UNIT-III

Isolation, Identification & Analysis of Phytoconstituents

Dr. A. R. Surana

Associate professor

K. K. Wagh College of Pharmacy

Nashik

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

Extraction

- Different methods are used for the extraction of the drugs i.e.
  - **Infusion**
  - **Decoction**
  - **Digestion**
  - **Maceration**
  - **Percolation**
  - **Distillation**

Isolation of compound

Isolation: Separation of a single compound from mixture of components present in the extract. Different methods are used for Isolation of compounds i.e.

- **Chromatography**
- Paper Chromatography
- Thin Layer Chromatography
- Column chromatography
- HPLC (High Performance Liquid Chromatography)
- GC (Gas Chromatography)

Identification:

Identification means to establish the class of compounds, their nature etc.

Compounds are identified by two basic way -

- **Qualitative identification;**
- **Quantitative identification:**

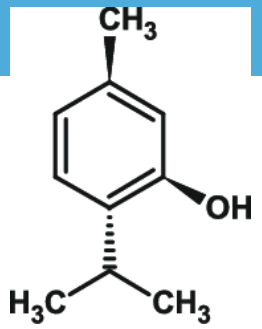
- The compounds are identified by different methods like Spectroscopic method, e.g. UV-Visible Spectroscopy, IR spectroscopy; Chromatography e.g. TLC, HPTLC, GC-MS etc.

Analysis of Phytoconstituents

After isolation of Phytoconstituents, these are analysed by different method likes

- Spectroscopic methods: UV-Visible Spectroscopy, IR Spectroscopy, NMR Spectroscopy, MS Spectroscopy etc.
- Chromatography methods: TLC, HPTLC, HPLC etc.

Menthol



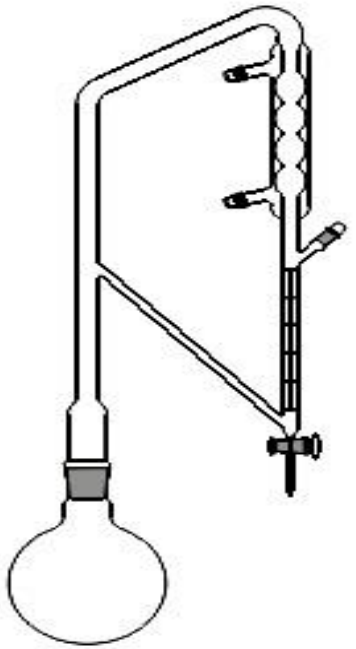
Synonym: Peppermint oil, Mint, Pudina, Brandy mint, Lamb mint.

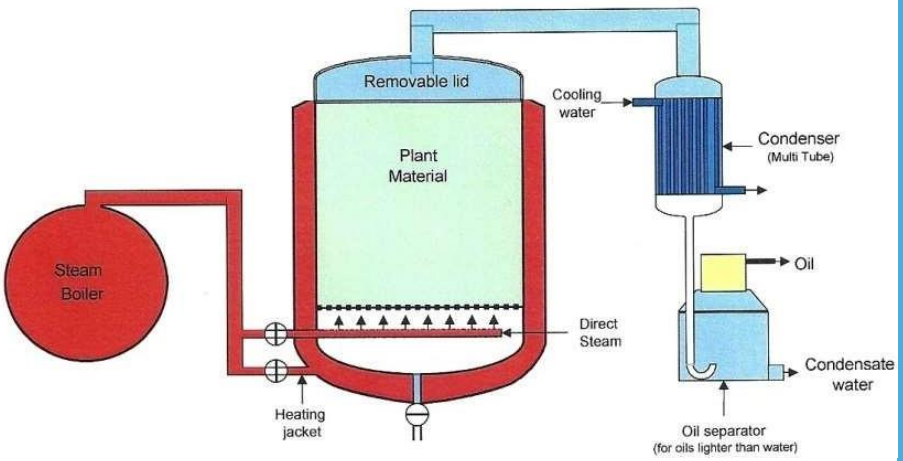
Biological source: It is volatile oil obtained by steam distillation of fresh flowering tops of plant *Mentha Piperita* Linn. Family: Labiatae

Isolation

Method 1

1. Take weigh quantity of coarse powder of leaves.
2. Extract oil by water distillation.
3. Separate the oil from water by using separating funnel and allow cooling.
4. Crystals of menthol will separate out.
5. Collect the crystals by centrifugation.
6. Recrystallize menthol from acetone or low boiling solvent to which menthol having solubility.





Method 2

1. Take air dried weighed quantity of mentha plant in stainless still container of assembly.
2. The steam under pressure, generated with the help of boiler.
3. Steam is then passed through the drug.
4. The plant material containing oils, releasing the plant's aromatic molecules and turning them into vapor.
5. It takes about 3-4 hours for distillation. Menthol comes over latter part of distillation.
6. Further it is passing through condenser where the vapor cools back into liquid form.
7. Essential oil floats on top of the water. From here, it is siphoned off.
8. Allow cooling, crystals of menthol will separate out.

Identification test

- Few drops of oil mix with 5 ml nitric acid & heat on waterbath. Within 5 min. solution develops blue colour further heating shows cooper colour fluorescence. After sometime its become yellow. which indicate the presence of menthol.
- 10 mg menthol crystals are dissolved in 4 drops of concentrated sulphuric acid and added a few drops of vanillin sulphuric acid reagent. It shows orange-yellow colour which changes to violet upon the addition of a few ml of water.
- **Properties:**

Appearance : White crystalline substances, which is solid at room temperature and melts slightly above (m.p. 41 to 43°C).
Odour : Characteristic and pleasant
Taste : Pungent followed by cooling sensation
Solubility : Soluble in 70% alcohol, ether and chloroform, insoluble in water

Menthol

Analysis

Sample preparation	:	1mg of sample is dissolved 1ml of methanol
Standard sample	:	1mg of Menthol is dissolved 1ml of methanol
Stationary phase	:	Silica gel –G
Mobile phase	:	1. Chloroform 2. Toluene:Ethyl acetate(93:7)
Detecting agent	:	1% vanillin – sulphuric acid reagent and heat the plate at 110° C for 10 minutes
Rf Value	:	1. 0.48-0.62 2. About 0.28

Analysis

Assay: Place about 10.0 g in an acetylation flask, add 10 ml of acetic anhydride and 1 g of anhydrous sodium acetate, attach a reflux condenser and boil for 2 hours. Cool, add 30 ml of water and warm on a water-bath for 15 minutes with occasional shaking. Transfer the contents of the flask to a separating funnel, reject the water layer and wash the remaining oil with water until the last washing no longer shows acid reaction. Dry the resulting oil by shaking with 2 g of anhydrous sodium sulphate, allow to stand for 30 minutes and filter through a dry filter paper. Weigh accurately about 1.5 g of the dry acetylated oil, add 3 ml of ethanol (95 per cent) and 0.1 ml of phenolphthalein solution and dropwise, 0.5 M ethanolic potassium hydroxide until the solution acquires a faint pink colour. Add a further 20.0 ml of the alkali, attach a reflux condenser and boil for 1 hour on a water-bath. Cool, add 1 ml of phenolphthalein solution and titrate the excess of alkali . with 0.5 M hydrochloric acid: Repeat the operation with the same quantities of the same reagents in the same manner without the oil and calculate the amount of total menthol from the following expression.

$$\textbf{Total menthol (\%)} = \frac{\textbf{(a-b)x7.813}}{\textbf{S -(a-b)xO.021}}$$

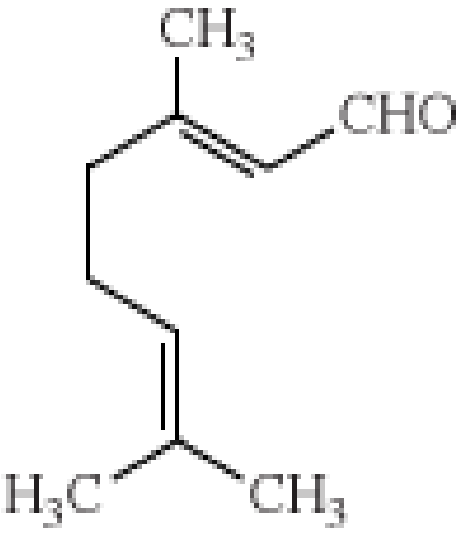
where,

S is the amount, in g, of the acetylated sample taken, a is the amount, in ml, of0.5 M hydrochloric acid consumed in the blank test, and b is the amount, in ml, of 0.5 M hydrochloric acid consumed in saponification of the acetylated oil

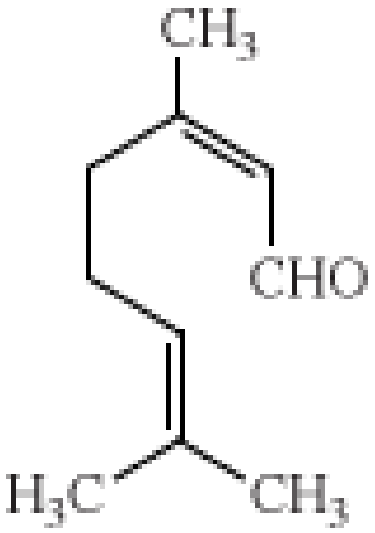
Citral

Synonym: Indian melissa oil, fever lemon-grass oil

Biological source: It is volatile distillation of leaves and aerial *Cymbopogon flexuous* or *Cym* Family: Graminae (Poaceae) oil sh 75% of aldehyde as citral



geranial
(citral a)



neral
(citral b)

Method 1: Fractional or vacuum fractionation

In vacuum fractionation the enrichment of citral happens and citral of 95% purity is generally obtained. Moreover removal of components like geranial, neral which have boiling points differing only by a few °C from that of citral is found to be difficult even when high efficiency fractionating columns are used. Being a mixture of α, β unsaturated aldehydes, citral is heat labile, and excessive heat treatment is likely to lead to rearrangements, polymerisation and eventual destruction of the material.

Method 2: Fractional crystallization Method

The fresh plant material is hydro- distilled to obtain lemon grass oil. It is purified by fractional crystallization. In the total oil, first Sodium sulphite is added, the citrals get converted into 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.

Identification

- Take sample to this add alcoholic solution of sudan red III, it shows red colour.
- Take sample to this add tincture alkane, it shows red colour .

Both above test indicate presence of citral.

Properties:

Appearance : Clear pale yellow liquid

Odour : Strong lemon like odour

Taste : Lemon like taste

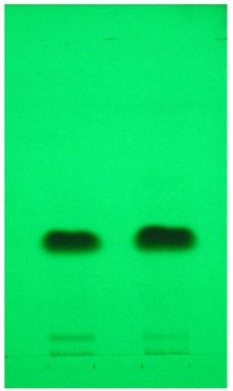
Solubility: Soluble in 3 parts of 70% alcohol, chloroform and fixed oil. Insoluble in water.

Boiling point : 224-228^{O} C

Analysis

A) Chromatographic analysis

Stationary phase: Silica gel G.
Mobile Phase- Toluene: Ethyl acetate (8.5:1.5)
Detecting agent: 2, 4, dinitrophenyl hydrazine reagent
Color spots : Yellow to orange
Rf value- 0.5



Sample preparation	:	1mg of sample is dissolved 1ml of methanol
Standard sample	:	1mg of Citrals dissolved 1ml of methanol
Stationary phase	:	Silica gel –G
Mobile phase	:	Toluene: Ethyl acetate(93:7)
Detecting agent	:	1% vanillin – sulphuric acid reagent and heat the plate at 110° C for 10 minutes
Rf Value	:	0.51

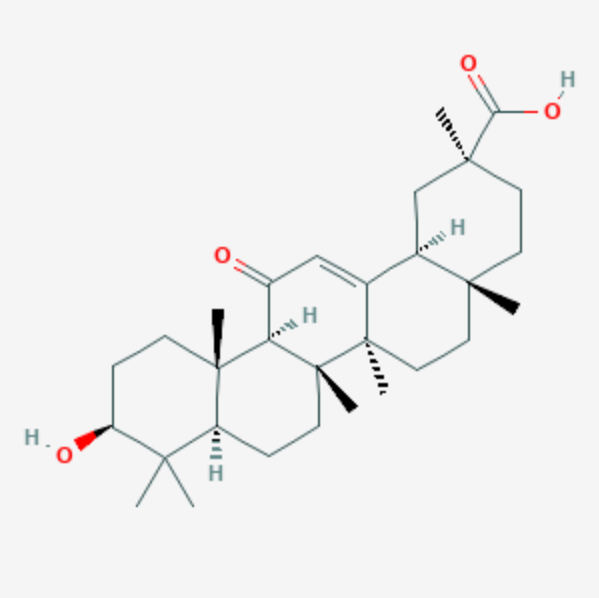
Analysis

B) Colorimetric Method

- The citral content of lemongrass oil has also been estimated by the coloring' agent-that of Ehrlich Miller consists of the solutions.
  - ❖ 5% p-dimethylaminobenzaldehyde solution in acetic acid.
  - ❖ 10% phosphoric acid solution in acetic acid.
- One ml each of the above solution is added to different amounts of citral in acetic acid, whereby a marked colour change from blue to pink can be observed.
- The percentage absorbance and extinction of the colored citral is then measured using colorimeter and calibration graphs are plotted.
- The amount of citral in solutions can be compared with that of known strength and thus the percentage of cirtal can be determined.

Biological Source

- This acid is obtained from the dried, peeled as well as unpeeled roots and stolons of Glycyrrhiza glabra var. Typica also known as Spanish liquorice belonging to the family Leguminosae (Fabaceae). It is a pentacyclic triterpenic acid and is obtained by hydrolysis of glycyrrhizin (a triterpene saponin glycoside).
- Glycyrrhetinic Acid



Isolation

1. Method I

- Take weighed quantity of Glycyrrhiza root powder and extract it with chloroform.
- Filter and separate the marc.
- Extract the marc with 0.5 M H2SO4 for 1 hour
- Filter and extract the filtrate with three portions of chloroform.
- Combine the chloroform layers.
- Distill off the chloroform extract to yield a dry residue of glycyrrhetinic acid.

2. Method II

(a) Liquorice powder is treated with boiling water which helps in the isolation of glycyrrhizin. The extract so obtained is evaporated to dryness.

(b) This extract is added to water and then hydrochloric acid (to obtain a pH of 3-3.4) which helps in the precipitation of glycyrrhetinic acid.

(c) The precipitate is filtered and is washed with water until a neutral pH is obtained and subjected to drying to obtain glycyrrhetinic acid.

Glycyrrhetinic Acid

- Commercially, pharmaceutical preparations utilise ammoniated glycyrrhizin which is prepared by treating the liquorice extract to obtain glycyrrhizic acid to which ammonia solution is added and the resulting solution is spread as a thin film on a glass plate. This plate is dried to obtain ammoniated glycyrrhizin in the form of dark brown shiny flakes

Identification

Sr. No	Chemical test	Observation	Inferences
1	Liebermenn test 3ml extract + 3 ml acetic anhydride, heat and cool. To this add drop of conc. Sulphuric acid.	Blue colour	Triterpenoids presents
2	Liebermenn- Burchard test 3 ml extract+ 2 ml chloroform + 1 ml acetic anhydride + one drop concentrated sulphuric acid	Blue-green to red orange colour	Triterpenoid / Steroids presents

Analysis

a) Glycyrrhetinic acid is analysed by TLC by the following method.

- Img of glycyrrhetinic acid is dissolved in a solvent mixture comprising of 1ml methanol-chloroform (1:1).
- This mixture is applied in the form of spots on silica gel G and eluted with solvent system comprising of toluene-ethyl acetate-glacial acetic acid (12.5: 7.5: 0.5). the plates are dried and sprayed with anisaldehyde sulphuric acid or 1 % vanillin- sulphuric acid heating at 105°C for 5 min.

3. The plate Glycyrrhetinic acid has an R_f value of 0.41 and gives for min. purplish spot.

b) Colorimetric method

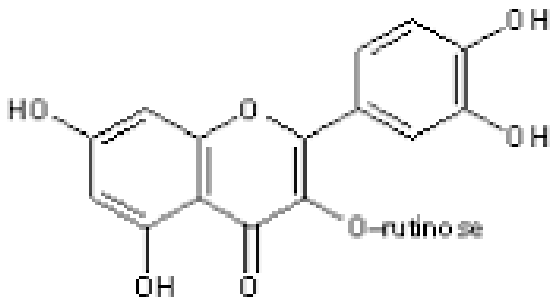
Sample + anisaldehyde +sulphuric acid shows purple colour. Measured intensity of color at 556 nm

Rutin

- **Synonym**: Rutin

Biological Source

Rutin is obtained from buck-wheat which comprises of dried ripe fruits of the plant *Fagopyrum esculentum*, family: Polygonaceae. Other sources of rutin include *Ruta graveolens*, from fresh leaves of tobacco plant *Nicotiana tabacum*, family: Solanaceae and also from cotton plant *Gossypium hirsutum* **Family**: Malvaceae.



Isolation

1. The dried plant material in powdered form is extracted with 80% v/v of 200 ml ethanol twice to obtain an hydro-alcoholic extract.
2. This extract is filtered and subjected to vacuum evaporation until it reduces to about 50-60 ml.
3. Equal volume of ether is added to this reduced extract and the ethereal layer is separated.
4. Again same volume of ether is added to the aqueous extract and this time also the ethereal layer is separated.
5. Only the aqueous layer is utilized which is subjected to evaporation under reduced pressure until it reaches 10 ml. The residual concentrated liquid is placed in a refrigerator overnight (0-5°C) to obtain rutin in the form of solid crystals that are separated from mother liquor.
6. Further purification of rutin is afforded using column chromatography where ethanol is used as mobile phase and magnesium silicate as solid phase.

Identification

Rutin+ Tollen's ragent (Ammonical silver nitrate) → Silver mirror

Rutin+ $FeCl_3$ → Greenish brown colour

Rutin+ Basic lead acetate → Greenish brown colour

Analysis

1. Chromatographic System

a. **Thin layer chromatography**

- Stationary phase: Pre-coated aluminium sheet with silica gel G
- Mobile phases:
- Ethyl acetate: Butanone: Formic acid: Water (50:30:10:10)
- Ethyl acetate: Formic acid : Acetic acid : Water (100:10:11:27)

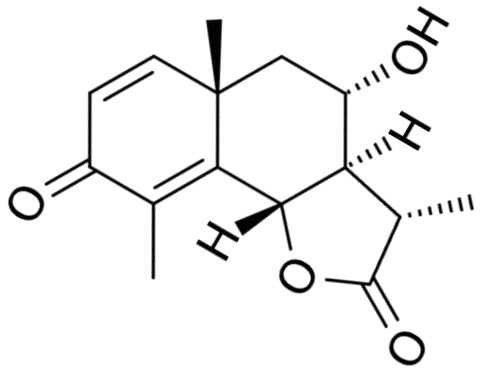
b. **Paper chromatography**

- Stationary phase: Watman paper No. 1 is used.
- Mobile Phases: Acetic acid: water (15:85)
- Isopropyl alcohol: Water (60:40)

2. **Spectrophotometric Analysis**

- The isolated rutin is dissolved in methanol and absorption peaks are determined under UV radiation
- By using KBr disk methodology IR spectrum of isolated rutin is determined.

Biological source: Artemisin is a sesquiterpenoid lactone, obtained from the unexpanded flower- heads of *Artemisia cina Berg*, *Artemisia brevifolia Wall*, *Artemisia maritime Linn.* and other species belongs to family Asteraceae.



Isolation

- Take 100 gm powder herb and macerated in methanol.
- Maceration performed in magnetic stirrer with speed of 700 rpm for 1 hour.
- Repeat process until methanol layer becomes colorless.
- Then extract was evaporated using rotavapour vacuum at temp. 40°c until volume reduce to 100 ml.
- The methanolic extract was partitioned using 50 ml hexane (Hexane: Methanol, 1:2) until colorless hexane layer was obtained. Separate hexane and methanol extract using separating funnel.
- To methanolic extract add 10 ml distilled water and 50 ml ethyl acetate partitioned until ethyl acetate layer become colorless.
- Again separate ethyl acetate and methanol-water extract.
- Each extract was concentrate using rotavapour vaccum at temp. 40° C.
- Artemisin was fractionated by coloumn chromatography using silica gel 60 as stationary phase and ethyl acetate: hexane (with increasing polarity) as mobile phase.

Test	Observation	Inference
Take drug sample+ Boil with 10ml alcohol and filter. To filtrate, add sodium hydroxide and heat again.	The red colour develops in liquid	Artemisin present

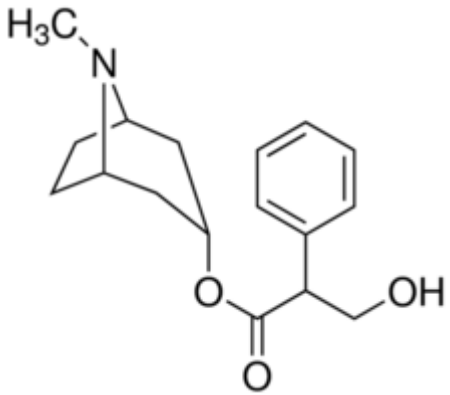
- ANALYSIS

  UV: 1 mg sample mix with 10 ml methanol and analysed λ 200-400 nm.

  IR: 2 mg sample mix with 98 mg of KBr which dried for 24 hr. at temperature 105° C Analysed at- 4000 cm-1 to 400 cm-1.

- ANALYSIS
  Analysis by TLC
- Sample preparation : 1mg of Artemisin is dissolved Chloroform
- Standard sample : Artemisin
- Stationary phase : Silica gel –G
- Mobile phase : Petroleum ether - Ethyl acetate (1:2)
- Detecting agent : p- dimethyl amino benzaldehyde and heat at 800C to produce color
- RF Value : Compare with standard Artemisin

- **Synonym**: Atropa, Datura, deadly nightshade leaf
- **Biological Source**: Atropine is obtained from fresh or dried leaves and aerial parts of the flowering plant *Atropa belladona* also called European belladonna (Family-Solanaceae). Other plants which are good source of atropine are *Atropa acuminate*, *Datura stramonium* and *Hyoscyamus niger*



Isolation

1. The drug material in powdered form is initially treated with sodium carbonate solution and is extracted with benzene or ether.
2. The solvent is treated with water that has been acidified using acetic acid, this helps to release alkaloidal free bases from the solvent.
3. The above mixture is then mixed with solvent ether and then shaken which helps in clearing off only colouring matter
4. Next step involves treatment with sodium carbonate which precipitates the alkaloids. This is followed by filtering washing and drying.
5. The dried mass so obtained is added to solvent ether or acetone and then treated with anhydrous sodium sulphate followed by filtration.
6. The filtrate so obtained is concentrated and then cooled due to which atropine which are separated by filtration.
7. The filtrate is in the form of crystalline mass to which alcohol is added followed by NaOH solution. The solution is kept aside for some time to enable the racemization of hyoscyamine to atropine which lacks optical activity.
8. The crude atropine so obtained is purified by crystallization.

Method-II

Dried coarsely powdered Belladona leaves are extracted with alcohol (95%) by soxhlet hot percolation method.

↓

Collect the Ethanolic extract

↓ Concentrate under vacuum

Syrupy mass

↓ Dissolved in dil Hcl & filter

Filtrate contains alkaloids in aqueous solution and it is extracted with petroleum ether ↓ to remove the chlorophyll pigments & other impurities

Make the aqueous solution alkaline with ammonia solution

↓ Extracted with CHCL3 * 3

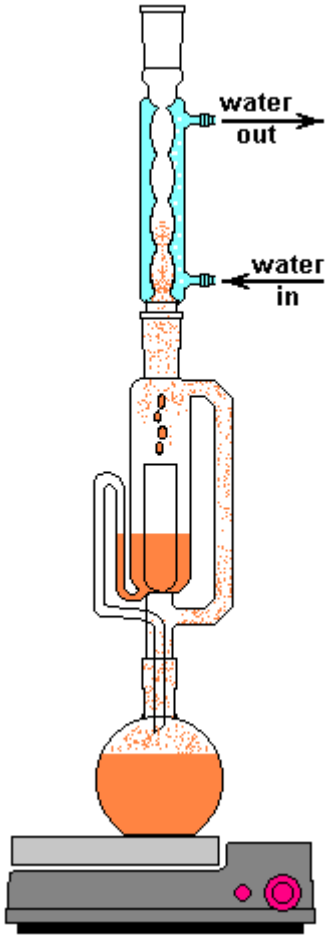
Combine the CHCL3 extracts

↓ Remove the CHCl3 under vaccum

Residue (Crude alkaloids)

↓ Extracted with dilute solution of oxalic acid

Alkaloids crystallized out (Fractional crystallization)



Identifications

- General Tests

1. Vitali-Morin's Test

The Alkaloid solution → (Evaporate to dryness on water bath) → Residue + Fuming HNO_3

↓

Evaporate to dryness on water bath

↓

Residue +3% methanolic KOH

↓

Deep purple colour

2. Rathenasinkam's Test

(a) In a water bath heat 0.5 mg atropine with HNO_3 for 10 min, add 30 ml water and extract with $CHCI_3$.

(b) Discard the $CHCI_3$ layer, to the residue add few drops of NH_3 and extract with $CHCI_3$

(c) Evaporate the $CHCI_3$ layer, to the residue add acetone and few drops of (10%) NaOH.

(d) Bluish-purple colour appears.

3.Schaer's Test

- Atropine solution in water + HCl + $AuCl_2$ - Lemon yellow precipitate

Analysis

Sample preparation : 1mg of Atropine is dissolved in 1ml of chloroform

Standard sample : Atropine

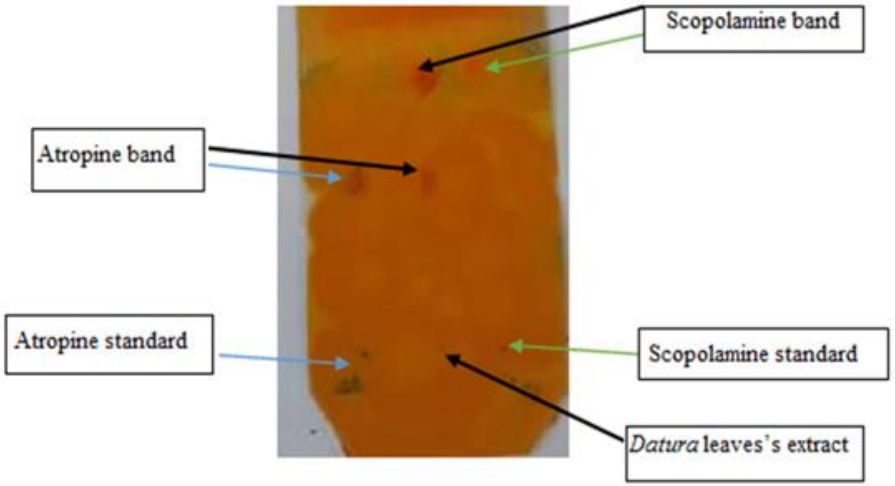
Stationary phase : Silica gel-G

Mobile phase : Toluene: Ethyl acetate: Diethyl amine (70:20:10)

Detecting agent : Dragendorff's reagent

Rf Value : 0.70

Colour spot : Yellow orange spot



Biological Source

Quinine is an quinoline alkaloid obtained from dried bark of *Cinchona calisaya*, *Cinchona succirubra*, *Cinchona officinalis*, *Cinchona ledgeriana*.

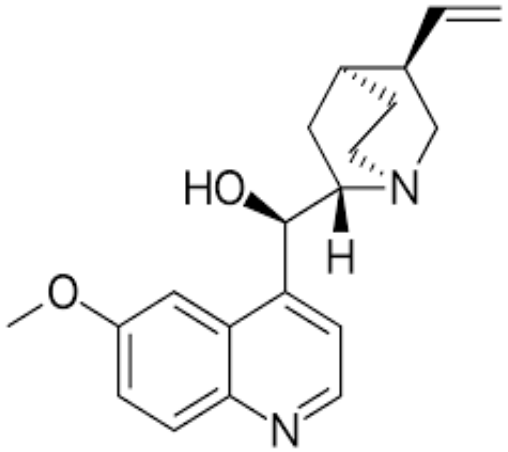
Family – Rubiaceae

Properties

- It is a white crystalline in colour.
- It is soluble in alcohol, acetone and chloroform.
- It is insoluble in water.

Uses

- Quinine is an antipyretic, anti-malerial agent.



Quinine

Powder of Cinchona bark

↓

Add alcoholic KOH (20%)

↓

Extract this mixture with benzene for 6 hrs. in Soxhlet apparatus

↓

Filter the benzene extract

↓

Shake with 5 % H_2SO_4

↓

Filter

Basify the filtrate with NH_3 solution

↓

Extract the filtrate with $CHCl_3$ (3X)

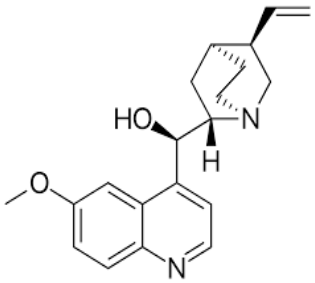
Combine & evaporate chloroform layer

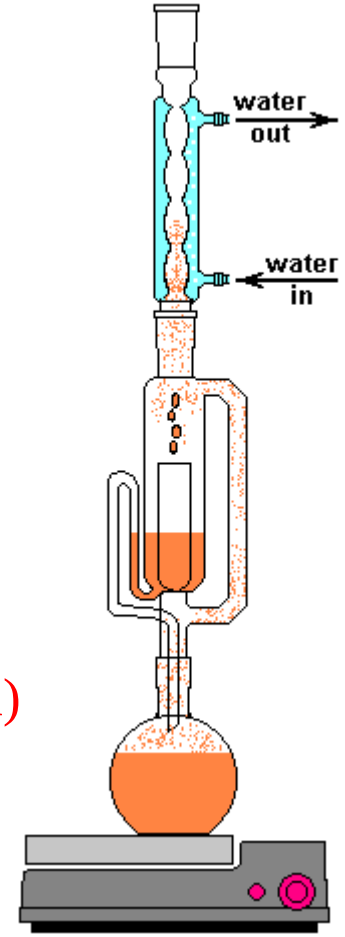
↓

Evaporate $CHCl_3$

↓

Crude Quinine, Recrytsallise it





Identification

General Tests

1. Thalleoquin test – substance + bromine water + dilute ammonia solution----------Emerald green colour

2 Powdered cinchona + Glacial acetic acid Heat Purple vapours

3. Quinidine solution + $AgNO_3$ solution, White precipitate, soluble in nitric acid

Specific Tests

1. Quinine + Dil. H_2SO_4 Blue fluorescence when observed under UV light

2. Quinine + Tartaric acid Formation of insoluble salts

Analysis

T.L.C Method

Sample preparation – Dissolved 1mg of quinine in 1ml ethanol

Stationary phase - Silica gel – G

Mobile phase – 1. Chloroform: :Diethyl amine (9:1)

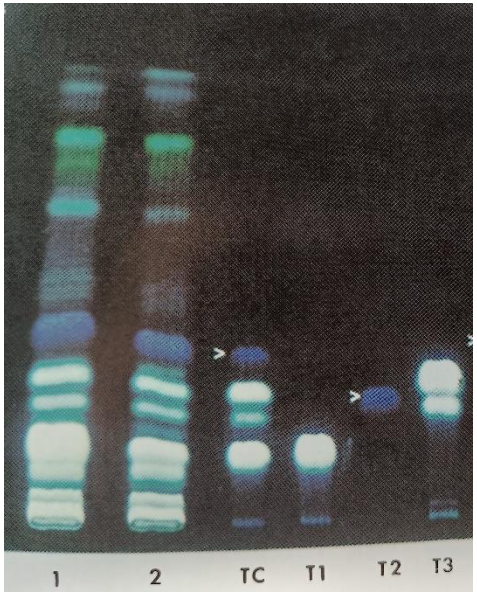
2. Chloroform : Acetone : Diethylamine (5:4:1)

Detecting agent – 1. Dragendroffs reagent

2. 10% Ethanolic H_2SO_4---UV 365nm

Rf Value – 1. 0.6

2. 0.17



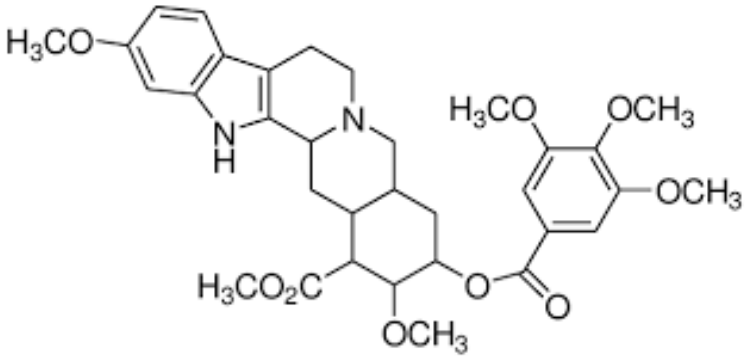
T1-Quinine
T2- Quinidine

Reserpine

Biological Source Reserpine is indole alkaloid obtained from dried roots of *Rauwolfia serpentina*.

Family: Apocynaceae

Uses It is used as antihypertensive drug.



Properties

Appearance: White or pale buff to slightly yellow crystalline powder, darkening slowly on exposure to light.

Odour: Odourless

Taste: Bitter taste

Solubility: Soluble in alcohol, chloroform and acetone, partially soluble in water, freely soluble in acetic acid

Reserpine

ISOLATION

- Take weighed quantity of root powder and moisten with 10% sodium bicarbonate
- Extract above mixture with benzene until it give positive reaction with HgI_2.
- Filter it and concentrate. Add ether and dilute hydrochloric acid to it. Shake well.
- Separate acid layer.
- Again washed with ether. Make alkaline with ammonia.
- Then extract above solution with chloroform.
- The chloroform extract washed with 10% sodium carbonate.
- Separate chloroform layer and evaporate to dry.
- Dissolve it in methanol to get crystals of reserpine.

Reserpine

Identification

1) Drug + Solution of vanillin in acetic acid -------Violet red colour is produced due to the presence of reserpine.

2) Tincture of rauwolfia root exposed to UV light ------- Blue fluorescence

3) Freshly cut surface of rauwolfia root + Concentrated HNO_3 (2 portions) + Water (1 portion) Medullary rays turn red in colour

Analysis

T.L.C Method

Satndard preparation – Dissolved 1mg of reserpine in 1ml ethanol

Sample preparation – 1 mg rauwolfia alkaloidal extract is dissolved in ethanol

Stationary phase – 1. Silica gel – G

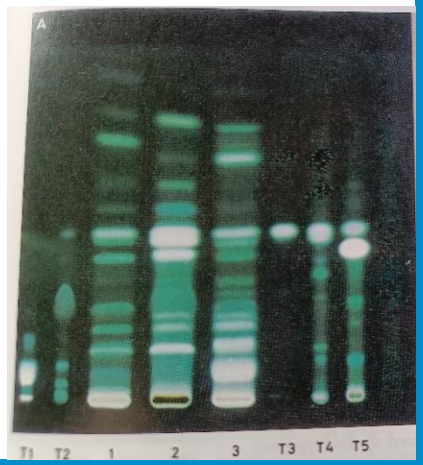
2. Alumina -G

Mobile phase – Chloroform-Acetone-Diethylamine (50:40:10)

Detecting agent – Dragendroffs reagent

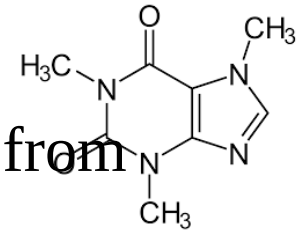
Rf Value – 1. 0.72

2. 0.35



T3-Reserapine
1 Rauwolfia Extract

Caffeine



Biological Source Caffeine is xanthine alkaloid obtained from coffee, cocoa beans, cola nuts and tea leaves.

It is obtained from prepared leaves of *Thea sinensis*. Family: Theaceae

It is obtained from dried ripe fruits of *Coffee arabica* and *Coffee liberica*. Family: Rubiaceae.

Uses: Caffeine is used as CNS stimulant

Properties.

Appearance : White powder or white glistering needles

Odour : Odourless

Taste : Bitter taste

Solubility : Soluble in hot water

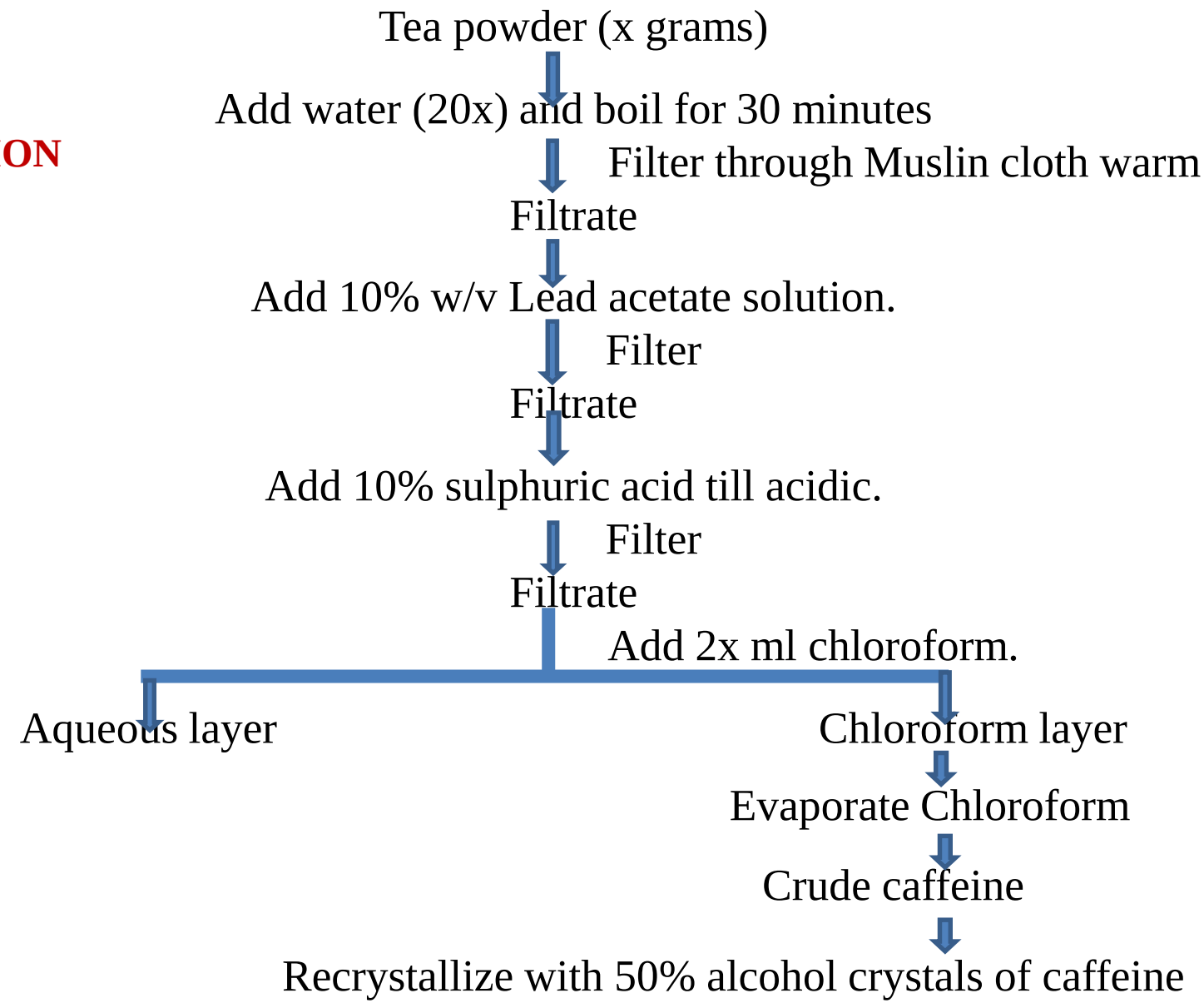
ISOLATION:

Method 1

- Take weighed quantity of above mention any drug powder and extract with ethanol in soxhlet assembly (6 hrs.). Filter it and to filtrate add magnesium oxide solution.
- Mixture is evaporated to dryness on water bath.
- Above residue washed with water multiple times. Collect the water layer.
- To aqueous extract add 10% sulphuric acid, boil it for 30 minutes.
- Once it gets lukewarm add 25 ml chloroform.
- For decolourization add sodium hydroxide/ potassium hydroxide.
- To above mixture add equal volume of water. Shake well.
- Separate the chloroform layer. Evaporate to dry.
- Dissolve it in warm water and evaporate.
- Needle shaped crystals of caffeine obtained.

Caffeine

ISOLATION
Method 2



Caffeine

- IDENTIFICATION
- Murexide test

  Take test sample to this add hydrochloric acid and potassium chloride. Heat it till it gets dry.

  Expose this powder to dilute ammonia. Purple colour indicates presence of caffeine.

- ANALYSIS

1) Thin layer chromatography

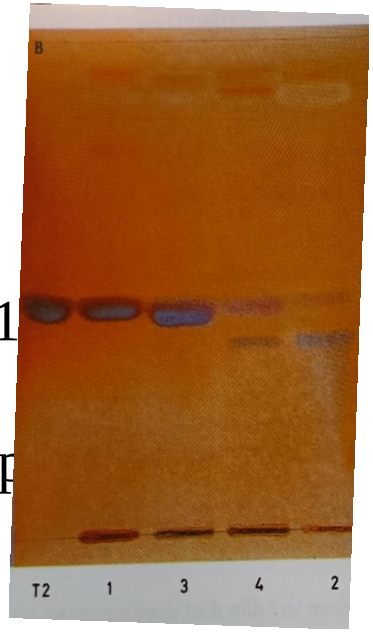
Stationary phase: Silica gel G

Mobile phase: Ethyl acetate: methanol: acetic acid (8:1:1

Chloroform: methanol (9:1).

Spraying reagent: In iodine chamber visible brown spot ap

- Caffeine has an Rf value of 0.41

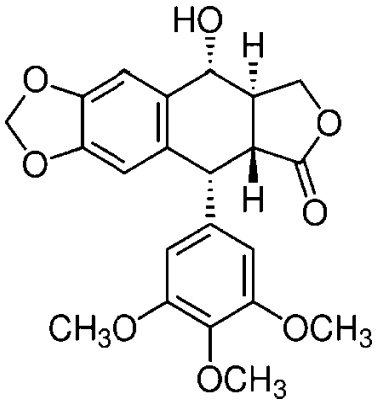


Podophyllotoxin

Biological source

Podophylotoxin is the lactone resin present in the root and rhizome of *Podophyllum hexandrum* and *P. emodi* Family – Berberidaceae. Podophyllum resin contains not less than 40% and not more than 50% of podophyllotoxin

Properties



Appearance: White to off-white solid

Odour: Characteristic

Taste: Bitter

Solubility: It is soluble in acetone, benzene;
very soluble in ethanol, chloroform;
slightly soluble in water; insoluble in ethyl ether

Uses

1) Podophyllotoxin is used as a cholagogue (agent that promotes bile flow),as an anticancer agent and to treat condylomata (warts caused due to human papilloma virus)

2) It is used in the synthesis of etoposide which is used for treating lymphomas, leukaemias, small-cell lung cancer and testicular cancer.

Isolation

1. Extract rhizomes or roots powder with methanol in soxhlet assembly.
2. Filter it and concentrate to semisolid mass.
3. To this add acidified water (1% v/v hydrochloric acid).
4. Allow mixture to stand for 2 hours to precipitate.
5. Filter under vacuum.
6. Filtrate washed with washed with cold water and then acidified water.
7. Dissolve residue in sufficient quantity of hot alcohol (90%). Filter and evaporate to dryness.
8. Recrystallise the residue in benzene to get podophyllotoxin.

IDENTIFICATION

- Treat podophyllotoxin with 50% sulphuric acid it 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 precipitate.

Analysis by TLC

Sample preparation : 1mg of Podophyllotoxin/ Podophyllum root or rhizome extract is dissolved in 1ml of methanol

Standard sample : Podophyllotoxin is dissolved in 1ml of methanol

Stationary phase: Silica gel-G

Mobile phase: Chloroform: Methanol (90:10) for about 6cm (Only glycosides are separated but aglycone like podophyllotoxin remains in the region of the front. The same plate is again eluted with more weakly polar Solvent Toluene : Acetone (65:35) upto 15 cm.

Detecting agent : Spray with methanol Sulphuric acid and heat 10 minutes at $110^{O}C$

RF Value : About 0.7

Colour spot : Violet-blue spot

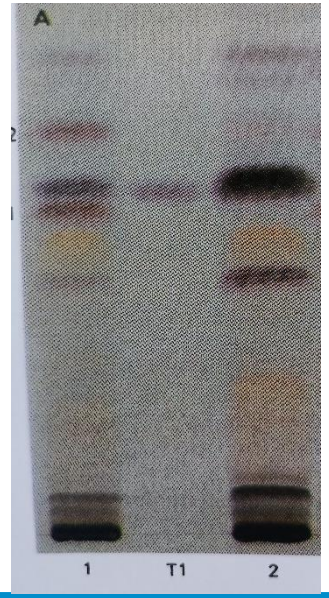
Analysis by HPLC

Method : Isocratic

Stationary phase : C18 column

Mobile phase : Methanol: water (6:4) at flow rate 0.8ml/min.

Detection : Photodiode detector at 283nm



1 *P. hexandrum* extract
***2. P. emodi* extract**
T1- Podophyllotoxin

Curcumin

Biological source: Curcumin or Curcuminoids are the diaryl hepnoid compounds obtained from the dried rhizomes of Turmeric, *Curcuma longa*, belongs to family – Zingiberaceae

Properties:

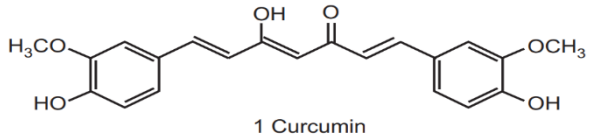
Appearance: Orange yellow crystalline powder

Odour: Characteristic

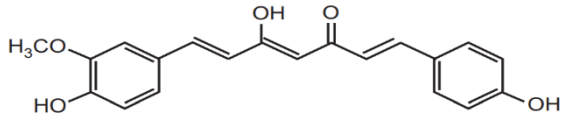
Taste: Slightly pungent bitter

Solubility: Insoluble in water and ether, but soluble in alcohol

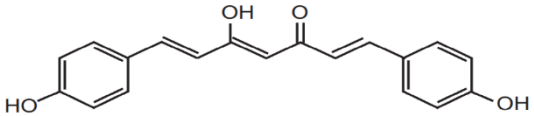
Uses: Curcumin shows anti-inflammatory, antioxidant & anticarcinogenic activity.



1 Curcumin



2 Demethoxycurcumin



3 Bis-demethoxycurcumin

ISOLATION

Method 1

- Take 20 gm of dried powder of turmeric rhizome and extract with petroleum ether in soxhlet assembly.
- Filter it. Residue again extract with methanol.
- Filter and concentrate extract.
- Take methanolic extract in separating funnel to this add 100 ml toluene and 100 ml 0.2 M sodium hydroxide solution. Shake it well.
- Separate aqueous phase and maintain pH 3 by addition of hydrochloric acid.
- Treat this acidified aqueous layer with diethyl ether (300 ml).
- Separate ethereal layer washed with 30 ml water and dried over magnesium sulphate.
- Yellow colour precipitate of curcumin obtained.

ISOLATION

Method 2:

- About 50gm of turmeric powder was extracted with 95% alcohol in Soxhlet assembly until all the coloring, the matter was extracted.
- Alcoholic extract was distilled off to a semi-solid brown colored mass (about 4.5%).
- The crude extract was dissolved in 50ml of benzene and extracted twice with an equal volume of0.1% sodium hydroxide solution.
- Combined the alkaline extracts and acidified with dilute hydrochloric acid. A yellow-colored precipitate was formed. Allowed to settle for about fifteen minutes.
- After setting of precipitate, concentrated the extract by boiling on a water bath and at the same time dissolving the precipitate in boiling water. During this process of boiling, the resinous material agglomerated and formed a lumpy mass.
- The solution was filtered in the hot condition and concentrated filtrate to a very small volume. It was set aside for slow precipitation of curcumin which was then dried, kept in a desiccator, and percentage yield was calculated.

Curcumin

- IDENTIFICATION

Treat powder drug with sulphuric acid it gives crimson colour.

Thin layer chromatography

Stationary phase: Silica gel 60F 254

Mobile phase: Chloroform: methanol (95:5).

Chloroform: ethanol: GAA (95:5:1).

UV detection: 420 nm.

254 nm

- ANALYSIS

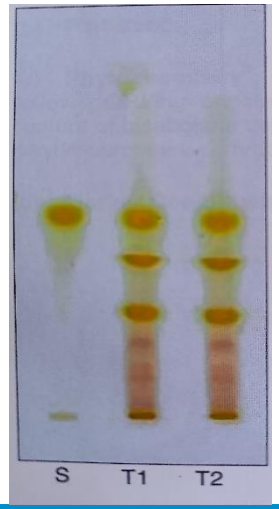
  1) HPLC

  Method: Isocretic

  Stationary phase: C18

  Mobile phase: Methanol, 2% acetic acid and acetonitrile

  Detection: UV-visible detection 425 nm.



Curcumin

2) Curcumin along with other curcurminoids can be analysed using TLC

- 1 mg curcumin is added to 1 ml methanol and spotted onto silica gel plate. Mobile phase: Chloroform-ethanol-glacial acid (94: 5:1).
- The plate is eluted and observed under 360nm light.
- Curcumin has an Rf value of 0.79 and yields a bright yellow fluorescent spot.
- Similarly desmethoxycurcumin has an Rf value of 0.60 while bisdesmethoxycurcumin has a value of 0.43

THANK YOU