



COMPLETE CONCEPT



B.PHARMA | 5 SEMESTER

**ISOLATION , IDENTIFICATION &
ANALYSIS OF PHYTOCONSTITUENTS**

**TERPENOIDS | GLYCOSIDES |
ALKALOIDS | RESINS**

UNIT - 3



B. PHARMA 5TH SEM ONE SHOT NOTES

UNIT-3

ISOLATION, IDENTIFICATION & ANALYSIS OF
PHYTOCONSTITUENTS

Isolation of Compound

- **Isolation:** Separation of a single compound from a mixture of components present in the extract. Different methods are used for Isolation of compounds.
- **Chromatography**
 - Paper Chromatography
 - Thin Layer Chromatography
 - Column chromatography
 - HPLC (High Performance Liquid Chromatography)
 - GC (Gas Chromatography)

Identification

- It is the process of identification or confirmation of the isolated compound.
- Many identifications test is used for identification of isolated compound.


Analysis of Phytoconstituents

- Phytoconstituents- these are chemical compound occur naturally & are medicinally active.
- These are dissolved out from cells and tissue from plant by process of extraction.
- Analysis- it is the process of determination of the concentration of extract (quality & quantity).
- Different technique used for this
 - TLC
 - HPLC
 - SPECTROSCOPY

Terpenoids

- Hydrocarbon compounds
- Available in volatile oil
- Easily oxidised
- colorless
- Insoluble in water
- Also known as isoprenoid & contain isoprene unit.

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Drug	Introduction	Isolation	Analysis of Phytoconstituents
Menthol	<p>Belong to class of monoterpene class</p> <p>Family- Labiatae.</p> 	<ul style="list-style-type: none"> Steam distillation – allow a fresh air dried mentha plant to distillation. cooled & convert back to liquid form i.e, mentha oil & water. Mentha oil float over the water Mentha oil is filtered & allow it to cool 	<ol style="list-style-type: none"> Sample preparation- 1mg of menthol is dissolve in 1mg of methanol (C₂H₅OH). Standard sample- menthol Stationary phase- silica gel G Mobile phase- pure chloroform Detecting agent- 1% vanillin (sulphuric acid H₂SO₄) reagent & heat the TLC plate at 110°c for 10min. RF value- 0.48.0.62

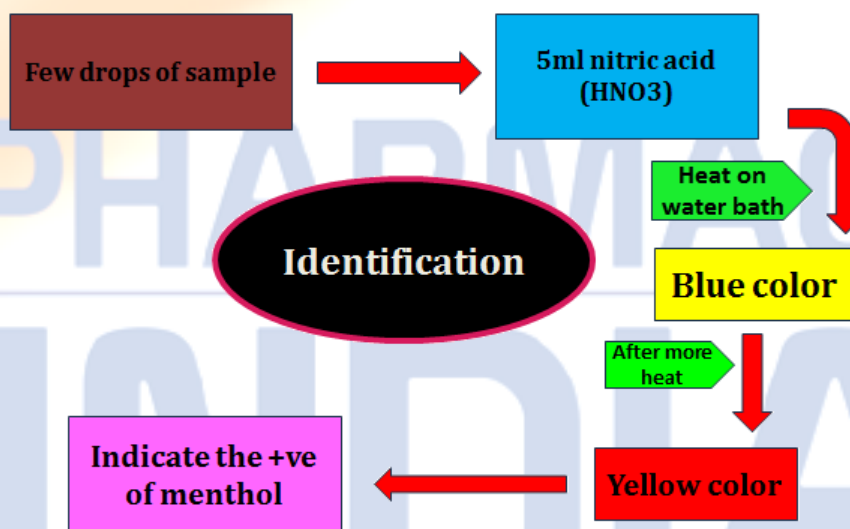



Figure: Identification of Menthol

Drug	Introduction	Identification	Analysis of phytoconstituents
Citral	<p>Belong to class of monoterpene class.</p> <p>Family - graminae</p> 	<ol style="list-style-type: none"> Sample + alcohol solution of Sudan red 3 -give red color which indicate presence of Citral. Sample + tincture- give red color which indicate the presence of Citral. 	<ol style="list-style-type: none"> Sample preparation- 1mg Citral in 1ml of methanol. Standard sample- citral Stationary phase- silica gel.G Mobile phase- pure chloroform Detecting agent- 2,4-dinitrophenyl hydrazine reagent. RF value- 0.51 Color spot- yellow to orange.

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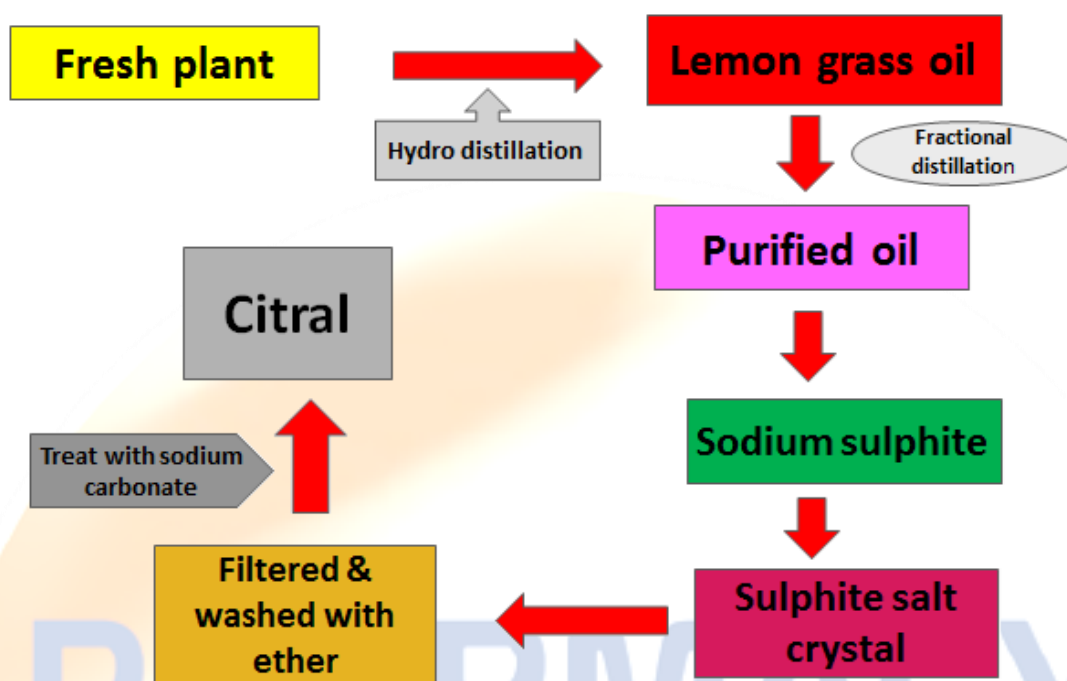



Figure: Isolation of Citral

Drug	Introduction	Identification	Analysis of phytoconstituents
Artemisin	<p>Belong to class of sesquiterpenes lactone. Plant – artemisia annua</p> 	<p>Sample 10ml → boil → Filter + sodium hydroxide → red color → indicate presence of artemisinin.</p>	<ol style="list-style-type: none"> 1. Sample preparation- 1mg of artemisinin in chloroform. 2. Standard sample- artemisinin 3. Stationary phase- silica gel.G 4. Mobile phase- ethyl acetate 5. Detecting agent- P-dimethylamino benzealdehyde7 heat 80c to produced color 6. RF value- compared with standard value.

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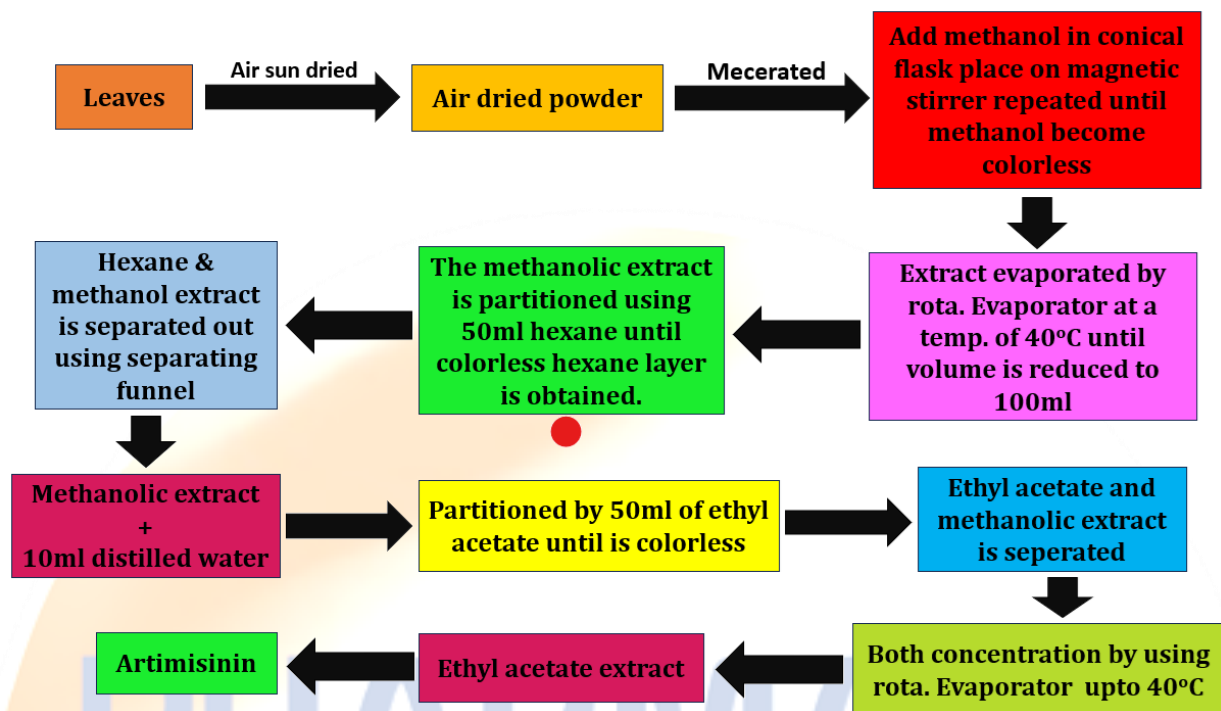


Figure: Isolation of Artemisinin

Glycoside

- Has sugar & non- sugar moieties
- Obtained from plant & animal source
- Organic compound
- Water soluble also soluble in alcohol

Uses

- Cardiotonic
- Analgesics
- Anti -ulcer
- Anti -rheumatics

Drug	Introduction	Identification	Analysis of phytoconstituents
Glycyrrhetic acid	It is a triterpenoids saponin glycoside. Family- Leguminosae	3ml extract + 3ml of acetic anhydride → Heat & and then cooled → Add conc. H ₂ SO ₄ → Blue color obtain → Indicate +ve of Glycyrrhetic acid	<ol style="list-style-type: none"> 1. Sample preparation- 1mg of glycyrrhetic acid in methanol: chloroform 2. Standard sample- glycyrrhetic acid 3. Stationary phase- silica gel.G 4. Mobile phase- toluene : ethyl acetate : glacial acetic acid 5. Detecting agent- 1% vanillin (sulphuric acid H₂SO₄) reagent

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			& heat the TLC plate at 110°C for 10min. 6. RF value- 0.41 7. Color – purplish spot
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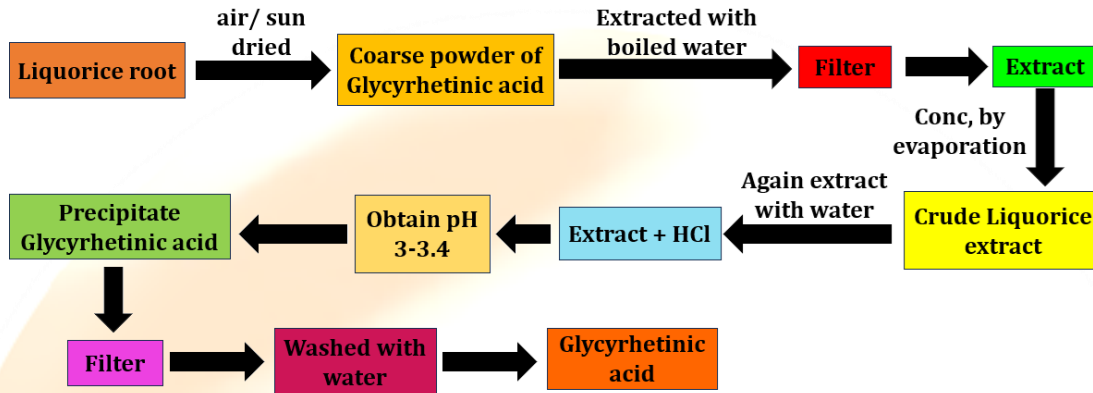



Figure: Isolation of Glycyrrhetic acid

Drug	Introduction	Identification	Analysis of phytoconstituents
Rutin	Family- polygonocae Obtained from various citrus fruit. Plant –fagopyrum esculation 	sample + Lead acetate → yellow color	1. Sample preparation- 1mg of rutin in 1ml of ethanol 2. Standard sample- rutin 3. Stationary phase- silica gel.G 4. Mobile phase- butane : ferric acid : water. 5. Detecting agent- anisaldehyde sulphuric acid reagent 6. Rf value- 0.43 7. Color – yellow spot

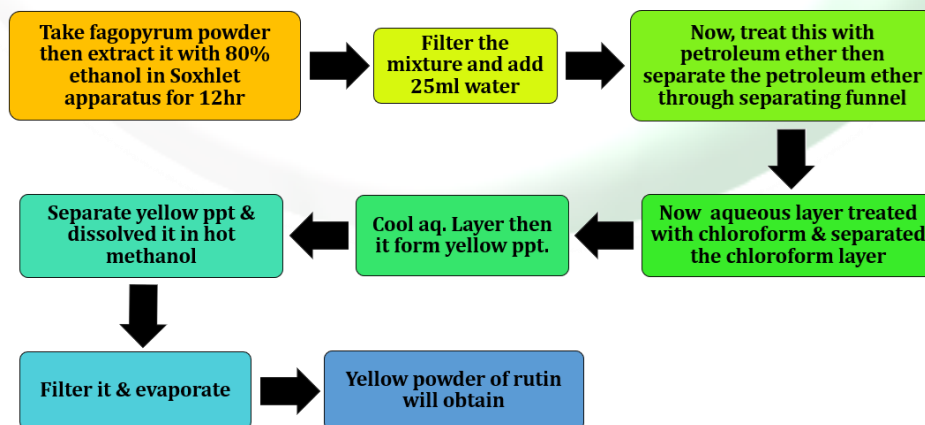



Figure: Isolation of Rutin

Alkaloids

- Nitrogenous compound
- Obtained naturally from plant & animal
- Derived from amino acid
- Contain one or more nitrogen

Drug	Introduction	Identification	Analysis of phytoconstituents
Atropine	It is a tropane alkaloids. Plant – Atropa belladonna Family- Solanaceae 	sample + conc. Nitric acid → Evaporate till dry → Residue +1ml acetone → Violet color	1. Sample preparation- 1mg atropine in chloroform 2. Standard sample- atropine 3. Stationary phase- silica gel.G 4. Mobile phase- toluene : ethyl acetate : diethyl amine. 5. Detecting agent- dragendroff's reagent. 6. RF value- 0.70 7. Color – yellow orange

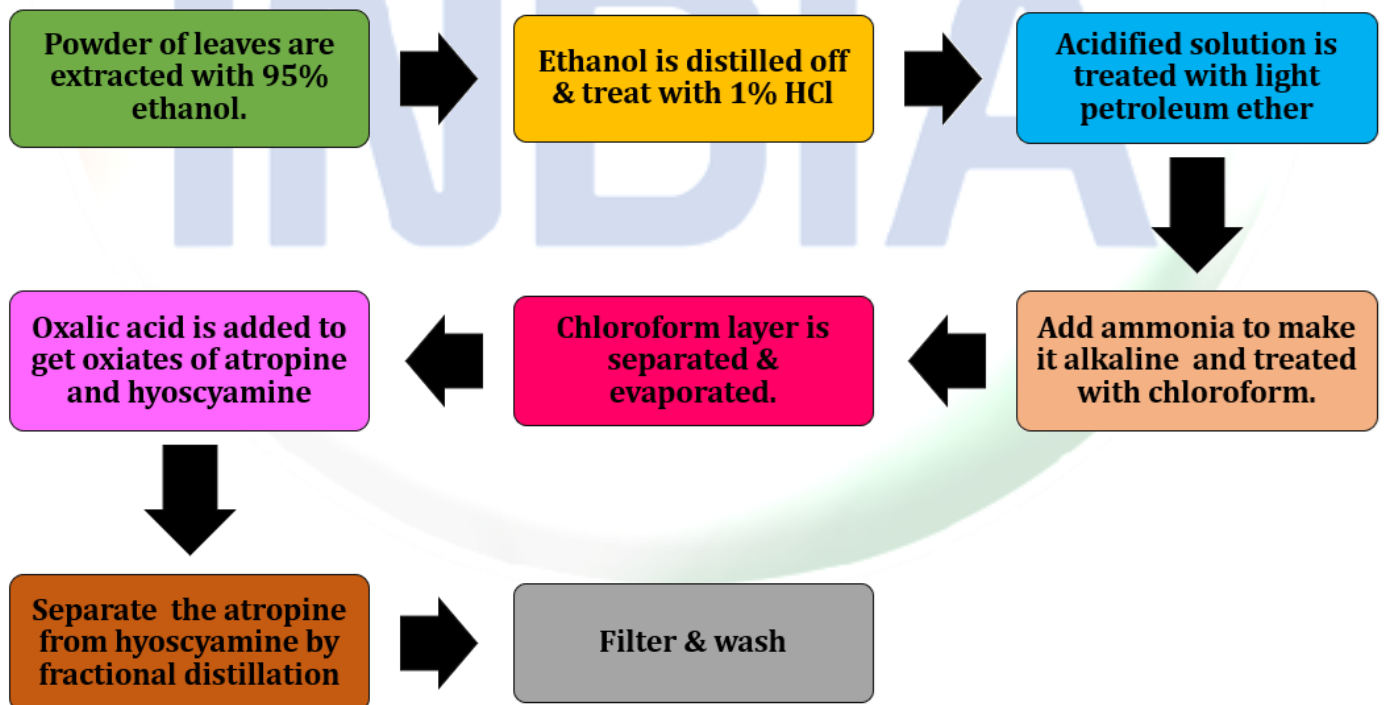



Figure: Isolation of Atropine

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Drug	Introduction	Identification	Analysis of phytoconstituents
Quinine	<p>It is a quinoline alkaloids</p> <p>Plant- cinchona officinalis</p> <p>Family- Rubiaceae</p> 	<p>sample + Ammonium solution + Bromine water → Green color</p> <p>Indicate +ve of quinine</p>	<ol style="list-style-type: none"> 1. Sample preparation- 1mg quinine in 1ml methanol 2. Standard sample- quinine 3. Stationary phase- silica gel.G 4. Mobile phase- chloroform: diethyl amine. 5. Detecting agent- dragendroff's reagent. 6. RF value- 0.17

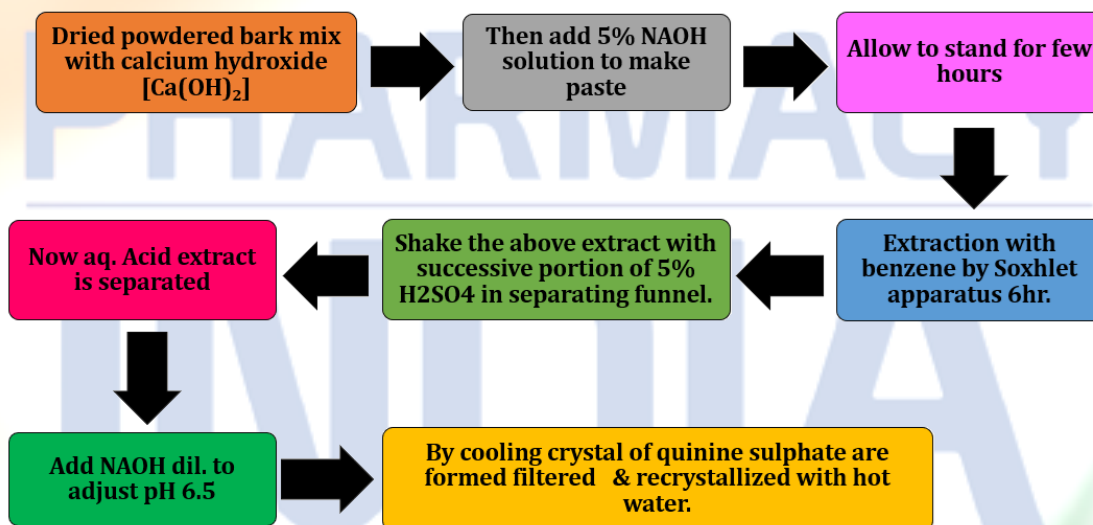



Figure: Isolation of Quinine

Drug	Introduction	Identification	Analysis of phytoconstituents
Reserpine	<p>It is a indole alkaloid.</p> <p>Plant- rauwolfia serpentina</p>	<p>sample + Solution of vanillin in acetic acid → Give red violet color</p>	<ol style="list-style-type: none"> 1. Sample preparation- 1mg reserpine in 1ml methanol 2. Standard sample- reserpine 3. Stationary phase- silica gel.G 4. Mobile phase- chloroform: diethyl ether: acetone. 5. Detecting agent- dragendroff's reagent.

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	Family- apocynaceae 		6. RF value- 0.72
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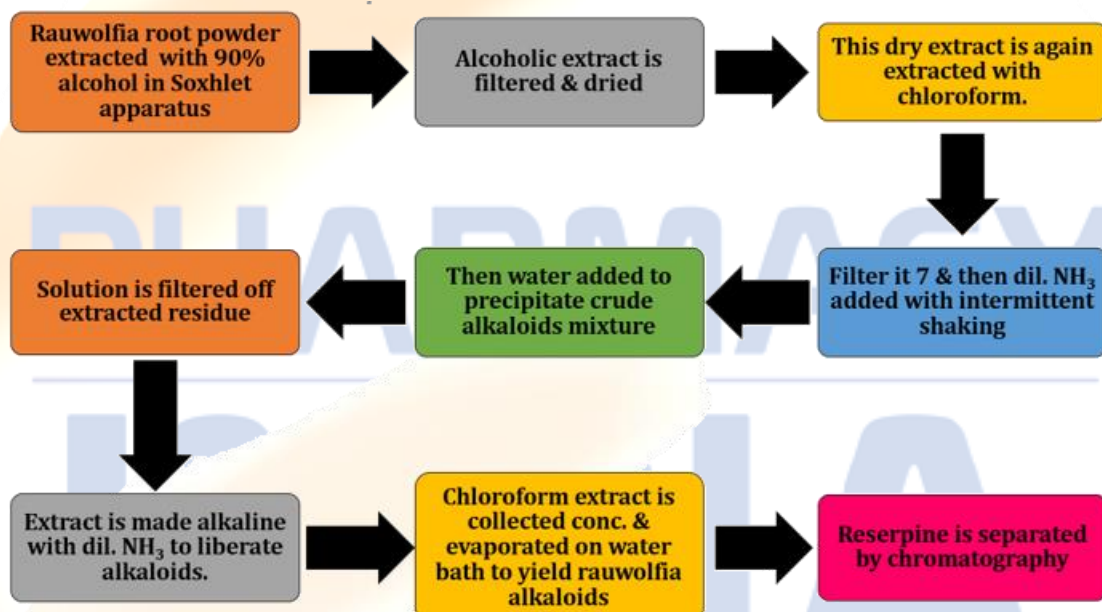


Figure: Isolation of Reserpine

RESINS

- Obtained from plant
- Amorphous compound
- Solid or semi- solid
- Soften & melt on heat
- Resin are secondary metabolites produce by higher plants

☐ Uses

- Cathartic
- Purgative
- Anti- tumor
- Anti-viral

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Drug	Introduction	Identification	Analysis of phytoconstituents
Podophyllotoxin	It is lactone resin. Plant- podophyllum hexadrum. Family- Berberidaceae	sample + 50% sulphuric acid → Violet blue color	<ol style="list-style-type: none"> 1. Sample preparation- 1mg podophyllotoxin in 1ml of ethanol 2. Standard sample- podophyllotoxin 3. Stationary phase- silica gel.G 4. Mobile phase- toluene : ethyl acetate 5. Detecting agent- spray with 50% sulphuric acid heated at 20c for 10 min.

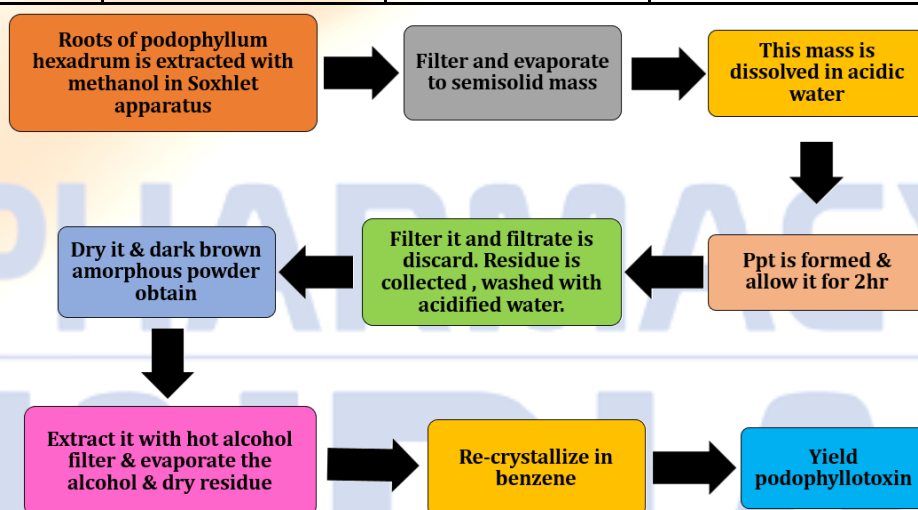

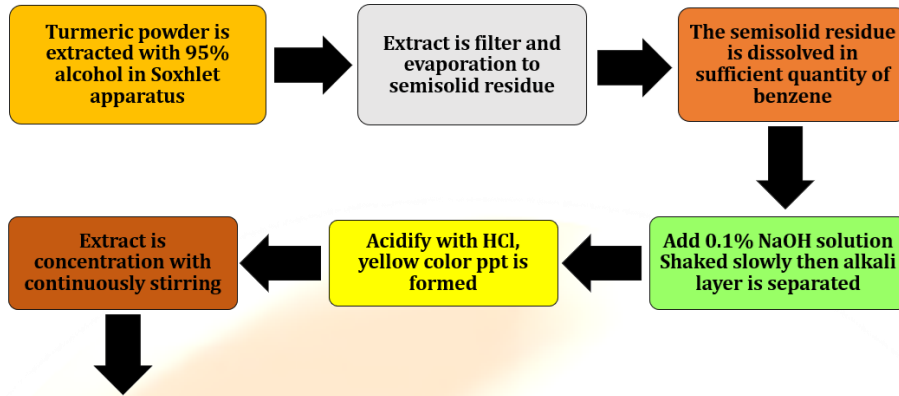


Figure: Isolation of Podophyllotoxin

Drug	Introduction	Identification	Analysis of phytoconstituents
Curcumin 	It is a diaryl hepnoid compound. Source- dried rhizomes of turmeric Family – Zingiberaceae	sample + Acetic anhydride + Conc. H ₂ SO ₄ → Violet blue color	<ol style="list-style-type: none"> 1. Sample preparation- 1mg curcumin in 1ml of methanol 2. Standard sample- curcumin 3. Stationary phase- silica gel.G 4. Mobile phase- chloroform: ethanol: glacial acetic acid. 5. Detecting agent- observed under uv light at 366nm 6. Rf value-0.79

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