UNIT-3

TERPENOIDS – 1.MENTHOL

- Menthol is a 10 – carbon mono cyclic terpene alcohol with a molecular weight of 156 and molecular formula of $C_{10}H_{20}0$.
- It is naturally produced in plants of Mentha genus and Lamiaceae family.
- One such plant is ***Mentha piperita*** (peppermint plant) which is a hybrid cross between ***Mentha aquatica*** (water mint) and ***Mentha spicata*** (spearmint).
- Menthol imparts the mint odour and taste to these plants and their essential oils.

ISOLATION

- Mentha oil is obtained by hydro distillation or steam distillation of fresh ground plant before flowering.
- For isolating (-) menthol from peppermint plant ,the peppermint oil is subjected to cooling.
- Menthol crystallises out from the oil and is separated by centrifugation.
- **Corn mint oil** is obtained by the steam distillation of the flowering herb,***Mentha arvensis***.
- This oil contains 70 – 80% of free (-)menthol which can be obtained by cooling the oil.

- The menthol crystals obtained are separated by centrifugation.
- Pure (-) menthol is obtained by re – crystallization using low boiling point solvents.
- De mentholised corn mint oil from which (-) menthol is removed by crystallization and which contains 40 – 50 % free menthol can also be re used for producing (-) menthol.

IDENTIFICATION

A few drops of menthol mixed with 5ml nitric acid solution

(prepared by adding 1ml nitric acid to 300ml glacial acetic acid) are heated on a water bath.within 5 minutes,a blue colour develops which deepens on further heating and produces copper colour fluorescence that turns golden yellow after some time.

ANALYSIS

- Accurately weighed 10gm of test sample is taken in an extraction flask.
- 10 ml acetic an hydride and 2gm sodium acetate an hydrous are added to the flask.
- A reflux condenser is attached to the flask and boiled for an hour.
- The contents are then cooled, 30 ml water is added, and the flask is again heated for 15 minutes in boilng water with occasional stirring.
- The contents are transferred to a separating funnel to separate the oil layer which is washed with water until it becomes neutral.

- 2 gm of anhydrous sodium sulphate is added with vigorous shaking and allowed to stand for 30 minutes.
- Then the mixture is filtered through a dried paper filter.
- Accurately weighed 2 gm of the obtained (acetylated) oil and 5 gm of the test sample (un acetylated oil) are taken in separate extraction flasks.
- 5ml of ethanol and 2 drops of phenolphthalein solution are added to each flask.
- 0.5ml/1ml of potassium hydroxide – ethanol solution is added to both the flasks for neutralizing the solutions.

- Then 25ml of 0.5/1 ml of potassium hydroxide ethanol solution is added.
- A Reflux condenser is attached to the flasks and the contents are boiled for an hour.
- After boiling, 25 ml of water is added immediately and stirred.
- The solution is cooled and the excess amount of potassium hydrate is titrated with 0.5 ml/1ml of hydro chloric acid.
- A blank test is performed by the same procedure.

2.CITRAL

- Citral is an acyclic mono terpenoid, and a major constituent
- (60 – 80%) of lemon grass oil.
- It is pale yellow liquid with a strong lemon like odour.
- It can be obtained from lemon grass oil by fractional distillation under reduced pressure.

ISOLATION

- It is isolated from lemon grass oil (contains 90% citral and 10% neral) by steam distillation.
- Steam distillation is carried out in a 5 ml short necked round bottom flask in which 0.5ml of lemon grass oil and 3 ml of water is added.

- Water is injected dropwise using a syringe through the septum in the flask to maintain a constant volume.
- The distillate is collected in an ice cooled 15ml centrifuge tube and then 2.5ml of tertiary butyl methyl ether is added.
- The centrifuge tube is then closed and shaken to dissolve the citral in to the upper ether layer.
- This ether layer is removed using a Pasteur pipette and placed in a clean reaction tube.
- The aqueous layer is extracted twice with 1.5ml of tertiary butyl methyl ether to remove all the citral.
- The ether extracts are combined and placed in a reaction tube.

- The ether layer is treated with 2ml of saturated sodium chloride solution to remove all traces of water and then the aqueous layer is discarded.

- The remaining traces of water are removed by treating the ether layer with anhydrous calcium chloride pellets.

- The mixture is shaken for 5 – 10 minutes in the stoppered reaction tube.

- The dry ether layer is placed in another clean, dry and tarred reaction tube with boiling stone.

- Fresh ether along with a drying agent is added to the tube to remove all traces of citral.
- The combined ether extract is evaporated using water bath.
- The reaction tube is connected to the water aspirator to remove the traces of ether.
- The percentage yield of citral is calculated from the product obtained (a clear fragrant oil).

IDENTIFICATION

- An alcoholic solution of sudan iii is added to the test sample. Red colour obtained.
- A drop of tincture alkane is added to the test sample.Red colour indicates the presence of citral in the volatile oil.

ANALYSIS

- Citral can be analysed in a shimadzu gas liquid chromatograph using a flame ionisation detector(FID).
- The instrument is connected to a computer having the CLASS GC 10 software that is used for data analysis.
- The percentage of each compound is calculated by dividing the peak area by the total area of component peaks.
- The detector and injection temperatures is maintained from 50 – 250 C with a temperature programme rate of 20 C/min starting at 2 minutes and finishing at 10 minutes.
- By comparing the RI(Retention indices) of standard and test compound,citral is confirmed.

3.ARTEMISIN

- Artemisin is a semi synthetic derivative of a drug that possesses the most rapid action against ***plasmodium falciparum*** malaria.
- It is isolated from the plant ***Artemisia annua*** (or sweet wormwood).
- This plant is widely used in Chinese medicine.
- Chemically Artemisin is a sesquiterpene lactone containing an un usual peroxide which is responsible for the drugs mechanism of action.
- The **WHO** has **banned the use of this drug** as a monotherapy,it can be used with combination of other anti malarial drugs.

ISOLATION

- 100 gms of powdered ***Artemisia annua*** is macerated with methanol in an flask with a magnetic stirrer rotating at a speed of 700 rpm for 1 hour. This process is repeated till the methanol layer becomes colourless.
- The extract obtained is evaporated in a rota vapor vacuum at 40 C Temperature till the extract volume reaches 100ml.
- To this extract solution 50ml of hexane is added and extracted several times till it becomes colourless.
- Two layers of methanol extracts and hexane extracts are obtained from this process.

- The methanol extract obtained is added with 10 ml of distilled water and again partitioned with 50ml of ethyl acetate.
- Partitioning is done several times till the ethyl acetate layer becomes colourless.
- By this process ethyl acetate extract (semi polar fraction) and methanolic extract (polar fraction) is obtained.
- Each extract is concentrated using a rotavapor at 40 C temperature.
- The most viscous extract containing artemisin is fractionated by column chromatography,using silica gel 60 as the stationary phase and a mixture of ethyl acetate – hexane as the mobile phase.
- Each fraction is collected and the presence of artemisin is identified.

IDENTIFICATION

1 gm of finely divided sample is boiled with 10 ml of alcohol and filtered. The filtrate is added with sodium hydroxide and again heated. The solution develops red colour indicating the presence of artemisin.

ANALYSIS

IR Spectrophotometer

- 2 mg of isolates are crushed and mixed with 98mg of KBr(Potassium bromide – dried for 24 hours at 105 C temperature).The isolates are analysed at wave number 4000-400cm using KBr as the base line.
- The spectrum of isolates obtained is compared with the spectrum of standard artemisin.

UV Spectrophotometer

1mg of isolates is dissolved in 10ml of methanol and analysed at wavelength of 200-400nm.The spectrum of isolates obtained is compared with the spectrum of standard artemisin.

Thin layer chromatography(TLC)

1 mg of isolates is dissolved in 5ml of ethyl acetate .Isolates are spotted with a solution using the capillary tube on a silica gel 60 F254 (the stationary phase).Ethyl acetate:hexane(3:97) and ethyl acetate:hexane(7:93) are used as the mobile phase.

HNMR Spectroscopy

Isolates are analysed using **CDC13** as the solvent and tetramethylsilane as the reference compound. The spectrum of isolates obtained is compared with the spectrum of STD artemisin.

Liquid chromatography – Mass spectrometry (LC – MS)

1 mg of sample is dissolved in 1ml of methanol and then 20µl is injected and eluted using methanol:water(9:1). With a flow rate of 1ml/min, separation is done through C 18 Column (RP 18). The samples are analysed separately as per their retention times.

GLYCOSIDES – 4. Glycyrrhetinic acid

- Glycyrrhetinic acid is an oleanolic acid obtained from glycyrrhiza,which possesses some anti allergic,anti bacterial and anti viral properties.
- It is used in allergic or infectious skin inflammation as a topical formulation,while it is used orally for its aldosterone effects in electrolyte regulation.
- It plays the role of an immunomodulator.
- Glycyrrhetinic acid is a pentacyclic triterpenoid, a cyclic terpene ketone, and a hydroxy mono carboxylic acid.
- It is a conjugate acid of a glycyrrhetinate.

ISOLATION
From a Glycyrrhizin salt

- Concentrated aqueous extract of liquorice root is treated with sulphuric acid to precipitate a brown sediment,which is washed with water and extracted with alcohol to isolate glycyrrhizin.
- The extract is added with KOH solution to precipitate the potassium salt of glycyrrhizin, which is filtered and crystallised twice in acetic acid.
- The salt is hydrolysed in sulphuric acid to obtain crude glycyrrhitinic acid that is acetylated by acetic an hydride.
- The cake obtained is purified with chloroform and acetic acid and hydrolysed with NaOH to obtain glycyrrhetinic acid.

By Enzymatic Reactions

- In this method glycyrrhitinic acid is produced by the hydrolysis of glycyrrhizin or its salts with their cultured preparations in the presence of nitrogen,phosphate and potassium.
- The yield of this method is 91% and the glycyrrhitinic acid obtained has an acceptable purity.
- This method is highly expensive.

By Hydrolysis of Liquorice Root

Liquorice root powder is hydrolysed in tri chloro acetic acid for 18 hours at 95 C temperature.Neutralized with lime solution and filtered,washed with water and extracted with ethanol.The extract is evapourated to form crystals of glycyrrhitinic acid.

IDENTIFICATION

Sulphuric acid (80%) is added to a thick section of the drug or powder.A deep yellow colour produced instantly indicating the presence of glycyrrhetinic acid.

ANALYSIS

- The extract to be analysed is prepared by continuous hot extraction method and dried using evaporating dish and heating mantle.
- Dilution is obtained to 5µgm/ml and scanned in UV range (200 – 400nm) in 10mm cell against solvent blank.At 254 nm it shows well defined wavelength. It is selected for spectrophotometric evaluation.

Preparation of standard stock solution

- 10 mg of glycyrrhitinic acid is dissolved in phosphate buffer (PH 6.8) ethanol (70:30 proportion) in 10 ml of volumetric flask.
- The final volume is adjusted using the same solvent to get a solution containing 1000µgm/ml concentration of glycyrrhitinic acid.
- Working stock solutions of 5-35µgm/ml conc of glygyrrhitinic acid also prepared and the absorbance of these solutions are measured at 254nm.

Analysis of the Herbal Extract

- Accurately weighed 20mg of herbal hydro alcoholic extract of liquorice is dissolved in phosphate buffer (pH 6.8) ethanol

  (70:30 proportion) in a 10 ml volumetric flask.
- The final volume is adjusted with a same solvent.
- The sample solution is filtered through whatman filter paper no 41.
- From this solution 0.1ml solution is taken and diluted to 10ml with phosphate buffer (Ph 6.8) ethanol (70:30 proportion) to get final concentration containing 20µgm/ml of glycyrrhitinic acid.

5.RUTIN

- Rutin is a bio flavonoid**.** Its pure form is yellow or yellow – green coloured, needle shaped crystal.
- It is a flavonol glycoside containing quercetin and the disaccharide rutinose(rhamnose and glucose).
- Rutin is found in many plants,fruits and vegetables, and the chief source is buckwheat.
- It is also found in citrus fruits,noni,black tea and apple peel.
- Rutin mostly metabolises to quercetin (its aglycone part) during digestion.
- Rutin has strong anti oxidant properties**.**

- It can also chelate metal ions, such as iron, there by reducing the Fenton reaction (production damaging oxygen radicals).
- It can stabilize vitamin C (ascorbic acid) thus on administering together, vitamin C activity will be strengthened.
- It has been proved to be effective in preventing venous oedema (an early sign of chronic venous disease of the leg).
- Rutin has anti inflammatory and healing properties.
- Rutin can inhibit some cancerous and pre – cancerous conditions.
- It can prevent atherogenesis and reduce the cytotoxicity of oxidized LDL – cholesterol.

ISOLATION

- 20 gm of powdered ***Ginkgo biloba*** (leaves and stem bark) is extracted with 250ml of 80% ethanol until exhaustion in a Soxhlet apparatus.
- The obtained extract is filtered and evaporated under vacuum till the volume comes down to 10 ml.
- To this residue,25ml water is added.
- The mixture is extracted with petroleum ether followed with chloroform.
- After extraction, the aqueous layer is collected and left undisturbed in a cold place for 3 days.

- A yellow precipitate separates out of the solution, which is filtered and washed with a combination of chloroform:ethyl acetate:ethanol (50:25:25).
- The un dissolved part of the precipitate is dissolved in hot methanol and filtered.
- The filtrate is evaporated to dryness to obtain 100mg yellow coloured powder (rutin) and its melting point is measured.

IDENTIFICATION

- Aqueous solution of rutin with ferric chloride gives a dark green colour appears.
- Aqueous solution of rutin with lead acetate gives an orange yellow precipitate.
- Aqueous solution of rutin with ammonium molybdate and antimony tri chloride an orange yellow colour precipitate.

ANALYSIS

TLC: The isolated rutin is compared with standard rutin using TLC method.

- An aluminium sheet coated with silica gel G is used as the stationary phase with ethyl acetate:butanone:formic acid:water (50:30:10:10).
- Ethyl acetate:formic acid:acetic acid:water (100:11:11:27) as the mobile phases.

Paper chromatography: Whatman No 1 filter paper is used as a stationary phase and acetic acid:water(15:85) and isopropyl alcohol:water (60:40) are used as mobile phases.

ALKALOIDS - 6. ATROPINE

- Atropine is the alkaloid obtained from plants of Solanaceae family such as ***Atropa belladonna*** (deadly night shade).
- ***Datura stramonium*** (thorn apple) and ***Hyoscyamus niger*** (henbane).
- It has a melting point of 115 – 116 C. It is optically in active.
- It is a strong poison and has a sharp bitter taste.
- It is used in ophthalmology due to its dilating action on the eye pupils.
- It stimulates and then depresses the CNS on internal administration.

ISOLATION

- Belladonna leaves powder is extracted with alcohol(95%) by Soxhlet hot percolation method.
- The obtained ethanolic extract is concentrated under vacuum till complete removal of alcohol.
- Syrupy mass obtained is dissolved in dilute HCL and filter.
- Filtrate containing alkaloids in aqueous solution is extracted with petroleum ether to remove the impurities.
- Then it is made alkaline with ammonia solution and extracted with chloroform three times.

- Chloroform extracts combined. Chloroform is removed under vacuum.
- Residue (Crude alkaloids) obtained is extracted with dilute solution of oxalic acid.
- Alkaloids crystallise out by fractional crystallization for both atropine.

IDENTIFICATION

Vitali - Morin Reaction: In this method, the tropane alkaloid is first treated with fuming nitric acid and then evaporated to dryness, methanolic potassium hydroxide solution is added to an acetone solution of nitrated residue. violet colour develops confirming the presence of tropane alkaloids.

Analysis

GC/MS Analysis: 0.5ml sample,5µl **IS** (Internal standard) solution and 1.0ml borate solution (0.1M,pH9.3) are mixed in a test tube and poured in to an column.

- After 15 minutes the target compounds are eluted with 4ml dichloromethane.The obtained elute is evaporated to dryness under nitrogen stream.
- The residue is mixed with 50µl of N,O – bis (tri methyl silyl) tri fluoroacetamide:trimethyl chlorosilane in 99:1 ratio and warmed at 45 C for 20 minutes.The solution obtained is mixed with 100µl dichloromethane, and a 1µl is injected in to GC/MS for analysis.

HPLC Analysis

- 0.5ml sample is poured in to an activated HLB cartridge,which is washed with 1ml of 5%methanol.
- The target compounds are eluted with 1ml methanol.
- The obtained elute is evaporated to dryness under reduced pressure.
- The residue is dissolved in 100µl of the mobile phase and 10µl of it is injected in to HPLC for analysis.

7.QUININE

- Quinine alkaloid is obtained from the bark of the plants ***Cinchona ledgeriana*** and ***Cinchona officinalis*** of **Rubiaceae** family.
- The barks of these trees contain about 8%quinine.
- Grafted varieties produce more than15% quinine (as the sulphate).
- It crystallises with 3 moles of water and melts at 177C under anhydrous conditions.
- It is laevo rotatory and bitter in taste.
- Its sulphate or dihydrochloride have been used as the most effective anti malarial agent since many years.

ISOLATION

- Cinchona bark powder is treated with 10%sodium carbonate solution and kept over night.
- Then refluxed with benzene for 6 hours.
- Filtered and hot filtrate is extracted with dilute sulphuric acid
- Made slightly alkaline (7.5pH) with sodium carbonate.
- Alkaloidal solution is boiled with activated charcoal and filtered and kept at 20 C for over night again filtered.
- Boil residue with water and make alkaline with sodium carbonate solution.
- Decant the solution.
- Precipitate of quinine settles down.

IDENTIFICATION

1.Thalleoquin Test: A Small amount of quinine is dissolved in dilute sulphuric acid and 1-2 ml of water. Add 2 -3 drops of bromine water and shake well. A drop of strong ammonia is added to the mixture produces emerald green colour.

2.Erythroquinine Test: A quinine solution in dilute acetic acid is added with 1-2 drops of bromine water and a drop of 10% solution of potassium ferric cyanide. A drop of strong ammonia solution is added produces red colour.

3.Quinine gives a strong blue fluorescence in dilute solutions of sulphuric, acetic, phosphoric or tartaric acids.

ANALYSIS

Standard Solution of Quinine

- Accurately weighed 0.1gm of quinine is dissolved in 0.05M sulphuric acid.
- 10 ml of this solution is diluted in 1000ml with 0.05 M sulphuric acid to get 0.00100mg/ml quinine.
- Using a calibrated burette 10.0,17.0,24.0,31.0,38.0,45.0,52.0 and 62.0 ml of the above dilute standard solution is run in to separate 100 ml graduated flasks and each solution is diluted up to the mark with 0.05 M Sulphuric acid.

Procedure

- The fluorescence of each of the above solutions is measured at 445nm using 62ml of dilute quinine solution as a standard for the fluorimeter.
- LF2 or an equivalent primary filter and gelatin are used as the secondary filters in a simple fluorimeter.
- The solutions containing 0.00025 and 0.00045mg/ml quinine are prepared.
- Their concentrations are determined by measuring the fluorescence on the instrument with the calibration curve.

8.RESERPINE

- Reserpine is the active principle of Rauwolfia species.
- Chemical investigation of around 60 different Rauwolfia species led to the isolation of 50 – 70 Rauwolfia alkaloids.
- The important alkaloids are reserpine, yohimbine, ajmalicine and ajmaline.
- The snake like roots of Rauwolfia are used for controlling hypertension, epilepsy, insomnia, fevers, cholera, dizziness and headaches.
- It has a melting point of 265 C.
- It is stable but gradually darkens on exposure to light.

ISOLATION

- Rauwolfia root powder is extracted with 90% alcohol by percolation.
- The alcoholic extract is concentrated and dried under reduced pressure below 60 C temperature to yield rauwolfia dry extract that contains 4% of total alkaloids.
- This dry extract is again extracted with proportions of ether:chloroform:alcohol (20:8:2.5).
- The extract obtained is added with little dilute ammonia with alternating shaking to convert the alkaloid in to water in soluble base.

- Water is added to the above mixture and the drug is allowed to settle after a few vigorous shakings.
- The solution is filtered and the residue is extracted with 4 volumes of 0.5 M NH_2SO_4 in a separating funnel.
- The total acid extract containing the alkaloidal salt is combined.
- The extract is filtered, made alkaline with dilute ammonia to liberate the alkaloid.
- The resulting alkaline solution is extracted with chloroform.
- The total chloroform extract is filtered and chloroform is removed by distillation.

- The total alkaloidal extract is dried under vacuum to yield total rauwolfia alkaloids (consists of the mixture of over 30 different components)
- This is subjected to column chromatographic fractionation for separating reserpine.

IDENTIFICATION

1.On treatment with solution of vanillin in acetic acid reserpine shows violet red colour.

2.Reserpine is colorimetrically determined by reaction between acidic solution of alkaloids and sodium nitrite.

Analysis

HPLC Analysis : water as solvent A and acetonitrile as solvent B are used as the mobile phase.

- Both the solvents are filtered through a millipore PTFE 0.45µm membrane.
- Separations are carried out using a linear gradient 0min 50% B,3 min 50%B, 6 min 55%B,7 min 100%B.
- Flow rate of mobile phase is 1.0 ml /min and the injection volume is 20µl.
- Chromatographic runs are carried out at 25 C UV detection is performed at 268nm.

9.CAFFEINE

- Caffeine is a naturally occurring xanthine alkaloid.
- It is the component of tea leaves(5%),coffee(1-2%) and kolanuts(1-2%).
- It is responsible for the stimulating action these beverages produce on the nerves and heart, and thus is employed in medicine.
- It has a melting point of 235C.It crystallises as silky needles.
- It has a bitter taste.it is sparingly soluble in water and alcohol.
- It is a weak base.it forms salts with strong acids,which gets easily decomposed by water.

- Its citrate and hydrochloride salts are used as diuretics and heart and nerve stimulants. its excessive use disturbs the digestion.

ISOLATION

Caffeine can be isolated by the following methods;

Method – 1

- Damaged tea leaves are powdered and boiled with water,and filtered hot.
- To the filtrate, lead acetate is added to precipitate out the tannins and albuminoids.
- sulphuric acid is used for removing the excess of lead (as lead sulphate) from the filtrate.

- The filtrate is decolourised with animal charcoal and caffeine is extracted with chloroform.
- The solvent is recovered by distillation and caffeine is purified by re – crystallisation with water.

Method – 2

- Finely powdered tea leaves are extracted with ethanol in Soxhlet extractor.
- The extract of caffeine obtained is absorbed on magnesium oxide.
- Caffeine is then treated with 10% H_2SO_4.
- It is then extracted with chloroform and re crystallised.

Method – 3

- Caffeine is extracted with coffee beans by leaching with water.
- Yield of about 90% can be obtained by extracting the coarse coffee powder with water 75 C.
- Extraction with water/coffee (9:1)takes about 90 minutes.

Method – 4

- Decaffeination of coffee is done with supercritical fluid extraction.
- This process was first developed by **k.zosel** who used liquefied carbon di oxide.
- The supercritical medium in a pressure vessel is circulated through moist coffee where it becomes charged with caffeine.

- It is then passed through second pressurised vessel containing an adsorbing medium (such as activated carbon,resin or water).
- This medium adsorbs the caffeine,which is then separated by extraction with chloroform.

IDENTIFICATION

Murexide test:

- caffeine is taken in a petri dish and added with HCl and potassium chlorate.
- The mixture is then heated to dryness.
- The residue is exposed to vapours of dilute ammonia.
- A purple colour appears, which disappears on adding fixed alkali.

- On treatment with tannic acid solution, caffeine produces a white coloured precipitate.

ANALYSIS

Caffeine can be analysed by,

- HPLC.
- UV/Visible spectrophotometry
- FTIR spectrophotometry methods.

10.PODOPHYLLOTOXIN

- Podophyllotoxin is a natural product isolated from ***Podophyllum peltatum*** and ***Podophyllum emodi.***
- It possesses many medicinal properties.
- **Etoposide** is a podophyllotoxin derivative and is used in the treatment of cancers mainly small cell lung carcinoma and testicular cancer.
- It exists as solvated crystals.It has a melting point of 183 C.
- It is insoluble in ethyl ether and ligroin, slightly soluble in water soluble in acetone and benzene and highly soluble in ethanol and chloroform.

ISOLATION

- The powdered rhizome or roots of ***P.emodi*** are extracted with methanol.
- The extract obtained is reduced under vacuum.
- The semi solid mass obtained is put in to acidulated water

  (10ml HCl in 100ml water).
- The precipitate formed is allowed to settle and filtered.
- The filtrate is decanted and washed with cold water
- The resin obtained is dried which gives a dark brown amorphous powder of podophyllin.

- This powder is extracted with chloroform and purified by repeated re - crystallisation from benzene (either alone or a mixture of alcohol and benzene).
- The resultant is washed with petroleum ether or hexane to yield podophyllotoxin.

Method – 2

- The chloroform soluble fraction is dissolved in alcohol.
- The resultant is refluxed with neutral aluminium oxide.
- This makes the solution light yellow in colour.
- To this alcoholic solution, benzene is added to obtain podophyllotoxin with a yield of 95 – 98%.

Method – 3

- The roots or rhizomes of ***P.emodi*** are extracted over a bed of neutral alumina with solvents like benzene, tolune,xylene etc for 1.5 – 4 hours.
- The resultant is re crystallised from organic solvents such as hot benzene, tolune and xylene to yield pure podophyllotoxin (95 – 97%).
- Podophyllotoxin is a tetrahydro naphthalene derivative having hydroxy and lactone groups.
- The attachment at ***cis*** – position is responsible for its purgative property and the attachment at ***trans*** - position responsible for its anti cancer property.

IDENTIFICATION

1. 0.5gm of the drug is macerated with 10ml of alcohol and filtered. The filtrate is added with 0.5ml of strong copper acetate solution. Formation of a brown precipitate confirms the presence of podophyllotoxin.

2. 0.5gm of powdered resin is mixed with 5ml 60% alcohol and 5ml 1N potassium hydroxide solution.

If on shaking the mixture, a stiff gelly is produced, the presence of Indian podophyllum resin is confirmed, while if no precipitate is formed, the presence of American podophyllum resin is confirmed.

ANALYSIS

HPLC Analysis

- Podophyllotoxin and its glycosides are identified by HPLC in which the retention time and UV spectrum of the test compound is compared with that of the reference compound.
- A standardized mixture of 2 marker compounds with known concentration of podophyllotoxin and podophyllotoxin – D glycoside is used to create calibration curves (percentage area with respect to the quantity of pure compounds).
- Both the marker compounds exhibit sufficient differences in their retention times, thus can be quantified easily.

11.CURCUMIN

- Curcumin is a diarylheptanoid obtained from the dried and fresh rhizomes of ***Curcuma longa***.
- It is the principal curcuminoid of turmeric.
- Curcumin exhibits potent anti inflammatory properties which is also protective against some form of cancer progression.
- Thus is under research for prevention and treatment of cancer.
- Curcumin has anti oxidant property it plays an important role in keeping curry for a long time without making it rancid.

ISOLATION

- 25gm of powdered dried turmeric rhizome is taken in a large beaker and mixed with 1000ml of distilled n-hexane using a magnetic stirring rod.
- The resultant suspension is stirred for 3 days then filtered and placed in a porous bag or ‘thimble’ of strong filter paper placed in the chamber of Soxhlet apparatus.
- 310ml of methanol is heated in a flask and its vapours are condensed in a condenser.
- The condensed extractor drips in to the thimble containing powdered turmeric rhizome and extracts it.

- When the liquid level in chamber rises to the top of siphon tube the liquid contents flood in to the flask.
- After 3 days the solvent is evaporated in a rotary apparatus and then dissolved in 100ml of toluene.
- The solution obtained is poured in to a separating funnel added with 100ml of 0.2 M NaOH and shaken for a few minutes.
- The aqueous phase is collected and acidified to Ph 3 using 0.2 M HCl.
- The brown extract turns yellow as it undergoes clarification.
- The filtrate is extracted with diethyl ether.In the final extraction the ether turns pale yellow coloured indicating the end of extraction.

- The combined ethereal phases are washed with 30ml water and dried over MgSO4.
- Ether is removed completely under vacuum to leave a yellow coloured solid curcuminoid which is purified by TLC.

IDENTIFICATION

1. Powdered drug with sulphuric acid gives a crimson colour.

2. Aqueous solution of turmeric with boric acid a reddish brown colour appears which turns greenish blue on adding alkali.

3. On treating the powdered drug with acetic anhydride and concentrated sulphuric acid a violet colour appears.

A red fluorescence appears when this test is observed under UV light.

ANALYSIS

Curcumin can be analysed by

- TLC Analysis
- UV/Visible spectroscopy
- HPLC Analysis