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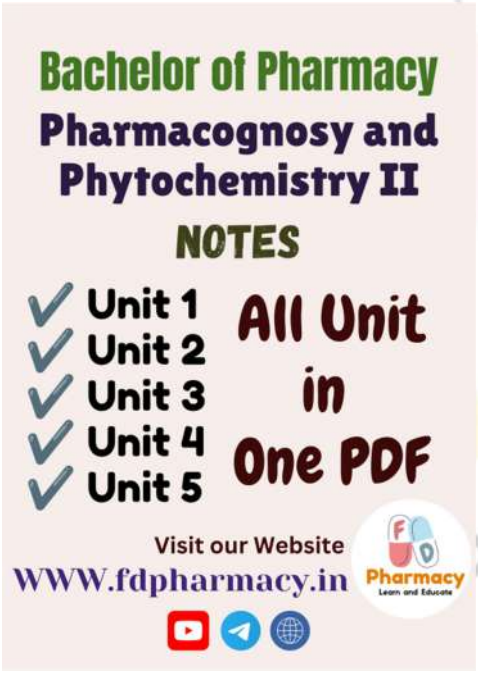
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PHARMACOGNOSY AND PHYTOCHEMISTRY - II

UNIT 3

TOPIC :

- **b) Glycosides:** Glycyrhetinic acid & Rutin

GLYCOSIDES

- Glycosides are naturally occurring organic compounds found mainly in plants.
  They are non-sugar compounds (aglycones or genins) combined with one or more sugar molecules (glycones).
- These compounds are biologically active and contribute to the therapeutic effects of many medicinal plants.
- A glycoside is a compound in which a sugar molecule is chemically bonded to a non-sugar molecule (aglycone or genin) through an oxygen or nitrogen or sulfur linkage.

General Structure:

Glycoside = Sugar part (Glycone) + Non-sugar part (Aglycone or Genin)

General Formula

$$\text{Glycoside} = \text{Glycone} + \text{Aglycone}$$

The bond between them is called a glycosidic bond.

Classification of Glycosides

Type	Based on Aglycone (Genin)	Example	Source	Uses
1. Cardiac Glycosides	Steroidal nucleus	Digoxin, Digitoxin	*Digitalis purpurea*	Used in heart failure
2. Anthraquinone Glycosides	Anthracene derivative	Aloe-emodin, Sennosides	*Aloe*, *Senna*	Laxative
3. Saponin Glycosides	Triterpenoid/steroidal	Dioscin, Glycyrrhizin	*Dioscorea*, *Liquorice*	Expectorant, anti-inflammatory
4. Cyanogenic Glycosides	Contain cyanide group (–CN)	Amygdalin	*Bitter Almond*	Source of hydrocyanic acid
5. Flavonoid Glycosides	Flavone derivative	Rutin, Hesperidin	*Orange peel*, *Buchu*	Antioxidant, capillary strengthener
6. Coumarin Glycosides	Coumarin nucleus	Umbelliferone	*Tonka bean*	Anticoagulant
7. Isothiocyanate Glycosides	Mustard oils	Sinigrin	*Black Mustard*	Rubefacient
8. Iridoid Glycosides	Monoterpenoid nucleus	Amarogentin, Loganin	*Gentian*, *Oleaceae plants*	Bitter tonic

Therapeutic Uses

- Cardiac Glycosides: Strengthen heart contraction (e.g., Digoxin).
- Anthraquinone Glycosides: Laxative effect (e.g., Senna, Aloe).
- Saponin Glycosides: Expectorant, anti-inflammatory, and hypocholesterolemic.
- Cyanogenic Glycosides: Source of hydrocyanic acid (used externally).
- Flavonoid Glycosides: Antioxidant, vasoprotective.
- Iridoid Glycosides: Bitter tonic, liver stimulant.

Commercial and Pharmaceutical Applications

- Used in cardiotonic preparations (*Digitalis*).
- Used as natural laxatives (*Senna*, *Aloe*).
- Used in cough syrups as expectorants (*Liquorice*).
- Employed as flavoring agents and antioxidants in food and cosmetics.
- Used in drug standardization and quality control of herbal medicines.

Isolation, Identification, and Analysis of Glycyrrhetinic Acid

- Glycyrrhetinic acid is a triterpenoid saponin aglycone obtained from the roots of *Glycyrrhiza glabra* (commonly known as Liquorice or Mulethi).
- It is derived from the hydrolysis of glycyrrhizin, the main sweet-tasting glycoside present in the plant.
  It possesses anti-inflammatory, anti-ulcer, antiviral, and hepatoprotective properties.

Chemical nature:

- Type: Pentacyclic triterpenoid
- Formula: $C_{30}H_{46}O_4$
- Structure: Consists of a lupane skeleton with carboxylic and hydroxyl groups

Isolation of Glycyrrhetinic Acid

Source:

- *Glycyrrhiza glabra* (Family: Fabaceae)
- Active constituent: Glycyrrhizin (converted to glycyrrhetinic acid by hydrolysis)
- Plant part used: Dried roots and stolons

Steps for Isolation:

1. **Collection and Drying:**
   - Roots of *Glycyrrhiza glabra* are collected, cleaned, and shade-dried.
2. **Powdering:**
   - The dried roots are coarsely powdered.
3. **Extraction:**
   - Powdered roots are extracted with hot water or ethanol-water mixture (70%) by reflux or maceration.
   - The extract mainly contains glycyrrhizin, a saponin glycoside.
4. **Filtration and Concentration:**
   - The extract is filtered and concentrated under reduced pressure to obtain a thick syrup.
5. **Hydrolysis of Glycyrrhizin:**
   - The concentrated extract is hydrolyzed using dilute hydrochloric acid (HCl) or enzymatic hydrolysis.
   - This breaks glycyrrhizin into glycyrrhetinic acid (aglycone) and glucuronic acid (sugar part).
6. **Purification:**
   - The organic extract is evaporated and recrystallized from ethanol to obtain pure glycyrrhetinic acid as colorless crystals.

Identification of Glycyrrhetinic Acid

Identification confirms the purity and chemical nature of glycyrrhetinic acid using physical, chemical, and instrumental methods.

A. Physical Characteristics:

- Appearance: Colorless crystalline powder
- Melting point: 280–290°C
- Solubility: Soluble in chloroform and ethanol; insoluble in water
- Taste: Bitter

B. Chemical Tests:

1. Liebermann–Burchard Test (for triterpenoids):
    - Dissolve in chloroform, add acetic anhydride and a few drops of concentrated sulfuric acid.
    - A green or blue-green color indicates the presence of triterpenoid structure.
2. Salkowski Test:
    - Add concentrated sulfuric acid to a chloroform solution of the compound.
    - A red or reddish-brown color appears, confirming triterpenoids.
3. Foam Test (for saponins):
    - Aqueous extract of liquorice produces persistent foam, due to the presence of saponins (glycyrrhizin, precursor of glycyrrhetinic acid).

C. Spectroscopic Identification:

- UV Spectroscopy: Absorption maxima (λmax) around 250 nm.
- IR Spectroscopy:
    - C=O stretching (carboxylic acid): 1700 cm^{-1}
    - O–H stretching: 3400 cm^{-1}
    - C–H stretching (CH_3 and CH_2 groups): 2850–2950 cm^{-1}
- NMR (^1H and ^{13}C): Confirms the triterpenoid skeleton with characteristic methyl and carboxylic carbon signals.
- Mass Spectrometry: Molecular ion peak at m/z 470, corresponding to $C_{30}H_{46}O_4$.

Analysis of Glycyrrhetinic Acid

A. Qualitative Analysis:

- TLC and chemical color tests are used to confirm presence of glycyrrhetinic acid in plant extracts.

B. Quantitative Analysis:

1. UV Spectrophotometric Method:
    - Glycyrrhetinic acid is dissolved in ethanol.
    - Absorbance measured at λmax 250 nm.
    - Concentration determined using Beer–Lambert's law.
2. HPLC (High-Performance Liquid Chromatography):
    - Column: C18 reverse-phase column
    - Mobile Phase: Acetonitrile : Water (80:20)
    - Flow rate: 1.0 mL/min
    - Detector: UV at 250 nm
    - Retention time: Approximately 7–8 minutes
    - Used for accurate estimation in extracts or formulations.
3. GC-MS (Gas Chromatography–Mass Spectrometry):
    - Used for molecular identification and purity check.
    - Confirms molecular ion peak at m/z 470.

Applications

- Anti-inflammatory – Acts similar to corticosteroids without their side effects.
- Anti-ulcer – Protects gastric mucosa by reducing acid secretion.
- Hepatoprotective – Useful in liver disorders.
- Antiviral – Effective against certain viral infections like hepatitis.
- Sweetening agent precursor – From glycyrrhizin (30–50 times sweeter than sucrose).

Isolation, Identification, and Analysis of Rutin

- Rutin (also known as vitamin P or rutoside) is a flavonoid glycoside composed of the flavonol quercetin and the disaccharide rutinose.
- It occurs widely in many plants and is known for its antioxidant, anti-inflammatory, and capillary-strengthening properties.

Chemical nature:

- Class: Flavonoid glycoside
- Chemical formula: $C_{27}H_{30}O_{16}$
- Molecular weight: 610.5
- Structure: Quercetin (aglycone) + Rutinose (sugar moiety)

Source

- Plants: *Fagopyrum esculentum* (Buckwheat), *Sophora japonica* (Japanese pagoda tree), *Eucalyptus*, *Tobacco*, *Tea*, *Citrus* fruits.
- Family: Polygonaceae or Leguminosae (depending on source plant).
- Plant part used: Flowers, leaves, and fruits.

Isolation of Rutin

Steps for Isolation:

1. **Collection and Drying:**
   - Flowers or leaves rich in rutin (e.g., *Sophora japonica* buds) are collected and shade-dried.
2. **Powdering:**
   - The dried material is coarsely powdered.
3. **Extraction:**
   - The powdered material is extracted using ethanol or methanol (70–80%) in a Soxhlet apparatus or by reflux for 4–6 hours.
   - The solvent extracts flavonoids including rutin and quercetin.

4. **Filtration and Concentration:**
    - The extract is filtered and concentrated under reduced pressure using a rotary evaporator.
5. **Precipitation and Purification:**
    - The concentrated extract is treated with lead acetate to precipitate flavonoids.
    - The precipitate is filtered, washed, and decomposed with hydrogen sulfide (H_2S) to remove lead.
    - The filtrate is again concentrated and allowed to crystallize.
6. **Crystallization:**
    - The purified extract is recrystallized from ethanol or methanol to obtain pure rutin crystals.
7. **Alternative Method:**
    - Rutin can also be isolated by column chromatography using silica gel as stationary phase and ethyl acetate:methanol:water (100:13.5:10) as mobile phase.

Identification of Rutin

A. Physical Characteristics:

- Appearance: Yellow crystalline powder
- Melting point: 190–192°C
- Solubility: Soluble in methanol, ethanol; slightly soluble in water
- Taste: Bitter

B. Chemical Tests:

1. Shinoda Test (for Flavonoids):
    - Add magnesium turnings and few drops of concentrated HCl to ethanolic solution of rutin.
    - A red or pink color appears — confirms presence of flavonoid nucleus.
2. Alkaline Reagent Test:
    - Add a few drops of sodium hydroxide solution to the alcoholic solution of rutin.

- Yellow color appears which becomes colorless on addition of acid — confirms flavonoids.

3. Lead Acetate Test:
   - Add lead acetate to aqueous extract — formation of a yellow precipitate indicates flavonoids.
4. TLC (Thin Layer Chromatography):
   - Stationary phase: Silica gel G
   - Mobile phase: n-Butanol : Acetic acid : Water (4:1:5, upper layer)
   - Detection: UV light (366 nm) or ammonia vapors — yellow-green fluorescent spot
   - Rf value: ≈ 0.3–0.4

C. Spectroscopic Identification:

1. UV-Visible Spectroscopy:
   - Rutin shows λmax around 256 nm and 355 nm, characteristic of flavonols.
2. IR Spectroscopy:
   - O–H stretching: 3400 cm^{-1}
   - C=O stretching (ketone): 1660 cm^{-1}
   - C–O–C stretching (ether): 1200 cm^{-1}
3. NMR (^{1}H and ^{13}C):
   - Shows characteristic signals for aromatic protons and sugar carbons.
4. Mass Spectrometry:
   - Molecular ion peak (M^{+}) at m/z 610, confirming molecular weight.

Analysis of Rutin

A. Qualitative Analysis:

- Confirmed using TLC and color reactions (Shinoda, lead acetate, alkaline reagent tests).
- Helps to detect rutin in crude plant extracts.

B. Quantitative Analysis:

1. UV Spectrophotometric Method:
    - Dissolve known quantity of rutin in methanol.
    - Measure absorbance at λmax 355 nm.
    - Concentration is calculated using Beer-Lambert's law.
2. HPLC (High-Performance Liquid Chromatography):
    - Column: C18 reverse-phase column
    - Mobile phase: Methanol : Water : Acetic acid (40:57:3)
    - Flow rate: 1.0 mL/min
    - Detection: UV detector at 355 nm
    - Retention time: ~5–6 minutes
    - Most accurate and reliable method for rutin estimation in herbal formulations.
3. HPTLC (High-Performance Thin Layer Chromatography):
    - Used for standardization of herbal drugs containing flavonoids.

Applications of Rutin

- Antioxidant: Scavenges free radicals and protects tissues from oxidative damage.
- Vasoprotective: Strengthens capillaries, used in treatment of varicose veins and hemorrhoids.
- Anti-inflammatory and anti-allergic activities.
- Cardioprotective and neuroprotective effects.