PHARMACOGNOSY-II

UNIT 3 NOTES

PHYTOCONSTITUENTS

- Terpenoids
- Glycosides
- Alkaloids
- Resins

NOTES AVAILABLE AT :

WWW.IMPERFECTPHARMACY.IN

General Introduction

Phytoconstituents

- Phytoconstituents are natural chemical compounds found in plants.
- These bioactive substances contribute to plant's therapeutic and pharmacological properties and are widely used in medicine, cosmetics & other industries.
- Examples: Alkaloid, Glycosides, Flavanoids etc.

Isolation

- Isolation of phytoconstituents involves the extraction and purification of bioactive compounds from plant materials such as leaves, roots, stems, seeds or flowers.
- The process is essential in pharmacognosy for studying the properties of these compounds & their potential uses in medicine, cosmetics and other industries.

Identification

- Identification of phytoconstituents involves determining the presence & characteristics of bioactive compounds in plant materials. using various physical, chemical and analytical techniques.

Analysis

- Analysis of phytoconstituents involves qualitative & quantitative examination of bioactive compounds in plants to understand their composition using various analytical techniques i.e chromatography.

TERPENOIDS

- Terpenoids, also known as isoprenoids, are large & diverse class of natural compounds derived from five carbon isoprene units (C_5H_8).
- They are largest group of phytoconstituents found in nature & are responsible for characteristic aromas, flavours and colors of many plants.
- Terpenoids are synthesized through Acetate - Mevalonate Pathway.

Classification Of Terpenoids

Terpenoids can be classified based on the number of isoprene units.

- Monoterpenoids (C_{10}) : Two isoprene units
- Sesquiterpenoids (C_{15}) : 3 " "
- Diterpenoids (C_{20}) : 4 " "
- Triterpenoids (C_{30}) : 6 " "
- Tetraterpenoids (C_{40}) : 8 " "
- Polyterpenoids ($C_{>40}$) : More than 8 isoprene units.

Function In Plants

- Help in defense mechanism
- Responsible for scents and flavours.
- Precursor to hormones like gibberellins
- Some are also part of plant cell membrane.

Pharmacological Properties

Terpenoids exhibit a wide range of biological activities, making them crucial in medicine.

- Antimicrobial : Artemisinin, thymol
- Anti-Inflammatory : boswellic acid
- Anticancer : taxol
- Antioxidant : carotenoids
- Hypolipidemic : squalene

TERPENOIDS IN SYLLABUS

- Menthol
- Citral
- Artemisinin

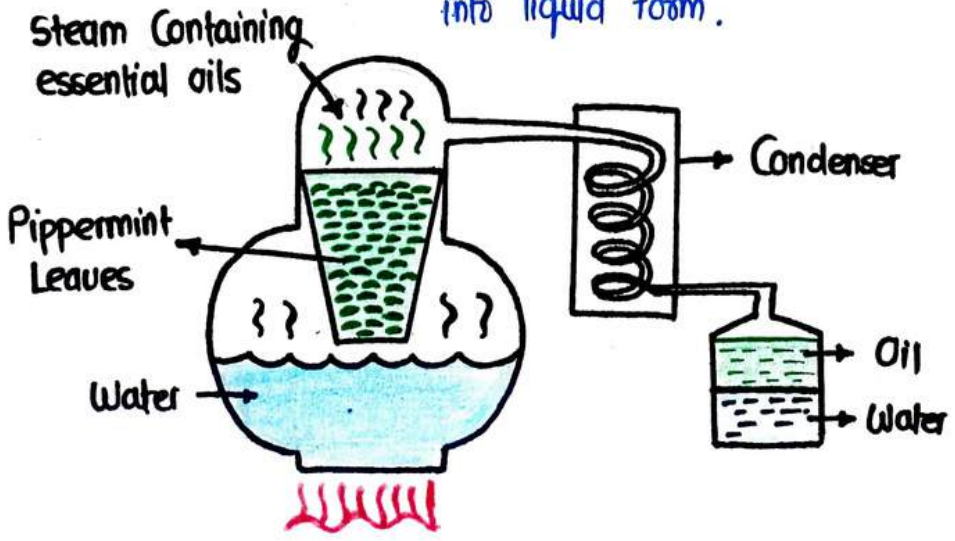
① MENTHOL

- Menthol is a naturally occuring compound found in the oil of certain mint species such as pippermint, spearmint etc.
- Biological Source: It is obtained from oils of Mentha Pipenta and Mentha spicata & belongs to Labiatae family

ISOLATION OF MENTHOL

- Menthol is typically isolated from essential oils obtained from mint species.
- It is isolated or extracted via 2 methods : Hydrodistillation or steam distillation.

Steam Distillation :
- Fresh or dried pippermint leaves are subjected to steam distillation to extract essential oils.
- This process involves passing steam through the plant material, which vaporizes the essential oils, including menthol.
- The vaporized oils are then condensed back into liquid form.



Crystallization :

- The condensed liquid, containing menthol, is cooled to low temperatures.
- As the temperature decreases, menthol crystalizes out of the solution.
- The crystals are separated from remaining liquid by techniques like filtration or centrifugation.

Purification (optional) : Further purification can be done using recrystallization in solvents like ethanol or acetone.

IDENTIFICATION OF MENTHOL

- Few drops of sample is mixed with 5 ml of nitric acid and heated on water bath.
- Blue colour is developed within 5 minutes
- After sometime it becomes yellow which indicates the presence of menthol.

ANALUSIS OF MENTHOL

- Menthol is mainly analysed using Thin Layer Chromatography.
- It involves following steps :

① Materials & Reagents

- TLC Plate: Silica gel coated plate
- Sample : Menthol
- Standard : Pure ~~Chloroform~~ Menthol
- Detecting Agent : ~~Pure Menthol~~ 1 % vanillin - sulphuric acid reagent
- Stationary Phase : Silica gel - G
- Mobile Phase : Pure Chloroform

② PROCEDURE

- Dissolve the menthol sample in a suitable solvent such as Methanol to prepare a dilute solution.
- Apply small, concentrated spots of sample & standard solution 1-2 cm above from ~~TLC plate~~ bottom of TLC plate.
- Place the TLC plate in a chamber containing solvent
- Allow the solvent to travel up the plate until it reaches 3/4th height of plate.
- Dry the plate.
- Expose it to detecting agent so that menthol spots will appear.

③ ANALUSIS

- Measure the Rf Value (Retention factor) of the spots :

$$Rf = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent}}$$

- Compare the Rf Value of sample & standard.
- Pure menthol typically have Rf Valu of 0.48- 0.62

② CITRAL

- Citral is a naturally occuring terpene compound mainly found in the oils of various plants, such as lemongrass & citrous fruits like lemon and oranges
- Biological Source : It is obtained from oils of Cymbopogan flexuosus, C. martini belongs to Graminae family.

ISOLATION OF CITRAL

- Citral is mainly isolated from lemon grass oil through fractional distillation.
- Lemongrass oil, which contains citral as a major component is subjected to fractional distillation.
- This technique separate the components based on their boiling points
- Citral, with a higher boiling point than many other components is collected in a separate fraction.

IDENTIFICATION OF CITRAL

The presence of citral can be detected by following two tests.

① An alcoholic solution of Sudan III is added to the test sample. An appearance of red colour indicates presence of citral.

② A drop of tincture alkane is added to test sample, red colour indicates presence of citral.

ANALYSIS OF CITRAL

Citral can be analysed using Thin Layer Chromatography

- Sample Preparation : 1 mg of citral is dissolved in 1 ml of methanol
- Standard Sample : Citral
- Stationary Phase : Silica gel - G
- Mobile Phase : Pure Chloroform
- Detecting Agent : 2,4 dinitrophenyl hydrazine reagent
- Colour spots : Yellow to orange
- Rf Value : 0.51

③ ARTEMISININ

Artemisinin is a potent antimalarial drug derived from the sweet wormwood plant.

Biological Source: It is derived from the herb Artemisia annua (sweet wormwood) & belongs to family Asteraceae.

ISOLATION OF ARTEMISININ

- Isolation of Artemisinin involves following steps:
- The leaves of artemisia annua are harvested and dried immediately at the peak of artemisinin content, often during flowering stage.
- A suitable solvent such as ethanol, methanol or hexane is used to extract artemisinin from dried leaves.
- Extraction is performed using Soxhlet Extraction or Supercritical fluid Extraction.
- After the crude extract is obtained, purification methods such as column chromatography or recrystallization are employed to isolate pure artemisinin.

IDENTIFICATION OF ARTEMISININ

- Artemisinin is mainly identified by spectroscopic techniques, however some chemical test like Peroxide Test is used for its identification.
- Add a small amount of sample to a test tube containing potassium-iodide solution. formation of yellow-brown colour due to release of iodine detect the presence of peroxide groups, which are characteristics of artemisinin.

ANALYSIS

Artemisinin are mainly analysed through Thin Layer Chromatography

- Sample Preparation : 1 mg. of Artemisinin dissolved in Methanol
- Standard Sample : Pure Artemisinin
- Stationary Phase : Silica - gel G
- Mobile Phase : Hexane : Ethyl Acetate (70:30)
- Detecting Agent : p- Anisaldehyde spray agent
- Rf Value : 0.3 - 0.4

GLYCOSIDES

- Glycosides are organic compounds in which a sugar molecule (glycone) is chemically bonded to a non-sugar component (aglycone) through a glycosidic bond.
- The glycone can consist of a single sugar group (monosaccharide) or several sugar groups (oligosaccharides).

GLYCOSIDES IN OUR SYLLABUS

- Glycyrrhetinic Acid
- Rutin

① GLYCYRRHETINIC ACID

Glycyrrhetinic Acid is a bioactive compound derived from hydrolysis of Glycyrrhizin, which possesses some anti-allergic, antibacterial and antiviral properties.

Biological Source: It is obtained from the roots of ~~Gly~~ Glycyrrhiza glabra belonging to Leguminosae family.

ISOLATION OF GLYCYRRHETINIC ACID

- The liquorice root (Glycyrrhiza Glabra) is dried, ground and extracted using a suitable solvent such as water, ethanol or methanol to obtain a coude extract rich in glycyrrhizin.
- The crude extract is then hydrolysed to give glycyrrhetinic acid.

IDENTIFICATION OF GLYCYRRHETINIC ACID

Glycyrrhetinic acid can be identified using following tests:

① It gives reddish-brown colour with sulfuric acid

② Libermann Test: Dissolve the sample in chloroform and add a few drops of acetic anhydride and concentrated sulfuric acid. A greenish-blue colour development indicates the presence of glycyrrhetinic acid

Analysis Of Glycyrrhetinic Acid

Glycyrrhetinic Acid can be analysed using Thin Layer Chromatography.

- Sample Preparation : 1 ml of glycyrrhetinic acid is dissolved in 1 ml of methol : Chloroform (1:1)
- Standard Sample : Glycyrrhetinic Acid
- Stationary Phase : Silica gel- G
- Mobile Phase : Toluene : Ethyl acetate : Glacial acetic acid (12.5 : 7.5 : 0.5).
- Detecting Agent : 1% vanillin - sulphuric acid reagent.
- Rf Value : 0.41

② RUTIN

Rutin is a flavanoid glycoside, a plant-derived compound that is commonly found in variety of fruits, vegetables and plants.

Biological Source: It is obtained from dried food grains of Fagopyrum esculentum belongs to Polygonaceae family.

ISOLATION OF RUTIN

- Dry & grind aerial parts or seeds of Fagopyrum esculentum into a fine powder
- Extract the powdered material using 70% ethanol or methanol in a soxhlet apparatus.
- Dissolve the crude extract in water and perform liquid-liquid extraction with ethyl acetate to separate impurities.
- Use column chromatography to isolate rutin.
- Concentrate rutin-rich fractions and recrystallize using ethanol or acetone-water mixtures to obtain pure rutin.

IDENTIFICATION OF RUTIN

Rutin can be identified by following tests:

① On treating an aqueous solution of rutin with ferric chloride solution, a dark green colour appears.

② On treating an aqueous solution of rutin with lead acetate, an orange yellow precipitate is formed.

ANALYSIS OF RUTIN

Rutin can be analysed using Thin Layer Chromatography.

- Sample Preparation : Dissolve 1 mg of Rutin in 1 ml of methanol
- Stationary Phase : Silica gel - G
- Mobile Phase : 10% aqueous sodium chloride solution
- Standard : Rutin
- Rf Value : 0.43

ALKALOIDS

- Alkaloids are large group of naturally occuring organic compounds that mostly contain basic nitrogen atoms.
- The term alkaloid was coined by W. Meissner in 1819.
- The found primarily in plants and known for their potent physiological effects in humans and ~~plants~~ animals.
- They include well known substances like caffiene, nicotine, morphine quinine & codiene.
- They often have bitter taste and are used in medicinal, agricultural and various other industries due to their pharmacological properties such as pain relief, stimulation and antibacterial effect.

ALKALOIDS IN OUR SYLLABUS

- Atropine
- Quinine
- Reserpine
- Caffiene

① ATROPINE

Atropine is a medication that is primarily used as an anticholinergic agent. It works by blocking the action of acetylcholine, a neurotransmitter, at muscarinic receptors in the body

Biological Source : Atropine is a tropane alkaloid obtained from fresh or dried leaves and flowering tops of Atropa belladona belongs to family solanaceae.

ISOLATION OF ATROPINE

- Harvest the leaves or roots of plant Atropa Belladona, as they contain the highest concentration of atropine.
- Now extract the dry powder with dilute acid (like HCl) to form water soluble atropine salts.
- Add an alkaline solution (eg- ammonia) to convert atropine to its free base.
- Extract the free base using an organic solvent like ether or chloroform.
- Evaporate the solvent and recrystallize atropine for purity.

IDENTIFICATION OF ATROPINE

- Atropine is identified through Vitalin - morin test
- Take small quantity of the solid atropine and add 2 drops of concentrated nitric acid in an evaporating dish.
- Evaporated to dryness on water bath.
- Now dissolve the residue in 1 ml of acetone.
- Add few drops of freshly prepared alcoholic potassium hydroxide solution. Violet colour development confirms presence of atropine.

ANALUSIS OF ATROPINE

Atropine can be analysed using Thin Layer Chromatography

- Sample Preparation : 1 mg of Atropine is dissolved in 1 ml of chloroform.
- Standard Sample : Atropine
- Stationary Phase : Pre-coated silica gel
- Mobile Phase : Toluene : Ethyl Acetate : Diethyl amine (70:20:10)
- Detecting Agent : Dragendroff's Reagent
- Rf Value : 0.70
- Colour Spot : Yellow orange spot

② QUININE

Quinine is a well known antimalarial agent, also used for its analgesic and anti-inflammatory properties.

Biological Source: Quinine is a natural product derived from bark of Cinchona tree (Cinchona calisaya, Cinchona officinalis etc) & belongs to family Rubiaceae.

ISOLATION OF QUININE

- Harvest the dried bark of Cinchona trees and grind it into a fine powder
- Extract quinine from the powdered bark using solvents like ethanol or hot water
- Filter out the solid plant material to obtain the liquid extract
- Evaporate the solvent under reduced pressure to concentrate the alkaloid extract.
- Add an acid to convert quinine into its salt form, which is more soluble in water.
- Purify the quinine through recrystallization or chromatography to obtain the pure compound.

IDENTIFICATION OF QUININE

- A small amount of quinine is dissolved in dilute sulphuric acid.
- To the resulting mixture, 2-3 drops of bromine water is added and shaken.
- A drop of strong ammonia is added.
  Production of emerald green colour indicates the presence of quinine.

ANALUSIS OF QUININE

Quinine is analysed by Thin Layer Chromatography :

- Sample Preparation : 1 mg of Quinine is dissolved in 1 ml of methanol
- Standard Sample: Pure Quinine
- Stationary Phase : Silica gel - G1
- Mobile Phase : Chloroform : Diethyl amine (9:1)
- Detecting Agent : Dragondroff's Reagent
- RF Value : 0.17

③ RESERPINE

Reserpine is a naturally occuring Alkaloid and widely used as antihypertensive and antipsychotic drug.

Biological Source : Reserpine is obtained from roots of Rauwolfia Serpentina & belongs to family Apocynaceae.

ISOLATION OF RESERPINE

- Harvest the dried roots of Rauwolfia serpentina and grind them into a fine powder
- Extract the alkaloids using a solvent like Ethanol or Methanol.
- Filter the extract to remove solid plant material.
- Evaporate the solvent to concentrate the extract.
- Add an acid to convert reserpine into its salt form, enhancing solubility
- Now purify it using methods like column chromatography or recrystallization.

IDENTIFICATION OF RESERPINE

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

ANALYSIS OF RESERPINE

Reserpine can be analysed through Thin Layer Chromatography.

- Sample Preparation : 1 mg of Reserpine is dissolved in 1 ml of methanol
- Standard Sample : Reserpine
- Stationary Phase : Silica gel-G
- Mobile Phase : Chloroform : Acetone : Diethyl ether (50 : 40 : 100)
- Detecting Agent : Dragendroff's Reagent
- RF Value : 0·72 - 0·35
- Colour Spot : Orange spot

④ CAFFINE

- Caffiene is a natural stimulant found in coffee, tea, chocolate energy drinks and some medications.
- It works by stimulating nervous system, helping to reduce fatigue, increase alertness and improve focus.

Biological Source: It is primarily obtained from leaves and leaf buds of Thea (camellia) sinesis belonging to Theaceae family and from dried ripe seeds of Coffea arabica belong to Rubiaceae family.

ISOLATION OF CAFFEINE

- Harvest and dry the plant material (e.g. coffee beans or tea leaves).
- Use a solvent like hot water, ethanol or chloroform to extract caffiene from plant material through maceration or soxhlet extraction.
- Filter the solution to remove solid plant waste.
- Evaporate the solvent to concentrate the caffiene extract
- Purify the caffiene using techniques like recrystallization or column chromatography.

IDENTIFICATION OF CAFFIENE

- Sample is taken in a petridish to which hydrochloric acid and potassium chlorate are added and heated to dryness
- The residue is exposed to vapours of dilute ammonia.
- A purple colour appears, which disappears on adding fixed alkali

ANALUSIS OF CAFFIENE

Caffiene can be analysed using Thin Layer Chromatography

- Sample Preparation : 1 mg of caffiene is dissolved in 1 ml of methanol
- Standard Sample : Pure Caffiene
- Stationary Phase : Silica - gel G
- Mobile Phase : Ethyl acetate : methanol : acetic acid (80 : 10 : 10)
- Detecting Agent : Expose to vapours of iodine
- RF Valu : 0.41
- Colour Spot : Brown

RESINS

- Resins are complex amorphous, solid or semi-solid plant-derived substances that are insoluble in water but soluble in organic solvents like alcohol, ether or chloroform.
- They are formed as secondary metabolites in plants, often as a result of plant defense mechanisms and are usually exuded from bark, leaves and other ~~plants~~ parts of plant.

Classification

Resins can be classified as follows:

- Oleoresins: Mixture of resins and essential oils
- Gum Resins: Combination of resins and gums
- Oleo-Gum Resins: Contain resin, gum and essential oils.
- Balsams: Resins containing benzoic or cinnamic acid

Resins In Our Syllabus

- Podophyllotoxin
- Curcumin

① PODOPHYLLOTOXIN

Podophyllotoxin is a naturally occuring product and used in the production of anticancer drugs like Etoposide.

Biological Source : It is obtained from roots and rhizomes of Podophyllum hexandrum and P. emodi belonging to family Berberidaceae.

ISOLATION OF PODOPHYLLOTOXIN

- Collect and dry the roots or rhizomes of Podophyllum species, then grind them into a fine powder.
- Extract the powder using solvents like ethanol or methanol through process like maceration or soxhlet extraction.
- Filter the extract to remove solid waste.
- Concentrate the extract by evaporating the solvent under reduced pressure.
- Purify the crude extract through chromatographic techniques such as column chromatography, which separates Podophyllotoxin from other compounds.

IDENTIFICATION OF PODOPHYLLOTOXIN

- Add 0.4 g. of powdered drug to 3 ml of 60% alcohol and then add 0.5 ml of 1 N potassium hydroxide and shake it
- Formation of a stiff jelly confirms the presence of Podophyllotoxin.

Analysis Of Podophyllotoxin

Podophyllotoxin can be analysed through Thin Layer Chromatography

- Sample Preparation : 1 mg of podophyllotoxin is dissolved in 1 ml methanol.
- Standard Sample : Podophyllotoxin
- Stationary Phase : Silica gel- G1
- Mobile Phase : Chloroform : Methanol (90:10)
- Detecting Agent : Vanillin - Sulphuric Acid
- RF Value : 0.65
- Colour Spot : Yellow spot

② CURCUMIN

- Curcumin is a natural polyphenolic compound found in rhizomes of turmeric.
- It is the principle bioactive compound responsible for turmeric's bright yellow colour and many of its medicinal properties.

Biological Source: It is found in rhizomes of Curcuma longa (turmeric) belongs to family Zingiberaceae.

ISOLATION OF CURCUMIN

- Dried turmeric rhizomes are powdered
- The powder is extracted using solvents like ethanol, methanol.
- Filter the extract to remove insoluble residues.
- Concentrate the filtrate by evaporating the solvent under reduced pressure.
- Use techniques like recrystallization or chromatographic methods to isolate pure curcumin.

IDENTIFICATION OF CURCUMIN

On treating the powdered drug with sulphuric acid, a crimson colour appears which confirms the presence of curcumin.

ANALYSIS OF CURCUMIN

Curcumin can be analysed through TLC.

- Sample Preparation : 1 mg of curcumin is dissolved in 1 ml methanol
- Standard Sample : Curcumin
- Stationary Phase : Silica gel - G
- Mobile Phase : Chloroform : Ethanol : Glacial acetic acid (94:5:1)
- Detecting Agent : Observed under UV light
- RF Value : 0.79

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