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From Plant Extract to Pure Molecules Modern Approaches to Phytochemical Isolation and Structural Elucidation of Bioactive Constituents

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INTRODUCTION

Plant extracts, whether pure or standardized, offer unrivalled chemical diversity, making them ideal for discovering novel drugs The World Health Organization (WHO) reports that over 80% of the global population relies on traditional medicine for primary healthcare. Herbal remedies have a long history of use in Asia, reflecting human relationships with nature. Traditional medicine plants contain many substances that can heal both chronic and infectious disorders Medicinal plants have long been used in traditional medicine to provide medicinal benefits. These resources have helped find and produce bioactive chemicals with various pharmacological properties. Medicinal plants have a diverse range of chemical ingredients that can provide health advantages. Medicinal plants include several bioactive compounds, including alkaloids, flavonoids, terpenoids, phenolics, and essential oils. These compounds' pharmacological properties, including antidiarrheal, analgesic, antibacterial, antioxidant, anti-inflammatory, anticancer, and immunomodulatory effects, have been extensively studied. Traditional methods have employed medicinal herbs as crude extracts since prehistoric times. The medicinal impact of plants is due to unique bioactive elements found in small amounts. Modern extraction techniques such as supercritical fluid extraction, microwave assisted extraction, column chromatography, HPLC, and HPTLC, as well as structural elucidation using UV, NMR, and IR MASS SPECTROSCOPY, are used to isolate pure molecules. Phytochemical screening of plant extracts identifies constituent elements through qualitative and quantitative testing and analysis. TLC, HPLC, GC-MS, NMR, and UV-Visible spectrophotometry are necessary to identify and quantify phytochemicals in extracts. To better comprehend the therapeutic potential of plants and create safe and effective herbal medications and natural health products, it's crucial to extract herbs and separate them chemically.

Plant resources have been used to cure human ailments for many years.

Several plant species, including opium (Papaver somniferum), myrrh (Commiphora species), and liquorice (Glycyrrhiza glabra), were mentioned on clay tablets from Mesopotamia in 2600 BC. These plants are still used in herbal formulations to treat diseases.

Organic compounds from natural sources have been used to treat diseases in their natural form and as lead molecules for developing synthetic and semisynthetic analogs with improved draggability. Some active ingredients used in therapeutic applications include morphine, codeine, noscapine, papaverine, quinine, artemisinin, and paclitaxel.

Innovative methods for extracting and isolating bioactive chemicals from plants are gaining popularity in research and development. Currently available and environmentally sustainable technologies include ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), and microwave-assisted extraction (MAE) greener technology as extraction methods that reduce energy consumption, use renewable natural products, and ensure a safe and high-quality extract/product. Natural compounds have distinct structural characteristics, such as more oxygen atoms, chiral centres, higher steric complexity and molecular rigidity, more hydrogen bond acceptors and donors, and lower aromatic ring atoms to total heavy atom ratio. Additionally, molecular parameters such as partition coefficient, molecular mass, and ring system diversity are more varied Developing analogs of natural substances to improve potency, pharmacokinetic qualities, or minimize toxicity can be challenging for medicinal chemists due to their distinct characteristics. This article discusses modern methods for discovering natural products from botanical sources, including candidate selection, bioactivity-guided extraction and fractionation, biological screening, phytochemical characterization to identify potential lead compounds, and

development of analogues through in-silico and virtual screening techniques.

Plant extracts are sources of natural and renewable materials and substances rich in bioactive ingredients for the synthesis of eco-friendly chemicals and products. Green chemistry can help to connect the power of plant-derived compounds through green chemistry methodologies and consequently produce environmental-friendly alternatives to the synthetic chemicals which will reduce the environmental impact and support the sustainable methods in different sectors. This combined study is of tremendous importance, since it might lead to the invention of safer, more sustainable and biodegradable products that will be the good for both the human health and the environment Bioactive substances Plants create a wide variety of phytochemicals during their existence. The first category is the PRIMARY METABOLITES: These are necessary for normal growth and development of a plant. These comprise nucleic acids, carbohydrates, fatty acids, proteins and the chemicals found in all plants for their growth and development such as growth regulators and cell wall components. SECONDARY METABOLITES: This is the second category: These are necessary to improve the plant's ability to thrive in its environment and to counteract threats in its niche. Simply said, secondary metabolites are substances produced by a plant, which allows a plant to adapt to its local environment. Important to remember that secondary metabolites have many functions in plant physiology and biochemistry. Any plant species reproducing in a non-favourable niche, e.g. tepid and aquatic tropical woodlands, will try to sustain itself by creating biomolecules that may have insecticide, fungicide, antibacterial or antiviral activities. However, considering that the plant's leaves do not present any symptom of invasion, they may include protective bioactive chemicals against insects and bacteria. Due to the abundant nature of pathogenic fungi in the soil, the roots may often release antifungal phytochemicals. These secondary metabolites may also show antifungal responses against human pathogenic fungus.

METHODOLOGY

EXTRACTION TECHNIQUES OF PLANT :

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use.

A typical extraction process may contain following steps

a) Collection and authentication of plant material for drying

b) Size reduction

c) EXTRACTION

d) Filtration

e) Concentration

f) Drying and Reconstitution

These include classes of preparations known as

A) Decoctions,

B) Infusions,

C) Maceration

D) Digetion

E) Percolation

F) Powdered Extracts.

Such preparations popularly have been called galenicals, named after Galen, the second century Greek physician.

The purposes of standardized extraction procedures for crude drugs are to attain the therapeutically desired portion and to eliminate the inert material by treatment with a selective solvent known as menstruum.

The extract thus obtained may be ready for use as a medicinal agent in the form of tinctures and fluid extracts, it may be further processed to be

incorporated in any dosage form such as tablets or capsules, or it may be fractionated to isolate individual chemical entities such as ajmalicine, hyoscine and vincristine, which are modern drugs. Thus, standardization of extraction procedures contributes significantly to the final quality of the herbal drug.

GENERAL METHODS FOR EXTRACTION OF PLANTS

A) CONVENTIONAL EXTRACTION METHOD :

1) Decoction

In this process, the crude drug is boiled in a specifi ed volumeof water for a defi ned time; it is then cooled and strained or fi ltered.This procedure is suitable for extracting water-soluble, heat-stableconstituents. This process is typically used in preparation of Ayurvedic extractscalled “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 its originalvolume by boiling during the extraction procedure. Then, the concentrated extract is fi ltered and used as such or processed further.

2) Infusion

Fresh infusions are prepared by macerating the crude drug for a shortperiod of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs.

3) Maceration

In this process, the whole or coarsely powdered crude drug isplaced in a stoppered container with the solvent and allowed to stand atroom temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, themarc (the damp solid material) is pressed, and the combined liquids areclarified by fi ltration or decantation after standing.

4) Percolation:

The solid ingredients are moistened with an appropriate amount of thespecific ed menstruum and allowed to stand for approximately 4 h in a wellclosed container, after which the mass is packed and the top of the percolator is closed. Additional menstruum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h.

5) SOXHLET EXTRACTION:

Soxhlet extraction is also known as continuous hot extraction. The apparatusused is known as Soxhlet extractor, which consists of a round bottom flask, extractionchamber, extraction thimble, siphon tube, and condenser. The finely powdered plantmaterial is placed inside a porous bag (thimble) and this thimble is placed in extractionchamber. The extraction solvent placed in the round bottom flask is continuouslyheated and its vapours condense in a condenser and drip into the plant material in thethimble and extracts it by contact. When the level of the solvent in thimble reachesthe top of siphon tube, the contents in the extraction chamber (extracted material andthe solvent) siphon into the round bottom flask. It is a most common method used for plant extraction, as the process consumes less time and solvent for extraction, and no filtration is required. The entire process is continued till the solvent flowing from extraction chamber does not leave any residue behind. The method is not suitable forthe extraction of thermo-labile bioactive components.

B) MODERN GREEN EXTRACTION METHOD :

1) MICROWAVE ASSISTED EXTRACTION TECHNIQUES:

one of the advanced techniques which uses mechanism of dipole rotation and ionictransfer by ion displacement in the solvent and the plant material. This method ismostly used to extract polar compounds.Microwave extraction has gained significantattention for its effectiveness and efficiency in extracting bioactive compounds fromplant materials (Lee et al. 2016). This technique utilizes electromagnetic waves,typically within the 300 MHz to 300 GHz frequency range, to heat and extractcompounds from plant matrices(Belwal et al. 2017). The process works by inducing aninteraction between these electromagnetic waves and the polar molecules present inboth the solvent and plant material (Pellati et al. 2013). This interaction transformselectromagnetic energy into thermal energy, generating heat that facilitates the extraction of bioactive compounds. The efficiency of microwave extraction isinfluenced by several key factors, including the power and frequency of the microwaveradiation, the solvent used, and the characteristics of the plant material High power and frequency enhance extraction efficiency by promoting rapid anduniform heating, whereas low power and frequency may result in incomplete extraction and potential thermal degradation of the compounds .Microwaves are electromagnetic waves with a frequency of 300 MHz (radioradiation) to 300 GHz. Two frequencies are used in research: 2.45 GHz for laboratory equipment and 915 MHz for industrial equipment (Woodhouse 2017). Two phenomena known as ionic conduction and dipole rotation are involved in producing microwave heating. The first is when the charge carriers develop an electrophoretic migration, among which are ions and electrons, which are subjected to the effect of the electric fieldof microwaves. The heating is the result of “friction” that is produced by the migration between the medium and the moving ions. Dipole rotation occurs when dipolarmolecules try to adapt to the alternating electric field. This phenomenon occurs in amicrowave environment. Collisions occur between the

dipoles and the surrounding molecules, resulting in the generation of heat Both phenomena takeplace at the same time. As a result, a change from microwave energy to thermal energy is generated In case of Methanol: Chloroform mixtures, former provides better overall heatingmefficiency because of its high dissipation factor. Because of low polarity Chloroform remains transparent . Microwave transparent solvents (eg.,hexane) are particularly suitable for extraction of thermoliable components.

ULTRASOUND ASSISTED EXTRACTION:

This process involves application of Ultrasound energy at a high-frequency Which disrupts the integrity of bioactive plant cell wall , henceincrease plant material surface area for solvent penetration. This leads to release ofsecondary metabolites into solvent. The extraction process is applicable to small,sample, reducing the time of extraction and amount of solvent used and maximizingyield.UAE involves application of high- frequency sound waves to a liquid mediumcontaining the sample of interest . The sound waves create high-pressure zones and low- pressure zones in liquid, which cause cavitation bubbles to form and collapserapidly These bubbles produce intense mechanical forces thatdisrupt cell walls of sample and enhance mass transfer of bioactive compounds fromsolid phase to liquid phase (kobus et al .,2022).This is a fast, cost-effective, and scalable technique for extracting compounds fromplant resources. The process involves using ultrasound waves, which enhance themass transport of bio-active constituents from the plant material to the solventmedia through cavitation, mechanical agitation, and thermology. UAE is a simpleand easy technique that consumes less solvent, has a shorter extraction time, andoperates at lower process temperatures, resulting in higher extraction efficiency.Therefore, UAE has potential applications in the chemical and food industries

SUPERCRITICAL FLUID EXTRACTION:

It is one of the advanced extraction techniques which utilizes the uniqueproperties of supercritical fluids. Supercritical fluid is a substance aboveits critical temperature and critical pressure and have properties between those ofa gas and a liquid, where distinct liquid and gaseous phase do not exist.Supercritical fluids are capable to diffuse quickly into the porous solid substance,hence dissolving the materials quickly. For plant extraction, supercritical carbondioxide is mainly used, as it is an ideal solvent with low viscosity, high diffusionrate, and high volatility. Due to its low critical temperature, it is also used toextract thermo-labile compounds.The supercritical carbon dioxide is inefficient to extract polar solutes dueto its non-polar nature, for which volatile polar modifiers such as methyl acetate,diethyl ether, methanol, or formic acid are added to extract polar compounds.This is a green technique for extracting compounds from plants that employsupercritical fluids, such as carbon dioxide, as a solvent. SFE offers severaladvantages over conventional extraction techniques because of thephysicochemical properties of supercritical solvents, which include improvedtransport characteristics that result in quicker extraction rates. The high-qualitextracts generated by SFE make it a hopeful method in the domains of food, pharmaceuticals, and cosmetics.

PRESSURIZED LIQUID EXTRACTION:

Pressurized liquid extraction (PLE) is an automated method ofextracting compounds from various materials using traditional solvents. PLEoperates under constant pressure and controlled parameters, includingtemperature, extraction time, and number of cycles. As the final extract is collected,it is automatically filtered, simplifying the process, and making it more efficient.PLE is an environmentally friendly technique that can utilize water andethanol to recover polar to medium polarity compounds. This aspect contributesto its eco-friendly properties, making it an attractive option for industries seekingsustainable and cost-effective extraction method. Pressurized liquid extraction is an advanced extraction technique, whichemploys solvent extraction at high temperatures and pressures, below the criticalpoints of the solvent; so that the solvent remains in the liquid state during thewhole extraction process. As high pressure and temperature is used during thprocess, a change in physicochemical properties of the solvent occur, leading to thedecrease in solvent surface tension and viscosity. This increases the solubility of analytes in the respective solvent and significantly higher extraction yields areobtained as compared with conventional extractions. Both SFE and PLE methodsare highly expensive and rarely used for plant extraction process.

ISOLATION AMD PURIFICATION TECHNIQUES:

The purification and isolation of bioactive compounds from plantshave reached new heights in recent years. Chromatographic processes are often used to separate a mixture by passing it through a specified mediumin which the components move at different rates. To isolate bioactive compounds, column chromatography techniques are often used. The greatest valuefor phytochemical separation is found in silica, alumina, cellulose, andpolyamide. Plants include phytochemicals, which are bioactive substances derived from plant components such as leaves, barks, seeds, seed coats, flowers,roots, and pulps. Extracts have been demonstrated to be physiologically active inboth in vitro and in vivo test systems in certain circumstances.Separation of chemicals is often followed by the assessment of thepresence of specific compounds within plant extracts using a range ofbioassays. Extraction of plant metabolites is essential for understanding theirfunction in disease prevention and treatment, as well as their harmful consequences.Purification and separation of bioactive chemicals from plants is atechnology that has shown considerable advancement, allowing for thesimultaneous development and availability of various complex bioassays on theone hand, while also providing accurate isolation, separation, and purificationprocedures on the other. Animal studies are more costly, require more time,and are prone to ethical problems, so in vitro procedures are frequentlyused. Plant material selection and collection are essential processes in isolating and characterizing a bioactive phytochemical. Extracts may be preparedusing a variety of solvents to separate and purify theactive chemicals responsible for the bioactivity.These include column chromatography, thin-layer chromatography (TLC),paper chromatography, gas chromatography, ion exchange chromatography, gel permeation chromatography, high-performance liquid chromatography, affinity

chromatography and many more. Below, we have discussed various chromatographictechniques in detail

STRUCTURAL ELUCIDATION:

In pharmaceutical companies are showing insignificant interest in naturalproduct research owing to their complex phytochemistry and difficulties in their accessand supply [75], a substantial amount of work related to the discovery of therapeutic agents from natural sources are being carried out in academic set-ups and the technical complexities regarding isolation and structural elucidation of bioactive phytoconstituents are being overcome with the cooperation of the chemists globally. At present,spectroscopic analyses have become the main approach in the structural determination of phytochemicals. After a preliminary biological screening of extracts ,the bioactive ones can be rapidly fractionated by high-performance liquid chromatography, subsequently, the chemical characterization of the fractions is performed by liquid chromatography-mass spectrometric (LC-MS) and nuclear magnetic resonance (NMR) spectroscopic analysis. After LC-MS analysis, the novel compounds in the isolates are first distinguished from the known compounds by comparing the MS data with those previously identified compounds available in the web libraries. Furthermore, adequate amounts of pure compounds can be readily isolated by HPLC through automated injection of extracts followed by the collection off reactions and their structural elucidation can be achieved through NMR and MS analysis. Consequently, the entire process starting from extraction to the isolation of pure natural compounds is now possible to achieve in days rather than months whichwas the case a few years ago .the development of efficient fractionation such as counter-current chromatography and emerging advancement in analytical techniques including NMR spectroscopy andmass spectrometry to isolate, purify and elucidate the structure of natural products is now allowing the natural product screening to fit in the timescale of high throughput screening [78–80]. It is now possible to isolate the bioactive compounds from the natural matrix and elucidate their structures within 4–5 weeks. Furthermore, complex structure-related issues can be solved with the use of significantly low quantities of a sample (sometimes less than 1 mg) . To further enhance the productivity of naturalproduct research several alternative techniques have also been explored.

CONCLUSION

Since bioactive compounds occurring in plant material consist of multi-component mixtures, their separation and determination still creates problems. Practically most of them have to be purified by the combination of several chromatographic techniques and various other purification methods to isolate bioactive compound(s).

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