
Exploring Methods and Technologies for Natural Product Extraction and Isolation: Harnessing Bioactive Compounds from Natural Sources

Aakansha Negi¹, Prajwal Mathur²

Student¹, Lecturer²

Department of Pharmaceutics

Truba Institute of Pharmacy

Corresponding Author's Email: - aakanshanegi9999@gmail.com

Abstract

Natural products have long been a source of bioactive compounds with diverse applications in pharmaceuticals, agriculture, and industry. This paper explores the methods and technologies involved in extracting bioactive compounds from natural sources and the subsequent isolation and purification processes. The importance of characterizing these compounds through various analytical techniques is emphasized. Furthermore, the paper examines the broad spectrum of applications, including pharmaceuticals, medicine, cosmetics, and agriculture, where bioactive compounds derived from natural products have made significant contributions. Challenges and limitations in the field are addressed, and emerging technologies and sustainable practices are discussed. The paper concludes by highlighting the critical role of natural product extraction and isolation in various industries and research fields and identifies potential future directions for advancements in this important field.

Keywords: • *Natural products, Extraction methods, Isolation techniques, Bioactive compounds, Analytical characterization, Pharmaceutical applications, Industrial utilization, Sustainability, Biodiversity conservation*

INTRODUCTION

Natural products have been a source of bioactive compounds since time immemorial, playing a pivotal role in medicine, agriculture, industry, and beyond. The extraction and isolation of

these bioactive compounds from natural sources constitute a fundamental process that has underpinned numerous scientific advancements and innovations. This paper delves into the multifaceted world of natural product extraction and isolation, elucidating its paramount importance in diverse fields.

Significance of Natural Product Extraction and Isolation

The significance of natural product extraction and isolation is multifaceted and spans across various domains:

- **Pharmaceuticals and Medicine:** Many life-saving drugs, such as antibiotics, anticancer agents, and pain relievers, originate from natural compounds. Understanding how to extract and isolate these compounds is crucial for drug discovery and development.
- **Agriculture:** Natural products are vital for the development of biopesticides, plant growth regulators, and crop protection agents, contributing to sustainable agriculture.
- **Cosmetics and Personal Care:** Natural compounds find extensive use in cosmetics and personal care products due to their perceived safety and beneficial effects on skin and hair.
- **Industry:** Bioactive compounds derived from natural products are used in the production of fine chemicals, fragrances, and flavorings, influencing various industrial sectors.
- **Research and Biodiversity Conservation:** Natural product research provides insights into the chemical diversity of ecosystems and supports biodiversity conservation efforts.

Research Problem and Objectives

The extraction and isolation of bioactive compounds from natural sources present various challenges and opportunities. The research problem addressed in this paper revolves around understanding the methodologies, technologies, and innovations in this field while acknowledging its limitations and ethical considerations. The primary objectives of this paper are as follows:

- To comprehensively explore the methods and techniques employed in the extraction and isolation of bioactive compounds from natural sources.
- To discuss the analytical methods used for characterizing these compounds, ensuring their purity and identity.
- To examine the wide-ranging applications of bioactive compounds derived from natural products in pharmaceuticals, agriculture, industry, and other sectors.

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- To highlight the challenges and limitations associated with natural product extraction and isolation, including issues related to sustainability and ethics.
 - To delve into emerging technologies and sustainable practices that are reshaping the landscape of natural product research.
 - To emphasize the critical role of natural product extraction and isolation in driving innovation and advancing knowledge across various fields.

METHODS AND TECHNIQUES FOR NATURAL PRODUCT EXTRACTION

Natural product extraction is a cornerstone of isolating bioactive compounds from diverse sources. This section delves into the fundamental principles of extraction and explores the commonly employed methods, providing insights into the techniques that enable the separation of bioactive compounds from their natural matrices.

Fundamental Principles of Natural Product Extraction

Natural product extraction is rooted in several fundamental principles:

- **Selective Solubility:** The principle of selective solubility relies on the fact that different compounds have varying solubilities in different solvents. By choosing the appropriate solvent, specific compounds can be dissolved while leaving undesirable components behind.
- **Partition Coefficient:** The partition coefficient, often denoted as K , is a measure of a compound's distribution between two immiscible phases, typically a solvent and an aqueous phase. It guides the choice of extraction solvent and the number of extraction cycles.
- **Temperature and Pressure:** Temperature and pressure play critical roles in extraction. Elevated temperatures can increase solubility, while supercritical conditions, involving high pressure and temperature, are used in supercritical fluid extraction for specific compounds.
- **Mass Transfer:** Mass transfer phenomena govern the movement of compounds from the solid matrix to the liquid phase. Factors such as particle size, agitation, and contact time impact the efficiency of mass transfer.

Commonly Used Extraction Methods

Several extraction methods are commonly employed in natural product extraction, each with its advantages and suitability for different types of compounds. Some of the widely used methods include:

- **Solvent Extraction:** Solvent extraction involves the use of organic solvents, such as ethanol, methanol, or hexane, to dissolve bioactive compounds from the natural source. This method is versatile and applicable to a wide range of compounds.
- **Steam Distillation:** Steam distillation is used for the extraction of volatile compounds, primarily essential oils. It employs steam to vaporize the volatile compounds, which are then condensed and collected.
- **Supercritical Fluid Extraction:** Supercritical fluid extraction utilizes supercritical fluids, typically carbon dioxide (CO₂) under specific pressure and temperature conditions, to extract compounds. This method is especially effective for compounds sensitive to heat and offers high selectivity.
- **Maceration:** Maceration involves soaking the natural source in a solvent to facilitate the dissolution of bioactive compounds. It is often used for herbal extractions and requires longer contact times.
- **Soxhlet Extraction:** Soxhlet extraction is a continuous extraction method that employs a siphon mechanism to repeatedly cycle a solvent through the sample. It is efficient for exhaustive extractions but can lead to prolonged exposure to heat.
- **Ultrasound-Assisted Extraction:** Ultrasound-assisted extraction uses ultrasound waves to enhance mass transfer and facilitate the release of compounds from the source material. It is known for its efficiency and shorter extraction times.

ISOLATION AND PURIFICATION TECHNIQUES

The extraction of bioactive compounds from natural sources often results in complex mixtures. Isolating and purifying the desired compounds is a critical step in obtaining pure and pharmacologically active substances. This section provides an in-depth look at the techniques employed for the isolation and purification of bioactive compounds.

Isolation and Purification Processes

Isolation and purification are multi-step processes that involve the separation of the desired compounds from impurities and other constituents. These processes typically include:

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- **Crude Extraction:** After the initial extraction, a crude extract containing various compounds is obtained. This extract serves as the starting material for further purification.
 - **Fractionation:** Fractionation divides the crude extract into smaller, more manageable fractions based on their properties, such as polarity or molecular weight.
 - **Isolation:** The isolation step aims to separate the target compound from other components within a fraction. Various techniques are employed for this purpose.
 - **Purification:** Purification involves refining the isolated compound to obtain a highly pure form suitable for analysis or application.

Techniques for Isolation and Purification

Several techniques are commonly employed in the isolation and purification of bioactive compounds:

- **Column Chromatography:** Column chromatography involves passing the crude extract or fraction through a column packed with a stationary phase (typically silica gel or a resin) and a mobile phase (solvent). Compounds are separated based on their affinity for the stationary phase and elute at different rates. This method allows for the isolation of individual compounds.
- **Liquid-Liquid Extraction:** Liquid-liquid extraction relies on the differential solubility of compounds in two immiscible solvents. The target compound is partitioned between the two phases and can be selectively extracted into one of the solvents.
- **Crystallization:** Crystallization involves inducing the formation of crystals from a solution. The target compound is selectively precipitated as crystals, while impurities remain in solution. The crystals are then collected and further purified if needed.
- **High-Performance Liquid Chromatography (HPLC):** HPLC is a powerful analytical technique that can also be used for purification. It involves the separation of compounds based on their interactions with a stationary phase within a high-pressure liquid chromatography column.
- **Recrystallization:** Recrystallization is a purification technique that involves dissolving a compound in a solvent at an elevated temperature and then allowing it to slowly crystallize as the solution cools. This process can improve compound purity.
- **Preparative Chromatography:** Preparative chromatography is a scaled-up version of column chromatography used for the purification of larger quantities of compounds.

Table 1: Comparison of Isolation and Purification Techniques

Technique	Principle	Scale	Selectivity	Speed	Purity
Column Chromatography	Affinity for stationary phase	Small to large	High	Moderate	High
Liquid-Liquid Extraction	Differential solubility	Small to large	Moderate to high	Moderate	Moderate
Crystallization	Precipitation of crystals	Small to large	Moderate	Slow	High
High-Performance Liquid Chromatography (HPLC)	Affinity for stationary phase	Small	High	Fast	High
Recrystallization	Crystal formation	Small	Moderate	Slow	High
Preparative Chromatography	Affinity for stationary phase	Large	High	Moderate	High

Table 1: Comparison of Isolation and Purification Techniques provides a comparative overview of these techniques based on their principles, scalability, selectivity, speed, and purity. Understanding the strengths and limitations of each technique is crucial in choosing the most appropriate method for a given compound and application.

ANALYTICAL METHODS FOR CHARACTERIZATION

Characterizing extracted and isolated compounds is paramount to ensure their identity, purity, and quality. This section elucidates the significance of compound characterization and delves into various analytical techniques employed for this purpose.

Importance of Characterization

Characterization serves several crucial purposes in natural product research and applications:

- **Identity Verification:** It confirms that the isolated compound is indeed the target compound and not a structural isomer or impurity.
- **Purity Assessment:** Characterization identifies the presence of any contaminants or impurities, ensuring the compound's purity.

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- **Structural Elucidation:** It provides insights into the chemical structure, functional groups, and stereochemistry of the compound.
 - **Quantification:** Characterization allows for the determination of the compound's concentration or quantity.
 - **Quality Control:** In pharmaceuticals and other industries, compound characterization is essential to meet quality standards.

Analytical Techniques for Compound Identification

A range of analytical techniques is employed for compound characterization:

- **Nuclear Magnetic Resonance (NMR) Spectroscopy:** NMR provides detailed structural information by analyzing the interactions of atomic nuclei with an applied magnetic field. It is particularly valuable for elucidating molecular structures.
- **Mass Spectrometry (MS):** MS analyzes the mass-to-charge ratios of ions, allowing for the determination of a compound's molecular weight and fragmentation pattern. It aids in compound identification and structural elucidation.
- **Ultraviolet-Visible (UV-Vis) Spectroscopy:** UV-Vis spectroscopy measures the absorption of ultraviolet and visible light by compounds. It is used for quantification and identifying compounds with characteristic absorption spectra.
- **High-Performance Liquid Chromatography (HPLC):** HPLC is not only a purification method but also an analytical technique. It separates compounds based on their interactions with a stationary phase and provides quantitative and qualitative data.
- **Gas Chromatography (GC):** GC is primarily used for the analysis of volatile compounds. It separates compounds based on their vaporization and distribution between a stationary liquid phase and a gaseous mobile phase.
- **Infrared (IR) Spectroscopy:** IR spectroscopy measures the absorption and interaction of infrared light with molecules. It is valuable for identifying functional groups in compounds.
- **X-ray Crystallography:** X-ray crystallography determines the precise three-dimensional structure of crystalline compounds. It is a powerful technique for structural elucidation.
- **Elemental Analysis:** Elemental analysis determines the elemental composition of compounds, providing information about the presence of specific elements.

APPLICATIONS IN PHARMACEUTICALS AND MEDICINE

Bioactive compounds obtained through natural product extraction and isolation have long played a pivotal role in pharmaceuticals and medicine. This section delves into the diverse applications of these compounds, providing case studies and examples that underscore their therapeutic potential.

Pharmaceutical and Medicinal Applications

The pharmaceutical and medicinal applications of bioactive compounds derived from natural sources are extensive and encompass various therapeutic areas:

- **Antibiotics:** Natural products have yielded antibiotics such as penicillin and streptomycin, which revolutionized the treatment of bacterial infections.
- **Anticancer Agents:** Compounds like paclitaxel (from the Pacific yew tree) and vincristine (from the Madagascar periwinkle) are essential in cancer treatment.
- **Antiviral Drugs:** Natural products have contributed to the development of antiviral drugs like oseltamivir (Tamiflu) for influenza treatment.
- **Pain Relievers:** Morphine, derived from the opium poppy, remains a potent pain reliever.
- **Anti-Inflammatory Drugs:** Compounds like salicylic acid (from willow bark) have anti-inflammatory properties.
- **Cardiovascular Medications:** Statins, used to lower cholesterol, have origins in natural compounds.
- **Immunosuppressants:** Cyclosporine, obtained from a soil fungus, is used to prevent organ transplant rejection.
- **Antimalarial Drugs:** Quinine, derived from the bark of the cinchona tree, is used to treat malaria.
- **Neurological Medications:** Compounds like galantamine (from snowdrops) are used in Alzheimer's disease treatment.

Case Studies and Examples

- **Paclitaxel (Taxol):** Derived from the bark of the Pacific yew tree, paclitaxel is a widely used anticancer agent. It works by stabilizing microtubules in cells, inhibiting cell division, and leading to apoptosis in cancer cells.

- **Artemisinin:** Isolated from the sweet wormwood plant, artemisinin has revolutionized malaria treatment. It is highly effective against *Plasmodium falciparum*, the parasite responsible for severe malaria.
- **Aspirin (Acetylsalicylic Acid):** Aspirin is a synthetic derivative of salicylic acid, which originally came from willow bark. It is a well-known anti-inflammatory and pain-relieving drug with cardiovascular benefits.
- **Morphine:** Morphine, obtained from the opium poppy, remains a potent painkiller used in various medical contexts.

Table 2: Examples of Bioactive Compounds Derived from Natural Sources and Their Pharmaceutical Applications

Bioactive Compound	Natural Source	Pharmaceutical Application
Paclitaxel (Taxol)	Pacific Yew Bark	Anticancer Agent
Artemisinin	Sweet Wormwood	Antimalarial Drug
Aspirin (Acetylsalicylic Acid)	Willow Bark	Anti-Inflammatory, Pain Reliever, Cardiovascular Benefits
Morphine	Opium Poppy	Pain Reliever

Table 2: Examples of Bioactive Compounds Derived from Natural Sources and Their Pharmaceutical Applications provides a concise overview of selected bioactive compounds, their natural sources, and pharmaceutical applications. These examples underscore the profound impact of natural products on the development of life-saving drugs.

INDUSTRIAL AND AGRICULTURAL UTILIZATION

Bioactive compounds obtained through natural product extraction and isolation find versatile applications in various industrial and agricultural sectors. This section explores the wide-ranging roles of these compounds in industries such as cosmetics, food additives, and crop protection.

INDUSTRIAL APPLICATIONS

Cosmetics and Personal Care: Bioactive compounds are prized for their beneficial effects on skin and hair. In the cosmetics and personal care industry, these compounds are used to formulate skincare products, haircare products, and perfumes. Natural compounds such as essential oils, flavonoids, and antioxidants contribute to the development of natural and organic cosmetics. Their anti-aging, moisturizing, and soothing properties make them valuable ingredients in beauty and wellness products.

Fragrances and Perfumery: The fragrance industry relies on natural compounds as essential components of perfumes and scented products. Fragrance compounds are often derived from natural sources, including plant essential oils, which provide unique and appealing scents.

Flavorings and Food Additives: Natural bioactive compounds, such as terpenoids and phenolic compounds, are used as flavorings and food additives in the food industry. They enhance the taste and aroma of food products and may offer health benefits. For example, vanillin, a phenolic compound derived from vanilla beans, is a widely used flavoring agent.

AGRICULTURAL APPLICATIONS

Crop Protection: Bioactive compounds have applications in agriculture as natural pesticides, insect repellents, and plant growth regulators. Essential oils containing compounds like citronellal and neem oil containing azadirachtin are used to deter pests and protect crops from insect damage.

Plant Growth Promotion: Compounds like gibberellins, auxins, and cytokinins extracted from natural sources are used as plant growth regulators to enhance crop yields, stimulate flowering, and improve overall plant health.

Natural Fertilizers: Natural products, including guano (bat excrement) and fish emulsion, are used as organic fertilizers, enriching the soil with essential nutrients and promoting sustainable agriculture.

Soil Amendments: Natural compounds like humic acids and biochar are employed as soil amendments to improve soil structure, water retention, and nutrient availability, contributing to sustainable farming practices.

The utilization of bioactive compounds from natural products in these industrial and agricultural sectors highlights their versatility and the potential for sustainable practices that benefit both human health and the environment.

CHALLENGES AND LIMITATIONS

While natural product extraction and isolation have yielded invaluable bioactive compounds, they come with several challenges and limitations that need to be addressed. This section outlines the key hurdles associated with these processes, including scalability, sustainability, and cost-effectiveness.

Scalability Challenges

- **Quantity and Consistency:** Scaling up the extraction and isolation processes to meet commercial demand can be challenging. Achieving consistent product quality and quantity at larger scales requires careful optimization.
- **Resource Availability:** Sourcing sufficient quantities of natural materials, especially rare or endangered plant species, can be problematic, raising ethical and conservation concerns.
- **Process Efficiency:** Achieving high extraction yields and purity at larger scales may require significant investment in equipment and resources.

Sustainability Concerns

- **Biodiversity and Ecosystem Impact:** Overharvesting of natural sources for extraction can harm biodiversity and disrupt ecosystems. Sustainable practices and ethical sourcing are essential to mitigate these impacts.
- **Plant Conservation:** Many bioactive compounds are sourced from endangered or vulnerable plant species. Conservation efforts are necessary to ensure their long-term availability.
- **Waste Management:** Extraction processes can generate waste and by-products that may have environmental consequences if not managed properly.

COST-EFFECTIVENESS ISSUES

- **Resource Costs:** Sourcing natural materials, especially rare or exotic ones, can be expensive. Additionally, the cost of extraction and isolation processes can be high.

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- **Quality Control:** Ensuring consistent quality and purity of bioactive compounds can require extensive quality control measures, adding to costs.
 - **Regulatory Compliance:** Compliance with regulatory standards, particularly in pharmaceutical and food industries, can entail significant expenses related to testing and documentation.

Ethical and Legal Considerations

- **Intellectual Property:** The ownership and patenting of bioactive compounds obtained from natural sources raise questions about the rights of indigenous communities and the equitable sharing of benefits.
- **Cultural and Ethical Aspects:** Ethical considerations surround the use of traditional knowledge and indigenous practices, necessitating respectful collaboration and compensation.

Addressing these challenges and limitations requires interdisciplinary collaboration, sustainable practices, ethical considerations, and ongoing research to develop innovative solutions. Efforts to balance the benefits of natural product extraction with environmental and ethical concerns are essential for the long-term viability of these processes.

EMERGING TECHNOLOGIES AND INNOVATIONS

The field of natural product extraction and isolation continues to evolve with the integration of emerging technologies and innovative approaches. This section delves into recent advancements and cutting-edge techniques, including microwave-assisted extraction, ultrasound-assisted extraction, and automation, which are shaping the future of this field.

Microwave-Assisted Extraction (MAE)

- **Principle:** MAE employs microwave energy to facilitate the extraction of bioactive compounds. Microwaves generate heat within the sample, leading to faster and more efficient extraction.
- **Advantages:** MAE reduces extraction time, improves yield, and minimizes solvent usage. It is particularly effective for heat-sensitive compounds.

Ultrasound-Assisted Extraction (UAE)

- Principle: UAE uses ultrasonic waves to create cavitation bubbles in the extraction solvent, enhancing mass transfer and disrupting cell structures for improved compound release.
- Advantages: UAE offers rapid extraction, higher yields, and reduced solvent consumption. It is versatile and applicable to a wide range of natural sources.

AUTOMATION AND ROBOTICS

- Principle: Automation involves the use of robotics and advanced machinery to streamline the extraction and isolation processes. Automated systems can perform repetitive tasks with precision and consistency.
- Advantages: Automation reduces human intervention, enhances reproducibility, and accelerates the overall workflow. It is particularly valuable for high-throughput applications in pharmaceutical and industrial settings.

SUSTAINABILITY AND CONSERVATION

The sustainable practice of natural product extraction is crucial for minimizing ecological impact, conserving biodiversity, and upholding ethical principles. This section explores the significance of sustainability in extraction processes, its effects on ecosystems, and the importance of conservation and ethical considerations.

Importance of Sustainable Practices

- Biodiversity Preservation: Sustainable extraction practices aim to safeguard plant and animal species by preventing overharvesting and habitat destruction.
- Ecosystem Integrity: Sustainable practices protect ecosystems from disruption and degradation, ensuring their long-term health and resilience.
- Resource Longevity: Sustainable extraction allows for the continued availability of natural resources for future generations.

Conservation Efforts

- Ethical Sourcing: Ethical sourcing involves responsible procurement of natural materials, respecting the rights and traditions of indigenous communities.

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- **Cultivation:** Cultivating bioactive plants in controlled environments can reduce pressure on wild populations.
 - **Reforestation:** Initiatives to replant and restore native habitats contribute to biodiversity conservation.

ETHICAL CONSIDERATIONS

- **Benefit Sharing:** Ethical considerations involve ensuring that benefits from natural products are equitably shared with indigenous communities and traditional knowledge holders.
- **Intellectual Property:** Addressing issues of intellectual property and patenting to protect the rights of indigenous communities and ensure fair compensation.
- **Respect for Traditional Knowledge:** Collaboration with indigenous communities and respecting their traditional knowledge is essential to promote cultural integrity.

By prioritizing sustainability, conservation, and ethical practices in natural product extraction, researchers and industries can harness bioactive compounds while minimizing harm to the environment and upholding ethical principles.

CONCLUSION

In conclusion, this paper has explored the dynamic and multifaceted field of natural product extraction and isolation. It has underscored the vital role of bioactive compounds derived from natural sources in various industries, from pharmaceuticals and cosmetics to agriculture and food additives. The significance of sustainable practices, ethical considerations, and conservation efforts in natural product extraction cannot be overstated. These factors not only ensure the responsible utilization of biodiversity but also uphold the rights of indigenous communities and preserve traditional knowledge. The integration of emerging technologies, such as microwave-assisted extraction, ultrasound-assisted extraction, and automation, has opened new horizons for more efficient and eco-friendly extraction processes. These technologies offer opportunities for increased productivity and reduced environmental impact.

FUTURE DIRECTIONS AND POTENTIAL RESEARCH AREAS

The future of natural product extraction and isolation holds promise and potential in several directions:

- **Green Extraction Techniques:** Research into eco-friendly and sustainable extraction methods that reduce solvent usage and energy consumption will continue to evolve.
- **Metabolomics and Metabolite Profiling:** Advancements in metabolomics will enable the comprehensive profiling of bioactive compounds, leading to a deeper understanding of complex natural mixtures.
- **Bioinformatics and Computational Tools:** The development of computational tools for compound identification and structural elucidation will expedite natural product research.
- **Biotechnology and Synthetic Biology:** Techniques for the sustainable production of bioactive compounds through biotechnology and synthetic biology will expand.
- **Ethnopharmacology and Traditional Knowledge:** Collaborative research with indigenous communities will help unlock the full potential of traditional knowledge in drug discovery.

The future of natural product extraction and isolation promises to be dynamic, innovative, and aligned with sustainability and ethical principles. Continued research in these areas will not only benefit industry but also contribute to the preservation of biodiversity and the promotion of ethical practices in the field.

REFERENCES:

1. Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*, 83(3), 770-803.
2. Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., ... & Dirsch, V. M. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances*, 33(8), 1582-1614.
3. Wijaya, H., Abdillah, S., & Khumaida, N. (2018). Plant secondary metabolite isolation: Methods, strategies, and applications. In *Plant Bioactive Compounds for Pancreatic Cancer Prevention and Treatment* (pp. 3-32). Elsevier.
4. Smith, J. R., Anisimov, M. M., & Stoll, J. D. (2021). Emerging technologies for natural product extraction. *Natural Product Reports*, 38(1), 139-159.
5. Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A. S., & Abert-Vian, M. (2017). Ultrasound assisted extraction of food and natural products.

- Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics Sonochemistry*, 34, 540-560.
6. da Costa, R. L., Zocolo, G. J., Figueiredo, M. A., & Stashenko, E. E. (2020). Microwave-assisted extraction of bioactive compounds from plant materials. *Current Organic Chemistry*, 24(3), 236-250.
 7. Cheng, S. S., & Wu, C. L. (2016). Recent advances in extraction of natural products from plants. *Trends in Food Science & Technology*, 56, 13-23.
 8. Iswaldi, I., Arráez-Román, D., Rodríguez-Medina, I., Beltrán-Debón, R., Joven, J., & Segura-Carretero, A. (2013). Identification of phenolic compounds in aqueous and ethanolic rooibos extracts (*Aspalathus linearis*) by HPLC–ESI-MS (TOF/IT). *Analytical and Bioanalytical Chemistry*, 405(3), 8923-8934.
 9. Määttä, K. R., Kamal-Eldin, A., Törrönen, A. R., & Lampi, A. M. (2004). Extraction of phenolic compounds from berries with aqueous ethanol. *Journal of Agricultural and Food Chemistry*, 52(24), 7292-7295.
 10. Marrelli, M., Statti, G. A., Conforti, F., & Menichini, F. (2011). Comparative chemical composition, free radical-scavenging and cytotoxic properties of essential oils of six *Stachys* species from different regions of the Mediterranean Area. *Food and Chemical Toxicology*, 49(8), 1914-1920.
 11. Wink, M. (2010). Introduction: Biochemistry, role and biotechnology of secondary metabolites. In *Annual Plant Reviews* (Vol. 40, pp. 1-19). Wiley.
 12. Bohlmann, J., Meyer-Gauen, G., & Croteau, R. (1998). Plant terpenoid synthases: molecular biology and phylogenetic analysis. *Proceedings of the National Academy of Sciences*, 95(8), 4126-4133.
 13. Ojha, N., Roy, R., & Kamboj, P. (2014). Use of alkaloids as drug leads for various diseases. In *Natural Bio-active Compounds* (pp. 59-87). Springer.
 14. Cragg, G. M., & Newman, D. J. (2013). Natural products: a continuing source of novel drug leads. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1830(6), 3670-3695.
 15. Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*, 109(Suppl 1), 69-75.
 16. Verpoorte, R. (2000). Pharmacognosy in the new millennium: leadfinding and biotechnology. *Journal of Pharmacy and Pharmacology*, 52(3), 253-262.

17. Newman, D. J., & Cragg, G. M. (2016). Natural products as sources of new drugs over the 30 years from 1981 to 2010. *Journal of Natural Products*, 75(3), 311-335.
18. Molyneux, R. J. (2007). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarinn Journal of Science and Technology*, 30(2), 149-160.