UNIT-IV

Industrial production, estimation and utilization of Phytoconstituents

(Forskolin, Sennoside, Artemisinin, Diosgenin, Digoxin, Atropine, Podophyllotoxin, Caffeine, Taxol, Vincristine and Vinblastine)

Presented By

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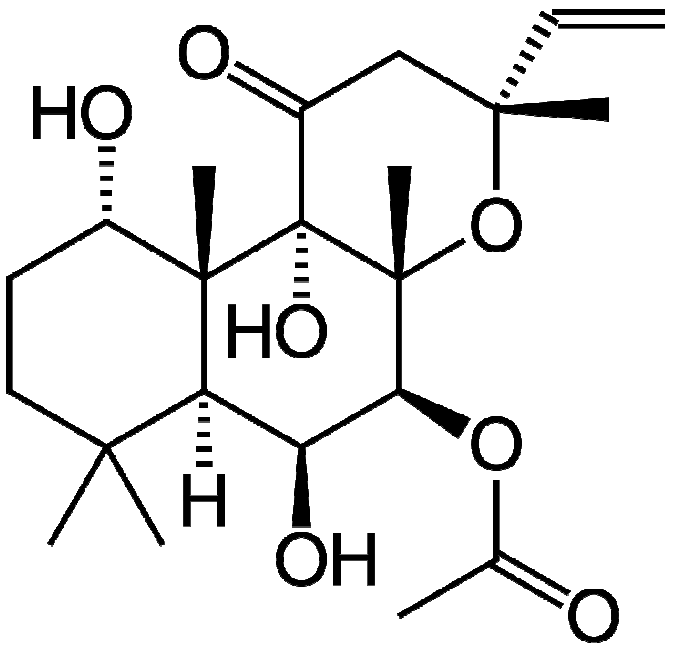
Forskolin

- Forskolin , a labdane diterpens is the active principle of the medicinal plant Coleus.
- It is off white crystalline solid and having antioxidant property.
- **Biological Source:** It is obtained from roots of of ***Coleus forskohlii,*** family- **Lamiaceae.**



Industrial Production Method

- **Method-1**
- Roots & bark powder extracted with **toluene** at 60˚C for 2 hours.
- Filtrate collected & concentrated at temperature not exceeding 40˚C.
- Concentrated extract mixed with **n-hexane**, yields crude **forskolin** in the form of brown ppt.
- Purified using column chromatography.

- **Method-2**
- Dried tubers root powder extracted with methanol by Soxhlet unit for 6 hrs.
- Filter and concentrate the extract and add chloroform and equal volume of water is added to this extract in separating funnel and shake it.
- Allow to stand and separate the chloroform layer.
- Ppt. the forskolin using ice cold n-hexane.
- A reddish brown colour powder is obtained.

Estimation

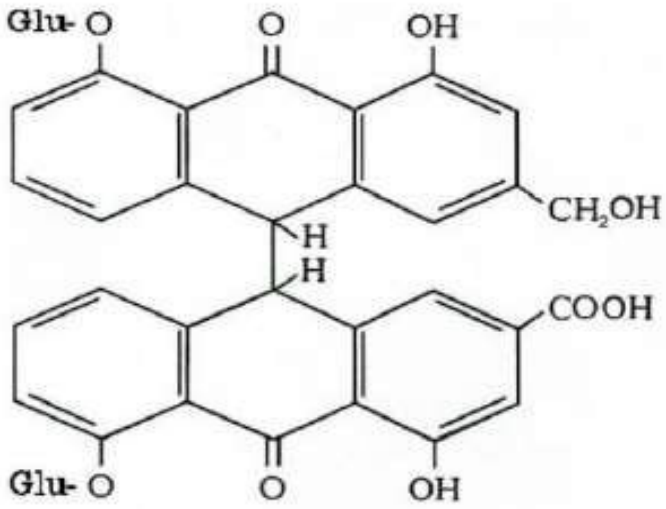
- **TLC & HPTLC**
- **Mobile phase:** Toluene: ethyl acetate (8.5: 1.5 v/v)
- **Stationary phase:** Silica gel F254
- **Visualizing agent:** 5% vanillin in glacial acetic acid and 10% sulphuric acid in water.

Utilization

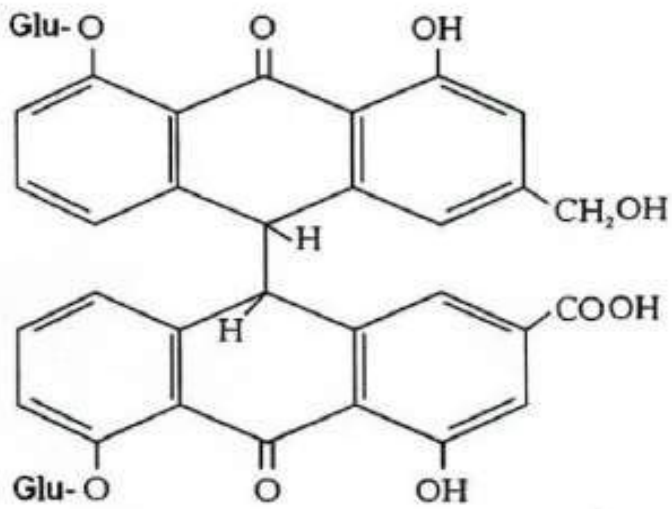
1. Antidepressant
2. Vasodilating
3. Antiobesity
4. In glaucoma
5. Antiasthmatic

Sennoside

- Senna glycoside, also known as sennoside or senna, is a medication used to treat constipation and empty the large intestine before surgery.
- **Biological Source:** It is obtained from dried leaflets of ***Cassia angustifolia*** **(Indian senna)** & ***C. acutifolia*** **(Alexandrian senna).** Family- Leguminosae.



Sennoside A



Sennoside B

Method of Isolation 1:

- Extract the senna leaves with 70 % methanol at room temp. by shaking for 4 hr
- Filter and concentrate to 1/8 th volume under vacuum
- Acidified with HCL (PH-3) followed by filtration and extraction of the solution with chloroform to remove aglycone if any.
- Then neutralize with ammonia and centrifuge to separate the sennoside.

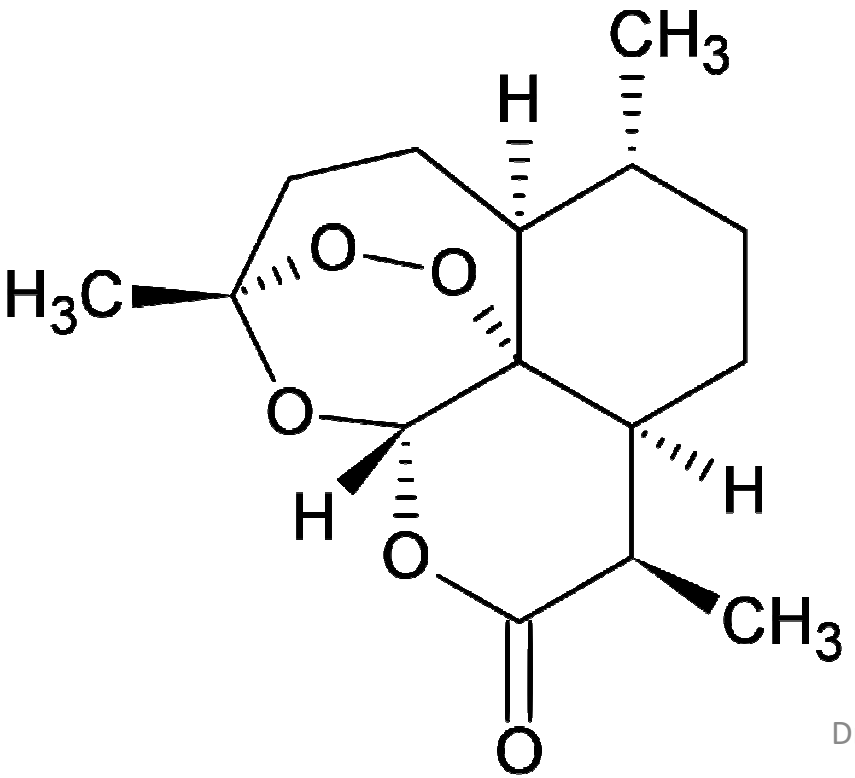
- **Method 2:**
- Dried senna leaves powder extracted with benzene for 2-3 hrs.
- Marc is dried and extracted with methanol for 4-6 hrs.
- Mix both the extracts and concentrated.
- pH of extract adjusted to 3.2 by HCl.
- Extract is mixed with hydrous calcium chloride in 25 ml denatured spirit.
- pH adjusted to 8 using ammonia & set aside for 2hrs, results into ppt of sennosides.

- **Estimation**
- Column- C18
- Mobile phase- 1% acetic acid in water: Acetonitrile (82:18)
- Flow rate- 1ml/min
- Detection- 350 nm

- **Utilization**
- The leaves and pod are used time immemorial in India and abroad as cathartic.
- It is used as purgative for either habitual constipation or occasional use.

Artemisinin

- Artemisinin is an active antimalerial constituents of herb ***Artemisia annua*** family Compositae.



- **Properties**
- It is white crystalline powder, soluble in most organic solvent. It is slightly soluble in oil

1-Method of Isolation :

- Leaves of *Artemisia annua* dried and powdered and subjected to extraction with pet ether
- The extract is concen., dried and redissolved in chloroform, to which acetonitrile is added to ppt inner plant const. such as sugar and wax.

- **Method 2:**
- 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.
- Concentrated at controlled temperature at 40˚C under vacuum.
- Artemisinin obtained as fine white crystals after recrystallization with cyclohexane.

Identification:

- Extract + dissolve in 1 ml chloroform
- Spot applied on silica gel plate
- Run with solvent system of petroleum ether: ethyl acetate (1:2)
- Dried plate sprayed with p-dimethyl-aminobenzaldehyde reagent and heated at 80 C for 10 min.

Utilization

- Uncomplicated malaria
- Severe malaria
- Helminthiasis
- Cancer
- In gastric infections

Diosgenin

- Diosgenin is obtained from dried rhizome and tubers of several species of ***Dioscorea*** **like** ***Dioscorea villosa*****,** ***Dioscorea prazeri*** Prain and Burk; *Dioscorea composite*; *Dioscorea spiculiflora; Dioscorea deltoidea* and *Dioscorea floribunda*, belonging to family Dioscoreaceae.

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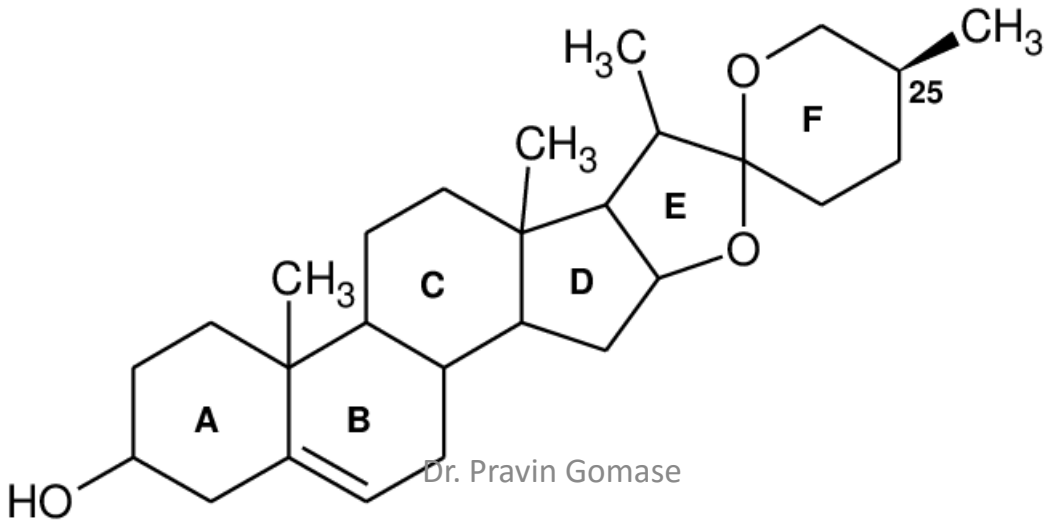
- **Method 1 of Isolation**
- Tubers of discorea wash with water dried and extracted with hot water or 95 % ethanol for several hrs.
- The alcoholic extract is concentrated under vacuum and the glycoside is ppt with solvent ether or by lead acetate followed by hydrolysis and extraction with petroleum ether.



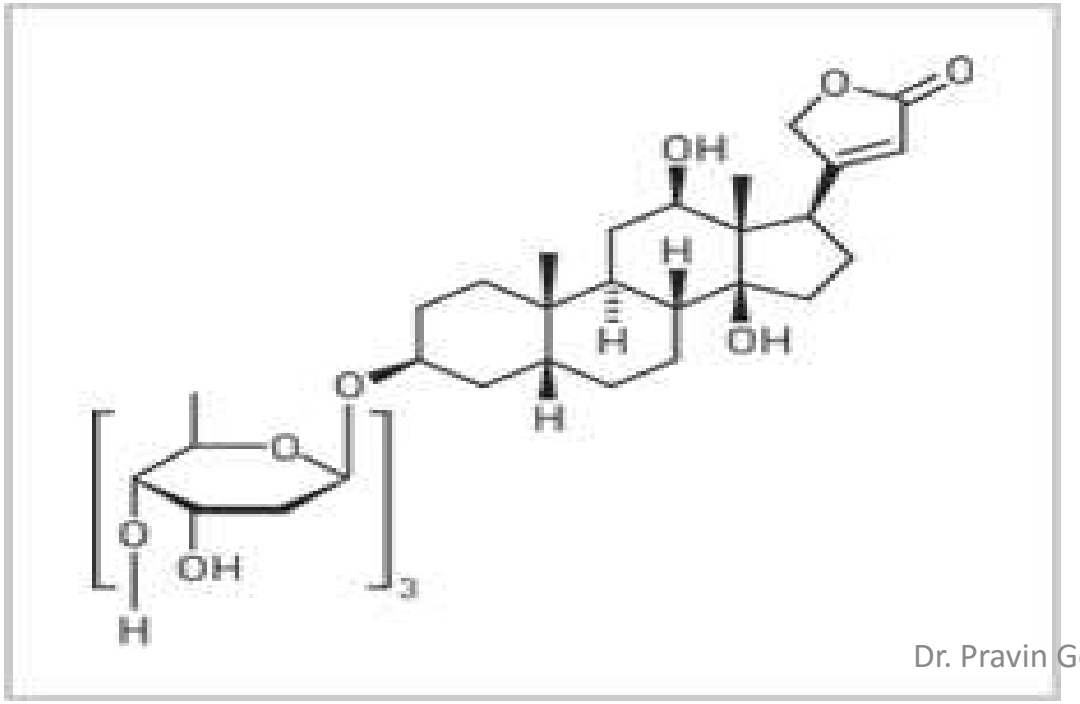
- **Method 2:**
- Dried powder hydrolyzed with 2.5N H_2SO_4 by reflux or autoclave.
- Marc washed with 10% sod. Bicarbonate to neutralize acid.
- Hydrolyzed powder extracted with benzene for 6-8 hrs.
- Benzene extract is filtered, residue dissolve in chloroform and concentrated by recystallization.

- **Estimation**
- HPTLC method
- Mob. Phase- toluene: ethyl acetate: formic acid
- (5:4:1)
- St. phase- Silica gel F 254

- **Utilization**
- Diosgenin is used as a precursor for the synthesis of many steroidal drugs like corticosteroids, sex hormones and oral contraceptives.
- It is also used in rheumatism.

Digoxin

- Digoxin is belongs to cardiac glycoside (Group of steroidal saponin).
- **Biological Source:**
- It is Cardiac glycoside obtained from leaves of ***Digitalis lanata*** Family- **Scrophularia**



- **1. Isolation Process**
- Pulverized drug with 50 % ethanol at low temp.
- Add lead acetate solution to remove impurities.
- Ppt is remove by centrifugation, the cardiac glycoside present in supernatant liquid
- Liquid extracted with chloroform, the chloroform extract is evaporated under vacuum and residue left behind is purified by chromatography.

- **Method 2:**
- Fresh leaves made into paste & treated with neutral salt.
- Paste is defatted with benzene & followed by extraction with ethyl acetate.
- Extract contain lanatoside C, which after hydrolysis yields digoxin.

- **Estimation**
- **Chemical test: Liebermann's Test:**
- Sample + Acetic anhydride + few drop of conc. Sulphuric acid gives initial reddish brown then turns to green.
- **Assay**
- 40 mg test & std solution of digoxin dissolve in sufficient ethanol.
- 5 ml of resulting solution, add 3ml picric acid solution.
- Measure absorbance at 495 nm.

- **Utilization**
- Digoxine is used as cardiac glycoside
- It also used to increase the force of myocardial contraction resulting in complete emptying of the ventricles without increasing the rate in congestive heart failure.

Atropine

- Atropine, poisonous crystalline substance belonging to a class of compounds known as alkaloids and used in medicine.
- Atropine occurs naturally in belladonna (***Atropa belladonna***), from which the crystalline compound was first prepared in 1831.

Isolation Method 1:

- Belladonna leaves extracted with 95 % alcohol in Soxhlet Apparatus
- Concentrated ethanolic extract in vacuum to remove alcohol
- Add dil HCL in solution and filter
- Again extracted with petroleum ether to remove impurities.
- Aqueous solution contain alkaloid and make it alkaline with ammonia solution.

- Extracted this liquid with chloroform three times
- Combine chloroform extract and concentrated under vacuum to obtained crude alkaloid
- Treat again with dilute solution of oxalic acid to obtained alkaloid crystals
- Recrystalise to obtained pure crystals

- **Method 2:**
- 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:

- **Chemical test: Vitali-Morin Reaction :**
- Atropin + Drop of Sulphuric acid------ Evaporated to dryness +3% solution of Met.KOH-----Bright purple colour which indicates the presence of Atropin.
- **TLC:**
- 1 % solution of Atropine + dissolve in 2N acetic acid
- Spot applied on silica gel plate
- Run with solvent system of Strong ammonia solution: methanol (1.5 : 100)
- Dried plate sprayed with acidified iodoplatinate reagent and heated at 110 C for 10 min.
- Rf value shows 0.18.

- **Utilization**
- Commonly used antispasmodic and anti cholinergic properties.
- It also used to remove pain

Podophyllotoxin

- The Podophyllotoxin molecule include a number of oxygen containing functional group such as an alcohol, a lactone, three methoxy group and an acetal.

- **Biological Source:**
- It is a resin obtained from roots & rhizomes of ***Podophyllum hexandrum, P. emodi & P. peltatum***. Family- **Berberidaceae.**

- **Industrial production**
- Dried roots & rhizomes extracted with methanol
- Evaporate the filtrate to semisolid mass
- Dissolve in acidic water results into pptn of podophyllotoxin

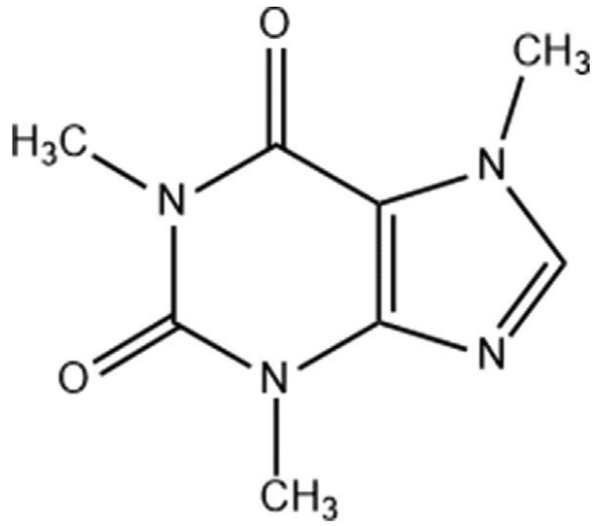
- **Estimation**
- **Chemical Test- Test for Lignan:**
- 0.5 ml aq. Solution of extract + 2 ml of 2 % furfuraldehyde in a test tube---- red colour indicate presence of lignan.
- **TLC:**
- Silica gel G plate
- Mob. Phase: Chloroform : Methanol (9:1)
- Detection: Iodine chamber
- Rf : 0.94

- **Utilization:**

1. Antitumour
2. Purgative
3. Emetic
4. Treatment of warts

Caffeine

- Caffeine is a central nervous system stimulant and is a group of the methylxanthin.
- The chemical classification of caffeine is xanthines.
- It is a purine alkaloid that occurs naturally in tea and coffee.



- **Biological Source:**
- It is obtained from beans of
- Coffee (*Coffee arabica,*
- *C. canephora*) and leaves of tea (*Thea sinensis*)

Method for isolation(Method 1)

- Weigh quantity of tea leaves transfer to 250 mi distil water
- Boil the water for 30 min with occasional stirring
- Allow to cool and filter the solution
- Take filtrate in separating funnel and to it add 100 ml chloroform. Shake vigorously so total caffeine will transfer to chloroform
- Separate chloroform layer and evaporate over water bath. To yield white caffeine crystals.

- **Method for isolation(Method 2)**
- Tea leaves powder boiled with water and filter the extract
- Treat it with lead acetate solution to ppt tanines
- Filter solution and add dil Sulhuric acid to remove excess lead acetate in the form lead sulphate.
- Filter and treat with charcoal to remove any coloring matter
- Take filtrate and add chloroform in separating funnel and shake
- Separate the chloroform layer and evaporate to yields white crystals of caffeine.

- **Method for isolation(Method 3)**
- 30 gm tea powder mix with 250 ml distilled water and 5 g sodium carbonate (removal of tannins) and boil for 10 min.
- Filter the content while hot
- Again take residue and add 100 ml water and boil
- Filter and combine all filtrate in 250 ml separating fennel and extract with 3 portion of dichloromethane without vigorous shaking.
- Drain the dichloromethane layer.
- Place in Petri dish for evaporation to yield needle shape caffeine.

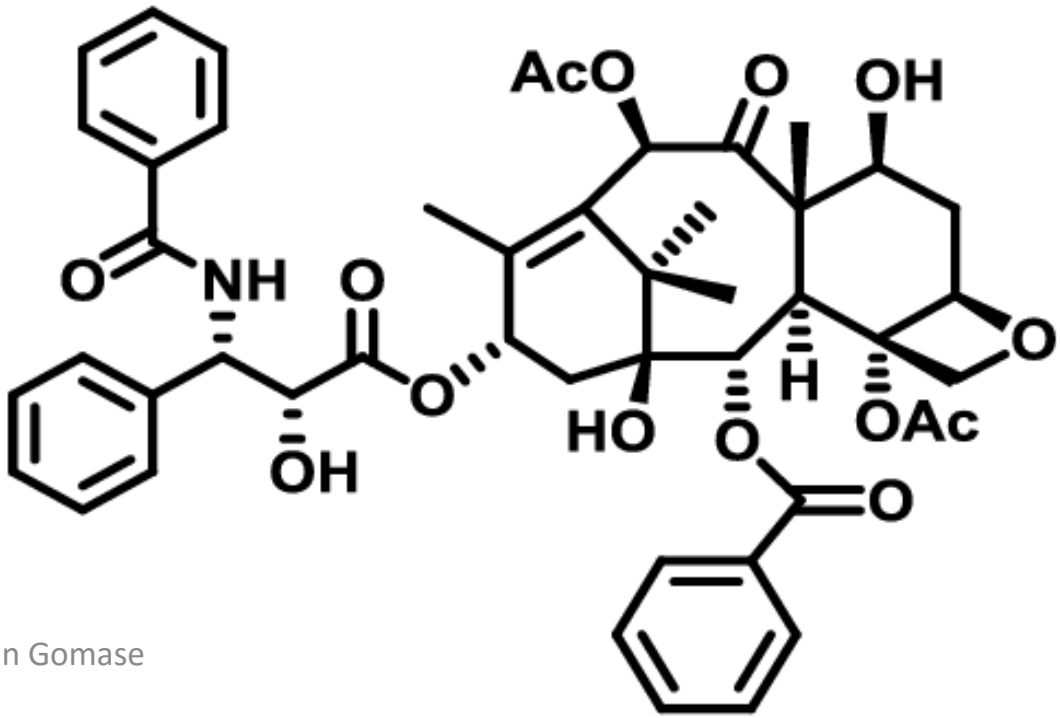
- **Identification and Analysis:**
- **Murexide test:**
- Take test sample add HCl and KCL. Heat it till it gets dry. Expose this powder to dil. Ammonia. Purple color indicates presence of caffeine.
- **TLC**
- Sta. phase: Silica gel G
- Mob. Phase: **Ethyl acetate: Methanol: acetic acid (8:1:1) or Chloroform : methanol (9:1)**
- Spraying reagent: Iodine chamber
- Rf 0.70

- **Estimation**
- HPLC method
- Mob. Phase- methanol: acetonitrile (65: 35
- v/v)
- Column- C18

- **Utilization:**
- Stimulant

Taxol

- Taxol, a natural diterpene alkaloid.
- Taxol was renamed to paclitaxel
- **Biological Source:**
- It nitrogen containing subs, obtained from bark of ***Taxus brevifolia,*** family **taxaceae.**



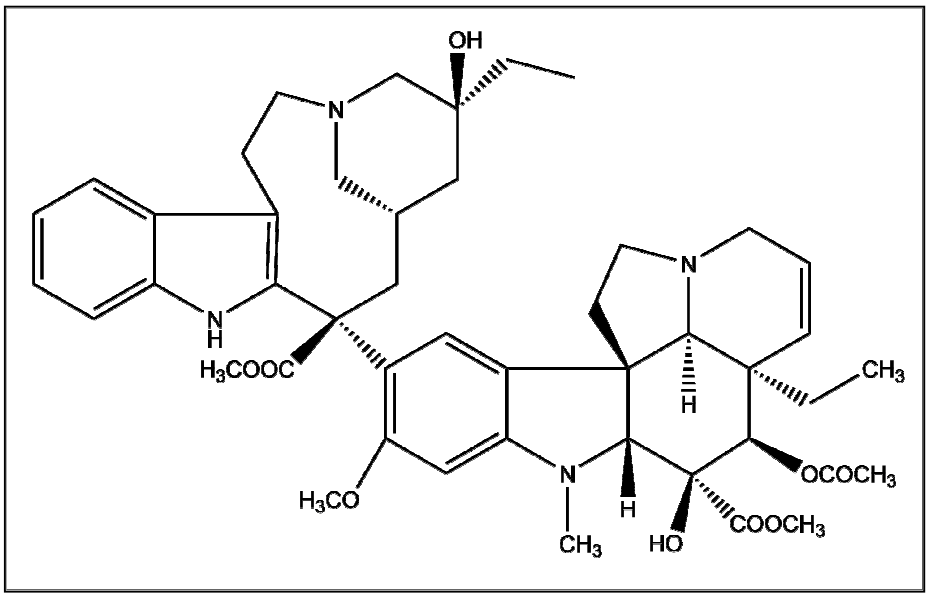
- **Production**
- Powdered bark extracted with methanol, filtered & evaporated to dryness.
- Partition with the mixture of carbon tetrachloride & water, filter & evaporated.
- Dried CCl_4 fraction again extracted with CCl_4 : methanol, evaporate to obtain crude taxol.

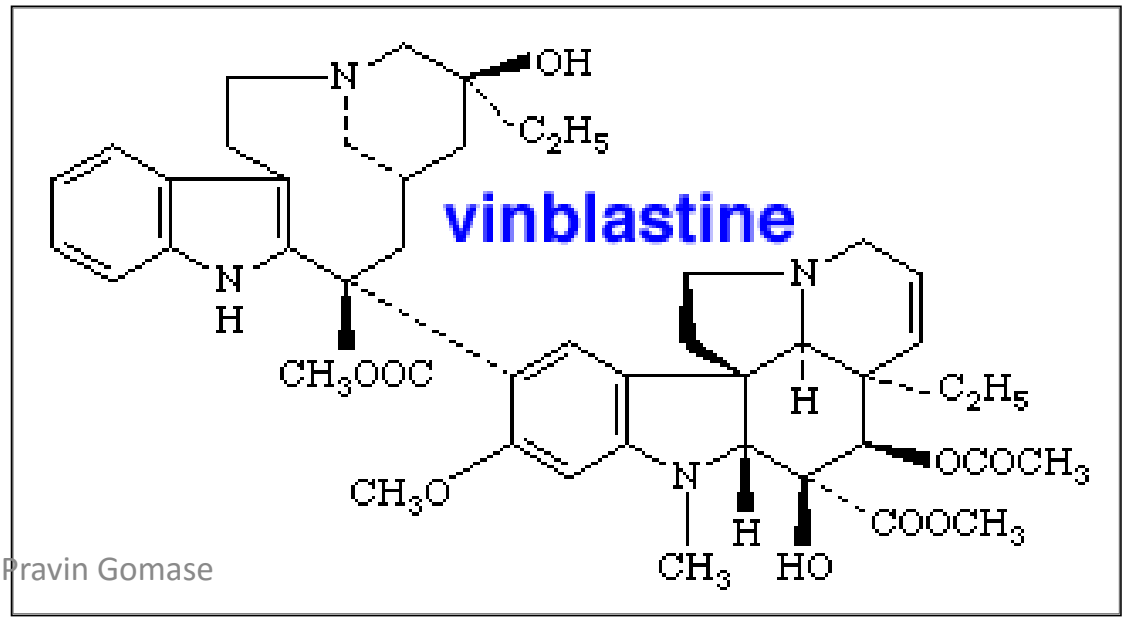
- **Estimation**
- **TLC:**
- **Silica Gel G Plate**
- **Mobile Phase:** Chloroform : Acetonitrile (7 :3)
- **Spraying Reagent:** Vanillin Sulphuric acid
- **Rf :** 0.59
- **HPTLC method**
- **Mob phase:** chloroform : methanol (7:1v/v)
- **Visualizing agent:** vanillin sulphuric acid.

- **Utilization**
- Treatment of ovarian, lung, bladder, esophageal & other types of cancers.
- Antiproliferative agent.

Vincristine and Vinblastine

- **Biological Source:** It is Indole alkaloid, obtained from ***Vica rosea***, **family-Apocynaceae.**





- **Isolation**
- Air dried Powder of vinca treated with ethanol
- Combine ethanolic extract and evaporated until concentrated to a gum
- Gum is acidified with 5 % HCl and washed with chloroform
- Shift to ice cold then shift this to basified with NH3 solution and extracted with chloroform
- Chloroform extract air dried over anhydrous sodium sulphate and conc. To a crude alkaloid.
- Crude dissolved in chloroform and again extracted with phosphate buffer

- Chloroform layer dried over anhydrous sodium sulphate conc. Under vaccum
- Dissolve in 250 ml chloroform and pet ether to ppt the alkaloid
- **Estimation**
- **Chemical test:** Vincristine and Vinblastine gives general test for alkaloid.
- **HPLC method**
- **Mob phase-** acetonitrile: 0.1 M phosphate buffer.
- **Wavelength-** 254nm.

- **TLC:**
- **Plate:** Precoated Silica gel GF254
- **Mobile Phase:** Toluene : Ethyle acetate : Benzene (6 : 3: 1)
- **Visualization:** Iodine chamber
- **Rf:** Vincristin (0.36), Vinblastin (0.48)

- **Utilization**
- Both drugs are still in use for treating cancer today, usually in combination with other chemotherapy agents.
- Childhood leukemia
- Immunosuppressant

Thank You