

# School of Medical and Allied Sciences

Course Code : PCY 410

Course Name: Standardization of Herbal Drug

## ISOLATION IDENTIFICATION AND ANALYSIS OF PHYTOCONSTITUENTS

The logo of Galgotias University is a stylized circular emblem. It features a central white swirl that resembles a 'G' or a flame, surrounded by concentric, overlapping bands of color: a light blue band, a yellow band, and a reddish-brown band. The entire emblem is set against a light grey circular background.

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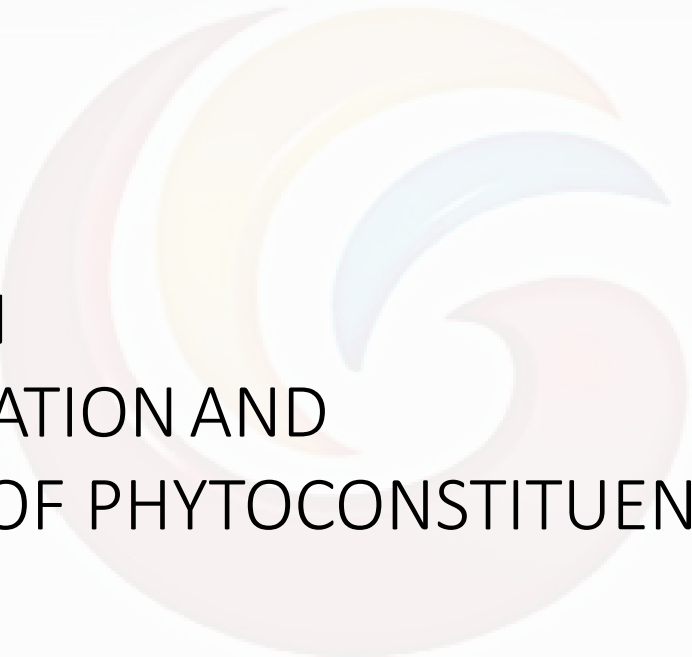

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# CURCUMIN

- Curcumin or Curcuminoids are the diaryl heptoid compounds obtained from the dried rhizomes of Turmeric, *Curcuma longa*, Family – Zingiberaceae
- Curcumin is the major colouring principle. It is a mixture of curcumin, monodesmethoxycurcumin and bisdesmethoxycurcumin

- It as an orange yellow, crystalline powder
- Insoluble in water and ether, but soluble in alcohol
- It is used as wound healing, ant-inflammatory, anti arthritic and antimicrobial activities
- Used against peptic ulcer

## EXTRACTION AND ISOLATION

- Curcumin can be obtained by different processes
- Turmeric powder is extracted with alcohol in Soxhlet extractor. Alcoholic extract is concentrated under reduced pressure and dried.
- **In another method** , Turmeric powder is extracted with is first extracted with hexane followed by acetone.
- The acetone extract is concentrated and dried to yield curcumin. It is recrystallized from hot ethanol to yield orange red needles.

## Identification and Analysis

### T.L.C Method

**Sample preparation** – Dissolved 1mg of Curcumin in 1ml of methanol

**Stationary phase** - Silica gel –G

**Standard sample** - Curcumin

**Detecting agent** – Observed under U.V light at 366nm

**Mobile phase** – Chloroform - Ethanol - Glacial acetic acid  
(94:5:1)

**RF Value** – Curcumin – 0.79

# ARTEMISIN

- **Synonym** – Santonica
- **Biological source** – Artemisin is a sesquiterpenoid lactone , obtained from the unexpanded flower- heads of *Artemisia annua*
- **Family** - Compositae
- **Medicinal use** – Antimalarial drug
- White crystalline powder, soluble in organic solvents



## EXTRACTION AND ISOLATION

- The leaves are air dried, coarsely powdered and extracted with petroleum ether (40-60).
- The extract is concentrated, dried and re-dissolved in chloroform. Add acetonitrile to precipitate sugars and waxes.
- Filter and collect the filtrate. Evaporate to dryness to yield residue

- The chromatographic fractionation of the concentrate on silica gel by eluting with Chloroform- ethyl acetate yields the fraction of artemisin
- The fractions containing artemesin could be crystallised from cyclohexane or 50% Ethanol

## Identification and Analysis

### T.L.C Method

**Sample preparation** – Dissolved 1mg of Artemisin in 1ml of Chloroform

**Stationary phase** - Silica gel –G

**Standard sample** - Artemisin

**Detecting agent** – p- dimethylaminobenzaldehyde and heat at 80°C to produce color

**Mobile phase** – Petroleum ether - Ethyl acetate (1:2)

**RF Value** – Compare with standard Artemisin

# ATROPINE

- Atropine is a tropane alkaloid obtained from *Atropa belladonna*, *Datura stramonium* and *Hyoscyamus niger*
- **Family** – Solanaceae
- Used as Antispasmodic, Mydriatic etc

## EXTRACTION AND ISOLATION

- Take weighed quantity of coarse powder and moisten with sodium carbonate solution.
- Extract the blended mixture in petroleum ether. Filter the petroleum ether extract
- Extract the filtrate with aqueous acetic acid ( alkaloids extracted in aqueous layer)
- Extract the aqueous fraction with solvent ether and separate both fraction. Discard solvent ether fraction

- Aqueous (Acidic fraction) made alkaline with sodium carbonate solution to obtain **precipitates of tropane alkaloids**. Filter the precipitate and dry to obtain residue.
- Dissolve the residue in diethyl ether .Filter and concentrate the filtrate. Atropine crystals will be separated out.
- Filter the crystals and dissolve in alcohol containing sodium hydroxide solution ( Hyocyamine is converted to atropine)
- Recrystallize the atropine sulphate from acetone. Separate the crystals of atropine.

## Identification and Analysis

➤ Chemical test –

➤ Vitalin –morin test -

➤ Take small quantity of the solid atropine and add 2 drops of Con.nitric acid in an evaporating dish and evaporated to dryness on water bath. Then dissolve the residue in 1ml of acetone. Add few drops of freshly prepared alcoholic potassium hydroxide solution.

➤ Violet colouration takes place due to tropane nucleus

## T.L.C Method

**Sample preparation** – Dissolved 1mg of Atropine in 1ml of Chloroform

**Stationary phase** - Silica gel –G

**Standard sample** - Atropine

**Detecting agent** – Drangendroffs reagent to produce yellow orange Color spots

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

**RF Value** – Compare with standard Atropine (0.70)



# CITRAL

- **Biological sources –**
- Citral is a monoterpene aldehyde found in variety of sources like lemon grass, lemon and orange peels etc
- *Cymbopogon fleuosus* ( Lemon grass) , Graminae . 75-85% of citral present in the drug
- Citral obtained from a natural source is a mixture of two geometric isomers geranial and neral

## ➤ **Properties**

- Geranial and Neral both are light oily liquids with lemon odour
- Citral is practically insoluble in water but miscible with alcohol, ether, benzyl benzoate etc

## EXTRACTION AND ISOLATION

- The fresh plant material is hydro- distilled to obtain lemon grass oil.
- **Purification by Fractional crystallization**
- To the total oil, first Sodium sulphite is added, the citral 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 and Analysis

### T.L.C Method

**Sample preparation** – Dissolved 1mg of Citral in 1ml of methanol

**Stationary phase** - Silica gel –G

**Standard sample** - Citral

**Detecting agent** – 2,4,dinitrophenyl hydrazine reagent to produce Yellow to orange Color spots

**Mobile phase** – Pure Chloroform

**RF Value** – 0.51

# MENTHOL

- Biological source –
- Menthol is a monoterpene alcohol obtained from different variety of mint oils or peppermint oils
- Biological source – It consists of the fresh flowering tops of *Mentha*
  - *piperita*, *Mentha officinalis*
- Family- Labiate
- Granular substance or crystalline with peppermint taste and odour, freely soluble in alcohol, chloroform, ether, petroleum ether

## EXTRACTION AND ISOLATION

- Take the accurately weighed 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.
- Re- Crystallize menthol from acetone or any other low boiling point solvent

## Identification and Analysis

### T.L.C Method

**Sample preparation** – Dissolved 1mg of menthol in 1ml of methanol

**Stationary phase** - Silica gel –G

Standard sample - Menthol

**Detecting agent** – 1% vanillin – sulphuric acid reagent and heat the plate 110 °C for 10 minutes

**Mobile phase** – Pure Chloroform

**RF Value** – 0.48-0.62

# CAFFEINE

- Caffeine is a purine alkaloid obtained from Tea leaves, Coffee seeds, cocoa, and other species
- **Biological source** -It consists of dried leaves of plant known as *Thea sinensis*
- **Family** – Theaceae
- It is chemically 1,3,7, trimethyl xanthine. It is isolated from tea and coffee seeds during decaffeination process.
- Tea leaves contains 1-4% of caffeine and coffee contains 1-2% of caffeine
- It is white powder or white ,glistering needles, odour less, bitter in taste, Soluble in hot water.
- Caffeine is a CNS stimulant and Diuretic



## EXTRACTION AND ISOLATION

- The powder tea leaves is extracted with boiling water and the aqueous extract is filtered while hot.
- The warm extract is treated with lead acetate to precipitate tannins and filtered.
- The filtrate is treated with excess of dilute sulphuric acid to precipitate lead in the form of lead sulphate.

- Filter and collect the filtrate
- The filtrate is boiled with Activated charcoal to remove colouring matter, if any and filtered to remove charcoal
- The filtered decolourized solution is extracted with chloroform successively .
- Combined the chloroform extracts evaporate on water bath to yield caffeine (white powder)
- It is recrystallized with alcohol

## Identification and Analysis

- **Chemical test –**
- **Murexide test –** To the caffeine add hydrochloric acid and potassium chlorate, heated to dryness. A purple colour is obtained by exposing the residue to vapours of dilute ammonia.
- **Thin layer chromatography (TLC)**

## T.L.C Method

**Sample preparation** – Dissolved 1mg of caffeine in 1ml of methanol or chloroform

**Stationary phase** - Silica gel –G

**Standard sample** - Caffeine

**Mobile phase** – Ethyl acetate: methanol : acetic acid  
(80:10:10)

**Detecting agent** – Expose to vapors of iodine

**RF Value** – 0.41

# RESERPINE

- **Biological source** – Reserpine is an indole alkaloid obtained from the roots of *Rauwolfia serpentina*
- **Family** – Apocyanaceae
- It is a white or pale buff to slightly yellow, odourless, crystalline powder
- It is soluble in alcohol, acetone and chloroform.
- Reserpine is an antihypertensive and antipsychotic agent

## EXTRACTION AND ISOLATION

- Rauwolfia root powder is exhaustively extracted with 90% alcohol by percolation
- The alcoholic extract is concentrated and dried under reduced pressure below 60°C to yield Rauwolfia dry extract.
- Rauwolfia dry extract is extracted with Ether-chloroform-90%alcohol (20:8:2.5)
- Collect the extract and add little dilute ammonia with intermittent shaking. Add water and allow the drug to settle after vigorous shaking.

- Filter off the solution and extract the residue with 4 volumes of 0.5N Ammonium sulphate in separating funnel. Combine all the extracts.
- The extract is made alkaline with dilute ammonia to liberate alkaloid. Finally it is extracted with chloroform.
- Collect the chloroform extract , concentrate and evaporate on water bath to yield total rauwolfia alkaloids.(30 different components)
- Residue is subjected to column chromatographic fraction for the separation of reserpine

## Identification and Analysis

### T.L.C Method

**Sample preparation** – Dissolved 1mg of Reserpine in 1ml of methanol or chloroform

**Standard sample** - Reserpine

**Stationary phase** - Silica gel – G

**Mobile phase** – Chloroform: acetone :diethyl amine  
(50:40:10)

**Detecting agent** – Dragendroffs reagent

**RF Value** – 0.72