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 - ✓ Unit 2
 - ✓ Unit 3
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PHARMACOGNOSY AND PHYTOCHEMISTRY - II

UNIT 3

TOPIC :

- **d) Resins: Podophyllotoxin, Curcumin**



Resins

- Resins are complex organic substances that are produced naturally in plants, especially in conifers and some other trees.
They are amorphous, non-crystalline, and insoluble in water but soluble in organic solvents such as alcohol, chloroform, and ether.
- Resins are usually formed in plants as a defensive secretion to protect against injury, infection, and pests.
- Resins are complex mixtures of volatile oils, resin acids, resin alcohols, esters, and resenes that occur naturally as oxidation products of essential oils.

Chemical Nature

- Resins contain carbon, hydrogen, and oxygen.
- They are non-nitrogenous substances.
- They are formed by polymerization and oxidation of volatile oils.
- They may contain small amounts of acidic (resinic acids) or neutral substances (resenes).

Classification of Resins

Resins can be classified as follows:

A. Based on occurrence

1. Natural Resins – obtained directly from plant exudations (e.g., Colophony, Benzoin).
2. Prepared Resins – produced by heating or treating natural substances (e.g., Resin from turpentine oil).

B. Based on chemical composition

1. Resin acids – e.g., Abietic acid (from Colophony).
2. Resin alcohols – e.g., Balsams.
3. Resin esters – e.g., Benzoin.
4. Resenes – neutral substances (e.g., Guaiac resin).
5. Glycoresins – resins combined with sugars (e.g., Jalap resin).
6. Oleo-resins – resins with volatile oils (e.g., Turpentine).
7. Gum-resins – resins with gum (e.g., Myrrh).
8. Balsams – resins containing cinnamic or benzoic acid (e.g., Benzoin, Peru balsam).

Therapeutic Uses

- Antiseptic and antimicrobial (e.g., Benzoin, Myrrh)
- Anti-inflammatory (e.g., Guggul)
- Antitumor and antiviral (e.g., Podophyllum resin)
- Expectorant and carminative (e.g., Asafoetida)
- Used in varnishes, adhesives, plasters, and cosmetics

Commercial Applications

- Used in pharmaceutical preparations (plasters, liniments, ointments).
- Used in paint and varnish industries.
- Employed in perfumery and incense.
- Used as binding agents in tablet formulation.

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Isolation, Identification and Analysis of Podophyllotoxin

- Podophyllotoxin is a lignan-type secondary metabolite obtained from the roots and rhizomes of *Podophyllum emodi* (Indian Podophyllum) and *Podophyllum peltatum* (American Mayapple).
- It is a cytotoxic compound used as a precursor for the synthesis of anticancer drugs such as etoposide and teniposide.
- It acts by inhibiting mitosis through binding to tubulin (spindle poison).

Molecular Formula: $C_{22}H_{22}O_8$

Molecular Weight: 414.41 g/mol

Chemical Class: Lignan

Isolation of Podophyllotoxin

Plant Source:

- Plant: *Podophyllum emodi* (Indian Podophyllum) or *P. peltatum*
- Family: Berberidaceae
- Part Used: Rhizomes and roots

Steps for Isolation:

1. Collection and Drying:
 - Rhizomes of *Podophyllum* are collected, washed, and dried under shade.
2. Powdering:
 - Dried rhizomes are coarsely powdered to increase the surface area for extraction.
3. Extraction:
 - The powdered rhizomes are extracted with ethanol (95%) or methanol using a Soxhlet apparatus for several hours.
 - The extract contains resinous mixture of lignans including podophyllotoxin.

4. Concentration:

- The alcoholic extract is filtered and concentrated under reduced pressure to obtain a resinous mass (known as *podophyllin*).

5. Purification:

- The crude podophyllin resin is dissolved in ethanol and treated with dilute ammonia to remove impurities.
- The solution is filtered and evaporated to dryness.

6. Isolation of Podophyllotoxin:

- The purified resin is dissolved in chloroform, and podophyllotoxin is separated by column chromatography using silica gel as the stationary phase.
- Eluent: Chloroform : Methanol (95:5).
- The fraction containing podophyllotoxin is collected and crystallized from ethanol or acetone to yield pure podophyllotoxin crystals.

Identification of Podophyllotoxin

Identification confirms the presence and purity of the compound using physical, chemical, and instrumental methods.

A. Physical Properties:

- Appearance: White crystalline powder
- Taste: Bitter
- Solubility: Soluble in alcohol, acetone, and chloroform; insoluble in water
- Melting point: 182–184°C

B. Chemical Tests:

1. Ferric Chloride Test:

- Dissolve a small amount of podophyllotoxin in alcohol and add ferric chloride solution → produces a green color, indicating phenolic group.

2. Sulfuric Acid Test:

- Add concentrated H_2SO_4 to podophyllotoxin → produces a reddish-brown color, indicating presence of lignan-type structure.

3. Vanillin Test:

- Add vanillin-HCl reagent → violet or red color appears, confirming aromatic lignan nucleus.

4. TLC Test:

- Used for qualitative identification (see below).

C. TLC Identification:

- Stationary Phase: Silica gel G
- Mobile Phase: Chloroform : Methanol (9:1)
- Detection: Under UV light (254 nm) or by spraying with vanillin-sulfuric acid reagent (reddish spot).
- R_f value: ≈ 0.42

D. Spectroscopic Identification:

1. UV-Visible Spectroscopy:

- $\lambda_{\text{max}} \approx 290 \text{ nm}$ (aromatic ring system).

2. IR Spectroscopy:

- Characteristic absorption bands:

- C=O (lactone): 1730 cm^{-1}
- O-H (phenolic): 3400 cm^{-1}
- Aromatic C=C: 1600 cm^{-1}

3. Mass Spectrometry:

- Molecular ion peak at $m/z = 414$ confirms molecular weight.

4. NMR Spectroscopy:

- Signals confirm presence of lactone ring, methoxy, and aromatic protons typical of lignans.

Analysis of Podophyllotoxin

A. Qualitative Analysis:

- Performed by TLC or HPTLC fingerprinting.
- Comparison of R_f value and color reaction with standard podophyllotoxin confirms its identity.

B. Quantitative Analysis:

1. UV Spectrophotometric Method:
 - Dissolve podophyllotoxin in ethanol and measure absorbance at $\lambda_{\text{max}} = 290 \text{ nm}$.
 - Use Beer-Lambert's law for concentration estimation.
2. HPLC Method:
 - Column: C₁₈ (reverse-phase)
 - Mobile phase: Acetonitrile : Water (70:30)
 - Detector: UV at 290 nm
 - Flow rate: 1.0 mL/min
 - Retention time: ~5–8 min
 - Provides accurate quantification in extracts and formulations.
3. HPTLC Method:
 - Used for simultaneous determination of lignans (podophyllotoxin, picropodophyllin).
 - Detection at 290 nm under UV.

Applications of Podophyllotoxin

- Anticancer agent precursor: Used to synthesize *etoposide* and *teniposide*.
- Antiviral agent: Used in topical preparations for treatment of *genital warts (condyloma acuminata)* caused by HPV.
- Antimitotic agent: Inhibits cell division by binding to tubulin.
- Cytotoxic agent: Used in research for cancer cell inhibition studies

Isolation, Identification and Analysis of Curcumin

- Curcumin is the principal bioactive compound found in the rhizomes of *Curcuma longa* (Turmeric).
- It is a polyphenolic compound responsible for the yellow color of turmeric.
- It possesses anti-inflammatory, antioxidant, antimicrobial, anticancer, and hepatoprotective properties.

Chemical name: 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione

Molecular formula: $C_{21}H_{20}O_6$

Molecular weight: 368.38 g/mol

Chemical class: Diarylheptanoid (Polyphenolic compound)

Isolation of Curcumin

Plant Source:

- Plant: *Curcuma longa* (Turmeric)
- Family: Zingiberaceae
- Part used: Dried rhizomes

Steps for Isolation:

1. Collection and Drying:
 - Fresh rhizomes of *Curcuma longa* are collected, cleaned, boiled, and dried in shade.
2. Powdering:
 - The dried rhizomes are coarsely powdered to increase extraction efficiency.
3. Extraction:
 - The powdered rhizomes are extracted using ethanol (95%) or acetone in a Soxhlet apparatus for 6–8 hours.

- The extract contains a mixture of curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin).
- 4. Concentration:
 - The solvent is evaporated under reduced pressure to obtain a thick concentrated extract.
- 5. Separation and Purification:
 - The concentrated extract is dissolved in ethyl acetate and filtered to remove insoluble matter.
 - The filtrate is concentrated, and petroleum ether is added to precipitate curcumin.
 - The precipitate is recrystallized from ethanol or acetone to obtain pure yellow crystalline curcumin.

Identification of Curcumin

Identification confirms the presence, purity, and structure of curcumin using chemical and instrumental tests.

A. Physical Properties:

- Appearance: Bright yellow-orange crystalline powder
- Odor: Slightly aromatic
- Solubility: Soluble in ethanol, methanol, acetone; insoluble in water
- Melting point: 179–183°C

B. Chemical Tests:

1. Borax Test:
 - Curcumin gives a reddish-brown color with borax solution, which turns greenish-blue on addition of acid.
→ Indicates curcuminoid structure.
2. Alkali Test:
 - Add a few drops of NaOH to ethanolic solution of curcumin → deep red color appears, which disappears on acidification.
→ Confirms phenolic nature.

3. Ferric Chloride Test:
 - Add FeCl_3 to ethanolic curcumin solution $\rightarrow$ gives green or brown color, showing phenolic OH groups.
4. Concentrated Sulfuric Acid Test:
 - With conc. $\text{H}_2\text{SO}_4 \rightarrow$ produces deep violet or reddish color, confirming conjugated double bonds.
5. TLC Test:
 - Spot test for qualitative identification (see below).

C. TLC Identification:

- Stationary phase: Silica gel G
- Mobile phase: Chloroform : Methanol (95:5)
- Detection: Under UV light at 366 nm or spraying with vanillin-sulfuric acid reagent (reddish spot).
- R_f value: 0.31–0.35 (for curcumin)

D. Spectroscopic Identification:

1. UV-Visible Spectroscopy:
 - $\lambda_{\text{max}} \approx 420\text{--}425 \text{ nm}$ (due to extended conjugation).
2. IR Spectroscopy:
 - Key absorption bands:
 - Phenolic O–H stretch: 3500 cm^{-1}
 - C=O (β -diketone): 1627 cm^{-1}
 - Aromatic C=C: 1600 cm^{-1}
 - C–O–C: 1020 cm^{-1}
3. Mass Spectrometry:
 - Molecular ion peak at $m/z = 368$ confirms molecular weight.
4. NMR Spectroscopy:
 - Characteristic peaks for aromatic protons and methoxy groups confirm curcumin structure.

Analysis of Curcumin

A. Qualitative Analysis:

- Performed by TLC or HPTLC fingerprinting to detect curcumin in turmeric extract.
- The R_f value and color reaction with standard curcumin confirm its identity.

B. Quantitative Analysis:

1. UV Spectrophotometric Method:
 - Prepare ethanolic solution of curcumin.
 - Measure absorbance at $\lambda_{\text{max}} = 420 \text{ nm}$.
 - Use Beer-Lambert's law to calculate concentration.
2. HPLC Method:
 - Column: C₁₈ (reverse-phase)
 - Mobile phase: Acetonitrile : Water (50:50 or 70:30)
 - Flow rate: 1 mL/min
 - Detection: UV at 425 nm
 - Retention time: ~7–9 min
 - Used for accurate quantification of curcuminoids in turmeric extracts and formulations.
3. HPTLC Method:
 - Mobile phase: Toluene : Ethyl acetate : Formic acid (6:6:1)
 - Detection wavelength: 425 nm
 - Used for simultaneous estimation of curcuminoids.

Applications of Curcumin

- Anti-inflammatory: Inhibits cyclooxygenase and lipoxygenase pathways.
- Antioxidant: Neutralizes free radicals and prevents oxidative stress.
- Anticancer: Suppresses tumor cell proliferation.
- Antimicrobial: Effective against bacteria, fungi, and viruses.

- Wound healing & hepatoprotective agent.

