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Beyond The Present: Future Research Directions On The Extraction And Fractionation Of Bioactives, With The Focus On Phytochemicals

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Abstract:
Bioactives are naturally occurring compounds that may have various beneficial effects on human health and well-being. These compounds, including phytochemicals, can be extracted and purified from a variety of sources using various methods and utilized as medicines or health-promoting products.
This review article explores the promising avenues for future research in the realm of bioactive compound extraction and fractionation. By undertaking a comprehensive study of the extraction and purification techniques, analytical methods, and related pharmaceutical affairs, this review discerns the intricacies of each method and underscores the gaps in knowledge that warrant further investigation. By shedding light on these gaps, we emphasize the need for an approach that combines engineering, chemistry, and biology.
We will explore the challenges faced in this field and highlight research directions. These challenges include optimizing process parameters, scaling up extraction systems, fractionating and purifying extracts, identifying and characterizing compounds, evaluating their activities, developing applications, investigating hybrid approaches, integrating analytical methods, advancing technology for bioactive production more efficiently and sustainably, and devising innovative strategies to reduce waste and utilize agricultural byproducts.
Overall, this article is a reference for researchers in the field, as it provides insights into the key challenges and changing dynamics of extracting and fractionating bioactive compounds.

Keywords: *bioactive, future studies, research directions, upcoming opportunities, extraction, fractionation, purification, plant, phytochemicals, methods, techniques*

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I. Introduction

In the evolving field of biotechnology, the discovery and utilization of active compounds, known as "bioactives", from plants (e.g. phytochemicals) and other organisms have become a significant focus of scientific exploration and innovation. These natural compounds go beyond being chemical substances, and manifest a profound impact on human health and well-being[1-7] . Some examples of classes of these active compounds are flavonoids, terpenes, alkaloids, phenolic acids subclasses like anthocyanins, polyphenol, quercetin, resveratrol, isoflavones, curcumin, flavonols, astaxanthin gallic acid, lycopene, flavones, apigenin, luteolin, kaempferol, stilbenes, myricetin, flavonoid, caffeic acids, naringenin, and vanillin. These compounds possess economic value as they can influence cellular processes in the body, and therefore there is strong interest in extracting and purifying these compounds from various plant sources using diverse methods for their use as medicines or products that promote health[8-17]. The extraction and purification process involves separating and isolating the extracts into fractions containing bioactive compounds or groups of compounds. Analytical methods such as chromatography mass spectrometry are employed to identify and characterize the compounds in each fraction. Evaluating the properties of fractions and compounds requires conducting bioassays such as measuring the capacity of each bioactive if it is an anti-oxidant, anti-inflammatory, antimicrobial, anticancer, or others. Creating applications for these fractions and compounds involves formulating products such as foods, nutraceuticals, cosmetics, pharmaceuticals and more[18-21].

The field of extracting and purifying bioactive compounds from plants is constantly evolving. It faces challenges and opportunities due to trends like population growth, urbanization, climate change, food security concerns, increased health awareness, environmental sustainability efforts and technological advancements. These trends drive the need for sustainable methods of producing bioactive substances while exploring diverse sources[22-25]. As biotechnologists, chemical engineers, researchers, and related specialists embark upon this intellectual voyage, it is critical to go through a future-oriented analysis and research exploration that not only includes the medical and health-promoting applications of bioactive compounds, but also navigates the network

of modern extraction and purification techniques, analytical methodologies, and the intricacies of pharmaceutical regulatory affairs.

Medical Applications and Health Benefits of Bioactive Compounds;

Bioactive compounds, sourced from a range of origins, have made significant contributions to the fields of medicine and health promotion. As we explore the world of phytochemicals, our attention is captivated by the applications of these compounds, specifically flavonoids and terpenes. This rich collection of compounds has the potential to act as a shield against diseases such as cardiovascular disorders and neurodegenerative conditions. Moreover, their anti-inflammatory characteristics offer hope for alleviating ailments beyond symptom relief[19, 26, 27]. Flavonoids; These natural polyphenolic compounds found in fruits, vegetables, and various plants have emerged as promising agents. Their remarkable antioxidant properties play a role, in combating free radicals and reducing oxidative stress. These natural compounds have the potential to act as a shield against ailments ranging from heart diseases to neurodegenerative conditions. Additionally, their anti-inflammatory properties offer hope for alleviating inflammation related disorders, beyond addressing symptoms[28-34]. Terpenes; Terpenes are intriguing and volatile constituents that give oils their fragrances. They hold potential in the field of therapeutics, from the calming effects of linalool found in lavender to the inflammatory and analgesic properties of β-caryophyllene terpenes offer a wide range of medicinal possibilities. Although they not hold promise for managing pain and anxiety, but instead they exhibit antimicrobial properties that open up new avenues in infectious disease treatment[35-39].

Modern Extraction Techniques

The process of extracting and purifying bioactive compounds from plants is an interdisciplinary field that requires the integration of multiple sciences including engineering, chemistry, and biology. To successfully extract these compounds it is important to choose the solvent or a combination of solvents, optimize processing parameters such as temperature, pressure, time flow rate, solvent to feed ratio and scale up the extraction system from laboratory to industrial settings. The extraction and purification of bioactive compounds have undergone advancements in main parts, driven by a combination of scientific disciplines[18, 22, 40, 41]. In the domain of bioactive compound extraction, modern methods such as Supercritical Fluid Extraction (SFE) have gained prominence. SFE has revolutionized extraction methodologies by allowing extraction of flavonoids from plant sources with improved efficiency. SFE relies on fluids such as carbon dioxide to achieve selective extraction of bioactives. Its ability to extract compounds without organic compound denaturation or contamination makes it highly desirable. However, a careful comparative analysis of SFE against contemporary extraction techniques is necessary to clarify their intricacies, advantages, and limitations comprehensively[42-47].

When it comes to unlocking the potential of herbs, Subcritical Water Extraction (SWE) emerges as a technique. It excels at extracting heat terpenes while preserving their bioactivity[23, 48-50]. In the field of biotechnology, researchers are increasingly turning to Pressurized Liquid Extraction (PLE) for isolating compounds from animal tissues[51, 52]. This method ensures yields of terpenes from organisms for pharmaceutical applications. Solid Phase Micro-extraction (SPME) is a technique particularly known for its precision in capturing volatile terpenes, and mostly applied for extracting bioactive compounds derived from microorganisms[53, 54]. The use of Ultrasound Assisted Extraction (UAE) has brought about a revolution in extracting flavonoids from plants by enhancing yield and reducing extraction time[43, 55] . In the quest for compounds, Soxhlet Extraction remains a method widely employed in extracting terpenes from aromatic herbs and botanicals[32, 56]. Supramolecular Solvent Based Extraction (SSE) has emerged as a technique for extraction of specific flavonoids from plant matrices, with high selectivity[57]. Countering the challenges for the extraction of heat-sensitive bioactive compounds, such as terpenes, Microwave-Assisted Extraction (MAE) is instrumental in obtaining them from delicate plant tissues[43, 58]. When it comes to extracting compounds from animal sources, Accelerated Solvent Extraction (ASE) has proven its effectiveness by isolating terpenes from animal fats[59, 60].

The pharmaceutical industry benefits from the use of Supercritical Carbon Dioxide (SC-CO2) extraction to obtain terpenes derived from sources ensuring both purity and bioactivity[42, 61]. Solid Phase Extraction (SPE) is considered a selective method widely preferred in research for the precise extraction of flavonoids from complex matrices[62, 63]. Additionally, Membrane Based Separation (MBS) processes are increasingly being utilized to concentrate and separate terpenes from fermentation broths of microorganisms, thereby enhancing processing efficiency[57, 64] . The development of Ionic Liquid Based Extraction (ILBE) has opened up possibilities, for extracting bioactive compounds, while minimizing environmental impact across various sources[65-67].

Liquid-Liquid Extraction (LLE) remains a technique for purifying flavonoids from extracts, offering both high selectivity and purity[68, 69]. In the field of phytochemical profiling, High Performance Liquid Chromatography (HPLC) is employed to accurately quantify and separate flavonoids in plant extracts[70].

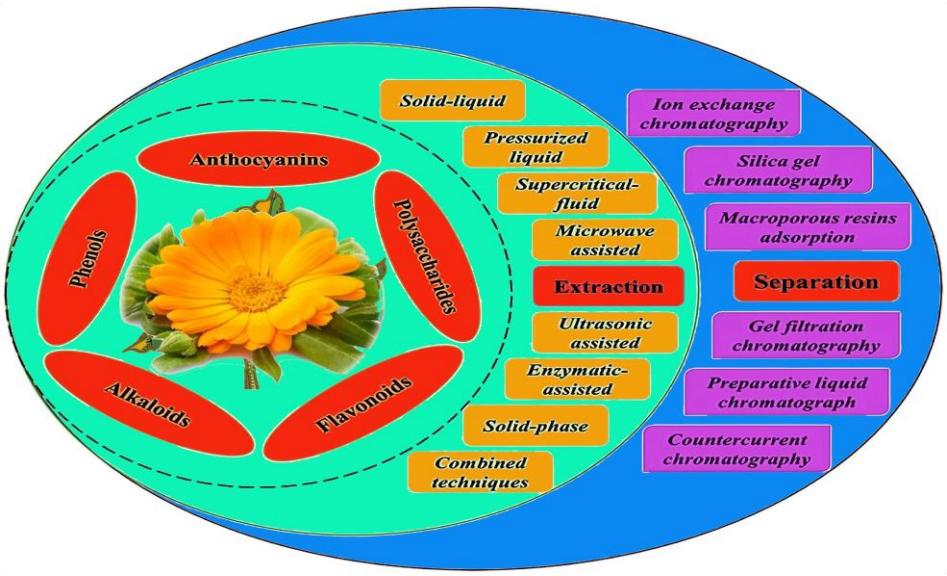

Figure 1: Commonly used extraction and fractionation methods described in the text

Co solvent and Co solvent Enhanced Subcritical Water Extraction (CSEWE) have proven to be effective in extracting flavonoids from plant materials, providing an alternative[50, 71, 72]. When it comes to extracting terpenes from organisms, Pulsed Electric Field (PEF) technology has shown enhancement by disrupting cell walls and aiding the release of compounds[73, 74]. In the field of biotechnology, Counter Current Chromatography (CCC) plays a role in purifying compounds like terpenes from fermentation broths[75-78]. The extraction of compounds from microorganisms is greatly facilitated through Pressurized Hot Water Extraction (PHWE) which ensures the retention of high bioactivity levels[79-81]. The combination of Artificial Intelligence (AI) and Machine Learning (ML) algorithms is revolutionizing the optimization of extraction processes, leading to yields of flavonoids, and terpenes from biological sources.

Modern Purification Techniques;

Purification techniques play an equally pivotal role in certifying the isolation of bioactive compounds in their refined and absolute forms, and is considered a critical step in ensuring their pharmaceutical and biotechnological relevance. Chromatography, crystallization, and membrane separation techniques represent the leaders of purification endeavors[22, 40, 82]. 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[83-86]. 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 separation[87]. Size-Exclusion Chromatography (SEC) is employed to isolate and purify high-molecular-weight flavonoid polymers from plant materials, enabling their subsequent analysis and characterization[88, 89]. 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. Liquid-Liquid Extraction (LLE) techniques, such as partitioning, remain essential for the isolation of terpenes from crude extracts, followed by subsequent purification steps. Solid-Phase Extraction (SPE) is increasingly favored for the purification of flavonoids from complex matrices, providing high selectivity and allowing for further concentration.

Supercritical Fluid Chromatography (SFC) is gaining recognition for its eco-friendly approach to purify terpenes and flavonoids, utilizing supercritical CO2 as the mobile phase[42, 43]. Ultrafiltration is a versatile technique used to concentrate and purify bioactive compounds, facilitating the removal of low-molecular-weight impurities[72, 90]. Dialysis plays a crucial role in the purification of flavonoids from crude plant extracts, effectively removing small impurities while retaining the compounds of interest[40, 91]. Membrane Filtration techniques, such as ultrafiltration and microfiltration, are invaluable for the separation and purification of bioactive compounds, especially from microbial fermentation broths. Precipitation methods, including salting-out and organic solvent precipitation, are utilized to purify terpenes and flavonoids from complex mixtures[64, 92]. Crystallization is another example of powerful purification techniques, enabling the isolation of highly pure bioactive compounds, including terpenes, through controlled crystalline growth[93]. Preparative Thin-Layer Chromatography (TLC) is employed to purify small quantities of flavonoids and terpenes, offering simple handling and rapid separation[82, 94].

Process Intensification strategies are continually developed to streamline the purification of bioactive compounds, offering improved efficiency and reduced environmental impact in the biotechnology and pharmaceutical sectors. Fractionation and purification methods empower researchers to unravel the complexity of plant extracts, offering pure bioactive compounds with high specificity. Yet, these techniques require much further research for process optimization, especially in the context of high-throughput applications.

Idea Generation

As it was initiated, one goal of this article is to summarize and evaluate the current state of knowledge on the certain discipline of emerging trends for advancements in extraction, fractionation, and purification of Natural Organic Compounds (NOCs), including bioactives. More importantly, this review also intends to provide a forecast for future directions as well as some educated 2023 research gaps (the new opportunities in the field) to be considered by the R&D researchers in biopharmaceutical sector.

The primary thread in the extraction and fractionation of NOCs is the idea generation. Idea generation is the process of creating, developing, and communicating new ideas that can be applied to troubleshoot problems or overcome challenges[95-98]. In the context of extraction, purification, and fractionation of bioactives, including herbal medicines, and plant-based Natural Health-promoting Products (NHPs), idea generation can put in place[99-104]:

Finding novel and effective methods, techniques, or strategies to isolate and characterize the desired compounds from various sources, such as plants, animals, fungi, bacteria, or marine organisms. This involves identifying the natural source of the compound, and the desired compound to be extracted.

Idea generation can also involve exploring new applications or functions of the extracted compounds in different fields, such as medicine, food, cosmetics, or agriculture.

The idea generation process can be aided by various techniques, including literature review, ethnobotanical surveys, and bioactivity-guided fractionation. Some examples of idea generation in this field are:

- Using biological approaches, such as enzymes, or microorganisms, to extract bioactive compounds from agro-industrial by-products, which can reduce the environmental impact and enhance the quality and bioactivity of the extracts
- Applying modern techniques, such as microwave-assisted extraction, ultrasound-assisted extraction, supercritical fluid extraction, or pressurized liquid extraction, to improve the efficiency and selectivity of the extraction process
- Combining different analytical methods, such as chromatography, spectroscopy, mass spectrometry, or nuclear magnetic resonance, to identify and quantify the isolated compounds and elucidate their structures and properties
- Developing new formulations, or delivery systems for the extracted compounds, such as nanoparticles, liposomes, microemulsions, or hydrogels, to enhance their stability, solubility, bioavailability, or targeting
- Screening the extracted compounds for various biological activities, such as antioxidant, anti-inflammatory, antimicrobial, anticancer, antidiabetic, or neuroprotective effects
- Investigating the mechanisms of action, and interactions of the extracted compounds with cellular and molecular targets
- Exploring the synergistic or antagonistic effects of different combinations of extracted compounds or with other agents

Determined from the simplicity and the availability of each technique to bioactive producers, its economical processing line, safety to environment, the quality and purity of the product, and several other criteria, we intend to spend more time on specific techniques including supercritical fluid extraction (SFE).

In the process of improvement of SFE for economic fractional extraction of NOCs, including bioactives idea generation can involve finding novel and effective methods, techniques, or strategies to optimize the extraction efficiency, selectivity, quality, and cost of the desired compounds from various sources such as plants, animals, fungi, bacteria, or marine organisms. SFE's Idea generation can also involve exploring new applications or functions of the extracted compounds in different fields, such as medicine, food, cosmetics, or agriculture. Some examples of idea generation in SFE process are:

Using different types of supercritical fluids (SCFs), such as water, ethanol, propane, or nitrous oxide, or their mixtures with carbon dioxide (CO2), to enhance the solubility and extraction of polar or non-polar compounds

Applying different modes of operation, such as batch, semi-batch, continuous, or recirculating, to improve the mass transfer and extraction yield.

Combining SFE with other techniques, such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), or pressurized hot water extraction (PHWE), to increase the extraction rate and reduce the energy consumption.

Developing new models or algorithms to simulate and optimize the SFE process parameters, such as temperature, pressure, flow rate, co-solvent concentration, and extraction time.
Designing new equipment or devices to perform SFE at different scales, from laboratory to industrial level.

Evaluating the environmental impact and sustainability of the SFE process compared to conventional solvent extraction methods.

Investigating the chemical composition and biological activity of the SFE extracts and fractions using advanced analytical methods and bioassays.

Regulatory Considerations And Quality Control

This section provides an overview of the regulations governing the production, extraction, purification, and the use of herbal medicine, plant-based medicaments and drugs. It also explores how progress and regulatory compliance interact, through exclusive examples. The discussion highlights the importance of adhering to bodies, standards, and guidelines to ensure product safety, effectiveness, and consistent quality[105-108]. The production and use of medicine, medicaments, and drugs are aspects of modern healthcare systems. Regulatory oversight is essential in ensuring their safety, quality, and also effectiveness. This article delves into the range of regulations that encompass these processes with a focus on idea generation, product development and commercialization[104, 109-117]:

1. Regulatory Bodies and Frameworks;

There are regulatory bodies that oversee the development and production of herbal based products. Notably in the United States there is the Food and Drug Administration (FDA) in Europe there is the European Medicines Agency (EMA) while globally there is the World Health Organization (WHO). These organizations establish guidelines and standards to guarantee product safety and effectiveness. These organizations have developed systems that cover stages of production, extraction, separation, purification and utilization.

2. Generating Ideas and Conducting Research;

The process of developing products often begins with exploring knowledge and practices. For example, the use of the medicine "Echinacea purpurea", in enhancing the system has gained popularity due to its traditional use. Research then focuses on identifying compounds, understanding their mechanisms of action and exploring therapeutic applications. Modern techniques like high performance liquid chromatography (HPLC) and mass spectrometry aid in identifying and quantifying constituents.

3. Ensuring Quality Control and Standardization;

Regulatory bodies place importance on quality control and standardization. The herbal medicine "Ginkgo biloba," renowned for its benefits, must adhere to set standards regarding the concentration of ginkgo flavonoids and terpene lactones. High performance thin layer chromatography (HPTLC) and nuclear magnetic resonance (NMR) spectroscopy are utilized to assess quality and ensure consistency across batches.

4. Conducting Preclinical and Clinical Trials;

The transition from laboratory research to clinical trials is carefully regulated. Herbal products, like "Hypericum perforatum" (St. John's Wort) undergo trials to validate their antidepressant properties.
Placebo controlled blind studies follow recognized guidelines to support claims of effectiveness.

5. Monitoring, after the product is on the market; Regulatory authorities go beyond approving products. Also monitor them after they are available to the public. They promptly. Report any side effects through adverse event monitoring and pharmacovigilance. The example of "Valeriana officinalis," a sedative, highlights the importance of post market surveillance in identifying potential liver toxicity.

The production, extraction, fractionation, purification and use of medicine remedies and medications involve processes that are closely regulated. Compliance with these regulations is crucial, for ensuring product safety, effectiveness and consistent quality. Researchers and manufacturers contribute to evidence based healthcare by following established guidelines and standards.

Future Directions And Research Opportunities

The field of research and practice related to extracting, fractionating, and purifying NOCs under various classification of bioactives, phytochemicals, and flavonoids, with health promoting or medical attributes, is constantly evolving. Despite a comprehensive study in various areas, there are still some unresolved aspects that require further investigation to improve in specific directions. Moreover, there are research challenges that have been overlooked or not yet recognized by researchers and practitioners worldwide. These challenges call for investigations, examinations, reviews, surveys, and evaluations of neglected problems.

When dealing with the extraction, fractionation or purification of NOCs; herbal medicines, flavonoids, natural health products or bioactives; researchers and practitioners face challenges. However, there are also opportunities and innovative approaches that can help overcome these challenges. Examples include developing intensified extraction techniques; utilizing biotechnology and synthetic biology for NOC production; as well as applying advanced analytical methods, and computational tools to facilitate the discovery and development of natural products[23, 118-122] .

Filling the research gap in this field can be quite complex and challenging. Fortunately, there have already been studies conducted around it. However, it is essential to recognize that there is still a need for extensive work and additional resources to tackle the research challenges in this area. Each challenge aims to pave the way for avenues of investigation[123-132]:

Standardization and Quality Control; One of the obstacles lies in the absence of standardized methods and protocols for extracting, fractionating and purifying natural products. This lack of standardization can result in outcomes and quality concerns. It is crucial to address this by ensuring quality and effectiveness of medicines and natural health products. The variability in plant sources, growth conditions and extraction techniques can lead to varying levels of compounds. Developing methods for extraction, fractionation and purification is crucial to ensure product quality.

Selectivity and Yield Optimization; Many natural products are mixtures that contain bioactive compounds. The challenge lies in achieving extraction and fractionation techniques that allow us to isolate compounds while maintaining high yields. Ongoing research focuses on developing methods of targeting compounds or classes of compounds with precision.

Solvent Choice and Environmental Impact; The choice of solvents used for extraction and purification has both process efficiency implications, as environmental consequences. Many traditional solvents are known to be toxic or environmentally harmful. Developing and improving eco solvents or alternative extraction techniques like fluid extraction is an area that requires attention.

Scaling up and Integrating Processes; While small scale extraction methods may work effectively, transitioning to industrial production levels can present challenges related to process efficiency, economics and scalability. It is crucial to develop methods that can be easily scaled up, while ensuring product quality and cost-effectiveness remain a priority.

Analyzing Complex Mixtures; Natural products often have compositions that make their identification, characterization and quantification quite challenging. Plant or microbial based natural products typically contain a mix of compounds, necessitating analytical techniques such as mass spectrometry, nuclear magnetic resonance (NMR) and chromatography for accurate identification and quantification of individual components within these mixtures.

Synergistic Effects and Holistic Approaches; In medicines and natural health products, the combined effects of compounds often contribute to their therapeutic benefits. Isolating compounds may not capture these synergies effectively. Hence, it is important to explore methods that preserve and study the interactions between compounds as an area of interest.

Enhancing Bioavailability and Formulation; Bioactive compounds exhibit solubility or stability issues, which can impact their absorption in the body and overall effectiveness.

Developing strategies to improve the solubility, stability, and absorption of these compounds in the body poses a challenge. When it comes to medicines, natural health products and bioactives there are hurdles and varying quality standards that differ by region. Navigating these variations while ensuring safety, effectiveness, and quality can be a task. However, there are ways to enhance the quality and efficacy of medicines;

Conduct standardized scientific studies on herbal medicines using appropriate methods and criteria. This will help evaluate their effects, mechanisms of action, safety profiles and potential interactions with drugs.

8.1.7.2 Implement manufacturing practices (GMP) along with stringent quality control standards for herbal medicines. This will ensure consistency, purity, potency and stability.

Provide accurate labeling information for medicines to educate consumers about their ingredients, dosage guidelines, indications for use or contraindications if any exist as well as warnings about possible adverse effects.

Educate healthcare professionals as consumers about proper usage of herbal medicines while highlighting potential risks associated with them; encourage reporting of any adverse events or interactions, to relevant authorities.

Encouraging the preservation and responsible utilization of resources that are rare or endangered while also respecting the traditional wisdom and cultural diversity of different communities reliant on herbal medicines is crucial. There are avenues to enhance the safety and effectiveness of herbal medicines. However, it's important to acknowledge that there is still work to be done in this field. To achieve progress, increased

collaboration and communication among researchers, practitioners, regulators, industry professionals, and consumers are essential.

Potential toxicity and negative effects; Certain natural products may possess toxicity or adverse effects, necessitating thorough safety assessments and regulation.

Bio-prospecting and Sustainability; The extraction of compounds from sources raises concerns about sustainability and the conservation of biodiversity. This is especially pertinent when dealing with scarce endangered or legally protected sources. It's crucial to adopt sourcing practices and bio-prospecting approaches that ensure the long term viability of plant species and ecosystems.

Lack of Comprehensive Databases; A database that encompasses extraction methods, fractionation techniques and purification strategies for natural products is currently lacking but highly needed. Such a resource would greatly facilitate knowledge sharing among experts in the field, while also aiding in the development of methods.

The Cost and Environmental Impact; Conventional extraction techniques like extraction and distillation can be costly both financially and environmentally due to their reliance on large quantities of organic solvents, energy consumption and time requirements.

The issue of yield and selectivity; Some extraction techniques have the drawback of yielding amounts of desired bioactive compounds or extracting unwanted impurities, which can result in a loss of valuable substances.

To tackle these emerging trends and advancements, in the extraction and separation of compounds, collaboration among chemists, pharmacologists, engineers and regulatory experts is essential. As technology progresses and our understanding of products deepens, researchers and professionals can strive for efficient, sustainable and standardized approaches when extracting, separating and purifying NOCs herbal medicines, flavonoids, natural health products as well as bioactives.

II. Concluding Remarks

The field of extracting and separating compounds from plants involves the integration of engineering, chemistry and biology. In this study, and throughout a series of analysis, we were able to discuss challenges, and identified areas for further research in this ever evolving field. One important area that requires attention in research is the optimization of process parameters. While there have been advancements in optimizing SFE conditions, there is still a need for exploration of the operational factors that affect extraction efficiency and selectivity. It would be beneficial to develop models that can help determine the conditions for extracting specific bioactive compounds from different plant materials, thereby improving the accuracy and effectiveness of extraction processes. Another crucial aspect that warrants investigation is scaling up extraction systems. Moving from small scale laboratory setups to scale processes presents unique challenges such as designing equipment suitable for large scale operations, managing high pressure systems effectively and optimizing energy consumption. Addressing these challenges will help facilitate the production of substances on a larger scale, meeting the increasing demand for natural products that promote health. Further research is needed to explore the fractionation and purification of extracts, as these processes have an impact on the quality and effectiveness of the products. Researchers should investigate techniques like chromatography and membrane based separation methods to achieve higher purity and better separation of bioactive compounds. Additionally, it is important to prioritize the development of purification methods that minimize usage and waste generation. Identifying and characterizing compounds are research goals.

Although advancements in techniques like mass spectrometry and nuclear magnetic resonance spectroscopy have helped with compound identification, ongoing efforts should focus on understanding the bioactivities of compounds and exploring how phytochemicals within complex mixtures work synergistically. To fully unlock the potential of compounds, future research should involve evaluations of their activities and applications across various fields such as pharmaceuticals, nutraceuticals and functional foods. Investigating extraction approaches that combine methods shows promise in improving extraction efficiency and selectivity. Sustainability plays a role in reducing the impact of producing bioactive compounds. Therefore, it is essential to adopt extraction practices and develop strategies for minimizing waste while utilizing agricultural byproducts.

Furthermore, it is important to explore the integration of techniques and technological advancements, in the production of compounds. Utilizing monitoring systems and real time analytics has the potential to improve process control and ensure high quality products. Additionally, we should focus on developing strategies that can minimize waste and maximize the use of byproducts, aligning with sustainability principles and promoting a circular economy.

To summarize, unlocking the potential of bioactives from plant sources requires continuous dedication to research, collaboration and innovation. As the demand for health enhancing products continues to rise, interdisciplinary research combining engineering, chemistry and biology becomes crucial. By addressing research gaps and embracing approaches, we can pave the way for a future where bioactive compounds

significantly contribute to health while minimizing their environmental impact during production. Researchers can make contributions by addressing these research gaps and adopting approaches that promote efficient, sustainable and economically viable methods for extracting and utilizing bioactives and phytochemicals from plants. This review article sheds light on the research gaps and challenges in the compound extraction and fractionation field. It serves as a resource for specialists and researchers navigating this evolving landscape.

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References

[1] Athikkavil FM, Aiswarya SU, Johny R, Et Al. A Potent Bioactive Fraction Against Colon Cancer From. Explor Target Antitumor Ther 2023; 4: 227-239. 20230424. DOI: 10.37349/Etat.2023.00131.
[2] Nekkaa A, Benaissa A, Mutelet F, Et Al. Plant: Extraction Of Bioactive Fractions And Evaluation Of Their Pharmacological And Phytochemical Properties. Antioxidants (Basel) 2021; 10 20210216. DOI: 10.3390/Antiox10020300.
[3] Xiang Q, Li M, Wen J, Et Al. The Bioactivity And Applications Of Pomegranate Peel Extract: A Review. J Food Biochem 2022; 46: E14105. 2022/02/08. DOI: 10.1111/Jfbc.14105.
[4] Saar-Reismaa P, Bragina O, Kuhtinskaja M, Et Al. Extraction And Fractionation Of Bioactives From. Pharmaceuticals (Basel) 2022; 15 20220112. DOI: 10.3390/Ph15010087.
[5] Silva-Stenico ME, Kaneno R, Zambuzi FA, Et Al. Natural Products From Cyanobacteria With Antimicrobial And Antitumor Activity. Curr Pharm Biotechnol 2014; 14: 820-828. Research Support, Non-U.S. Gov't 2014/01/01.
[6] Coulston AM, Boushey C And Ferruzzi M. Nutrition In The Prevention And Treatment Of Disease. 3rd Ed. Oxford: Elsevier/Academic, 2013, P.Xvi, 903 P.
[7] Tedeschi P, Nigro M, Travagli A, Et Al. Therapeutic Potential Of Allicin And Aged Garlic Extract In Alzheimer's Disease. Int J Mol Sci 2022; 23 2022/07/10. DOI: 10.3390/Ijms23136950.
[8] Bagchi D. Biotechnology In Functional Foods And Nutraceuticals. Hoboken: CRC Press, 2010.
[9] Fattorusso E, Gerwick WH And Taglialatela-Scafati O. Handbook Of Marine Natural Products. New York: Springer, 2012.
[10] Hohtola A. Bioactive Compounds From Northern Plants. Adv Exp Med Biol 2010; 698: 99-109. 2010/01/01.
[11] Szajdek A And Borowska E. Bioactive Compounds And Health-Promoting Properties Of Berry Fruits: A Review. Plant Foods For Human Nutrition 2008; 63: 147-156.
[12] Chen CY And Blumberg JB. Phytochemical Composition Of Nuts. Asia Pac J Clin Nutr 2008; 17 Suppl 1: 329-332. Research Support, U.S. Gov't, Non-P.H.S.
Review 2008/05/28.
[13] Venugopal V. Marine Products For Healthcare Functional And Bioactive Nutraceutical Compounds From The Ocean. Functional Foods And Nutraceuticals Series. Boca Raton: CRC Press/Taylor & Francis, 2009.
[14] Gilbert J And Enyuva H. Bioactive Compounds In Foods. John Wiley & Sons, 2009.
[15] Mayer AM And Gustafson KR. Marine Pharmacology In 2005-2006: Antitumour And Cytotoxic Compounds. Eur J Cancer 2008; 44: 2357-2387. Research Support, N.I.H., Intramural
Research Support, Non-U.S. Gov't
Review 2008/08/15. DOI: 10.1016/J.Ejca.2008.07.001.
[16] Kirakosyan A And Kaufman PB. Recent Advances In Plant Biotechnology. Dordrecht ; New York: Springer, 2009, P.Xiv, 409 P.
[17] Fingerman M And Nagabhushanam R. Biomaterials From Aquatic And Terrestrial Organisms. Enfield, NH: Science Publishers, 2006, P.Xi, 610 P.
[18] Wang W, Liu Z, Liu Y, Et Al. Plant Polypeptides: A Review On Extraction, Isolation, Bioactivities And Prospects. Int J Biol Macromol 2022; 207: 169-178. 20220304. DOI: 10.1016/J.Ijbiomac.2022.03.009.
[19] Das G, Patra JK, Kang SS, Et Al. Pharmaceutical Importance Of Some Promising Plant Species With Special Reference To The Isolation And Extraction Of Bioactive Compounds: A Review. Curr Pharm Biotechnol 2022; 23: 15-29. DOI: 10.2174/1389201022666210122125854.
[20] Tiwari BK, Brunton N And Brennan CS. Handbook Of Plant Food Phytochemicals : Sources, Stability And Extraction. Oxford: Wiley-Blackwell, 2013, P.Xv, 510 Pages.
[21] Advanced Analysis Of Nutraceuticals. Elsevier, 2011.
[22] Castro-Muñoz R, Boczkaj G And Cabezas R. A Perspective On Missing Aspects In Ongoing Purification Research Towards. Foods 2023; 12 20230508. DOI: 10.3390/Foods12091916.
[23] Kumar M, Dahuja A, Tiwari S, Et Al. Recent Trends In Extraction Of Plant Bioactives Using Green Technologies: A Review. Food Chem 2021; 353: 129431. 20210303. DOI: 10.1016/J.Foodchem.2021.129431.
[24] Kozlowska A And Szostak-Wegierek D. Flavonoids--Food Sources And Health Benefits. Rocz Panstw Zakl Hig 2014; 65: 79-85. Research Support, Non-U.S. Gov't
Review 2014/10/03.
[25] Mohammad Azmin SNH, Sulaiman NS, Mat Nor MS, Et Al. A Review On Recent Advances On Natural Plant Pigments In Foods: Functions, Extraction, Importance And Challenges. Appl Biochem Biotechnol 2022; 194: 4655-4672. 20220706. DOI: 10.1007/S12010-022-04050-Z.
[26] Zitek T, Bjelic D, Kotnik P, Et Al. Natural Hemp-Ginger Extract And Its Biological And Therapeutic Efficacy. Molecules 2022; 27 2022/11/27. DOI: 10.3390/Molecules27227694.
[27] Das G, Shin HS And Patra JK. Key Health Benefits Of Korean Ueong Dry Root Extract Combined Silver Nanoparticles. Int J Nanomedicine 2022; 17: 4261-4275. 2022/09/23. DOI: 10.2147/IJN.S357343.

[28] He CH, Lv JM, Khan GJ, Et Al. Total Flavonoid Extract From Dracocephalum Moldavica L. Improves Pulmonary Fibrosis By Reducing Inflammation And Inhibiting The Hedgehog Signaling Pathway. Phytother Res 2023; 37: 2745-2758. 2023/02/17. DOI: 10.1002/Ptr.7771.

[29] Feng H, He Y, La L, Et Al. The Flavonoid-Enriched Extract From The Root Of Smilax China L. Inhibits Inflammatory Responses Via The TLR-4-Mediated Signaling Pathway. J Ethnopharmacol 2020; 256: 112785. 2020/03/31. DOI: 10.1016/J.Jep.2020.112785.

[30] Yusoff IM, Mat Taher Z, Rahmat Z, Et Al. A Review Of Ultrasound-Assisted Extraction For Plant Bioactive Compounds: Phenolics, Flavonoids, Thymols, Saponins And Proteins. Food Res Int 2022; 157: 111268. 20220422. DOI: 10.1016/J.Foodres.2022.111268.

[31] Zeng Z, Chen C, Situ Y, Et Al. Anoectochilus Roxburghii Flavonoids Extract Ameliorated The Memory Decline And Reduced Neuron Apoptosis Via Modulating SIRT1 Signaling Pathway In Senescent Mice. J Ethnopharmacol 2022; 296: 115361. 2022/05/25. DOI: 10.1016/J.Jep.2022.115361.

[32] Wasihun Y, Alekaw Habteweld H And Dires Ayenew K. Antibacterial Activity And Phytochemical Components Of Leaf Extract Of Calpurnia Aurea. Sci Rep 2023; 13: 9767. 20230616. DOI: 10.1038/S41598-023-36837-3.

[33] Toul F, Moussouni S, Ghembaza N, Et Al. Identification Of Phenolic Compounds In The Buds Of Algerian. J Complement Integr Med 2022; 19: 219-224. 20210906. DOI: 10.1515/Jcim-2021-0336.

[34] Gabr GA, Hassan HMM, Seshadri VD, Et Al. Comparative Study Of Phenolic Profile, Antioxidant And Antimicrobial Activities Of Aqueous Extract Of White And Green Tea. Z Naturforsch C J Biosci 2022; 77: 483-492. 2022/05/23. DOI: 10.1515/Znc-2021-0321.

[35] Wu QG, Huang LY, Fan MH, Et Al. Anti-Inflammatory Activities Of Monoterpene And Sesquiterpene Glycosides From The Aqueous Extract Of Artemisia Annua L. Chem Biodivers 2023; 20: E202201237. 2023/02/06. DOI: 10.1002/Cbdv.202201237.

[36] Mannino G, Iovino P, Lauria A, Et Al. Bioactive Triterpenes Of Protium Heptaphyllum Gum Resin Extract Display Cholesterol-Lowering Potential. Int J Mol Sci 2021; 22 2021/04/04. DOI: 10.3390/Ijms22052664.

[37] Pardo-Rodriguez D, Lasso P, Mateus J, Et Al. A Terpenoid-Rich Extract From Clethra Fimbriata Exhibits Anti-Trypanosoma Cru Zi Activity And Induces T Cell Cytokine Production. Heliyon 2022; 8: E09182. 2022/04/05. DOI: 10.1016/J.Heliyon.2022.E09182.

[38] Ads EN, Hassan SI, Rajendrasozhan S, Et Al. Isolation, Structure Elucidation And Antimicrobial Evaluation Of Natural Pentacyclic Triterpenoids And Phytochemical Investigation Of Different Fractions Of. Molecules 2022; 27 20220310. DOI: 10.3390/Molecules27061805.

[39] De Vita S, Finamore C, Chini MG, Et Al. Phytochemical Analysis Of The Methanolic Extract And Essential Oil From Leaves Of Industrial Hemp Futura 75 Cultivar: Isolation Of A New Cannabinoid Derivative And Biological Profile Using Computational Approaches. Plants (Basel) 2022; 11 2022/07/10. DOI: 10.3390/Plants11131671.

[40] Laszlo C, Kaminski K, Guan H, Et Al. Fractionation And Extraction Optimization Of Potentially Valuable Compounds And Their Profiling In Six Varieties Of Two. Molecules 2022; 27 20221121. DOI: 10.3390/Molecules27228105.

[41] Awad AM, Kumar P, Ismail-Fitry MR, Et Al. Green Extraction Of Bioactive Compounds From Plant Biomass And Their Application In Meat As Natural Antioxidant. Antioxidants (Basel) 2021; 10 20210915. DOI: 10.3390/Antiox10091465.

[42] Arumugham T, K R, Hasan SW, Et Al. Supercritical Carbon Dioxide Extraction Of Plant Phytochemicals For Biological And Environmental Applications - A Review. Chemosphere 2021; 271: 129525. 20210102. DOI: 10.1016/J.Chemosphere.2020.129525.

[43] Weremfo A, Abassah-Oppong S, Adulley F, Et Al. Response Surface Methodology As A Tool To Optimize The Extraction Of Bioactive Compounds From Plant Sources. J Sci Food Agric 2023; 103: 26-36. 20220728. DOI: 10.1002/Jsfa.12121.

[44] Mur R, Pardo JI, Pino-Otín MR, Et Al. Supercritical Antisolvent Fractionation Of Antioxidant Compounds From. Int J Mol Sci 2021; 22 20210828. DOI: 10.3390/Ijms22179351.

[45] Yousefi M, Rahimi-Nasrabadi M, Mirsadeghi S, Et Al. Supercritical Fluid Extraction Of Pesticides And Insecticides From Food Samples And Plant Materials. Crit Rev Anal Chem 2021; 51: 482-501. 20200415. DOI: 10.1080/10408347.2020.1743965.

[46] Shukla A, Naik S, Goud VV, Et Al. Supercritical CO2 Extraction And Online Fractionation Of Dry Ginger For Production Of High-Quality Volatile Oil And Gingerols Enriched Oleoresin. Industrial Crops And Products 2019; 130: 352-362.

[47] Mishra RC, Kumari R And Yadav JP. Comparative Antidandruff Efficacy Of Plant Extracts Prepared From Conventional And Supercritical Fluid Extraction Method And Chemical Profiling Using GCMS. J Dermatolog Treat 2022; 33: 989-995. 20200728. DOI: 10.1080/09546634.2020.1799919.

[48] Kaczmarska A, Pieczywek PM, Cybulska J, Et Al. A Mini-Review On The Plant Sources And Methods For Extraction Of Rhamnogalacturonan I. Food Chem 2023; 403: 134378. 20220921. DOI: 10.1016/J.Foodchem.2022.134378.

[49] Lu X, Li W, Wang Q, Et Al. Progress On The Extraction, Separation, Biological Activity, And Delivery Of Natural Plant Pigments. Molecules 2023; 28 20230712. DOI: 10.3390/Molecules28145364.

[50] Rebaque D, López G, Sanz Y, Et Al. Subcritical Water Extraction Of Equisetum Arvense Biomass Withdraws Cell Wall Fractions That Trigger Plant Immune Responses And Disease Resistance. Plant Mol Biol 2023 20230502. DOI: 10.1007/S11103-023-01345-5.

[51] Santos PH, Kammers JC, Silva AP, Et Al. Antioxidant And Antibacterial Compounds From Feijoa Leaf Extracts Obtained By Pressurized Liquid Extraction And Supercritical Fluid Extraction. Food Chem 2021; 344: 128620. 20201113. DOI: 10.1016/J.Foodchem.2020.128620.

[52] Rodríguez De Luna SL, Ramírez-Garza RE And Serna Saldívar SO. Environmentally Friendly Methods For Flavonoid Extraction From Plant Material: Impact Of Their Operating Conditions On Yield And Antioxidant Properties. Scientificworldjournal 2020; 2020: 6792069. 20200828. DOI: 10.1155/2020/6792069.

[53] Sampaio KL, Biasoto AC And Da Silva MA. Comparison Of Techniques For The Isolation Of Volatiles From Cashew Apple Juice. J Sci Food Agric 2015; 95: 299-312. 20140530. DOI: 10.1002/Jsfa.6718.

[54] Zhang X, Oakes KD, Luong D, Et Al. Solid-Phase Microextraction Coupled To LC-ESI-MS/MS: Evaluation And Correction For Matrix-Induced Ionization Suppression/Enhancement For Pharmaceutical Analysis In Biological And Environmental Samples. Anal Chem 2011; 83: 6532-6538. Research Support, Non-U.S. Gov't 2011/07/21. DOI: 10.1021/Ac200718d.

[55] Gutiérrez-Sánchez MDC, Aguilar-Zárate P, Michel-Michel MR, Et Al. The Ultrasound-Assisted Extraction Of Polyphenols From Mexican Firecracker (%B Molecules. 2022; 27 20221213. DOI: 10.3390/Molecules27248845.

[56] Ho TC And Chun BS. Extraction Of Bioactive Compounds From Pseuderanthemum Palatiferum (Nees) Radlk. Using Subcritical Water And Conventional Solvents: A Comparison Study. J Food Sci 2019; 84: 1201-1207. 20190403. DOI: 10.1111/1750-3841.14501.

[57] Knoll W. Handbook Of Biofunctional Surfaces. Boca Raton: Crc Press, 2012.

[58] Choommongkol V, Punturee K, Klumphu P, Et Al. Microwave-Assisted Extraction Of Anticancer Flavonoid, 2',4'-Dihydroxy-6'-Methoxy-3',5'-Dimethyl Chalcone (DMC), Rich Extract From. Molecules 2022; 27 20220218. DOI: 10.3390/Molecules27041397.

[59] López-Hortas L, Pérez-Larrán P, González-Muñoz MJ, Et Al. Recent Developments On The Extraction And Application Of Ursolic Acid. A Review. Food Res Int 2018; 103: 130-149. 20171016. DOI: 10.1016/J.Foodres.2017.10.028.

[60] Krakowska A, Rafińska K, Walczak J, Et Al. Comparison Of Various Extraction Techniques Of Medicago Sativa: Yield, Antioxidant Activity, And Content Of Phytochemical Constituents. J AOAC Int 2017; 100: 1681-1693. 20170713. DOI: 10.5740/Jaoacint.17-0234.

[61] Dos Reis Gasparetto B, Chelala Moreira R, Priscilla França De Melo R, Et Al. Effect Of Supercritical CO. Food Res Int 2022; 162: 111900. 20220906. DOI: 10.1016/J.Foodres.2022.111900.

[62] Attallah OA, Nady M, Al Gendy AM, Et Al. Preparation And Optimization Of A Molecularly Imprinted Polymers - Solid Phase Extraction System For The Extraction Of Bioactive Sesquiterpene Lactones Of. Nat Prod Res 2023; 37: 1844-1850. 20220902. DOI: 10.1080/14786419.2022.2118744.

[63] Hu Q, Lan L, Li W, Et Al. Low-Temperature Cleanup Followed By Dispersive Solid-Phase Extraction For Determination Of Nine Polar Plant Growth Regulators In Herbal Matrices Using Liquid Chromatography-Tandem Mass Spectrometry. Chromatographia 2023; 86: 483-495. 20230413. DOI: 10.1007/S10337-023-04254-3.

[64] Buyel JF. Strategies For Efficient And Sustainable Protein Extraction And Purification From Plant Tissues. Methods Mol Biol 2022; 2480: 127-145. DOI: 10.1007/978-1-0716-2241-4_9.

[65] Khan S, Rauber D, Shanmugam S, Et Al. Efficient Lignin Fractionation From Scots Pine (%B Polymers (Basel). 2022; 14 20221031. DOI: 10.3390/Polym14214637.

[66] Zhang J, Zong A, Xu T, Et Al. A Novel Method: Ionic Liquid-Based Ultrasound-Assisted Extraction Of Polyphenols From Chinese Purple Yam. Nat Prod Res 2018; 32: 863-866. 20170824. DOI: 10.1080/14786419.2017.1361955.

[67] Lovejoy KS, Davis LE, Mcclellan LM, Et Al. Evaluation Of Ionic Liquids On Phototrophic Microbes And Their Use In Biofuel Extraction And Isolation. J Appl Phycol 2013; 25: 973-981. DOI: DOI 10.1007/S10811-012-9907-0.

[68] Mahmoudi S, Chaichi MJ, Shamsipur M, Et Al. Determination Of Atropine By HPLC In Plant Of Datura By Liquid-Liquid Extraction And Magnet Solid-Phase Extraction. J Chromatogr Sci 2023 20230614. DOI: 10.1093/Chromsci/Bmad041.

[69] Truong DH, Ta NTA, Pham TV, Et Al. Effects Of Solvent-Solvent Fractionation On The Total Terpenoid Content And In Vitro Anti-Inflammatory Activity Of. Food Sci Nutr 2021; 9: 1720-1735. 20210127. DOI: 10.1002/Fsn3.2149.

[70] Danchana K, Yamashita N, Umeda MI, Et Al. Separation And Fractionation Of Glutamic Acid And Histidine Via Origami Isoelectric Focusing. J Chromatogr A 2023; 1706: 464247. 20230727. DOI: 10.1016/J.Chroma.2023.464247.

[71] Yilmaz-Turan S, Jiménez-Quero A, Moriana R, Et Al. Cascade Extraction Of Proteins And Feruloylated Arabinoxylans From Wheat Bran. Food Chem 2020; 333: 127491. 20200706. DOI: 10.1016/J.Foodchem.2020.127491.

[72] Carrier DJ, Ramaswamy S And Bergeron C. Biorefinery Co-Products. Chichester, West Sussex ; Hoboken: John Wiley & Sons, 2012, P.Xix, 361 P.

[73] Bocker R And Silva EK. Pulsed Electric Field Assisted Extraction Of Natural Food Pigments And Colorings From Plant Matrices. Food Chem X 2022; 15: 100398. 20220721. DOI: 10.1016/J.Fochx.2022.100398.

[74] Pattnaik M, Pandey P, Martin GJO, Et Al. Innovative Technologies For Extraction And Microencapsulation Of Bioactives From Plant-Based Food Waste And Their Applications In Functional Food Development. Foods 2021; 10 20210130. DOI: 10.3390/Foods10020279.

[75] De Souza Wuillda ACJ, Das Neves Costa F, Garrett R, Et Al. High-Speed Countercurrent Chromatography With Offline Detection By Electrospray Mass Spectrometry And Nuclear Magnetic Resonance Detection As A Tool To Resolve Complex Mixtures: A Practical Approach Using Coffea Arabica Leaf Extract. Phytochem Anal 2023 20230801. DOI: 10.1002/Pca.3271.

[76] Fontana A And Schieber A. Preparative Fractionation Of Phenolic Compounds And Isolation Of An Enriched Flavonol Fraction From Winemaking Industry By-Products By High-Performance Counter-Current Chromatography. Plants (Basel) 2023; 12 20230607. DOI: 10.3390/Plants12122242.

[77] Khadhraoui B, Ummat V, Tiwari BK, Et Al. Review Of Ultrasound Combinations With Hybrid And Innovative Techniques For Extraction And Processing Of Food And Natural Products. Ultrason Sonochem 2021; 76: 105625. 20210611. DOI: 10.1016/J.Ultsonch.2021.105625.

[78] Xue H, Zhu X, Tan J, Et Al. Counter-Current Fractionation-Assisted Bioassay-Guided Separation Of Active Compound From Blueberry And The Interaction Between The Active Compound And A-Glucosidase. Foods 2021; 10 20210301. DOI: 10.3390/Foods10030509.

[79] Boateng ID. A Critical Review Of Ginkgolic Acids In Ginkgo Biloba Leaf Extract (Egb): Toxicity And Technologies To Remove Ginkgolic Acids And Their Promising Bioactivities. Food Funct 2022; 13: 9226-9242. 2022/09/07. DOI: 10.1039/D2fo01827f.

[80] Mandura Jarić A, Šeremet D, Vojvodić Cebin A, Et Al. The Multiple-Response Modeling Of Heat-Assisted, Microwave-Assisted And Subcritical Water Extraction On Selected Phenolics From Traditional Plant Species. Prep Biochem Biotechnol 2022; 52: 809-822. 20211111. DOI: 10.1080/10826068.2021.1998111.

[81] Mašković PZ, Veličković V, Đurović S, Et Al. Biological Activity And Chemical Profile Of Lavatera Thuringiaca L. Extracts Obtained By Different Extraction Approaches. Phytomedicine 2018; 38: 118-124. 20171129. DOI: 10.1016/J.Phymed.2017.11.010.

[82] Kalsi PS And Jagtap S. Pharmaceutical, Medicinal And Natural Product Chemistry. Oxford: Alpha Science, 2013, P.Xxii, [608] P.

[83] Karpitskiy DA, Bessonova EA, Shishov AY, Et Al. Selective Extraction Of Plant Bioactive Compounds With Deep Eutectic Solvents: Iris Sibirica L. As Example. Phytochem Anal 2023 20230806. DOI: 10.1002/Pca.3272.

[84] Zhang Y, Kiyohara H, Matsumoto T, Et Al. Fractionation And Chemical Properties Of Immunomodulating Polysaccharides From Roots Of Dipsacus Asperoides. Planta Med 1997; 63: 393-399. DOI: 10.1055/S-2006-957720.

[85] Trivedi P, Kumar JK, Negi AS, Et Al. HPLC Method Development And Validation Of Cytotoxic Agent Phenyl-Heptatriyne In Bidens Pilosa With Ultrasonic-Assisted Cloud Point Extraction And Preconcentration. Biomed Chromatogr 2011; 25: 697-706. 20100901. DOI: 10.1002/Bmc.1505.

[86] Sidana J And Joshi LK. Recycle HPLC: A Powerful Tool For The Purification Of Natural Products. Chromatography Research International 2013; 2013.

[87] Conlon JM. Purification Of Naturally Occurring Peptides By Reversed-Phase HPLC. Nature Protocols 2007; 2: 191-197.

[88] Umaña MM, Dalmau ME, Eim VS, Et Al. Effects Of Acoustic Power And Ph On Pectin-Enriched Extracts Obtained From Citrus By-Products. Modelling Of The Extraction Process. J Sci Food Agric 2019; 99: 6893-6902. 20191004. DOI: 10.1002/Jsfa.9975.

[89] Aznar R And K. Rai D. Purification And Isolation Techniques For Enrichment Of Bioactive Phytochemicals From Herbs And Spices. Herbs, Spices And Medicinal Plants: Processing, Health Benefits And Safety 2020: 177-206.

[90] Sritongtae B, Karami Z, Morgan MRA, Et Al. Fractionation Of Foam-Mat Dried Rice Bean Hydrolysates Using Membrane Filtration And Solid Phase Extraction: Peptide- And Phenolic-Based Fractions With Bioactive Potential. Food Res Int 2022; 157: 111274. 20220420. DOI: 10.1016/J.Foodres.2022.111274.

[91] Cermeño M, Kleekayai T, Amigo-Benavent M, Et Al. Current Knowledge On The Extraction, Purification, Identification, And Validation Of Bioactive Peptides From Seaweed. Electrophoresis 2020; 41: 1694-1717. 20200714. DOI: 10.1002/Elps.202000153.
[92] Li H, Yuan Z, Shang X, Et Al. Application Of Gradient Acid Fractionation Protocol To Improve Decolorization Technology By Lignin-Based Adsorbent. Int J Biol Macromol 2021; 172: 10-18. 20201231. DOI: 10.1016/J.Ijbiomac.2020.12.206.
[93] Kim KH, Lee KW, Kim DY, Et Al. Optimal Recovery Of High-Purity Rutin Crystals From The Whole Plant Of Fagopyrum Esculentum Moench (Buckwheat) By Extraction, Fractionation, And Recrystallization. Bioresour Technol 2005; 96: 1709-1712. 20050225. DOI: 10.1016/J.Biortech.2004.12.025.
[94] Jug U, Vovk I, Glavnik V, Et Al. Off-Line Multidimensional High Performance Thin-Layer Chromatography For Fractionation Of Japanese Knotweed Rhizome Bark Extract And Isolation Of Flavan-3-Ols, Proanthocyanidins And Anthraquinones. J Chromatogr A 2021; 1637: 461802. 20201215. DOI: 10.1016/J.Chroma.2020.461802.
[95] Zhu E And Sambath S. Information Technology And Agricultural Engineering. Advances In Intelligent And Soft Computing,. Berlin ; New York: Springer,, 2012, P. 1 Online Resource (Xvii, 991 P.).
[96] Zhang H. Characterization Of Bioactive Phytochemicals From The Stem Bark Of African Mahogany Khaya Senegalensis (Meliaceae). 2008.
[97] Jagatap VR, Ahmad I And Patel HM. Recent Updates In Natural Terpenoids As Potential Anti-Mycobacterial Agents. Indian Journal Of Tuberculosis 2022; 69: 282-304.
[98] Tyagi U And Sarma AK. Perspectives Of Biomass Based Lignin To Value Added Chemicals In Biorefineries: Challenges, Extraction Strategies And Applications. Biofuels, Bioproducts And Biorefining 2022; 16: 1869-1892.
[99] Jain D, Meena M, Singh D, Et Al. Isolation, Development And Validation Of HPTLC Method For The Estimation Of B-Carotene From Gymnosporia Senegalensis (Lam.) Loes. Plant Physiology And Biochemistry 2023: 107843.
[100] Haghighi M And Rezaei K. Designing An All-Apple-Pomace-Based Functional Dessert Formulation. British Food Journal 2013; 115: 409-424. DOI: 10.1108/00070701311314228.
[101] Gershwin ME, Greenwood MRC And New York Academy Of Sciences. Foods For Health In The 21st Century : A Roadmap For The Future, Http://Www3.Interscience.Wiley.Com/Journal/123323016/Issue Http://Www.Loc.Gov/Catdir/Enhancements/Fy1210/2012405118-D.Html (2010).
[102] Smith J And Charter E. Functional Food Product Development. Functional Food Science And Technology. Chichester, West Sussex ; Ames, Iowa: Wiley-Blackwell, 2010.
[103] Khan RS, Grigor J, Winger R, Et Al. Functional Food Product Development–Opportunities And Challenges For Food Manufacturers. Trends In Food Science & Technology 2013; 30: 27-37.
[104] Barrajon E. Natural Compounds As New Cancer Treatments. MDPI, 2019.
[105] Zhao Y-Y. Traditional Uses, Phytochemistry, Pharmacology, Pharmacokinetics And Quality Control Of Polyporus Umbellatus (Pers.) Fries: A Review. Journal Of Ethnopharmacology 2013; 149: 35-48.
[106] Bhosale VV And Banerjee D. Scientific Validation Of Herbal Medicine. Herbal Medicine In India: Indigenous Knowledge, Practice, Innovation And Its Value 2020: 573-579.
[107] Madhu M, Kumar D, Sirohi R, Et Al. Bioactive Peptides From Meat: Current Status On Production, Biological Activity, Safety, And Regulatory Framework. Chemosphere 2022: 135650.
[108] Chalamaiah M, Ulug SK, Hong H, Et Al. Regulatory Requirements Of Bioactive Peptides (Protein Hydrolysates) From Food Proteins. Journal Of Functional Foods 2019; 58: 123-129.
[109] Fabrowska J, Łęska B, Schroeder G, Et Al. Biomass And Extracts Of Algae As Material For Cosmetics. Marine Algae Extracts: Processes, Products, And Applications 2015: 681-706.
[110] Kim S-K. Handbook Of Marine Macroalgae : Biotechnology And Applied Phycology. Hoboken, NJ: John Wiley & Sons Inc., 2012, P.Xx, 567 P.
[111] He J-Y, Ma N, Zhu S, Et Al. The Genus Codonopsis (Campanulaceae): A Review Of Phytochemistry, Bioactivity And Quality Control. Journal Of Natural Medicines 2015; 69: 1-21.
[112] Katiyar CK. Phytopharmaceuticals: Unique Regulatory Category With Immense Potential For Medical Unmet Needs. Natural Medicines. CRC Press, 2019, Pp.223-233.
[113] Jiangang F, Ling D, Zhang L, Et Al. Houttuynia Cordata Thunb: A Review Of Phytochemistry And Pharmacology And Quality Control. Chinese Medicine 2013; 2013.
[114] Li J, Yuan C, Pan L, Et Al. Bioassay-Guided Isolation Of Antioxidant And Cytoprotective Constituents From A Maqui Berry (Aristotelia Chilensis) Dietary Supplement Ingredient As Markers For Qualitative And Quantitative Analysis. Journal Of Agricultural And Food Chemistry 2017; 65: 8634-8642.
[115] Looney M, Kehoe S, Abraham R, Et Al. Regulatory Aspects Of Bioactive Glasses: A Basic Primer On "Design Control" For Academic Researchers. Bioactive Glasses. Elsevier, 2018, Pp.181-199.
[116] Bauer TW And Smith ST. Bioactive Materials In Orthopaedic Surgery: Overview And Regulatory Considerations. Clinical Orthopaedics And Related Research (1976-2007) 2002; 395: 11-22.
[117] Phelan M, Aherne A, Fitzgerald RJ, Et Al. Casein-Derived Bioactive Peptides: Biological Effects, Industrial Uses, Safety Aspects And Regulatory Status. International Dairy Journal 2009; 19: 643-654.
[118] Castro-Muñoz R, Boczkaj G And Cabezas R. A Perspective On Missing Aspects In Ongoing Purification Research Towards Melissa Officinalis. Foods 2023; 12 2023/05/13. DOI: 10.3390/Foods12091916.
[119] Castro-Muñoz R, Gontarek-Castro E And Jafari SM. Up-To-Date Strategies And Future Trends Towards The Extraction And Purification Of Capsaicin: A Comprehensive Review. Trends In Food Science & Technology 2022; 123: 161-171.
[120] Carocho M, Barros L, Calhelha RC, Et Al. Melissa Officinalis L. Decoctions As Functional Beverages: A Bioactive Approach And Chemical Characterization. Food & Function 2015; 6: 2240-2248.
[121] Yuan D, Guo Y, Pu F, Et Al. Opportunities And Challenges In Enhancing The Bioavailability And Bioactivity Of Dietary Flavonoids: A Novel Delivery System Perspective. Food Chemistry 2024; 430: 137115. DOI: Https://Doi.Org/10.1016/J.Foodchem.2023.137115.
[122] Council NR. Separation & Purification: Critical Needs And Opportunities. 1930.
[123] Valencia-Arredondo JA, Hernández-Bolio GI, Cerón-Montes GI, Et Al. Enhanced Process Integration For The Extraction, Concentration And Purification Of Di-Acylated Cyanidin From Red Cabbage. Separation And Purification Technology 2020; 238: 116492.
[124] Bohn T, Mcdougall GJ, Alegría A, Et Al. Mind The Gap—Deficits In Our Knowledge Of Aspects Impacting The Bioavailability Of Phytochemicals And Their Metabolites—A Position Paper Focusing On Carotenoids And Polyphenols. Molecular Nutrition & Food Research 2015; 59: 1307-1323.

[125] Bohn T, Mcdougall G, Alegría A, Et Al. Mind The Gap-Deficits In Our Knowledge Of Aspects Impacting The Bioavailability Of Phytochemicals And Their Metabolites-A Position Paper Focusing On Carotenoids And Polyphenols. Molecular Nutrition & Food Research 2015; 59. DOI: 10.1002/Mnfr.201400745.
[126] Sosa-Hernández JE, Escobedo-Avellaneda Z, Iqbal HM, Et Al. State-Of-The-Art Extraction Methodologies For Bioactive Compounds From Algal Biome To Meet Bio-Economy Challenges And Opportunities. Molecules 2018; 23: 2953.
[127] Damman CJ. Perspective: Nutrition's Next Chapter – Bioactive Gaps And The Microbiome–Mitochondria Axis. Advances In Nutrition 2023; 14: 420-425. DOI: Https://Doi.Org/10.1016/J.Advnut.2023.03.016.
[128] Uemura D. Exploratory Research On Bioactive Natural Products With A Focus On Biological Phenomena. Proceedings Of The Japan Academy, Series B 2010; 86: 190-201.
[129] Bai X, Zhou L, Zhou L, Et Al. The Research Progress Of Extraction, Purification And Analysis Methods Of Phenolic Compounds From Blueberry: A Comprehensive Review. Molecules 2023; 28: 3610.
[130] Du Z And Li Y. Review And Perspective On Bioactive Peptides: A Roadmap For Research, Development, And Future Opportunities. Journal Of Agriculture And Food Research 2022: 100353.
[131] Chemat F, Rombaut N, Fabiano-Tixier AS, Et Al. Green Extraction: From Concepts To Research, Education, And Economical Opportunities. Green Extraction Of Natural Products: Theory And Practice 2015: 1-36.
[132] Putra NR, Yustisia Y, Heryanto B, Et Al. Advancements And Challenges In Green Extraction Techniques For Indonesian Natural Products: A Review. South African Journal Of Chemical Engineering 2023.