International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Extraction and Isolation Techniques for Natural Products: A Comprehensive Review

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Abstract:
It summarizes recent advances in extraction and isolation techniques for natural products, with emphasis on green and sustainable approaches. It discusses the shift from conventional solvent intensive methods, such as maceration and Soxhlet extraction, to modern techniques including ultrasound-, microwave- and supercritical fluid–assisted extraction that improve efficiency and reduce toxic solvent use. Particular focus is placed on deep eutectic solvents (DES) and natural deep eutectic solvents (NADES) as tunable, low-toxicity media for recovering bioactive phytonutrients like terpenoids, alkaloids, and polyphenols. It also covers solid–liquid extraction optimization, chromatographic separation (LPLC, preparative HPLC, TLC, GC), and complementary purification operations such as recrystallization and trituration for obtaining analytical-grade isolates. Analytical characterization using HPLC, LC–MS, NMR, IR and UV–Vis is outlined to confirm purity and structural identity of isolated compounds. Case studies, including bakuchiol, Artemisinin and paclitaxel, illustrate how optimized extraction and isolation workflows translate into successful pharmaceutical products. Finally, the article highlights current challenges in scalability, reproducibility and cost, and points to future integration of automation, microfluidics and artificial intelligence to design smarter, more sustainable extraction processes and accelerate natural product drug discovery.

Keywords: Extraction, Isolation, Chromatography, HPLC, Spectroscopic, HPLC, LC–MS, NMR, IR, UV–Vis, Green technologies.

1 Introduction:

Current advancements of naturally occurring Des (deep eutectic solvents) and NADES (Natural Deep Eutectic Solvents) when utilizing contemporary environmentally conscious technologies will be discussed in relationship to how to obtain bioactive phytonutrients. The main focus of interest will be on bioactive compounds, that are considered of major economic value; such as: terpenoids, alkaloids, nitrogen/sulphur-based compounds, especially. Polyphenols are widely used in food, pharmaceutical and cosmetic industries. Traditional methods used to extract a particular phytonutrient typically require the use of organic solvents in conjunction with many days' of various extraction techniques. The use of these organic solvents has many disadvantages such as high consumption levels of solvents, cost of energy for extraction methods. The current industry trend is a focused on creating safe and more sustainable methods - in turn

reducing the use of toxic solvents and waste during extraction. The use of DES solvent compliant with the principles of green analytical chemistry and reduce extraction method requirements for i.e. execute extraction with water in conjunction with the extraction solvent. The above-mentioned opportunities available with employing either the DES or NADES solvents include their application to extract specific bioactive compounds by modifying their density, viscosity and polarity by changing; base component chemical composition, molar ratios, temperature, and volume ratios of water. When compared to traditional ionic liquids used in biological systems, DES or NADES are generally less expensive, less toxic, and derived from naturally occurring chemicals.(1)

One justifiable alternative is the creation and use of more environmentally friendly extraction agents. Water is the cleanest of all possible solvents it can be used industrially in all industries and sectors including those with the strictest limitations imposed by rule such as food and pharmaceutical. The physical and chemical properties of water prevent it from being utilized effectively in the extraction process. Water can extract polar biologically active compounds even when the polar biologically active compound can be extracted with water, water's ability to extract polar biologically active compounds is generally very much lower than that which can be accomplished using organic solvents like methanol or ethanol however, with respect to less polar, non-polar, and hydrophobic compounds, water has very poor extracting capability. (2)

2 Extraction techniques:

Extraction is the process of separating out the desired components from a chemical mixture. Extraction methods separate compounds, broadly categorized by phases (liquid-liquid, solid-liquid) or techniques like Soxhlet (continuous solvent), Maceration (soaking), Percolation (solvent flow), Distillation(heating/vaporizing), Supercritical Fluid (using supercritical CO2), and modern assisted methods like Microwave-Assisted (MAE) and Ultrasound-Assisted (UAE)Solid-liquid extraction techniques are frequently utilized during the initial stages of creating a product in many industrial processes. Solid-liquid extraction is a separation technique that can be performed both in laboratory settings and on an industrial scale to extract a particular compound or group of compounds from a solid material using a solvent. There are several factors that must be taken into consideration to optimize the solid-liquid extraction process which include understanding the physical and chemical properties of the solid matrix, including melting point (temperature), mechanical properties and solubility. Granulometry of the solid matrix must be examined and possibly altered for extraction; for example, if the particle size is too small, it will facilitate solvent dispersion but could result in clogging of the extraction system, so finding an ideal particle size for each process is necessary. Additionally, selecting an appropriate solvent is critical as well and must be based on the solute that you are attempting to extract. (3)

Likewise, identifying a solvent that meets all of previously mentioned considerations can be quite difficult, as there are many factors that must be taken into account in order to satisfy the design of the extraction process, the characteristics of the solid matrix and the desired target compounds to obtain.(4)

The primary focus is how efficiently extract green methods of isolating and Characterizing bioactive compounds in a variety of matrices, including what is typically viewed as waste materials, therefore creating opportunities for improved recycling and utilization. Some of the publications described the identification of bioactive compounds extracted by various chromatographic methods using both Traditional detection Methods (UV/visible) and Advanced methods such as mass spectrometry and nuclear magnetic resonance. Optimizing extraction, quantifying and determining the inhibition of

bakuchiol by P coryfolia have also been extensively studied in traditional Chinese and Ayurvedic medicine. Various extraction techniques have been applied to the seeds of P. Cory folia including maceration, reflux, Soxhlet and UAE with the extracted solutions analysed using HPLC. The effect of extracted bakuchiol UAE method produced the largest amount of bakuchiol, which significantly reduced levels of circulating biomarkers when administered to experimental animals. (4)

2.1 Isolation and purification techniques:

Isolation procedures are described as methods that separate the compound of interest from the reaction mixture, and these include liquid-liquid extraction of aqueous and organic phases, with acid-base chemistry, solid-phase extraction passage of a solution through a solid material that retains the compound or impurities, accelerated solvent extraction for solid/semisolid samples at high temperature and pressure, and sample work-ups to remove solvents or reagents such as pyridine, DMF, thionyl chloride, and metal catalysts. These paragraphs usually follow this order: first, the general idea of the method, then detailed stepby-step treatment of the reaction mixture, and finally remarks on limitations or dangers such as pyridine toxicity, flammability of dry Pd. (5). The expression of 'natural products' generally affects to natural chemical compounds that have certain distinctive pharmacological or biological activities. Natural products encompass a broad spectrum of compounds such as alkaloids, terpenoids, flavones, lignans, and coumarins, among others. At present, owing to the documented biodiversity in the world, natural products are acting as a valuable source of chemical diversity, structural diversity, and bioactive diversity. Natural products have been the principal source for the production of pharmaceuticals, cosmetics, flavours, and dietary supplements. (5)(2)

Methods of purification are also mentioned that it describes the procedure of purifying the crude isolated substance to render it more amenable to analysis or further use. Recrystallization is mentioned as a procedure that involves the dissolution of the crude solid in a hot solvent in which it is soluble, followed by cooling to enable the formation of pure crystals while leaving the impurities undissolved or dissolved in the solvent, with instructions on how to select the solvent and the procedure for determining solubility. Trituration is mentioned as a procedure that involves grinding the crude solid with a non-polar solvent such as n-hexane or diethyl ether to remove non-polar impurities in the form of sticky substances, while sublimation is used to purify substances that have the ability to easily change from solid to vapour and back to solid form as crystals on a cold surface when their vapour pressure is high compared to that of the impurities.

The different chromatographic techniques are mention in, starting with a general explanation of why their components distribute differently between stationary and moving phases and then describing each technique. Thin Layer HPLC will be used as a method for evaluating purity and determining Rf values; Column HPLC and Flash HPLC will be used as methods of preparative separation on silica or alumina; and HPLC, HPTLC, UPLC, FPLC, GC, SCF and capillary electrophoresis will be used as advanced techniques for high-resolution separation based on polarity, volatility or charge. Each of these techniques describes how to isolate crude product from mixture and then how to purify that compound to meet criteria established for purpose of analysis or to develop material that can be utilized for biological research. (5)(1)

2.2 Chromatographic Techniques:

2.2.1 Preparative High-performance Liquid Chromatography:

Preparative High-Performance Liquid Chromatography, preparative HPLC is a type of liquid chromatography that is particularly used for the isolation and purification of large quantities of certain

compounds, rather than their analysis. Unlike HPLC analysis, where only a very small quantity of sample is injected in order to get a well-resolved peak, preparative HPLC involves a larger injection volume and a larger column load in order to isolate milligrams to grams of a compound. The basic principle, however, remains the same in both cases: the separation of the solutes based on their differential interactions with the stationary phase packed inside the column and the mobile phase that is passed through it under high pressure. As each compound passes out of the column and through the detector, the corresponding effluent fraction is diverted into a separate container, where the chemist can isolate relatively pure samples of the compound for further analysis. (6) (15)

In HPLC, method development often begins with an analytical-scale method, which is then scaled up by adjusting the column size, particle size, flow rate, and injection load while keeping similar chromatographic selectivity. The columns used are usually larger in internal diameter and filled with similar stationary phases, like reversed-phase C18, to those found in analytical systems. However, they need to handle higher sample loads and, at times, higher flow rates. The mobile phase composition and gradient are improved to find a balance between resolution and throughput. Better separation results in higher purity, while faster runs increase productivity. Detection typically uses UV-Vis or other detectors. The main goal of detection is to signal when a desired peak appears, allowing for precise synchronization of fraction collection. Collected fractions can later be pooled, concentrated, and rechecked by analytical HPLC to confirm purity. (6) (16)

It is applied in pharmaceutical and biomedical research, natural product isolation, peptide and protein separation, and purification of synthetic intermediates. It is especially useful when the desired compound is heat-sensitive, non-volatile, or difficult to crystallize. In such instances, gas chromatography or simple recrystallization may not be very effective. While HPLC is more costly and solvent-intensive than many traditional methods of purification, it offers high resolution, purity, and the flexibility to modify the conditions of separation for similar impurities or isomers. It is thus considered one of the most useful methods when efficient separation and recovery of purified material are required on a larger scale. (6)

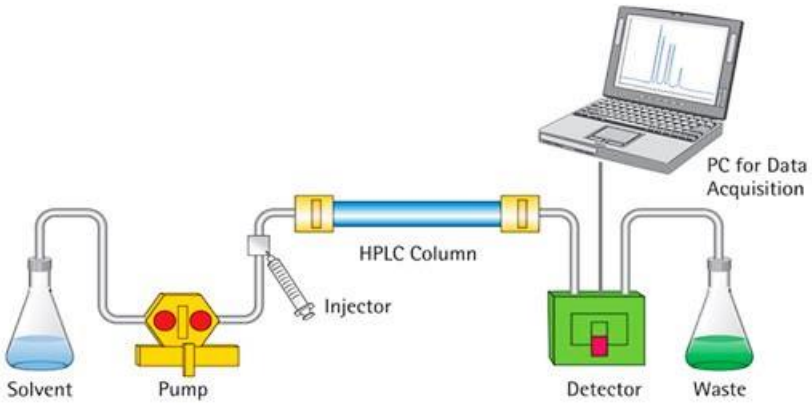

Figure 1: High-performance Liquid Chromatography (HPLC)

2.2.2 Low-pressure Liquid Column Chromatography (LPLC)

Low-pressure liquid column chromatography (LPLC) is a simple variant of liquid chromatography that employs large particles of the stationary phase typically 40-200 µm in size and is conducted at near to atmospheric pressure, so that the system does not need high-pressure pumps. The stationary phase is packed in a glass or plastic column, and the mobile phase (solvent system) passes through the stationary phase either by gravity or slight positive pressure/vacuum, depending on the size of the particles and the desired flow rate. Since the particles are large and the pressure is low, the efficiency of separation is lower

compared to HPLC, and it takes longer, but it is cheap and useful for handling large amounts of crude extracts and preparing multiple fractions for further purification.(7)

In the Liquid - Liquid Partition Chromatography separation is usually done in one of two ways by using adsorption or by separating things based on size. This depends on what type of the stationary phase's like. The Silica gel is a material that has a charge and is a bit acidic which makes it work well for separating things that are also charged or basic like certain kinds of medicines. There are also kinds of silica like C18, C8 or amino propyl silica that are used when we want to separate things in a different way like when we are using reversed-phase or intermediate-polarity separations, in Liquid Partition Liquid Chromatography. Other adsorbents include alumina, which may be acidic, basic, or neutral depending on the method of preparation, and resins derived from polystyrene, which have varying selectivity's. The mobile phases vary from non-polar solvents such as n-hexane to chloroform, dichloromethane, ethyl acetate, methanol, and water. Normal-phase LPLC normally employs mixtures of n-hexane, ethyl acetate, and methanol, while reversed-phase LPLC employs mixtures of water, methanol, and acetonitrile. (7) (20)

Operationally, if the size of the packing particles is larger than 60 µm, gravity elution alone is sufficient: solvent is added to the top of the open column and allowed to drain by gravity. When particle sizes are in the 40- to 60-µm range, positive pressure at the top of the column or vacuum at the bottom may be used to speed up the separation, resulting in modifications such as flash chromatography (using pressurized gas) and vacuum liquid chromatography (VLC). LPLC is thus largely a preparative technique in natural products chemistry: it is well suited to the initial fractionation of plant or microbial extracts into groups of compounds, from which specific fractions can then be purified on a higher-resolution technique such as preparative HPLC or

High-speed counter-current chromatography. (7) (18)

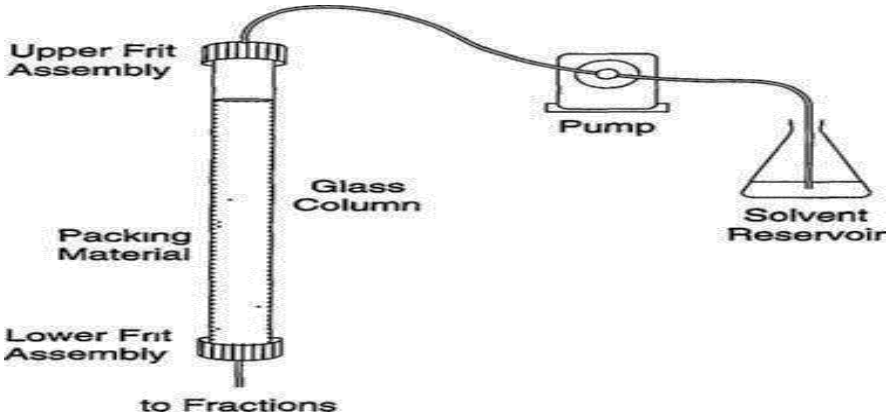

Figure 2: Low-pressure Liquid Column Chromatography (LPLC)

2.2.3 Thin-layer chromatography (TLC):

Thin-layer chromatography (TLC) is a technique used to separate components of a mixture on a flat plate coated with a thin layer of adsorbent like silica gel, alumina, or cellulose. This layer serves as the stationary phase. A small spot of the sample solution is applied near the bottom of the plate. The plate is then placed in a closed chamber with a shallow pool of solvent or solvent mixture below the origin line. The solvent rises up the plate through capillary action, dissolves the sample, and carries its components at different rates. This depends on how much they stick to the stationary phase compared to the mobile phase. As a result, the components separate into distinct spots at various heights (8)

The size of the packing particles is larger than 60 µm, gravity elution alone is sufficient: solvent is added to the top of the open column and allowed to drain by gravity. When particle sizes are in the 40- to 60-µm

range, positive pressure at the top of the column or vacuum at the bottom may be used to speed up the separation, resulting in modifications such as flash chromatography (using pressurized gas) and vacuum liquid chromatography (VLC). LPLC is thus largely a preparative technique in natural products chemistry: it is well suited to the initial fractionation of plant or microbial extracts into groups of compounds, from which specific fractions can then be purified on a higher-resolution technique such as preparative HPLC or high-speed counter-current chromatography. (8)

In TLC plates can be bought pre-coated or made by layering a slurry of adsorbent material, like silica gel suspended in a binder like calcium sulfate, on glass, aluminium, or plastic surfaces. This layer is then dried and activated by heating. The samples are applied as small concentrated spots using capillary tubes. It is critical to keep the spot diameter between 2 and 5 mm, not disturb the adsorbent surface, and to dissolve the samples in volatile, preferably non-polar solvents to prevent spreading. After development in a saturated chamber, the solvent front is marked. The plate is then dried, and the spots are directly visible if colored. Otherwise, illumination with UV light, iodine vapor, or spray reagents is used. For example, ninhydrin is used for amino acids, potassium permanganate for oxidizable compounds, and chromic reagents for sugars. Difficulties with large spots, irregular solvent fronts, and streaks can be reduced by using the right chamber size, solvent volume, spotting technique, and dilution of the sample. TLC finds numerous applications as a quick, inexpensive, and simple analytical technique for following the course of a reaction, testing the purity of samples, identifying substances by matching them with known samples, and carrying out preliminary separations prior to more advanced techniques such as HPLC. In the pharmaceutical industry, it is used for impurity profiling, analysis of active components, and separation of multicomponent drugs; in biochemistry, it is applied to amino acids, peptides, steroids, and other metabolites; and in food and environmental analysis, it is used for the detection of pesticides, dyes, vitamins, and mycotoxins. On a semi-preparative level, preparative TLC enables the isolation of tens to hundreds of milligrams of material by applying the sample in the form of a band, developing it to create horizontal regions, scraping off the desired region, and then eluting the compound with an appropriate solvent, providing a convenient alternative to column chromatography for small-scale separations. (8)

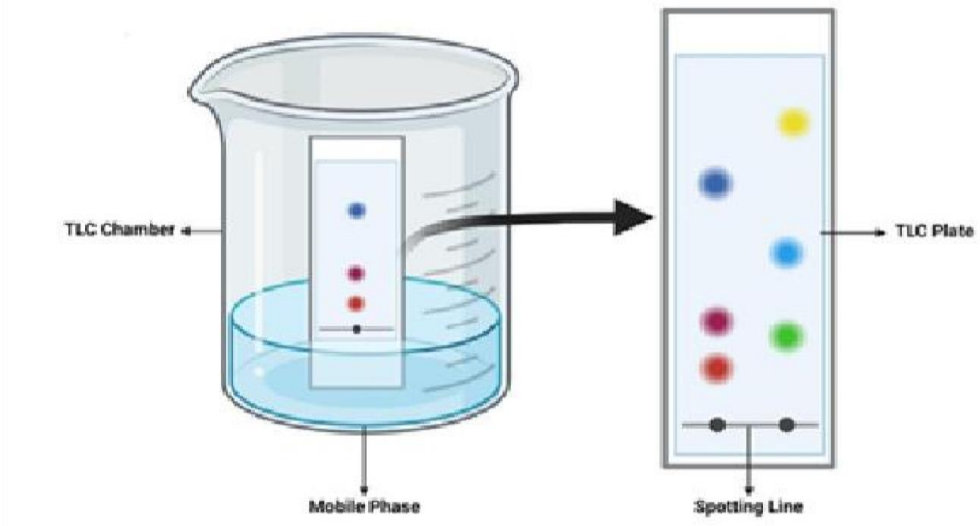

Figure 3: Thin-layer chromatography (TLC):

2.2.4 Gas Chromatography:

Gas Chromatography is a type of gas dissolved gas chromatography procedure where the stationary phase is a liquid layer coated on a solid, inert substrate in a column, and the mobile phase proceeds through the column as a gas carrier He or N_2 under high pressure; therefore, before the experiments begin, the samples must be gas-evaporated so they will travel through the gas column with the gas carrier. (9)(22)(23)

As the gas-evaporated sample passes through the column, the separating factors will depend on the physical and chemical characteristics of each individual component as they partition between the gas phase (I.E., evaporation) and the liquid stationary phase (i.e., adsorption). In addition, each individual component in the mixture will have a varying degree of interaction with the stationary phase of the column, resulting in varying times for each component to pass through the column, which is a function of its relative degree of interaction with the stationary phase (i.e., hold on to the station phase).

As a result of varying levels of interaction with the stationary phase, those components with more interaction will take longer to exit the column and have a longer retention time. Conversely, those components with little or no interaction with the stationary phase will move with the inert gas carrier, respectively, more quickly than those components with more interaction with the stationary phase; this is due to the larger mass of those components with more interaction having a smaller inertia than the lower-mass components with less interaction; therefore, the varying levels of interaction will make gas chromatography a very useful technique for separating individual components from a sample, even at trace levels.(9)(25)Gas chromatography can be applied to gases, mixtures of volatile liquids, and volatile components of solid materials. In biomedical and medical laboratories, gas chromatography is employed to analyze the quantity of substances like steroids, barbiturates, and lipids in biological materials. Gas chromatography can also be employed to separate small molecules like alcohols, esters, lipids, and compounds with amino groups. Gas chromatography can be employed to analyze the connection between enzymes and volatile substrates or products. Compared to liquid chromatography, which is more suitable for thermally unstable and non-volatile compounds, gas chromatography is more preferred if the compounds can be vaporized without decomposing.(9)(22)

Gas chromatography uses an inert gaseous mobile phase, a liquid stationary phase on an inert support, and high pressure. The combination of these three factors makes gas chromatography highly efficient in a short time. Its high sensitivity, speed, and capacity to analyse very small amounts of samples have made gas chromatography a widely accepted technique in analytical chemistry, medical diagnostics, pharmaceutical analysis, environmental analysis.(9)

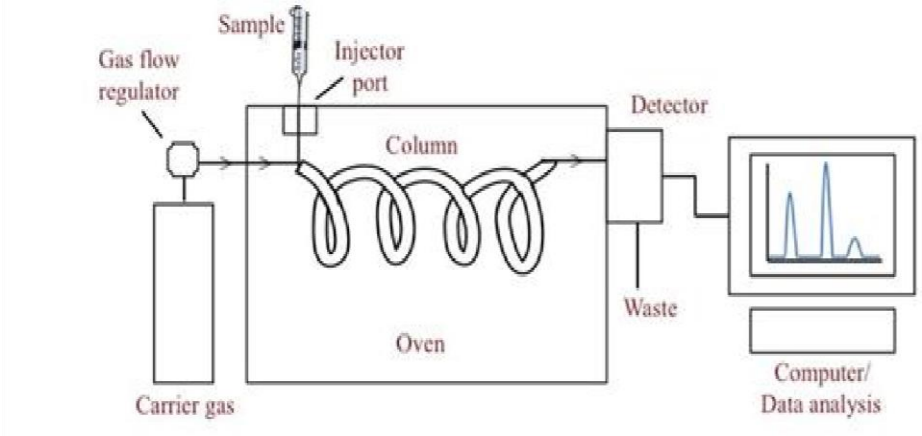

Figure 4: Gas Chromatography

Technique	Principal	Best For	Advantage	Limitation
Soxhlet Extraction	Continuous reflux of solvent through solid sample.	Thermostable Non- volatile solid sample.	High efficiency less solvent needed than maceration.	Time-consuming; not for heat sensitive compounds.
Maceration/Soaking	Soaking in solvent at room temperature.	Heat-sensitive compounds.	Simple, low cost.	Slow, Low efficiency.
Percolation	Constant flow of fresh solvent Throng sample	Rapid, high-yield extraction.	More efficient than Maceration.	Requires Specialized setup.
MAE(Microwave)	Microwave energy heats solvent/sample.	Rapid extraction of phenolic compounds.	Fast Low solvent consumption.	Not suitable for non-polar/Volatile compound.
UAE(Ultrasound)	Cavitation bubbles disrupt cell walls.	Thermolabile Compounds.	High exception rate at lower temps.	Potential for compound degradation.
SFE(Supercritical)	Using supercritical CO2 under high pressure.	Essential oils, non-polar compound.	Eco-friendly, high purity high yield.	High capital cost.
Distillation	Separation by boiling point.	Volatile organic compounds.	Simple, good for essential oils.	Only for volatile components.
Chromatography	Differential migration of components.	Isolation/purification of compounds.	High resolution and purity.	Expensive, Low throughput.

Table 1: List of Extraction Techniques

3 Factors Affecting Extraction and Isolation:

Compounds from plant materials. These are the sample preparation methods (prewashing, drying or freeze-drying to keep volatile actives), particle size grinding (i.e., generally 40-200 μ) to keep surface area/solvent contact maximal, and prevent channelling or degradation of heat-labile components, the polar/non-polar nature of the chosen solvent based on compound type (hydrophilic=flavonoid=polar solvent; lipophilic=non-polar dichloromethane/hexane/cache chlorophyll) as well as the extraction method used. For example, maceration is time-consuming (3 days at room temp) and variable volume solvents; however, recovery is 100% complete but has difficulty determining percent recovery due to using variable volumes of solvent. Soxhlet (good recovery) uses a 150/200 ml volume of solvent. Reflux time ranges from 3-18 hours, sonication (enhancement.) (10)

Use of caviation: 1 hr. explodes air bubbles throughout liquid-similar to boiling-saves time/only takes >30% less liquid than maceration. More importantly, highly advanced techniques (supercritical fluid

extraction {SFE}, or microwave-assisted extraction) utilize less solvent; decrease thermal degradation; provide rapid kinetics; increase solubility and selectivity via changes in pressure/temperature. All, however, require special equipment and/time to set up those systems. (10) (2)

4 Analytical Characterization of Isolated Compounds:

Analysing isolated natural compounds is important to assess their purity and provide definitive structure elucidation. This involves the use of sophisticated analytical tools, particularly spectroscopic and spectrometric. Initial purity assessment and fractionation monitoring are usually performed by chromatographic methods like HPLC with detectors such as ultraviolet (UV) – and visible (Vis) spectrophotometry. Using UV-Vis, absorption spectra provided via chromophores aid in determining the class of compound and checking purity; whereas, high-resolution mass spectrometry (generate molecular weight and elemental composition) and pattern of peptide fragmentation are helpful in determining the partial structure of the compound. The power of the integrated approach of separation and detection demonstrated in LC-MS allows the simultaneous separation of individual components in a crude chemistry or extract and the detection (mass-based identification) based on mass/charge of a molecule and structural prediction even though there is no prior full isolation. Additionally, infrared spectrometry can provide information on the functional groups present in molecules supporting the information obtained through the other methods. (11)

5 Green and Sustainable Approaches:

The rise of green and sustainable methods for the extraction and isolation of natural products has occurred due to increased awareness of the environmental ramifications that result from using conventional methods. The "Six Principles of Green Extraction" dictates these processes will use green or environmentally friendly solvents, minimize energy usage, and make use of all co-products. In addition, there is a major push towards using alternative safer solvents, especially agro-solvents (solvents derived from renewable agricultural material) or water as opposed to the hazardous volatile organic solvents such as dichloromethane or hexane. The use of energy-efficient methods will also reduce the overall energy usage in the extraction process. Non-conventional extraction techniques such as Supercritical Fluid Extraction (SFE), Pressurized Hot Water Extraction (PHWE), Microwave-Assisted Extraction (MAE), and Ultrasound-Assisted Extraction (UAE) have been documented to have shorter extraction periods, reduce the usage of solvents during extraction, and greater selectivity and efficiency compared to traditional techniques such as Soxhlet extraction or maceration. Finally, the environmental aspects of the extraction process will be considered in a "zero waste" philosophy whereby the entire natural matrix is utilized for the production of co-products or energy, thus fulfilling the principles of the circular economy and bio refinery. (12)

6 Applications and Case Studies:

Natural products have served as an important source of medicinal compounds for many years. The extensive use of natural product drugs, such as Artemisinin and Paclitaxel, has demonstrated the therapeutic potential of these products. For example, Artemisinin has become the basis for Artemisinin-based Combination Therapies (ACTs), which are now the standard treatment for uncomplicated Plasmodium falciparum malaria. In addition to its effectiveness in treating malaria, there are now studies describing the anticancer properties of both Artemisinin and its derivatives in vitro and in vivo, due to

their ability to induce apoptosis, arrest the cell cycle, and inhibit angiogenesis in various types of human cancers. The information presented here supports the idea of drug repurposing. Paclitaxel (Taxol) is an additional example of the medicinal value of natural products. Paclitaxel is a diterpenoid isolated from the bark of the Pacific Yew tree. . It is considered to be one of the most successful anticancer drugs derived from natural products because of its broad-spectrum activity against various types of cancers, including ovarian, breast, and non-small cell lung cancer, The mechanism of action of this drug includes selective binding to beta-tubulin, induction of tubulin polymerization, and stabilization of microtubules, which ultimately results in the prevention of spindle formation, resulting in mitotic arrest in the G2/M phase and subsequent death of cancer cells, Because of the low natural abundance of Paclitaxel, commercial-scale production of this drug is currently being carried out through semi-synthesis using a more readily available natural precursor, 10-deacetylbaccatin III, obtained from the needles of Taxus baccata.(13) (3)

7 Challenges, Limitations, and Future Perspectives:

Natural product extraction and isolation are challenged in terms of scalability, reproducibility, and cost. The results are unpredictable because natural products have varying properties depending on the plant species, geographical distribution, climate, and time of harvest. The industrial-scale production processes are faced with economic and technological challenges because natural products are present in low concentrations, which scientists must isolate from laboratories. The traditional processes of isolating compounds are time-consuming since they require large amounts of solvents, which can damage heat-sensitive materials in the process. (14) (2), To address these challenges, we require extraction techniques that employ automated systems and artificial intelligence. The combination of high-throughput automation and microfluidics technology enables faster extraction and fractionation procedures, which saves time, reduces the use of solvents, and lowers labour costs. This increases efficiency. AI algorithms, together with data management technology, will revolutionize the way we select the optimal extraction conditions. Additionally, they will enable the linking of chemical structures to their biological roles and simplify the process of identifying existing compounds, thereby accelerating the discovery of new chemical entities. (14)

8 Conclusion:

In short, this work shed light on the gradual change of the extraction processes that rely heavily on solvents and are harmful to the environment rather than bioactive natural compounds isolation through the use of eco, friendly methods. Some of them are the use of DES/NADES, solid, liquid extraction that is enhanced, and separation and analysis by advanced chromatographic and spectroscopic methods. All these methods not only provide higher efficiency but also reduce the use of toxic solvents and energy consumption. They allow for the selective extraction of the main components of secondary metabolism like terpenoids, alkaloids, polyphenols, and other biologically active substances that are the basis of pharmaceuticals, cosmetics, food, and nutraceutical products.

However, the large, scale utilization of green extraction technologies still has certain limitations. Besides the issues of scaling up and cost, various biological materials, and low concentration of the target compound are some of the factors that can affect the repeatability and industrial use of these green extraction processes. Future research should be focused on combining automated high, throughput systems, microfluidics, and artificial intelligence for the process optimization and structure, activity relationships to speed up the natural product discovery.

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