Moringa oleifera Extraction, Isolation, Quantification and Its Application in Functional Foods: A review

Rolla Al-Shalabi[1], Nozlena Abdul Samad[1*]

Abstract

Background: M. oleifera, a rapidly growing and drought-resistant tree belonging to the Moringaceae family, originates from the Indian subcontinent. Commonly referred to as Moringa, drumstick tree, horseradish tree, or ben oil tree, it is cultivated worldwide for its nutrient-dense seed pods and leaves, which serve as vegetables and are utilized in traditional medicinal practices. Furthermore, Moringa is admitted for its capacity to purify water. The rising interest in natural products has heightened the significance of Moringa, owing to its nutritional and therapeutic properties, thereby establishing it as an essential element in health promotion and disease prevention. Objective: This article intends to explore the diverse extraction techniques employed for the isolation of bioactive compounds from M. oleifera, with a focus on the effectiveness and specificity of various methodologies for understanding Moringa's role in nutritional health and disease prevention. Methodology: This study examines various extraction techniques for M. oleifera, such as ultrasound-assisted extraction, microwave-assisted extraction, aqueous two-phase system, pressurized hot water extraction. Results: The findings indicate that each extraction technique possesses distinct benefits, with high-performance liquid chromatography playing a vital role in the precise identification and quantification of various compounds. The combination of advanced extraction techniques and high-performance liquid chromatography significantly improves the effectiveness of isolating the essential nutrients and bioactive compounds found in Moringa components, such as carbohydrates, proteins, vitamins, and essential minerals. Conclusion: M. oleifera is recognized as a significant source of both nutritional and therapeutic compounds. The application of current extraction techniques, along with high-performance liquid chromatography, enables the efficient extraction of its health-enhancing constituents, highlighting its potential role in fostering general health and aiding disease prevention. As the demand for natural products increases, Moringa remains an essential focus in the fields of nutritional and medicinal research.

Keywords: Extraction methods, Functional foods, Isolation techniques, M. oleifera, Quantification.

Significance | Moringa oleifera is significant for its adaptability, nutritional value, and bioactive compounds, offering immense potential in sustainable extraction methods.

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Editor Muhammad Asif, Ph.D., And accepted by the Editorial Board May 15, 2024 (received for review Feb 28, 2024)

Introduction

M. oleifera, belonging to the *Moringaceae* family, is a fast-growing, softwood tree native to the sub-Himalayan regions of northern India. It is one of thirteen species in its genus and has spread widely to tropical and subtropical regions, due to its remarkable adaptability, *M. oleifera* has found a home in drought-prone areas, making it valuable in regions facing climate challenges

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Please Cite This:
Rolla Al-Shalabi, Nozlena Abdul Samad (2024). "Moringa oleifera Extraction, Isolation, Quantification and Its Application in Functional Foods: A review", Australian Herbal Insight, 7(1),1-10, 9958

(Abdull Razis et al., 2014).

The extraction of bioactive compounds from *M. oleifera* has gained attention due to the plant's extensive nutritional and medicinal properties. Various extraction methods have been explored to maximize the yield and quality of these compounds. Among them, ultrasonic-assisted extraction (UAE) has emerged as a quick, efficient, and environmentally friendly technique. By utilizing ultrasonic waves, UAE enhances solvent movement, accelerates mass transfer, and significantly reduces extraction time and cost. This method is practical for large-scale applications and offers high-quality extraction, particularly in the case of *M. oleifera* oils (Dhanani et al., 2017).

Another extensively used approach is microwave-assisted extraction (MAE), which uses microwave radiation to raise the temperature of both the solvent and the plant material, improving bioactive component extraction. MAE's quick heating capacity for the solvent and sample matrix makes it ideal for extracting a wide range of analytes, including those that are susceptible to thermal degradation. The success of MAE depends on optimizing several factors such as solvent polarity, sample size, extraction temperature, and microwave power, all of which influence the efficiency of the process (Albarri et al., 2021).

The Aqueous Two-Phase System (ATPS), a liquid-liquid fractionation method, is gaining recognition as a green extraction technique for *M. oleifera*. It has shown immense potential in the extraction and purification of biomolecules, with applications extending to industrial and environmental sectors. ATPS offers high recovery rates, scalability, and cost-effectiveness, making it an attractive option for sustainable extraction processes (Gu et al., 2012).

Pressurized Hot Water Extraction (PHWE), also known as subcritical or superheated water extraction, uses water as the solvent at high temperatures but below its critical point. Originally developed for environmental analysis, PHWE has proven to be an efficient method for extracting bioactive compounds from *M. oleifera,* offering an environmentally friendly alternative to conventional extraction methods (Gbashi et al., 2017).

To analyse and separate the extracted compounds, High-Performance Liquid Chromatography (HPLC) plays a critical role. This technique uses a pressurized liquid solvent to move the *M. oleifera* sample through an adsorbent column, allowing the separation of its components based on their interactions with the adsorbent. The method's precision and reliability make it ideal for analysing *M. oleifera*'s complex chemical makeup (Chokwe et al., 2020).

As health consciousness grows, the demand for natural, plant-based products has increased, particularly those offering functional benefits beyond basic nutrition. *M. oleifera*, rich in essential macro and micronutrients, as well as bioactive compounds, holds significant promise as a functional food with potential health benefits, including disease prevention and overall wellness enhancement (Abdull Razis et al., 2014).

2. Extraction Methods for *M. Oleifera*

2.1 Ultrasonic-Assisted Extraction

This technique has gained widespread use for extracting bioactive compounds from *M. oleifera* due to its numerous advantages, including fast processing times, low energy consumption, and minimal damage to sensitive compounds (Petigny et al., 2013). UAE is particularly preferred over conventional methods like soxhlet extraction, which requires prolonged extraction times, up to 8 hours, and in which the yield is highly dependent on the condition of the thimble (Buddin et al., 2018). In contrast, UAE offers shorter extraction durations and higher yields, making it a more efficient method. Common solvents used in UAE for *M. oleifera* include absolute ethanol, 50% ethanol, and deionized water as shown in (Figure 1, Figure 2 and Figure 3) respectively. The technology behind UAE leverages ultrasonic waves, high-frequency sound waves above 20 kHz, sometimes reaching up to 100 MHz, which penetrate the solvent, initiating a series of processes that enhance extraction efficiency (Lu et al., 2012).

Several mechanisms contribute to the improved extraction of *M. oleifera* compounds, such as swelling and hydration of the plant material, disruption of the cell walls, and increased solvent penetration. The ultrasonic waves induce rapid acceleration and vibration of both solid and liquid particles, causing the solutes from *M. oleifera* to quickly migrate from the solid phase into the solvent. The intensity of the ultrasonic waves weakens the intermolecular forces within *M. oleifera*, leading to the breakdown of its molecular structure. Cavitation, the formation and collapse of bubbles generated by the ultrasound, further damages the plant's cell membranes, facilitating the release of bioactive compounds. This process enhances mass transfer and promotes deeper solvent penetration into the cellular structures of *M. oleifera*, thereby improving the overall extraction efficiency (MS et al., 2018; Panzella et al., 2020; Poobalan et al., 2018).

2.2 Microwave-Assisted Extraction

It has emerged as a highly efficient and cost-effective method for extracting bioactive compounds from *M. oleifera*, offering significant advantages over traditional extraction techniques (MS et al., 2018). MAE is recognized for its reduced extraction time and lower solvent consumption, making it an ideal approach for sustainable extraction (Taamalli et al., 2012). Operating within a frequency range of 300 MHz to 300 GHz and wavelengths between one meter and one millimetre, microwaves efficiently transfer energy to both the solvent and plant material (Rehman et al., 2020). Key parameters influencing the optimization of *M. oleifera* extraction via MAE include the choice of solvent, solid-to-solvent ratio, microwave power, extraction temperature, and duration.

Ethanol is one of the most commonly used solvents in MAE of *M. oleifera* due to its strong microwave absorption capabilities and high solubilisation efficiency for phenolic compounds. It can be used alone or in combination with water, depending on the desired extraction outcome. Ensuring the proper amount of solvent fully submerges the plant material during irradiation is crucial for maximizing extraction efficiency, while any excess solvent must be removed to fully recover the extracted compounds (Panzella et al., 2020). MAE utilizes both microwave energy and solvents to extract bioactive compounds, with the added advantage of simultaneously heating the plant material and inactivating weak hydrogen bonds within the matrix (Dhanani et al., 2017). There are two distinct modes of operation in MAE: pressurized microwave-assisted extraction (PMAE), which utilizes a closed vessel under controlled temperature and pressure, and focused microwave-assisted extraction (FMSE), which operates in an open vessel at atmospheric pressure (Albarri et al., 2021).

Ultimately, the effectiveness of MAE for *M. oleifera* is influenced by the polarity, structure, and behaviour of both the solvents and the target compounds, which must be carefully optimized to achieve maximum extraction yield and efficiency (Prasetyaningrum et al., 2021).

2.3 Aqueous Two-Phase System

Using this extraction method for *M. oleifera* is highly preferred due to its rapid processing time, low energy requirements, ease of alcohol recovery through evaporation, cost-effectiveness, and scalability. The method is known for its low interfacial tension, high resolution, yield, and capacity, making it a practical choice for large-scale extractions (Djande et al., 2018; X. Liu et al., 2013). ATPS extraction of *M. oleifera* can be achieved using short-chain alcohols or hydrophilic solvents combined with inorganic salts, creating an efficient and adaptable system (Djande et al., 2018). These short-chain alcohols, such as ethanol, methanol, and 2-propanol, when paired with inorganic salts like phosphate or sulphate, form a stable ATPS due to the salting-out effect and the limited solubility of the salt in alcohol. This results in two distinct phases: a top alcohol-rich phase and a bottom salt-rich phase, both containing at least 80% water and exhibiting low surface tension (F. Liu et al., 2013).

The ATPS of *M. oleifera* is driven by hydrophobic interactions, hydrogen bonding, and ionic forces, enhancing the extraction process (Gu et al., 2012). The incorporation of affinity ligands into the ATPS can significantly improve recovery yields and purification efficiency. The ATPS system is typically composed of two immiscible phases, which may be formed from two polymers, a polymer and a salt, an ionic liquid and a salt, or a low molecular weight alcohol and a salt (Ruiz-Ruiz et al., 2012). The type of inorganic salt used is critical in determining the stability and partitioning efficiency of the system for *M. oleifera* extraction. However, care must be taken when using inorganic salts like ammonium sulphate, as they may pose potential health risks (Zhang et al., 2013).

2.4 Pressurized Hot Water Extraction

The ATPS has emerged as a highly preferred method for extracting bioactive compounds from *M. oleifera* due to its fast processing time, low energy consumption, efficient alcohol recovery via evaporation, cost-effectiveness, and scalability. This method offers several advantages, including low interfacial tension, high resolution, increased yield, and enhanced capacity, making it a highly practical choice for large-scale extractions (Djande et al., 2018; F. Liu et al., 2013; Plaza et al., 2013). ATPS extraction of *M. oleifera* is typically performed using short-chain alcohols or hydrophilic solvents combined with inorganic salts, resulting in an efficient and flexible extraction system (Djande et al., 2018). Short-chain alcohols such as ethanol, methanol, and 2-propanol, when paired with inorganic salts like phosphate or sulphate, create a stable ATPS through the salting-out effect and the limited solubility of the salt in alcohol. This process generates two immiscible phases: an alcohol-rich top phase and a salt-rich bottom phase, both containing at least 80% water and exhibiting low surface tension (X. Liu et al., 2013).

The extraction efficiency of ATPS for *M. oleifera* is enhanced by hydrophobic interactions, hydrogen bonding, and ionic forces, which drive the partitioning of bioactive compounds (Gu et al., 2012). Incorporating affinity ligands into the ATPS can further enhance recovery yields and improve purification efficiency. The system can be composed of various combinations, including two polymers, a polymer and a salt, an ionic liquid and a salt, or a low molecular weight alcohol and a salt, creating two immiscible phases (Ruiz-Ruiz et al., 2012). The choice of inorganic salt plays a crucial role in determining the stability and partitioning efficiency of the ATPS system during *M. oleifera* extraction. However, caution is necessary when using salts like ammonium sulphate, as they can pose potential health risks (Zhang et al., 2013).

3.Quantification and Characterization of Bioactive Compounds

3.1 HPLC Separation and Quantification Methods

This method utilize HPLC finely packed stationary phase particles (3 µm, 5 µm, and 10 µm) to achieve efficient separation. The stationary phase can operate based on various principles such as adsorption, partitioning, ion exchange, size exclusion, or gel permeation. HPLC is highly regarded for its speed, efficiency, automation, precision, and reproducibility. Several types of detectors can be employed with HPLC, including diode array, fixed or variable ultraviolet/visible (UV-VIS), fluorescence, mass spectrometry (MS), refractive index, and evaporative light scattering detectors (Chokwe et al., 2020). UV detectors are among the most commonly used due to their ability to monitor multiple

wavelengths simultaneously, enabled by programmable multiple wavelength scanning (Siddiqui et al., 2017).

In one of the studies reported by Rodríguez-Pérez et al., (2015), HPLC was performed using an Agilent 1200 Series Rapid Resolution LC system, equipped with a vacuum degasser, binary pump, auto sampler, and diode array detector. Chromatographic separation was carried out on a Zorbax Eclipse Plus C18 column (1.8 µm, 150 mm x 4.6 mm). The mobile phases consisted of acetonitrile (A) and acidified water (0.5% formic acid, v/v) (B). The gradient elution was as follows: 0 min, 5% B; 10 min, 35% B; 65 min, 95% B; 67 min, 5% B, with a final 3-minute conditioning cycle under initial conditions. The flow rate was maintained at 0.50 mL/min throughout the gradient, and the effluent from the column was split using a T-type splitter (1:2 split ratio) before being introduced into the mass spectrometer. The injection volume was 10 µL.

3.2 Ultraviolet/Visible Spectroscopy

Ultraviolet/Visible Spectroscopy is a useful tool for both qualitative and quantitative analysis, enabling the identification of various compounds in pure and biological mixtures. It is particularly effective in assessing compounds with strong UV chromophores, such as phenolic compounds (e.g., anthocyanin, tannins, and phenols), which form complexes with metals like iron. UV-Vis spectroscopy has shown that phenolic extracts absorb light at specific wavelengths: flavones at 280 nm, phenolic acids at 320 nm, anthocyanin at 360 nm, and other compounds at 520 nm. This method is preferred for its rapidity and cost-effectiveness compared to other analytical techniques, although it is less selective and primarily provides information on the general polyphenol content (Rehman et al., 2020; Taamalli et al., 2012).

3.3 Infrared Spectroscopy

This method relies on the vibrational changes that occur within molecules when they are exposed to infrared light. As infrared light passes through a sample, certain frequencies are absorbed depending on the types of bonds present in the molecule. This absorption leads to vibrational transitions, making IR spectroscopy particularly useful for detecting specific bond types in organic molecules. Fourier Transform Infrared Spectroscopy (FTIR) is a high-resolution technique that allows for the rapid and non-destructive analysis of substances, such as *M. oleifera* extracts and other natural products, including honey (Cozzolino, 2015; Rehman et al., 2020).

3.4 Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance Spectroscopy based on the magnetic properties of atomic nuclei, particularly those of hydrogen, carbon, and carbon isotopes. Nuclear Magnetic Resonance (NMR) spectroscopy provides detailed information about the molecular structure by measuring the interactions of magnetic nuclei, offering insights into the types and number of atoms in different molecular environments. LC-NMR is particularly useful for identifying and characterizing a wide range of compounds such as acids, alkaloids, flavonoids, xanthones, saponins, and quinones. Previous studies have successfully employed techniques such as preparative or semi-preparative thin-layer chromatography, liquid chromatography, and column chromatography to isolate individual phenolic compounds, which were then structurally identified using offline NMR (Altemimi et al., 2017).

4 Applications of *M. oleifera* in Functional Foods

4.1 Nutritional Properties of M. oleifera

M. oleifera stands out not only for its rich nutritional composition and medicinal benefits but also for its versatility in extraction, isolation, and quantification of bioactive compounds, which have made it a valuable ingredient in functional foods. The plant's nutrient-dense profile, combined with advancements in extraction techniques, has facilitated the efficient isolation of these beneficial compounds, making *M. oleifera* a prime candidate for inclusion in various health-promoting food products (Prasetyaningrum et al., 2021).

Extraction methods such as UAE, MAE, ATPS, and PHWE allow for the effective recovery of *M. oleifera*'s bioactive components, including vitamins, minerals, amino acids, antioxidants, and anti-inflammatory agents (Buddin et al., 2018; MS et al., 2018; Panzella et al., 2020; Petigny et al., 2013). These compounds are essential for various therapeutic applications and functional foods due to their roles in promoting health and preventing diseases. Advanced techniques like HPLC, UV-VIS spectroscopy, and NMR spectroscopy further enable the precise quantification and characterization of these compounds, ensuring the purity and consistency of *M. oleifera* extracts used in functional food formulations (Altemimi et al., 2017; Chokwe et al., 2020; Cozzolino, 2015; Rehman et al., 2020; Siddiqui et al., 2017).

The application of *M. oleifera* in functional foods, such as *Moringa*-fortified bread, snacks, and beverages, leverages the plant's exceptional nutrient content, including its high levels of vitamins, minerals, and essential fatty acids (Abdull Razis et al., 2014; Joshi & Varma, 2017; Sengev et al., 2013). For instance, *Moringa* seed and leaf powders have been incorporated into various products to enhance their nutritional value. *M. oleifera* leaves, rich in protein and essential amino acids, are particularly beneficial for addressing malnutrition and promoting overall health (Thapa et al., 2019). The calcium content in *M. oleifera* is crucial for bone and teeth health, and its protein-rich leaves can help combat protein-energy malnutrition, especially in undernourished populations (Gopalakrishnan et al., 2016; Singh & Prasad, 2013).

Additionally, the bioactive compounds isolated from *M. oleifera*, such as glucosinolates, isothiocyanates, and other anti-cancerous agents, contribute to the plant's medicinal value in treating

conditions like diabetes, anemia, and inflammation (Sahay et al., 2017; Thapa et al., 2019). These therapeutic benefits further enhance *M. oleifera*'s application in functional foods aimed at not only providing basic nutrition but also preventing and managing chronic diseases.

Moreover, the low-calorie content of *M. oleifera* leaves makes them ideal for functional foods targeting weight management (Gopalakrishnan et al., 2016). The plant's application extends to animal nutrition as well, where its bioactive compounds, such as essential oils, saponins, and tannins, improve feed utilization and animal performance, enhancing the nutritional quality of dairy and meat products in the agricultural sector (Kholif et al., 2016, 2019).

The efficient extraction, isolation, and quantification of *M. oleifera*'s bioactive compounds play a critical role in its application in functional foods. With its vast array of essential nutrients and medicinal properties, *M. oleifera* offers immense potential in promoting health and wellness, both in human food systems and in livestock feed, making it a vital resource for the food and agricultural industries (Sengev et al., 2013).

The therapeutic, nutritive, and medicinal benefits of a plant are greatly influenced by its nutritional makeup. Research has shown that the *M. oleifera* has a wide range of essential elements, including vitamins, minerals, amino acids, beta-carotene, antioxidants, anti-inflammatory chemicals, and omega 3 and 6 fatty acids (Abdull Razis et al., 2014). The *Moringa* plant may be used as a functional food product in the food production industry due to its greater nutritional content. For instance, *Moringa* seed and leaf powders are dried and used to make a range of functional meals, including cookies, wheat bread, and snack foods (Joshi & Varma, 2017; Sengev et al., 2013). *M. oleifera'*s leaves are rich in protein, minerals, and all the essential amino acids (Thapa et al., 2019). Calcium is a mineral that helps develop bones and teeth, and a lack of it can result in rickets, bone pain, osteoporosis, etc. (Gopalakrishnan et al., 2016). Additionally, *M. oleifera*'s protein-rich leaves may aid in the fight against protein and energy deficiency among the world's undernourished (Singh & Prasad, 2013). Along with tannin, sterols, terpenoids, flavonoids, saponins, anthraquinones, and alkaloids, *M. oleifera* also contains considerable levels of anti-cancerous chemicals such glucosinolates, isothiocyanates, glycoside compounds, and glycerol-1-9-octadecanoate (Thapa et al., 2019). These chemicals have a variety of important medicinal uses, such as treating diabetes, anaemia, skin conditions, blood pressure, and inflammation (Sahay et al., 2017). *M. oleifera* also contains vitamins C, D, and E as well as the beta-carotene found in vitamin A, pyridoxine, nicotinic acid, and folic acid in vitamin B, as well as other vitamins. Additionally, *Moringa* leaves have a low calorie count, making them suitable for an obese person's diet (Gopalakrishnan et al., 2016). Additionally, the leaves of *M. oleifera* trees may contain nutrients that can be used as innovative feed additives to enhance animal performance in Egypt and other developing countries (Kholif et al., 2016). Ruminants can employ the bioactive compounds contained in *M. oleifera* plant seeds and leaves, including essential oils, saponins, and tannins, some of which have antibacterial to increase feed utilisation, animal performance, and the nutritious content of their milk (Kholif et al., 2019).

4.2 Advantages and Disadvantages of UAE, MAE, ATPS and PHWE of M. oleifera

The process of extracting bioactive compounds from *M. oleifera* is essential for its utilization in functional foods and nutraceuticals. A range of advanced methodologies is employed to enhance extraction efficiency, selectivity, and yield. Notably, techniques such as UAE, MAE, ATPS, and PHWE present distinct benefits, but they also have certain restrictions as shown in Table 1.

5. Discussion

This review highlights four key modern extraction techniques commonly applied to *M. oleifera* and their effectiveness in preserving the plant's bioactive properties. The choice of extraction technique is crucial for maximizing yield while minimizing alterations to the functional properties of the extracted compounds (Dhanani et al., 2017). When selecting an extraction process and solvent, factors such as the sample matrix, chemical properties of the analytes, matrix-analyte interactions, and the desired efficacy and quality of the extract must be carefully considered. UAE is widely recognized for its efficiency in reducing both extraction time and solvent consumption. By employing high-frequency sound waves, UAE facilitates the breakdown of *M. oleifera* cell walls, enhancing the mass transfer of bioactive compounds into the solvent. However, the potential for free radical generation at frequencies above 20 kHz poses a risk to the stability of some phytochemicals. Despite this, UAE's time-saving and solvent-reducing advantages make it a leading technique for *M. oleifera* extraction (Panzella et al., 2020; Petigny et al., 2013).

The MAE similarly offers significant advantages over traditional methods such as maceration and soxhlet extraction. The use of microwave radiation accelerates the extraction process and reduces solvent consumption, making MAE an efficient alternative for *M. oleifera* extraction. However, careful optimization of microwave power, extraction time, and temperature is essential to avoid thermal degradation of sensitive compounds. Despite these challenges, MAE has been shown to improve both the recovery and reproducibility of *M. oleifera* extracts, making it a valuable tool for large-scale extraction efforts (Azwanida, 2015; Dhanani et al., 2017).

The ATPS extraction is a versatile liquid-liquid fractionation method that allows for the recovery and purification of *M. oleifera* bioactive compounds. ATPS systems are created using

Figure 1. *M. oleifera* leaf extract of ethanol using UAE

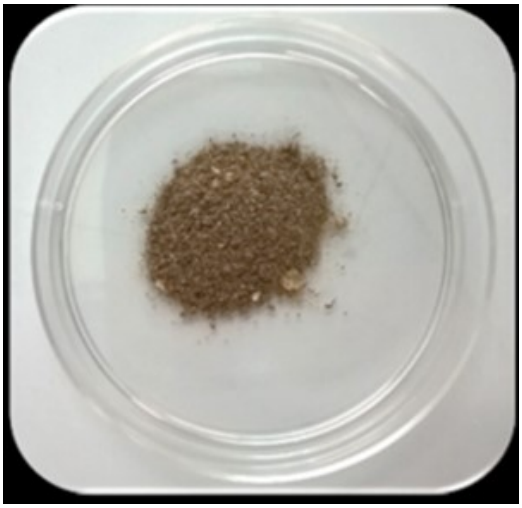

Figure 2. *M. oleifera* leaf extract of 50% ethanol using UAE

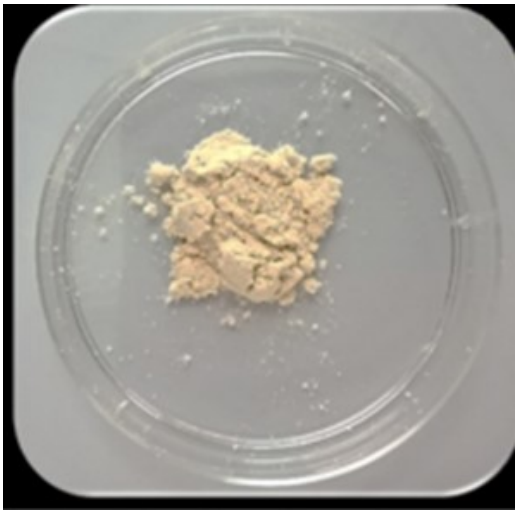

Figure 3. *M. oleifera* leaf extract of deionised water using UAE

Table 1. Advantages and disadvantages of the UAE, MAE, ATPS and PHWE extraction techniques of *M. oleifera*

Extraction Method	Advantages	Disadvantages	Citation
UAE	- Fast extraction of M. oleifera - Low energy consumption during extraction of *M. oleifera* - Less time required for extracting *M. oleifera* - Less active *M. oleifera* compound damage - High extraction yields of *M. oleifera*	-Use of a large amount of organic solvents in Ultrasonic assisted extraction (UAE) of *M. oleifera* is dangerous for human health and environment	(Kazemi et al., 2016; Lu et al., 2012; Petigny et al., 2013)(1, 38, 39)
MAE	- Faster heating to extract bioactive materials of *M. oleifera* - Reduced equipment size - Reduced thermal gradients - Increase in extract yield of *M. oleifera* - Shorter time for extracting *M. oleifera*	- High equipment cost - Filtration required - Very poor efficiency for volatile compounds	(Panzella et al., 2020; Rehman et al., 2020)
ATPS	- Versatility - High efficiency - High yield - Improved purification factor - Selectivity - Low cost - Fast mass transfer rate - Environment-friendly - Capable of continuous operation - Ease of scaling-up	- Difficult to predict the partitioning of biomolecules in ATPS in *M. oleifera* - Lack of understanding of the systems or engineering involved - Struggled with large scale applications due to other issues, such as achieving the necessary selectivity during protein extraction, the price of the components forming the phase, and the required wastewater treatment associated with ATPS of *M. oleifera*	(de Brito Cardoso et al., 2013; Iqbal et al., 2016)
PHWE	- Shorter times required for extracting *M. oleifera* - More efficient - Environment friendly - Less expensive - Eco-friendly and inexpensive solvent used (H_2O) to extract *M. oleifera* - Limits the use of organic solvents	- Not suitable for heat sensitive compounds - No commercial or high-throughput system for PHWE of *M. oleifera*	(Gbashi et al., 2017; Gołębiewska et al., 2022; Heng et al., 2013; Liang & Fan, 2013; Plaza & Turner, 2015)

combinations of polymers, salts, or alcohol-salt mixtures to form two immiscible phases. While ATPS is scalable and cost-effective, its lack of selectivity remains a key limitation. Adjustments to parameters such as pH, temperature, and component concentration can enhance selectivity, but complete separation of target molecules from contaminants is often challenging. Nevertheless, ATPS remains a highly effective method for bulk extraction due to its environmental benefits and ease of use (Ruiz-Ruiz et al., 2012). PHWE offers an environmentally friendly alternative by using water at elevated temperatures and pressures as a solvent. This technique enhances the solubility of medium-polar compounds and eliminates the need for harmful organic solvents, making it particularly attractive for sustainable extraction of *M. oleifera*. PHWE's ability to extract bioactive compounds efficiently while maintaining a low environmental impact underscores its potential for wide adoption in industries focused on sustainability (Gołębiewska et al., 2022; Khoza et al., 2014).

In addition to these extraction techniques, HPLC is a critical tool for the precise analysis and quantification of *M. oleifera* bioactive compounds. HPLC's high specificity and accuracy make it indispensable for confirming the purity of extracts, though the cost and complexity of ensuring system compatibility must be considered (Siddiqui et al., 2017). Moreover, spectroscopy methods such as UV-VIS and NMR provide detailed insights into the chemical structure of *M. oleifera* compounds, offering additional validation of the extracts' functional properties (Altemimi et al., 2017; Rehman et al., 2020).

Finally, the exceptional medicinal and nutritional profile of *M. oleifera* has made it the subject of increasing scientific interest. Its leaves, seeds, and other parts are packed with antioxidants, vitamins, minerals, and bioactive compounds like quercetin and kaempferol, which are linked to anti-inflammatory, anti-cancer, and anti-diabetic effects (Abdull Razis et al., 2014; Thapa et al., 2019). The nutrient density of *M. oleifera*, with vitamin C, vitamin A, iron, and protein levels far surpassing common foods like oranges and spinach, further highlights its potential as a functional ingredient in both food and pharmaceutical applications (Abdull Razis et al., 2014; Gopalakrishnan et al., 2016). Its therapeutic use in treating a variety of conditions, from diabetes to anemia and skin disorders, reinforces its value as a medicinal plant (Sahay et al., 2017; Thapa et al., 2019).

Overall, the modern extraction techniques discussed in this review provide critical tools for maximizing the extraction and application of *M. oleifera*'s bioactive compounds. These advanced methods allow for greater scalability, efficiency, and environmental sustainability, making them well-suited for the growing interest in *M. oleifera* as both a functional food ingredient and a source of medicinal compounds.

6. Conclusion

The extraction of *M. oleifera* plays a vital role in isolating its various chemical constituents. Contemporary methods such as UAE, MAE, ATPS, and PHWE have proven effective, with the choice of extraction solvent being a key factor influencing outcomes. UAE stands out for its simplicity and cost-effectiveness, while MAE offers repeatability despite challenges with volatile compounds. ATPS and PHWE also provide high-quality yields. Additionally, techniques like HPLC and spectroscopic methods offer efficient ways to analyse *M. oleifera.* Utilizing the nutrients from *M. oleifera* can address nutritional and health-related issues, with further research offering potential for pharmaceutical development

Author contributions

R.A.S and N. A.S wrote the manuscript, supervised, edited the manuscript, and reviewed the study.

Acknowledgment

The authors were grateful to their department.

Competing financial interests

The authors have no conflict of interest.

References

Abdull Razis, A. F., Ibrahim, M. D., & Kntayya, S. B. (2014). Health benefits of Moringa oleifera. Asian Pacific Journal of Cancer Prevention, 15(20), 8571–8576.

Albarri, R., Toprakçı, İ., Kurtulbaş, E., & Şahin, S. (2021). Estimation of diffusion and mass transfer coefficients for the microwave-assisted extraction of bioactive substances from Moringa oleifera leaves. Biomass Conversion and Biorefinery, 1–8.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants, 6(4), 42.

Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants, 4(196), 412–2167.

Buddin, M., Rithuan, M. Z. A., Surni, M. S. A., Jamal, N. H. M., & Faiznur, M. F. (2018). Ultrasonic assisted extraction (UAE) of Moringa oleifera Seed Oil: Kinetic study. ASM Sci. J, 11(3), 158–166.

Chokwe, R. C., Dube, S., & Nindi, M. M. (2020). Development of an HPLC-DAD method for the quantification of ten compounds from Moringa oleifera Lam. and its application in quality control of commercial products. Molecules, 25(19), 4451.

Cozzolino, D. (2015). Infrared spectroscopy as a versatile analytical tool for the quantitative determination of antioxidants in agricultural products, foods and plants. Antioxidants, 4(3), 482–497.

de Brito Cardoso, G., Mourão, T., Pereira, F. M., Freire, M. G., Fricks, A. T., Soares, C. M. F., & Lima, Á. S. (2013). Aqueous two-phase systems based on acetonitrile and carbohydrates and their application to the extraction of vanillin. Separation and Purification Technology, 104, 106–113.

Dhanani, T., Shah, S., Gajbhiye, N. A., & Kumar, S. (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. Arabian Journal of Chemistry, 10, S1193–S1199.

Djande, C. Y. H., Piater, L. A., Steenkamp, P. A., Madala, N. E., & Dubery, I. A. (2018). Differential extraction of phytochemicals from the multipurpose tree, Moringa oleifera, using green extraction solvents. South African Journal of Botany, 115, 81–89.

Gbashi, S., Njobeh, P., Steenkamp, P., & Madala, N. (2017). Pressurized hot water extraction and chemometric fingerprinting of flavonoids from Bidens pilosa by UPLC-tandem mass spectrometry. CyTA-Journal of Food, 15(2), 171–180.

Gołębiewska, E., Kalinowska, M., & Yildiz, G. (2022). Sustainable use of apple pomace (AP) in different industrial sectors. Materials, 15(5), 1788.

Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). Moringa oleifera: A review on nutritive importance and its medicinal application. Food Science and Human Wellness, 5(2), 49–56.

Gu, F., Gao, J., Xiao, J., Chen, Q., Ruan, H., & He, G. (2012). Efficient methods of purification of α-galactosidase from Aspergillus niger: aqueous two-phase system versus three-phase partitioning. Rom Biotechnol Lett, 17, 7853–7862.

Heng, M. Y., Tan, S. N., Yong, J. W. H., & Ong, E. S. (2013). Emerging green technologies for the chemical standardization of botanicals and herbal preparations. TrAC Trends in Analytical Chemistry, 50, 1–10.

Iqbal, M., Tao, Y., Xie, S., Zhu, Y., Chen, D., Wang, X., Huang, L., Peng, D., Sattar, A., & Shabbir, M. A. B. (2016). Aqueous two-phase system (ATPS): an overview and advances in its applications. Biological Procedures Online, 18, 1–18.

Joshi, P., & Varma, K. (2017). Preparation of Value Added Products from Dehydrated Drumstick Leaves. Online Int. Interdiscip. J, 7, 41–48.

Kazemi, M., Karim, R., Mirhosseini, H., & Hamid, A. A. (2016). Optimization of pulsed ultrasound-assisted technique for extraction of phenolics from pomegranate peel of Malas variety: Punicalagin and hydroxybenzoic acids. Food Chemistry, 206, 156–166.

Kholif, A. E., Gouda, G. A., Galyean, M. L., Anele, U. Y., & Morsy, T. A. (2019). Extract of Moringa oleifera leaves increases milk production and enhances milk fatty acid profile of Nubian goats. Agroforestry Systems, 93, 1877–1886.

Kholif, A. E., Morsy, T. A., Gouda, G. A., Anele, U. Y., & Galyean, M. L. (2016). Effect of feeding diets with processed Moringa oleifera meal as protein source in lactating Anglo-Nubian goats. Animal Feed Science and Technology, 217, 45–55.

Khoza, B. S., Chimuka, L., Mukwevho, E., Steenkamp, P. A., & Madala, N. E. (2014). The Effect of Temperature on Pressurised Hot Water Extraction of Pharmacologically Important Metabolites as Analysed by UPLC-qTOF-MS and PCA. Evidence-Based Complementary and Alternative Medicine, 2014(1), 914759.

Liang, X., & Fan, Q. (2013). Application of sub-critical water extraction in pharmaceutical industry. Journal of Materials Science and Chemical Engineering, 1(05), 1.

Liu, F., Ong, E. S., & Li, S. F. Y. (2013). A green and effective approach for characterisation and quality control of Chrysanthemum by pressurized hot water extraction in combination with HPLC with UV absorbance detection. Food Chemistry, 141(3), 1807–1813.

Liu, X., Mu, T., Sun, H., Zhang, M., & Chen, J. (2013). Optimisation of aqueous two-phase extraction of anthocyanins from purple sweet potatoes by response surface methodology. Food Chemistry, 141(3), 3034–3041.

Lu, C., Wang, H., Lv, W., Ma, C., Lou, Z., Xie, J., & Liu, B. (2012). Ionic liquid-based ultrasonic/microwave-assisted extraction combined with UPLC–MS–MS for the determination of tannins in Galla chinensis. Natural Product Research, 26(19), 1842–1847.

MS, U., Ferdosh, S., Haque Akanda, M. J., Ghafoor, K., AH, R., Ali, M. E., Kamaruzzaman, B. Y., MB, F., Shaarani, S., & Islam Sarker, M. Z. (2018). Techniques for the extraction of phytosterols and their benefits in human health: A review. Separation Science and Technology, 53(14), 2206–2223.

Panzella, L., Moccia, F., Nasti, R., Marzorati, S., Verotta, L., & Napolitano, A. (2020). Bioactive phenolic compounds from agri-food wastes: An update on green and sustainable extraction methodologies. Frontiers in Nutrition, 7, 60.

Petigny, L., Périno-Issartier, S., Wajsman, J., & Chemat, F. (2013). Batch and continuous ultrasound assisted extraction of boldo leaves (Peumus boldus Mol.). International Journal of Molecular Sciences, 14(3), 5750–5764.

Plaza, M., Abrahamsson, V., & Turner, C. (2013). Extraction and neoformation of antioxidant compounds by pressurized hot water extraction from apple byproducts. Journal of Agricultural and Food Chemistry, 61(23), 5500–5510.

Plaza, M., & Turner, C. (2015). Pressurized hot water extraction of bioactives. TrAC Trends in Analytical Chemistry, 71, 39–54.

Poobalan, K., Lim, V., Kamal, N. N. S. N. M., Yusoff, N. A., Khor, K. Z., & Samad, N. A. (2018). Effects of ultrasound assisted sequential extraction (UASE) of Moringa oleifera leaves extract on MCF 7 human breast cell line. Malaysian Journal of Medicine & Health Sciences, 14.

Prasetyaningrum, A., Rokhati, N., Dharmawan, Y., & Prinanda, G. R. (2021). Comparison study for extraction of bioactive flavonoids from moringa oleifera, apple, onion, and ascorbic acid (orange) by using microwave-assisted, ultrasound-assisted and maceration methods. IOP Conference Series: Materials Science and Engineering, 1053(1), 12123.

Rehman, M. U., Khan, F., & Niaz, K. (2020). Introduction to natural products analysis. In Recent advances in natural products analysis (pp. 3–15). Elsevier.

Rodríguez-Pérez, C., Quirantes-Piné, R., Fernández-Gutiérrez, A., & Segura-Carretero, A. (2015). Optimization of extraction method to obtain a phenolic compounds-rich extract from Moringa oleifera Lam leaves. Industrial Crops and Products, 66, 246–254.

Ruiz-Ruiz, F., Benavides, J., Aguilar, O., & Rito-Palomares, M. (2012). Aqueous two-phase affinity partitioning systems: current applications and trends. Journal of Chromatography A, 1244, 1–13.

Sahay, S., Yadav, U., & Srinivasamurthy, S. (2017). Potential of Moringa oleifera as a functional food ingredient: A review. Magnesium (g/Kg), 8(9.06), 4–90.

Sengev, A. I., Abu, J. O., & Gernah, D. I. (2013). Effect of Moringa oleifera leaf powder supplementation on some quality characteristics of wheat bread. Food and Nutrition Sciences, 4(3), 270.

Siddiqui, M. R., AlOthman, Z. A., & Rahman, N. (2017). Analytical techniques in pharmaceutical analysis: A review. Arabian Journal of Chemistry, 10, S1409–S1421.

Singh, Y., & Prasad, K. (2013). Moringa oleifera leaf as functional food powder. Charact Uses, 4, 317–324.

Taamalli, A., Arráez-Román, D., Barrajón-Catalán, E., Ruiz-Torres, V., Pérez-Sánchez, A., Herrero, M., Ibañez, E., Micol, V., Zarrouk, M., & Segura-Carretero, A. (2012).

Use of advanced techniques for the extraction of phenolic compounds from Tunisian olive leaves: Phenolic composition and cytotoxicity against human breast cancer cells. Food and Chemical Toxicology, 50(6), 1817–1825.

Thapa, K., Poudel, M., & Adhikari, P. (2019). Moringa oleifera: A review article on nutritional properties and its prospect in the context of Nepal. Acta Sci. Agric, 3(11), 47–54.

Zhang, D.-Y., Zu, Y.-G., Fu, Y.-J., Wang, W., Zhang, L., Luo, M., Mu, F.-S., Yao, X.-H., & Duan, M.-H. (2013). Aqueous two-phase extraction and enrichment of two main flavonoids from pigeon pea roots and the antioxidant activity. Separation and Purification Technology, 102, 26–33.