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PHARMACOGNOSY AND PHYTOCHEMISTRY - II

UNIT 5

TOPIC :

- **Basics of Phytochemistry**

  Modern methods of extraction, application of latest techniques like Spectroscopy, chromatography and electrophoresis in the isolation, purification and identification of crude drugs.

Basics of Phytochemistry

- Phytochemistry is the branch of chemistry that deals with the study of chemical substances (phytoconstituents) that are derived from plants.
- These naturally occurring compounds are responsible for the plant's physiological and therapeutic properties.
- Phytochemistry involves extraction, isolation, purification, identification, and structural elucidation of bioactive compounds present in medicinal plants.

Objectives of Phytochemistry

- To identify bioactive chemical constituents present in plants.
- To isolate and purify the active principles from crude plant materials.
- To determine the chemical structure and biological role of isolated compounds.
- To standardize herbal drugs based on chemical markers.
- To understand biosynthetic pathways and mechanisms of action of phytoconstituents.

Classification of Plant Constituents

Phytochemicals are classified based on their chemical nature and biosynthetic origin:

Class	Examples	Therapeutic Uses
Alkaloids	Morphine, Atropine, Quinine	Analgesic, antimalarial
Glycosides	Digoxin, Sennosides	Cardiotonic, laxative
Terpenoids	Menthol, Taxol	Anticancer, antimicrobial
Flavonoids	Quercetin, Rutin	Antioxidant, anti-inflammatory
Phenolics	Tannins, Eugenol	Antiseptic, astringent
Steroids	Diosgenin	Hormonal precursor
Volatile oils	Clove oil, Eucalyptus oil	Antiseptic, carminative
Resins and	Myrrh, Asafoetida	Antimicrobial, expectorant

Gums		

Modern Methods of Extraction

Extraction is the process of separating active constituents from plant material. Modern methods are designed for efficiency, selectivity, and minimal degradation.

A. Solvent Extraction

Maceration

- Maceration is one of the classical methods of extraction used in phytochemistry to extract bioactive constituents from plant materials.
- Maceration is the process of soaking coarsely powdered plant material in a suitable solvent at room temperature for a specific period to dissolve the soluble constituents.

Principle

- Based on the solubility of plant constituents in the chosen solvent.
- The solvent diffuses into the plant material, dissolves the active constituents, and allows gradual extraction.

Procedure

1. Selection of Plant Material:
    - Use dried and powdered plant parts (leaves, roots, bark, seeds).
2. Choice of Solvent:
    - Depends on the polarity of the constituents (e.g., water, ethanol, methanol, chloroform).
3. Soaking:
    - Place plant powder in the solvent container.
    - Stir occasionally to enhance extraction.
4. Duration:
    - Usually several hours to days, depending on the plant and constituent.
5. Filtration:
    - Separate the solvent extract from plant residue.

6. Concentration:
    - Evaporate the solvent to obtain the crude extract.

Advantages

- Simple and easy to perform.
- Requires minimal equipment.
- Suitable for thermolabile compounds (does not use heat).
- Can be scaled up for small to medium production.

Limitations

- Time-consuming (may take days).
- Less efficient compared to modern techniques (e.g., Soxhlet, UAE).
- Risk of microbial growth if prolonged soaking occurs.
- Requires large volumes of solvent for complete extraction.

Applications

- Used in extraction of alkaloids, flavonoids, glycosides, and essential oils for:
    - Pharmacological studies
    - Quality control
    - Preparation of tinctures and herbal extracts

Percolation

- Percolation is a classical phytochemical extraction method used to obtain active constituents from plant materials more efficiently than maceration.
- Percolation is a process in which a solvent is passed continuously through a column of coarsely powdered plant material to extract soluble constituents.

Principle

- Based on solvent percolation through plant material.
- Solvent dissolves the bioactive constituents as it trickles through the powder, which is tightly packed in a percolator.
- More efficient than simple soaking because fresh solvent contacts the plant material continuously.

Apparatus

- Percolator: Usually a glass or stainless steel cylinder with:
  1. Stopcock at the bottom for collecting extract
  2. Funnel or filter to hold plant material
  3. Lid to prevent evaporation

Procedure

1. Preparation of Plant Material:
   - Powdered and dried plant material is used.
2. Packing the Percolator:
   - Plant powder is packed loosely in layers to allow uniform solvent flow.
   - A filter is placed at the bottom to prevent clogging.
3. Wetting:
   - Pre-wet the powder with the chosen solvent (maceration for a few hours may be done initially to moisten).
4. Percolation:

- Solvent is added to the top, passes through the material, and extract is collected from the stopcock at the bottom.
- Continuous addition of fresh solvent may be done to complete extraction.

5. Concentration:
   - The collected extract is concentrated under reduced pressure or gentle heat to obtain the crude extract.

Advantages

- Faster and more efficient than maceration.
- Requires less solvent due to continuous percolation.
- Suitable for thermolabile compounds when done at room temperature.
- Provides uniform extraction of constituents.

Limitations

- Requires special apparatus (percolator).
- Packing errors can lead to channeling, reducing efficiency.
- Not suitable for very fine powders as it may clog the percolator.

Applications

- Extraction of alkaloids, glycosides, flavonoids, and essential oils.
- Preparation of tinctures, fluid extracts, and herbal formulations.
- Widely used in pharmacognosy and herbal medicine manufacturing.

Soxhlet Extraction

- Soxhlet Extraction is a classical continuous extraction method commonly used for thermostable phytoconstituents. It is more efficient than maceration or percolation.
- Soxhlet Extraction is a method in which a solvent is continuously refluxed over a solid plant material to extract soluble constituents.

Principle

- Solvent refluxes in a siphon system, dissolving plant constituents repeatedly.
- Fresh solvent continuously contacts the material, improving extraction efficiency.
- Ideal for compounds stable at the solvent's boiling point.

Apparatus

The Soxhlet apparatus consists of:

1. Round-bottom flask: Contains the extraction solvent.
2. Soxhlet extractor: Holds the plant material in a thimble.
3. Condenser: Condenses vaporized solvent to percolate over the plant material.
4. Thimble: Usually made of cellulose or filter paper, holding the powdered plant.

Procedure

1. Preparation of Plant Material:
    - Dry and coarsely powder the plant part.
    - Place in thimble in the Soxhlet extractor.
2. Selection of Solvent:
    - Choose based on the solubility of target constituents (e.g., ethanol, methanol, chloroform, hexane).
3. Assembly:

    - Connect flask, Soxhlet extractor, and condenser.
4. Extraction:
    - Heat the solvent in the flask, which vaporizes and condenses over the thimble.
    - Extracts accumulate in the flask after siphoning.
    - Repeat for several cycles until the material is exhausted.
5. Concentration:
    - Evaporate the solvent to obtain the crude extract.

Advantages

- Efficient extraction with minimal solvent.
- Suitable for thermostable compounds.
- Continuous extraction ensures complete recovery of constituents.
- Can be used for large-scale extraction in industrial applications.

Limitations

- Not suitable for heat-labile compounds (may degrade at boiling solvent temperature).
- Requires heating apparatus and careful monitoring.
- Time-consuming for some plant materials.

Applications

- Extraction of alkaloids, glycosides, essential oils, fatty acids, and lipophilic compounds.
- Commonly used in pharmacognosy laboratories for preparation of extracts.
- Also used in industrial phytochemical extraction.

B. Advanced Extraction Techniques

Ultrasound-Assisted Extraction (UAE)

- Ultrasound-Assisted Extraction (UAE) is a modern, efficient extraction technique used to isolate bioactive compounds from plant materials.
- UAE is a method in which ultrasonic waves (high-frequency sound waves) are used to disrupt plant cell walls, enhancing the release of phytochemicals into the solvent.

Principle

- Ultrasound generates cavitation bubbles in the solvent.
- The collapse of bubbles produces micro-jets that break plant cell walls.
- This enhances solvent penetration and facilitates rapid extraction of active constituents.

Apparatus

- Ultrasonic bath: Plant material immersed in solvent; ultrasound transmitted via water bath.
- Ultrasonic probe/horn: Direct immersion for higher intensity and faster extraction.

Procedure

1. Preparation of Plant Material:
    - Dried, coarsely powdered plant parts are used.
2. Selection of Solvent:
    - Based on polarity of target compounds (e.g., ethanol, methanol, water).
3. Ultrasonic Treatment:
    - Plant material is placed in solvent and exposed to ultrasonic waves (20–40 kHz) for a specific duration.
4. Filtration and Concentration:

- After extraction, filter the solution and concentrate the extract under reduced pressure.

Advantages

- Rapid extraction compared to conventional methods.
- Requires less solvent.
- High extraction efficiency and yield.
- Suitable for thermolabile compounds as it uses moderate temperature.
- Can be used on small to medium scale.

Limitations

- Limited scalability for large industrial production.
- Requires special ultrasonic equipment.
- Prolonged exposure may cause degradation of some sensitive compounds.

Applications

- Extraction of alkaloids, flavonoids, glycosides, essential oils, and phenolics.
- Used in quality control, phytochemical research, and herbal medicine preparation.
- Also applied in food and cosmetic industries for bioactive compound extraction.

Microwave-Assisted Extraction (MAE)

- Microwave-Assisted Extraction (MAE) is a modern, rapid extraction technique used to isolate bioactive compounds from plant materials using microwave energy.
- MAE is a method in which microwave radiation is used to heat the plant-solvent mixture, causing cell rupture and faster release of phytochemicals into the solvent.

Principle

- Microwave energy causes polar molecules in the plant matrix and solvent to oscillate, generating heat internally.
- This internal heating disrupts cell walls, releasing intracellular constituents.
- Extraction is enhanced by increased mass transfer between plant and solvent.

Apparatus

- Microwave extraction unit: Includes a microwave generator, extraction vessel, and condenser.
- Can be closed-vessel (high pressure) or open-vessel system depending on solvent volatility.

Procedure

1. Preparation of Plant Material:
    - Dried and coarsely powdered plant material is used.
2. Selection of Solvent:
    - Polar solvents (e.g., ethanol, methanol, water) are preferred to absorb microwave energy.
3. Microwave Treatment:
    - Mix plant material with solvent in extraction vessel.
    - Expose to microwave radiation for a short period (minutes).
4. Filtration and Concentration:

- Filter the extract and evaporate solvent under reduced pressure to obtain the crude extract.

Advantages

- Rapid extraction (minutes instead of hours).
- Higher yield of bioactive compounds.
- Less solvent consumption.
- Suitable for thermolabile compounds with controlled temperature.
- Environmentally friendly and energy-efficient.

Limitations

- Requires special microwave extraction equipment.
- High-power microwaves may degrade sensitive compounds.
- Scaling up for industrial production is challenging.

Applications

- Extraction of alkaloids, flavonoids, terpenoids, glycosides, and essential oils.
- Used in quality control, phytochemical analysis, and herbal drug preparation.
- Applied in food, cosmetic, and nutraceutical industries for rapid extraction.

Supercritical Fluid Extraction (SFE)

- Supercritical Fluid Extraction (SFE) is a modern, advanced extraction technique that uses supercritical fluids as solvents to extract bioactive compounds from plant materials.
- SFE is a method in which supercritical fluids—substances at a temperature and pressure above their critical point—are used to dissolve and extract phytochemicals from plant matrices.

Principle

- A supercritical fluid exhibits properties of both a liquid and gas, with high diffusivity and low viscosity.
- Carbon dioxide (CO_2) is most commonly used due to:
  - Non-toxicity
  - Non-flammability
  - Moderate critical temperature (31.1°C) and pressure (73.8 bar)
- The supercritical fluid penetrates plant material, dissolves bioactive compounds, and is later separated by reducing pressure.

Apparatus

- Extraction vessel: Contains plant material.
- Pump: Pressurizes CO_2 to supercritical state.
- Separator: Reduces pressure to precipitate the extracted compounds.
- Heating unit & pressure controller: Maintain required temperature and pressure.

Procedure

1. Preparation of Plant Material:
   - Dried and powdered plant parts are used for optimal contact with supercritical fluid.
2. Loading:
   - Plant material is placed in the extraction vessel.
3. Supercritical Fluid Extraction:

- CO_2 is pressurized and heated to reach supercritical conditions.
- Supercritical CO_2 passes through plant material, dissolving lipophilic constituents.

4. Separation:
   - Pressure is reduced in the separator; compounds precipitate and are collected.
5. Recovery:
   - CO_2 can be recycled, making the process eco-friendly.

Advantages

- High selectivity for lipophilic compounds.
- No toxic solvent residues; safe and environmentally friendly.
- Suitable for thermolabile compounds due to mild extraction temperature.
- High efficiency and purity of extracts.
- Reusable solvent (CO_2), reducing cost and waste.

Limitations

- Requires expensive high-pressure equipment.
- Not suitable for polar compounds without co-solvents.
- Industrial scaling requires expertise in pressure handling.

Applications

- Extraction of essential oils, terpenoids, alkaloids, fatty acids, and carotenoids.
- Used in pharmaceutical, nutraceutical, food, and cosmetic industries.
- Ideal for high-value, sensitive compounds requiring pure extracts.

Pressurized Liquid Extraction (PLE)

- Pressurized Liquid Extraction (PLE) is a modern, high-efficiency extraction technique that uses high pressure and elevated temperature to extract phytochemicals from plant materials.
- PLE is a method in which plant constituents are extracted using a solvent at elevated temperature and pressure, below its critical point, to increase solubility and mass transfer.

Principle

- High temperature increases solute solubility and diffusion rate.
- High pressure keeps the solvent in liquid state above its boiling point.
- Combination of temperature and pressure enhances extraction efficiency, reduces extraction time, and minimizes solvent use.

Apparatus

- Extraction cell: Holds powdered plant material.
- Pump: Delivers solvent under high pressure.
- Heater: Maintains elevated temperature of the solvent.
- Collection system: Collects extract after passage through plant material.
- Optional automation: Modern PLE systems allow programmed cycles.

Procedure

1. Preparation of Plant Material:
    - Dry and finely powdered plant material is preferred.
2. Solvent Selection:
    - Polar or non-polar solvents depending on target compounds (e.g., water, ethanol, methanol, hexane).
3. Loading:
    - Place plant material in extraction cell.
4. Extraction:
    - Solvent is pumped at high pressure and temperature through the cell.
    - Extracted compounds dissolve in the solvent and are collected.
5. Concentration:
    - Evaporate solvent to obtain the crude extract.

Advantages

- Rapid extraction compared to conventional methods.
- Requires less solvent.
- High yield and purity of bioactive compounds.
- Suitable for thermolabile compounds with controlled temperature.
- Can be automated for reproducibility and large-scale extraction.

Limitations

- Requires expensive high-pressure equipment.
- Not suitable for extremely sensitive compounds at high temperatures.
- Operator must have knowledge of pressure handling and safety.

Applications

- Extraction of alkaloids, flavonoids, glycosides, terpenoids, and phenolics.
- Used in pharmacognosy, herbal medicine production, and quality control.
- Applied in nutraceutical, cosmetic, and food industries for high-value plant extracts.

Isolation, Purification, and Identification of Phytochemicals: Spectroscopy

- Spectroscopy is a fundamental analytical technique used in phytochemistry to identify, characterize, and quantify bioactive compounds based on their interaction with electromagnetic radiation.
- It is applied after extraction and preliminary purification to confirm the chemical structure and functional groups of plant constituents.

Principle of Spectroscopy

- Molecules interact with electromagnetic radiation by absorption, emission, or scattering.
- The resulting spectrum provides information about molecular structure, functional groups, and chemical environment.
- Different types of spectroscopy provide different levels of information:
  - UV-Vis: Electronic transitions, conjugated systems
  - IR: Functional groups
  - NMR: Carbon-hydrogen framework, molecular structure
  - MS: Molecular weight, fragmentation pattern

Types of Spectroscopy

A. UV-Visible (UV-Vis) Spectroscopy

- Principle: Molecules absorb UV or visible light, causing electronic transitions ($\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$).
- Applications:
  - Identification and quantification of flavonoids, phenolics, alkaloids.
  - Estimation of total phenolic or flavonoid content in extracts.
- Example: Determining quercetin concentration in plant extract.

B. Infrared (IR) Spectroscopy

- Principle: Molecules absorb infrared radiation causing vibrational transitions of chemical bonds.
- Applications:
  - Identification of functional groups like –OH, –NH_2, –C=O, –COOH.
  - Characterization of glycosides, terpenoids, and alkaloids.
- Example: Confirming –OH group in flavonoid glycosides.

C. Nuclear Magnetic Resonance (NMR) Spectroscopy

- Principle: Certain nuclei (^1H, ^{13}C) resonate in a magnetic field, giving chemical shift data.
- Applications:
  - Detailed molecular structure elucidation.
  - Identification of isomers.
  - Determines carbon-hydrogen framework in alkaloids, steroids, and terpenoids.
- Example: Structure elucidation of morphine or digitoxin.

D. Mass Spectrometry (MS)

- Principle: Molecules are ionized, and the mass-to-charge ratio (m/z) of ions is measured.
- Applications:
  - Determines molecular weight.
  - Provides fragmentation patterns for structural confirmation.
- Example: Identifying molecular weight of alkaloids, flavonoids, and terpenoids.

Role of Spectroscopy in Phytochemistry

1. Identification: Confirms the presence of target compounds.
2. Structural Elucidation: Provides detailed information on functional groups, connectivity, and stereochemistry.
3. Purity Assessment: Detects impurities in crude extracts.
4. Quantification: Measures concentration of active constituents.

Advantages of Spectroscopy

- Non-destructive in many cases.
- Requires small sample amounts.
- Highly sensitive and accurate.
- Rapid and reproducible.
- Can be combined with chromatography for enhanced identification.

Chromatography

- Chromatography is a powerful analytical technique used in phytochemistry to separate, purify, and identify bioactive compounds based on their differential migration between stationary and mobile phases.
- It is used after extraction to isolate individual constituents from crude plant extracts.

Principle of Chromatography

- Separation occurs due to differences in affinity of compounds for the stationary phase (solid or liquid) and solubility in the mobile phase (liquid or gas).
- Compounds with higher affinity for the stationary phase move slower, while those with higher solubility in mobile phase move faster.
- The separation allows identification, purification, and quantification of individual phytochemicals.

Types of Chromatography

A. Thin Layer Chromatography (TLC)

- Principle: Separation based on adsorption on a thin layer of silica gel or alumina.
- Procedure:
  1. Spot extract on TLC plate.
  2. Develop in solvent system (mobile phase).
  3. Visualize spots under UV light or by spraying with detecting reagents.
- Applications:
  - Identification of alkaloids, flavonoids, glycosides.
  - Monitoring purity of extracts.

B. Column Chromatography

- Principle: Separation of compounds as they pass through a column packed with stationary phase (silica, alumina).
- Procedure:
    1. Pack column with adsorbent.
    2. Load crude extract on top.
    3. Elute with appropriate solvent or gradient.
    4. Collect fractions and analyze by TLC.
- Applications:
    - Isolation and purification of individual phytochemicals.
    - Large-scale separation for herbal drug preparation.

C. High Performance Liquid Chromatography (HPLC)

- Principle: Separation occurs under high pressure using a liquid mobile phase and fine particle stationary phase.
- Applications:
    - Quantitative analysis of alkaloids, flavonoids, glycosides, phenolics.
    - Quality control of herbal formulations.
- Advantage: Highly sensitive, reproducible, and precise.

D. Gas Chromatography (GC)

- Principle: Separation of volatile compounds in a gas mobile phase through a column with stationary phase.
- Applications:
    - Analysis of essential oils, fatty acids, and terpenoids.
    - Identification of volatile phytochemicals in plant extracts.

Role of Chromatography in Phytochemistry

1. Separation: Isolates individual components from complex mixtures.
2. Identification: Compares retention time or Rf value with standards.
3. Purification: Collects pure fractions for further analysis.
4. Quantification: HPLC and GC allow accurate measurement of constituents.

Advantages

- High resolution and sensitivity.
- Applicable to complex mixtures.
- Can be analytical (identification) or preparative (purification).
- Can be combined with spectroscopy (TLC-MS, HPLC-MS) for structural elucidation.

Limitations

- Requires suitable solvents and stationary phases.
- Some methods are expensive (HPLC, GC).
- GC is only for volatile or derivatized compounds.

Electrophoresis

- Electrophoresis is a modern analytical technique used to separate and identify biomolecules based on their charge, size, and mass under an electric field.
- It is often applied in phytochemistry for proteins, glycoproteins, nucleic acids, and charged secondary metabolites.

Principle

- Molecules in a solution or gel matrix move under an electric field according to their net charge and size.
- Cations migrate towards the cathode, and anions migrate towards the anode.
- Separation occurs due to differences in mobility, which is influenced by molecular weight, shape, and charge.

Types of Electrophoresis

A. Paper Electrophoresis

- Matrix: Filter paper soaked with buffer.
- Applications: Separation of small ionic compounds like alkaloids, amino acids, and sugars.
- Detection: Use dyes or reagents to visualize separated components.

B. Gel Electrophoresis

- Matrix: Agarose or polyacrylamide gel.
- Applications:
  - Agarose gel: Separation of nucleic acids (DNA, RNA).
  - Polyacrylamide gel (PAGE): Separation of proteins and glycoproteins.
- Detection: Staining with Coomassie Blue, silver stain, or ethidium bromide.

C. Capillary Electrophoresis (CE)

- Principle: Separation of molecules in a capillary tube under high voltage.
- Applications:
  - High-resolution analysis of alkaloids, amino acids, flavonoids.
  - Quantitative and reproducible separation in small sample volumes.

Role of Electrophoresis in Phytochemistry

1. Separation: Resolves complex mixtures of biomolecules or charged metabolites.
2. Purification: Isolates individual compounds for further analysis.
3. Identification: Based on mobility, staining pattern, and comparison with standards.
4. Quality control: Confirms the presence or absence of specific bioactive compounds in extracts.

Advantages

- High resolution for charged molecules.
- Can separate very small quantities.
- Compatible with further analysis (spectroscopy, mass spectrometry).
- Rapid and reproducible, especially in capillary electrophoresis.

Limitations

- Limited to charged molecules; neutral compounds require modification.
- Requires specialized equipment and buffers.
- Detection often needs staining or derivatization.