Ultrasonic-Assisted and Enzyme-Assisted Extraction Techniques for the Identification of Antioxidant Compounds in Natural Plants

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Ultrasonic-Assisted and Enzyme-Assisted Extraction Techniques for the Identification of Antioxidant Compounds in Natural Plants

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Abstract— This literature review synthesis recent advancements and findings in the extraction of antioxidant compounds using two innovative techniques: ultrasonic-assisted extraction (UAE) and enzyme-assisted extraction (EAE). Antioxidants are molecules that assist in shielding the body's cells from the damaging effects of free radicals, which are unstable molecules produced by internal activities like metabolism and external influences like pollution and UV radiation. Free radicals have the ability to produce oxidative stress, which can result in ageing, cell damage, and a number of diseases, including cancer, heart disease, and neurological problems. The review offers a comprehensive exploration of the principles and applications of these methods in identifying antioxidant compounds from various sources, including plants, fruits, and agricultural by-products. Additionally, it critically assesses studies that compare the effectiveness of ultrasonic-assisted extraction and enzyme-assisted extraction in isolating antioxidant compounds, particularly phenolic compounds. The review discusses the advantages and limitations of each technique in terms of extraction yield, purity, and specificity. Furthermore, it highlights the synergistic effects observed when combining ultrasonic and enzymatic treatments. Overall, this review serves as a valuable resource for researchers, industry professionals, and stakeholders interested in leveraging ultrasonic-assisted and enzyme-assisted extraction methods for the identification and extraction of antioxidant compounds. By synthesizing current knowledge and outlining future research directions, the review aims to advance the field of antioxidant compound extraction and contribute to the development of novel functional ingredients with significant health benefits.

Keywords—Ultrasound-assisted extraction, Enzyme-assisted extraction method, Antioxidant

I. INTRODUCTION

Scientists have identified numerous antioxidant compounds, employing various extraction methods to isolate these bioactive substances. Extraction involves removing desired components from solid or liquid materials by dissolving them in a suitable solvent. This process allows for selective interaction between the solvent and the target compound, enabling separation from the original material. Following extraction, filtration typically separates the extracted substance from remaining components, which may undergo further processing or purification for various applications in chemistry, biology, and other fields.

This review focuses primarily on two extraction methods: Ultrasonic Assisted Extraction (UAE) and Enzyme Assisted Extraction. UAE employs high-intensity ultrasonic pulses. Antioxidants are molecules that assist in shielding the body's cells from the damaging effects of free radicals, which are unstable molecules produced by internal activities like metabolism and external influences like pollution and UV radiation. Free radicals have the ability to produce oxidative stress, which can result in ageing, inflammation, cell damage, and a number of diseases, including cancers, heart diseases, and neurological problems. to induce acoustic cavitation in the extraction medium, breaking down cell walls and enhancing the release of bioactive compounds. This method is renowned for its rapid extraction speed, preservation of labile chemicals, versatility in solvent use, and ecological benefits due to reduced solvent usage [1]. Enzyme Assisted Extraction involves using enzymes such as cellulases, hemicellulases, and pectinases to break down cell wall components and release bioactive substances from natural sources like plants, bacteria, fungi, algae, and animals. This technique is valued for its environmental sustainability, efficiency, and ability to selectively extract desired compounds while maintaining their original potency [2].

The extraction techniques play a crucial role in obtaining bioactive chemicals from natural sources, impacting efficiency, selectivity, and environmental sustainability. This review aims to advance knowledge and promote the development of functional ingredients with potential health benefits.

II. LITERATURE REVIEW

A. *Ultrasonic-Assisted Extraction Method (UAE)*

Ultrasonic extraction theory posits that high-frequency sound waves can enhance the extraction of bioactive compounds from plant materials. Bioactive chemicals are released into the solvent due to the breakdown of plant cell walls and membranes. Cavitation bubbles create localized high pressures and temperatures, further aiding in the extraction process by increasing the surface area available for mass transfer.

Ultrasonic-assisted extraction (UAE) stands out as a preferred method for extracting bioactive chemicals from plant materials due to its numerous advantages. UAE is also noted for its speed, minimizing the degradation of thermosensitive chemicals, and its environmentally friendly approach with reduced solvent usage [3].

UAE finds wide application across various industries. In the food sector, it extracts bioactive substances and antioxidants from fruits, vegetables, and herbs, thereby enhancing nutritional profiles and functional qualities of food products. In pharmaceuticals, UAE facilitates the extraction of active pharmaceutical ingredients (APIs) from medicinal plants, aiding in the formulation of pharmaceuticals, vitamins, and herbal remedies. The cosmetics industry utilizes UAE to extract antioxidant and anti-aging compounds from natural sources for skincare, haircare, and makeup products.

In research, UAE is pivotal for extracting and analyzing a diverse range of chemicals, contributing to advancements in biology, pharmacology, and chemistry. Its ability to enhance extraction efficiency, reduce extraction time, and minimize solvent use makes it a preferred choice among scientists and researchers. Optimizing UAE parameters plays a crucial role in improving the extraction efficiency of bioactive compounds from natural sources. Factors such as vessel diameter, sample-to solvent ratio, extraction temperature and sonication time are carefully selected and managed to maximize the yield of target compounds [4].

For instance, the fruit of Melastoma sanguineum sims was subjected to UAE to extract antioxidants. Fruit powder was mixed with an ethanol aqueous solution and treated with ultrasound under controlled conditions of power, temperature, and time. Mechanical effects produced by ultrasonic energy improved mass transfer and solvent penetration into the sample matrix, enhancing internal and eddy diffusion. Ultrasonic cavitation aided in breaking down cell walls, facilitating the release of antioxidant compounds from the fruit material. In order to optimize the extraction parameters ethanol concentration, solvent-to-material ratio, extraction time, temperature, and ultrasonic power the study successfully applied response surface methodology (RSM). The best extraction conditions for ultrasound-assisted extraction (UAE) were found by using this method, which enabled a thorough investigation of the relationship between these factors.

As compared to traditional techniques like maceration and Soxhlet extraction, the study's findings showed how effective UAE is. In particular, compared to maceration and Soxhlet extraction techniques, UAE showed a notable increase in extraction efficiency. The study also showed how UAE is a more advantageous extraction technique due to its shorter extraction times and lower need for organic solvents [5].

Rhynchosia minima root represents another example where UAE is beneficial. This medicinal herb, shown in figure 1.[6] known for its bioactive components with potent anticancer and antioxidant properties, can be effectively extracted and concentrated using UAE. Optimization of extraction parameters such as exposure time, solvent-to-material ratio, and extraction temperature enhances the yield of bioactive polysaccharides from the root, maximizing extraction efficiency. The bioactive chemicals extracted from Rhynchosia minima which is a small, perennial herbaceous plant belonging to the family Fabaceae (the legume or pea family). Root finds applications in functional foods, nutritional supplements, and pharmaceutical formulations, highlighting its potential to improve human health and well-being [7].

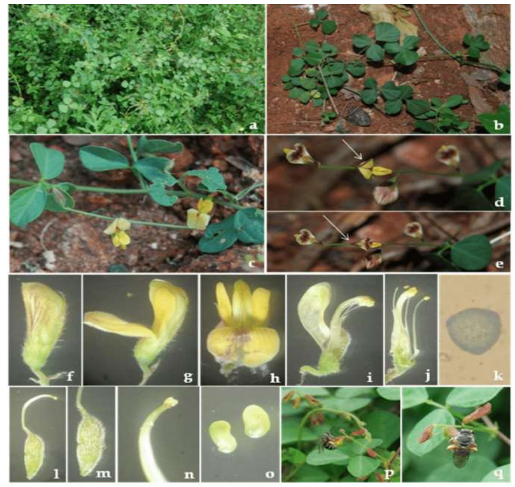

Fig.1. Pollination of Rhynchosia minima (L.) (Aluri and Kunuku, 2019, 116)

The Mediterranean plant *Lavandula stoechas*, known locally as “Ladastacho”, has long been prized in countries like Morocco for its therapeutic benefits. Rich in phenolic substances such as flavone glycosides are a type of naturally occurring compound consisting of flavone aglycone such as apigenin glycosides, disomic and phenolic acids, this plant is associated with a variety of health benefits, including antibacterial, antifungal, anti-inflammatory, and antioxidant properties. Utilizing UAE for *Lavandula stoechas* enhances both the yield and total phenolic content of the plant extract. The abundant phenolic compounds extracted from *Lavandula stoechas* are crucial for their positive impacts on human health, making them valuable in fields such as nutrition, medicine, and healthcare. This approach facilitates the development of products enriched with enhanced anti-inflammatory, antioxidant, and other health-promoting qualities from *Lavandula stoechas* extracts [8].

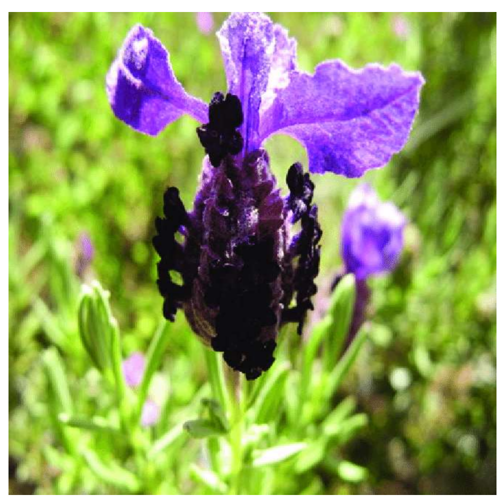

Fig.2. Flower of Lavandula stoechas (Zeynep et al., 2021, 264)

Mahua seeds are particularly well-suited for ultrasonic extraction techniques due to their richness in antioxidants and phytonutrients, which supports the employment of ultrasonic extraction procedures for them. In comparison to traditional techniques, it describes the benefits of ultrasonic-assisted extraction (UAE). A higher oil yield was obtained with the UAE technique, outperforming traditional extraction methods with an oil yield of 56.97% and more than 99% oil recovery. In comparison to Soxhlet and mechanical extractions, oil extracted using UAE showed the highest antioxidant capacity. Multiple antioxidant assays, such as ABTS, DPPH, and FRAP assays, verified this higher efficacy.

The UAE used light energy, a 35-minute extraction period, and a low temperature of 35°C to obtain these remarkable results. Maintaining the bioactive components in the oil was made easier by using a binary mixture of acetone and isopropanol (11 v/v). The research found important phytonutrients in the mahua oil, including tocopherols, phytosterols, terpenoids, and tocotrienols. These compounds have been linked to health benefits like antidiabetic, anti-inflammatory, and anticancer effects. The document presents a consistent comparison between the oil yield and antioxidant capacity of the UAE process and traditional Soxhlet and mechanical extraction methods. Together, these results show how successful ultrasonic extraction methods are for mahua seeds, especially when it comes to obtaining oil that is rich in antioxidants and improved in bioactive compounds, which is ideal for a range of industrial uses.

This process highlights the potential of repurposing and adding value to these underutilized seeds to produce antioxidant-rich mahua seed oil, which is highly valuable for the food and nutraceutical industries. The application of UAE for extracting mahua seed oil has shown higher oil recovery and yield compared to traditional methods like Soxhlet and mechanical extraction, while consuming less energy, requiring shorter extraction times, and operating at lower temperatures. UAE's gentle processing conditions also help preserve the bio actives in the oil. Although mahua oil is currently used primarily in traditional Asian cuisine, advanced extraction techniques like UAE could expand its applications in the food and nutraceutical sectors. The superior performance of UAE in co-extracting antioxidants and improving oil yield suggests it is a viable option for industrial scale-up [9], [10].

According to [11], the UAE method yielded the highest extraction value from the peel and pericarp of Punica granatum L. (Nimali variety). These yields were superior to those obtained through traditional boiling, microwaving, and water bath methods. Additionally, UAE extracts demonstrated the highest flavonoid content, "DPPH" free radical scavenging activity, and capacity to reduce ions from $Fe3+$ to $Fe2+$ for both peel and pericarp extracts [11].

Ultrasonic extraction can also be applied to pepper leaves to extract bioactive components. Using ultrasonic waves with an appropriate solvent helps break down cell walls and release desired chemicals from the plant material. Pepper leaves hold significant potential for ultrasonic extraction due to their effectiveness in extracting bioactive components. Compared to conventional methods, ultrasonic extraction allows for higher yields of bioactive chemicals from the leaves, increasing both production and efficiency. Additionally, this method is more sustainable, as it uses fewer harsh chemicals and is considered a green technology [12].

B. Enzyme - Assisted Extraction Method (EAE)

Enzyme-assisted extraction utilizes enzymes to break down cell wall material and release bioactive compounds from plant materials. Enzymes catalyse biochemical reactions under mild conditions, making them suitable for selectively breaking down target compounds. These plant-derived enzymes work by rupturing cell walls, thereby releasing the desired substances. This technique is commonly used to extract bioactive molecules, polyphenols, antioxidants, and other valuable substances from natural sources, offering an efficient way to obtain these chemicals without compromising their purity or bioactivity [13].

In the case of extracting phenolic compounds from murucizeiro leaves (Byrsonima crassifolia), enzymes are used to break down the leaf cell walls, facilitating the release of phenolic compounds. The process typically begins with selecting the appropriate enzymes, such as cellulase, pectinase, or hemicellulase, to target the leaf cell wall components. The leaves are pretreated with the selected enzymes under controlled pH, temperature, and duration conditions to ensure optimal enzymatic activity. This pretreatment weakens the cell walls, aiding the extraction of phenolic compounds. Following enzymatic pretreatment, an appropriate solvent is used to extract the phenolic chemicals from the plant material. The combined action of enzymes and solvent extraction enhances the efficiency and yield of phenolic compounds from murucizeiro leaves. These leaves are rich in phenolic compounds with anti-inflammatory and antioxidant properties, making them valuable for various applications. Furthermore, enzyme-assisted extraction of murucizeiro leaves provides a platform for research and development, allowing for the discovery of new bioactive compounds and applications in the natural products industry.

Fig. 3. Pplant of O murucizeiro (Moura et al., 2016, 20)

However, there are challenges to consider when using murucizeiro leaves for enzyme-assisted extraction. The variable concentration of phenolic components in the leaves may impact the consistency and yield of the extraction process. Standardizing the extraction method may require specialized knowledge and adjustments to achieve effective and consistent results. Additionally, the cost of enzymes and extraction equipment could be a factor, especially for large-scale extraction processes. The overall quality of the extracted phenolic compounds may be affected if specific compounds are degraded or if the bioactive properties of the plant material are altered during enzymatic treatment. These challenges underscore the importance of optimizing and carefully considering the use of murucizeiro leaves for enzyme-assisted extraction to maximize benefits and minimize potential drawbacks [14].

Avocado peel is a valuable source of polyphenols with strong antioxidant properties, making it an excellent candidate for enzyme-assisted extraction processes. Researchers aim to utilise this extraction method to harvest the beneficial polyphenols from avocado peel, which are renowned for their potent antioxidant effects. The antioxidant activity of avocado peel is attributed to a variety of phenolic compounds present in the peel. Enzyme-assisted extraction is particularly effective at isolating these compounds. This method extracted a higher concentration of polyphenols compared to traditional extraction techniques, making it a promising approach for obtaining natural antioxidants from avocado peel.

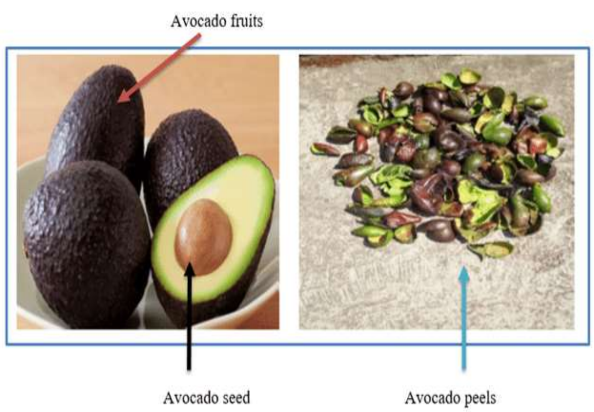

Fig. 4. Avocado-Peels (Tafere 2021, 55)

Overall, the application of avocado peel in enzyme-assisted extraction highlights its potential as a rich source of bioactive chemicals for creating antioxidant-rich extracts for various uses in the food and pharmaceutical industries [15].

Sea cucumbers are also valuable in enzyme-assisted extraction processes, particularly due to their high polysaccharide content. Enzymes such as the cysteine protease papain can effectively release polysaccharides from sea cucumbers. Compared to other extraction methods, this technique offers a more efficient and sustainable way to extract valuable components from sea cucumbers.

Using enzyme-assisted extraction for sea cucumbers has several advantages. It is highly efficient, easy to implement, and environmentally friendly.

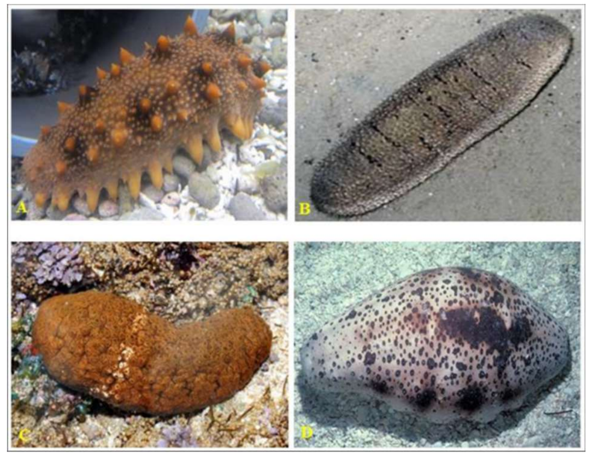

Fig.5. Commercially important species of sea cucumbers in aquaculture (Rahman et al., 2022, 421)

This method also helps preserve the bioactivity and potential health benefits of the extracted compounds by maintaining the polysaccharide structure. By efficiently lysing cells and releasing bioactive components, enzyme-assisted extraction ensures a more comprehensive extraction process, resulting in higher yields of essential compounds from sea cucumbers. Additionally, compounds recovered from sea cucumbers using enzyme-assisted extraction have demonstrated potential antioxidant effects. Sea cucumber polysaccharides exhibit anti-free radical properties, including the ability to scavenge radicals such as superoxide, hydroxyl, "DPPH", and ABTS 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid). These antioxidant capabilities suggest that compounds extracted from sea cucumbers could have significant antioxidation potential [16].

Habanero chili pepper seeds, rich in bioactive compounds like capsaicinoids and phenolic compounds, are valuable for enzyme-assisted extraction techniques. Enzyme-assisted extraction (EAE) enhances the efficiency of extracting these beneficial components from seeds. Using enzymes such as cellulase, the extraction process can be optimized to yield higher concentrations of these compounds from habanero chili pepper seeds. This environmentally friendly method of obtaining essential anti-inflammatory chemicals highlights the potential of habanero seeds as a raw material for extracting bioactive compounds with anti-inflammatory properties.

This demonstrates the significance of habanero chili pepper seeds as a valuable source of bioactive substances for developing nutraceuticals and functional foods. Additionally, extracting anti-inflammatory chemicals from habanero chili pepper seeds using enzymes creates opportunities for utilizing by-products that would otherwise be discarded. This research not only increases the value of habanero chili pepper seeds but also helps reduce food waste in the food industry [17].

Raspberry pomace, a by-product of industrial processing, holds significant potential in the food and nutraceutical industries through enzyme-assisted extraction. Raspberry pomace is a valuable source of bioactive compounds such as tocopherols, polyphenols, and polyunsaturated fatty acids, which are often discarded. Enzyme-assisted extraction effectively isolates these compounds from the pomace, providing a viable method for recovering valuable materials [18].

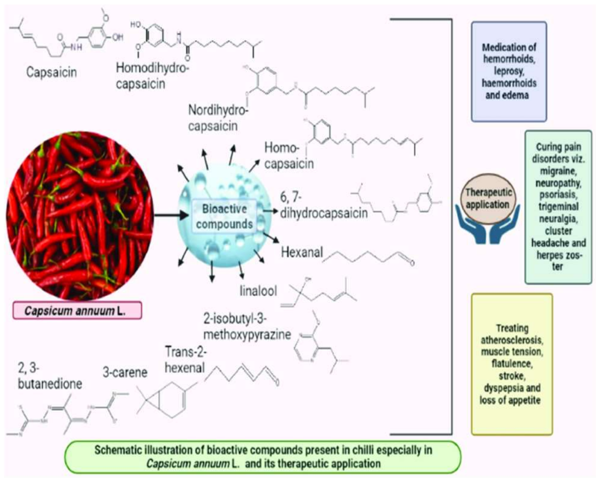

Fig. 6. Bal, Solanki & Sharangi, Amit & Upadhyay, Tarun & Khan, Fahad & Pandey, Pratibha & Siddiqui, Samra & Saeed, Mohd & Lee, Hae-Jeung & Yadav, Dharmendra. (2022). Biomedical and Antioxidant Potentialities in Chilli: Perspectives and Way Forward. Molecules. 27. 6380. 10.3390/molecules27196380.

One major application of enzyme-assisted extraction from raspberry pomace is in the production of nutraceuticals. The extracted bioactive substances, such as ellagitannins, tocopherols, phytosterols, and essential fatty acids, have shown potential health benefits. When incorporated into nutraceutical products, these antioxidant-rich substances may offer various health advantages. This process also enables the efficient recovery of these beneficial molecules, making it a valuable technique for creating functional food ingredients. The isolated bioactive components can be used as industrial additives like antioxidants prevent oxidation in fuel, plastics and rubber (butylated hydroxytoluene-BHT), Plasticizers-Improve flexibility in plastics (phthalates, adipates), Anti-foaming Agents - Reduce foam in industrial processes (silicone oils, polydimethylsiloxane) or ingredients to enhance the nutritional value and functional properties of food products like Flavour Enhancers to improve or intensify the taste (monosodium glutamate - MSG). For instance, the antioxidant properties of the extracted compounds can help extend the shelf life of food products by preventing oxidation. Their natural origins also align with the growing consumer demand for natural and clean-label food ingredients [18].

A systematic approach is essential for identifying antioxidant compounds in brown seaweeds using enzyme-assisted extraction. The selection of appropriate enzymes is crucial. Enzymes like carbohydrase and proteases are typically used to break down the complex polysaccharides in brown seaweeds' cell walls. Preparing the brown seaweeds for extraction involves cleaning and processing them for enzymatic treatment. During the enzymatic extraction process, the selected enzymes hydrolyze the seaweeds, facilitating the release of bioactive substances, including antioxidants. The enzymatic extracts are then analyzed for antioxidant activity using various techniques. Metal chelating (the process of binding chemicals, known as chelating agents, to metal ions to create a stable, ring-like complex is known as metal chelation) , reducing power, radical scavenging, and inhibition of lipid oxidation. These tests help evaluate the antioxidant potential of the isolated compounds. Further fractionation and characterization of the enzymatic extracts can identify specific antioxidant compounds. Analytical techniques like mass spectrometry, spectroscopy, and chromatography are used to study and identify the antioxidant compounds present in the fractions. The cosmetic and personal care industries can also benefit from compounds derived from brown seaweeds. Research has shown that phenolic compounds and antioxidants from seaweeds have anti-aging and skin-protective properties. Incorporating these natural elements into skincare products can offer benefits such as skin hydration, protection against environmental stresses, and anti-inflammatory effects [19].

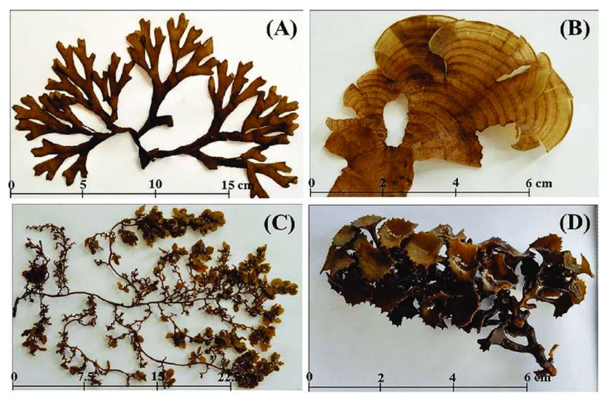

Fig. 7. Brown Seaweeds (Heriyanto et al., 2017, 325)

In the study, the recovery of plant protein and bioactive compounds from sesame bran was enhanced using ultrasonic extraction and enzyme-assisted extraction methods. For the ultrasonic extraction, sesame bran was subjected to ultrasonic waves under vacuum conditions. The key variables in this method include vacuum pressure, vacuum time, and restoration time after vacuum application. These factors were carefully selected and optimized to maximize extraction efficiency, while maintaining constant ultrasonic power and temperature throughout the process. In contrast, the enzyme-assisted extraction method utilized the enzyme alcalase to facilitate the extraction. The sesame bran and water mixture were treated with the enzyme, and the pH was adjusted to enhance enzyme activity. Similar to ultrasonic extraction, this method was also conducted under vacuum conditions to optimize extraction efficiency.

Both techniques, ultrasonic extraction and enzyme-assisted extraction, were employed to extract plant protein and bioactive components from sesame bran. The ultrasonic extraction process disrupted the cellular structure of sesame bran under vacuum using ultrasound waves. This disruption facilitated the release of intracellular compounds, enhancing extraction efficiency. The ultrasonic waves softened plant tissues, making it easier to extract proteins and other beneficial substances. On the other hand, the enzyme-assisted extraction method used the enzyme alcalase to aid in the extraction process. Enzymes are known to break down complex molecules into simpler forms, making proteins and other bioactive components more accessible.

The enzyme concentration, temperature, pH level, extraction time and enzyme to substrate ratio were optimized to maximize extraction efficiency, targeting specific compounds in sesame bran. While the ultrasonic extraction method focused on physically breaking down the cellular structure, the enzyme-assisted extraction method relied on enzymatic reactions to enhance extraction. The ultrasonic method used mechanical means to disrupt plant tissues, whereas the enzyme-assisted method used enzymatic breakdown to facilitate extraction. Both methods were conducted under vacuum to improve solvent penetration into the cellular components and increase extraction efficiency. Overall, the ultrasonic extraction method and the enzyme-assisted extraction method complemented each other in the study, employing different mechanisms to enhance the recovery of plant protein and bioactive compounds from sesame bran. The goal of combining these techniques under vacuum was to maximize the yields of valuable components from agricultural byproducts such as sesame bran by optimizing the extraction process [20].

ACKNOWLEDGMENT

I would like to express my sincere gratitude to everyone who contributed to the successful completion of this literature review. First and foremost, I extend my heartfelt thanks to my supervisor, Dr. Udeshika Yapa Bandara, for her invaluable guidance, continuous support, and insightful feedback throughout this research process. Their expertise and encouragement were instrumental in shaping this review. I am also grateful to the staff of faculty of science, NSBM Green University, Pitipana, Sri Lanka for providing the necessary resources and a conducive environment for my research. Special thanks to the library staff for their assistance in accessing relevant academic journals and articles.

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