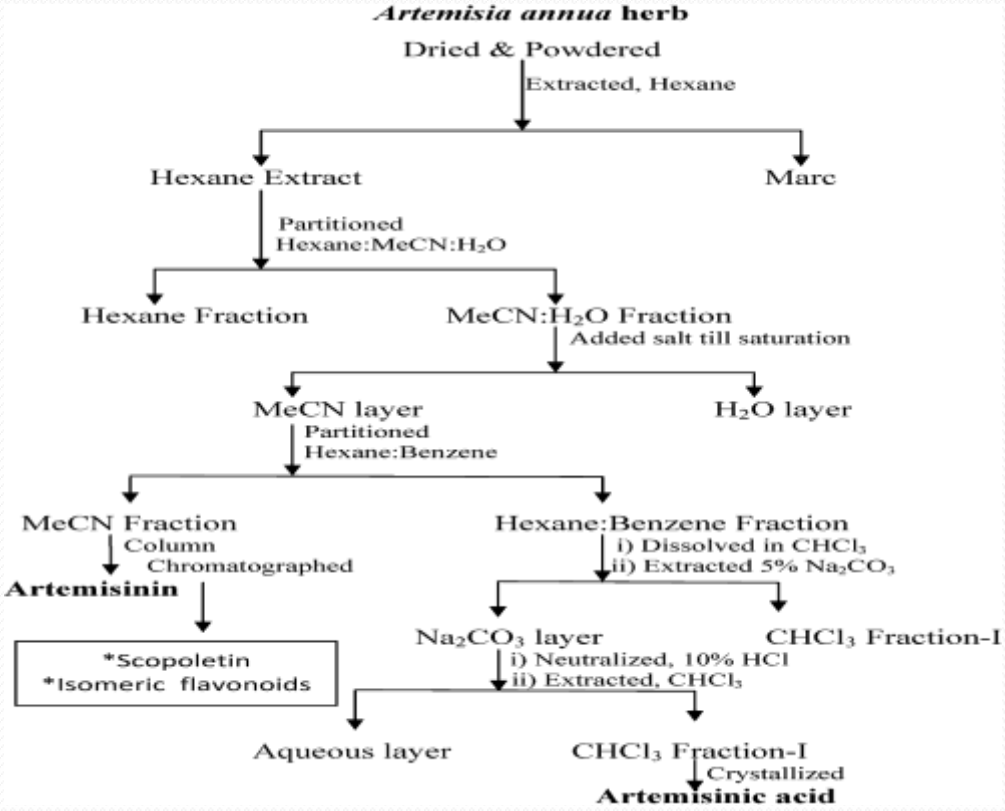
Artemisinin

Artemisinin is an active antimicrobial constituent of the traditional Chinese medicinal herbs *Artemisia annua* belonging to family compositae.



Artemisinin

Isolation of Artemisinin:



Melting point: 156-157 ^{o}C

Identification and analysis of Artemisinin: The pre determined concentration of *Artemisia annua* test extracts along with standard artemisinin applied over the pre coated silica gel 60 TLC plates. This plate then developed using mobile phase hexane: ethyl acetate: acetone (16:1:1).After the development , the plate is dried and exposed to saturated ammonia vapour for approx . 2 hrs later, the plate is scanned at 320 nm using TLC densitometer. Artemisinin shows the R_f value of about 0.75.

Glycosides

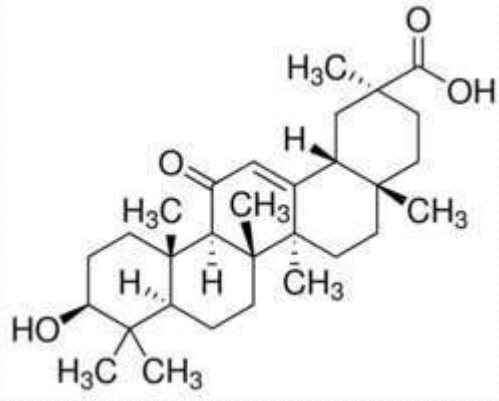
Glycoside can be defined as the organic compound mainly of plant origin and rarely of animal which on enzymatic or acidic hydrolysis yields one or more sugar moieties (Glycone) and a non sugar moiety (Aglycone or genin).

Glycoside →Aglycone (genin) + Glycone (Sugar)

Glycosides are considered to be sugar ethers or acetals and they are formed by condensation of hydroxyl group of non group and hemiacetal hydroxyl group of sugar. The sugar(glycone) present in glycosides are mono saccharides like glucose and rhamnose or more rarely deoxy sugars such as cymarose found in cardiac glycosides. The aglycone part may be alcohol, phenol or amines. The linkages between glycone and aglycone is known as glycosidic linkage.

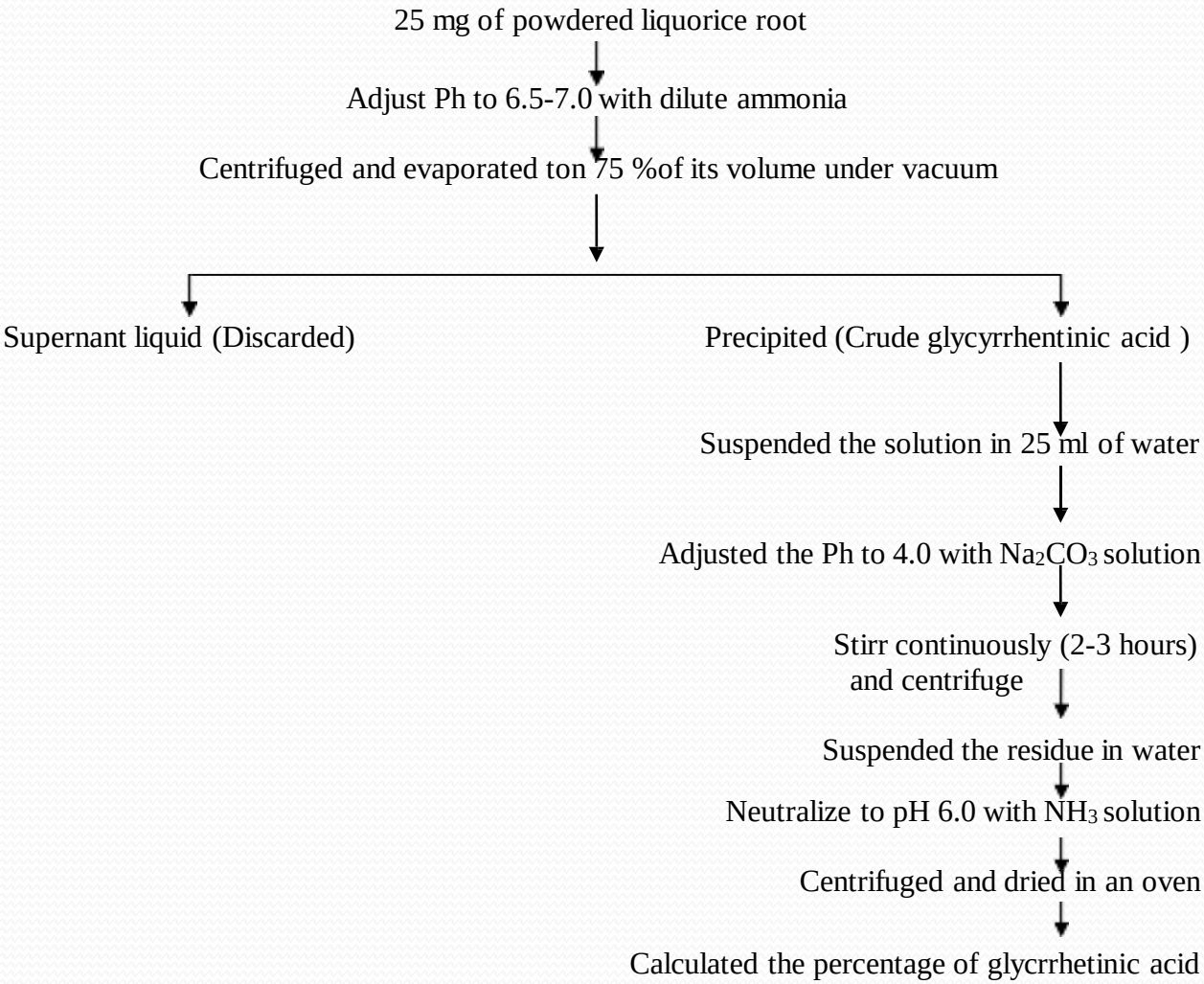
Glycyrhetinic acid

It is obtained from liquorice which consists of dried peeled or unpeeled roots and stolon of *Glycyrrhiza glabra* belonging to family Leguminosae.



Glycyrhetinic acid

Isolation of Glycyrhetinic acid:



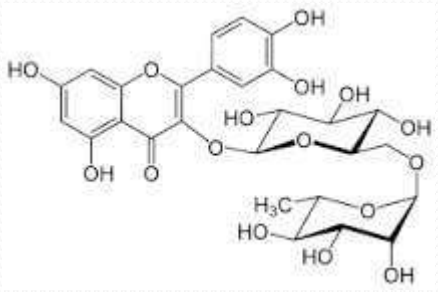
Melting point: 200-214 ^oC

Identification and analysis of Glycyrhetinic acid

The TLC fingerprint shows that licorice extract contained glycyrrhetinic acid. 1 mg of Glycyrhetinic acid is dissolved in 1ml of methanol and the spots are applied over silica gel-G plate. The TLC plates are eluted in chloroform: methanol: formic acid, (9:1:0.1 v/v) The dried plates are sprayed with anisaldehyde/sulphuric acid reagent. Glycyrhetinic acid shows R_f value 0.42.

Rutin

Rutin is the class of flavonoids glycoside obtained from buck wheat (*Fagophrum esculentum*) belonging to family Polygonaceae.



Rutin

Isolation of Rutin: Freshly harvested buck wheat leaves are transferred in a stainless steel vessel and sufficient quantity of 85% isopropyl alcohol is added so as to cover the plant material. The whole of the contents are rapidly heated and maintained at boiling for about 10 min. The hot extract is filtered rapidly. The marc left behind is further extracted with isopropyl alcohol in a similar manner and filtered. The combined extract is then concentrated to one fourth of the original volume and filtered. The marc is washed with boiling water. The filtrate and the washing are combined and immediately cooled to a temperature of about 5 ^{o}C. After cooling, the crude rutin crystallizes out and allowed to stand for about 1 hr for complete crystallization. The Crystals are filtered out followed by washing with cold water. The isolated rutin is dried to a constant weight at 110 ^{o}C.

Melting point: 214-215 ^{o}C.

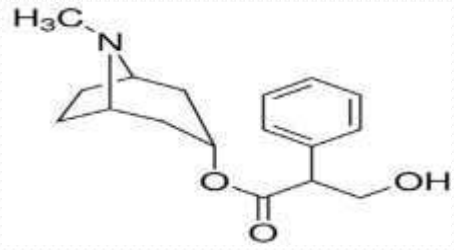
Identification and analysis of Rutin: 1 mg of rutin is dissolved in 1ml of methanol. The silica gel G plate is spotted with the sample and eluated in the solvent system ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26). The dried plate are sprayed with natural product polyethylene glycol reagent under observed UV 365nm. Rutin gives yellow spot with R_f value 0.40.

Alkaloids

Alkaloids are a class of basic, naturally occurring organic compounds that contain at least one nitrogen atom. This group also includes some related compounds with neutral and even weakly acidic properties. They are very potent in nature and found generally in plants.

Atropine

Atropine belongs to the class of tropane alkaloids. It is obtained from the dried leaves and other aerial parts of *Atropa belladonna* belonging to family Solanaceae.



Atropine

Isolation of Atropine:

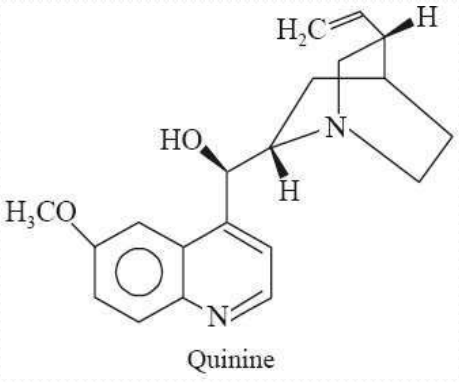
The powdered belladonna leaves are first extracted with ethanol followed by solvent evaporation to yield syrupy residue. The syrupy residue is then treated with 1% hydrochloric acid in order to form alkaloid salts and to precipitate out resinous matter. The acidic solution is filtered to remove resinous matter. In a separating funnel, the acidic solution is treated with petroleum ether and chloroform followed by the removal of acidic layer. The acid layer is treated with ammonia solution and further extracted with chloroform in a separating funnel. The solvent is removed by distillation to get the crude alkaloid mixture which is further treated with oxalic acid. The oxalates of atropine and hyoscyamine are separated by fractional crystallization from acetone and ether which results in the formation of atropine oxalate crystals.

Melting point: 115-116 ^{o}C.

Identification and analysis of Atropine: 1ml solution of atropine dissolved in 2N acetic acid is spotted over silica gel G plate and eluted in the solvent system of strong ammonia solution: methanol (1.5:100).TLC plate is spread with acidified iodoplatinate solution. Atropine gives the R_f value 0.70.

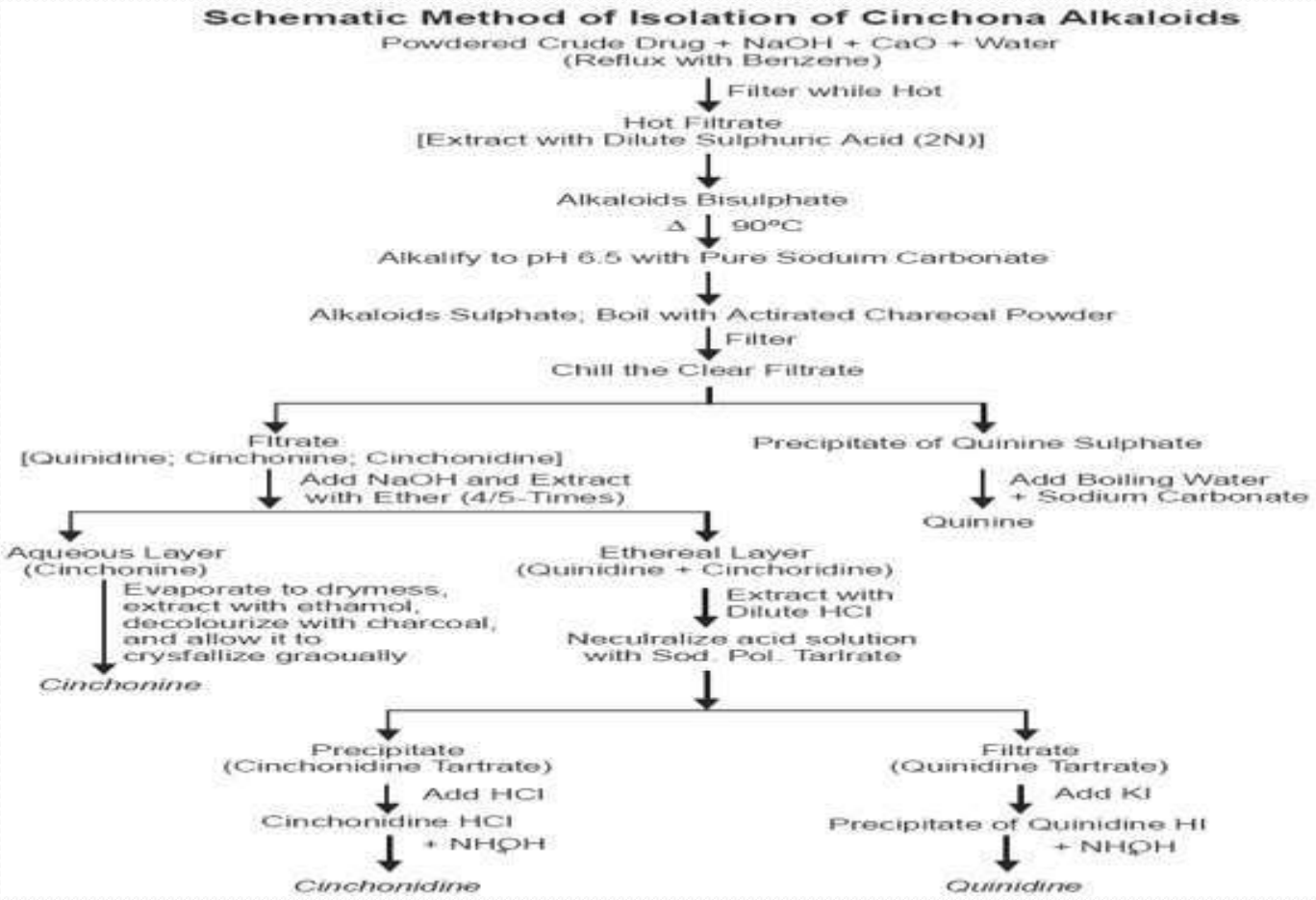
Quinine

It is a quninoline alkaloid present in bark of *Cinchona officinalis* belonging to family Rubiaceae.



Quinine

Isolation of Quinine:

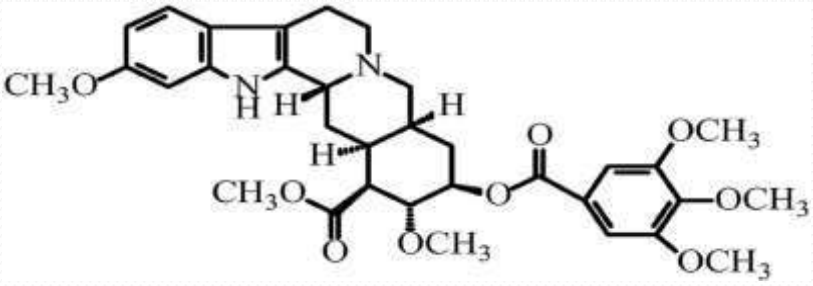


Melting point: 177 °C.

Identification and analysis of Quinine: 1mg quinine dissolved in 1 ml of chloroform is spotted over silica gel G plate and eluted in the solvent system of chloroform: diethylamine (90:10). TLC plate is spread with acidified iodoplatinate solution. Quinine gives the R_f value 0.65.

Reserpine

Reserpine is an indole alkaloid obtained from the roots of *Rauwolfia serpentina* belonging to family Apocyanaceae.



Reserpine

Isolation of Reserpine: The powdered and sieved roots are allowed to swell in a $NaHCO_3$ solution (10% w/v) for a period of 10-12 hours. The resulting solution is extracted with benzene, until the extracts give a weak positive reaction with HgI_2. The combined benzene extracts are concentrated and ether is added to the benzene solution. The resulting mixture is extracted with dilute HCl. The combined acidic solution is washed with ether, filtered and extracted with chloroform in a successive manner. The combined chloroform extract is washed subsequently with 10% (w/v) sodium carbonate solution and followed by water so as to get rid of any *free acids* present. The resulting extract is finally evaporated to dryness under vacuum. The residue is dissolved in anhydrous methanol and seeded with a pure crystal of reserpine and allowed to cool gradually when reserpine will crystallize out.

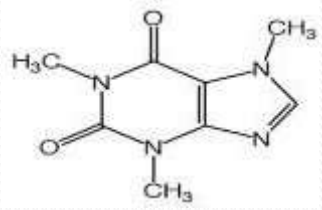
Note: The chloroform will specifically extract the weakly basic alkaloids, such as: Reserpine and Rescinnamine.

Melting point: 265^oC.

Identification and analysis of Reserpine: 1 mg of pure Reserpine is dissolved in methanol. The spots are applied over the silica gel G TLC plate, elute the plate in solvent system chloroform: acetone: diethylamine (50:40:10).The eluted plated are dried and sprayed with Dragendorff's reagent. Reserpine gives R_f value 0.72.

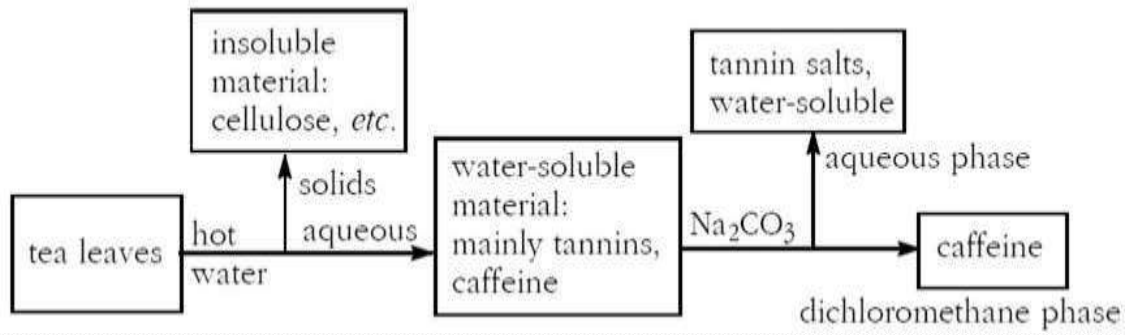
Caffeine

Caffeine is purin alkaloid obtained from the leaves and leaf buds of *Thea sinensis* belonging to family Theaceae.



Caffeine

Isolation of Caffeine:



Melting point: 235-237°C.

Identification and analysis of Caffeine:

Murexide test: Take few crystals of caffeine in porcelain dish, add 4 to 5 drops of concentrated nitric acid and evaporate to dryness. The residue left behind; add few drops of ammonium hydroxide. Formation of purple colour indicates the presence of caffeine.

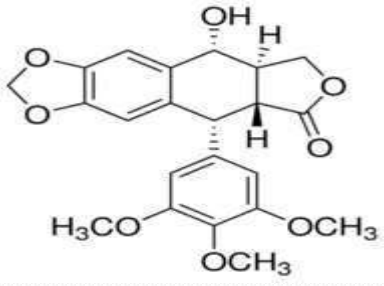
Small quantity of isolated caffeine is dissolved in dichloromethane and spotted on pre coated silica gel G plate. The plate is the developed in TLC chamber previously saturated for 30 min with the mobile phase ethyl acetate: methanol: water in the ratio of 100: 13.5:10.The developed plate is sprayed with potassium iodide solution (1g potassium iodide and 1 g iodine dissolved in 100 ml ethanol) followed by spraying with 1:1 mixture of 25 % hydrochloric acid and 96 % ethanol. The presence of dark brown spot confirms the presence of caffeine gives the R_f value 0.79.

Resins

Resins are defined as amorphous, non nitrogenous, heterogenous plant extractor exudates with complex chemical nature. Resins are natural or synthetic organic compound consisting of a non crystalline or viscou liquid substance. Natural resins are typically fusible and flammable organic substances that are transparent or translucent and are yellowish to brown in colour.

Podophyllotoxin

It is obtained from dried rhizome and root of *Podophyllum hexandrum*. American podophyllum consists of dried rhizomes and roots of *P. peltatum* belonging to family Berbiridiaceae.



Podophyllotoxin

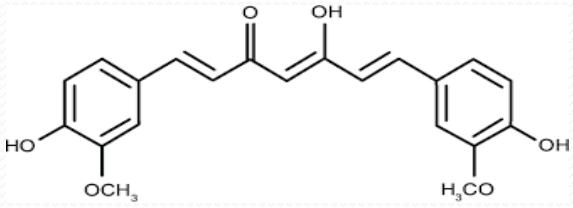
Isolation of Podophyllotoxin: The powdered Podophyllum is extracted with alcohol and filtered. The alcoholic extract is evaporated to form a syrupy mass. The syrupy mass is treated with acidified with HCL then cooled and filtered. The residue is washed with acidified water and dissolved in hot alcohol. The extract is evaporated to form a residue (Podophyllum resin or Podophyllin).This Podophyllum resin is further purified to yield podophyllotoxin.

Melting point: 183-184^{o}C

Identification and analysis of Podophyllotoxin: 1 mg of Podophyllotoxin is dissolved in 2ml methanol. The spots are applied over the silica gel G TLC plate, elute the plate in solvent system Toluene: Ethyl acetate (5:7 v/v).The eluted plated are dried and sprayed with 50% sulphuric acid reagent. Podophyllotoxin gives R_f value 0.39.

Curcumin

Curcumin or curcuminoids are diaryl heptanoid, bright yellow coloured compounds obtained from the dried rhizomes of *Curcuma longa* belonging to the family Zingiberaceae.



Curcumin

Isolation of Curcumin:

Turmeric powder is extracted with alcohol in soxhlet extractor. The alcoholic extract is concentrated under reduced pressure and dried. In another procedure, turmeric powder is first extracted with hexane followed by acetone. The acetone extract is concentrated and dried to yield curcumin.

Melting point: 183^{o}C

Identification and analysis of Curcumin:

Curcumin gives yellow red colour with concentration sulphuric acid. With sodium hydroxide curcumin gives deep brown colour.

Dissolve 1 mg of Curcumin in 1 ml methnol. Apply the spot on silica gel G plate and elute the plate in the solvent system chloroform:ethanol:glacial acetic acid (94:5:1 v/v) Dry the eluted plate and visualize under 366nm light. Curcumin exhibits a bright yellow fluorescent sopt at R_f value 0.79.

Thank you