Innovative Techniques for Natural Product Isolation: Bridging Tradition and Modernity

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. The low efficiency of conventional isolation processes leads to increased processing time, higher energy consumption, and more solvent usage, which can make the process less sustainable. The use of organic solvents in conventional extraction can pose environmental and health risks, as many solvents are toxic and harmful 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.

INTRODUCTION

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 antiinflammatory, 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"(Komal et al., 2019). "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"(Zhang et al., 2018). “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” (Zhang et al., 2018).

“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”(Komal et al., 2019). “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”(Ishwari et al., 2014). “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(Fotsing et al., 2022). 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" (Fotsing et al., 2022).

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

"The Soxhlet apparatus is a specialized glass device designed for organic solvent extractions. It consists of a thimble made from filter paper, which holds the powdered solid material and is placed within the Soxhlet extractor"(Yogeshri et al., 2023). "The setup is connected to a round-bottom flask containing the solvent and a reflux condenser. During operation, the solvent in the flask is gently heated to produce vapor, which travels through a side tube, condenses in the condenser, and drips onto the material in the thimble"(Yogeshri et al., 2023). "The Soxhlet gradually fills with solvent, and once it reaches the top of the attached tube, the solvent siphons back into the flask, carrying with it the extracted components from the material" (Yogeshri et al., 2023).

SteamDistillation

"Steam distillation is a simple vaporization technique commonly used to extract volatile oils from crude plant materials. This method involves passing steam directly through the material, causing the volatile essential oil to evaporate. The vapor is then condensed, and the essential oil is separated from the water by decantation" (Souiy, 2024).

HydroDistillation

"This method is the most commonly used for extracting essential oils. The plant material, after being soaked in water, is heated using a heating mantle. The heat causes the essential oil to be released from the oil glands within the plant tissues, and it travels along with the steam"(Zhou et al., 2023). "The steam-oil mixture is then condensed, separating the oil from the water. The condensed water is recycled using a standard glass apparatus known as a Clevenger apparatus" (Zhou et al., 2023).

Expression

Citrus essential oils are obtained through a process called expression, also referred to as cold pressing. In the past, this method was performed manually using sponge pressing. The oil released during the process was collected by squeezing the sponge. According to Park et al. (2023), oils produced through this method retain a stronger fruit aroma compared to those extracted by other methods.

Enfluerage

This method is used to capture the delicate fragrances of flowers. Flower petals are placed on a layer of refined fat, which absorbs their scent. The saturated fat is then treated with a solvent, usually alcohol, to dissolve the aromatic compounds(Dey et al., 2020). Cooling the alcohol extract to 20°C allows any dissolved fat to be removed. The pure oils are then obtained by evaporating the alcohol under reduced pressure (Dey et al., 2020).

Supercritical Fluid Extraction (SFE).

Supercritical Fluid Extraction (SFE) represents the most advanced technology for extraction. This process involves compressing gases, typically CO_2, into a dense liquid form. The liquid is then passed through a cylinder containing the material to be extracted(Akanda et al., 2012). The extract-laden liquid is directed into a separation chamber, where the extract is separated, and the gas is recovered for reuse. By adjusting temperature and pressure, the solvent properties of CO_2 can be precisely controlled. A key advantage of SFE is that CO_2 completely evaporates, leaving no solvent residues behind (Akanda et al., 2012).

UltrasonicExtraction

In this process, high-frequency sound waves disrupt the cell walls of plant tissues, releasing natural compounds. Immiscible solvent mixtures, such as hexane with methanol and water, can be used in ultrasound-assisted extraction(Carreira-Casais et al., 2021). The process generates heat, which can lead to the breakdown of heat-sensitive compounds. To prevent this, the extraction container is placed in an ice bath to maintain a lower temperature (Carreira-Casais et al., 2021).

MicrowaveAssistedExtraction(MAE)

Microwave extraction, also referred to as microwave-assisted extraction, combines traditional solvent extraction with microwave technology. Microwave-Assisted Organic Syntheses (MAOS), which rapidly build small molecules into large polymers, have transformed the synthesis of organic compounds(Delazar et al., 2012). This method enhances the extraction kinetics by using microwave heating to interact with the solvents and plant material, facilitating the transfer of analytes from the sample matrix into the solvent(Delazar et al., 2012). When microwave radiation interacts with the dipoles of polar or polarizable materials, heat is generated near the surface through conduction. The dipole rotation caused by microwave electromagnetic fields breaks hydrogen bonds, increases ion mobility, and improves solvent penetration into the matrix. However, in non-polar solvents, heating is minimal as energy transfer relies solely on dielectric absorption (Delazar et al., 2012).

Solid Phase Extraction (SPE)

This method utilizes various cartridges and disks with different sorbents that selectively bind solute molecules to the stationary phase. It is a fast, cost-effective, and sensitive technique that allows for both sample preparation and concentration in a single step(Fotsing et al., 2022). Solid-phase extraction units are available in ion exchange, normal phase, and reverse phase configurations. For example, Sep-Pak C18 cartridges (reverse phase) can remove polar components, while less polar components can be eluted afterward (Fotsing et al., 2022).

Counter-current Extraction

Counter-current extraction (CCE) involves creating a fine slurry by pulverizing wet raw material using toothed disc disintegrators. In this method, the material to be extracted moves in a single direction, typically as fine slurry, through a cylindrical extractor, where it comes into contact with the extraction solvent(Jeenu and Girisa, 2020). As the material progresses, the extract becomes increasingly concentrated. By optimizing the quantities and flow rates of both the solvent and the material, complete extraction can be achieved. This process is highly efficient, quick, and operates without exposure to extreme temperatures. The final product is a sufficiently concentrated extract that exits one end of the extractor, while the marc (nearly free of visible solvent) is discharged from the other end (Jeenu and Girisa, 2020).

Aqueous Alcoholic Extraction by Fermentation

The extraction process involves soaking the crude drug, either in powder form or as a decoction (kasaya), for a specific duration. During this time, the drug ferments and produces alcohol, which facilitates the extraction of active compounds from the plant material(Purnendu et al., 2022). The alcohol also serves as a preservative. If fermentation occurs in an earthen jar, the water should first be boiled in the vessel to ensure proper fermentation. For large-scale production, metal vessels, porcelain jars, or wooden vats are used in place of clay pots (Purnendu et al., 2022).

ISOLATION AND PURIFICATION

The extracts obtained from the previously mentioned techniques consist of a complex mixture of different natural product types with varying polarities. To obtain a pure bioactive molecule, further separation and purification are necessary(Chakanaka et al., 2024). Identifying and characterizing pure bioactive natural products remains challenging due to the complexity of their separation. Recent advancements have been made in the isolation and purification of natural products. Various separation techniques, such as TLC, HPTLC, paper chromatography, column chromatography, gas chromatography, OPLC, and HPLC, have been used to isolate and purify numerous bioactive natural compounds (Chakanaka et al., 2024). Column chromatography and thin-layer chromatography (TLC) remain the most widely used methods due to their affordability, ease of use, and availability of different stationary phases. Additionally, non-chromatographic methods involving monoclonal antibodies (MAbs), such as immunoassays and phytochemical screening assays, are also employed (Sasidharan et al., 2011). The structure and biological activity of the purified compounds are then determined. Below is a discussion of some of the commonly used methods for separating natural products(Sasidharan et al., 2011).

Thin Layer Chromatography (TLC)

Thin-layer chromatography (TLC) is the most commonly used planar chromatographic technique for analyzing natural products. It is a simple, cost-effective method suitable for isolation, analysis, and optimizing column chromatography parameters(Waksmundzka-Hajnos et al., 2022). In TLC, less polar organic solvents are typically used as the mobile phase, while the stationary phase is usually made of silica or alumina, which are more polar. This setup is referred to as normal phase chromatography(Waksmundzka-Hajnos et al., 2022). Alternatively, reverse phase TLC can be used, where the mobile phase consists of a polar solvent like water or alcohol, and the stationary phase is alkyl-bonded silica or alumina, which are less polar (Waksmundzka-Hajnos et al., 2022).

Column Chromatography (CC)

Column chromatography is the most effective method for separating crude plant extracts into their individual components in a pure form. In this preparative chromatographic technique, the extract is applied to a stationary phase (typically silica gel) packed into a column, and a mobile phase (eluent) is passed through it. The natural products in the mixture are carried through the column at different rates by the mobile phase, based on their affinities for the stationary and mobile phases (Susanti et al., 2024).

Gas Chromatography (GC)

Gas chromatography (GC) is an analytical technique that separates substances based on their volatilities. It provides both qualitative and quantitative information for each unique component in a sample. In GC, the stationary phase is liquid, while the gas phase flows through(Bishnu and Masoud, 2018). The rate of migration of a chemical species is determined by its distribution between the gas phase and the stationary phase(Bishnu and Masoud, 2018). A species that is fully distributed in the stationary phase will not migrate, while one fully distributed in the gas phase will migrate at the same rate as the flowing gas (Bishnu and Masoud, 2018). A species that partially distributes itself in both phases will migrate at an intermediate rate. In gas chromatography, the sample is vaporized and injected at the head of the chromatographic column, where it is carried through the column by an inert gaseous mobile phase. The column contains a liquid stationary phase adsorbed onto the surface of an inert solid (Ahmed, 2024; Coskun, 2016).

High Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is a robust, versatile, and widely used method for separating natural compounds(Mesud et al., 2024). It is an analytical technique designed to separate and identify both organic and inorganic solutes in various samples, particularly in fields like biology, pharmaceuticals, food, environmental science, and industry(Mesud et al., 2024). HPLC is becoming increasingly popular for fingerprinting studies in medicinal plant quality control. The first step in using HPLC is selecting a suitable detector(Mesud et al., 2024). The choice of stationary and mobile phases plays a crucial role in determining the separation efficiency. Modern HPLC typically uses a polar liquid phase, often a mixture of water and another solvent, and a non-polar solid phase like C18 (Ahmed, 2024). The analyte is eluted through the column under high pressures, up to 400 bars, before passing through a diode array detector (DAD). The DAD analyzes the absorption spectra of the analytes to assist in their identification. HPLC is particularly useful for substances that cannot be vaporized or that decompose at high temperatures. It also complements gas chromatography in compound detection (Mesud et al., 2024).

Thin Layer Chromatography with High Performance (HPTLC)

Natural compound separation is achieved using planar chromatography with high-performance layers, which include detection and data collection(Mesud et al., 2024). These high-performance layers consist of pre-coated plates with a layer thickness of 150–200 microns and a sorbent particle size of 5–7 microns. Reducing the thickness of the layer and the particle size enhances plate efficiency and improves the quality of the separation(Mesud et al., 2024). While high-performance thin-layer chromatography (HPTLC) plates are significantly more expensive—ranging from 4 to 6 times the cost of regular plates—they offer a more efficient option when high sensitivity, accuracy, and precision are required in performance-demanding applications (Mesud et al., 2024).

Optimum Performance Laminar Chromatography (OPLC)

OPLC (open planar chromatography) is an innovative approach in parallel chromatography that combines the advantages of TLC and HPTLC. It offers both analytical and preparative capabilities, making it beneficial for research and quality control laboratories. OPLC is a powerful liquid chromatography method that merges the multidimensionality and flash chromatography features of TLC with the user-friendly interface and high resolution of HPLC. The basic principle of OPLC mirrors that of other chromatographic techniques: a liquid mobile phase is pushed through a stationary phase, such as silica, using a pump (Emil and Ernő, 2011). Flat planar columns are used similarly to cylindrical glass or stainless-steel columns, with the flat column being pressurized up to 50 bars. The mobile phase is then forced through the column at a constant linear velocity by a solvent delivery pump (Attimarad et al., 2011).

Preparative Planar Chromatography

Although less commonly used than column chromatography, PPC (planar partition chromatography) is a popular technique due to its low cost and simplicity, particularly for isolating nanoparticles (NPs)(Abdelmohsen et al., 2022). One of PPC's notable advantages is its ability to reveal a broad range of chemical compositions for NPs, which can be achieved on a small portion of the plate, leaving the remaining compounds intact and easy to isolate(Abdelmohsen et al., 2022). To improve the efficiency of the traditional TLC mobile phase, forced flow methods such as centrifugal planar chromatography and over-pressured layer chromatography have been developed. These techniques help control flow rates, facilitating compound elution and real-time detection (Abdelmohsen et al., 2022).

Chiral Chromatographic

Once chiral compounds are isolated, a process to determine their absolute configuration is typically necessary. For enantio-selective separation at an analytical scale, techniques like GC, HPLC, SFC, or CE are commonly used, with HPLC being the most frequently applied(Usama et al., 2022). This separation method can isolate enantiomers either directly, using chiral stationary or mobile phases, or indirectly, through the use of chiral derivatization agents or additives (Usama et al., 2022).

Preparative Gas Chromatography

PGC (Packed Gas Chromatography) is an alternative method for extracting volatile oils. Typically, packed columns with larger sample capacities but lower peak resolution are used(Usama et al., 2022). However, in recent years, there has been a growing use of advanced techniques involving thick-phase film full-bore capillaries in conjunction with capillary GC equipment, which offer improved performance (Usama et al., 2022).

STRUCTURE DETERMINATION

The structure of natural products is determined using data from various spectroscopic techniques, such as UV-Visible, Infrared (IR), Nuclear Magnetic Resonance (NMR), and Mass Spectroscopy(Altemimi et al., 2017). The basic principle of spectroscopy involves passing electromagnetic radiation through an organic compound, which absorbs some of the radiation without absorbing it entirely. By measuring the absorption levels of the radiation, a spectrum is generated. These spectra are characteristic of specific bonds in the compound(Altemimi et al., 2017). Based on these spectra, the structure of the natural compound can be identified. Scientists typically rely on spectra from regions like Ultraviolet (UV), Visible, Infrared (IR), Radio frequency (FTIR), and electron beam for structural analysis (Altemimi et al., 2017).

UV-Visible Spectroscopy

UV-visible spectroscopy is commonly used for qualitative analysis and identifying various compounds in both pure substances and biological mixtures. It is especially effective for quantitative analysis, as aromatic molecules act as strong chromophores in the UV range(Masarrat et al., 2021). Natural compounds can be identified using UV-visible spectroscopy, which provides information on the overall polyphenol content. This technique is not only cost-effective but also relatively quick, making it a more affordable option compared to other methods, although it may be less selective (Masarrat et al., 2021).

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectroscopy is an important technique for identifying the functional groups in plant extracts. It helps in the identification and structural determination of molecules. FTIR is a high-resolution analytical tool that allows for the identification of chemical constituents and the elucidation of their structures. It provides a quick, non-destructive method for fingerprinting herbal extracts or powders, as noted by Kassem et al. (2023).

Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear Magnetic Resonance (NMR) Spectroscopy provides insights into the physical, chemical, and biological properties of matter. While one-dimensional NMR techniques are commonly used, two-dimensional NMR methods can reveal more complex molecular structures(Reif et al., 2021). Solid-state NMR is employed to determine the molecular structure of solids, while radio-labeled 13C NMR is used to identify the different types of carbon present in a compound. Additionally, 1H-NMR is useful for identifying the types of hydrogen atoms and understanding the connectivity of hydrogen atoms within the compound (Reif et al., 2021).

Mass Spectroscopy (MS)

Mass spectrometry (MS) is a powerful analytical method used to identify unknown compounds, quantify known substances, and determine the structure and chemical properties of molecules.

The MS spectrum provides information on the molecular weight of a sample(Ma, 2022). This technique is commonly used for structural elucidation of organic compounds, peptide or oligonucleotide sequencing, and for detecting the presence of previously characterized compounds in complex mixtures with high specificity. MS achieves this by simultaneously defining the molecular weight and diagnostic fragments of the molecule (Ma, 2022).

Molecular Distillation (MD)

Molecular distillation is a technique that separates molecules by distilling them under a vacuum at a temperature significantly lower than their boiling point. This method is particularly effective for separating thermo-sensitive and high-molecular-weight compounds (Zhang et al., 2018; Ketenoglu and Tekin, 2015).

CONCLUSION

This paper primarily focused on a comprehensive evaluation of the various techniques used in the extraction and separation of natural products. In recent years, the role of natural products in drug development has seen significant growth. Although isolation processes can be time-consuming, they have often limited the use of natural products. However, with the growing demand for natural product isolation and drug synthesis, numerous more efficient techniques have been developed to enhance the extraction and separation of these compounds.

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 the writing or editing of this manuscript.

REFERENCES

Abdelmohsen U. R., Sayed A. M., Elmaidomy A. H., (2022). Natural Products' Extraction and Isolation-Between Conventional and Modern Techniques. *Frontiers in Natural Products*. 1. DOI=10.3389/fntpr.2022.873808

Abubakar AR, Haque M. (2020) Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. J Pharm Bioallied Sci. 12(1):1-10. doi: 10.4103/jpbs.JPBS_175_19. Epub 2020 Jan 29. PMID: 32801594; PMCID: PMC7398001.

Ahmed, R. (2024). High-Performance Liquid Chromatography (HPLC): Principles, Applications, Versatality, Efficiency, Innovation and Comparative Analysis in Modern Analytical Chemistry and In Pharmaceutical Sciences. Preprints. https://doi.org/10.20944/preprints202409.0057.v1

Akanda M J., Sarker M. Z., Ferdosh, S., Manap M. Y., AbRahman N. N., AbKadir M. O., (2012). Applications of supercritical fluid extraction (SFE) of palm oil and oil from natural sources. Molecules. 10;17(2):1764-94. doi: 10.3390/molecules17021764. PMID: 22328076; PMCID: PMC6268233

Altemimi A., Lakhssassi N., Baharlouei A., Watson D. G., Lightfoot D. A., (2017). Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants (Basel). 6(4):42. doi: 10.3390/plants6040042. PMID: 28937585; PMCID: PMC5750618.

Attimarad M, Ahmed K. K, Aldhubaib B. E, Harsha S., (2011). High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. Pharm Methods. 2(2):71-5. doi: 10.4103/2229-4708.84436. PMID: 23781433; PMCID: PMC3658041.

Bishnu P. R., and Masoud A., (2018). *Analytical Chemistry90* (22), 13133-13150DOI: 10.1021/acs.analchem.8b01461

Budniak L., Vasenda M., Marchyshyn S, Kurylo K., (2020). Determination of the optimum extraction regime of reducing compounds and flavonoids of Primula denticulata Smith leaves by a dispersion analysis. Pharmacia 67(4): 373–378. https:// doi.org/10.3897/pharmacia.67.e54170

Carreira-Casais A, Otero P, Garcia-Perez P, Garcia-Oliveira P, Pereira A. G, Carpena M, Soria-Lopez A, Simal-Gandara J, Prieto MA. (2021). Benefits and Drawbacks of Ultrasound-Assisted Extraction for the Recovery of Bioactive Compounds from Marine Algae. Int J Environ Res Public Health. 18(17):9153. doi: 10.3390/ijerph18179153. PMID: 34501743; PMCID: PMC8431298.

Chakanaka P. M., Cecil K. K., Placxedes S., Babatunde A. O., (2024). Conventional and modern techniques for bioactive compounds recovery from plants: Review, *Scientific African*, 27, 2025, e02509, https://doi.org/10.1016/j.sciaf.2024.e02509.

Coskun O. (2016). Separation techniques: Chromatography. North Clin Istanb. 11;3(2):156-160. doi: 10.14744/nci.2016.32757. PMID: 28058406; PMCID: PMC5206469.

Delazar A, Nahar L, Hamedeyazdan S, Sarker SD. (2012). Microwave-assisted extraction in natural products isolation. Methods Mol Biol. 864:89-115. doi: 10.1007/978-1-61779-624-1_5. PMID: 22367895.

Dey P, Kundu A, Kumar A, Gupta M, Lee BM, Bhakta T, Dash S, Kim H. S., (2020). Analysis of alkaloids (indole alkaloids, isoquinoline alkaloids, tropane alkaloids). Recent Advances in Natural Products Analysis. :505–67. doi: 10.1016/B978-0-12-816455-6.00015-9. Epub 2020 Mar 20. PMCID: PMC7153348.

Ekor M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 10;4:177. doi: 10.3389/fphar.2013.00177. PMID: 24454289; PMCID: PMC3887317.

Emil M., and Ernő T., (2011). Overpressured Layer Chromatography (OPLC) – AFlexible Tool of Analysis and Isolation. *Natural Product Communications.* 6(5): 719-732.

Fotsing Y. S., Kezetas J. J., El-Saber, B., Ali, I., and Ndjakou B. L., (2022). Extraction of Bioactive Compounds from Medicinal Plants and Herbs. IntechOpen. doi: 10.5772/intechopen.98602 https://doi.org/10.2478/rtuect-2023-0031

Ishwari R. R., Sakshi S. M., Komal D. S., Rupali R. B., (2024). A review on Extraction Techniques used in advanced herbal drug technology, *International Journal of Novel Research and Development.* 9(2):470-473.

Jeenu J., and Girisa C., (2020). General Techniques of Extraction of Active Constituents In Herbal Drugs. *World Journal of Pharmaceutical Research.* 9(4):1900-1908.

Kassem A, Abbas L, Coutinho O, Opara S, Najaf H, Kasperek D, Pokhrel K, Li X, Tiquia-Arashiro S. (2023). Applications of Fourier Transform-Infrared spectroscopy in microbial cell biology and environmental microbiology: advances, challenges, and future perspectives. Front Microbiol. 21;14:1304081. doi: 10.3389/fmicb.2023.1304081. Erratum in: Front Microbiol. 2023 Dec 13;14:1342406. doi: 10.3389/fmicb.2023.1342406. PMID: 38075889; PMCID: PMC10703385.

Ketenoglu, O., and Tekin, A., (2015). Applications of Molecular Distillation Technique in Food Products. *Ital. J. Food Sci.,* 27. 277-281.

Komal P., Namrata P., and Pradnya I., (2019). Techniques Adopted for Extraction of Natural Products Extraction Methods: Maceration, Percolation, Soxhlet Extraction, Turbo distillation, Supercritical Fluid Extraction. *International Journal of Advanced Research in Chemical Science (IJARCS)* 6(4):1-12 DOI: http://dx.doi.org/10.20431/2349-0403.0604001 www.arcjournals.org

Kumar A. P., Kumar M, Jose A, Tomer V, Oz E, Proestos C, Zeng M, Elobeid T. K, Oz F. (2023). Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. Molecules. 28(2):887. doi: 10.3390/molecules28020887. PMID: 36677944; PMCID: PMC9862941.

Luksta , I., Spalvins, K., (2023). Methods for Extraction of Bioactive Compounds from Products: A Review. *Environmental and Climate Technologies*. 27(1):422–437

Ma, X. (2022). Recent Advances in Mass Spectrometry-Based Structural Elucidation Techniques. *Molecules*, *27*(19), 6466. https://doi.org/10.3390/molecules27196466

Masarrat M., Neha A. M., Sumaiya K., Afeefa K., (2021). Uv-Vis Spectroscopy in Analysis of Phytochemicals. *International Journal of Pharmaceutical Research and Applications.* 6(5):482-499. DOI: 10.35629/7781-0605482499

Mesud, M. H., Ahmet C., Seyda Y., Sibel A. O., (2024). Advanced sample preparation and chromatographic techniques for analyzing plant-based bioactive chemicals in nutraceuticals,.*Journal of Chromatography Open.* 5,100131. https://doi.org/10.1016/j.jcoa.2024.100131.

Mohammed G. R. (2018). Extraction, Isolation and Characterization of Natural Products from Medicinal Plants. *International Journal of Basic Sciences and Applied Computing (IJBSAC)*. 2 (6):1-6.

Nazari, M., Shokoohizadeh, L. & Taheri, M. (2025). Natural products in the treatment of diabetic foot infection. *Eur J Med Res* **30**, 8. https://doi.org/10.1186/s40001-024-02255-y

Park MK, Cha JY, Kang MC, Jang HW, Choi YS. (2023). The effects of different extraction methods on essential oils from orange and tangor: From the peel to the essential oil.

Food Sci Nutr. 12(2):804-814. doi: 10.1002/fsn3.3785. PMID: 38370058; PMCID: PMC10867503.

Purnendu P., Indu. S., Banamali D., Bhuyan, G. C., Rao M. M., (2022). Therapeutic importance of *Asava* and *Arista* (Fermentative Formulation) in Ayurveda: A review*Res J. Pharmacology and Pharmacodynamics.2022;14(4):273-276.*DOI: 10.52711/2321-5836.2022.00047

Reif B, Ashbrook SE, Emsley L, Hong M. (2021). Solid-state NMR spectroscopy. Nat Rev Methods Primers. 1:2. doi: 10.1038/s43586-020-00002-1. Epub 2021 Jan 14. PMID: 34368784; PMCID: PMC8341432.

Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med. 8(1):1-10.

Souiy, Z. (2024). Essential Oil Extraction Process. IntechOpen. doi: 10.5772/intechopen.113311.

Susanti, I., Pratiwi, R., Rosandi, Y., & Hasanah, A. N. (2024). Separation Methods of Phenolic Compounds from Plant Extract as Antioxidant Agents Candidate. *Plants, 13*(7), 965. https://doi.org/10.3390/plants13070965

Svetlana I., Stanislav S., Alexander P., Olga S., Ilia N., Elena K., Olesia K., Viktoria L., Svetlana N., Olga B., (2024). Medicinal plants: A source of phytobiotics for the feed additives. *Journal of Agriculture and Food Research,* 16, 101172, https://doi.org/10.1016/j.jafr.2024.101172.

Usama R. A., Ahmed M. S., and Abeer H. E., (2022). Natural Products' Extraction and Isolation-Between Conventional and Modern Techniques. Front. Nat. Prod. 1:873808. doi: 10.3389/fntpr.2022.873808

Waksmundzka-Hajnos, M., Hawrył, M., Hawrył, A., Jóżwiak, G. (2022). Thin Layer Chromatography in Phytochemical Analysis. In: Buszewski, B., Baranowska, I. (eds) Handbook of Bioanalytics. Springer, Cham. https://doi.org/10.1007/978-3-030-95660-8_24

Yogeshri j. J., Abhijit p. A., Lohiya R. T., Milind J. U.,, Atul T. H., and krishna R. G., (2023). *International Journal of Science and Research Archive*. 2023, 10(01):555–568. DOI: https://doi.org/10.30574/ijsra.2023.10.1.0768

Zhang Q. Q., Li‐Gen L., and Wen‐Cai Y., (2018). Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine.* 13:20 https://doi.org/10.1186/s13020-018-0177-x

Zhou, W., Li, J., Wang, X., Liu, L., Li, Y., Song, R., Zhang, M., & Li, X. (2023). Research Progress on Extraction, Separation, and Purification Methods of Plant Essential Oils. *Separations*, *10*(12), 596. https://doi.org/10.3390/separations10120596