UNIT-IV

INDUSTRIAL PRODUCTION, ESTIMATION AND UTILIZATION OF VARIOUS PHYTOCONSTITUENTS

Points to be covered in this topic

- Forskolin
- Sennoside
- Artemisinin
- Diosgenin
- Digoxin
- Atropine
- Podophyllotoxin
- Caffeine
- Taxol
- Vincristine and vinblastine

PHYTOCONSTITUENTS

- **Phytoconstituents** are **biologically significant chemical compound** that occur naturally in the plant body.
- They are **formed through various primary And secondary metabolite** in plant body
- Dealing with phytoconstituents is known as phytochemistry. There are various groups of **phytoconstituents such as phenolic, glycosidic, alkaloid, saponins, Terpenes, tannins etc.**

FORSKOLIN

Biological Source: Diterpenoid extracted from roots of *Coleus forskohlii*, family- Lamiaceae

Industrial Production

i. Roots & bark powder extracted with toluene at 60°C for 2 hours
ii. Filtrate collected & concentrated at temperature not exceeding 40°C
iii. Concentrated extract mixed with n- hexane, yields crude forskolin in the form of brown ppt.
iv. Purified using column chromatography

Identification

TLC Method

Plate – silica gel 60 F254
Mob. Phase – Benzene : methano
Detection – UV at 366 nm
Rf – 0.25

Estimation

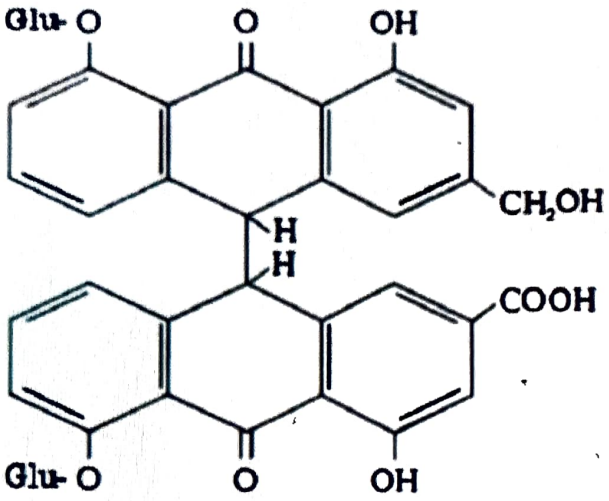
HPLC
Column – HIQ sil C8
Mob. Phase - Acetonitrile : water
Detection – 210 nm

Utilization:

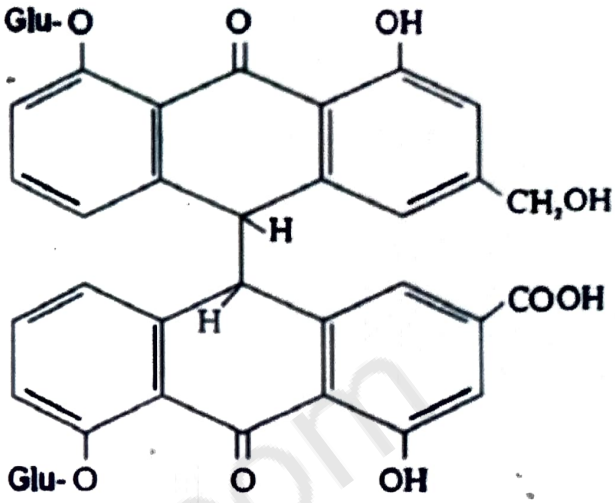
- ✓ Antidepressant
- ✓ Vasodilating
- ✓ Antiobesity
- ✓ In glaucoma
- ✓ Antiasthmatic

SENNA

Biological Source: Dianthrone glycosides, leaflets of *Cassia angustifolia* (Indian senna) & *C. acutifolia* (Alexandrian senna). Family- Leguminosae



Sennoside A



Sennoside B

- **Industrial production**

Dried senna leaves powder extracted with **benzene for 2-3 hrs**

↓

Marc is dried and extracted with **methanol for 4-6 hrs.**

↓

Mix both the extracts and concentrated

↓

pH of extract adjusted to **3.2 by HCl**

↓

Extract is mixed with **hydrous calcium chloride in 25 ml denatured spirit**

↓

pH adjusted to 8 using **ammonia & set aside for 2hrs, results into ppt. of sennosides**

Identification

- ✓ Extracted sennoside is identified using chemical test
- ✓ TLC method

Chemical Test

Borntrager's test

TLC method

- ✓ **Plate** – precoated silica gel -60 F254
- ✓ **Mob. Phase** – 2 propanol : ethyl acetate : water : formic acid
- ✓ **Rf**- 0.52 (sennoside-A)
  0.32 (sennoside-B)

Estimation

➢ **HPTLC**

- ✓ **Plate** – precoated silica gel 60 F254
- ✓ **Detection** – UV at 366 nm
- ✓ **Rf** – 0.52 (Sennoside A)
  0.32 (Sennoside B)

➢ **HPLC Method**

- ✓ **Column** – C18
- ✓ **Column temp** - 40°C
- ✓ **Mobile phase** – Acetonitrile : water :phosphoric acid
- ✓ **Flow rate** - 1.2 ml / min
- ✓ **Detection** – UV at 380 nm
- ✓ **Rt** – 77min

Utilization

1. Treatment of **constipation**
2. In **skin diseases**
3. Useful in **loss of appetite, indigestion, rheumatism & anaemia**

ARTEMISININ

Biological Source: sesquiterpene lactone obtained from the **leaves & unexpanded flower** heads of ***Artemisia annua.*** **Family- Asteraceae**

Industrial preparation:

Fresh leaves are **dried below 60°C**, powder is extracted with methanol by maceration

↓

Methanol extract partitioned with **hexane**

↓

The hydro alcoholic extract partitioned with ethyl acetate until the colourless

↓

Contentrated at controlled temperature at **40°C** under **vacuum**

↓

Artemisinin obtained as fine white crystals after recrystallization with cyclohexane

Identification

Chemical test

Sample + $FeCl_2$ or NaI → **Deep violet** colour

Utilization

- Antimalaria
- In **gastric infection**
- Suppress **inflammatory immune reaction**
- **Anticancer**

DIOSEGNIN

Biological Source: Aglycone obtained after the hydrolysis of steroidal saponin glycoside dioscin present in *Dioscorea deltoidea*, *D. composite.* **Family- Dioscoreaceae**

Industrial production

Dried powder hydrolyzed with 2.5N H_2SO_4 by reflux or autoclave

↓

Marc washed with 10% sod. Bicarbonate to neutralize acid

↓

Hydrolyzed powder extracted with benzene for 6-8 hrs.

↓

Benzene extract is filtered, residue dissolve in chloroform and concentrated by recrystallization

Identification

Chemical test

Sample + Acetic anhydride → Heat and cooled + conc. Sulphuric acid

↓

Formation of green colour

- **TLC method**
  - ✓ **Plate –** Precoated silica gel 60F254
  - ✓ **Mob. Phase –** n-heptane : ethyl acetate
  - ✓ **Rf –** 0.47 green spot
  - ✓ **Standard –** Std. Diosgenin is dissolve in chloroform and applied on TLC plat
  - ✓ **Sample :** 1 mg of dried extract is dissolved in 50ml of chloroform and then applied on TLC plate

Estimation :

- **HPTLC**
  - ✓ **Plate –** Silica gel GF_{254}
  - ✓ **Mob. Phase –** Toluene : ethyl acetate : acetic acid : formic acid
  - ✓ **Detection –** UV at 366nm
  - ✓ **Rf –** 0.83

Std and sample preparation – The std diosgenin (100mcg/ml) and sample (50mg /ml) Were prepared in methanol and applied on TLC plate

- **HPLC method**
- ✓ **Column – C18**
- ✓ **Mob. Phase –** Methanol : water
- ✓ **Detection –** UV at 203nm
- ✓ **Flow rate –** 0.4 ml /min
- ✓ **Rt –** 6.5 min
- ✓ **Utilization :** Diosgenin is used for commercial synthesis of cortisone, progesterone and other steroid production. Treatment of rheumatism

DIGOXIN

Biological source : Cardiac glycoside obtained from leaves of *Digitalis lanata*.

Family- Scrophulariaceae

Industrial production:

- Fresh leaves made into paste & treated with neutral salt
- Paste is defatted with benzene & followed by extraction with ethyl acetate
- Extract contain lanatoside
- Which after hydrolysis yields digoxin

Identification

- **Chemical test**

Lieberman's test

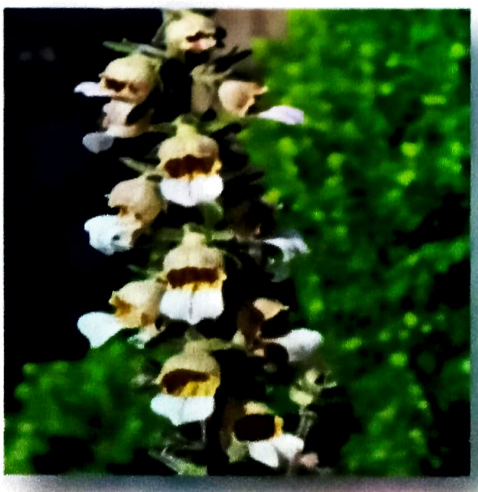
Sample + acetic anhydride + few drops of conc. sulphuric acid

↓

Initially **reddish violet**

↓

Turn to **green**



➢ **TLC Method**

Plate – precoated silica gel C254

Mob. Phase – ethyl acetate : methanol : water

Rf- 0.65

Std – A std sol is prepared with 1 mg of drug to 1 ml of methanol and applied on TLC plate

Sample – 250 mg of leaf powder treated with 25 ml of 50% methanol and ultrasonicate for 1.5 hr. in an ice water bath the extract was filtered and then was dissolved in 3 ml of methanol followed by in 50 ml of ethyl acetate

- **Estimation :**

➢ **HPLC method**

- **Column –** cosmosil 5C-AR-300
- **Mob phase –** Acetonitrile : Methanol : pure water
- **Detection –** UV at 220 nm
- **Flow rate –** 0.5 ml/min
- **Rt –** 12.9 min.

- **Utilization :**

- Treat heart failure
- Decrease the risk of blood clot

ATROPINE

Biological source : Tropane alkaloid, **flowering tops** of ***Atropa belladonna, Datura stramonium*** & ***Hyoscyamus niger***

Family- Solanaceae

Industrial production :

Powdered drug extracted with **ether or benzene**

↓

Concentrate the **non-polar extract & partitioned with acetic acid**

↓

Add **sodium bicarbonate** leading to ppt alkaloid

↓

Dry the ppt & **crystallized by dissolving in solvent ether**

Identification

➢ **TLC Method**

Plate – precoated silica gel G_{254}

Mob . Phase – Ammonia : water : acetone

Detection spraying agent – **Dragondroff's reagent**

R_f – 0.18

Sample prepⁿ – 10mg of extract dissolve in 10 ml of methanol and then applied on TLC plate

Std. – Atropine sulphate dissolved in 10 ml of methanol and applied in volume of 10 µl

Utilization:

1. As **preanesthetic medication**
2. Antispasmodic

PODOPHYLLOTOXIN

Biological source : resin, roots & rhizomes of ***Podophyllum hexandrum, P. emodi & P. peltatum***, **Family- Berberidaceae**

Industrial production

Dried root powder → Extracted with **ethanol by soxhlet method for 6 hr.** →

Concentrate the syrup mass

↓

Added water and **dilute HCl**

↓

Cooled and allowed to stand for **two hr.**

↓

Filter under vaccum ← **Washed residue with acidified water**

Cooled below 5°C → Residue + 90% Alcohol → Filtered and evaporate

↓

Light brown powder of podophyllotoxin

- **Identification**

Test for lignan – 0.5 ml of aq. Sol^{n} extract + 2ml of 2% (v/v) furfuraldehyde in test tube

↓

Red colour indicate the presence of lignan

➢ **TLC**

Plate – silica gel G

Mob. Phase – chloroform : methanol

Detection - ionic chamber

R_f . 0.94

➢ **Sample preparation :**

1 mg of sample dissolved in 10 mg of methanol and applied on plate

Std – 1mg of drug is dissolved in 1ml of methanol and applied on TLC plate

- **Utilization**

✓ Antitumor

✓ Purgative

✓ Emetic

CAFFEINE

Biological source : ***xanthine alkaloid***, leaves of ***Camellia sinesis*** (Theaceae), seeds of ***Coffea arabica*** (Rubiaceae)

- **Identification :**

➢ **TLC**

✓ **Plate** - Precoated silica gel 60

✓ **Mob phase** – ethyl acetate : methanol : Ammonium hydride

✓ R_f – 0.64

✓ **Sample** – 1 mg of isolated sample dissolved in 50 ml of methanol

✓ **Std.** – 1 mg of std caffeine citrate is dissolved in 1 ml of methanol and applied on TLC plate

- **Utilization**

Stimulate nervous system. Cause **Relaxation of cardiac and respiratory muscles**

TAXOL

Biological Source: Nitrogen containing subs, bark of *Taxus brevifolia,* fam- **Taxaceae**

- **Industrial Production:**

Powdered bark extracted with methanol, filtered & evaporated to dryness.

↓

Partition with the mixture of carbon tetrachloride & water, filter & evaporated.

↓

Dried CCl_4 fraction again extracted with **CCl_4 : methanol, evaporate** to obtain crude taxol

- **Identification:**

➢ **TLC**

✓ Plate – silica gel GF_{254}

✓ Mob. Phase – Chloroform : acetonitrile

✓ R_f – 0.59

- **Estimation :**

➢ **HPLC**

✓ Column – C_{18}

✓ Mob – Acetonitrile : water

✓ Detection - UV at 227 NM

✓ Flow rate - 1.0 ml /min

✓ Rt – 15.1 min

➢ **HPTLC**

✓ Plate – precoated silica gel GF_{254}

✓ Mob. Phase – Chloroform : methanol

✓ Detection – UV at235nm

✓ R_f - 0.25

Utilization

1. Treatment of **ovarian, lung, bladder, esophageal & other types of cancers**
2. **Antiproliferative agent**

VINCRISTINE & VINBLASTINE

Biological source : Vinca is dried whole plant of ***catharanthus roseus***

Family : Apocynaceae

These both are chemical constituent obtained from vinca plant . Basically they are **indole group of alkaloid**

Vinblastine : White solid powder soluble in organic solvent but insoluble in water

Vincristine : White crystalline solid , odourless , hygroscopic in nature . Soluble in organic solvent but insoluble in water

Industrial production :

- Air dried leaves of **vinca are finely crushed with ethanol**
- The crushed material is filtered and washed throughly with the ethanol
- The ethanolic filtrates are evaporate until it is concentrated to a gum
- Gum is **acidified with 5% HCl** and washed with **chloroform**
- Chloroform extract are **dried over anhydrous sod. Sulphate** and concentrated to a crude alkaloid gum
- Then alkaloid are dissolved in 400 ml of **chloform** and extracted with one litre of phosphate buffer
- The chloroform layer is dried **over Na_2SO_4** and filtered and then petroleum ether is added which causes some alkaloid to precipitate out
- The vinblastine containing fraction is loaded on flash chromatography column **packed with alumina**

- The column is eluted with ethyl acetate in petroleum ether and concentrated to gum
- Subsequent elution with ethanol in ethyl acetate afford a vinblastine rich formation

Identification :

Chemical test

Both Vincristine & vinblastine gives general test for alkaloids

- **TLC**
- ✓ **Plate-** precoated silica gel GF254
- ✓ **Mob . Phase** – **Toluene : ethyl acetate : benzene**
- ✓ **Rf -0.36(vincristine), 0.48 (vinblastine)**
- ✓ **Sample** – 1 mg of extract dissolve in 10 ml of ethanol and applied in TLC plate
- ✓ **Std** – 1 mg of std vincristine and vinblastine are separately dissolve in 5 ml of ethanol and applied in TLC plate

Estimation:

HPTLC method

- ✓ **Plate** – precoated silica gel aluminum plates
- ✓ **Mob . Phase** – **Toluene : methanol : diethyl amine**
- ✓ **Detection** – 3.7 nm for VC and 235 for VB
- ✓ **Rf** – 0.39(VC)

0.49 (VB)

HPLC

- ✓ **Column** – chromolith performance RP-18c
- ✓ **Detection** – UV at 254 nm
- ✓ **Rf** – 16.97 min(VC)

26.93 min(VB)

Utilization

1. **In chemotherapy regimens**
2. **Childhood leukemia**
3. **Immunosuppressant**