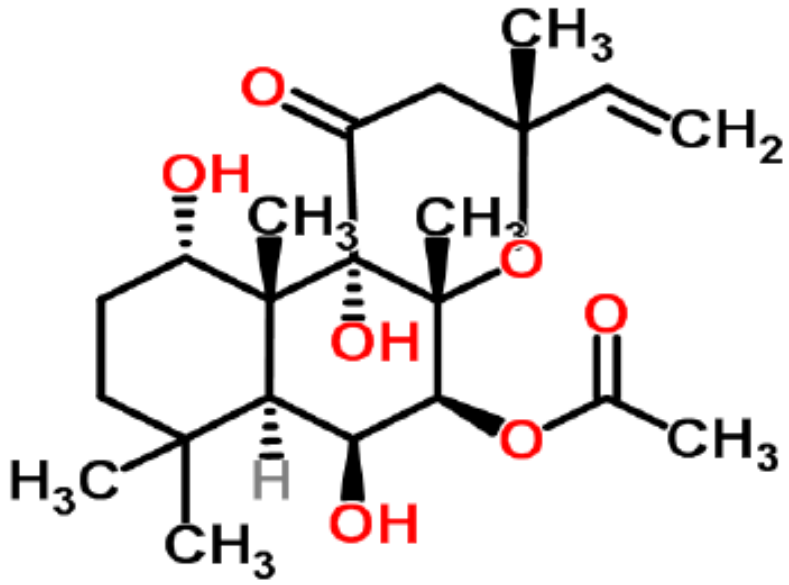
Industrial production, estimation and utilization of Phytoconstituents

Dr. A. R. Surana
Associate professor
Department of Pharmacognosy

Syllabus

- Forskolin (*Coleus forskohlii*)
- Artemisinin (*Artemisia* cina Berg)
- Sennoside (*Cassia angustifolia Vahl*)
- Diosgenin(*Dioscorea composita*)
- Digoxin(*Digitalis lanata*)
- Atropine (*Atropa acuminata*)
- Podophyllotoxin (*Podophyllum hexandrum*)
- Caffeine (*Thea sinensis*)
- Taxol (*Taxus brevifolia*)
- Vincristine (*Catharanthus roseus*)
- Vinblastine (*Catharanthus roseus)*

Biological Source: Labdane diterpenoid extracted from roots of *Coleus forskohlii*, family- Lamiaceae.



Industrial Production

The dried roots of *C. forskohlii* were made into coarse powder by using pulverizer (for decreasing the moisture content)

↓

Material was subjected to the Soxhlet apparatus (continuous hot percolation) for extraction process using toluene as a solvent in 1:4 (raw materials to solvent) ratio

↓

The extract was collected & concentrated

↓

Hexane was added to the concentrate

↓

To it equal volume of water was added

↓

Than transferred to separating funnel and was shaken well

↓

Industrial Production

Continue..

The mixture was allowed to settle and the hexane layer was separated

Hexane layer was concentrated and wet cake was formed

It was dried followed by milling and sieving

Thus, obtained product forskolin was used to analyze the quality and the solubility properties.

Estimation:

1. **Liquid Column chromatography (HPLC)**
   - Column: made up of stainless steel with dimensions of 25cm X 4.6mm
   - Column packing: It is done with silica of 5µm in size
   - Mobile phase: Acetonitrile : water(45:55)
   - Flow rate: 1.8mil/min
   - Sample injected in volume of 20 µl
   - Detector: Spectrophotometer at 220nm

- **Procedure**:

  Both sample i.e. test & standard are injected in column to determine the content of Forskolin. Fractions are collected and subjected for Spectrophotometer at 220nm

2. **TLC & HPTLC**
   - Mobile phase: Toluene:ethyl acetate (8.5:1.5 v/v)
   - Stationary phase- Silica gel F_{254}
   - Visualizing agent- 5% vanillin in glacial acetic acid and 10% sulphuric acid in water.

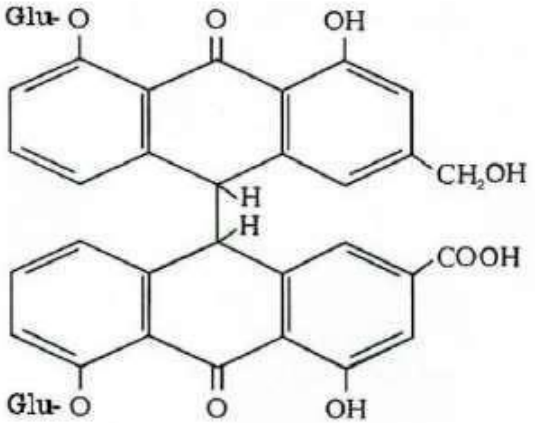
- **Identification test:**

  Copper acetate test: Aq. Extract + Copper acetate solution gives Emerald green color

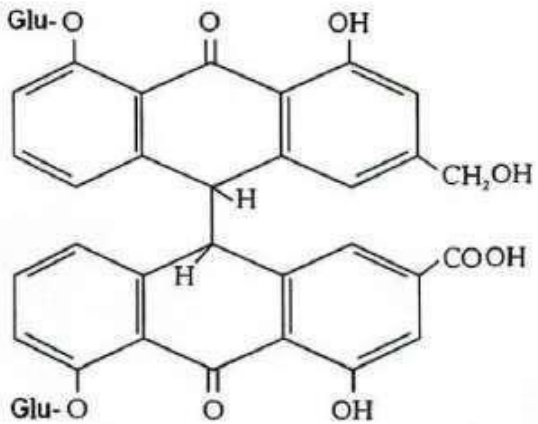
Utilization:

- Antidepressant agent
- Vasodilating- Used in hypertention
- Antiobesity agent
- It reduces intra-occular pressure- Used In glaucoma
- Antiasthmatic agent
- Used as antiplatelet agent

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



Sennoside A



Sennoside B

Industrial production

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

Industrial Production

Coarsely powdered seena leaves are extracted with benzene & 1-5% ethanol to remove pigments & resins

↓

The dried marc is further extracted with ethanol

↓

Alcoholic extract is concentrated in vaccum below 40°C

↓

Dry alcoholic extract is mixed with solution of calcium chloride in methanol & the solution is filtered

↓

Filterate is added with methanolic ammonia till red brown color disappears

↓

Precipitate material is filtered & washed with methanol & dried

Calcium sennoside is suspended in methanol & acidified with gluconic acid at 40°C

↓

Acidified extract after filtration yields a precipitate containing yellow mass of Sennoside A

↓

Filterate treated with methanolic hydrobromic acid & evaoporated, it yields Sennoside B

Method II

Drug + methanol (80-90%) at pH 2-9 + gluconic acid

↓

Extract is filtered & adjusted to pH of 3.7-3.9 with ammonia

↓

Stirred & allowed to stand for 1 hr

↓

Precipitate is obtained & removed by filteration

↓

Again stirred for 1 hr with 10% solution of methanolic calcium chloride & added with 30% ammonia to reach pH of 6.5-6.8

↓

Calcium sennosides are precipitated within an hour & filtered & washed with methanol

↓

Estimation

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

Chemical Test: Brontagers test

Utilization:

1. Treatment of constipation
2. In skin diseases
3. As an anthelmintic
4. Useful in loss of appetite, dysentery, indigestion, malaria, jaundice, gout,rheumatism & anaemia.

Assay

Determine by liquid chromatography (HPLC).

Test solution. Weigh accurately 1.0 g of the coarsely powdered substance in a round bottom flask, add about 10 ml of 1 per cent v/v acetic acid and 25 ml of methanol and reflux on a water bath for about 30 minutes. Cool to room temperature; make up the volume up to 50.0 ml with methanol and filter.

Reference solution. A 0.004 per cent w/v solution of sennasides RS in methanol.

Chromatographic system –

Column: a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm)

Mobile phase: 1% acetic acid in water: Acetonitrile (82:18)

Flow rate: 1ml per minute,

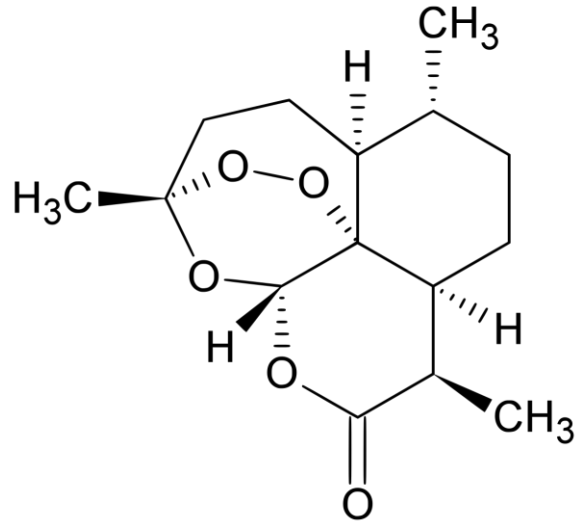
Detection: spectrophotometer set at 350 nm,

Injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent. Inject the reference solution and the test solution. Calculate the content ofsennoside A and B.

Biological source: Sesquiterpene lactone obtained from the leaves & unexpanded flower heads of *Artemisia annua*.

Family- Asteraceae.



Industrial production:

1. Fresh leaves are dried below 60˚C, powder is extracted with methanol by maceration.
2. Methanol extract partitioned with hexane
3. The hydro alcoholic extract partitioned with ethyl acetate until the colourless.
4. Contentrated at controlled temperature at 40˚C under vacuum.
5. Artemisinin obtained as fine white crystals after recrystallization with cyclohexane.

Industrial production

Leaves are collected & dried

↓

They are coarsely powdered & subjected for isolation using petroleum ether as a solvent

↓

Filter the extract & concentrate to dryness

↓

Residue obtained is dissolved in chloroform

↓

Acetonitrile added to precipitate out impurities like sugar & waxes

↓

Filter it and discard the precipitate

↓

Again concentrate to dryness

Estimation

HPLC

Assay: Determine by liquid chromatography (HPLC)

Test solution. Dissolve 100 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution. A 0.. 1 pyr cent wIv solution of artemisinin RS in the mobile phase.

Column: C18Column (a stainless steel column 10 cmx 4.6mm, packed with octadecylsilane bonded to porous silica 3µm)

Mobile phase: Isocratic acetonitrile : water(60:40)

Isocratic, Acetonitrile :water : methanol (50:30:20)

Flow rate: 0.6mL/min

Sample injected in volume of **20 µl**

Detector: UV at 216 nm

Inject the test solution and the reference solution. Calculate the content ofClsH220s.

TLC

Sample preperation: Drug is dissolved in chloroform

Stationary Phase: Silica gel G

Mobile phase: Pet. Ether: ethyl acetate (1:2)

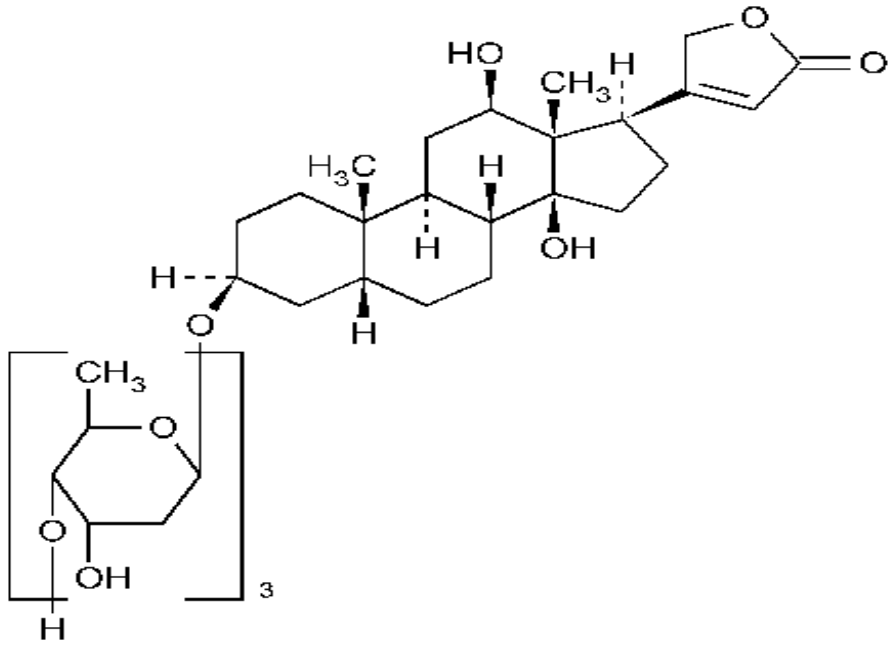
Detection: 1. p-dimethylaminobenzaldehyde & heating at 80^0C to produce color

2. Anisaldehyde sulphuric acid reagent followed by heating to 110˚C.

Utilization

- Strong anthelmintic, specially for round worms
- Used as antimalarial drug
- Suppress inflammatory immune reactions

Biological Source: Digoxin consists of cardenolide cardiac glycoside obtained from dried leaves of *Digitalis lanata*. belonging to family Scrophulariaceae



Industrial Production

Take accurately weighed quantity of fresh leaves

↓

Defat with petroleum ether

↓

Defatted mass grinded with neutral salt to inactivate the enzymes

↓

Above grinded mass is extracted with ethyl acetate for 2 hours

↓

Filter it & concentrate the filtrate to dryness

↓

Column chromatographic seperation is done for above residue to yield Lanatoside A, Lanatoside B & Lanatoside C

↓

Lanatoside C fractions are further treated with dil. HCL to get hydrolysis

↓

Shake with Ethyl acetate, Distill off solvent gives Digoxin

Industrial Production

Take accurately weighed quantity of fresh leaves

↓

Extraction with 50% ethanol at low temperature, filter

↓

In filtrate add Lead acetate solution to remove impurities

↓

The ppt are remove by centrifugation

↓

Supernatant is extracted with Chloroform

↓

Evaporate Chloroform extract (Cardiac glycoside)

↓

Column chromatographic separation is done for above extract to yield Lanatoside A, Lanatoside B & Lanatoside C

↓

Lanatoside C fractions are further treated with dil. HCL to get hydrolysis

↓

Shake with Ethyl acetate, Distill off solvent gives Digoxin

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 C

Alkaline hydrolysis → Deacetyl Lantoside

Enzymatic hydrolysis → Acetyl Digoxin

Deacetyl Lantoside — Enzymatic hydrolysis → Digoxin

Acetyl Digoxin — Alkaline hydrolysis → Digoxin

Estimation:

1. Colourimetric analysis

a) Assay-40 mg test & std solution of digoxin dissolve in sufficient 95% ethanol to produce 50 mL

b) Dilute 5 ml of above solution to 100 mL with 95% ethanol

c) 5 ml resulting solution, add 3ml Alkaline picric acid solution. All to stand for 30 min.

d) Measure absorbance at 495 nm against balnk (5 ml 95% alcohol, add 3ml Alkaline picric acid solution).

2. Thin layer chromatography

Sample preparation: 1mg of sample in 1ml of solvent (alcohol)

Stationary Phase: Silica gel g

Mobile phase: Cyclohexane: Acetone: Acetic acid (49:49:2)

Chamber Saturation time : 15mins

Detection: Detected by spraying reagent i.e. 5% aqueous sulphuric acid

Blue spots are observed under UV at 385nm

Estimation

3. Assay- Determine by liquid chromatography (HPLC)

Test solution. Dissolve 50 mg of the substance under examination in 200 ml of ethanol (95 per cent).

Reference solution. A 0.025 per cent w/v solution of digoxin RS in diluted ethanol (95 per cent).

Chromatographic system - a stainless steel column 25 cm x 4.2 mm packed with octadecylsilane bonded to porous silica (5 µm), -

Mobile phase: Water :Acetonitrile (37:13)

Flow rate. 3 ml per minute

Detection spectrophotometer set at 218 nm, - I

Injection volume. 10 µL.

Procedure :Inject the reference solution. The test is not valid unless the theoretical plates is not less than 1200 and tailing factor for the principal peak is not more than 2.0. The resolution between the peaks due to digoxin and digoxigenin analogue is not less than 4.0. The relative standard deviation for the replicate injections is not more than 2.0 per cent. Inject the test solution and the reference solution. Calculate the content ofC41H64014'

Utilization

- Used as a cardiotonic & is the most widely used drug in treating congestive heart failure.
- Also used to treat atrial flutter, Atrial fibrillation
- Diuretic

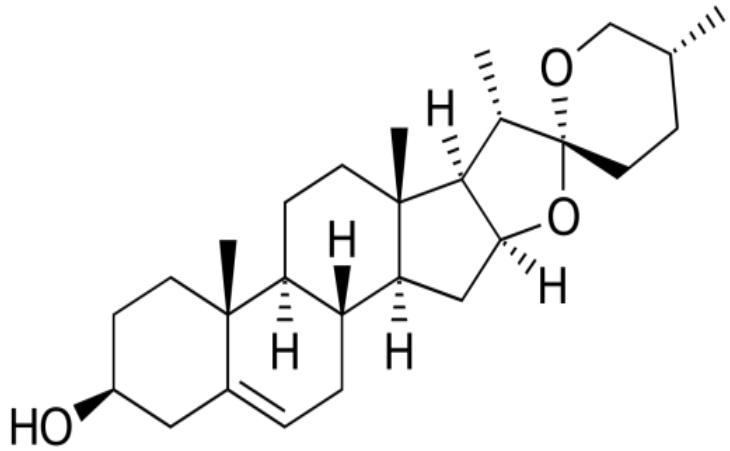
- Digoxin Injection (250 µg per mL.)
- Digoxin Paediatric Solution (50 µg per mL.)
- Digoxin Tablets (62.5 µg; 125 µg; 250 µg.)

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

Family- Dioscoreaceae.

Diosgenin a steroidal aglycone obtained from a dried fruits of *Tribulus terrestris*

Family- _Zygophyllaceae).



Industrial production

Method I
Acid Hydrolysis method

Dried powder hydrolyzed with 2N/4N H_2SO_4/HCl by reflux or autoclave.

↓

Marc washed with 10% sod. Bicarbonate to neutralize acid

↓

Washed with water to make neutral

↓

Hydrolyzed Marc/powder extracted with benzene/ Toluene for 6-8 hrs.

↓

Filtrate, concentrate to reduce volume

↓

Crystals of Diosgenin

Industrial production

Method II
Alcoholic extraction method

Pulverized Sliced dried tuber to make a powder form.

↓

Extracted the fine powder is with ethanol, two times for 6-8 hours

↓ Filter

Filtrate concentrated to make a syrupy liquid.

↓

Hydrolyzed Concentrated extract with 2N HCI or H_2SO_2 for 6-12 hours.

↓

Precipitate crude diosgenin (about 85%)

↓ Filter and residue washed with water

Crude diosgenin

↓

Purified by recrystallization with alcohol

This method has the following disadvantages:
1. Unsaturated sapogenin quickly decomposes to spirostadiene.
2. A gummy material is formed alongside.
3. It is time consuming.

These shortcomings can be overcome by treating the hydrolyzed extract with lime or other alkali: and coloured impurities.

Industrial production

Method III
Fermentation method

Fresh dioscorea tubers & smash it in the hammer mill

↓

The smashed product is fermented for 4-10 days

↓

Fermented material is air dried & hydrolysed with mineral acid

↓

Extraction is done by using heptane

↓

Filter & concentrate slowly to obtain crystals of diosgenin

↓

Hydrolyzed Marc/powder extracted with benzene/ Toluene for 6-8 hrs.

↓

Filtrate, concentrate to reduce volume

↓

Crystals of Diosgenin

Chemical test:

Libermann-Burchard test

Sample + Chloroform + Acetic anhydride + One drop of concentrated Sulphuric acid--Blue green to red orange colour

Salkowski reaction

Sample+ Chloroform + Sulphuric acid concentrated--Chloroform layer shows red colour and acid layer shows green fluorescence

Estimation

HPTLC

Stationary Phase: Silica Gel F254

Mobile Phase: Toluene: ethyl acetate: formic acid (5:4:1)

Sample and Standard preparation: Six gradual increased concentration of standard 450 nm. diosgenin were prepared in chloroform. Isolated diosgenin solution prepared and used as test diosgenin

Detection: P-anisaldehyde- sulphuric acid detecting reagent. Densitometric scanning wavelength 450 nm. Diogenin developed pinkish blue colour spot. The derivatised plate scanning Was performed using tungsten halogen source at 450nm

The calibration curve of peak AUC versus concentration of standard diosgenin used to quantify it in test extract.

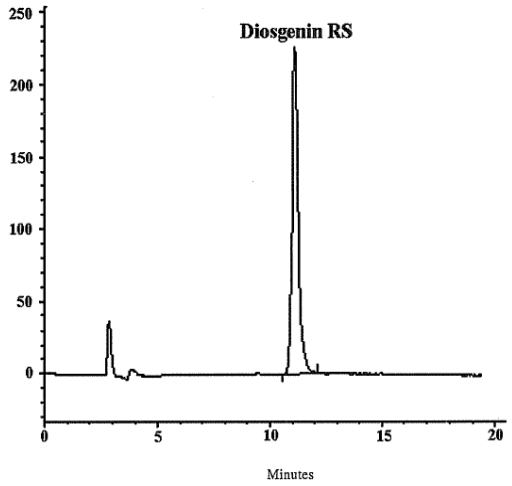
UV standard curve method

Prepare the solution A (0.5 ml p-anisaldehyde in 99.5 ml ethyl acetate) and solution B (50 ml sulphuric acid with 50 ml ethyl acetate).

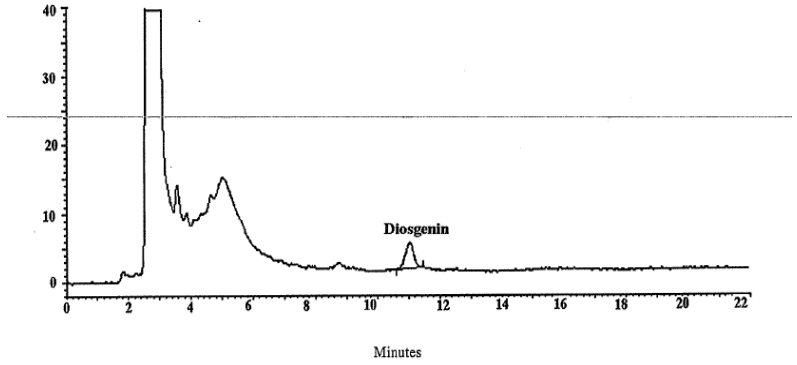
The test samples is dissolved in 2 ml ethyl acetate and add 1ml of reagent A and B. Stirred well and maintain the temperature 60^{o} C for 10 minutes to develop the colour. Allow sample. to cool at 25°C. Measure the absorbance at 430 nm using ethyl acetate as blank. The calibration curve of standard diosgenin (2-70 µg) in ethyl acetate was made and determine the concentration of unknown

Estimation

Assay. Determine by liquid chromatography (HPLC)
Test solution. Reflux 5.0 g ofthe substance under examination with 50 m1 of sulphuric acid (10 per cent) for 4 hours. Cool and transfer to separating funnel. Extract with 50 ml of ethyl acetate. Repeat the extraction 3 times. Pass the ethyl acetate layer through sodium sulphate and evaporate. Dissolve the residue with 50 mL of methanol.
Reference solution. A 0.1 per cent w/v solution of diosgenin RS in methanol.
Chromatographic system
Column: A stainless steel column 25 cm x 4.6 rom packed with octadecylsilane bonded to porous silica (5 µm)
Mobile phase: Acetonitrile:methanol (8:2)
flow rate. 1ml per minute,
injection volume. 20 µl.
Detection: spectrophotometer set at 210 nm,
Inject the reference solution. The relative standard deviation for the replicate injections is not more than 2.0 per cent. Inject the test solution. Calculate the content of diosgenin.



HPLC Chromatogram of diosgenin RS



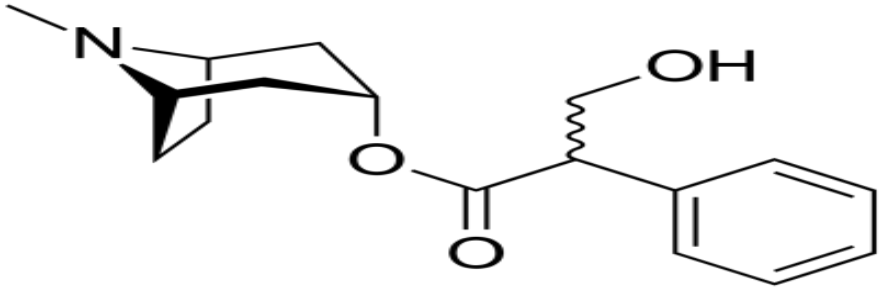
- **Utilization:**

1. As a precursor for steroidal synthesis like several corticosteroids, sex- harmones & oral-contraceptives
2. In treatment of rheumatic arthritis

Atropine

Source: tropane alkaloid, flowering tops of *Atropa belladonna*, *Datura stramonium & Hyoscyamus niger*.

Family- Solanaceae.



Atropine

Industrial production

Take accurately weighed quantity of belladonna leaves & powder to obtain coarse sized particles

↓

Extract it with 95% ethanol, filter & distill off solvent

↓

Add sufficient quantity of 1% HCL to remove resinous matter

↓

Add petroleum ether, shake well & made alkaline with ammonia

↓

Extract this mixture with chloroform layer

↓

filter

Separate chloroform layer & add dilute acid

↓

Shake & separate chloroform layer

Add ammonia to make solution alkaline

↓

Evaporate chloroform layer & add oxalic acid which will separate out crystals of atropine & Hyoscyamine oxalate

↓

Atropine & hyoscyamine oxalate are separated by dissolving crystals in acetone /ether

↓

filter

Filtrate contain Hyoscyamine oxalate crystals & residue contain atropine oxalate

Estimation:

Stationary Phase: silica gel GF254.
Mobile phase. Acetoene: water :strong ammonia solution.(90:7:3)
Test solution
Reference solution. 1% of Atropine solution in 2N acetic acid

Examine in ultraviolet light at 254 nm and 365 nm, spray with 10 ml of modified **potassium iodobismuthate solution** until the bands become visible as orange or brown on a yellow background. The bands in the chromatogram obtained with test solution have similar

1)Detected by spraying reagent i.e. acidified iodoplatinate solution.
2) Dragendorff's reagent

Utilization:

1. As preanesthetic medication
2. Antispasmodic

Atropine Eye Ointment

Atropine Injection

Atropine Methonitrate

Atropine Sulphate

Atropine Sulphate Eye Ointment

Atropine Sulphate Injection

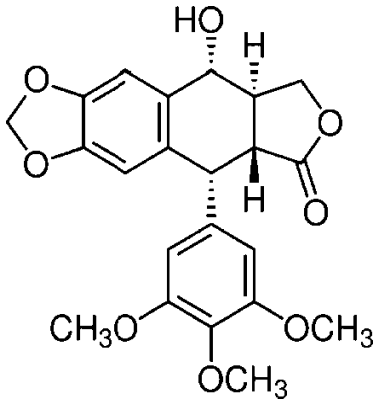
Atropine Sulphate Tablets

Atropine Sulphate and Morphine Sulphate Injection

Atropine Tablets

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



Industrial Production

Take accurately weighed quantity of rhizomes/roots of podophyllum with methanol

↓ filter

Evaporate to semisolid mass

↓

Dissolve semi-solid mass into acidic water

↓

Precipitate formed is allowed to settle for 2 hrs

↓ filter & wash with cold water

Collect the residue, wash with acidified water

↓

Dry it to obtain dark brown amorphous powder

Extract the residue with hot water

↓

Filter & evaporate to dryness

↓

Re-crystallise the residue in benzene to yield podophyllotoxin

Estimation

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.

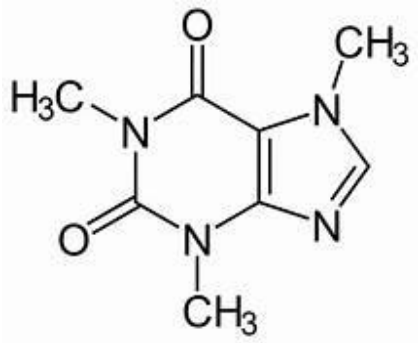
Detection : Photodiode detector at 283nm

Utilization

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.

- **Biological source:**

  It is methylated xanthine alkaloid derivative found in coffee bean i.e. *Coffea arabica*, family Rubiaceae *Thea sinensis*, family Theaceae, *Theobroma cacao*, family Sterculiaceae



Industrial Production

- **Method I:**

Weigh tea leaves transfer to distilled water

↓ boil for 30 min

After complete decoction, allow it to cool

↓ filter

Take filtrate to separating funnel add chloroform

↓ shake vigorously

Separate the chloroform layer

↓

Evaporate the chloroform layer

↓

White color crystals are collected

CAFFEINE

Weigh tea leaves transfer to distilled water & boil for 30 min

↓ filter

Treat with Lead acetate to precipitate tannins

↓ filter

Add sulphuric acid to remove excess of lead acetate

↓ filter

Add charcoal to remove coloring matter

↓ filter

Take filtrate is separating funnel

↓

Extract with 3 portions of chloroform

↓

Separate the chloroform layers & combine them

↓

Evaporate to precipitate white crystals & re-crystallise with alcohol

- **Method III:**

Weigh tea leaves & extract with 5% aq. Sodium carbonate solution in a beaker for 30 min

↓ filter

Neutralise the filtrate with 10 % sulphuric acid

↓ filter

Wash marc with dichloromethane

↓

Take mixture in separating funnel to separate dichloromethane layer

↓

Evaporate layer to obtain caffeine crystals

- Method IV: (Sublimation)

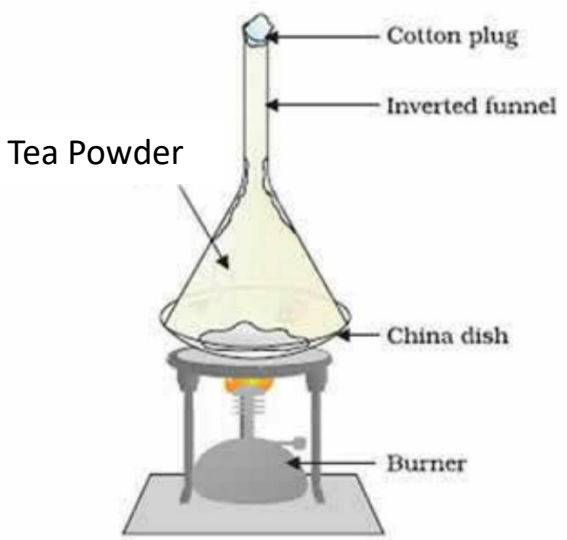
Powder of tea leaves, heat in beaker covered with funnel having closed end

↓

White caffeine crystals are deposited at end glass of funnel

↓

Collect the crystals of caffeine



Estimation

1. **Thin layer chromatography**

- Sample preparation: 1mg of sample in 1ml of solvent (methanol/chloroform)
- Stationary Phase: Silica gel g
- Mobile phase: Ethyl acetate: Methanol: Acetic acid (80:10:10)
- Chamber Saturation time : 15mins
- Detection: Detected by exposure to Iodine vapours (R_f 0.41)

2. HPLC method

- Mob. Phase- methanol: acetonitrile (65: 35 v/v)
- Column- C18
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm, injection volume.20 ,.ti

Assay

Weigh accurately about 0.18 g and dissolve with warming in 5 rnl of anhydrous glacial acetic acid. For Caffeine Hydrate, use material previously dried at 100° to 105°. Cool, add 10 rnl of acetic anhydride and 20 rnl of toluene. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically.

1 rnl 0.1 M perchloric acid is equivalent to 0.01942 g of CSHllN40 2

Uilizations

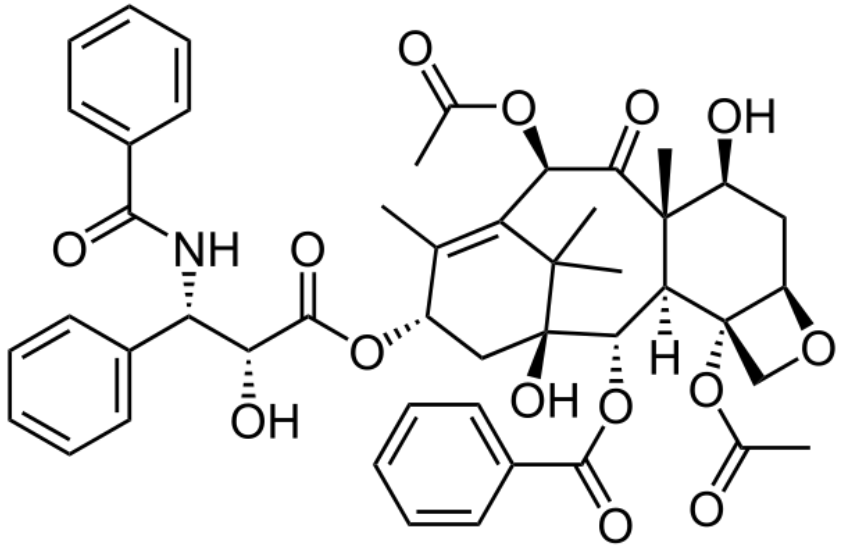
- Used as a beverage
- cns stimulant
- Strong diuretic
- Mood elevator
- Antitussive
- Vasodilator
- involuntary muscle relaxant

Synonyms:

Yew, Talispatra, Himalayan yew, Birmi

Biological source:

This consists of dried leaves, bark & roots of various species of *Taxus* i.e. *T. baccata*, *T. brevifolia*, *T. canadensis*, *T. cuspidate*, *T. wallichiana*, belonging to family Taxaceae



INDUSTRIAL PRODUCTION

Take the accurately weighed quantity of powder of bark of Taxus plant

↓

Extract the powder with ethanol

↓ filter

Evaporate at 40^{o}C to dryness

↓

Dissolve this residue again in methanol

↓ filter & evaporate

Dissolve this residue in mixture of carbon tetrachloride & water

↓ filter & centrifuge

Separate & combine carbon tetrachloride

↓ evaporate

Dissolve this residue in mixture of methanol & CCl_4 (1:1)

↓ filter

Evaporate to obtain dry residue of taxol alkaloids

↓

Purification is done by TLC

Estimation

It is estimated by Thin layer chromatography

- Sample preparation: 1mg of sample in 1ml of solvent (methanol)
- Stationary Phase: Silica gel g
- Mobile phase: CCl_4 : methanol (95:5)
- Chamber Saturation time : 15mins
- Detection: Detected at R_f 0.35 to 0.37

Utilizations

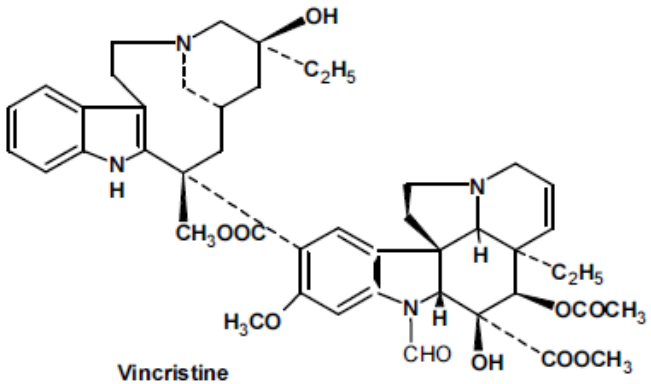
- Anticancer
- Analgesic
- Anti-inflammatory
- Antipyretic
- Anti-convulgant
-

- **Synonym:**

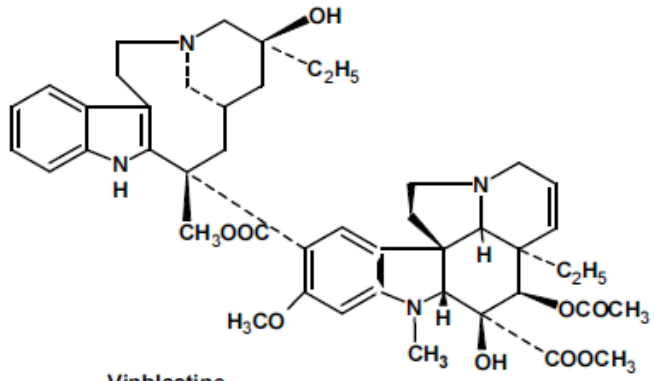
  vinca rosea, rosy periwinkle, sadabahar, catharanthus, sadaphuli

- **Biological source:**

  It consists of dried parts of the whole plant *Catharanthus roseus* family Apocynaceae



Vincristine



Vinblastine

Industrial production

Pulverized dried entire vinca plant

↓

Extract with mixture of aqueous alcoholic acetic acid (9:1)

↓

filter

Evaporate to dryness & dissolve in 2% hot HCL

↓

Adjust the pH of filtrate to 4 with NaOH

↓

Extract it with benzene & adjust pH of aqueous solution is raised to 7

↓

Extract again with benzene

↓

separate benzene layer

↓

Evaporate benzene layer, crude alkaloidal residue is obtained

Further from alkaloidal residue Vincristin & Vinblastine are seperated by Column Chromatography

1) Benzene : methylene chloride (63:35)

Vinblastine fractions are collected and evaporated to dryness, yields vinblastine sulphate & crystalised from alcohol

2) At pH 4.9 to 5.9 fractions afford vincristine & crystallised from methanol

Estimation

It is estimated by Thin layer chromatography

- Sample preparation: 1mg of sample in 1ml of solvent (methanol)
- Stationary Phase: Silica gel G
- Mobile phase: Acetonitrile : Benzene (30:70)
- Chamber Saturation time : 15 mins
- Detection: Detected by spraying reagent with 1% solution of Ceric ammonium sulphate in 85% phosphoric acid at R_f 0.39

Vinblastine

Chromatographic system - a stainless steel column 25 cm x 4.2 mm packed with octadecylsilane bonded to porous silica (5 µm),
Guard column packed with a suitable silica gel placed between the pump and the injection device
Mobile phase: methanol :1.5 per cent v/v solution of diethylamine adjusted to pH 7.5 with phosphoric acid: acetonitrile (50:38:12)
Flow rate. 3 ml per minute
Detection spectrophotometer set at 262 nm
Injection volume. 10 µL.

Vincristine

Chromatographic system - a stainless steel column 25 cm x 4.2 mm packed with octadecylsilane bonded to porous silica (5 µm),
Guard column packed with a suitable silica gel placed between the pump and the injection device
Mobile phase: A) 1.5 per cent v/v solution of diethylamine adjusted to pH 7.5 with phosphoric acid
B). methanol
Separation Gradient elution

Time	Mobile A	Mobile B
0-12	38	62
12-27	38.8	62.92
27-29	8.38	92.62
29-34	38	62

Flow rate. 2 ml per minute
Detection spectrophotometer set at 297 nm
Injection volume. 20 µL.

Utilization

- Antitumour action used in malignant conditions

- **Vincristine:** in treatment of acute lymphocytic leukaemia, wilm's tumour, cancer of mammary glands, cervical cancer, lung cancer & reticulum cell sarcoma.

-**Vinblastine:** reduces proportion of leukocytees in blood has suppressant action on immune system.

- Hypoglyceamic action

- Used in hypertention

- To treat sore throat, cough and bronchial congestion

- Treat asthma

- Haemostatic agents

THANK YOU