Estimation of active compounds, Antioxidant and antimicrobial activity of Extracts from *Moringa Oleifera* Leaves cultivated in Syria

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Received: 18/ 10/ 2025 **Accepted:** 8/04 /2026

Abstract

Moringa Oleifera has received increased attention in recent years, due to its rich effective plant compounds, antioxidant properties and multiple therapeutic effects. This study aims to investigate the bioactive components, antioxidant activity, and antimicrobial effect of Moringa Oleifera leaf extracts grown on Syrian soil. Two extracts, one aqueous and the other alcoholic, were prepared from the powder of the dried leaves of this plant, and the content of phenolic compounds and macro-flavonoids in each of them was determined. The antioxidant activity was also evaluated by the method of inhibition of free radicals DPPH•, while the antibacterial effect on various bacterial strains, Gram-positive and Gram-negative, was tested by the method of diffusion on the agar medium. The results of the study revealed that the ethanol extract surpassed its aqueous counterpart in the content of phenols and total flavonoids, reaching values of 0.17 mgGAE/g DW and 0.16 mgQE/g DW in ethanol extract, compared to 0.14 mgGAE/g DW and 0.13 mgQE/g DW in the aqueous extract, respectively. The ethanol extract also recorded an inhibition rate of 93.5% compared to 92.88% for the aqueous extract. Both extracts showed greater effectiveness against Gram-negative bacteria than positive ones. The study concludes that the plant compounds contained in the aqueous and ethanol extracts of Moringa Oleifera leaves, have a high ability to resist oxidation and inhibit bacteria, which confirms that they are of extreme nutritional and therapeutic value.

Keywords: Antibacterial, Antioxidant, Phenols, Flavonoids, Moringa Oleifera.

Introduction:

Moringa is a tropical plant from the Moringaceae family, commonly found growing across tropical regions worldwide. The genus Moringa consists of 13 species (Mangundayao& Yasurin, 2017) of which only Moringa Oleifera has been accorded research and development attention. Moringa is a highly valuable multipurpose tree, recognized for its essential roles in nutrition, industry, and healthcare (Gopalakrishnan et al., 2016). The leaves are edible and serve as a dietary staple in many regions, and are known to possess remarkably high antioxidant activity (Ntshambiwa et al., 2023). It is widely used as a vegetable, functional food and medicinal plant that retains rich nutritional composition with diverse pharmacological activities (Niziol-Lukaszewska et al., 2020). It has been demonstrated that Moringa Oleifera exhibits pharmacological properties such as antioxidant

(Gopalakrishnan et al., 2016), anti-inflammatory, anti-cancer, anti-hyperglycemic and anti-hyperlipidemic properties (Meduri et al., 2022). Moringa Oleifera has traditionally been used in the treatment of malaria, parasitic diseases, skin diseases (Vergara-Jimenez et al., 2017) hypertension and diabetes.

The efficacy of this plant relies on identifying therapeutically and an industrially valuable compound having medicinal significance (Srivastava et al., 2023). Therefore, the present investigation was an attempt for the exploration of phyto-compounds of Moringa Oliefera leaf extracts, evaluation of their bioactive and antioxidant potential that may lead to the development of the promising therapeutic agent for the treatment of various ailments (Oleg et al., 2018). Studies have identified the bioactive profile of Moringa Oleifera to comprise vitamins, carotenoids, polyphenols, flavonoids, essential amino acids, and phenolic acids. (Gothai et al., 2017) found that aquatic, hydroalcohol or alcohol extracts of Moringa Oleifera exhibited various biological activities including antioxidant, tissue protective, and analgesic properties (Suganandam et al., 2022), whereas the leaf extracts were shown to be safe to use based on safety studies on animals (Fernandes et al., 2021).

All over the globe since the beginning of life to combat various diseases, Antioxidants contribute towards a plethora of significance in preventing stress by stabilizing or deactivating free radicals caused by several degenerative diseases (Natsir et al., 2019). Antioxidants present in food are important in lowering oxidative damage to proteins, lipids and nucleic acids induced by free radicals. This effect may be due to the presence of phytochemicals and antioxidants present in them including flavonoids and anthocyanins (Sayyed et al., 2019).

Identifying new antimicrobial agents is crucial for combating pathogenic microbes, particularly in treating infections caused by resistant strains. Medicinal herbs possessing antimicrobial properties are regarded as a promising source of novel antimicrobial compounds (Tariq et al., 2023). Antimicrobial activity has been extensively studied in Moringa Oleifera. Crude extracts of different tissues of Moringa Oleifera display different antimicrobial activities. Moreover, several molecules with antimicrobial activities have been identified from different tissues of Moringa Oleifera (Khalid et al., 2023).

This study aimed to assess the antioxidant and antibacterial properties of both aqueous and ethanolic extracts derived from Moringa oleifera leaves, moreover, determination of total phenolic and flavonoid content as the main phytochemical groups responsible of plant extracts bioactivity. Such information will help create awareness about the benefits of Moringa leaves for possible utilization and future improvement.

Materials and Methods

The research was carried out in the department of medical biotechnology, national commission for biotechnology, Damascus-Syria from September 2024 to May 2025. Moringa Oleifera leaves were collected in spring from Syrian coast country side, dried in 40°C temperature for 24 hr and grinded.

Preparation of Aquatic Extract:

250 ml Distilled water was added to 50g of dried grinded M. Oleifera leaves, with stirring for 24 hours (Jahally& Puchooa, 2017), followed by filtration and rotary evaporation (BioBase China) (Water bath temperature of 90 °C, vacuum level adjusted for 200 mbar, and rotation speed of 100 rpm).

Preparation of Alcoholic Extract:

250 ml Ethanol was added to 50g of dried grinded M. Oleifera leaves, with stirring for 24 hours, followed by filtration and rotary evaporation (BioBase China), (Water bath temperature of 40 °C, vacuum level adjusted for 200 mbar, and rotation speed of 100 rpm) according to (Abebe, 2023).

Extraction yield:

The extraction yield of aquatic and alcoholic extracts was calculated by the Equation:

Extraction yield% =A1\A2 *100 (1) (Mehganathan& Rosli, 2022).

A1 the weight of the extract concentrated by the rotary evaporator.

A2 the weight of the dried plant used for extraction.

Determination of Total Phenolic Content:

The amount of total phenols was determined by the Folin - Ciocalteu reagent method as described by Gedikoglu et al (2019) with modification. 50 µL volume of extract 20% was mixed with 1 ml of Folin - Ciocalteu's reagent. After 10 min, 2 ml of 15% Na_2CO3 solution was added and the mixture was incubated for 30 min in the dark. The reaction mixture absorbance was measured at 740 nm, and the reaction mixture without the sample was used as a blank.

Gallic acid was selected as a standard, and a standard curve was prepared of different concentrations ranging (0.1-1 mg/L). The total polyphenol content of the plant extract was expressed as Gallic acid equivalents (mg GA/g) for dry powder. All samples were analyzed in triplicate.

Determination of Total Flavonoid Content:

To determine the total flavonoid content, 50 µL volume of extract 20% was mixed with 1 ml of 2% AlCl3 ethanolic solution. The reaction mixture was incubated at room temperature for 15 minutes, after which absorbance was measured at 430 nm. Quercetin was selected as a standard, and a standard curve was prepared (0-50 mg/ml). The total flavonoid content was expressed as mg quercetin equivalents/g for dry weight Gedikoglu *et al* (2019) with modification. All samples were analyzed in triplicate.

Determination of Antioxidant Activity:

1 ml of 6×10^{-5} M ethanolic solution of DPPH was added to 50 µl of the plant extract, (Odak et al., 2015). The reaction progress absorbance of the mixture was measured at 517 nm after 30 minutes. All determinations were performed in triplicate.

The percentage of DPPH Inhibition was calculated according to the Equation: Inhibition% = $A_{C\,(0)}$ - $A_{A(t)}$/ $A_{C\,(0)}$ * 100 (2) (Kulisica et al., 2004).

$A_{C\,(0)}$ is the absorbance of the control at t = 0 min,

$A_{A\,(t)}$ is the absorbance of the sample at t = 30 min

Antibacterial Activity Estimation:

The agar diffusion method was used to determine the antibacterial activity according to the method of (NCCLS, 2000). Briefly, the nutrient agar medium in Petri dish was inoculated with 50 µl of 10^5–10^6 cuf/ml bacteria Gram-positive Bacillus subtilis, Bacillus cereus, and Staphylococcus aureus and Gram-negative Escherichia coli, Pseudomonas aeruginosa, and klebsiella pneumonia, all tested bacterial strains were obtained from the department of biodiversity, national commission for biotechnology, Damascus, Syria. Then sterile filter discs were impregnated with 50 µl of plant extract and placed on the agar surface. Petri dishes were incubated at 37 °C for 24 h (Amabye& Tadesse, 2016). The diameter of the zone of inhibition (mm) was measured using a caliper Singh and (Tafida, 2014). All tests were performed in triplicate.

Data analysis:

The results were reported as the mean ± standard deviation for triplicate measurements. Statistical analysis of the data was carried out via independent samples t-test to compare the treatment means at P-value<0.05.

Results and Discussion:

1. ***Extraction yield:***

The aquatic and alcoholic extracts of M.Oleifera showed an extraction yield of 7.5% and 10% respectively.

Table (1): The extraction yield of tested *M. Oleifera* extracts.

Extract	Aquatic	Alcoholic
Extraction yield%	7.5±0.1	10±0.1

* Values represent means ± standard deviation of triplicate readings P-value<0.05.

2. *Total Phenolic and Flavonoid Content:*

The tested extracts were investigated for their total phenolic and flavonoid content (Fernandes *et al*., 2021). The results clearly indicated high total phenolic and flavonoid content in alcoholic extract compared to aquatic extract as shown in Table 2, which consistent with what mentioned by (Kashyap *et al*., 2022).

Table (2): Total phenolic and flavonoid content in tested *M. Oleifera* extracts.

Extract	Total phenolic Content (mg.GAE/g.DW) *	Total Flavonoid Content (mg QE/g DW) *
Aquatic	0.14 ± 0.001^{b}	0.13 ± 0.227^{b}
Alcoholic	0.17 ± 0.002^{a}	0.16 ± 0.176^{a}

* Values represent means ± standard deviation of triplicate readings P-value<0.05.

Both the qualitative and quantitative estimation in our study also revealed the presence of high phenols and flavonoids quantities, in the leaf extracts of M. Oleifera. Similar results were reported by (Vazquez‑Leon et al., 2017) stating the potential antioxidant activity by the mature M. Oleifera leaves.

Phenols and flavonoids are secondary metabolites known to have antioxidant activity (Puspitasari et al., 2023); In particular, flavonoids are also well recognized for their anti-inflammatory, anti-allergic and anti-cancer activities (Al-Jaber, 2017).

3. *Antioxidant Activity:*

The tested M. Oleifera extracts were evaluated for antioxidant activity using DPPH radical scavenging method (Kumar et al., 2012), with higher antioxidant activity of alcoholic extract compared to aquatic Table (3).

Table (3): The inhibition percent of the DPPH of tested M. Oleifera extracts.

Extract	% DPPH Inhibition
Aquatic	92.88 ± 0.014^{b}
Alcoholic	93.5 ± 0.141^{a}

* Values represent means ± standard deviation of triplicate readings P-value<0.05.

Burt (2004) showed that M. Oleifera is one of such medicinal plants highly rich in secondary metabolites like phenols, sterols, glycosides, flavonoids and terpenoids. Many of these phytocompounds have been reported to possess potential antioxidants activity (Younis et al., 2022). The potential scavenging abilities present in the different extracts of M. Oliefera may be due to the appreciable presence of polyphenols and flavonoids which prevents oxidative damage and retains the internal uniformity in the plants.

Antibacterial Activity:

The antimicrobial activity of M. Oleifera extracts against six bacterial species was qualitatively assessed by the presence or absence of the inhibition zone (Wang et al., 2016). The antibacterial activity is summarized in Table 4. The results revealed that the M. Oleifera alcoholic extract presented significant antibacterial effect against the tested bacteria in particular Escherichia coli,

Staphylococcus aureus and Pseudomonas aeruginosa while klebsiella pneumonia appeared to be the most resistant to tested extracts, as presented by (Dodiya& Amin, 2015).

Table (4): Antibacterial activity of tested M. Oleifera extracts.

* Mean inhibition zone diameter (mm) after 24 h of incubation			
Extract	Gram positive		
	B. subtilis	B. cereus	S. aureus
Aquatic	11 ± 0.141[a]	11 ± 0.071[b]	15 ± 0.283[b]
Alcoholic	14 ± 0.071[b]	15 ± 0.141[a]	18 ± 0.354[a]
Extract	Gram negative		
	E. coli	P. aeruginosa	k. pneumoniae
Aquatic	16 ± 0.354[b]	16 ± 0.283[b]	10 ± 0.354[a]
Alcoholic	18 ± 0.495[a]	18 ± 0.424[a]	16 ± 0.212 [b]

* The diameter of the filter paper disc (6 mm) is included, No inhibition (< 6 mm) diameter.

Similar to Khouj et al., (2020) findings Moringa Oleifera is reported to have an antimicrobial effect on pathogenic bacteria. The antimicrobial properties of Moringa oleifera have been attributed to different parts of the plant, such as the leaves, seeds, pods and stems which are known for their antibacterial activity and are counted as rich source of antimicrobial agents.

Conclusion:

The results indicated that Moringa Oleifera leaf extracts contained significant levels of bioactive compounds, such as phenols and flavonoids. These compounds are known for their antioxidant properties, beside higher antibacterial activity against Gram negative bacteria compared to Gram positive bacteria. The bioactive components identified in Moringa oleifera leaves suggest their potential application in the development of nutraceuticals and functional foods with health-promoting properties. However further research is required to isolate individual components to develop antioxidants for food and pharmaceutical industries.

Acknowledgment:

The authors would like to thank the Syrian national commission for biotechnology (NCBT) for support.

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تقدير المركبات الفعالة والنشاط المضاد للأكسدة والميكروبي لمستخلصات أوراق *Moringa Oleifera* المزروعة في سورية

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تاريخ الاستلام: 18/ 10/ 2025 **تاريخ القبول:** 8/ 04/ 2026

الملخّص

حظيت نبتة *Moringa Oleifera* باهتمامٍ متزايدٍ في السنوات الأخيرة، ويعود ذلك إلى ما تذخر به من مركبات نباتية فعّالة وخصائص مضادة للأكسدة وتأثيرات علاجية متعددة. تهدف هذه الدراسة إلى استقصاء المكونات الحيوية النشطة، والفاعلية المضادة للأكسدة، والتأثير المضاد للميكروبات في مستخلصات أوراقMoringa Oleifera المزروعة على الأراضي السورية. جرى تحضير مستخلصَين، أحدهما مائي والآخر كحولي، من مسحوق الأوراق المجففة لهذه النبتة، وتمّ تحديد محتوى كلٍّ منهما من المركبات الفينولية والفلافونويدات الكلية. كذلك قيم النشاط المضاد للأكسدة بطريقة تثبيط الجذور الحرة DPPH•، فيما اختُبر التأثير المضاد للبكتيريا على سلالات بكتيرية متنوعة، موجبة وسالبة الغرام، بطريقة الانتشار على وسط الآغار. كشفت نتائج الدراسة أن المستخلص الإيثانولي تفوّق على نظيره المائي في محتوى الفينولات والفلافونويدات الكلية، إذ بلغت القيم 0.17 mgGAE/gDW و0.16 mgQE/gDW للمستخلص الايثانولي مقارنة ب 0.14 mgGAE/gDW و0.13 mgQE/gDW للمستخلص المائي وعلى التوالي. كما سجّل المستخلص الإيثانولي نسبة تثبيط بلغت 93.5% بينما كانت في المستخلص المائي 92.88%. وأبدى كلا المستخلصَين فاعليةً عالية في تثبيط البكتيريا سالبة الغرام مقارنةً بالموجبة. تخلص الدراسة إلى أن المركبات النباتية الموجودة في المستخلصات المائية والإيثانولية لأوراق Moringa Oleifera، تتمتع بقدرة عالية في مقاومة الأكسدة وتثبيط البكتيريا، مما يؤكد بأنها بالغة القيمة الغذائية والعلاجية.

الكلمات المفتاحية: مضاد للبكتيريا، مضاد للأكسدة، فينولات، فلافونيدات، *Moringa Oleifera*.