UNIT-III

* **Isolation** :- Isolation is a separation technique in which we can obtain a purified compound from the mixture of component present in extract.

* **Identification**:- It is the process in which we have to identify that the isolated compound is correct or not.

- compounds are identified by two basic way -

i) qualitative identification

ii) quantitative identification

1) **Qualitative identification** :-

It is a step to identify different class of compound presents in extracts by chemical test.

- Test for alkaloid
- Test for Glycoside
- Test for protein & amino acid
- Test for tannin
- Test for carbohydrates
- Test for saponin

ii) Quantitative identification :-

The compound are identified by different methods like - UV visible spectroscopy, IR spectroscopy, chromatography - TLC, HPTLC, HPLC, column.

Analysis of Phytoconstituents

- After isolation of phytoconstituents, analysis process is used for determination of concentration of extract.
- These are analysed by different methods.

→ Spectroscopy – IR, UV, NMR, Mass

→ chromatography – TLC, HPTLC, HPLC, column.

TLC → Thin-layer chromatography.
HPTLC → High-performance thin-layer chromatography
HPLC → High-performance liquid chromatography.

A. TERPENOIDS

* Properties :-

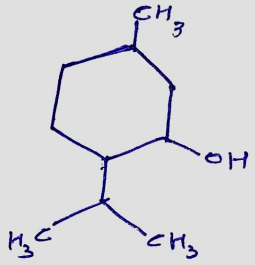
- Most of the terpenoids are colourless.
- Terpenoids are insoluble in water, but soluble in organic solvent.
- Terpenoids are either open chain or cyclic unsaturated compound having one or more double bond.
- They easly get oxidised by oxidizing agents.

Uses :-

- Terpenoids are used in preparation of perfumes, cosmetics, soaps, foods and pharmaceutical beverage industries.
- Apart from food & pharmaceutical industries, They are also used in insecticides, pesticides, and deodrants.
- They also act as antiseptic, stimulant, carminative, diuretic, anthelmentic, analgesic & aromatic.

Menthol

Menthol is a monocyclic, monoterpenes containg alcohol group.



* **Synonyms :-** Mentha oil

* **Biological source :-**

The oil is obtained by steam distillation of the fresh flowering tops of the plant known as Mentha piperita

* **family :-** Labiatae.

- Menthol is isolated by two methods—
  - → Hydro distillation
  - → Steam distillation.

Isolation of Menthol—

Taken fresh plant leaf of peppermint plant.

↓

extraction was done by steam distillation.

↓

Then obtained peppermint oil is passed through a bed of anhydrous sodium sulphate to remove moisture.

↓

The moisture free oil frozen to about -60°c for about 7-days.

↓

The menthol in the oil form flaky crystal and menthol separated by filtration.

↓

The motherliquor still contains menthol along with menthone and other constituents.

↓

For removing menthone added boric acid and boiled for 3-hours under distillation and remove menthone.

↓

Remaining borate of menthol is subjected for saponification with 50 ml of NaOH by heating under reflux for about 1 hour.

↓

The resultant solution is allowed to cool to separate remaining menthol.

Identification of Menthol

- **Identification Test**

1. On heating the crystal of menthol in watch glass on water bath, the entire material evaporates without leaving any residue.

2.) Small quantity of menthol is taken in a test tube and added equal quantity of camphor → Liquifaction of contents of the test tube indicates the presence of Menthol.

3.) Few gm of Menthol + few drops of conc sulphuric acid + few drops of vanillin → orange yellow colour.

Added water colour changes to violet.

Analysis of Menthol

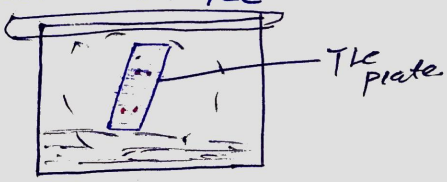
1. **TLC Method :-**

- This method is based on adsorption chromatography.
- It is an important analytical tool for qualitative & quantitative of a number of natural products.

i) Taken silica Gel (column) as stationary phase

Sample prepared in menthol is applied to TLC plate.

ii) Then TLC plate is subjected in TLC chamber.



iii) TLC chamber previously saturated for 30 mins with mobile phase, hexane : Ethylacetate (8:2).

iv) Then the plate are dried at room temperature for 15 minutes.

v) Then, spread with anisaldehyde sulphuric acid (spraying reagent).

vi) Spray Reagent followed by heating at 105°c for 10 minutes

vii) The menthol shows RF value about 0.34.

2. HPLC Method

i) Column :- Shim-Pack, VP-ODS

ii) Mobile phase :- Ethyl acetate : isooctane (30 : 70).

iii) Detection :- 322 nm.

iv) Flow Rate :- 3ml/minute.

v) column temp. :- 25°c.

vi) Sample inject :- 40 µL.

vii) Retention time :- 8.2 minutes.

viii) Sample preparation :- 1 mg of isolated sample is dissolved in 50 ml of n-hexane and applied in HPLC chromatograph.

ix) standard preparation : 1 mg of standard menthol is dissolved in 25 ml of n-hexane and applied in HPLC chromatograph.

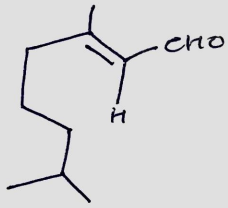
Citral

* **Synonyms:-** Lemon grass

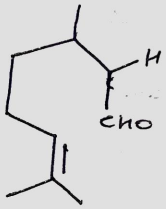
It is chemically aldehyde group of mono terpenoids.

* **Biological source:-** It is obtained by steam distillation from the leaves of cymbopogan Citratus

* **family:-** Graminae



citral-A



citral-B

Isolation of citral

citral is isolated mainly from lemongrass oil by steam distillation.

Leaf of Lemon grass is taken.

↓ Hydrodistillation

oil of Lemon Grass

↓

oil is separated from water by separating funnel.

↓

Pure oil obtain by added diethyl ether.

↓

↓

Ether layer collected slowly to avoid formation of emulsion.

↓ Boil at low temperature.

Ether layer slowly evaporate.

↓

yellow citral liquid separated.

Identification of citral

1.) citral + alcoholic solution of sudan Red-III

↓

Red colour appear.

2) citral + Tincture of Alkane

↓

Red colour appear.

Analysis/Estimation of citral

Analysis by TLC method:-

- Standard sample:(citral)
- Sample preparation:- 1mg of citral is mixed in 1ml of hexane (methanol)
  - Add spot on TLC Plate
- Stationary phase:- Silica Gel - G.
- Mobile phase:- Toluene : Ethyl acetate. (97 : 3)
- Spraying Reagent :- Vanillin sulphuric acid reagent.

- colour spot :- yellow to orange.
- RF value :- 0.38.

HPLC Method

- Column :- ODS (octadecylsilane) hypersil.
- Mobile phase :- Methanol : acetic acid (60:40).
- flow rate :- 1 ml/minute.
- Detection :- 254 nm.
- Sample inject :- 20 µL.
- Retention time :- 10.7 minute (citral-A)
  12.2 minute (citral-B)
- Sample preparation :- 1 ml citral oil is mixed in 1 ml of Toluene and applied on HPL column.
- standard preparation :- 1 ml of standard citral mixed in 1 ml of Toluene and applied on HPLC column.

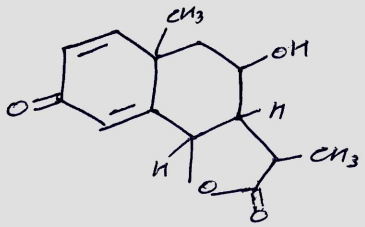
Artemisin

It is belong to class of sesquiterepene Lactone.

* Synonyms :- sweet annine, wormweed.
* Biological Source :- It is obtain from the leaves & unexpanded flower heads of Artemisia Ammua
* family :- Asteraceae.

* It contains 2-3 % of essential oil and two crystalline substances.

example- Santonin, Artimitin.



Isolation of Artemisin

* Fresh leaves of Artemisia Annua are dried at control temperature Not more than 60°C temperature

↓

* Dried powdered material is extracted with methanol using **maceration method**.

↓

* The maceration is repeated till the macerate become colourless.

↓

* The methanol extract partitioned with hexane several time till last hexane fraction becomes colourless.

↓

* The methanol extract mixed with little quantity of water to produce hydroalcoholic extract.

↓

* This hydroalcoholic extract is partioned with **ethyl acetate** for several time until the ethyl acetate layer is colourless.

↓

↓

* Then — ethyl acetate fraction & methanol water fraction concentrated at controlled temperature (40°c under vaccuum)

↓

* Purified using column chromatography on silica gel adsorbent.

↓

* The fraction loaded silica gel column eluted with 7.5 % ethyl acetate in chloroform and elutes.

↓

* Artemisin obtained as fine white crystalls by using cyclo-hexane.

Identification of Artemisin

1. Sample + Alcohol + NaOH

↓ Heat

Red colour.

Analysis / Estimation of Artemisin

Analysis by TLC Method:

- preparation of sample - The known concentration of extract are dissolved in hexane., filter if necessary and use for analysis.
- preparation of standard solution - Predetermined concentration of standard artemesinin dissolve in hexane, use for analysis.

- Stationary phase – Silica Gel G.
- Mobile phase – hexane: Ethyl acetate: acetone (16:1:1)
- Spraying reagent – Vaniline
- RF value – 0.34.

By HPTLC Method -

- Plate – Precoated silica Gel 60 F_{254}.
- Mobile phase – N-hexane : ethyl acetate
- Detection – UV at 540 nm.
- Spraying reagent – anisaldehyde sulphuric ā
- Sample inject – 10 µL.
- RF value – 0.28.

HPLC Method -

- Column – ODS
- Mobile phase – acetonitril: water (65:35).
- Detection – UV at 250 nm.
- Flow rate – 0.5 ml/min.
- Sample inject – 10 µL.
- Retention time – 7.2 min
- Utilization – antimalarial

GLYCOSIDES

It is an organic compounds obtained from plants and animals sources, which on enzymatic or acid hydrolysis yields one or more sugar moieties (glycone) and a non-sugar moiety (aglycone).

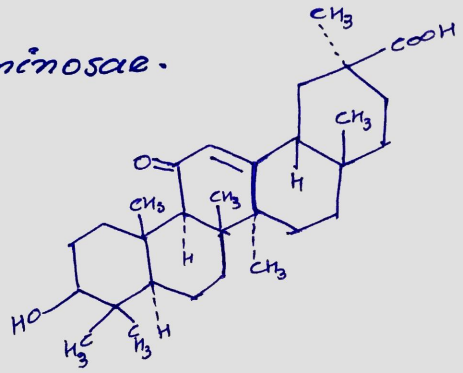
Properties :-

- These are colourless but some of them are colored (e.g; flavinoids - yellow, Anthracene - red).
- Crystalline or amorphus substance.
- Soluble in water & Alcohols, but insoluble in chloroform and ether.
- They are easily hydrolyzed by mineral acids, water and enzymes.

Drugs → I) Glycyrhetinic acid
II) Rutin.

Glycyrhetinic acid

* Synonyms :- Liquorice Root, Mulethi
* Biological Source :- It is a triterpenoid saponin glycoside obtained from the roots and stolons of Glycyrrhiza glabra.
* family :- Leguminosae.



* Isolation of Glycyrhetinic acid

* Take the glycyrrhiza Glabra roots and converted into powder form.

↓

* Transferred about 20gm of liquorice powder in stoppered conical flask.

↓

* Add 50 ml of acetone and 2ml of dil. Nitric acid.

↓

* Cork the flask and macerate about 2 hours with intermittent shaking.

↓

* filter the content of the flask and transfer the marc into another conical flask.

↓

* Add 20 ml of acetone, warm & filter.

↓

* Combined both filterates and concentrated.

↓

* Add sufficient quantity of dil. ammonia solution for precipitation.

↓

* Separate out the precipitate by filtration and washing with 5 ml acetone twice.

↓

* Drying and weighing the crystals of glycyrhetinic acid.

Identification of Glycyrrhetinic acid

1. By chemical Test :-

* Libermann's Test :-

3ml extract + 3ml acetic anhydride

↓

heated & then cooled

↓

Added concentration H_2SO_4 drops

↓

Blue colour is observed.

* Libermann's Burchard Test

3ml of extract + 2ml of chloroform

↓

Added 1 ml of acetic anhydride & 1 drop of H_2SO_4.

↓

Blue green to Red orange colour.

Analysis / Estimation of Glycyrrhetinic

* Analysis by TLC Method :-

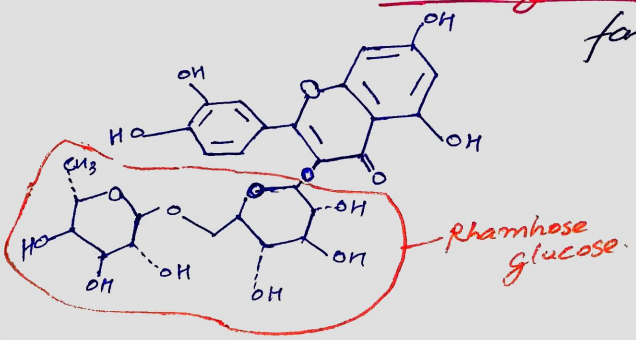
- Sample preparation — 1 mg of sample is dissolved in 1 ml methanol : chloroform. (1:1).
- Stationary phase — Silica Gel-G.
- Mobile phase — chloroform : diethyl ether : formic acid. (80:30:5).
- Spraying reagent — 10% w/v H_2SO_4 in ethanol.
- RF value — 0.72.

By HPTLC Method:-

- Plate — precoated silica gel $60F_{254}$.
- Mobile phase — ethyl acetate: ethanol: water: ammonia (6:5:2:.4:0.1)
- Detection — UV at 254 nm.
- RF value — 0.42.
- Sample inject — 10 µL.
- Standard Drug preparation — 2mg of drug (glycyrhetinic acid) dissolved in methanol. and volume make upto 25 ml with methanol.
- preparation of sample solution — 2gm of concentrated extract was dissolved in methanol and vol. was made upto 25 ml with methanol.

Rutin

* Synonyms:- Buck wheat.
* Biological source:- It is the powder of dried food grains of Fagopyrum Esculentum.
* family:- polygonaceae
  - It is also obtained from herb of Ruta graviolens. family- Rutaceae.



Isolation of Rutin

Freshly buck wheat leaves are transferred in stainless steel vessel.

↓

Added 85% of IPA (Isopropyl Alcohol)

↓ Boil for 10 minutes

Hot extract is filtered

↓

Marc left behind is further extracted with IPA in similar manner and filtered.

↓

The combined extract is then concentrated to 1/4 of original volume.

↓

washed with boiling water

↓

cold to a temperature of about 5°c.

↓

After cooling the crude rutin crystallized out & allow to stand for about 1 hour and for complete crystallization.

↓ yellow powder of Rutin will be obtained.

Identification Tests

1. Rutin + lead acetate

↓

yellow ppt.

2. Rutin + ferric chloride

↓

Green brown colour / dark green.

Analysis / Estimation of Rutin

By TLC method :-

- **Sample preparation** — 1 mg of rutin is dissolved 1 ml of methanol.
- Stationary phase — silica gel G.
- Mobile phase — ethyl acetate : formic acid : butanone : water (50 : 10 : 30 : 10).
- Detecting agent — Anisaldehyde Sulphuric acid reagent.
- colour — yellow spot.
- RF value — 0.53.

By UV Spectroscopy :-

- **Sample solution** — Dissolve 0.5 gm of sample in 50 ml of HPLC grade methanol and filter.

↓

Take 2 ml of filtrate in a 50 ml vol. flask.

↓

Add 2 ml distilled water and 5 ml ammonium molibdade.

↓

Adjust volume upto the mark with distilled water.

- **Standard solution** — Dissolve 0.02 gm of Rutin in 50 ml HPLC grade methanol.

↓

Take 1 ml of this solution in 50 ml volume flask and added 2 ml distilled water & 5 ml ammonium molibdade.

↓

↓

Adjust volume upto the mark with distilled water.

↓

measure the absorbance of standard & sample solution.

$$\boxed{\frac{A_{sample} \times C \times 50 \times 100}{A_{standard} \times W \times Z}}$$

where,

A_{sample} → Absorbance of sample

$A_{standard}$ → Absorbance of std. sol^n.

C → concn of std. sol^n of Rutin.

W → weight of sample in gm.

Z → Volume of sample.

ALKALOIDS

- These are basic nitrogenous compound of plants or animals origin possesses a pharmacological action.
- They contain nitrogen and mainly in heterocyclic ring.
- Mainly derived from amino acids.
- founds in animals, fungi & bacteria.

Types of Alkaloids :-

True Alkaloids

Nitrogen found in the heterocyclic ring and originates from amino acids.

e.g.; Atropine, morphine

Proto-Alkaloids

Nitrogen found outside the heterocyclic ring and derived from amino acids.

e.g.; Ephedrine

Pseudo Alkaloids

Not originated from amino acids.

e.g.; caffiene, Theobromine etc...

Properties

- colourless, crystalline, non-volatile, solids (some are liquids & volatiles).
- Generally bitter in taste.

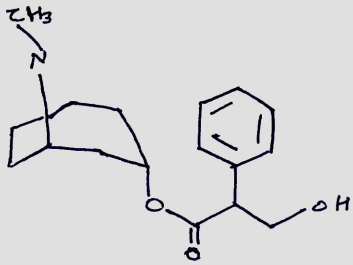
Drugs → Atropine, Quinine, Reserpine, caffiene.

Atropine

- It is a tropane alkaloids obtained from the fresh or dried leaves and flowering tops of Atropa Belladona.

* family :- Solanaceae.

- It is insoluble in ether, easily soluble in chloroform, alcohol and sparingly soluble in water.



Atropine

Isolation of Atropine

* powdered drug material (belladona leaves) is taken and moistens with sodium carbonate solution.

↓

* Mixture is extracted in petroleum ether then filter it.

↓

* In filterate, add aqueous acetic acid then extract it with ether.

↓

* Both are separated through separating funnel and separate aqueous fraction.

↓

* In aqueous fraction, add sodium carbonate to make solution alkaline.

↓

* this forms precipitates of tropane alkaloids

↓

filter, dry → residue.

↓

dissolved in diethyl ether / oxalic acid.

filter it & concentrated the filtrate & cooled.

↓

Hyoscyamine & Atropine will be separated out.

The crystals are filtered and dissolved in alcohol containing NaOH solution (Hyoscyamine is converted to Atropine).

↓

Again Atropine recrystallize from acetone and crystals of Atropine are separated.

Identification Tests

* **Vitali-Morin Test:**

Small quantity of the solid Atropin is taken and added 2 drops of concn Nitric acid in an evaporating dish and evaporated to dryness on water bath.

↓

Then residue is dissolved in 1ml of acetone and few drops of freshly prepared alcoholic potassium Hydroxide solution is added.

↓

Violet coloured residue is obtained.

OR

Sample (Atropin) + concn. HNO_3

↓

Evaporated till dryness

↓

Residue + 1ml acetone + few drops. KOH

↓

Violet colour obtained.

Analysis / Estimation of Atropin

By TLC :-

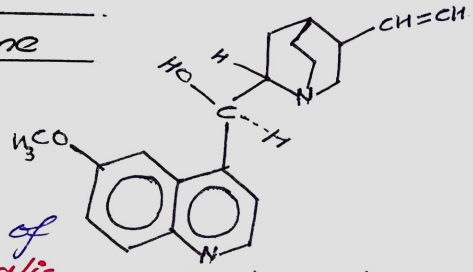
- Sample preparation – 1 mg of Atropine is dissolved in 1 ml of chloroform.
- Standard sample – Atropin.

- Stationary phase — Pre-coated Silica Gel
- Mobile phase — Toluene : Ethyl acetate : Diethyl amine (70:20:10).
- Detecting agent — Dragendroff's reagent.
- RF value — 0.70.
- colour spot — yellow orange spot [4, 5].

Uses :-

- It is used as antispasmodic, mydriatic
- and antidote in opium poisioning.

Quinine



* Biological source:-
It is an quinone alkaloids obtained from the dried bark of cinchona officinalis, cinchona ledgeriana, and Cinchona succirubra
* family :- Rubiaceae.

Isolation of Quinine

Dried powder bark (15-20 gm) of cinchona officinalis.

↓

Mixed with calcium hydroxide [$Ca(OH)_2$].

↓

Add 5% NaOH solution to make paste.

↓

Allow to stand for few hours.

↓

Extraction with Benzene by soxhlet continuous extraction process. (about for 6 hours).

↓

Shake the above extract with successive portion of 5% H_2SO_4 (sulphuric acid) in Separating funnel.

↓

Now, the aqueous acid extract is separated.

↓

Adjust the pH 6.5 with dil. NaOH

↓ cooled.

crystals of quinine Sulphate are formed and recrystalized with hot water.

Identification Tests

Thalleoquin Test:

Sample + Ammonium solution + Bromine water

↓

Emerald green indicates the presence of quinine.

Analysis of Quinine

By TLC :-

- Sample preparation — 1 mg of quinine is dissolved in 1 ml of methanol.
- Standard sample — Quinine.
- Stationary phase — Silica gel-G.
- Mobile phase — chloroform : Diethylamine (9:1).
- Detecting agent — Dragendorff's Reagent.
- RF value — 0.17 [4,5].

Analysis By HPLC

- Method — Isocretic
- Stationary phase — C18 colum.
- Mobile phase — Methanol: Acetonitrile - 0.1 mol/L: ammonia: acetone (45:15:40).
- Detection — Fluorescense at excitation 325nm.
- Emission — 375 nm [5].
- Retention time — 25.6 minutes.
- flow rate — 1 ml/min.
- sample inject — 15 µL.

Uses:-

- It is an antimalarial drugs
- It also have cardiac depressant activity used to treat cardiac arrhythimias.

Reserpine

* Biological sources:-
It is an indole alkaloids obtained from the dried roots of Atropa Belladona, Rauwolfia Serpentina, belongs to
* family:- Apocynaceae.

Alternate:

* Isolation of Reserpine

Powdered drug is moisten with 10% Sodium bicarbonate solution

↓

Extracted with Benzene in Soxhlet apparatus.

↓

Extract is concentrated and to the residue ether is added

↓

Ether extract is transferred in a separating funnel and extracted with dil. HCl and acid layer is separated.

↓

Added ammonia solution until the layer become alkaline

↓

Further extracted with chloroform.

↓

Chloroform layer is washed with 10% Solution sodium carbonate and evaporated to dryness.

↓

Residue is dissolved in methanol and allowed to cooled.

↓

Crystals of Reserpine are obtained.

Identification of Reserpine

By chemical Test

* Reserpine solution + dil H_2SO_4

↓

Expose to light

↓

Distinct yellow colour with fluorescence.

* Reserpine solution + solution of vanillin in acetic $\bar{a}$

↓

Violet Red colour is produced.

Analysis of Reserpine

Analysis by TLC :-

- Test solution — 1 mg of Reserpine is dissolved in 1 ml of ethanol.
- Stationary phase — Silica Gel-G.
- Mobile phase :- Chloroform : Acetone (7:3).
- Detecting Agent :- Dragendroff's Reagent.
- RF value :— 0.96.
- Colour spot :— Orange.

By HPTLC Method :-

- Plate :- Precoated Silica Gel 60 F_{254}
- Mobile phase — Chloroform : Toluene : Ethyl acetate : diethyl amine (7:7:4:1).
- Detection — 254 nm.
- Sample inject — 15 µm
- RF value — 0.36

Standard preparation: — A stock solution of Reserpine (0.1 mg/ml) is prepared in methanol. Different volumes (2, 4, 6, 8) µl are applied to TLC plate.

Caffiene

* **chemical class** :- Purine alkaloid

* **Biological Source** : Found in various part of the plant leaves, seed & fruits.

Sources of caffiene	caffiene content
• Coffee seed – Caffea Arabica • Family – Rubiaceae	1-2%
• Tea leaves – Camellia sinensis family – Theaceae	1-5%

Isolation of caffiene

Taken the dry leaves of Tea and crushed into powder form.

↓

40 gm of tea leaves (powdered) placed in 500 ml beaker.

↓

Added 300 ml distilled water and 6gm of anh. Sodium carbonate.

↓

Boiled for 10-15 minutes with intermittent shaking.

↓ filter into conical flask.

Residue back into the beaker and add 150 ml distilled water & boil again.

↓

Transfer the filtrate in conical flask.

↓

cool and transfer the content into separating funnel.

↓

Add dichloromethane in separating funnel without shaking.

↓

Dichloromethane layer is separated and collect in conical flask.

↓

flask is gently heated for evaporation of dichlorometane.

↓

Dried residue (crystals) of caffiene is obtained.

Identification of Caffiene

* Identification By chemical Test :-

i) Murexide Test –

caffiene crystal + drop of conc. HCl

↓

Add potassium chlorate

↓

Red colour formed

↓ added ammonia solution

Turns to violet colour.

* Analysis By TLC Method –

- Sample preparation – 1 mg of caffiene is dissolved in 1 ml of methenol or dichloro methane.
- Stationary phase – Silica Gel - G.
- Mobile phase – Ethyl acetate : Methenol : Ammonium hydroxide (85 : 10 : 5).
- Detecting / spraying agent – ferric chloride : iodine.
- colour spot – Brown.
- RF value – 0.63.

* By HPLC

- Sample preparation – 1 ml filtrate make up Volume 25 ml with HPLC water. then filtered (microfilter) and filled into HPLC Voils for analysis.
- Column – C_{18}.
- Mobile phase – water.
- Detection – 275 nm.
- flow rate – 1 ml/min.
- Sample inject – 10 µL.
- Retention time – 2.6 minutes.

RESINS

They are solid/semi-solid, amorphus products derived from natural living sources and mostly from the plant origin.

- They are mixture of essential oils, oxygenated products of terpene and carboxylic acid.
- Found as exudations from the trunk of various trees.

Properties –

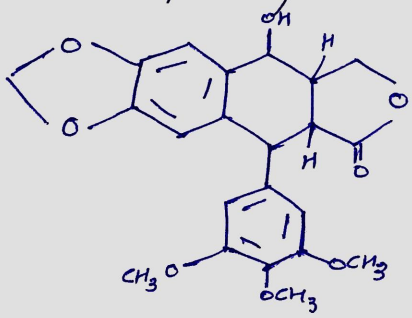
- These are translucent, or transplant solid, semisolids, amorphus or sometimes liquids. [carbon compounds].
- Most of the resins are heavier than water.
- These are water insoluble but mostly soluble in alcohol, volatile oil, fixed oil and also some non-polar aqueous solvents like Benzene or Ether.

▶ DRUGS – • Podophylloton, • Curcumin

Podophyllotoxin

* Biological source:– It consists of dried rhizome & roots of Podophyllum hexandrum,

family – Berberidaceae



Isolation of Podophyllotoxin

Podophyllum powder is extracted with methanol by using soxhlet apparatus.

↓

After extraction filter the content and evaporate to get a semi-solid mass.

↓

Semi-solid mass poured into distilled water containing HCl.

↓

Cooled at 5°c with constant stirring

↓

Allow the mixture, stand for 2 hour.

↓

Filter the content & wash the residue on the filter paper with cold acidified water.

↓

Dissolve this residue in hot 90% ethanol

↓

Filter and evaporate to dryness

↓

Recrystallized the residue in benzene to yield podophyllotoxin.

Identification of Podohyllotoxin

1) Sample + 50% H_2SO_4

↓

Violet Blue colour.

2) Drug + Alcohol ⟶ filtrate + copper acetate
↓
Brown ppt.

3) Alcoholic extract + Potassium hydroxide
↓
stiff jelly is produced.

Analysis of Podophyllotoxin

* By TLC Method :-

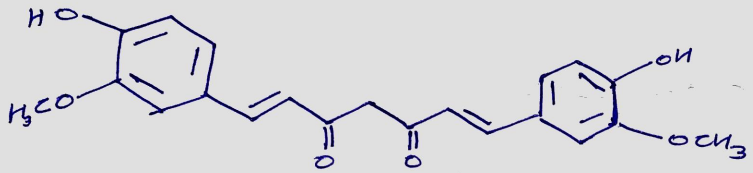
- Sample preparation — 1 mg of extract dissolve in 50 ml of methanol & applied on TLC plate.
- standard preparation — 1 mg of standard sample dissolve in 50 ml of methanol and applied on TLC plate.
- stationary phase — Silica Gel-G.
- Mobile phase — Chloroform : Methanol (9:1)
- Spraying reagent — Iodine
- R.F value — 0.94.

* By HPLC Method :-

- Sample Preparation — Extract sample is prepared with mobile phase (methanol & water) - (62:38) filtered and injected in HPLC for analysis.
- column — C_{18} RP
- Mobile phase — Methanol : water
- Detection — 280 nm.
- flow Rate — 0.9 ml/min
- Sample inject — 20 µL.
- Retention time — 9.45 min.

Curcumin

* **Synonyms:-** Haldi, turmeric, Indian Saffron.
* **Biological source:-** Curcumin is a bright yellow chemical compound produced by fresh as well as dried rhizomes of the plant **curcuma longa**
  family- Zingiberaceae.



curcumin

Isolation of Curcumin

Turmeric powder extracted with 95% Alcohol in Soxhlet apparatus.

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Evaporate the alcohol to get the semi-solid mass.

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Semi-solid mass dissolved in benzene and further extracted with 0.1% NaOH solution in separating funnel.

↓

Alkaline extract is acidified by addition of dil. HCl.

↓

formation of yellow colour precipitate.

↓

Boil the extract on water bath.

|

↓

During this process, the resinous material will separate out and will form lumpy mass.

↓

Filter and concentrated the filtrate.

↓

Finally cool to get curcumin.

Identification of Curcumin

* **Identification By chemical Test**

1) Drug + H_2SO_4 ⟶ Crimson colour.

2) Aqueous solution of turmeric with Boric acid

↓

Reddish Brown.

↓

Addition of Alkali, changes to Greenish.

Analysis of Curcumin

* **Analysis By TLC Method:-**

- Sample Preparation — Extract 1 gm of the coarsely powdered substance with 5 ml methanol. Filter filterate applied on the TLC plate.
- Stationary phase — Silica gel - G.
- Mobile phase — Chloroform : Ethanol : Glacial acetic acid (9.4 : 5 : 0.1)
- observation — under UV light
- RF value — 0.84.

* **Analysis By HPLC Method:-**

- Column — C_{18}.
- Mobile phase — acetonitrile : acetic acid : Water (50 : 1 : 49).
- Detection — 425 nm.
- Flow Rate — 1 ml/min
- Sample inject — 20 µL.
- Retention time — 7.04 min.