



Shree H.N.Shukla Institute of Pharmaceutical Education & Research Rajkot

B.Pharm Semester V

Subject Name: Pharmacognosy and Phytochemistry II

Subject code: BP503TP

**UNIT III: Isolation, Identification and Analysis of
Phytoconstituents**

Contents

UNIT-III

Sl. No.	Topic	Page No.
3.	General Introduction	3
	Extraction	3
	Isolation	3
	Identification	3
	Analysis of Phytoconstituents	4
3.1	Volatile oil and Terpenoids	4
3.1.1	Menthol	6
3.1.2	Citral	8
3.1.3	Artemisin	10
3.2	Glycosides	11
3.2.1	Glycyrrhetic acid	13
3.2.2	Rutin	15
3.3	Alkaloids	17
3.3.1	Atropine	19
3.3.2	Quinine	20
3.3.3	Reserpine	22
3.3.4	Caffeine	24
3.4	Resins	25
3.4.1	Podophyllotoxin	27
3.4.2	Curcumin	28
3.5	References	30

3. General Introduction

Extraction:

It is the process in which the animal or plant tissues are treated with specific solvents (Petroleum ether, Chloroform, Ethyl acetate, Alcohol, Water etc.) whereby the medicinally active compound (Phytoconstituents) are dissolved out from the cells and tissues.

The purpose of extraction is to separate the soluble plant metabolites like Carbohydrate, Glycoside, Alkaloids, Terpenoids, Flavonoids etc.

Different methods are used for the extraction of the drugs i.e.

A. Infusion

B. Decoction

C. Digestion

D. Maceration: Simple, Modified etc.

E. Percolation: Simple, Hot continuous percolation (Soxhletation), etc

F. Distillation: Water distillation for oils, Steam distillation for oils

Isolation of compound:

Isolation: Separation of a single compound from mixture of components present in the extract. Different methods are used for Isolation of compounds i.e.

Chromatography

A. Paper Chromatography

B. Thin Layer Chromatography

C. Column chromatography

D. HPLC (High Performance Liquid Chromatography)

E. GC (Gas Chromatography)

Identification:

Identification means to establish the class of compounds, their nature etc.

Compounds are identified by two basic ways i.e. Qualitative and Quantitative identification.

A. Qualitative identification;

It is a step to identify the different class of compounds present in extracts by chemical test. Different tests are

a. Test for Alkaloid: Mayer's test, Dragendorff's test, Wagner's test, Hager's test etc.

b. Test for Carbohydrates: Molish's test, Fehling's test, Barfoed's test, Benedict's test etc.

- c. **Test for Glycoside:** Raymond's test, Borntrager's test, Legal's test, Baljet's test, Keller Killani test etc.
- d. **Test for Saponin:** Foam test, Hemolytic test
- e. **Test for Proteins and Amino Acids:** Millon's test, Biuret test, Ninhydrin test etc.
- f. **Test for Phenolic Compounds:** Ferric chloride test, Lead acetate test, Alkaline reagent test etc.
- g. **Test for Tannins:** Gold bladders test, Matchstick test etc.
- h. **Test for Phytosterols:** Libermann-Burchard's test, Salkowskireaction

B. Quantitative identification:

The compounds are identified by different methods like Spectroscopical method, e.g. UV-Visible Spectroscopy, IR spectroscopy; Chromatography e.g. TLC, HPTLC, GC-MS etc.

Analysis of Phytoconstituents

After isolation of phytoconstituents, these are analysed by different method likes

- A. Spectroscopical methods: UV-Visible Spectroscopy, IR Spectroscopy, NMR Spectroscopy, MS Spectroscopy etc.
- B. Chromatography methods: TLC, HPTLC, HPLC etc. [1,2].

3.1 Volatile oils and Terpenoids

The odorous, volatile principle of plant and animal source are known as volatile oil. As they evaporate when exposed to air at ordinary temperature, they are also called ethereal oil. Volatile oils are also called essential oil because they produce essence. E.g. Peppermint oil, Lemon grass oil, Clove oil, Citronella oil, Caraway oil, etc.

Terpenoids are hydrocarbons of plant origin of the general formula $(C_5H_8)_n$ as well as their oxygenated, hydrogenated and dehydrogenated derivatives. Terpenoids are abundantly available in volatile oils. They consist of a complex mixture of terpenes or sesquiterpenes, alcohols, aldehyde, ketones, acids and esters. E.g. Menthol, Citral, Eugenol, Citronellol Geraniol, Limonene, etc.

Distribution:

These are commonly found in the species of Labiateae, Rutaceae, Piperaceae, Zingiberaceae, Umbelliferae etc.

Physical Properties:

Terpenoids are colourless liquid

Soluble in organic solvents and insoluble in water

Most of the terpenoids are optically active

Volatile in nature

Boiling point 150 – 180⁰ C

Chemical Properties:

They are unsaturated compounds

They undergo addition reaction with hydrogen, halogen, halogen acids to form addition products like NOCl, NOBr and hydrates.

They undergo polymerization and dehydrogenation in the ring.

On thermal decomposition, terpenoids gives isoprene as one of the product.

Isolation:**A) Isolation of essential oils from plantparts**

- a) Steam distillationmethod
- b) Solvent extraction
- c) Maceration
- d) Adsorption in purified fats/Enfluerage

B) Separation of terpenoids from essential oils

- a) Chemical methods
- b) Physicalmethods

Classification:

The terpenoids have general formula (C₅H₈)_n, based on the value of 'n' the terpenoids are classified into following [3].

Table 1: Classification of terpenoids

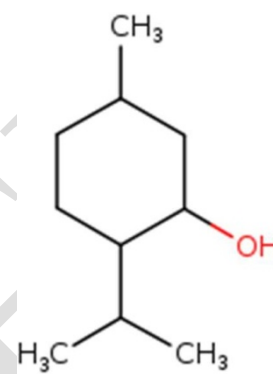
Sr. No.	Name of the class of terpenoids	No. of Isoprene units	Molecular formula	Example
1	Monoterpenes or Terpenes	2	C ₁₀ H ₁₆	Peppermint, Camphor, Eucalyptus, Lemon grass, Turpentine etc.
2	Sesquiterpenes	3	C ₁₅ H ₂₄	Artemisia, Clove, Sandal wood, Acorous etc.
3	Diterpenes	4	C ₂₀ H ₃₂	Taxus, Coleus

4	Triterpenes	6	$C_{30}H_{48}$	Ambergris
5	Tetraterpenes or Carotenoids	8	$C_{40}H_{64}$	Annatto, Crocus, Chlorophyll,
6	Polyterpenes	n	$(C_5H_8)_n$	Rubber

3.1.1 Menthol



Fig. 1: *Menthapiperita*(Mentha)



Menthol

Synonym:

Menthol

Biological source:

Menthol is a monoterpene alcohol obtained from oils of *Mentha piperita* var. *Vulgaris* (Black peppermint) and *Mentha piperita* var. *officinalis* (White peppermint), belongs to family- Labiate. *Mentha* species contains about 1-3% of volatile oils and oil contains not less than 44% [3].

Isolation:

Menthol is isolated by two methods i.e

Method 1: Hydro-distillation

Method 2: Steam distillation

Method 1: Hydro-distillation

Required quantity of coarse powder leaves of *Mentha piperita*. The peppermint oil is extract by hydrodistillation method by using clevenger apparatus. The oil is separated from water and allow to cooling. After cooling crystals of (-) menthol will separate out. The crystals are collected by centrifugation and re- crystallized the menthol by acetone or any other low boiling point solvent [2].

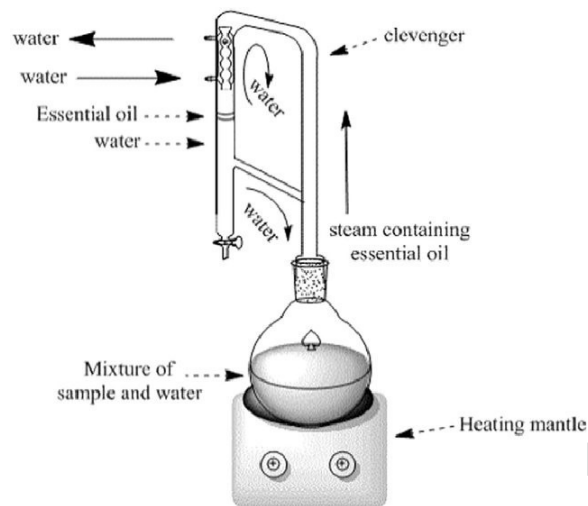


Fig. 2: Hydro-distillation by Clevenger apparatus system

Method 2: Steam distillation

Required quantity of air dried mentha plant is charged into stainless steel still having perforated bottom. The steam under pressure is generated with the help boiler and steam is passed through the drug. The mixture vapour (water and volatile oil) are passed through the condenser where vapour is cooled and back to liquid form. The mentha oil is collected in separating can. Mentha oil is floated on top of the water due to lighter than water. The oil is then decanted and filtered. The oil is allowed to cool; crystal of menthol will separate out [2].

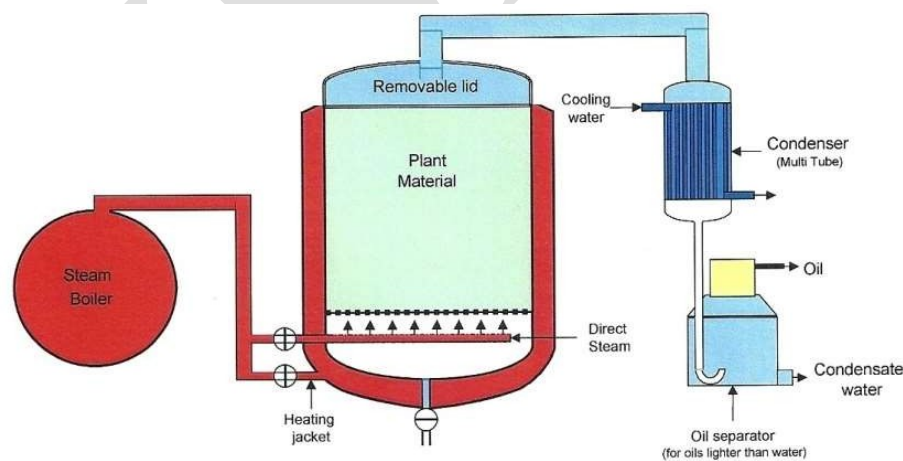


Fig. 3: Steam distillation

Properties:

- Appearance : White crystalline substances, which is solid at room temperature and melts slightly above (m.p. 41 to 43°C).
- Odour : Characteristic and pleasant
- Taste : Pungent followed by cooling sensation
- Solubility : Soluble in 70% alcohol, ether and chloroform, insoluble in water

Identification by chemical test:

Few drops of sample is mixed with 5ml of nitric acid and heated on water bath. Blue colour is developed within 5 minutes, after some time it becomes yellow which indicate the presence of menthol.

Analysis by TLC

Sample preparation	: 1mg of Menthol is dissolved 1ml of methanol
Standard sample	: Menthol
Stationary phase	: Silica gel –G
Mobile phase	: Pure Chloroform
Detecting agent	: 1% vanillin – sulphuric acid reagent and heat the plate at 110 ⁰ C for 10 minutes
RF Value	: 0.48-0.62

Utilization:

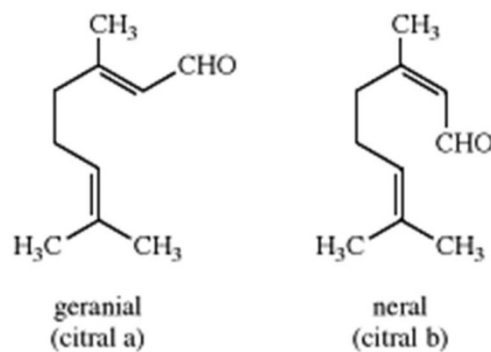
It is used as in various dosage forms for its cooling sensation, flavouring property, carminative, antispasmodic and antipruritic.

Storage condition:

It should be store in well closed and air-tight containers protected from light and in cool place.

3.1.2 Citral

Fig. 4: Cymbopogon flexuosus (Lemongrass)



Citral

Synonym:

Citral

Biological source:

Citral is a monoterpene aldehyde obtained from oils of *Cymbopogon flexuosus*, *C. martini* belongs to family- Graminae. It contains not less than 75% of aldehyde calculated as

Citral. It also present in Lemon oil (*Citrus limonus*) and Orange oil (*Citrus aurantium*), familyRutaceae.

Isolation:

Citral is isolated by following methods i.e.

Method 1: Hydro-distillation

Method 2: Fractional crystallization Method

The fresh plant material is hydro- distilled to obtain lemon grass oil. It is purified by fractional crystallization. To the total oil, first Sodium sulphite is added, the citrals 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. Citral is found in two form i.e Citral-a (Geranial) and Citral-b (Neral) [4, 5].

Properties:

Appearance : Clear pale yellow liquid

Odour : Strong lemon like odour

Taste : Lemon like taste

Solubility : Soluble in 3 parts of 70% alcohol, chloroform and fixed oil.

Insoluble in water.

Boiling point : 224-228^o C

Identification by chemical test:

Alcoholic solution of Sudan red III is added to the sample. Red colour is appeared which indicate presence of citral.

Tincture alkane is added to the sample. Red colour is appeared which indicate presence of citral[6]

Analysis by TLC

Sample preparation : 1mg of Citral is dissolved 1ml of methanol

Standard sample : Citral

Stationary phase : Silica gel –G

Mobile phase : Pure Chloroform

Detecting agent : 2, 4, dinitrophenyl hydrazine reagent

Color spots : Yellow to orange

RF Value : 0.51 [4,5]

Utilization:

It is used as a flavouring agent and perfumery. Commercially citral is act as precursor for the synthesis of β -ionone. β -ionone is used as starting material for the synthesis of Vitamin A [7].

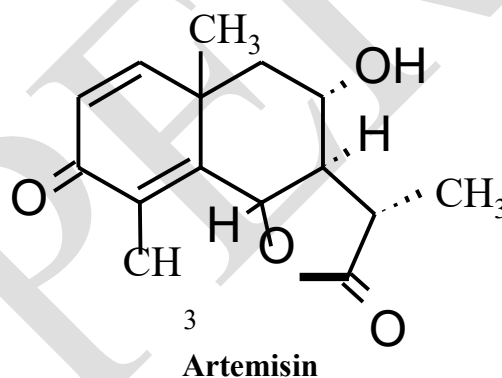
Storage condition:

It should be store in well closed and air-tight containers protected from light and in cool place.

3.1.3 Artemisin



Fig. 5: *Artemisiacina*(Santonica)



Synonym

Artemisin

Biological source:

Artemisin is a sesquiterpenoid lactone, obtained from the unexpanded flower-heads of *Artemisia cina* Berg, *Artemisia brevifolia* Wall, *Artemisia maritime* Linn. and other species belong to family Asteraceae. It contains 2-3% of essential oil and two crystalline substances i. e. Santonin and Artemisin [3].

Isolation:

The air dried powdered of herb is macerated in methanol for 1 hour with magnetic stirrer having speed of 700 rpm. This process is repeated until methanol layer become colorless. The extract is evaporated by rota-evaporator at a temperature of 40°C until volume is reduced to 100ml. The methanolic extract is partitioned using 50ml hexane (Hexane: Methanol, 1:2) until colorless hexane layer is obtained. Hexane and methanol extract is separated by using separating funnel. 10ml of distilled water is added to methanolic extract and partitioned by 50ml of ethyl acetate until ethyl acetate layer become colorless. Again ethyl acetate and methanolic extract is separated. Both extracts are concentrated by using

rotaevaporater at a temperature of 40⁰C. Artemisin is fractionated by column chromatography using silica gel 60 as stationary phase and ethyl acetate: hexane as mobile phase [6].

Identification by chemical test:

Sample is boiled with 10ml of alcohol and filtered. Sodium hydroxide is added to filtrate and heated. Red colour is appeared in liquid [3, 6].

Analysis by TLC

Samplepreparation	: 1mg of Artemisin is dissolved Chloroform
Standardsample	:Artemisin
Stationaryphase	: Silica gel-G
Mobile phase	: Petroleum ether - Ethyl acetate(1:2)
Detectingagent	: p- dimethyl amino benzaldehyde and heat at 80 ⁰ C to produce color
RFValue	: Compare with standard Artemisin [4,5]

Analysis by UV

1mg of Artemisin is mixed with 10ml of methanol and analysed λ 200-400nm [4, 6].

Utilization:

It is used as antimalarial. It acts both against chloroquine sensitive and resistant *P. falciperum* and *P. vivax* malarial parasites [3].

Storage condition:

It should be store in well closed and air-tight containers protected from light and in cool place.

3.2 Glycosides

Glycosides is a organic compound obtain from plants and animal source, which on enzymatic hydrolysis gives one or more sugar moieties along with a non sugar moiety, which are attached by Glycosidal linkage. Sugar moiety is called glycone and non sugar moiety is called aglycone or genin.

Glycosidal linkage: This is formed by condensation of OH group of sugar part and hydrogen from -CH, -OH, -SH, -NH present on aglycone part. Depending upon linkage, the glycoside may be called as O-glycoside, C-glycoside S-glycoside and N-glycoside.

Distribution:

Found in various families like Apocynaceae, Scrophulariaceae, Leguminosae, Liliaceae

Properties:

Glycosides are crystalline, amorphous substances which are soluble in water, and dilute alcohol with exception of resin glycoside, but soluble in organic solvent like chloroform or ether. The aglycones moiety is insoluble in non polar solvent like benzene or ether. Glycosides are easily hydrolyzed by mineral acids, water and enzyme. They show optical activity normally they are levorotatory. Glycoside contains sugar but still the physical, chemical and therapeutic activity is based on aglycone portion.

Isolation of Glycoside

The method by which glycoside are isolated is called Sta-Otto method.

Extraction:

Finely powdered (drug containing glycoside) is extracted by successive extraction in a Soxhlet apparatus with alcohol as solvent (Various enzyme deactivated due to heating). The thermolabile glycoside should be extracted at below 45⁰C. The extract is treated with lead acetate to precipitate tannins (eliminate non glycosidal impurities) and filtered it. To the filtrate H₂S gas is passed, the excess of lead acetate is precipitate as lead sulphide.

The extract is again filtered and concentrated to get crude glycoside.

Isolation:

The filtrate is subjected to fractional crystallization, distillation or chromatography to obtain pure component.

Characterization:

Pure component is determined by the spectroscopy method i.e. UV, IR, NMR, MS, etc. [3].

Classification:

Glycoside may be classified into following points

- A. Based on the chemical nature of non sugar moiety
- B. Based on the glycosidallinkage
- C. Based on the nature of sugar moiety
- D. Based on the therapeutic activity

A. Based on the chemical nature of non sugar moiety

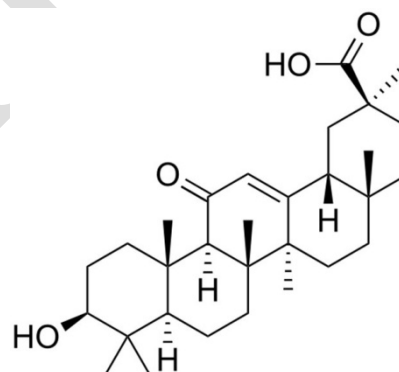
- a. **Anthraquinone glycoside:** Ex: Senna, Aloe, Rhubarb, Cascara, Cochineal, Hypericum.

- b. **Steroidal or Cardiac glycoside:** Ex: Digitalis, Squil (Indian & European), Stropanthus.
- c. **Saponine glycoside:** Ex. Yasti (Glycyrrhizin), Liquorice, Ginseng, Dioscorea, Sarsaparila, Senega, Bramhi, Gokhru
- d. **Cyanogenic glycoside:** Ex. Bitter almond, Wild cherry bark.
- e. **Isothiocyanate glycoside** Ex. Blackmustard.
- f. **Flavonoid glycoside:** Ex Citris fruits, Gingko, Milk-Thistel (Silymarin), Buck wheat (Rutin).
- g. **Coumarin glycoside or furano coumarine glycoside:** Ex Ammi, Psoralea fruit, Visnaga, Cantharides.
- h. **Aldehyde glycoside:** Ex. Anantmul (Sariva), Vanillapods.
- i. **Phenol glycoside:** Ex. Bearberry.
- j. **Steroidal glycoside:** Ex. Solanum.
- k. **Glucosidal bitter or miscellaneous glycoside:** Ex. Gentian, Saffron, Chirata, Quassia, Guduchi, Hena, Gudmar.

3.2.1 Glycyrrhetic acid



Fig 6: *Glycyrrhiza glabra* (Liquorice)



Glycyrrhetic acid

Synonym:

Glycyrrhetic acid

Biological source:

Glycyrrhetic acid is a triterpenoid saponin glycoside obtained from the roots and stolons of *Glycyrrhiza glabra* belongs to family- Leguminosae. The chief constituent of liquorice is Glycyrrhizin (Glycyrrhizin acid) which on hydrolysis yields Glycyrrhetic acid (Glycyrrhetic acid) [3].

Isolation:

The isolation of glycyrrhizin from *Glycyrrhiza* is based on its solubility.

Method 1: Required quantity of coarse powder of Glycyrrhiza roots is extracted with boiling water, filtered and concentrated the extract to obtain a crude liquorice extract. Then this extract is again extracted in water and acidifies with HCl to obtain a pH 3-3.4 to precipitate Glycyrrhetic acid and filtered. The residue is washed with water to yield Glycyrrhetic acid [2].

Properties:

Appearance : White crystalline powder
Odour : Characteristic
Taste : Characteristic
Solubility : In soluble in water\ but freely soluble in alcohol, chloroform, benzene, ether etc.[3].

Identification by chemical test:

Libermann test: 3ml of extract and 3ml of acetic anhydride is heated and cooled. To this a drops of conc. H_2SO_4 is added. Blue colour is observed which indicate the presence of triterpenoid.

Libermann-Burchard test: In 3ml of extract, 2ml of chloroform, 1ml of acetic anhydride and one drop of conc. H_2SO_4 is added. Blue-green to red orange colour is observed which indicate the presence of triterpenoid or steroids [6].

Analysis by TLC

Samplepreparation : 1mg of Glycyrrhetic acid is dissolved in 1ml of methanol:
Chloroform(1:1)
Standardsample : Glycyrrhetic acid
Stationaryphase : Silica gel-G
Mobile phase : Toluene: Ethyl acetate: Glacial acetic acid (12.5:7.5:0.5)
Detectingagent : 1% vanillin-sulphuric acid reagent or Anisaldehyde-sulphuric acid and heated for 10minutes at $110^{\circ}C$
RFValue : 0.41
Coloursport : Purplish spot[5]

Analysis by colorimetric method

In the sample anisaldehyde and sulphuric acid is added, which shows purple colour. The intensity of colour is measured in colorimeter at 556nm [6].

Utilization:

It is used in Rheumatoid Arthritis, Inflammation and Addison's disease [3].

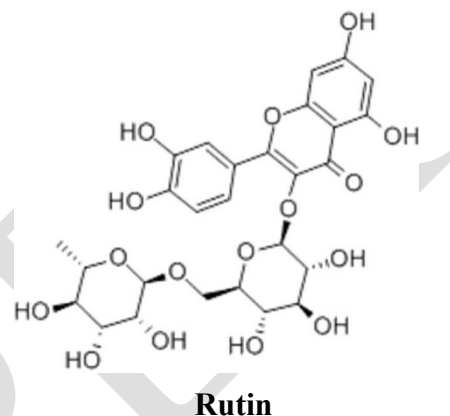
Storage condition:

It should be stored in well closed and air-tight containers protected from light and in cool place.

3.2.2 Rutin



Fig 7: *Fagopyrum esculentum* (Buck wheat)



Synonym:

Rutin

Biological source:

Rutin is a flavonoid glycoside obtained from the powder of dried food grains of *Fagopyrum esculentum* belongs to family- Polygonaceae. It is also obtained from various citrus fruit [3].

Isolation:

Required quantity of *Fagopyrum* powder is defatted with n-hexane and filtered. Then the marc is extracted with 78% of alcohol for 1 hour, filtered it and evaporated to obtain dried residue. Residue is dissolved in sufficient quantity of 30% acetone, filtered and evaporated the solvent to 1/4th of its original volume. Sufficient quantity of 5% aqueous solution of borax is added (until resulting pH is 7.5) with continuous stirring. Sufficient quantity of solid NaCl is added with stirring. Mixture is filtered and acidified with phosphoric acid to bring pH to 5.5 and stirred for 15 minutes. Then the mixture is filtered and residue is washed with 20% NaCl solution. Again mixture is filtered and evaporated the filtrate to 500^oC to 1/4th of its original volume. HCl is added to the mixture in hot condition to bring pH to 1.5, cooled and kept in a refrigerator overnight. Crystal of rutin is separated out [2].

Properties:

Appearance	: Greenish yellow powder
Odour	: Odourless
Taste	: Tasteless
Solubility	: Sparingly soluble in water but its solubility increase in boiling water soluble in methyl alcohol, isopropyl alcohol, pyridine and solution of alkali hydroxides[3].

Identification by chemical test:

Shinoda test: 3ml of ethanol and few drops of sulphuric acid are added to the sample. To this 0.5g of magnesium turnings is added. Pink colour is developed which indicate the presence of flavonoids.

Lead acetate is added to the sample. Yellow coloured precipitate is observed which indicate the presence of flavonoids.

Ferric chloride is added to the sample. Dark green colour is observed which indicate the presence of flavonoids [3, 6].

Analysis by TLC

Samplepreparation	: 1mg of Rutin is dissolved 1ml of methanol
Standardsample	:Rutin
Stationaryphase	: Silica gel-G
Mobile phase	: Ethyl acetate: butanone: formic acid: water (50:30:10:10) Ethyl acetate: butanone: formic acid: water(100:10:11:27)
Detectingagent	: Anisaldehyde sulphuric acidreagent
RFValue:	0.43 (10% aqueous sodium chloridesolution)
Colourspot	: Yellow spot [5,6]

Utilization:

It is used to treat capillary bleeding along with increased capillary fragility and thereby useful in treatment of retinal haemorrhages. It is also used as Antioxidant[3].

Storage condition:

It should be store in well closed and air-tight containers protected from light and in cool place.

3.3 Alkaloids

Alkaloids, which mean alkali-like substances, are basic nitrogenous compounds of plant or animal origin and generally possessing a marked physiological action on man or animals, when used in small quantities. The nitrogen is usually contained in a heterocyclic ring system and it mainly derived from amino acids. Alkaloids are found in animals, fungi & bacteria.

Type:

True Alkaloids: Nitrogen founds in the hetrocyclic ring and originates from amino Acids. E.g. Nicotine, Atropine and Morphine.

Proto – alkaloids: These are also called amino alkaloids; these are the compounds that lack one or more of the properties of typical alkaloids. In protoalkaloids the nitrogen is outside the ring and derived from amino acids. E.g. Hordenine, Ephedrine, Colchicines, Mescaline, Cathinone.

Pseudo-Alkaloids: Do not originate from amino acids. E.g. Caffeine, Theobromine, Conessine, Theophylline, Steroidal Alkaloids etc.

Distribution:

Found in various families like Papaveraceae, Solanaceae, Ranunculaceae, Rubiaceae, Leguminosae etc.

General properties:

Alkaloids are colorless, crystalline, non-volatile, solids; a few such as coniine and nicotine are liquids and volatile in nature. Some volatile are coloured in nature, viz. berberine is yellow, betanidin is red and salt of sanguinarine are copper red in colour. The free bases (i.e. alkaloids themselves) are insoluble in water but soluble in most of the organic non-polar solvents. The salt of most alkaloids are soluble in water. Most of the alkaloids are laevorotatory (optically active), although a few are dextrorotatory (e.g. coniine), while a few are even optically inactive, viz. papaverine. Generally, the alkaloids are bitter in taste and have pronounced physiological activity [3].

Extraction and Isolation:

The extraction of alkaloids is based on their basic character and solubility pattern. The normal procedures followed are to treat the moistened drug with alkali so as to set free the base as it exists in salts form and then separate free base with organic solvent. This is known as Stas Otto process.

General Method

The plant material is powdered and treated with hexane or petroleum ether to separate fixed oils, fats and waxy substance, if any. Then extracted with boiling ethanol (hot extraction in soxhlet apparatus). Solvent is distilled off and residue is treated with inorganic acid, whereupon the bases are extracted as their soluble salts. The free bases are liberated by addition of sodium carbonate and extracted with organic solvents like chloroform. The mixture of bases thus obtained are separated by various methods into individual compounds like chromatography (Column chromatography, Thin layer chromatography, Gas chromatography etc.) for separation of vast number of alkaloids, steam distillation for volatile liquid alkaloids like coniine, sparteine, nicotine etc.

Classification:

Alkaloids may be classified in any of the following ways.

- A. Chemical classification
- B. Pharmacological classification
- C. Taxonomic classification
- D. Biosynthetic classification

A. Chemical classification:

The criteria for chemical classification are the presence of a basic chemical structure. This is probably the most widely accepted and common mode of classification of alkaloids.

Examples are;

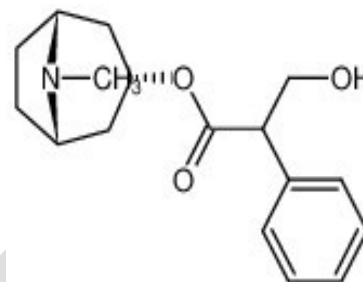
- a. Pyrrole and Pyrrolidine alkaloids e.g Hygrine, cocaine etc.
- b. Pyridine and piperidine alkaloids e.g Arecoline, lobeline, nicotine etc.
- c. Pyrrolizidine alkaloids e.g. echimidine, senecionine etc.
- d. Tropane alkaloids e.g Atropine, hyoscyamine, hyoscyne etc.
- e. Quinolones alkaloids e.g Quinine, quinidine, cinchonine, cinchonidine etc.
- f. Isoquinoline alkaloids e.g d-tubocurarine, emetine, papaverine, narcotine etc.
- g. Indole alkaloids e.g Ergometrine, Ergotamine, reserpine, vincristine, strychnine etc.
- h. Imidazole alkaloids e.g Pilocarpine, Pilocarpine etc.
- i. Purine bases e.g Caffeine, theobromine etc.
- j. Steroidal alkaloids e.g Protoveratrine, solanidine etc.
- k. Diterpene e.g. Aconitine, aconine etc.

- l. Alkalmine e.g. ephedrine, colchicinesetc.
- m. Phenanthrene e.g Morphin, Codineetc.
- n. Aporphine e.g.Boldine
- o. Norlupinane e.g. Cytisine, laburnine etc [1,3].

3.3.1 Atropine



Fig 8: *Atropa belladonna* (Belladonna)



Atropine

Synonym:

Atropine

Biological source:

Atropine is a tropane alkaloid obtained from the fresh or dried leaves and flowering tops of *Atropa belladonna*, *Datura stramonium* (Not less than 0.25%) and *Hyoscyamus niger* (Not less than 0.05%), belongs to family – Solanaceae [3].

Isolation:

Required quantity of coarse powder is taken and moistens with sodium carbonate solution. The blended mixture is extracted in petroleum ether and filters it. To the filtrate aqueous acetic acid is added and further the aqueous fraction is extracted with ether. Both fraction are separated by separating funnel and discard solvent ether fraction. Aqueous (Acidic fraction) is made alkaline with sodium carbonate solution to obtain precipitates of tropane alkaloids. The precipitate is filtered and dry to obtain residue. The residue is dissolved in diethyl ether, filtered it and concentrated the filtrate. Atropine crystals will be separated out. The crystals are filtered and dissolve in alcohol containing sodium hydroxide solution (Hyocyanine is converted to atropine). The atropine sulphate is recrystallized from acetone and crystals of atropine are separated[2].

Properties:

Appearance: Colourless crystal or white crystalline powder with and.

Odour: Odourless

Taste: Bittertaste

Solubility: Easily soluble in water, soluble in ethanol, but insoluble in ether and chloroform

Identification by chemical test:

Vitali–Morin test: Small quantity of the solid atropine is taken and added 2 drops of Conc. nitric acid in an evaporating dish and evaporated to dryness on water bath. Then the residue is dissolved in 1ml of acetone and few drops of freshly prepared alcoholic potassium hydroxide solution is added. Violet coloration takes place due to tropane nucleus [3].

Analysis by TLC

Samplepreparation : 1mg of Atropine is dissolved in 1ml of chloroform

Standardsample :Atropine

Stationary phase : Pre-coated Silica gel

Mobile phase : Toluene: Ethyl acetate: Diethylamine
(70:20:10)

Detectingagent : Dragendorff's reagent

RFValue : 0.70

Colourspot : Yellow orange spot [4,5]

Utilization:

It is used as antispasmodic, mydriatic and antidote in opium poisoning.

Storage condition:

It should be store in well closed and air-tight containers protected from light and in cool place.

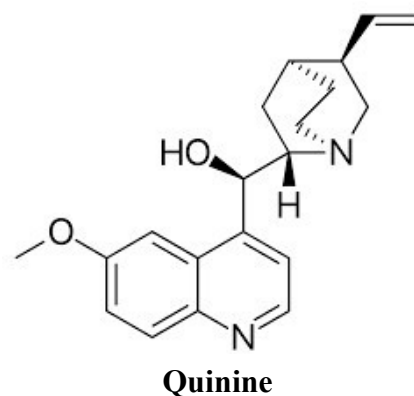
3.3.2 Quinine



Fig 9: *Cinchona calisaya* (Peruvian)

Synonym:

Quinine



Biological source:

Quinine is a quinoline alkaloid obtained from the dried bark of *Cinchona calisaya*, *Cinchona officinalis*, *Cinchona ledgeriana* and *Cinchona succirubra*, belongs to family – Rubiaceae. Quinine and quinidine are stereo-isomers. Quinine is levorotatory and quinidine is dextrorotatory [3].

Isolation:

Required quantity of dry powder bark material is first well mixed with about 30% of its weight of alcoholic calcium hydroxide or calcium oxide or calcium oxide (20%) and sufficient quantity of sodium hydroxide solution (5%) to make a paste. It is allowed to stand for few hours so that alkali can convert cinchona alkaloids to free bases. The mass is then transferred to a Soxhlet apparatus and extraction is carried out with benzene for 6 hours. After completion of extraction the benzene extract is shaken with successive portions of 5% sulphuric acid in separating funnel. The aqueous acid extract is separated from benzene layer and adjusted the pH 6.5 with dilute sodium hydroxide, cooled. Crystals of quinine sulphate are formed, filtered and recrystallized with hot water [2,4].

Properties:

Appearance:	Colourless crystal or white crystalline powder
Odour:	Odourless
Taste:	Intensely bitter taste
Solubility:	Sparingly soluble in water, readily soluble in chloroform, alcohol and ether[5].

Identification by chemical test:

Thalleoquin test: Bromine water and ammonia solution is added in small quantity of powdered the sample. Emerald green colour takes place which indicates the presence of quinine [3].

Analysis by TLC

Sample preparation:	1mg of Quinine is dissolved in 1ml of methanol
Standard sample:	Quinine
Stationary phase:	Silicagel-G
Mobile phase:	Chloroform: Diethyl amine (9:1)
Detecting agent:	Dragendorff's reagent
R _F Value:	0.17 [4, 5]

Analysis by HPLC

Method	: Isocratic
Stationary phase	: C18 column
Mobile phase	: Methanol: Acetonitrile-0.1mol/L: ammonia: acetone (45:15:40)
Detection	: Fluorescence at excitation 325nm.
Emission	: 375nm[5]

Utilization:

Quinine is antimalarial. Quinidine is a cardiac depressant therefore used in cardiac arrhythmias.

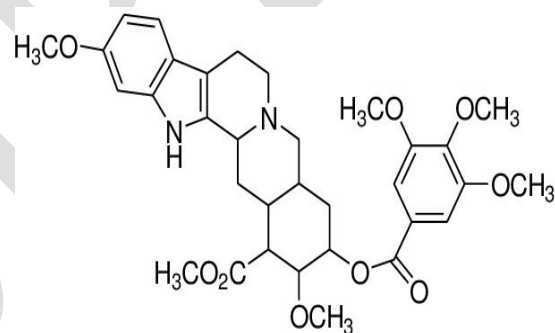
Storage condition:

It should be stored in well closed and air-tight containers protected from light and in cool place.

3.3.3 Reserpine



Fig 10: *Rauwolfia serpentina* (Sarpagandha)



Reserpine

Synonym:

Reserpine

Biological source:

Reserpine is an indole alkaloid obtained from the dried roots of *Atropa belladonna*, *Rauwolfia serpentina*, belongs to family – Apocynaceae. Sarpagandha contains not less than 0.15% of reserpine and ajmalicine [3].

Isolation:

Rauwolfia root powder is exhaustively extracted with 90% alcohol in Soxhlet apparatus. The alcoholic extract is filtered, concentrated and dried under reduced pressure below 60⁰ C to yield dry extract. The dry extract is extracted with ether-chloroform- 90% alcohol (20:8:2.5) and filtered. In filtrate dilute ammonia is added with intermittent shaking. Then water is added to precipitate the crude alkaloids mixture and allowed the drug to settle

after vigorous shaking. The solution is filtered off and extracted the residue with 4 volumes of 0.5N Ammonium sulphate in separating funnel and combined all the extracts. The extract is made alkaline with dilute ammonia to liberate alkaloid. Finally it is extracted with 3 portion of chloroform. Chloroform extract is collected, concentrated and evaporated on water bath to yield total rauwolfia alkaloids. Residue is subjected to column chromatographic fraction for the separation of reserpine [2,5]

Properties:

- Appearance: White or pale buff to slightly yellow crystalline powder, darkening slowly on exposure to light.
- Odour: Odourless
- Taste: Bitter taste
- Solubility: Soluble in alcohol, chloroform and acetone, partially soluble in water, freely soluble in acetic acid [4,5].

Identification by chemical test:

When sample is treated with solution of vanillin in acetic acid, a violet red colour is produced which indicates the presence of reserpine [3].

Analysis by TLC

- Sample preparation : 1mg of Reserpine is dissolved in 1ml of methanol
- Standard sample : Reserpine
- Stationary phase : Silicagel-G
- Mobile phase : Chloroform: Acetone: Diethyl ether (50:40:10)
- Detecting agent : Dragendorff's reagent
- R_F Value : 0.72-0.35
- Colour spot : Orange spot [4,5]

Utilization:

It is used as antihypertensive and antipsychotic agent [3].

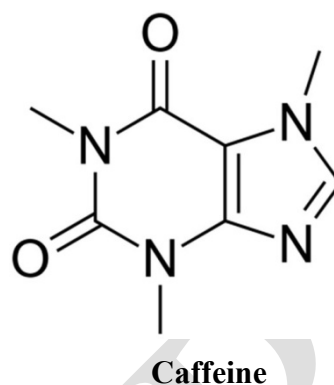
Storage condition:

It should be stored in well closed and air-tight containers protected from light and in cool place.

3.3.4 Caffeine



Fig 11: *Thea sinensis*(Tealeaves)



Synonym:

Caffeine

Biological source:

Caffeine is a purine alkaloid obtained from Tea leaves, Coffee seeds, cocoa, and other species. It is chemically 1, 3, 7, trimethyl xanthine which is isolated from tea and coffee seeds during decaffeination process [3].

It is obtained from the prepared leaves and leaf buds of *Thea sinensis*, belongs to family – Theaceae and dried ripe seeds of *Coffea Arabica*, *C. liberica*, belongs to family Rubiceae. Tea leaves contains 1-4% of caffeine and coffee contains 1- 2% of caffeine.

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. 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 and evaporated on water bath to yield caffeine (white powder). It is recrystallized with alcohol [2, 4].

Properties:

- Appearance : White powder or white glistering needles
- Odour :Odourless
- Taste : Bittertaste
- Solubility : Soluble in hot water [4,5]

Identification by chemical test:

Murexide test: Sample is taken in a petridish to which hydrochloric acid and potassium chlorate are added and heated to dryness. A purple colour is obtained by exposing the residue to vapours of dilute ammonia. The purple colour is lost on addition of fixed alkali [3].

Analysis by TLC

Sample preparation	: 1mg of Caffeine is dissolved in 1ml of methanol and chloroform
Standard sample	: Caffeine
Stationary phase	: Silicagel-G
Mobile phase	: Ethyl acetate: methanol: acetic acid (80:10:10)
Detecting agent	: Expose to vapours of iodine
R _F Value	: 0.41
Colour spot	: Brown spot [4,5]

Analysis by HPLC

Method	: Isocratic
Stationary phase	: C18 column
Mobile phase	: Methanol: Water (25:75)
Detection	: UV-Visible detection 254nm [6].

Utilization:

Caffeine is a CNS stimulant and Diuretic. It is used in beverage.

Storage condition:

It should be stored in well closed and air-tight containers protected from light and in cool place.

3.4 Resins

Resins are amorphous products of complex chemical nature. These are mixtures of essential oils, oxygenated products of terpenes and carboxylic acids found as exudations from the trunk of various trees. These are normally produced either in schizogen or in schizolysigenous ducts or in cavities and are regarded as the end products of metabolism.

Distribution:

Resins and resinous substances are distributed throughout the entire plant kingdom, specifically the *Spermatophyta i.e.*, the seed plants. Resin-containing families are Pinaceae

(Colophory or Rosin); Leguminosae (Tolu Balsam and Balsam of Peru); Dipterocarpaceae (Balsam substitute for copaiba); Burseraceae (Myrrh) and Umbelliferae (Asafoetida).

General properties:

They are found to be translucent or transparent solid, semisolid or liquid substances containing large number of carbon atoms. Most of the resins are heavier than water. That are invariably water insoluble but mostly soluble in alcohol, volatile oil, fixed oils, chloral hydrate and non polar organic solvents like benzene or ether. They are hard, electrically non-conductive and combustible masses. When heated, they first get softened and ultimately melt.

Chemically, they contain organic acids, alcohols, esters, and natural resins.

Extraction and Isolation:

Pharmaceutical resins are obtained from the plants and animals by following methods.

Method 1: A Powdered drug is extracted with alcohol, filtered and concentrated. Then concentrated extract is shaken with excess of water to get precipitated. This method is used for extraction of Jalap, Podophyllum, ipomoea, etc.

Method 2: By distillation for separation of oil, e.g. Colophony, copaiba, etc.

Method 3: By heating the plant part, e.g. Guaiacum etc.

Method 4: As plant exudates by incision, e.g. Myrrh, Balsam, etc.

Classification:

It may be classified into different way i.e.

A. On the basis of occurrence with other secondary metabolites (Resin combinations)

- a. **Oleo-resins:** The homogenous mixtures of volatile oil and resins, e.g. Turmeric, Capsicum, Canada balsam, Copaiba, etc.
- b. **Gum-resins:** The homogenous mixtures of gum and resins, e.g. Cannabis, Colophony, etc.
- c. **Oleo-gum-resins:** The homogenous mixtures of volatile oil, gum and resins, e.g. Guggule, Asafoetida, Myrrh, etc.
- d. **Glyco-resins:** The homogenous mixtures of sugars and resins, e.g. Jalap, Ipomoea, Podophyllum, etc.
- e. **Balsam-resins:** Resin containing benzoic acid and/or cinnamic acid, e.g. Balsam of Tolu, Balsam of Peru, Storax, etc.

B. On the basis of chemical constituents

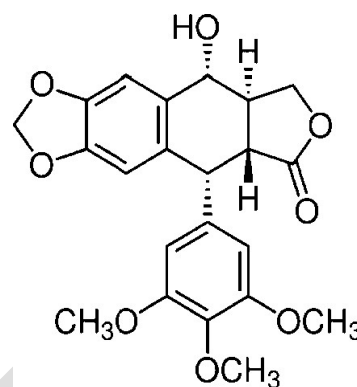
- a. **Acid resins:** Resin along with their acids, e.g. Colophony (Abietic acid)
- b. **Ester resins:** Ester as the chief constituents of the resins, e.g. Benzoin (Coniferyl benzoate) and Storax (Cinnamylcinnamate)

- c. **Resin alcohol:** The contents are the complex alcohols of high molecular weight. They are found in free state or as esters, e.g. Balsum of peru [3, 8,9].

3.4.1 Podophylotoxin



Fig 12: *Podophyllum hexandrum* (Podophyllum)



Podophylotoxin

Synonym:

Podophylotoxin

Biological source:

Podophylotoxin is the lactone resin present in the root and rhizome of *Podophyllum hexandrum* and *P. emodi*, belongs to family – Berberidaceae. Podophyllum resin contains not less than 40% and not more than 50% of podophyllotoxin [3].

Isolation:

Required quantity of rhizomes or roots of *P. emodi* is taken with methanol, filtered it and evaporated to semisolid mass. Semisolid mass is dissolved into acidic water. Precipitate is formed which should be allowed for at least for 2hrs., filtered it and the filtrate is washed with cold water. The residue is collected, washed with acidified water and dry to obtain dark brown amorphous powder. Then the residue is extract with hot alcohol, filtered and evaporated to dryness. Finally residue is re- crystallise in benzene to yield podophyllotoxin [2].

Properties:

- Appearance: White to off-white solid
Odour: Characteristic
Taste: Bitter
Solubility: It is soluble in acetone, benzene; very soluble in ethanol, chloroform; slightly soluble in water; insoluble in ethyl ether [4,5].

Identification by chemical test:

Sample drug is treated with 50% Sulphuric acid. It will show violet-blue colour [3].

Analysis by TLC

Sample preparation : 1mg of Podophyllotoxin is dissolved in 1ml of methanol

Standard sample : Podophyllotoxin

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 Chloroform: Acetone (65:35) upto 12cm.

Detecting agent : Spray with methanol Sulphuric acid and heat 10 minutes at 110^o C

RF Value : 0.65

Colour spot : Yellow spot [4,5]

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[6].

Utilization:

Podophyllotoxin and its derivatives are used as cathartic, purgative, antiviral agent, vesicant, antihelminthic, and antitumor agents (anti-proliferative agent).

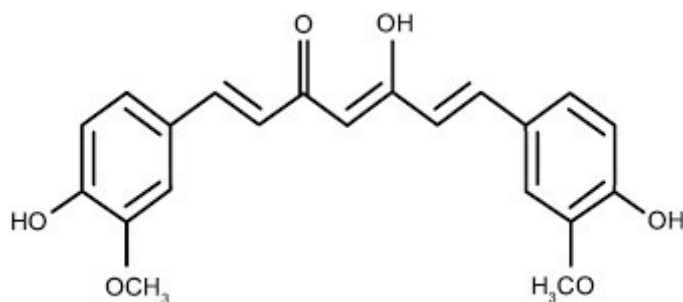
Storage condition:

It should be stored in well closed and air-tight containers protected from light and in cool place.

3.4.2 Curcumin



Fig 13: *Curcuma longa* (Turmeric)



Curcumin

Synonym:

Curcumin or Curcuminoids

Biological source:

Curcumin or Curcuminoids are the diaryl heptoid compounds obtained from the dried rhizomes of Turmeric, *Curcuma longa*, belongs to family – Zingiberaceae. Curcumin is the major colouring principle present upto 5% in the rhizomes. It is a mixture of curcumin, monodesmethoxycurcumin and bisdesmethoxycurcumin [3].

Isolation:

Curcumin can be obtained by different processes.

Method 1: Turmeric powder is extracted with n-hexane for 2hrs., filtered it and discarded n-hexane extract. Then marc is extracted with acetone for 2hrs. The acetone extract is distilled off and dried the crystals of curcumin. It is recrystallized from hot ethanol to yield orange red needles.

Method 2: Turmeric powder is extracted with pure alcohol by Soxhlet extractor until colouring matter is removed. Alcoholic extract is filtered and concentrated under reduced pressure to semisolid residue. This residue is dissolved in sufficient quantity of benzene. Benzene portion is transferred to separating funnel and 0.1% NaOH solution is added, shaken slowly. The alkali layer is separated and repeated it twice. Combined alkali layer and acidify with HCl, yellow colour precipitate is formed. The extract is concentrated with continuous stirring; lumpy mass of resin will be separated out. The extract is filtered and evaporated the filtrate to get crystal of pure curcumin [2].

Properties:

Appearance: Orange yellow crystalline powder

Odour: Characteristic

Taste: Slightly pungentbitter

Solubility: Insoluble in water and ether, but soluble in alcohol [4,5].

Identification by chemical test:

Sample is treated with acetic anhydride and conc. H₂SO₄, it gives violet colour. When this test is observed under UV light, red fluorescence is seen [3].

Analysis by TLC:

Samplepreparation : 1mg of Curcumin is dissolved in 1ml of methanol

Standardsample :Curcumin

Stationaryphase : Silicagel-G

Mobile phase	: Chloroform: Ethanol: Glacial acetic acid (94:5:1)
Detecting agent	: Observed under U.V light at 366nm
RF Value	: 0.79 [4,5]

Analysis by HPLC

Method	: Isocratic
Stationary phase	: C18 column
Mobile phase	: Methanol: 2% Acetic acid and Acetonitrile
Detection	: UV-Visible detection 425nm [6]

Utilization:

It is used as anti-inflammatory, anti-arthritic, antimicrobial and antioxidant. It is also used against peptic ulcer, wound healing.

Storage condition:

It should be stored in well-closed and air-tight containers protected from light and in a cool place.

3.5 References

1. Ansari SH. Essential of Pharmacognosy. 6th ed. Birla Publication Pvt. Ltd. Delhi; 2013.
2. Khadabadi SS, Deore SL, Baviskar BA. Experimental Phytopharmacogy. 2nd ed. Nirali Prakashan, Pune; 2013.
3. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 2nd ed. Nirali Prakashan, Pune; 2016.
4. <https://www.slideshare.net/USrinivasa/isolationidentification-and-analysis-of-phytoconstituents>.
5. Sudha T, Rajeswari R., Ravikkumar VR, Nimbekar TP. Pharmacognosy and Phytochemistry-II. 1st ed. S. Vikash and Company, Jalandhar; 2019.
6. Chakraborty AK, Charde MS, Manekar S, Rathod SM. Pharmacognosy and Phytochemistry-II. 1st ed. S. Vikash and Company, Jalandhar; 2019.
7. Kalia AN. Textbook of Industrial Pharmacognosy, 1st ed. CBS Publishers and Distributors, New Delhi; 2009.
8. Deore SL, Khadabadi S, Baviskar BA. Pharmacognosy and Phytochemistry: A Comprehensive Approach. PharmaMed Press/BSP Books; 2014.
9. Ali M. Pharmacognosy and Phytochemistry. 1st ed. CBS Publishers & Distribution Pvt. Ltd. New Delhi, 1; 2009.