



Isolation And Purification Techniques Of Advancement Herbal Technology

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ABSTRACT:

Natural Products from medicinal plants either as pure or as a standardized extracts provide unlimited opportunities for new drugs because of the unmatched availability of chemical constituents. Since ancient times, natural products have been utilized to treat and cure chronic diseases like cancer, diabetes, asthma, anti-inflammatory, analgesic and as alternatives for hormone replacement therapy worldwide. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Despite their widespread existence, the variety of bioactive natural products in natural medicines are not sufficient. Today, it is very crucial to develop effective and selective methods for the extraction and isolation of new natural products. The focus of this review paper is to provide a comprehensive view on the analytical methodologies, which include extraction, isolation and characterization of the natural products from medicinal plants and common phytochemical screening assays.

KEYWORDS: Bioactive Compounds, Extraction Methods, Medicinal plants, Natural Products.

OBJECTIVES:

- Isolation techniques aim to improve the purity of a substance by removing unwanted impurities or contaminants,
- Ensuring the isolated compound is suitable for further analysis or applications.

- Chromatography is a technique used to separate and identify individual components in a mixture based on their differential partitioning between a stationary phase and a mobile phase.
- HPTLC improves upon traditional TLC by providing higher resolution and sensitivity, making it suitable for more complex mixtures and trace analysis.
- Purification methods are designed to improve the purity of a substance, often achieving a level of purity necessary for specific analytical or industrial applications.

I. General Isolation Techniques

• Extraction Methods: -

Extraction can be defined as the removal of soluble material from an insoluble Residue, either liquid or solid, by treatment with a liquid solvent. It is therefore, a solution Process and depends on the mass transfer phenomena. The controlling factor in the rate of Extraction is normally the rate of diffusion of the solute through the liquid boundary layer at The interface. The principal methods of extraction are –

Methods of extraction:

a) Traditional Methods

- Maceration.
- Digestion.
- Decoction.
- Infusion.
- Percolation.
- Continuous hot extraction (Soxhlet extraction).

b) Modern Methods

- Supercritical fluid extraction.
- Microwave assisted extraction.
- Ultrasonic assisted extraction.

A. Maceration: -

- In this process, solid plants parts are placed in a stoppered container with the whole of the solvent and allowed to stand for a period of at least 3 days (3 - 7 days) with frequent agitation, until soluble matter is dissolved.
- The mixture is then strained (through sieves / nets), the marc pressed and the combined liquids clarified (cleaned by filtration) or by decantation, after standing. When the solvent is water and the period of maceration is long, a small quantity of alcohol may be added to prevent microbial growth.

B. Digestion: -

- This is a form of maceration in which gentle heat (40- 60oC) is applied during the process of extraction. It is used when moderately elevated temperature is not objectionable.
- The process may be modified by mixing the material with the solvent using magnetic stirrer, mechanical stirrer or by shaking occasionally by hand. After 8 to12 hours, the extract is filtered, and fresh solvent is added and the process repeated till all the desired products are extracted.

C. Decoction: -

- In this process, the powdered plant materials is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered.
- This procedure is suitable for extracting water-soluble, heat-stable constituents. This process is typically used in preparation of Ayurvedic extracts called 'quath' or 'kawath'.
- The starting ratio of crude drug to water is fixed, e.g. 1:4 or 1:16; the volume is then brought down to one-fourth to its original volume by boiling during the extraction procedure.
- Then, the concentrated extract is filtered and used as such or processed further.

D. Infusion: -

- In this extraction process, the plant material is macerated for a short period of time with either cold or boiling water.
- It is a dilute solution of the readily soluble components of the crude drugs.

E. Percolation: -

- This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts.

- The plant material is taken in a percolation tube plugged with cotton or fitted with a filter and a stopcock.
- Solvent is added into the plant material allowed to stand for approximately 4 hour in a well closed container, after which the mass is packed and the top of the percolator is closed.
- The whole system is kept for 24 hour at room temperature and the solvent along with the extracted material is collected by opening the stopper below and mixed liquid is clarified by filtration or by standing followed by decanting.

F. Soxhlet Extraction: -

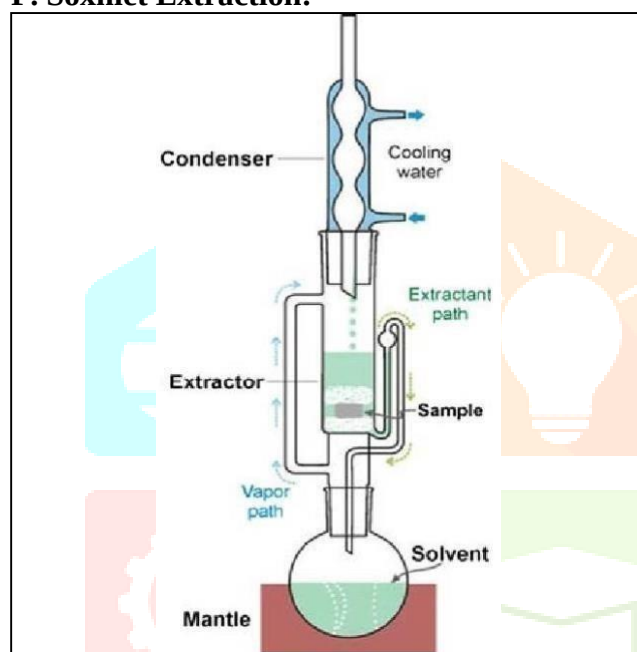


Figure: Soxhlet Extraction Method

- Named after 'Franz Ritter von Soxhlet', a German agricultural chemist, it is the best method for the continuous extraction of a solid by a hot solvent.
- Soxhlet apparatus is a specialized glass refluxing unit mainly used for organic solvent extractions.

The apparatus is fitted to a round bottomed (RB) flask containing the solvent and to a reflex condenser.

The solvent in the RB flask is boiled gently, the vapor passes up through the side tube, condensed by the condenser and falls into the thimble containing the material and slowly fills the soxhlet.

- When the solvent reaches the top of the attached tube it siphons over into the flask, thus removes the portion of the substance, which it has extracted.

G. Supercritical Fluid Extraction: -

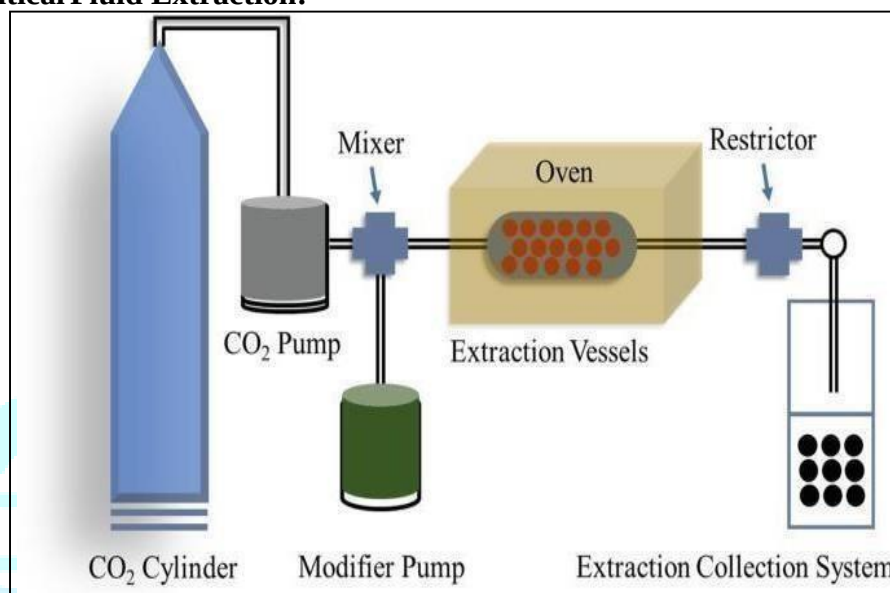


Figure: Supercritical Fluid Extraction

The critical point of a pure substance is defined as the highest temperature and pressure at which the substance can exist in vapour-liquid equilibrium i.e. physical and thermal properties that are between those of pure liquid and gas.

- SCF's offers liquid like densities, gas like viscosities, gas like compressibility properties and higher diffusivities than liquid.
 - 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.
- The most commonly used supercritical fluids are carbon dioxide (CO₂) and water, which are used for decaffeination and power generation, respectively. The critical point of a pure substance is defined as the highest temperature and pressure at which the substance can exist in vapour- liquid equilibrium i.e. physical and thermal properties that are between those of pure liquid and gas.
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Advantages: -

- Dissolving power of the SCF is controlled by pressure and/or temperature.
- SCF is easily recoverable from the extract due to its volatility.
- Non-toxic solvents leave no harmful residue.
- High boiling components are extracted at relatively low temperatures.
- Separations not possible by more traditional processes can sometimes be effected.
- Thermally labile compounds can be extracted with minimal damage as low temperatures can be employed by the extraction.

Disadvantages: -

- Elevated pressure required.
- Compression of solvent requires elaborate recycling measures to reduce energy costs.
- High capital investment for equipment.

Applications: -

- Recovery of organics from oil shale.
- Separations of biological fluids.

Bioseparation.

Petroleum recovery.

- Crude dewaxing.
 - Coal processing (reactive extraction and liquefaction).
 - Selective extraction of fragrances, oils and impurities from agricultural and food products
- Pollution control.
- Combustion and many other.

H. Microwave Assisted Extraction: -

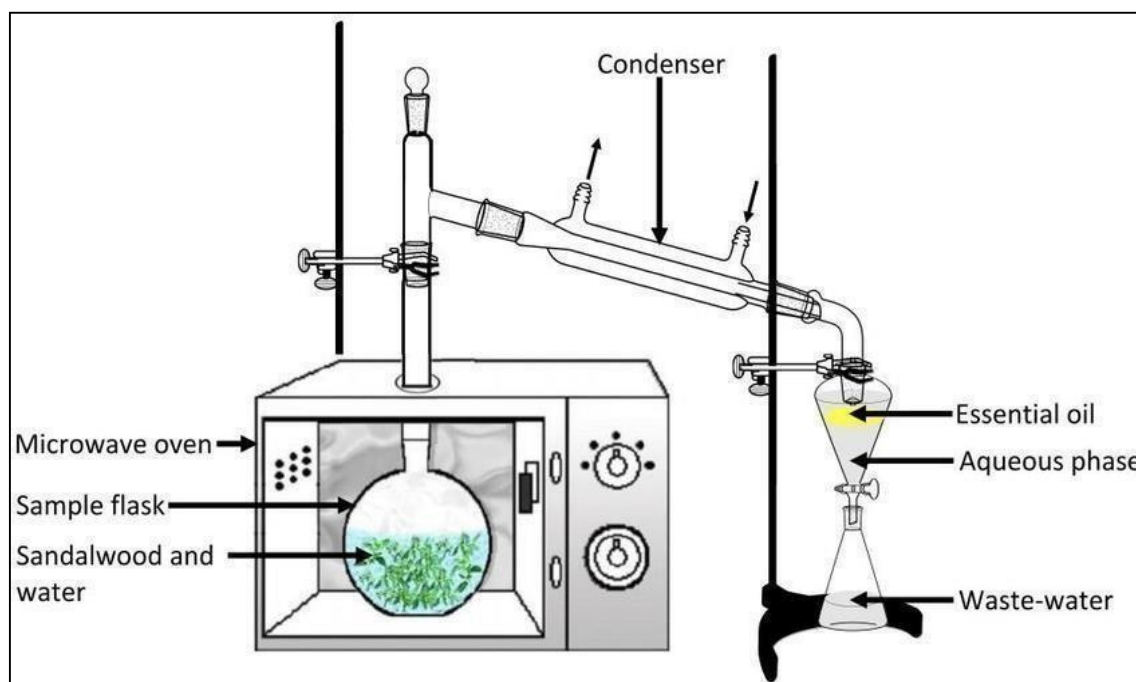


Figure: Microwave Assisted Extraction

Microwaves (frequency 300MHz to 300GHz) are non-ionizing 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.
- Mostly the dried plant materials are used for extraction; however, the plant cells containing microscopic traces of moisture also serve as the target for microwave heating.

As a result, the plant cells swell up and exert pressure on the cell wall.

Under the influence of this pressure, the cells stretch and ultimately rupture, thus leaching out the active constituents into the surrounding solvent and improving the yield of phytoconstituents.

- Common solvent mixture is hexane acetone.

Advantages: -

- It is less time consuming as it takes only a few seconds to few minutes (15-20 minutes) to complete the extraction process.
- It requires less solvent (only a few milliliters). It improves the extraction yield.
- Since it is an automatic process, it provides better accuracy and precision.
- It can be used for the extraction of thermolabile constituents.

- It can be used for extracting minute traces of constituents including heavy metals and pesticide residues from a few milligrams of plant sample.
- It provides agitation during extraction, which improves the phenomenon of mass transfer.
- Since its instrumental set up (like Soxhlet) combines the features of Soxhlet as well as benefits of microwave, it makes extraction even more attractive.

Disadvantages: -

- An A more filtration or centrifugation is essential to withdraw the solid residues during MAE.
- Furthermore, the efficiency of microwaves can be extremely poor when either the mark compounds or the solvents are non-polar, or when they are volatile.

Application: -

Extracted logically involved mixtures extracted by microwave- enabled Technique.

- Extraction of taxanes from *Taxus brevifolia* needles.
 - Azadiractin related luminous from *Azadirachta indica* seed kernels.
- Extraction of mycorrhizal acid from *Glycyrrhizia glabra* origins.

Extraction of artemisinin from *Artemisia annual*.

An increased microwave temperature and a short extraction time are better effective in removing antioxidative phenolic compounds from tomato usinug MAE.

- MAE was verified as a potential to alternative to conventional methods for extraction of phenols such as chlorogenic acids from green coffee beans.

I. Ultrasound Assisted Extraction: -

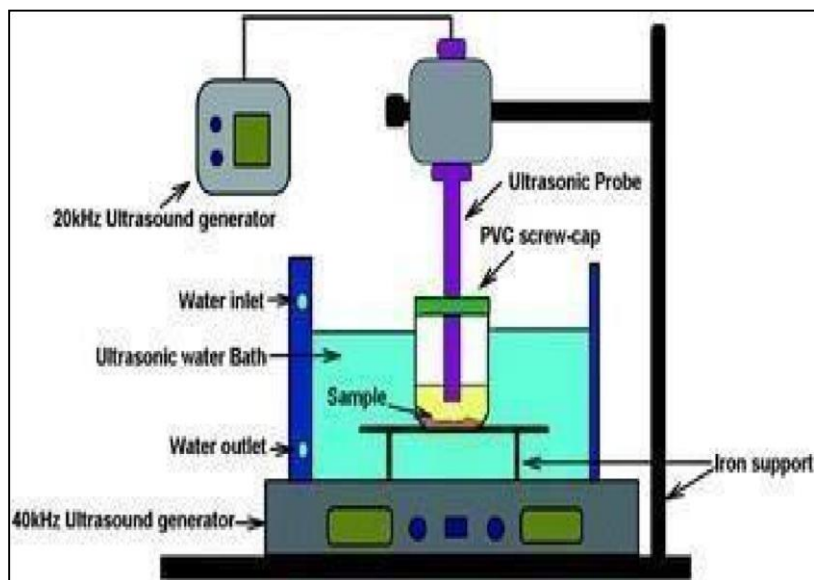


Figure: Ultrasound assisted extraction

The commonness above the 20,000 Hz are known as ultrasound.

- Ultrasonic waves are using in ultrasonic extraction.
 - These waves reason cavitations consequence on the dry cell and destruct the cell wall and release the active members.
 - When ultrasonic waves are passed through the liquid media it compresses (produce high pressure) and reaction (low pressure) to the liquid media.
 - Due to this process small holes or vacuum beads are formed in the solvent.
 - After specific duration these bubbles are not able to soak more power produce by microwave and they burst.
 - At the high tension cycle they burst which is known as cavitation.
- Due to this cavitation cell wall destructed and functional chemical constituent are extracted.

Advantages: -

- It is an a reasonable, easy and efficient choice to traditional extractions technique.
- It contain the increase of extraction result and quicker kinetics.
- It decrease the operating temperature permitting the extraction of thermolabile compounds.
- Compared with different novel extraction techniques such as microwaves assisted extraction, the ultrasound machine is cheaper and its process is easier.

Disadvantages: -

- The active constituents of medicinal plants through formation of free revolutionaries and consequently unpleasant changes in the drug molecules.

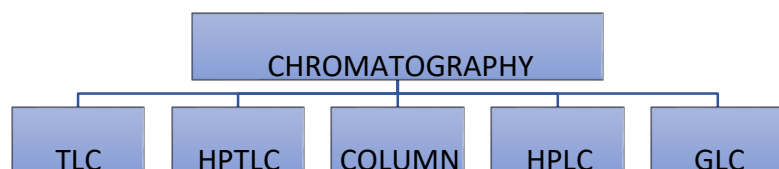
- The free revolutionaries may have undesirable side effect.

Applications: -

- Used to extract nutraceuticals from plants such as essential oils and lipids dietary complements. e.g. oils from almond, apricot and rice bran.
- Extraction of saponins from ginseng, the experimental total result and saponin yield increased by 15 and 30%, respectively.
- It was found that rice bran oil extraction can be efficiently conducted in 30 min under high intensity ultrasound either utilizing hexane or a fundamental aqueous explanation..
- e.g. oils from almond, apricot and rice bran.
- Extraction of saponins from ginseng, the empirical total yield and saponin earnings increased by 15 and 30%, respectively.

II. Chromatographic Techniques

- Chromatography represents the most versatile separation technique and readily available. Chromatography is Defined as technique of isolation and identification of components or compounds or mixture of it's into individual Components by using stationary phase and mobile phase.
- Plant materials are separated and purified by using various Chromatographic techniques. Herbal medicine is a complicated system of mixtures.
- Thus, the methods of choice for Identification of ' botanical drug' are mainly intended to obtain a characteristic fingerprint of a specific plant that Represent the presence of a particular quality defining chemical constituents.
- Chemical fingerprints obtained by chromatographic technique and especially by hyphenated chromatography, are Strongly recommended for the purpose of quality control of herbal medicines, since they might represent Appropriately the "chemical integrities" of the herbal medicines and therefore be used for authentication and Identification of the herbal products.
- Thin layer chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC).



A. Thin Layer Chromatography (TLC): -

- TLC is the most commonly used planar chromatographic method in natural product research.
- This is the easiest and cheapest technique and can be applied in the analysis, isolation and setting the parameters for column chromatography.
- Usually, silica or alumina (more polar) is used as the stationary phase and organic solvents (less polar) are used as the mobile phase.
- This situation is categorized as normal phase chromatography. In contrast to this, reverse phase TLC is available, in which stationary phase is alkyl bonded silica or alumina (less polar) and mobile phase is polar solvent like water, alcohol etc.

A. Column Chromatography (CC): -

- Column chromatography is the most effective technique used in separation of crude plant extracts into its components in pure form.
- This is a preparative chromatographic method and the stationary phase (silica gel) is packed in a column and then the mobile phase (eluent) is passed through the column after loading the extracts on the top of the stationary phase.
- The mobile phase carries the natural products present in the mixture at different rate based on their affinities to the stationary and mobile phase.
- Separated compounds can be collected along with the mobile phase.

B. High Performance Thin Layer Chromatography (HPTLC): -

- It is a planar chromatography where separation of natural compounds is achieved on high performance layers with detection and data acquisition.
- These high-performance layers are pre-coated plates coated with a sorbent of particle size 5-7 microns and a layer thickness of 150-200 microns.

- HPTLC plates are substantially more expensive (4- to 6-times more) than normal plates but are an efficient alternative when high sensitivity, accuracy and precision are required in situations demanding high performance.

C. High Performance Liquid Chromatography (HPLC): -

- D. It is a versatile, robust, and widely used technique for the isolation of natural products.
- E. HPLC is an analytical technique for the separation and determination of organic and inorganic solutes in any samples especially biological, pharmaceutical, food, environmental, industrial etc.
- F. Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of medicinal plants.

In order to identify any compound by HPLC, a detector must first be selected, The extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase.

- G. Modern HPLC uses a non-polar solid phase, like C18 and a polar liquid phase, generally a mixture of water and another solvent.
- H. High pressure up to 400 bars is required to elute the analyte through column before they pass through a diode array detector (DAD).
- I. A DAD measures the absorption spectra of the analytes to aid in their identification.
- J. HPLC is useful for compounds that cannot be vaporized or that decompose under high temperature and it provides a good complement to gas chromatography for detection of compounds.

IV. Purification Technique Of Phytoconstituent's

The separation of phytochemicals is a process of isolating the constituents of plant extracts or effective parts one by one and purifying them into monomer compounds by physical and chemical methods. Classical isolation methods, including solvent extraction, precipitation, crystallization, fractional distillation, salting out, and dialysis, are still used commonly at present. On the other hand, modern separation technologies such as column chromatography, high performance liquid chromatography, ultrafiltration, and high-performance liquid drop counter current chromatography also play an important role in the separation of phytochemicals. This section describes the common methods and their specific applications in isolation of phytochemicals.

• Solvent method:

A. Acid and basic solvent method: -

- It is carried out according to the different acidity and alkalinity of each component in the mixture.
- Water insoluble alkaline organic components, such as alkaloids, could react with inorganic acids and form salts, which can be separated from non-alkaline and water-insoluble components.
- Acid components with carboxyl or phenolic hydroxyl groups can be salted by bases and dissolved in water.
- Components with lactone or lactam substructures can be saponified and dissolved in water and then isolated from other water-insoluble components.
- The total extract can be dissolved in lipophilic organic solvents (ethyl acetate is commonly used) and extracted respectively with acid water and alkali water, and then the total extract would be divided into acidic, alkaline, and neutral parts.
- Of course, the total extract can also be dissolved in water and extracted with organic solvents after adjusting the pH value.
- The alkalinity or acidity of the fractions are different and can be separated further by pH gradient extraction.
- When using the acid and basic solvent method, attention should be paid to the strength of acidity or alkalinity, the contact time with the separated components, heating temperature, and time, so as to avoid the structural changes of some compounds under severe conditions or the chemical structures cannot be restored to the original states.

B. Polarity gradient extraction method: -

- This method is to achieve the separation aim based on the different polarity of each component in plant extracts and the different partition coefficients in two-phase solvents.
- Generally, different two-phase solvent systems are selected according to the polarity of components in plant extracts.
- For example, the components with strong polarity can be separated by n-butanol-water system, the components with medium polarity can be separated by ethyl acetate-water system, and the components with weak polarity can be separated by chloroform (or ether)water system.
- During the operation, the plant extract should be dissolved by water firstly, and then the solution or suspension is extracted in a separating funnel with different organic solvent which is not miscible with water based on the polarity difference.

- Usually, the extract was extracted with petroleum ether (or cyclohexane) firstly, then ethyl acetate (or chloroform), and finally with water saturated n-butanol, as shown in Petroleum ether layer contains lipid-soluble compounds with low polarity.

Ethyl acetate layer contains medium polar compounds such as monoglycosides, flavonoids, and compounds with more polar functional groups.

- N-butanol layer contains compounds with strong polarity, such as oligoglycosides and other water-soluble components.
- Compounds in water layer possess strongest polarity, such as glycosides with more glycosyl groups, carbohydrates, amino acids, proteins, and other water-soluble compounds.

C. Precipitation method: -

- It is a method based on the formation of precipitation of some phytochemicals by reaction with specific reagents, or the precipitation of some components from the solution by adding specific reagents, which can reduce the solubility of some components in the solution.
- The precipitation reaction must be reversible if the target components are required to form precipitation.
- While if the components are nontarget, the precipitation generated will be removed, so the precipitation reaction can be irreversible.
- According to the addition of reagents or solvents, this method could be classified as follows onents in the mixed component solution can be changed by adding a specific solvent that can be mutually soluble with the solution, so it can be precipitated from the solution.
- For example, using water as an extracting solvent to extract phytochemicals, ethanol is added to the water extracting concentrate to make its alcohol content more than 80%, and then polysaccharides, proteins, starch, gum, and so on will be precipitated and removed after filtration.
- The preceding procedure is called water extraction and ethanol precipitation. Crude polysaccharides from plants are often separated with this method.

V. Introduction To Different Techniques Of Characterization Of Bioactive Constituents

Determination of the structure of natural products uses data from a wide range of spectroscopic techniques such as UV-Visible, Infrared (IR), Nuclear Magnetic Resonance (NMR) and Mass spectroscopy. The basic principle of spectroscopy is passing electromagnetic radiation through an organic compound that absorbs some of the radiation, but not all. By measuring the amount of absorption of electromagnetic radiation, a spectrum can be produced. The spectra are specific to certain bonds in a compound. Depending on these spectra, the structure of the natural compound can be identified. Scientists mainly use spectra produced from either three or four regions—Ultraviolet (UV), Visible, Infrared (IR), Radio frequency (FTIR), and electron beam for structural clarification.

A. UV-Visible Spectroscopy: -

- UV-visible spectroscopy can be performed for qualitative analysis and for identification of certain classes of compounds in both pure and biological mixtures.
- Preferentially, UV-visible spectroscopy can be used for quantitative analysis because aromatic molecules are powerful chromophores in the UV range.
- Natural compounds can be determined by using UV-visible spectroscopy.
- Moreover, spectroscopic UV-Visible techniques were found to be less selective and give information on the composition of the total polyphenol content

B. Fourier Transform Infrared Spectroscopy (FTIR): -

- Fourier- transform infrared spectroscopy is a valuable tool for the identification of functional groups present in the plant extract.
- It helps for identification and structure determination of the molecule. It is a high-resolution analytical tool to identify the chemical constituents and elucidate the structural compounds.
- FTIR offers a rapid and non-destructive investigation to fingerprint herbal extracts or powders.

C. Nuclear Magnetic Resonance Spectroscopy (NMR):-

Nuclear Magnetic Resonance Spectroscopy gives physical, chemical and biological properties of matter.

- One dimensional technique is routinely used but the complicated structure of the molecules could be achieved through two-dimensional NMR techniques.

- Solid state NMR spectroscopy is used for the determination of molecular structure of solids. Radiolabelled ^{13}C NMR is used to identify the types of carbon are present in the compound.
- ^1H -NMR is used to find out types of hydrogen are present in the compound and to find out how the hydrogen atoms are connected.

D. Mass Spectroscopy: -

- Mass spectrometry is a powerful analytical technique for the identification of unknown compounds, quantification of known compounds and to elucidate the structure and chemical properties of molecules. • Through MS spectrum, the molecular weight of sample can be determined.
- This method mostly employed for the structural elucidation of organic compounds, for peptide or oligonucleotide sequencing and for monitoring the existence of previously characterized.

Outcomes:

1. Covering fundamental methods for separating compounds from natural sources.
2. Exploring separation methods based on differences in components' distribution between a stationary and a mobile phase.
3. Delving into specific chromatographic techniques like Thin-Layer Chromatography, High-Performance Thin-Layer Chromatography, and High-Performance Liquid Chromatography.
4. Understanding methods to refine and purify bioactive compounds obtained through isolation processes.

Conclusion:

Since natural products from plant extracts usually contain various component mixtures with different polarities, their separation creates a big challenge for the process of identification and characterization. Extraction plays an important role in separation and characterization of different natural products. Practically most of them have to be purified by the combination of several chromatographic as well as non-chromatographic techniques and various other purification methods to isolate natural product and different techniques of characterization of bioactive constituents. In conclusion, the isolation and purification techniques employed in herbal drug technology play a pivotal role in enhancing the quality, safety, and efficacy of medicinal plant-derived products.

Through these processes, impurities are removed, leading to increased purity and the concentration of bioactive compounds essential for therapeutic effects. This not only ensures batch-to-batch consistency and adherence to regulatory standards but also contributes to the standardization of herbal drugs. The identification and isolation of active compounds enable a better understanding of the pharmacological properties, allowing for the development of targeted and efficacious formulations.

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