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Diploma in Pharmacy 1st Year
Pharmacognosy
Chapter 11 : Phytochemical Investigation of Drugs

Chapter 11

Phytochemical Investigation of Drugs

→ Phytochemistry involves the study of chemicals (mainly the secondary metabolites) the plants produce as a measure to protect themselves from insects, pests, pathogens, herbivores, UV exposure, and environmental hazards.

→ Phytochemistty includes the structural compositions, the biosynthetic pathways, functions, mechanism of actions in the living systems, and the medicinal, industrial, and commercial applications of secondary metabolites.

→ Phytochemicals (derived from the Greek word phyto meaning plant) are naturally occurring and biologically active chemical compounds present in plants.

→ They provide health benefits for humans in addition to those provided by the macronutrients and micronutrients.

Extraction of Drugs

- The process of separating medicinal By active constituents of plant and animal tissues with the help of selective solvents and standard procedures is termed extraction.
- The extracted products of plant tissues obtained in liquid or semisolid state (after removing the solvent) or in dry powdered form are complex mixtures of metabolites; these products are meant for oral or external use.
- The extracted preparations include decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts, or powdered extracts; these preparations are named as galenicals after Galen (a Greek physician of 2^{nd} century)
- The standardised extraction procedures involve treatment with a selective solvent (menstruum) to yield the therapeutically active constituents of crude drugs, removing the inactive ones.
- The undissolved residue left behind is termed marc.
- The menstruum used for extraction should have the following properties:
    - Chemically and physically inert,
    - Non-toxic,
    - Inexpensive, and
    - Selective, i.e., it should dissolve the desired active constituents with a minimum of the inert material
- Water, ethanol, and their mixtures are most commonly used as they fulfil the above mentioned considerations.
- The drug extraction process is divided into the following four steps:
    - The solvent penetrates the drug,
    - The drug constituents dissolve in the solvent,
    - The solution within the cells diffuses out, and
    - The dissolved portion separates from the exhausted drug.

Modern Methods of Extraction

→ Extraction of crude drugs can be carried out by various processes, and the selection of process depends on the chemical properties of the drug's active constituents.

→ Various extraction methods employed are:

1. Maceration,
2. Digestion,
3. Percolation,
4. Continuous hot extraction (Soxhlet extraction),
5. Supercritical fluid extraction,
6. Counter current extraction,
7. Microwave assisted extraction,
8. Ultrasonic assisted extraction,
9. Infusion and decoction, and
10. Pressure cooker extraction.

Maceration

- The word maceration denotes softening. The maceration process (or Process M) is used for producing tinctures, extracts, and concentrated infusions.
- It is the simplest method of crude drug extraction, which was official in I.P., 1966.

Digestion

- Digestion is a modified maceration process.
- It involves extraction at such a high temperature which does not put adverse effects on the active ingredients.
- Higher temperature enhances the solvent action of menstruum and constant mechanical agitation of the system speed up the attainment of equilibrium.
- If at the used temperature the menstruum gets volatilised easily, a reflux condenser should be attached to the vessel in which the digestion process is being performed; this facilitates the condensation of menstruum, so that it can be recovered and returned back to the container.

Percolation

- The term percolation has been derived from the Greek word percolare which means to pass through.
- Percolation (or Process P) is also termed lixivation.
- It involves extracting the constituents of granulated or powdered drug by slowly passing down through it a suitable menstruum.
- The menstruum while travelling down the drug column under the influence of gravity, extracts the drug particles layer-wise, which are further replaced with the layers above as it moves downwards.
- Percolation method achieves complete drug extraction.

Supercritical Fuid Extraction (SFE)

- The process of SFE involves separating one component (i.e., the extractant) from another (i.e., the matrix) using supercritical fluids (i.e., the extracting solvent).
- The extraction is generally from a solid matrix or from liquids.
- Supercritical fluid is a substance at temperature and pressure above its critical point. It can diffuse through solids like a gas and dissolve materials like a liquid.
- It can be suitably used as a substitute for organic solvents in various industries and laboratory processes. The most Commonly used supercritical fluid are carbon dioxide and water which are used for decaffeination and power generation , respectively How ever Modified Co- solvent such as ethanol or methanol can also be used

Advantage

- ✓ SFE determines the rate at which the extraction can be performed.
- ✓ The SFE process completes within 20-60 minute
- ✓ SFE produces less waste solvent

Disadvantages

- ♦ Carbon dioxide (the most commonly used solvent in SFE) cannot be used for extracting polar compounds due to its low polarity.
- ♦ Presence of water in SFE process may cause problems.
- ♦ In SFE, the matrix effect is unpredictable.
- ♦ SFE process requires specialised/expensive equipment.

Counter Current Extraction (CCE)

→ In CCE, toothed disc disintegrators are used to pulverise the wet raw material to be extracted.
→ Fine slurry is obtained which is moved in one direction within a cylindrical extractor.
→ Here the slurry comes in contact with the extraction solvent.
→ The farther the slurry moves, the more concentrated the extract becomes.
→ When the quantities of solvent and material and their flow rates are optimised, complete extraction is concentrated extract is received at one end of the extractor while the marc (which is free of visible solvent) falls out from the other end.

Microwave Assisted Extraction (MAE)

- Microwaves are non-ionising electromagnetic waves present in the electromagnetic spectrum between X-rays and infrared rays.
- They are made up of the electric and magnetic field (two oscillating perpendicular fields), of which the former is responsible for heating

Infusion and Decoction

- These methods are now rarely used. Infusions were prepared from vegetable drugs with water-soluble and easily extractable constituents; and decoction process was used for extracting vegetable drugs with water-soluble and heat soluble constituents. In the infusion process, the drug was moistened with water, macerated with boiling water, the liquid was strained, and desired volume was made.)

Pressure Cooker Extraction

In this method, the drug is initially macerated with the menstruum and then is held for 5-15 minutes in a pressure cooker at 151b/sq. inch pressure.

The cooker is then cooled and the extract is removed by straining and pressing the marc.

This method achieves complete drug extraction in comparatively less time.

However, it cannot be used for drugs with thermolabile constituents.

Isolation, Purification, and Identification of Drugs

- The progress in the isolation and analysis techniques has led to the identification of many unknown compounds.
- For isolating a particular compound from its plant material, various processes are involved.
- These isolation processes depend on the nature of the active constituents present in the crude drug.
- After isolation, identification, and purification of a plant constituent, the class of the compounds and the particular substance within that class should also be determined.
- Complete identification depends on measuring other properties (such as melting point, boiling point, optical, rotation, and retention factor) and comparing the data with those in standard literature with techniques of Ultraviolet Uv), Infrared(IR), Nuclear Magnetic Resonance (NMR). and Mass Spectral (MS) measurements.
- For the final confirmation of a known plant compound that has been identified on the above basis is compared with the standard sample, while the identity of new compounds is confirmed by chemical degradation or by the synthesis of compound in laboratory

Spectroscopy

- Spectroscopy involves the study of interaction of electromagnetic radiation with matter.
- The various spectroscopic methods that are utilised for identification of natural compounds are:
- Ultraviolet (UV) and Visible Spectroscopy

- Ultraviolet (UV) and visible absorption techniques involve the analytical methods which measure the light absorption lying in the wavelength region from 190-900nm by different substances.
- The wavelength region from 190- 380nm is the UV region and that from 380-900nmis the visible region of the electromagnetic spectrum.
- Absoption in the UV-visible region results from the electronic transitions within the molecule.
- The separation step and absorption measurement in UV- Visible region is the most widely used analytical procedure in the field of pharmacy.
- The UV-spectrum along with other analytical data often serves as a tool for identity Confirmation and is also used as a detector in HPLC.
- The absorption spectra of every plant constituent are different n specific range of visible light specimen.
- A very dilute Solution should be used, and the wavelength should be recorded in nanometres with maximum and minimum of the peak.

Chromatography

- Chromatography is the most versatile separation technique and involves separation of two or more substances by distribution between a fixed (or stationary) phase and amoving (or mobile) phase.
- Chromatography is used for separation, isolation, purification, and identification of components in a mixture.
- Plant materials can be separated and purified using variouis chromatographic complex system of mixtures.
- Thus, the identification methods for botanical drugs obtain a characteristic fingerprint of a specificplant that shows the presence of a particular Chromatographic techniques like High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Gas Chromatography-Mass Spectrometry (GC-MS), and Thin Layer Chromatography (TLC) are widely used for such purpose.

Electrophoresis

- The technique of electrophoresis involves migration of a charged particle under the influence of electric field (electro-charged particle and phoresis-movement).
- For separating mixtures by electrophoresis, a filter paper strip saturated with an electrolyte solution (a buffer buffer (a solution)is supportedin the centre and its two ends are dipped into solutions having immersed electrodes.
- After placing a spot of the material to be fractionated on the paper, the whole apparatus is sealed and a potential difference of 2-10volts/cm is applied along the paper.
- Some mixtures are separated at higher voltages.

- The solutes move towards the anode or cathode based on the nature of charge on the ions of solute mixture.
- Thus, the amino acids can be separated into either groups into either groups (acidic, neutral or basic group) or individual amino acids.
- A substance's migration velocity magnitude of ionic charge and the size and shape of the depends on the particular molecule.
- The filter paper used can also be replaced with thin layers of gels, and the process is then termed gel filtration.
- Many alkaloidal mixtures, plant acids, component sugars of cardiac glycosides, and anthraquinone derivatives have been separated by this method.

THANK YOU