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Therapeutic Bioactives and Their Progressive Extraction, Fractionation, and Analytical Techniques: A Comprehensive Review

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Abstract:
This comprehensive review thoroughly evaluates the state-of-the-art techniques employed in the extraction, fractionation, and analytical profiling of therapeutic bioactives sourced from a diverse array of natural origins. Addressing specialists and researchers in the field, this article offers a systematic examination of advanced methodologies that are integral to the current landscape of bioactive compound research.
The review commences by providing an extensive examination of extraction methodologies, highlighting critical parameters such as solvent selection, temperature, and duration to optimize the yield and purity of bioactive compounds. It proceeds to search through advanced fractionation techniques, presenting a detailed comparison of column chromatography, solid-phase extraction, and preparative HPLC, with a focus on their efficacy in the isolation and purification of target compounds.
A distinctive feature of this review is its exclusive chapter, titled "Exploring the Power of Nature's Pharmacy." This chapter explores the diverse pharmacological attributes and clinical health benefits offered by bioactives sourced from plants and microorganisms. It studies the potential of these natural compounds in addressing a range of medical conditions and their role in promoting overall health and well-being.
Furthermore, this review emphasizes the urgency to develop sustainable and eco-friendly extraction practices, expand the exploration of undiscovered bioactive resources, and advance multidimensional analytical techniques for a comprehensive characterization of compounds.
In conclusion, this comprehensive review serves as an invaluable resource for specialists and researchers, offering a detailed analysis of progressive techniques in extraction, fractionation, and analytical profiling. The specialized chapter on medical applications and health benefits underscores the profound significance of bioactives in modern healthcare, marking a crucial advancement in the field.
Keywords: *bioactives, plant, phytochemicals, flavonoids, terpenes, extraction, fractionation, purification, modern methods, green techniques, Supercritical, SFE, HPLC*

I. Introduction

Natural compounds derived from plants, algae, and terrestrial or marine microorganisms, including bioactives and flavonoids, have been utilized for centuries in medicine[1-7]. These compounds possess a range of properties such as anti-cancer, anti-inflammatory, anti-diabetic, anti-oxidant, anti-microbial[8], cardiovascular protection, neuroprotective, anti-allergic effects. Terpenes and flavonoids are examples that highlight the roles they play in herbal remedies and other health related products[8-17]. The extraction and fractionation of these compounds from sources are crucial for creating medicine, medicaments, and drugs[18-25]. The process of fractionating and purifying NOCs, including plant-based bioactives, from plant materials and microorganisms involves a series of methodical steps. These methods are supported by techniques to ensure the production of high quality compounds with therapeutic applications. By integrating precision with techniques, valuable compounds can be extracted with enhanced purity and efficacy. This contributes to the progress of natural products' chemistry as the associated industries[26-29]. However, the extraction and isolation processes for products have traditionally been labor-intensive and time-consuming, which has hindered their application in drug development. In years, though, there has been a pressing need to develop selective methods for extracting and isolating bioactive natural products[30, 31]. This review seeks to offer a perspective on the approaches employed for extracting and isolating natural products, including specific illustrations of products and methods, as well as a strong literature review backing this information.

Extraction plays a critical role in industries because it serves as the initial step in isolating valuable compounds. During the extraction process, desired compounds are separated from materials like plant materials and microorganisms. These extracted compounds find applications in areas such as developing nutraceutical products, health promoting products, functional foods, food additives, or treatments for diseases like diabetes, infections, cancer, and inflammatory diseases[32-37]. To aid the extraction process, different techniques come into play. Solvent extraction, steam distillation and Supercritical Fluid Extraction (SFE) are some of the methods used. Chromatographic techniques like paper chromatography, thin layer chromatography, column chromatography and high performance liquid chromatography (HPLC) are employed for fractionation and purification of substances[5, 18, 38-44].

The choice of extraction method depends on factors such as the compound sought after the source material being used and also the intended application. There are a variety of methods for extraction, and we will review the most practical techniques in this review. These include distillation, pressing, sublimation, infusion, decoction, maceration, percolation, Soxhlet extraction and reflux extraction. For example, pressurized liquid extraction is a method used to extract natural compounds from complex matrices. It boasts selectivity and efficiency while also being compatible with processes within an intensification, or biorefinery strategy. Microwave assisted extraction is another method employed in extracting natural products. It offers advantages such, as reduced solvent usage, shorter extraction periods and increased selectivity. Various techniques are employed to fractionate and purify substances. These techniques include paper chromatography, thin layer chromatography, column chromatography and high performance liquid chromatography (HPLC)[44-53]. The journey towards bioactive compounds' restraints demands meticulous quality control, often mediated through the lens of analytical methodologies, and regulatory affairs. Mass spectrometry, with its ability to unveil molecular weights and structural intricacies, emerges as a sentinel in the arsenal of characterization tools. Nuclear magnetic resonance (NMR) spectroscopy offers a three-dimensional elucidation of molecular structures, unveiling the cryptic identity of bioactive compounds. High-performance liquid chromatography (HPLC) stands as the linchpin in compound separation and quantification within complex mixtures. Pharmaceutical regulatory affairs introduces an umbrella of compliance and safety. The surveillance and inspection of manufacturing practices, documentation, and quality control resonates with an emphasis on ensuring the integrity of bioactive compounds as therapeutic or health promoting entities[54-59].

The quest to unveil the full spectrum of bioactive compound potentials is far from being entirely charted. Substantial cleavage in our knowledge about bioactives, their advantages or disadvantages, and their production processes appears large, and it is demanding our attention and intellect. The optimization of process parameters for scalable extraction methodologies, coupled with the precise characterization of enigmatic bioactives, constitutes a principal research frontier. The success into the future of research of bioactive compounds depends on a multidisciplinary convergence of engineering, chemistry, and biology. It is this fusion that will unleash the alchemy of innovation, bridging research gaps and igniting the flame of transformative ideas. As we tread the intricate path ahead, the amalgamation of these disciplines will herald a renaissance in the extraction and fractionation of bioactive compounds, redefining the boundaries of human health and well-being. There has been an increasing demand to develop selective methods for economic extracting and efficient isolating bioactive natural products from different sources, either various parts of plants or animals, algaes and microalgae, fungi and micro fungi, or bacteria[46, 48, 51, 60, 61]. Herbal Medicine draws upon the accumulated knowledge of cultures that have identified plants and their components for their healing abilities. In the interim that certain aspects of medicine remain rooted in beliefs and practices, modern scientific research has deepened our understanding of the mechanisms behind these therapeutic effects.

II. The Therapeutic Properties And Potential Applications

Thanks to the advancements in technology, we now have the ability to discover, isolate, and represent pure natural health compounds from plants and microorganisms. Bioactives are a diverse group of naturally occurring compounds found in various plants, microorganisms, even in animals that have the potential to exert positive effects on human health. Here are some examples of therapeutic bioactives commonly found in plants[62, 63-66]**:**
Flavonoids: A class of polyphenolic compounds with antioxidant, anti-inflammatory, and anti-cancer properties. Examples: quercetin (found in apples, onions), catechins (found in green tea), and anthocyanins (found in berries). **Carotenoids:** Pigments in many fruits and vegetables with antioxidant properties and are precursors to vitamin Examples: beta-carotene (found in carrots), lycopene (found in tomatoes), and lutein (found in leafy greens). **Phenolic Acids:** Compounds with antioxidant and anti-inflammatory activities. Examples: caffeic acid (found in coffee), rosmarinic acid (found in rosemary), and ellagic acid (found in berries). **Saponins:** Glycosides with potential immune-modulating and cholesterol-lowering effects. They are found in various legumes and some herbs. **Alkaloids:** Nitrogen-containing compounds with diverse biological activities. Examples include caffeine

(found in coffee and tea), nicotine (found in tobacco), and capsaicin (found in chili peppers). **Terpenes:** A large group of compounds responsible for the aroma and flavor of many plants. Examples include limonene (found in citrus fruits), menthol (found in mint), and curcumin (found in turmeric). 7- Glucosinolates: Sulfur-containing compounds found in cruciferous vegetables like broccoli, cabbage, and kale. They have potential anti-cancer properties. **Lignans:** Polyphenolic compounds with potential antioxidant and hormone-balancing effects. They are found in seeds, particularly flaxseeds. **Resveratrol:** A polyphenol with antioxidant and potential cardiovascular benefits, found in red grapes, berries, and peanuts. **Gingerol and Curcumin:** Compounds found in ginger and turmeric, respectively, with anti-inflammatory and potential anti-cancer effects. Bioactives serve as the building blocks for developing potent and efficient health promoting compounds and medicaments that target health conditions. Herbal medicine, also known as herbalism or botanical medicine, is a healing practice that utilizes plants' bioactives to treat health conditions. This ancient wisdom has been passed down through generations across cultures, and continues to hold significance in modern medicine.

In this chapter, we delve into the therapeutic applications of bioactives and explore examples that highlight their therapeutic properties and potential uses[67-76]**:**

Antibiotics; From remedies to modern antibiotics, these microbial, fungal, or plant-based compounds have brought about a revolution in medicine by specifically targeting microorganisms responsible for causing illnesses.

Malaria Treatment; For centuries quinine, extracted from the bark of the Cinchona tree, has been utilized as a treatment for malaria. Its antimalarial properties disrupt the life cycle of Plasmodium parasites, thereby reducing their ability to cause disease.

Anticancer Agents; Paclitaxel, obtained from the bark of Pacific yew trees (brevifolia) holds importance as a chemotherapy drug. It interferes with cancer cell division, impeding tumor growth and progression.

Pain Management; Compounds extracted from plants have been found to possess inflammatory properties that can help alleviate discomfort associated with various conditions. Salicylates, and Aspirin, are a type of compound found in plants like Salix (willow) which have been used for pain relief since times.

Relief for discomfort; Peppermint (Mentha piperita) is commonly used to provide relief for discomfort. Its essential oils are known to have an effect on the muscles in the tract, which can help alleviate symptoms associated with irritable bowel syndrome (IBS).

Heart Health and Vascular Health; For health support, Hawthorn plant leaves (Crataegus spp.) have been traditionally utilized. Research suggests that extracts from hawthorn may improve blood circulation and potentially aid in managing conditions such as heart failure. Another example is Digitalis compounds derived from the plant (Digitalis purpurea).

Antidepressant effects; St. John's Wort (Hypericum perforatum) is renowned for its antidepressant effects. Its active compounds, hypericin and hyperforin are thought to influence neurotransmitter levels offering relief for mild to depression symptoms.

Immune boosting properties; Echinacea (Echinacea spp.) has gained popularity due to its immune boosting properties and its ability to reduce the duration and severity of colds and upper respiratory infections.

Preventing organ rejection; Cyclosporine derived from the fungus Tolypocladium inflatum is used to suppress responses in organ transplant patients.

Anticancer Agents; Bioactive compounds have also made progress in cancer treatment, offering avenues for inhibiting tumor growth and improving patient outcomes.

Chemotherapy; Sourced from the Pacific yew tree (Taxus brevifolia) paclitaxel disrupts cell division and has been instrumental in chemotherapy regimens for different types of cancers.

Vinblastine and Vincristine; Extracted from the Madagascar periwinkle (Catharanthus roseus) these compounds have played a role in treating leukemia and lymphoma. They inhibit cell division while suppressing tumor growth.

Neuroactive Compounds; Certain extracted compounds influence function with applications in health disorders as well as neurological conditions.

Hypericin and Depression; Hypericin and hyperforin derived from Hypericum perforatum (St. Johns Wort) have been studied for their antidepressant effects by modulating neurotransmitter levels. Caffeine, derived from coffee beans and other sources, is a recognized stimulant for the nervous system. It boosts alertness and cognitive function.

In summary, the extracted compounds have opened up applications in medicine due to their therapeutic properties. These compounds have greatly improved healthcare outcomes by serving as analgesic agents, cardiovascular aids and even anticancer medications. However, it is crucial to conduct research, clinical trials and safety assessments to ensure their effectiveness and safety. The collaboration between knowledge and advanced scientific methods continues to shape the development of medicines that harness the healing power of nature.

III. Extraction Methods

The initial step, in separating the desired products from materials, is called extraction.
In this chapter, we are classifying various traditional and novel techniques used for extracting bioactives from a variety of natural sources[63, 66, 75, 77-95]**:**

3.1 Solvent extraction; This method widely Relies on solvents like ethanol, methanol and hexane to separate desired compounds from plant material.

3.2 Steam distillation; This approach focuses on obtaining compounds like oils from plant materials.

3.3 Supercritical fluid extraction; This technique employs fluids such as carbon dioxide to extract the desired compounds from plants. Supercritical fluid extraction (SFE) is gaining popularity as an alternative for extracting products because of its efficiency and environmentally friendly nature. In SFE, a supercritical fluid refers to a substance maintained at temperatures and pressures above its point exhibiting properties that lie between those of a liquid and a gas[96-99].

3.3.1 Advantages of employing SFE in plant-based bioactive extraction are as follows;

3.3.1.1 Swift process; It only takes around 30–60 minutes per sample, making it a speedy and efficient method.

3.3.1.2 Eco-friendly characteristics; Carbon dioxide is commonly used as the fluid in SFE. Being non-toxic, non-flammable and readily available, it poses harm to the environment.

3.3.1.3 Extraction; SFE can selectively extract desired compounds from materials while leaving unwanted substances behind.

3.3.1.4 Enhanced antioxidant yields; Studies have shown that SFE yields amounts of antioxidants compared to SFE methods. The resulting extracts also exhibit purity. Retain more bioactivity.

3.3.1.5 Approach; SFE serves as an alternative to traditional extraction methods by utilizing a non-toxic and easily obtainable solvent.

Overall, the efficiency, selectivity, and sustainability make SFE a promising technique for the extraction of products.

3.3.2 There are drawbacks to using fluid extraction (SFE) for extracting NOCs. These include;

3.3.2.1 Expensive equipment; The cost of the equipment for SFE can be quite high, making it impractical for many industries and their extraction necessitates.

3.3.2.2 Dependence on matrix; The efficiency of SFE is heavily influenced by the matrix and its particle size, and may require optimization when working with specific matrices. Consequently, this can impact the effectiveness of the extraction process.

3.3.2.3 High pressure requirement; SFE requires operating at high pressures (1,000. 5,000 PSIA) to keep the solvent in its state. This demand for high pressure can pose a disadvantage for a variety of SFE applications.

3.3.2.4 Solubility; the solubility of desired compounds in supercritical solvents is crucial for an efficient extraction process. However, some compounds may have limited solubility in these solvents, affecting extraction yield.

3.3.2.5 Selectivity; SFE might not offer selectivity to solely extract desired compounds while leaving unwanted ones behind.

The choice of extraction method ultimately depends on factors, like the desired compound(s) as final product, the intended application, the routes of delivery to human body, and the feedstocks for the processing line. Although SFE has advantages like its speed, environmental friendliness, and better selectivity; it also comes with some downsides such as cost, reliance on matrix properties, and limitations regarding solubility and selectivity, therefore it may not always be the most suitable choice for all applications.

3.3.3 Advantages of other extraction techniques compared to supercritical fluid extraction (SFE);

3.3.3.1 Variety of solvents; Traditional solvent extraction techniques offer a wide range of solvents to choose from, providing flexibility in selecting the most suitable one for extracting the desired compound.

3.3.3.2 Equipment cost; In comparison to SFE equipment, traditional solvent extraction techniques require expensive equipment, making them more accessible and cost-effective for certain applications.

3.3.3.3 Improved selectivity; Traditional solvent extraction techniques can exhibit selectivity when it comes to extracting compounds, allowing for targeted extraction and purification.

3.3.3.4 Established methods; Traditional solvent extraction techniques have been extensively used and studied for years, resulting in established protocols and methodologies.

3.3.3.5 Compatibility with a variety of matrices; Traditional solvent extraction techniques are generally compatible with different types of matrices, including complex and diverse samples.

While SFE offers advantages, it may not always be the choice for certain compounds. It's crucial to take into account the characteristics of the compounds and the requirements of the extraction process when deciding between SFE and traditional solvent extraction techniques. The selection should be based on factors such as the application, desired compounds and available resources. Each method has its strengths and limitations; thus, making an informed decision is essential based on the needs of the extraction process.

3.3.4 There are some types of compounds that may not be suitable, for extraction using SFE;

3.3.4.1 Heat sensitive compounds; It is not recommended to use SFE for heat compounds due to the extraction temperature. Some examples of heat compound that may not be suitable for extraction using SFE include oils, enzymes and proteins.
3.3.4.2 Polar compounds; SFE may have limited solubility for compounds, which can affect the extraction yield.
3.3.4.3 Hydrophilic compounds; Compared to extraction techniques, SFE may not be as effective in extracting hydrophilic compounds.
3.3.4.4 Compounds with solubility in fluids; Some compounds may have limited solubility, in supercritical fluids, which can impact the extraction yield.
3.3.5 Here are a few alternative techniques, for extraction of heat sensitive compounds;
3.3.5.1. Microwave assisted extraction (MAE); MAE is a method of extraction commonly used in extracting products. It offers advantages, such as reducing the amount of solvents needed, shortening the extraction time and increasing selectivity.
3.3.5.2. Pressurized liquid extraction (PLE); PLE is another highly efficient method of extraction that can be combined with processes for an intensified or biorefinery approach.
3.3.5.3. Ultrasound assisted extraction; This is an eco efficient method used in extracting products. It provides benefits like yield, shorter extraction time and reduced solvent consumption.
3.3.5.4. Traditional solvent extraction techniques; For heat compounds, traditional methods like steam distillation and solvent extraction may be more suitable compared to fluid extraction (SFE).

In the extraction process, conventional methods often encounter some challenges. Firstly, they may require an amount of time which can be both time-consuming and costly for an industry. Secondly, a substantial quantity of solvents is often needed, posing environmental concerns. These techniques sometimes exhibit poor efficiency in extracting desired compounds, resulting in lower yields. Additionally, there's a risk of decomposition for thermolabile compounds during the extraction process, potentially impacting the quality of extracted substances. Volatility and toxicity are also amongst the drawbacks associated with methods that use solvents for natural product extractions. Conventional methods often involve the use of toxic solvents, posing potential risks, to both workers health and the environment. Lastly, conventional solvent extraction techniques may not be highly selective in their extraction process. Can inadvertently extract compounds. Counter-Current Chromatography (CCC) is a valuable method for the preparative purification of terpenes, particularly from fermentation broths, ensuring high bioactivity retention. Ion-Exchange Chromatography (IEC) assists in the purification of charged flavonoids, exploiting their differing ionic properties for separation. Flash Chromatography accelerates the purification of terpenes from plant extracts by reducing solvent consumption and improving separation efficiency. Centrifugal Partition Chromatography (CPC) aids in the isolation of bioactive compounds from natural sources, particularly terpenes, through liquid-liquid partitioning.

To summarize this section, although conventional solvent extraction techniques have long been employed for natural product extraction purposes, they do come with limitations and deficiencies such as volatility and toxicity issues along with prolonged extraction times significant solvent requirements, low efficiency in extracting desired compounds, thermal decomposition risks for thermolabile substances, during the process and limited selectivity during the overall extraction procedure.

IV. Fractionation Methods

In this section, we explore the techniques used for fractionation and purification, and highlight some complexities involved in their processes, by providing few examples. When it comes to the production of phytochemicals, bioactives, and other organic compounds in pharmaceutical grade, with no doubt semi-purification or complete fractionation considered as an important step in the processing line. Purification plays a crucial role in achieving plant-based medicaments in their highest efficient form, with lowest or no toxicity and side effects. These active compounds, such as terpenes and flavonoids, may hold great importance due to their therapeutic applications as herbal medicines, nutraceuticals, and pharmaceuticals. Various fractionation techniques such as chromatography and crystallization are employed for this purpose. Fractionation techniques such as column chromatography, preparative high performance liquid chromatography (HPLC), and liquid partitioning are commonly used to separate mixtures into groups of compounds. Preparative HPLC is particularly effective for fractionating mixtures. For example, one way to isolate flavonoids from plant extracts is through the application of a reversed phase HPLC column. This allows for the isolation of a fraction of many compounds existed in a mixture, ensuring exceptional purity of the product. As another instance, terpenes like artemisinin, which has medical properties, can be purified using flash chromatography with elution. Crystallization is also utilized to enhance the purity of terpene compounds like menthol extracted from peppermint oil.

Some common purification methods include[72, 92, 97, 100-112]**:**

4.1 Solid phase extraction (SPE); This method involves using a material like silica gel or activated carbon to selectively adsorb the desired compounds from a mixture. The adsorbed compounds can then be eluted using a solvent. Collected for further analysis or use.

4.2 Supercritical fluid extraction (SFE); This method employs a fluid such as carbon dioxide or ethanol above its temperature and pressure to dissolve and extract target compounds from either liquid matrices. The advantages of SFE include reduced reliance on solvents, increased extraction efficiency and preservation of the quality and bioactivity of the extracted compounds.

4.3 Chromatography; The process of fractionating and purifying substances involves the use of chromatographic techniques, like paper chromatography, thin layer chromatography, column chromatography and high performance liquid chromatography (HPLC). Chromatography is a term that refers to a group of methods used to separate the components of a mixture based on their varying affinities for a phase (such as a column or paper) and a mobile phase (such as gas or liquid). It is a technique that separates the components of a mixture based on their chemical properties. Paper chromatography and thin layer chromatography are utilized for compound separation, while column chromatography and HPLC are employed for compound separation.

Preparative planar chromatography helps separate quantities of compounds, whereas preparative column chromatography is more suitable for quantities. The used types of purification methods for bioactive compounds include paper chromatography, thin layer chromatography, gas chromatography and high performance liquid chromatography.

4.4 Fermentative Production by Recombinant Cells: This method involves using microorganisms, like bacteria or fungi, to produce organic compounds (NOCs) or convert bioactive compounds from plant materials or other substrates. The method offers advantages, including the potential to improve the production, variety and stability of compounds. Additionally, it enables the introduction of functionalities and properties.

These techniques can be applied to a range of plant materials and microorganisms. Examples include by-products, medicines, fruits, vegetables, beverages, medicinal plants, marine algae, actinomycetes and endophytes. These sources yield an array of compounds such as phenolic compounds, flavonoids, terpenes, alkaloids, saponins, glucosides, antibiotics, anticancer agents, antidiabetic agents and neuroprotective agents. These compounds exhibit effects including antioxidant properties, anti-inflammatory activity, antimicrobial activity, anticancer potential, antidiabetic effects and neuroprotective properties.

4.5 Supramolecular Solvent-Based Extraction (SSE) can be adapted for preparative-scale purification, capitalizing on its ability to selectively isolate specific bioactive compounds.

4.6 Evaporative Techniques, such as Rotary Evaporation and Freeze Drying, are applied for the concentration and purification of bioactive compounds following liquid-liquid extractions or chromatography.

4.7 Solid-Phase Microextraction (SPME) is instrumental in the purification of terpenes from microbial cultures, offering rapid and selective extraction.

4.8 Electrophoresis techniques, such as Capillary Electrophoresis (CE), enable the purification of charged flavonoids through their differential migration under an electric field.

4.9 Centrifugation-based methods, including Density Gradient Centrifugation, are useful for the purification of terpenes and flavonoids from cellular debris and impurities.

4.10 Precipitation with Polymers is employed to selectively separate and purify bioactive compounds, especially from fermentation broths of microorganisms.

4.11 Supramolecular Chromatography makes use of host-guest interactions to purify specific bioactive compounds, making it an adaptable tool for separation and purification.

4.12 Aqueous Two-Phase Extraction (ATPE) offers an environmentally friendly-approach to the purification of flavonoids and terpenes, with two immiscible phases isolating the target compounds.

4.12 Advanced Filtration techniques, including Tangential Flow Filtration (TFF), are applied for the purification, and concentration of bioactive compounds, as well as minimizing product loss.

4.13 Crystalline Sponge X-ray Crystallography aids in the purification and structural elucidation of bioactive compounds, ensuring the highest purity and also confirming their identity.

4.14 Multi-Dimensional Chromatography is employed for the purification of complex mixtures of terpenes and flavonoids, offering improved resolution and purity.

4.15 Supercritical Fluid Extraction (SFE) is adapted for preparative purposes, enabling the isolation and purification of terpenes and flavonoids under supercritical conditions.

4.16 High-Speed Counter-current Chromatography (HSCCC) is a versatile technique for the preparative purification of terpenes and flavonoids, offering efficient separation as well as scalability.

4.17 Ultra-High-Pressure Liquid Chromatography (UHPLC) is utilized for rapid and high-resolution purification of bioactive compounds, reducing purification time and solvent usage.

4.18 Cryogenic techniques, such as Liquid Nitrogen Fractionation, aid in the isolation and purification of thermolabile terpenes and flavonoids from plant materials.

4.19 Supercritical Anti-Solvent Precipitation (SAS) is employed to precipitate and purify bioactive compounds, selectively , achieving high purity and controlled particle size.
4.20 High-Performance Liquid Chromatography (HPLC) is an indispensable tool for researchers, allowing for the separation and purification of flavonoids from plant extracts with exceptional precision and purity.
4.21 As another example, Reverse-Phase Chromatography (RPC) within HPLC systems is ideal for the purification of hydrophobic terpenes, as it exploits differences in polarity for efficient separation87.
4.22 Size-Exclusion Chromatography (SEC) is employed to isolate and purify high-molecular-weight flavonoid polymers from plant materials, enabling their subsequent analysis and characterization.
4.23 Liquid-Liquid Extraction (LLE) techniques, such as partitioning, remain essential for the isolation of terpenes from crude extracts, followed by subsequent purification steps.
4.24 Solid-Phase Extraction (SPE) is increasingly favored for the purification of flavonoids from complex matrices, providing high selectivity and allowing for further concentration.
4.25 Supercritical Fluid Chromatography (SFC) is gaining recognition for its eco-friendly approach to purify terpenes and flavonoids, utilizing supercritical CO2 as the mobile phase[91, 98].
4.26 Ultrafiltration is a versatile technique used to concentrate and purify bioactive compounds, facilitating the removal of low-molecular-weight impurities[113, 114].
4.27 Dialysis plays a crucial role in the purification of flavonoids from crude plant extracts, effectively removing small impurities while retaining the compounds of interest[69, 111].
4.27 Membrane Filtration techniques, such as ultrafiltration and microfiltration, are invaluable for the separation and purification of bioactive compounds, especially from microbial fermentation broths.
4.28 Precipitation methods, including salting-out and organic solvent precipitation, are utilized to purify terpenes and flavonoids from complex mixtures[69].
4.29 Crystallization is another example of powerful purification techniques, enabling the isolation of highly pure bioactive compounds, including terpenes, through controlled crystalline growth[115].
4.30 Preparative Thin-Layer Chromatography (TLC) is employed to purify small quantities of flavonoids and terpenes, offering simple handling and rapid separation[75, 106].
4.31 Continuous Chromatography systems are gaining traction for large-scale purification of terpenes and flavonoids, providing a continuous and efficient process.
4.32 In situ crystallization techniques are applied within Chromatographic systems to facilitate the simultaneous purification and crystallization of bioactive compounds.
4.33 Affinity Membrane Chromatography utilizes ligand-functionalized membranes for the high-affinity purification of specific bioactive compounds, increasing yield and purity.
4.34 Dynamic Axial Compression Chromatography (DACC) is a preparative technique that aids in the efficient purification of terpenes and flavonoids from complex mixtures[116].
4.35 Vacuum Distillation is employed for the purification of volatile terpenes, allowing for the collection of high-purity fractions at reduced temperatures.
4.36 Microscale Purification techniques, such as Microscale Solid-Phase Extraction (µSPE), are used for the purification of small quantities of bioactive compounds with high precision[117].
4.37 Hybrid Chromatography systems combine multiple separation mechanisms to enhance the purification of flavonoids and terpenes, achieving better purity and yield.
4.38 There is an increase in the application of Continuous Crystallization processes for the purification of bioactive compounds, allowing for the continuous production of highly pure bioactives.
4.39 Simulated Moving Bed Chromatography (SMB) is utilized for large-scale purification, offering significant improvements in separation efficiency and solvent utilization[63].
4.40 Membrane Chromatography systems exploit membranes with specific adsorption properties to purify bioactive compounds, ensuring high selectivity and rapid processing.
4.41 Supramolecular Liquid Chromatography (SLC) leverages host-guest interactions for the selective purification of bioactive compounds, providing a multipurpose approach[118].
4.42 Ultra-High-Performance Supercritical Fluid Chromatography (UHPSFC) is instrumental in achieving ultra-high-purity terpenes and flavonoids, catering to pharmaceutical standards.
4.43 Electrochromatography combines electrophoresis and chromatography principles to enhance the purification of charged bioactive molecules, particularly flavonoids.
4.44 Simulated Countercurrent Chromatography (SCCC) is employed for the preparative purification of bioactives, delivering excessive purity and bigger capacity.
4.45 Affinity Chromatography. In the purification of animal-derived bioactive compounds, Affinity Chromatography offers specificity, isolating terpenes or flavonoids with high affinity to ligands immobilized on the chromatographic matrix.
4.46 Immobilized Metal Ion Affinity Chromatography (IMAC) is applied for the purification of bioactive compounds containing specific metal-binding motifs, ensuring high yield and purity[106, 111, 119, 120].

To illustrate the extraction and fractionation processes of compounds effectively, specific examples of products and methods have been provided. With advancements, new automatic and rapid techniques are being developed to extract and separate natural products efficiently in order to meet the requirements of high throughput screening.

V. Analytical Techniques

In the field of chemical engineering and biotechnology, ensuring the quality and authenticity of NOCs such as bioactives, flavonoids, and terpenes obtained from sources like medicinal herbs, microalgae, and plants is of utmost importance. During the processes of extraction, fractionation, and purification, scientists utilize methods to maintain the integrity and reliability of these compounds. By employing these analytical techniques, they can verify the genuineness and effectiveness of products intended for applications. Some common tools used in this regard include resonance (NMR) spectroscopy, mass spectrometry (MS) and high resolution gas chromatography mass spectrometry (GC MS). For instance, NMR spectroscopy plays a role in confirming the structure of compounds. It helps identify structures like quercetin through NMR analysis to validate their identity.

This section provides an overview of techniques utilized for Fractionation Quality Control and quality assurance, ensuring the purity and safety of the final product after the extraction and purification phases[91, 121-138]**:**
HPLC remains an indispensable tool in the array of analytical methods. It allows for separation, identification, and quantification of bioactive compounds present in complex mixtures.HPLC, which employs detectors, like UV-VIS and MS is capable of distinguishing compounds with specificity. It can effectively identify flavonoids, terpenes and other substances. In the case of herbs like Ginkgo biloba HPLC plays a role in analyzing flavonoid glycosides to ensure consistent product quality and effectiveness.
Gas Chromatography Mass Spectrometry (GCMS) is a tool for analyzing compounds, particularly terpenes. Terpenes often contribute to the aroma and therapeutic properties of natural health products. By identifying terpene profiles found in oils extracted from aromatic plants like Lavandula angustifolia, GC MS guarantees the authenticity and therapeutic potential of the products.
Nuclear Magnetic Resonance Spectroscopy (NMR) is a technique for elucidating compound structures without causing any damage. It provides information by examining the atomic nuclei of molecules. In the realm of flavonoids, NMR confirms the presence of moieties. Enables differentiation between classes of flavonoids, in tea extracts. This ensures labeling and assessment of content.
Thin Layer Chromatography (TLC); Thin Layer Chromatography is a tool used in the process of fractionation, allowing for the separation of compounds based on their chemical properties. It plays a role in the extraction of bioactives from plant extracts by helping researchers identify fractions enriched with distinct subclasses of flavonoids. When applied to Camellia sinensis leaves, this technique ensures the isolation of a range of bioactive compounds contributing to the characteristics of the final extract.
Mass Spectrometry Imaging (MSI); Mass Spectrometry Imaging is an approach used to examine the distribution of bioactive compounds within samples. This technique has proven useful in studying herbs as it reveals how various bioactives like flavonoids are localized within plant tissues. By mapping the distribution patterns of these compounds in Ginkgo biloba leaves researchers gain insights into the roles that specific compounds play in different physiological processes.
High Performance Thin Layer Chromatography (HPTLC); High Performance Thin Layer Chromatography enhances separation efficiency and sensitivity beyond what TLC offers. It serves as a method for achieving purification by ensuring levels of precision and accuracy in separating compounds. When it comes to terpene extracts, the use of HPTLC ensures that individual terpenes are pure, by isolating bands. This method has been demonstrated in the purification of oils from Mentha piperita.
Liquid Chromatography Mass Spectrometry (LCMS); LCMS combines separation capabilities with detection, enabling high resolution analysis of mixtures. In the case of compounds derived from microalgae, LC MS aids in purifying substances like carotenoids. This promotes product purity. Unlocks the potential of these compounds as colorants and antioxidants.

Innovation and idea generation play a role in chemical engineering and biotechnology. Metabolomics offers an approach to studying all metabolites within samples, which allows for the discovery of compounds and their synergistic effects. By utilizing metabolomics, researchers can identify previously bioactives within matrices leading to innovative ideas for future natural health products. In the realm of chemical engineering and biotechnology, it is essential to ensure the quality and identity of compounds during extraction, fractionation and purification processes. The combination of methods, such as HPLC, GC MS, NMR, and MSI ensures the genuineness, effectiveness and safety of bioactive compounds, flavonoids, terpenes and other natural health products. As technology progresses, these techniques continue to empower scientists in understanding the intricacies of nature and opening doors for solutions and enhanced health products in the future.

VI. Concluding Remarks

In this comprehensive review, we have systematically explored the majority of progressive techniques in the extraction, fractionation, and analytical profiling of therapeutic bioactives, essential components of the contemporary landscape of bioactive compound research. Our endeavor has catered to specialists and researchers, providing a systematic analysis of advanced methodologies and their critical roles in this evolving field. Our journey began with a meticulous examination of extraction methodologies, acknowledging the pivotal parameters that dictate the outcome of these processes, such as solvent choice, temperature, and extraction time. The foundation of any successful bioactive compound research lies in the efficiency and effectiveness of extraction. With the selection of appropriate solvents and optimized conditions, we are better equipped to ensure high yields and the utmost purity, prerequisites in the isolation and subsequent study of bioactives. Continuing our exploration, we investigated advanced fractionation techniques, critically evaluating column chromatography, solid-phase extraction, and preparative high-performance liquid chromatography (HPLC). These are instrumental tools for the isolation and purification of target compounds. Our review underscores the versatility, selectivity, and efficiency of these methods in different contexts, offering guidance to researchers seeking the ideal approach to fractionate and purify bioactives. A distinctive highlight of this review was the exclusive chapter titled "Exploring the Power of Nature's Pharmacy." This chapter delved into the abundant pharmacological attributes and health benefits inherent in bioactives sourced from plants and microorganisms. It is undeniable that nature serves as a vast reservoir of potent compounds with significant medical applications, and our examination revealed the profound potential of these bioactives in addressing a wide range of medical conditions. This exploration serves as a testament to the invaluable contributions that bioactives offer to modern healthcare, showcasing the diverse applications and health benefits that await further exploration and development.

Furthermore, our review underscored the urgency of addressing certain research gaps within the field. The quest for sustainable and eco-friendly extraction practices has emerged as a pressing concern in light of growing environmental consciousness. The expansion of our understanding of uncharted bioactive resources presents an enticing avenue for future exploration, unearthing novel therapeutic compounds that may redefine the landscape of medicine. Additionally, the advancement of multidimensional analytical techniques remains a critical frontier, enabling comprehensive characterizations that lay the foundation for further applications in drug development, nutraceuticals, and more. In conclusion, our comprehensive review stands as an invaluable resource for specialists and researchers alike, offering a detailed and up-to-date analysis of progressive techniques in the realms of extraction, fractionation, and analytical profiling. The specialized chapter dedicated to the medical applications and health benefits of bioactives derived from plants and microorganisms signifies a critical milestone in modern healthcare, and it is our hope that this review will serve as an impetus for further exploration and innovation in the field. We have navigated the multifaceted domain of bioactives, unveiling their profound potential in the realm of therapeutic applications and health benefits, a testament to the enduring power of nature's pharmacy in advancing medical science and human well-being.

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