Method of Natural Product Isolation: A Review

ABSTRACT

Naturally occurring substances from prebiotic, microbial, plant and animal sources have always piqued human curiosity. Plant extracts of various parts have been used extensively in traditional medicines, perfumes, food flavorings, and preservatives. They are also more frequently used in both common and chronic illnesses. Natural products are essential sources for medication production. The development of efficient and selective techniques for the extraction and isolation of those bioactive natural compounds is crucial nowadays. The thorough assessment of the many techniques utilized in the extraction and separation of natural products was the main emphasis of this paper. The traditional and contemporary methods used in natural products research are also presented in this paper. Some isolation processes are time consuming, in efficient and however have hindered the application of natural products isolation and synthesis. Due to tremendous increase in application of different extraction methods for natural product isolation/drug synthesis, more efficient techniques have been developed to extract and separate natural products.

Key word: Natural, isolation, techniques, characterization, maceration, medicine.

BACKGROUND

Naturally occurring substances from prebiotic, microbial, plant and animal sources have always piqued human curiosity. Numerous plant extracts have been used extensively in traditional treatments, fragrances, food flavorings, and preservatives. They are also more frequently used in both common and chronic illnesses (Svetlana et al., 2024). Alkaloids, steroids, tannins, glycosides, volatile and fixed oils, resins, phenols, and flavonoids are among the active substances found in plants and are deposited in various regions of the plant. The combination of these active compounds gives the plant its beneficial and medicinal effects. Numerous plant extracts have been used extensively as food flavorings, preservatives, folk remedies, and fragrances (Kumar et al., 2023).

As a remedy for gastropathy, hepatitis, nephritis, edema, chest pain, fever and cough of pneumonia, bronchitis, and arthritis, bioactive natural products are more frequently used in both chronic and infectious diseases such as cancer, diabetes, and asthma, as well as anti-

inflammatory, analgesic, and antipyretic solutions, and as alternatives to hormone replacement therapy (Nazari et al., 2025). Today, natural medicines not only meet the primary health-care needs of the majority of the population in developing countries, but they have also gained increasing attention in developed countries due to their low or nonexistent side effects. More than 80% of the worldwide population solely depends on traditional medicine for their primary healthcare, most of which involve use of natural products from plant. In the USA, roughly 49% of the population has tried natural medicines for disease prevention and treatment (Ekor, 2014).

Identification and characterization are made more difficult by the separation of natural products derived from plant extracts, which typically contain multiple component combinations with varying polarity. In order to separate and characterize various natural compounds, extraction is crucial. In order to isolate natural products, the majority of them must be purified using a mix of many chromatographic and non-chromatographic procedures as well as other purification methods (Zhang et al., 2018).

EXTRACTION

Using selective extraction procedures, extraction is the initial stage in separating the potential portion or substance from its sources, which include plants and animals. Both good and undesirable chemicals are produced in a pure state during extraction. To extract desired components from natural products, some contemporary or environmentally friendly extraction techniques, such as pressurized liquid extraction (PLE), microwave assisted extraction (MAE), and super critical fluid extraction (SFC), are used in addition to more traditional techniques like maceration, percolation, and reflux extraction. In order to assess the biological activity of secondary metabolites, make herbal medications, or separate known mixtures of substances, natural products must be extracted (Komal et al., 2019).

Extraction is the process of employing standard and selective methods to separate the parts of a plant that have medicinal activity. Because the desired chemical components must be extracted from the plant materials for additional separation and characterization, it is the most important initial stage in the investigation of medicinal plants (Abubakar and Haque, 2020).

There are various techniques for extracting natural products from existing plants. These techniques fall into two categories: traditional (long-standing) and modern (more recent). Modern methods use pressure and/or higher temperatures, whereas conventional methods use organic solvents or water and are typically conducted at atmospheric pressure. According to the

extraction principle, extraction techniques include pressing, sublimation, solvent extraction, and distillation (Zhang et al., 2018). The approach that is most frequently utilized is solvent extraction. The following steps are involved in the extraction of natural products: the solvent enters the solid matrix, the solute dissolves in the solvents, the solute diffuses out of the solid matrix, and the extracted solutes are gathered. Solvents such as water, ethanol, chloroform, dichloromethane, hexane, ethyl acetate, methanol, etc. are most frequently employed for the extraction processes.

Conventional extraction techniques typically include the use of organic solvents, necessitate a significant amount of solvents, and take a considerable amount of time to complete. Modern extraction techniques have also been used to extract natural products, and they have several benefits, including improved extraction yield, reduced consumption of organic solvents, and shorter extraction times (Luksta and Spalvins, 2023).

Maceration

The lengthy extraction time is a drawback of this really straightforward extraction technique. Maceration is a cold extraction technique that is isocratic. It works well for extracting chemicals that are thermoliable. By submerging a plant sample in a specific solvent, this technique extracts the constituent elements from plants in a solvent. It is carried out in a steady condition at room temperature (Komal et al., 2019).

Percolation

When making tinctures and fluid extracts, this is the method most commonly employed to extract the active components. The plant material is placed in a percolation tube that has a stop cock and filter or is plugged with cotton. After adding the solvent and letting the plant material stand in a tightly sealed container for around four hours, the mass is packed and the percolator's top is sealed. After a 24-hour period at room temperature, the solvent and extracted material are collected by opening the stopper below, and the mixture is either filtered or allowed to stand before being decanted (Ishwari et al., 2014).

Digestion

This type of maceration involves applying mild heat (between 40 and 60^0C) while the extraction is taking place. When a somewhat higher temperature is acceptable, it is employed. The procedure can be changed by stirring the mixture by hand occasionally or by combining the material and solvent with a mechanical or magnetic stirrer. The extract is filtered after 8 to 12

hours, and new solvent is added. This process is continued until all of the desired compounds have been extracted (Fotsing et al., 2022).

Infusion

The plant material is macerated with either cold or boiling water for a brief length of time during this extraction procedure. It is a diluted mixture of the crude medications' easily soluble ingredients (Abubakar and Haque, 2020).

Decoction

This method involves boiling the plant material in a given amount of water for a predetermined amount of time, cooling, and then straining or filtering it. This process works well for extracting components that are heat-stable and soluble in water (Abubakar and Haque, 2020).

Reflux

Boiling solvent is used to treat the material in this hot extraction procedure. A condenser installed on top of the container preferably a flask with a circular bottom recycles the solvent vapor. The extraction of thermolabile natural products is not possible with it (Mohammed, 2018).

Tincture

It is an alcohol-based plant material extract. Fresh plant material and ethyl alcohol are often consumed at 1:5 ratios. The alcohol in the tinctures prevents them from breaking down when kept at room temperature (Budniak et al., 2020).

Liquid Extraction under Pressure (PLE)

The enhanced solvent extraction system (ESE) and accelerated solvent extraction system (ASE) are other names for the technique. The technique uses high temperatures and pressures; the higher temperature speeds up the extraction process by making the solvent more diffusive, while the higher pressure keeps the organic solvent liquid without boiling and forces it to pass through the matrix pores (Budniak et al., 2020).

Soxhlet Extraction

Soxhlet apparatus is a specialized glass refluxing unit mainly used for organic solvent extractions. The powdered solid material is added in to a thimble made up of filter paper and is placed inside the soxhlet extractor. The apparatus is fitted to a round bottomed flask containing the solvent and to a reflex condenser. The solvent in the flask is boiled slowly, 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/washes the portion of the substance, which it has extracted (Yogeshri et al., 2023).

SteamDistillation

Steam distillation, which is a straightforward vaporization process that is accomplished by passing steam directly through the material, is the standard procedure used to isolate volatile oil from crude plant material. In this process, the steam volatile essential oil is recovered by condensation, where oil separates out of water by decantation. (Souiy, 2024).

HydroDistillation

This is the method that is most frequently used to separate essential oils. A heating mantle is used to boil the plant material after it has been soaked in water. The essential oil is released from the plant tissues' oil glands and travels with the steam as a result of the hot water's action. The steam oil combination is condensed, the oil is separated from the water, and the condensed water is recycled using a common glass device called a Clevenger apparatus (Zhou et al., 2023).

Expression

Citrus essential oils are extracted via a process called expression, sometimes known as cold pressing. Sponge pressing, which was actually done by hand, was the method of expression used in earlier times. Squeezing the sponge allowed the oil that was released during this procedure to be collected. According to Park et al. (2023), oil made this way has a higher concentration of the fruit odor character than oil made any other way.

Enfluerage

The exquisite smells of flowers are extracted using this process. The petals of the flowers are put on top of a layer of refined fat, which absorbs the scent of the flowers. The saturated fat is then treated with a solvent, typically alcohol, which dissolves the fragrant ingredients. When the alcohol extract is cooled to 20 ^{0}C, the remaining fat that has dissolved in it can be extracted. Pure oils are produced by evaporating the alcohol under lower pressure (Dey et al., 2020).

Supercritical Fluid Extraction (SFE).

This is the most cutting-edge extraction system in terms of technology. Supercritical Fluid Extraction (SFE) is the process of compressing gases typically CO_2 into a thick liquid. After that, the liquid is forced through a cylinder that holds the substance that has to be removed. The liquid containing the extract is then pushed into a separation chamber, where the gas is collected for further use and the extract is separated from it. By altering the temperature and pressure, the

solvent characteristics of CO_2 can be controlled. One of SFE's benefits is that CO_2 evaporates entirely, leaving no solvent residues behind (Akanda et al., 2012).

UltrasonicExtraction

High frequency sound causes natural chemicals to be released from plant tissues during this process, damaging the cell wall. Hexane with methanol and water are examples of immiscible solvent mixes that can be employed with ultrasound-assisted extraction. Heat is produced throughout the process, allowing heat-labile chemicals to break down. To lower the temperature in these situations, the extraction container is submerged in an ice bath (Carreira-Casais et al., 2021).

MicrowaveAssistedExtraction(MAE)

It is simply known as microwave extraction, and it blends classical solvent extraction with microwave extraction. Microwave assisted organic syntheses (MAOS), which build up tiny molecules into massive polymers in a short amount of time, have revolutionized the synthesis of organic compounds. Analyte partitioning from the sample matrix into the solvent is facilitated by increasing the kinetics of extraction using microwave heating of the solvents and plant tissue. Heat is transported by conduction when microwave radiation interacts with the dipoles of polar and polarizable materials, causing heating close to the materials' surface. Hydrogen bonds are broken by the dipole rotation of molecules caused by microwave electromagnetic fields, which increases dissolved ion movement and facilitates solvent penetration into the matrix. In non-polar solvents, poor heating occurs as the energy is transferred by dielectric absorption only (Delazar et al., 2012).

Solid Phase Extraction (SPE)

Various cartridges and disks with a range of sorbents where the solute molecules are preferentially bonded over the stationary phase are used in this quick, affordable, and sensitive method. It is possible to complete sample preparation and concentration in a single step. There are ion exchange solid phase extraction units, normal phase, and reverse phase units available. For instance, polar components can be eliminated using Sep-Pak C18 cartridges (reverse phase), while the low polar components that are left can be eluted at a later time (Fotsing et al., 2022).

Counter-current Extraction

Counter-current extraction (CCE) creates fine slurry by pulverizing wet raw material using toothed disc disintegrators. This method involves moving the material to be extracted in a single

direction typically as fine slurry inside a cylindrical extractor until it comes into contact with the extraction solvent. The extract gets more concentrated the farther the initial material travels. Therefore, optimal solvent and material quantities and flow rates allow for complete extraction. The procedure is quite effective, takes very little time, and is safe from extreme temperatures. Finally, sufficiently concentrated extract comes out at one end of the extractor while the marc (practically free of visible solvent) falls out from the other end (Jeenu and Girisa, 2020).

Aqueous Alcoholic Extraction by Fermentation

The extraction process entails soaking the crude drug, either as a powder or as a decoction (kasaya), for a predetermined amount of time. This allows the drug to ferment and produce alcohol in the process, which makes it easier to extract the active ingredients from the plant material. The resulting alcohol also acts as a preservative. It should not be new if the fermentation is to be done in an earthen jar; instead, the water should be boiled in the vessel first. In large-scale production, clay pots are substituted by metal vessels, porcelain jars, or wooden vats (Purnendu et al., 2022).

ISOLATION AND PURIFICATION

The extracts from the aforementioned techniques contain a complicated mixture of distinct natural product types with varying polarity. Additional separation and purification are required to achieve a pure bioactive molecule. The process of identifying and characterizing pure bioactive natural products still faces significant challenges due to their separation. The process of isolating and purifying natural products has advanced recently. Several separation techniques, including TLC, HPTLC, paper chromatography, column chromatography, gas chromatography, OPLC, and HPLC, have been used to separate and purify a large number of bioactive natural compounds. Because of their affordability, ease of use, and availability in a variety of stationary phases, column chromatography and thin-layer chromatography (TLC) remain the most popular methods. In addition, non-chromatographic methods that employ monoclonal antibodies (MAbs), including immunoassay, phytochemical screening assay (Sasidharan et al., 2011). The structure and biological activity of the pure chemicals are subsequently ascertained. Below is a discussion of a few popular methods for separating natural products:

Thin Layer Chromatography (TLC)

The most used planar chromatographic technique for studying natural products is TLC. This is the simplest and least expensive method that can be used for isolation, analysis, and column

chromatography parameter tuning. Organic solvents, which are less polar, are typically utilized as the mobile phase, while silica or alumina which is more polar, are typically utilized as the stationary phase. Normal phase chromatography is the term used to describe this circumstance. As an alternative, reverse phase TLC is available, where the mobile phase is a polar solvent such as water, alcohol, etc., and the stationary phase is alkyl-bonded silica or alumina (less polar) (Waksmundzka-Hajnos et al., 2022).

Column Chromatography (CC)

The most efficient method for separating unrefined plant extracts into their constituent parts in a pure state is column chromatography. In this preparative chromatographic procedure, the extracts are loaded onto the stationary phase (silica gel), which is then packed in a column, and the mobile phase (eluent) is passed through the column. Depending on their affinities to the stationary and mobile phases, the natural products in the mixture are carried by the mobile phase at varying rates (Susanti et al., 2024).

Gas Chromatography (GC)

This analytical method separates substances mainly according to their volatilities. For each unique component contained in a sample, GC offers both qualitative and quantitative information. The liquid phase is stationary while the gas phase is flowing. The distribution of the chemical species in the gas phase determines its rate of migration. In contrast to a species that distributes itself 100% into the stationary phase, which will not migrate at all, a species that distributes itself 100% into the gas phase will migrate at the same rate as the flowing gas. A species will migrate at an intermediate rate if it distributes itself partially in both phases. In gas chromatography, a sample is vaporized and then injected onto the chromatographic column's head. The sample is then transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase, which is adsorbed onto the surface of an inert solid (Coskun, 2016).

High Performance Liquid Chromatography (HPLC)

It is a durable, adaptable, and popular method for separating natural ingredients. An analytical method for separating and identifying organic and inorganic solutes in any sample, particularly those from the biological, pharmaceutical, food, environmental, and industrial sectors, is high-performance liquid chromatography (HPLC). This method is currently becoming more and more well-liked among other analytical techniques as the primary option for fingerprinting studies for

medicinal plant quality control. The first step in using HPLC to identify any component is choosing a detector. The selection of the stationary phase and mobile phase largely dictates the degree or extent of separation. A polar liquid phase, typically a combination of water and another solvent, and a non-polar solid phase, such as C18, are used in modern HPLC. The analyte must be eluted through a column at high pressures of up to 400 bars before it may pass through a diode array detector (DAD). To help identify the analytes, a DAD examines their absorption spectra. For substances that cannot be vaporized or that break down at high temperatures, HPLC is helpful. It is also an excellent addition to gas chromatography for compound detection (Mesud et al., 2024).

Thin Layer Chromatography with High Performance (HPTLC)

Natural compound separation is accomplished using planar chromatography on high-performance layers with detection and data collection. These high-performance layers are pre-coated plates that have a 150–200 micron layer thickness and a sorbent with a particle size of 5-7 microns. The reduction in thickness of layer and particle size results in increasing the plate efficiency as well as nature of separation. HPTLC plates are substantially more expensive (4-6 times more) than normal plates but are an efficient alternative when high sensitivity, accuracy and precision are required in situations demanding high performance (Mesud et al., 2024).

Optimum Performance Laminar Chromatography (OPLC)

The benefits of TLC and HPTLC are combined in OPLC, a novel idea in parallel chromatography. Research and quality control labs can benefit from the analytical and preparative capabilities of OPLC. It is a potent liquid chromatography separation method that combines the multidimensionality and flash chromatography capabilities of TLC with the use friendly interface and resolution of HPLC. The fundamental idea of OPLC is the same as other chromatographic methods: a liquid mobile phase is forced through a stationary phase, like silica, by use of a pump. Flat planar columns can be employed in the same manner as cylindrical glass or stainless steel columns. The flat column is pressurized up to 50 bars and mobile phase is forced through it at constant linear velocity via a solvent delivery pump (Attimarad et al., 2011).

Preparative Planar Chromatography

Although it has less uses than column chromatography, PPC has been a frequently used technique because of its low cost and simplicity in isolating nanoparticles (NPs). PPC's wide range of chemical disclosure approaches for NPs, which may be accomplished on a small portion

of the plate and leave the majority of compounds consistent and easy to isolate, is an intriguing feature. Forced flow techniques like centrifugal planar chromatography and over pressured layer chromatography have been developed to lower the uncontrolled flow rates for the traditional TLC mobile phase, allowing for compound elution and online disclosure (Abdelmohsen et al., 2022).

Chiral Chromatographic

After chiral compounds' isolation, generally a process to detect absolute configuration is required. For enantio separation at an analytical scale, GC, HPLC, SFC, or CE have universally been applied. However, HPLC is used most frequently. This separation procedure provides insulating enantiomers directly with chiral stationary or mobile-phase, or indirectly with chiral derivatization agents additives (Usama et al., 2022).

Preparative Gas Chromatography

PGC is an alternative choice for the extraction of volatile oils. Mostly, filled columns with greater sample capacity but lower peak resolution are established. However, there have been an expanding number of outstanding operations of thick-phase film full-bore capillaries with capillary GC apparatus during the recent years (Usama et al., 2022).

STRUCTURE DETERMINATION

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 amount of radiation, but not complete. By measuring the amount of absorption of electromagnetic radiation, a spectrum can be created. The spectra are specific to certain bonds in a compound. Depending on these spectra, the structure of the natural compound can be established. Scientists mainly use spectra produced from three or four regions Ultraviolet (UV), Visible, Infrared (IR), Radio frequency (FTIR), and electron beam for structural elucidation (Altemimi et al., 2017).

UV-Visible Spectroscopy

UV-visible spectroscopy can be performed for qualitative analysis and for identification of various types 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-Vis techniques were found to be less selective and give information on the composition of the total polyphenol content. This technique is not time consuming, and presents reduced cost compared to other techniques (Masarrat et al., 2021).

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy is a valuable tool for the identification of functional groups present in plant extracts. It aids for identification and structure determination of molecules. It is a high resolution analytical tool to identify chemical constituents and elucidate their structures. FTIR offers a rapid and nondestructive investigation to fingerprint herbal extracts or powders according to Kassem et al., (2023).

Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear Magnetic Resonance Spectroscopy produces physical, chemical and biological properties of matter. One dimensional technique is routinely used but the complicated structure of the molecules could be gained through two dimensional NMR techniques. Solid state NMR spectroscopy is applied for the determination of molecular structure of solids. Radio labeled ^{13}C NMR is used to identify the types of carbons present in the compound. ^{1}H-NMR is used to find out types of hydrogen present in the compound and to find the connectivity of hydrogen atoms (Reif et al., 2021).

Mass Spectroscopy (MS)

Mass spectrometry is a strong 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, peptide or oligonucleotide sequencing and for monitoring the existence of previously characterizes compounds in complex mixtures with a high specificity by defining both the molecular weight and a diagnostic fragment of the molecule at the same time (Ma, 2022).

Molecular Distillation (MD)

Molecular distillation separates the molecules by distillation under vacuum at a temperature much below its boiling point. It is a suitable distillation method for separating thermo-sensitive and high-molecular-weight compounds (Zhang et al., 2018; Ketenoglu and Tekin, 2015).

CONCLUSION

The thorough assessment of the many techniques utilized in the extraction and separation of natural products was the main emphasis of this paper. The contribution of natural products to drug development in past few years has been increasing tremendously. The isolation processes are time consuming and however have hindered the application of natural products. Due to tremendous increase in application of different extraction methods for natural product isolation/drug synthesis more and more efficient techniques have been developed to extract and separate natural products.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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