OPEN ACCESS
Bioactivities Vol. 3 No. 2 (2025)
https://doi.org/10.47352/bioactivities.2963-654X.457
Check for updates
Research Article

A Comparative Study on Bioactivities of Aqueous Extracts from Various Parts of *Moringa Oleifera*

Annissa Annissa*, Hanif Amrulloh, and Hamed Kioumarsi

Received : August 6, 2025	Revised : September 23, 2025	Accepted : October 29, 2025	Online : December 30, 2025

Abstract
Moringa oleifera (*M. oleifera*) is widely recognized as a rich source of natural antioxidants; however, systematic comparisons of the phytochemical composition and biological activities of aqueous extracts obtained from different plant organs remain limited. This study aimed to comparatively evaluate the phytochemical profiles, antioxidant capacity, antibacterial activity, and antifungal activity of aqueous extracts prepared from the leaf, twig, bark, and wood of *M. oleifera*. Qualitative phytochemical screening, determination of total phenolic and total flavonoid contents, Fourier-transform infrared spectroscopy, 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay, and resazurin-based microdilution assays against representative bacterial and fungal pathogens were performed. Leaf and twig extracts exhibited the greatest phytochemical diversity, whereas the wood extract showed the simplest composition. The leaf extract contained the highest total phenolic content (683.95 ± 16.74 µg gallic acid equivalents mL^{-1}) and total flavonoid content (514.08 ± 26.12 µg catechin equivalents mL^{-1}), followed by the twig, bark, and wood extracts. Consistently, the leaf extract demonstrated the strongest antioxidant activity, with 64.60 ± 0.69% radical scavenging activity, approaching that of the reference antioxidant (71.72 ± 0.56%). Fourier-transform infrared spectroscopy confirmed the presence of hydroxyl, carbonyl, aromatic, and ether functional groups associated with phenolic and flavonoid compounds, particularly in leaf and twig extracts. Antibacterial and antifungal activities followed the same overall trend, with leaf extracts exhibiting the greatest inhibitory effects, followed by twig, bark, and wood extracts. These findings demonstrate that the distribution of bioactive phytochemicals is strongly organ-dependent and closely associated with antioxidant and antimicrobial activities. The study identifies *M. oleifera* leaf as the most promising source of water-extractable antioxidant compounds and supports their potential application in the development of sustainable functional foods, nutraceuticals, and phytopharmaceutical products.

Keywords: aqueous extract, antibacterial activity, antifungal activity, antioxidant activity, flavonoids, *Moringa oleifera*, phenolic compounds, phytochemicals

1. INTRODUCTION

Herbal medicine has long attracted global scientific interest due to its historical significance and therapeutic potential in traditional healthcare systems. Traditional medicinal practices are grounded in a holistic philosophy that emphasizes maintaining overall well-being, preventing disease, regulating immune responses, and providing individualized treatment approaches [1]-[3]. In recent decades, medicinal plants have also become an important focus of modern pharmacological research, particularly in the discovery of bioactive compounds for drug development. It has been estimated that more than 60% of currently used synthetic drugs are derived directly or indirectly from plant sources, highlighting the significant role of natural products in pharmaceutical innovation [4]. Plant-based medicines are often regarded as relatively safer alternatives because they typically exhibit lower toxicity, fewer adverse effects, and greater accessibility compared with many synthetic pharmaceuticals. Moreover, medicinal plants not only contribute to healthcare but also serve as an important source of livelihood for a substantial portion of the global population, particularly in developing countries where traditional remedies remain widely used [5]-[7].

Among the numerous medicinal plants investigated, *Moringa oleifera* Lam. is one of the most widely cultivated and studied species within the family *Moringaceae* and the genus *Moringa* [8]-[10]. The species is native to the sub-Himalayan regions of Northern India, Pakistan, Afghanistan, Bangladesh, and Nepal, but it is now extensively cultivated in tropical and subtropical regions worldwide. *M. oleifera* is a fast-growing, drought-resistant tree capable of tolerating a wide range of environmental conditions, including arid climates and moderate frost, which contributes to its adaptability and global distribution [11]. Almost

Table 1. Phytochemical analysis of *Moringa Oleifera* aqueous extract.

Chemical constituents	Testing methods	*M. olifera* aqueous extract			
		Leaf	Twig	Bark	Wood
Alkaloids	Dragendroff's test	+	+	+	-
Flavonoids	$AlCl_3$ test	+	+	+	+
Saponins	Foam test	+	+	-	-
Carbohydrates	Anthrone test	+	+	+	+
Polyphenols	Puncal-D	+	+	-	-
Proteins	Ninhydrin test	+	+	+	-
Amino Acids	Millon's test	+	+	+	-
Phenolics	Follin-Ciocalteu test	+	+	+	+
Triterpenes	Salkowski test	-	-	-	-
Anthraquinones	Borntragges test	-	-	-	-

Note: + present ; - absence

every part of the plant—including leaves, flowers, seeds, pods, and roots—has recognized commercial, nutritional, or medicinal value [12]-[16]. In particular, the leaves are considered highly nutritious due to their rich composition of macro- and micronutrients such as proteins, lipids, calcium, phosphorus, magnesium, iron, potassium, and essential vitamins including vitamin B complex, vitamin C, and vitamin E [17]. In addition to these nutrients, *M. oleifera* leaves contain a wide array of phytochemicals, including tannins, sterols, saponins, terpenoids, phenolic compounds, alkaloids, and flavonoids such as quercetin, isoquercitrin, and kaempferol derivatives, as well as bioactive compounds like isothiocyanates and glycosides [18]-[20]. The abundance of these compounds contributes to the growing scientific interest in this plant as a potential source of natural bioactive molecules.

M. oleifera has been widely used in traditional medicine systems across Asia and Africa for the treatment of various diseases and infections. Different parts of the plant have been consumed in the form of infusions, decoctions, or fresh preparations such as salads, reflecting its dual role as both a medicinal and nutritional resource [20]. Beyond traditional medicine, *M. oleifera* has also gained attention in modern industries due to its diverse applications. In the food sector, it is utilized for food preservation and as an ingredient in functional foods and nutraceutical products intended for human consumption [21]. The plant has also found applications in pharmaceutical formulations, cosmetic products, and agricultural practices due to its rich phytochemical profile and biological activities [22]. Numerous studies have reported that *M. oleifera* exhibits a wide range of pharmacological properties, including antimicrobial (antibacterial, antifungal, and antiviral), anticancer, antioxidant, anti-inflammatory, and immunomodulatory activities [23]-[25]. Additionally, the seeds of *M. oleifera* have been extensively applied in water treatment processes as a natural and environmentally friendly coagulant, demonstrating the plant's versatility beyond biomedical applications [26].

Several studies have explored the chemical composition and biological activities of *M. oleifera* extracts from different plant parts. For instance, Shih et al. investigated the phytochemical composition and antioxidant activity of *M. oleifera* cultivated in Taiwan using methanolic extracts derived from leaves, stems, and stalks [27]. Their findings demonstrated a strong radical-scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals as well as significant reducing power. Among the evaluated plant parts, the antioxidant activity followed the order leaf > stem > stalk, indicating that the leaves contain the highest concentration of antioxidant compounds. Furthermore, the extracts exhibited notable hydrogen peroxide scavenging activity and elevated

superoxide dismutase (SOD) activity, particularly in the leaf and stem fractions. Similarly, Farooq and Koul conducted a comparative study examining the antioxidant and antibacterial properties of aqueous and ethanolic extracts derived from the leaves and seeds of *M. oleifera* [18]. Their results demonstrated strong antibacterial activity against several pathogenic microorganisms, including Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, and Staphylococcus aureus, as determined by the disc diffusion method. The study also reported high total phenolic content (TPC) and total flavonoid content (TFC), which were strongly correlated with the observed antioxidant and antibacterial activities.

Despite the growing body of literature describing the pharmacological potential of *M. oleifera*, variations in phytochemical composition and bioactivity may occur depending on plant parts, geographic origin, and environmental conditions. The purpose of this work was to enrich the research on the Moringa oleifera through performing phytochemical screening and evaluate the biological activities of different parts of *M. oleifera*, including leaves, twigs, stem bark, and wood collected in Metro, Lampung, Indonesia.

2. MATERIALS AND METHODS

2.1. Materials

Moringa oleifera samples, including leaf, twig, bark, and wood, were collected from Metro, Lampung, Indonesia, between February and April 2025. The plant materials were harvested from mature trees and transported to the laboratory for further processing and analysis. All chemical reagents used in this study were of analytical grade and were employed without further purification. Dragendorff's reagent, anthrone reagent, sulfuric acid (H_2SO_4), ninhydrin reagent, Millon's reagent, ferric chloride ($FeCl_3$), perchloric acid ($HClO_4$), 3-indoleacetic acid, Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid, catechin, aluminum chloride ($AlCl_3$), sodium nitrite ($NaNO_2$), and sodium hydroxide (NaOH) were purchased from Merck Sigma-Aldrich Reagent Pte. Ltd. (Singapore). Distilled water and other laboratory consumables, including filter papers and glassware, were obtained from standard laboratory suppliers and used according to established laboratory protocols.

2.2. Methods

2.2.1. Preparation of Aqueous Extract

The extraction procedure was performed based on previously reported methods by Amrulloh et al. [9], with minor modifications. Fresh samples of *M. oleifera* (leaf, twig, bark, and wood) were thoroughly washed with running tap water to remove dust and surface contaminants. The cleaned samples were then dried under direct sunlight until a constant weight was obtained to minimize moisture content. The dried plant materials were subsequently ground into fine powder using a laboratory grinder and stored in airtight containers at room temperature until further use. For the preparation of aqueous extracts, 4 g of powdered plant material from each plant part was weighed and mixed with 100 mL of distilled water. The mixture was heated at 60 °C for 20 min with continuous stirring to facilitate the extraction of

Table 2. Total phenolic content, flavonoid content, and antioxidant activity of *M. oleifera* aqueous extract.

Sample	Total phenolic content, flavonoid content and antioxidant activity		
	Total phenolic content (µg Gallic acid · ml^{-1})	Total flavonoid content (µg Catechin · ml^{-1})	DPPH scavenging activity (%)
Ascorbic acid	-	-	71.72 ± 0.56
Wood	5.21 ± 1.20	8.92 ± 3.98	10.01 ± 1.56
Bark	112.04 ± 5.38	38.41 ± 5.43	23.76 ± 2.31
Twig	628.38 ± 26.63	78.97 ± 9.87	51.42 ± 1.24
Leaf	683.95 ± 16.74	514.08 ± 26.12	64.60 ± 0.69

water-soluble phytochemical constituents. After heating, the extract was allowed to cool to room temperature and then filtered through Whatman filter paper to remove solid residues. The resulting filtrate was collected and stored at 4 °C prior to further phytochemical and bioactivity analyses.

2.2.2. Phytochemical Analysis

The aqueous extracts of *M. oleifera* obtained from different plant parts were subjected to qualitative phytochemical screening to detect the presence of major classes of bioactive compounds. Alkaloids were identified using Dragendorff's test [10], while flavonoids were detected by the aluminum chloride ($AlCl_3$) colorimetric test [10]. Saponins were determined using the foam test [10], and carbohydrates were identified using the anthrone method [9]. Polyphenolic compounds were analyzed using the Puncal-D method [9], whereas proteins and amino acids were evaluated using the ninhydrin test and Millon's test, respectively [9] [10]. In addition, triterpenoids and anthraquinones were determined using the Salkowski test and Bornträger's test, respectively [9]. Quantitative determination of total phenolic content (TPC) was performed using the Folin–Ciocalteu method [10], with gallic acid as the calibration standard. The results were expressed as micrograms of gallic acid equivalents per milligram of extract (µg GAE mg^{-1}). Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric assay with catechin as the standard reference compound, and the results were expressed as micrograms of catechin equivalents per milligram of extract (µg CE mg^{-1}) [10].

2.2.3. FTIR Analysis

Fourier-transform infrared (FTIR) spectroscopy was employed to identify functional groups present in the aqueous extracts obtained from different parts of *M. oleifera*. The FTIR spectra were recorded using an FTIR spectrophotometer (Shimadzu IRPrestige-21, Kyoto, Japan). Prior to analysis, the extracts were prepared according to standard sample preparation procedures suitable for infrared spectroscopy. Spectral measurements were conducted within the wavenumber range of 4000–500 cm^{-1}, allowing for the identification of characteristic absorption bands corresponding to functional groups associated with phytochemical constituents such as phenolics, alcohols, amines, and carbonyl compounds. The obtained spectra were subsequently analyzed to interpret the chemical functionalities present in each extract.

2.2.4. Bioactivity Test

2.2.4.1. Antioxidant Activity

The antioxidant activity of the *M. oleifera* extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay following the method described by Amrulloh et al. [9], with ascorbic acid used as the reference antioxidant standard. A 0.1 mM DPPH solution was prepared by dissolving DPPH in ethanol. The ascorbic acid stock solution was prepared by dissolving 1 mg of ascorbic acid in 1 mL of methanol, followed by serial dilution to obtain concentrations ranging from 50 to 500 µg. For each assay tube, 200 µL of the ascorbic acid solution was mixed with 1 mL of 0.1 mM DPPH solution and 800 µL of 50 mM Tris-HCl buffer (pH 7.4). The final volume was adjusted to 4 mL with ethanol.

For the extract samples, stock solutions were prepared by dissolving 1 mg of each extract in 1 mL of methanol. Aliquots corresponding to concentrations ranging from 50 to 500 µg were transferred into separate tubes, and the final volume was adjusted to 2 mL using ethanol. Subsequently, 1 mL of 0.1 mM DPPH solution and 800 µL of 50 mM Tris-HCl buffer (pH 7.4) were added to each tube. A control solution was prepared by mixing 1 mL of 0.1 mM DPPH, 800 µL of 50 mM Tris-HCl buffer (pH 7.4), and 2 mL of ethanol without extract. All reaction mixtures were incubated at room temperature for 30 min in the dark to ensure complete reaction. The absorbance was measured at 517 nm using a UV–Vis spectrophotometer (Analytik Jena Specord 200 Plus). The percentage of DPPH radical scavenging activity was calculated using Equation (1):

$$\%\text{DPPH scavenging activity} = (Ac - As) / Ac \times 100\% \quad (1)$$

where Ac represents the absorbance of the control solution and As represents the absorbance of the sample. All measurements were performed in triplicate, and the results were expressed as mean

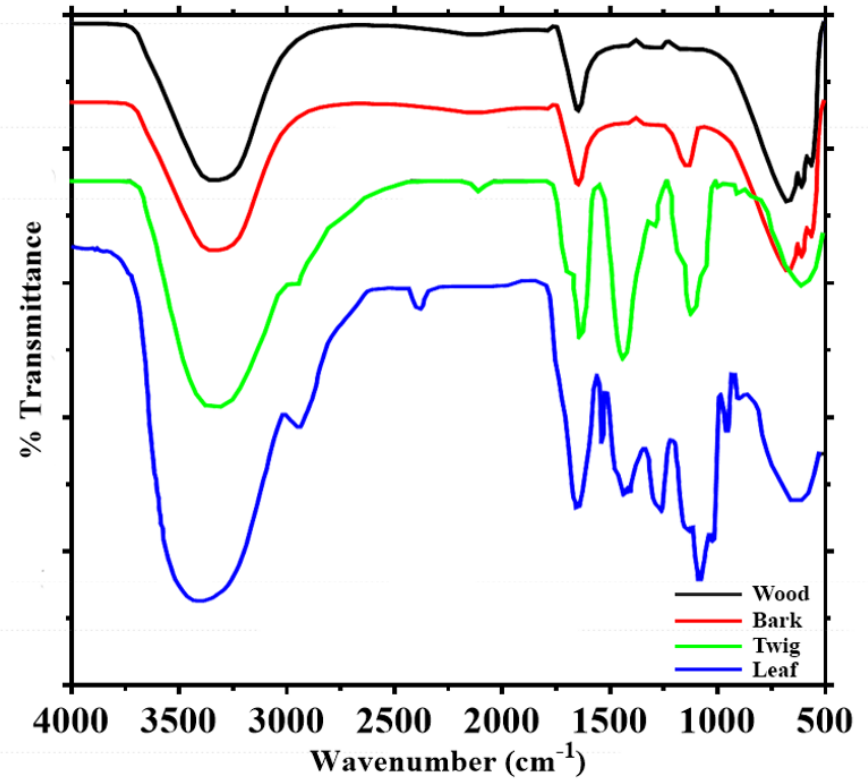

Figure 1. FTIR spectrum of *Moringa oleifera* aqueous extract.

values ± standard deviation [9].

2.2.4.2. Antibacterial and Antifungal Activity

2.2.4.2.1. Microorganism and Inoculum Preparation

The antibacterial activity of the extracts was evaluated against both Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative bacteria (*Escherichia coli* and *Shigella dysenteriae*), obtained from the Microbiology Laboratory of Airlangga University, Indonesia. For antifungal testing, cultures of *Aspergillus flavus*, *Aspergillus niger*, and *Candida albicans* were also obtained from the same laboratory. Prior to testing, bacterial and fungal strains were cultured on nutrient agar (NA) slants and incubated for 24 h. A single colony from Mueller–Hinton agar (MHA) plates was selected and transferred into Mueller–Hinton broth (MHB) using a sterile inoculating loop. The cultures were incubated overnight at 37 °C with shaking at 100 rpm to obtain actively growing microbial populations. The optical density of the bacterial or fungal suspension was then adjusted to match the 0.5 McFarland turbidity standard by adding sterile MHB. This standardization resulted in an inoculum concentration of approximately 10^6–10^7 colony-forming units per milliliter (CFU · mL^{-1}) for use in antimicrobial assays [10].

2.2.4.2.2. Minimum Inhibitory Concentration (MIC) Determination

The minimum inhibitory concentration (MIC) of the extracts was determined using the resazurin-based microtiter assay, which provides a rapid and cost-effective method for screening antimicrobial activity against multiple microbial strains. A resazurin indicator solution was prepared by dissolving a 270 mg resazurin tablet in 40 mL of sterile distilled water. The assay was conducted under aseptic conditions using sterile 96-well microtiter plates [28]. Each well initially received 100 µL of extract solution at a concentration of 600 mg · mL^{-1}, followed by serial dilution across the wells to obtain a range of extract concentrations. Subsequently, 50 µL of standardized bacterial or fungal suspension was added to each well. Afterward, 10 µL of resazurin indicator solution was added to each well to monitor microbial metabolic activity. The microtiter plates were sealed with sterile film to prevent evaporation and

incubated at 37 °C for 24 h. Microbial growth was visually assessed based on the color change of the resazurin indicator from blue to pink, which indicates metabolic activity and cell viability. The MIC value was defined as the lowest concentration of extract that prevented the color change, indicating inhibition of microbial growth. Streptomycin (10 µg · 500 μL^{-1}) and ketoconazole (10 µg · 500 μL^{-1}) were used as positive controls for antibacterial and antifungal assays, respectively, while sterile distilled water mixed with nutrient broth served as the negative control .

3. RESULTS AND DISCUSSION

3.1. Phytochemical Analysis

The extraction of natural compounds from different parts of Moringa oleifera, namely leaves, twigs, stem bark, and wood, was carried out using an aqueous extraction approach to evaluate their phytochemical composition. Since different plant tissues are known to accumulate distinct classes of secondary metabolites, qualitative phytochemical screening was performed to identify the major groups of bioactive compounds present in each extract. The presence of alkaloids was determined using Dragendorff's reagent, which consists of potassium iodide and bismuth(III) nitrate under acidic conditions. Alkaloids typically contain tertiary amine groups that can interact with tetraiodobismuthate ions to form a stable complex, resulting in the formation of an orange precipitate. The appearance of this characteristic precipitate therefore indicates the presence of alkaloid compounds in the extract, confirming the occurrence of nitrogen-containing secondary metabolites commonly associated with biological activities such as antimicrobial and antioxidant effects [29].

The presence of flavonoids was evaluated using the aluminum chloride ($AlCl_3$) colorimetric test. In this method, flavonoid compounds form stable complexes with Al^{3+} ions, producing a yellow coloration that indicates a positive reaction. This complex formation occurs due to the interaction between aluminum ions and the hydroxyl groups present in the flavonoid structure [30]. Saponins were detected using the foam test, which relies on the surfactant-like properties of saponins. When the extract solution is vigorously shaken with water, the presence of saponins is indicated by the formation of persistent foam that remains stable for several minutes. These qualitative assays provide preliminary evidence of the presence of major classes of phytochemicals that may contribute to the biological activities of the plant extracts [31].

Carbohydrates in the extracts were identified using the anthrone test, which is based on the dehydration of carbohydrates under acidic conditions to produce furfural derivatives that subsequently react with anthrone reagent to form a bluish-green complex [32]. The presence of polyphenolic compounds was evaluated using the Puncal-D test, where the formation of a blue-colored complex indicates the presence of polyphenols. Protein detection was carried out using the ninhydrin test, in which free amino groups of proteins react with ninhydrin reagent to produce a characteristic blue–purple color, commonly referred to as Ruhemann's purple [33]. Meanwhile, amino acids were detected using Millon's test. Millon's reagent, prepared by dissolving mercury in nitric acid, reacts specifically with phenolic hydroxyl groups of aromatic amino acids such as tyrosine, producing a red precipitate or coloration [34]. These qualitative assays collectively provide a preliminary assessment of the biochemical composition of the extracts.

The presence of phenolic compounds was further examined using the Folin–Ciocalteu test. The Folin –Ciocalteu reagent contains phosphomolybdate and phosphotungstate complexes that undergo reduction in the presence of phenolic compounds, leading to the formation of a blue-colored solution [9]. In addition, triterpenoids were evaluated using the Salkowski test, where the formation of a golden-yellow coloration after the addition of concentrated sulfuric acid in chloroform indicates the presence of triterpenes. Anthraquinones were analyzed using the Bornträger's test, in which a positive reaction is characterized by the appearance of a cherry-red color in the aqueous phase due to the presence of anthraquinone derivatives [35]. These tests are widely used in phytochemical studies to provide rapid screening of secondary metabolites prior to more advanced chemical characterization.

The results of the qualitative phytochemical screening of aqueous extracts from different parts

of *M. oleifera* are summarized in Table 1. The analysis revealed that flavonoids, carbohydrates, and phenolic compounds were present in all examined extracts. Alkaloids, proteins, and amino acids were detected in the leaf, twig, and bark extracts but were absent in the wood extract. Furthermore, saponins and polyphenols were detected only in the leaf and twig extracts. In contrast, triterpenes and anthraquinones were not detected in any of the analyzed extracts. These findings clearly demonstrate that the phytochemical composition varies significantly among different plant parts of *M. oleifera*, which may influence their biological activities and potential applications.

Based on the data presented in Table 1, both the leaf and twig extracts exhibited the richest phytochemical composition, containing alkaloids, flavonoids, saponins, carbohydrates, polyphenols, proteins, amino acids, and phenolic compounds. In contrast, the stem bark extract showed a comparatively simpler phytochemical profile, consisting mainly of alkaloids, flavonoids, carbohydrates, and phenolic compounds. The aqueous extract obtained from the wood portion exhibited the most limited phytochemical diversity, where only flavonoids, carbohydrates, and phenolic compounds were detected. These results suggest that metabolite accumulation is more prominent in metabolically active tissues such as leaves and young branches compared with structural tissues such as wood.

The phytochemical screening revealed that flavonoids and phenolic compounds were consistently present in all extracts. Because these compounds are widely recognized as major contributors to antioxidant activity, further quantitative analyses were performed to determine the total phenolic content (TPC) and total flavonoid content (TFC) of the extracts. The total phenolic content was determined using the Folin–Ciocalteu method, with the results expressed as micrograms of gallic acid equivalents per milliliter of extract (µg GAE mL^{-1}) [36]. Meanwhile, the total flavonoid content was determined using the aluminum chloride colorimetric method, with catechin used as the standard reference compound. The results were expressed as micrograms of catechin equivalents per milliliter of extract (µg CE mL^{-1}) [10]. These quantitative measurements allow for a more precise evaluation of the contribution of phenolic and flavonoid compounds to the biological activity of the extracts.

The results for total phenolic and flavonoid contents obtained from different parts of *M. oleifera* are summarized in Table 2. The total phenolic content of the leaf, twig, stem bark, and wood extracts was 684 ± 16.7, 628 ± 26.6, 112 ± 5.38, and 5.21 ± 1.20 µg GAE mL^{-1}, respectively. These

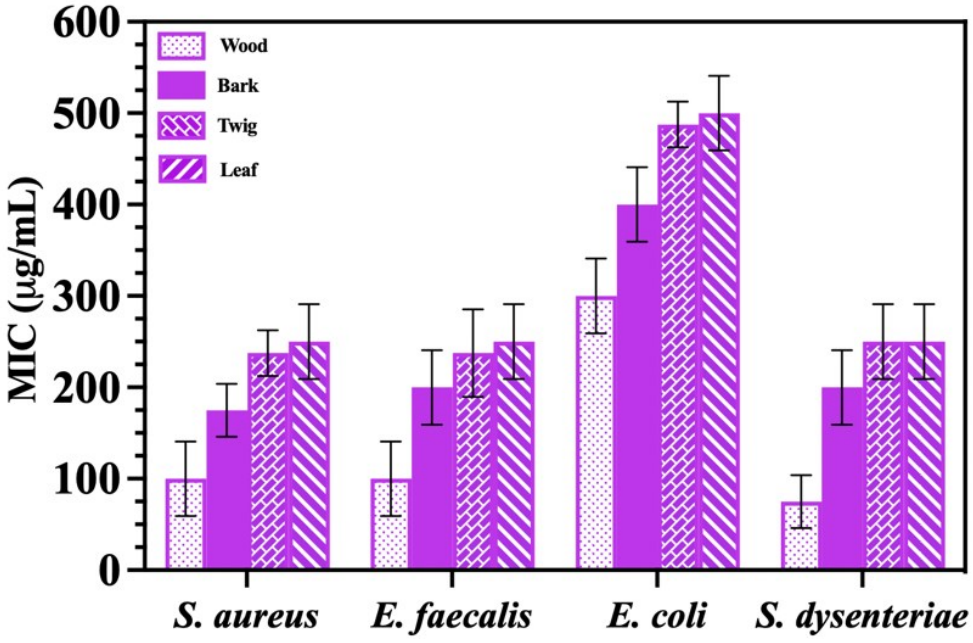

Figure 2. Antibacterial activity of *M. oleifera* aqueous extract.

results indicate that the leaf extract contains the highest phenolic concentration, followed closely by the twig extract, whereas the stem bark and wood extracts contain significantly lower phenolic levels. The remarkably low phenolic content observed in the wood extract suggests that the accumulation of phenolic metabolites is strongly associated with the metabolically active parts of the plant.

A similar trend was observed for the total flavonoid content. The flavonoid concentrations in the leaf, twig, stem bark, and wood extracts were 514 ± 26.1, 79.0 ± 9.87, 38.4 ± 5.43, and 8.92 ± 3.98 µg CE mL^{-1}, respectively. The leaf extract exhibited a substantially higher flavonoid content compared with the other plant parts, whereas the wood extract again displayed the lowest value. The high flavonoid concentration in the leaf extract may be attributed to the role of flavonoids in plant defense mechanisms and photoprotection, as these compounds are frequently synthesized in photosynthetically active tissues.

3.2. FTIR Analysis

The aqueous extracts from different parts of *M. oleifera* exhibited varying phytochemical compositions, Fourier-transform infrared (FTIR) spectroscopy was employed to further characterize the functional groups present in the extracts. FTIR analysis provides valuable information regarding the chemical functionalities of organic compounds and can help confirm the presence of functional groups associated with phytochemical constituents. The FTIR spectra obtained from leaf, twig, stem bark, and wood extracts are presented in Figure 1. As expected, the spectra displayed notable differences among the plant parts, reflecting variations in their chemical composition.

The FTIR spectra of the leaf and twig extracts displayed similar spectral features, characterized by a broad absorption band around 3400 cm^{-1} and several distinct peaks in the regions of 1650–1750, 1400–1500, approximately 1250, and around 1100 cm^{-1}. The similarity between these spectra is consistent with the phytochemical screening results, which indicated that the leaf and twig extracts contain comparable classes of bioactive compounds. In contrast, the stem bark extract exhibited a simpler spectral profile, showing a broad band near 3400 cm^{-1} and sharper peaks around 1650–1750 and 1100 cm^{-1}. The simplest spectrum was observed for the wood extract, which displayed only a broad band at approximately 3400 cm^{-1} and a peak near 1650–1750 cm^{-1}, reflecting its lower phytochemical diversity.

The broad absorption band observed near 3400 cm^{-1} in all extracts corresponds to O–H stretching vibrations, which are characteristic of hydroxyl groups present in phenolic compounds, alcohols, and carbohydrates. The presence of this band may also be influenced by the use of water as the extraction solvent. The sharp peaks in the region of 1650–1750 cm^{-1} correspond to C=O stretching vibrations, indicating the presence of carbonyl-containing compounds such as flavonoids, phenolic acids, or other oxygenated metabolites. Additionally, the peaks observed near 1100 cm^{-1} are attributed to C–O stretching vibrations commonly associated with alcohols, ethers, and glycosidic bonds found in carbohydrate and phenolic structures [37].

Further analysis of the spectra revealed peaks in the regions of 1400–1500 cm^{-1} and around 1250 cm^{-1}, which can be attributed to C–C stretching vibrations in aromatic rings and aliphatic C–H bending vibrations, respectively. These spectral features support the presence of aromatic compounds such as phenolics and flavonoids in the extracts. Overall, the FTIR results are consistent with the phytochemical screening data and confirm that leaf and twig extracts contain a broader range of functional groups compared with the stem bark and wood extracts.

The phytochemical screening indicated that the leaf and twig extracts contained diverse secondary metabolites, including alkaloids, flavonoids, saponins, carbohydrates, polyphenols, proteins, amino acids, and phenolic compounds. These compounds typically possess functional groups such as hydroxyl (O–H), carbonyl (C=O), and ether (C–O) groups, as well as aromatic and aliphatic carbon structures. Accordingly, these functional groups were clearly reflected in the FTIR spectra of the leaf and twig extracts. In contrast, the stem bark and wood extracts contained fewer classes of compounds—mainly flavonoids, carbohydrates, and phenolic compounds—which explains the relatively simpler FTIR spectral patterns observed for these samples.

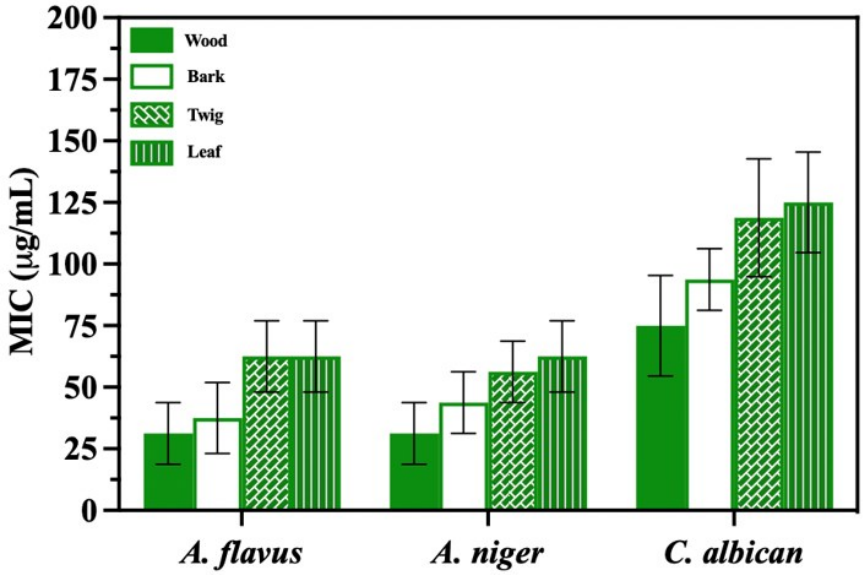

Figure 3. Antifungal activity of *M. oleifera* aqueous extract.

3.3. Bioactivity Test

3.3.1. Antioxidant Activity Test

Evaluation the potential biological application of *M. oleifera* extracts as natural antioxidant agents, the antioxidant activity of the aqueous extracts obtained from different plant parts was assessed using the in vitro DPPH radical scavenging assay. This method is widely used to evaluate the free radical scavenging ability of plant extracts due to its simplicity, reliability, and reproducibility. The antioxidant activity results are summarized in Table 2. The DPPH scavenging activity values for the leaf, twig, stem bark, and wood extracts were 64.6 ± 0.69%, 51.4 ± 1.24%, 23.8 ± 2.31%, and 10.0 ± 1.56%, respectively.

These results indicate that the antioxidant activity varies significantly among the different plant parts. The leaf extract exhibited the highest antioxidant activity, followed by the twig extract, while the stem bark and wood extracts showed considerably lower activities. This trend closely corresponds to the measured total phenolic and total flavonoid contents of the extracts, suggesting a strong correlation between phenolic concentration and antioxidant capacity. Phenolic and flavonoid compounds are well known for their ability to donate hydrogen atoms or electrons to neutralize free radicals, thereby preventing oxidative damage.

Similar findings have been reported in previous studies. For example, Vongsak et al. reported that *M. oleifera* leaf extracts exhibited high phenolic and flavonoid contents, which were directly associated with strong antioxidant activity [38]. The present results are therefore consistent with earlier reports and further confirm that the leaf portion of *M. oleifera* is the richest source of antioxidant compounds. Moreover, the observed trend in antioxidant activity—leaf > twig > stem bark > wood—suggests that the distribution of antioxidant compounds within the plant is not uniform and is concentrated primarily in metabolically active tissues.

The antioxidant activity of the leaf extract approached that of the reference antioxidant, ascorbic acid, which exhibited a DPPH scavenging activity of 71.72 ± 0.56%. This finding highlights the significant antioxidant potential of *M. oleifera* leaf extract and suggests that it may serve as a promising natural source of antioxidant compounds. The high antioxidant activity observed in the leaf extract is likely attributed to its high concentration of phenolic and flavonoid compounds containing free hydroxyl groups capable of scavenging reactive oxygen species. These results further support the potential use of *M. oleifera* as a valuable natural antioxidant for applications in food, pharmaceutical, and nutraceutical industries.

3.3.2. Antibacterial Activity

Antibacterial activity of the aqueous extracts obtained from different plant parts was evaluated

against gram-positive bacteria (*S. aureus* and *E. faecalis*) and gram-negative (*E. coli* and *S. dysenteriae*) clinically isolated in vitro. The evaluation was carried out using the method of resazurin microtiter assay plate, the antibacterial activities of the samples are compiled in Figure 2. Antibacterial activity varied considerably among the different plant parts, with the leaf extract exhibiting the highest activity, followed by the twig, bark, and wood extracts. Against *S. aureus*, the antibacterial activity increased progressively from wood (100–150 µg/mL) and bark (50–200 µg/mL) to twig (150–250 µg/mL) and leaf (200–300 µg/mL). A similar pattern was observed for *E. faecalis*, where wood extracts displayed the lowest activity (50–150 µg/mL), while bark, twig, and leaf extracts exhibited progressively higher activities ranging from 100 to 300 µg/mL. Among the tested bacteria, *E. coli* demonstrated the lowest susceptibility, requiring the highest inhibitory concentrations, with values ranging from 250–350 µg/mL for wood, 350–450 µg/mL for bark, 450–500 µg/mL for twig, and 450–550 µg/mL for leaf extracts. In contrast, *S. dysenteriae* was more susceptible, with antibacterial activity increasing from 50–100 µg/mL in the wood extract to 150–250 µg/mL, 200–300 µg/mL, and 200–300 µg/mL in the bark, twig, and leaf extracts, respectively. Collectively, these findings indicate a consistent increase in antibacterial activity following the order of leaf > twig > bark > wood across all bacterial species.

The good antibacterial activity observed in the leaf and twig extracts is likely associated with the higher accumulation of secondary metabolites in metabolically active aerial tissues compared with woody organs. Previous studies have demonstrated that leaves generally contain greater concentrations of phenolic compounds, flavonoids, tannins, and terpenoids, which exert antibacterial effects through disruption of bacterial cell membranes, inhibition of nucleic acid and protein synthesis, and induction of oxidative stress [39]. Similar tissue-dependent antibacterial activity has been reported in numerous medicinal plants, where leaf extracts consistently exhibited stronger inhibitory effects than stem or wood extracts due to their richer phytochemical composition. Furthermore, the relatively lower susceptibility of *E. coli* compared with the Gram-positive bacteria is in agreement with previous reports attributing this phenomenon to the presence of an outer lipopolysaccharide membrane that restricts the penetration of antimicrobial phytochemicals. Conversely, Gram-positive bacteria such as *S. aureus* and *E. faecalis* possess a simpler cell wall architecture that facilitates interaction between bioactive compounds and intracellular targets, resulting in greater antibacterial susceptibility [40]. Therefore, the present findings are consistent with previous investigations demonstrating that both plant organ specificity and bacterial cell wall structure play crucial roles in determining the antibacterial efficacy of plant-derived extracts.

3.3.3. Antifungal Activity

The antifungal activities of the wood, bark, twig, and leaf extracts against *Aspergillus flavus*, *Aspergillus niger*, and *Candida albicans* are presented in Figure 3. Overall, the antifungal activity increased from wood to leaf extracts, indicating that the aerial parts of the plant possessed greater inhibitory potential than the lignified tissues. Against *A. flavus*, the wood extract exhibited the lowest activity (25–50 µg/mL), followed by bark (25–50 µg/mL), while twig and leaf extracts showed higher activities ranging from 50–75 µg/mL. A similar trend was observed against *A. niger*, with wood extracts displaying inhibitory values of 25–50 µg/mL, bark extracts ranging from 25–50 µg/mL, and twig and leaf extracts exhibiting stronger activities of 50–75 µg/mL. Among the tested fungal strains, *C. albicans* required the highest inhibitory concentrations, with wood extracts showing activities of 50–100 µg/mL, bark extracts 75–100 µg/mL, twig extracts 100–150 µg/mL, and leaf extracts 100–150 µg/mL. Collectively, these results demonstrate that leaf and twig extracts consistently exhibited superior antifungal activity compared with bark and wood extracts, whereas *C. albicans* was less susceptible than the filamentous fungi *A. flavus* and *A. niger*.

The enhanced antifungal activity of the leaf and twig extracts is likely attributable to the higher accumulation of bioactive secondary metabolites, including phenolic compounds, flavonoids, tannins, alkaloids, and terpenoids, which are predominantly synthesized in photosynthetically active tissues [9].

Previous studies have shown that these phytochemicals inhibit fungal growth through multiple mechanisms, such as disrupting membrane integrity by interacting with ergosterol, increasing membrane permeability, inhibiting cell wall biosynthesis, suppressing mitochondrial function, and inducing intracellular oxidative stress [41]. Similar organ-dependent antifungal activity has been widely reported in medicinal plants, where leaf extracts frequently exhibit greater inhibitory effects than stem or wood extracts because of their richer phytochemical profiles. The comparatively lower susceptibility of *C. albicans* observed in the present study is also consistent with previous reports describing the adaptive resistance of this opportunistic yeast, which possesses efficient stress -response systems, biofilm-forming capability, and membrane-associated defense mechanisms that reduce the efficacy of antimicrobial compounds [41]. Therefore, the present findings support previous evidence that variations in antifungal activity are strongly influenced by both the distribution of secondary metabolites among plant organs and the intrinsic structural and physiological characteristics of the target fungal species.

4. CONCLUSIONS

This comparative investigation demonstrated that the phytochemical composition and biological activities of *Moringa oleifera* are strongly influenced by the plant organ used for extraction. Among the evaluated tissues, the leaf extract exhibited the richest phytochemical composition, the highest total phenolic and flavonoid contents, and the greatest antioxidant, antibacterial, and antifungal activities, followed by the twig, bark, and wood extracts. FTIR analysis further confirmed the presence of functional groups characteristic of phenolic and flavonoid compounds, supporting the phytochemical screening results. The strong agreement between phytochemical abundance and biological activities indicates that phenolic- and flavonoid-rich extracts are the primary contributors to the observed antioxidant and antimicrobial effects. The present findings identify *M. oleifera* leaf as the most promising source of water-extractable bioactive compounds among the plant parts evaluated. The use of aqueous extraction also highlights the feasibility of developing safe, sustainable, and environmentally friendly natural products without relying on organic solvents. These results provide a scientific basis for the utilization of *M. oleifera* leaf extracts in food, pharmaceutical, and nutraceutical applications. Future studies should focus on the isolation and structural characterization of the major active constituents, quantitative metabolomic profiling, elucidation of molecular mechanisms underlying their biological activities, and in vivo validation to facilitate the development of standardized plant-based therapeutic and functional products.

AUTHOR INFORMATION

Corresponding Author

Annissa Annissa — Department of Public Health, Universitas Faletehan, Serang-42161 (Indonesia);
Email: annissa@uf.ac.id
orcid.org/0009-0002-7251-3561

Author

Hanif Amrulloh — Department of Islamic Primary School Teacher Education, Universitas Ma'arif Lampung, Metro-34111 (Indonesia);
orcid.org/0000-0001-7458-9258

Hamed Kioumarsi — Department of Animal Science Research, Agricultural Research, Education and Extension Organization (AREEO), Rasht-4915685915 (Iran);
orcid.org/0000-0002-0783-0934

Author Contributions

A. A.: Methodology, Research, Writing - Original Draft; H. A. and H. K.: Conceptualization, Supervision, Writing, Review and Edition. All authors read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES

[1] M. S. Aslam and Y. J. Kim. (2025). "Herbal medicine in the modern age: The era of personalized precision". *Journal of*

Integrative Medicine. **23** (6): 591-604. 10.1016/j.joim.2025.08.005.

[2] W. Abdelmoumene, N. Belyagoubi-Benhammou, Z. Kaid, A. Dich, and L. Belyagoubi. (2025). "Synergistic antioxidant properties of phenolic bioactives in algerian sahara herbal remedies for cancer treatment: A focus on Moringa oleifera, Ephedra alata, Anabasis articulata, and Atriplex halimus". *South African Journal of Botany.* **186** : 37-48. 10.1016/j.sajb.2025.08.034.

[3] D. Duryat, R. Rodiani, and T. Maryono. (2025). "Acute Toxicity Study of the Leaf and Fruit Extracts of Avicennia marina (Forssk.) on Wistar White Male Mice". *Journal of Multidisciplinary Applied Natural Science.* **5** (1): 288-304. 10.47352/jmans.2774-3047.247.

[4] C. A. Dehelean, I. Marcovici, C. Soica, M. Mioc, D. Coricovac, S. Iurciuc, O. M. Cretu, and I. Pinzaru. (2021). "Plant-Derived Anticancer Compounds as New Perspectives in Drug Discovery and Alternative Therapy". *Molecules.* **26** (4): 10.3390/molecules26041109.

[5] S. Parthasarathy, S. H. Aly, S. V. Tharumasivam, B. Giridharan, J. Chandran, P. Thirumurthy, M. S. Abd El Hafeez, and M. El-Shazly. (2025). "Unlocking nature's secrets: a review on the pharmacokinetics of plant-based medicines and herbal remedies". *Natural Product Research.* 1-25. 10.1080/14786419.2025.2513583.

[6] M. Fan, X. Xu, W. Lang, W. Wang, X. Wang, A. Xin, F. Zhou, Z. Ding, X. Ye, and B. Zhu. (2023). "Toxicity, formation, contamination, determination and mitigation of acrylamide in thermally processed plant-based foods and herbal medicines: A review". *Ecotoxicology and Environmental Safety.* **260** : 115059. 10.1016/j.ecoenv.2023.115059.

[7] S. K. Banerjee, M. M. Lahiri, D. Agarwal, and H. Agrawal. (2025). "Addressing Awareness and Affordability of Generic Medicines in India: A Data Driven Strategic Framework". *Journal of Multidisciplinary Applied Natural Science.* 10.47352/jmans.2774-3047.285.

[8] X. Kou, B. Li, J. B. Olayanju, J. M. Drake, and N. Chen. (2018). "Nutraceutical or Pharmacological Potential of Moringa oleifera Lam". *Nutrients.* **10** (3). 10.3390/nu10030343.

[9] H. Amrulloh, A. Fatiqin, W. Simanjuntak, H. Afriyani, and A. Annissa. (2021). "Bioactivities of nano-scale magnesium oxide prepared using aqueous extract of Moringa Oleifera leaves as green agent". *Advances in Natural Sciences: Nanoscience and Nanotechnology.* **12** (1). 10.1088/2043-6254/abde39.

[10] H. Amrulloh, A. Fatiqin, W. Simanjuntak, H. Afriyani, and A. Annissa. (2021). "Antioxidant and Antibacterial Activities of Magnesium Oxide Nanoparticles Prepared using Aqueous Extract of Moringa Oleifera Bark as Green Agents". *Journal of Multidisciplinary Applied Natural Science.* **1** (1): 44-53. 10.47352/jmans.v1i1.9.

[11] R. S. N. Brilhante, J. A. Sales, V. S. Pereira, D. Castelo-Branco, R. A. Cordeiro, C. M. de Souza Sampaio, M. de Araujo Neto Paiva, J. Santos, J. J. C. Sidrim, and M. F. G. Rocha. (2017). "Research advances on the multiple uses of Moringa oleifera: A sustainable alternative for socially neglected population". *Asian Pacific Journal of Tropical Medicine.* **10** (7): 621-630. 10.1016/j.apjtm.2017.07.002.

[12] P. V. Viotti, W. M. Moreira, O. A. A. d. Santos, R. Bergamasco, A. M. S. Vieira, and M. F. Vieira. (2019). "Diclofenac removal from water by adsorption on Moringa oleifera pods and activated carbon: Mechanism, kinetic and equilibrium study". *Journal of Cleaner Production.* **219** : 809-817. 10.1016/j.jclepro.2019.02.129.

[13] K. Gharsallah, L. Rezig, K. Msaada, A. Chalh, and T. Soltani. (2021). "Chemical composition and profile characterization of Moringa oleifera seed oil". *South African Journal of Botany.* **137** : 475-482. 10.1016/j.sajb.2020.11.014.

[14] V. Kalaiselvi, R. Mathammal, S. Vijayakumar, and B. Vaseeharan. (2018). "Microwave assisted green synthesis of Hydroxyapatite nanorods using Moringa

oleifera flower extract and its antimicrobial applications". *International Journal of Veterinary Science and Medicine.* **6** (2): 286-295. 10.1016/j.ijvsm.2018.08.003.

[15] S. Suresh, A. S. Chhipa, M. Gupta, S. Lalotra, S. S. Sisodia, R. Baksi, and M. Nivsarkar. (2020). "Phytochemical analysis and pharmacological evaluation of methanolic leaf extract of Moringa oleifera Lam. in ovalbumin induced allergic asthma". *South African Journal of Botany.* **130** : 484-493. 10.1016/j.sajb.2020.01.046.

[16] Y. A. Soltan, N. M. Hashem, A. S. Morsy, K. M. El-Azrak, A. N. El-Din, and S. M. Sallam. (2018). "Comparative effects of Moringa oleifera root bark and monensin supplementations on ruminal fermentation, nutrient digestibility and growth performance of growing lambs". *Animal Feed Science and Technology.* **235** : 189-201. 10.1016/j.anifeedsci.2017.11.021.

[17] S. J. Granella, T. R. Bechlin, D. Christ, S. R. M. Coelho, and C. H. d. O. Paz. (2021). "An approach to recent applications of Moringa oleifera in the agricultural and biofuel industries". *South African Journal of Botany.* **137** : 110-116. 10.1016/j.sajb.2020.10.006.

[18] B. Farooq and B. Koul. (2020). "Comparative analysis of the antioxidant, antibacterial and plant growth promoting potential of five Indian varieties of Moringa oleifera L". *South African Journal of Botany.* **129** : 47-55. 10.1016/j.sajb.2018.12.014.

[19] B. Das, S. Moumita, S. Ghosh, M. I. Khan, D. Indira, R. Jayabalan, S. K. Tripathy, A. Mishra, and P. Balasubramanian. (2018). "Biosynthesis of magnesium oxide (MgO) nanoflakes by using leaf extract of Bauhinia purpurea and evaluation of its antibacterial property against Staphylococcus aureus". *Materials Science & Engineering C-Materials for Biological Applications.* **91** : 436-444. 10.1016/j.msec.2018.05.059.

[20] E. Fachriyah, M. A. Ghifari, and K. Anam. (2018). "Isolation, Identification, and Xanthine Oxidase Inhibition Activity of Alkaloid Compound from Peperomia pellucida". *IOP Conference Series: Materials Science and Engineering.* **349**. 10.1088/1757-899x/349/1/012017.

[21] L. N. Ariani, T. Estiasih, W. B. Sunarharum, and A. Khatib. (2023). "Potential of moringa (Moringa oleifera) leaf powder for functional food ingredients: A review". *Czech Journal of Food Sciences.* **41** (1): 8-20. 10.17221/221/2022-cjfs.

[22] A. Pareek, M. Pant, M. M. Gupta, P. Kashania, Y. Ratan, V. Jain, A. Pareek, and A. A. Chuturgoon. (2023). "Moringa oleifera: An Updated Comprehensive Review of Its Pharmacological Activities, Ethnomedicinal, Phytopharmaceutical Formulation, Clinical, Phytochemical, and Toxicological Aspects". *International Journal of Molecular Sciences.* **24** (3). 10.3390/ijms24032098.

[23] R. Liu, J. Liu, Q. Huang, S. Liu, and Y. Jiang. (2022). "Moringa oleifera: a systematic review of its botany, traditional uses, phytochemistry, pharmacology and toxicity". *Journal of Pharmacy and Pharmacology.* **74** (3): 296-320. 10.1093/jpp/rgab131.

[24] S. Divya, V. K. Pandey, R. Dixit, S. Rustagi, T. Suthar, D. Atuahene, V. Nagy, D. Ungai, A. E. M. Ahmed, B. Kovacs, and A. M. Shaikh. (2024). "Exploring the Phytochemical, Pharmacological and Nutritional Properties of Moringa oleifera: A Comprehensive Review". *Nutrients.* **16** (19). 10.3390/nu16193423.

[25] M. T. Arshad, S. Maqsood, A. Ikram, and K. T. Gnedeka. (2025). "Recent Perspectives on the Pharmacological, Nutraceutical, Functional, and Therapeutic Properties of Moringa oleifera Plant". *Food Science & Nutrition.* **13** (4): e70134. 10.1002/fsn3.70134.

[26] L. Horn, N. Shakela, M. K. Mutorwa, E. Naomab, and H. M. Kwaambwa. (2022). "Moringa oleifera as a sustainable climate-smart solution to nutrition, disease prevention, and water treatment challenges: A review". *Journal of Agriculture and Food Research.* **10**. 10.1016/j.jafr.2022.100397.

[27] M. C. Shih, C. M. Chang, S. M. Kang, and M. L. Tsai. (2011). "Effect of different parts (leaf, stem and stalk) and seasons (summer and winter) on the chemical compositions

and antioxidant activity of Moringa oleifera". *International Journal of Molecular Sciences.* **12** (9): 6077-88. 10.3390/ijms12096077.

[28] K. M. Patel, B. B. Parmar, K. A. Sadariya, and S. K. Bhavsar. (2022). "Assessment of in vitro antibacterial activity and MIC of cinnamon bark powder ethanolic and aqueous extracts against bacteria". *The Journal of Phytopharmacology.* **11** (5): 324-329. 10.31254/phyto.2022.11502.

[29] H. S. Elshafie, I. Camele, and A. A. Mohamed. (2023). "A Comprehensive Review on the Biological, Agricultural and Pharmaceutical Properties of Secondary Metabolites Based-Plant Origin". *International Journal of Molecular Sciences.* **24** (4). 10.3390/ijms24043266.

[30] Z. Fu, X. Jiang, D. Kong, Y. Chen, J. Zhuang, M. Han, Y. Shi, S. Lai, Y. Liu, L. Gao, and T. Xia. (2022). "Flavonol-Aluminum Complex Formation: Enhancing Aluminum Accumulation in Tea Plants". *Journal of Agricultural and Food Chemistry.* **70** (43): 14096-14108. 10.1021/acs.jafc.2c04963.

[31] Z. Fan, Z. Deng, T. Wang, W. Yang, and K. Wang. (2018). "Synthesis of Natural Tea-Saponin-Based Succinic Acid Sulfonate as Anionic Foaming Agent". *Journal of Surfactants and Detergents.* **21** (3): 303-312. 10.1002/jsde.12027.

[32] D. Haldar, D. Sen, and K. Gayen. (2017). "Development of Spectrophotometric Method for the Analysis of Multi-component Carbohydrate Mixture of Different Moieties". *Applied Biochemistry and Biotechnology.* **181** (4): 1416-1434. 10.1007/s12010-016-2293-3.

[33] G. Dhamodharan and S. Mirunalini. (2013). "A detail study of phytochemical screening, antioxidant potential and acute toxicity of Agaricus bisporus extract and its chitosan loaded nanoparticles". *Journal of Pharmacy Research.* **6** (8): 818-822. 10.1016/j.jopr.2013.07.025.

[34] S. Begum, S. Mittapally, and A. U. Esmat. (2020). "GC-MS Analysis of Bioactive Compounds and Phytochemical Evaluation of the Ethanolic Extract of Gomphrena globosa L. Flowers". *Journal of Drug Delivery and Therapeutics.* **10** (2): 53-58. 10.22270/jddt.v10i2.3914.

[35] M. Zwerger, A. Deisl, F. Hammerle, and M. Ganzera. (2024). "Determination of anthraquinones in Frangula alnus by Supercritical Fluid Chromatography". *Journal of Chromatography A.* **1737** : 465432. 10.1016/j.chroma.2024.465432.

[36] A. C. Famurewa, N. T. Asogwa, P. M. Aja, G. G. Akunna, J. N. Awoke, C. A. Ekeleme-Egedigwe, E. K. Maduagwuna, A. M. Folawiyo, E. E. Besong, E. U. Ekpono, and P. A. Nwoha. (2019). "Moringa oleifera seed oil modulates redox imbalance and iNOS/NF-κB/caspase-3 signaling pathway to exert antioxidant, anti-inflammatory and antiapoptotic mechanisms against anticancer drug 5-fluorouracil-induced nephrotoxicity in rats". *South African Journal of Botany.* **127** : 96-103. 10.1016/j.sajb.2019.08.038.

[37] P. Viogenta, L. Susanti, and L. Megasari. (2023). "Antibacterial Activity and Bioautography of the Chloroform Fraction of Morel Berry (Physalis angulata L.) Root Against Staphylococcus epidermidis and Pseudomonas aeruginosa". *Journal of Multidisciplinary Applied Natural Science.* **3** (1): 90-99. 10.47352/jmans.2774-3047.148.

[38] B. Vongsak, P. Sithisarn, S. Mangmool, S. Thongpraditchote, Y. Wongkrajang, and W. Gritsanapan. (2013). "Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringa oleifera leaf extract by the appropriate extraction method". *Industrial Crops and Products.* **44** : 566-571. 10.1016/j.indcrop.2012.09.021.

[39] N. Jubair, M. Rajagopal, S. Chinnappan, N. B. Abdullah, and A. Fatima. (2021). "Review on the Antibacterial Mechanism of Plant-Derived Compounds against Multidrug-Resistant Bacteria (MDR)". *Evidence-Based Complementary and Alternative Medicine.* **2021** : 3663315. 10.1155/2021/3663315.

[40] T. D. Tavares, J. C. Antunes, J. Padrao, A. I. Ribeiro, A. Zille, M. T. P. Amorim, F. Ferreira, and H. P. Felgueiras. (2020). "Activity of Specialized Biomolecules against Gram-Positive and Gram-Negative

Bacteria". *Antibiotics (Basel).* **9** (6). 10.3390/antibiotics9060314.

[41] M. S. A. Aboody and S. Mickymaray. (2020). "Anti-Fungal Efficacy and Mechanisms of Flavonoids". *Antibiotics (Basel).* **9** (2). 10.3390/antibiotics9020045.