

Original Research Article

<https://doi.org/10.20546/ijcmas.2026.1504.023>

Evaluation of Antimicrobial Activity of Aqueous, Methanolic, and Ethanolic Extracts of *Moringa oleifera* Leaves (Lam.) in Cape Girardeau, Missouri, USA

Bridget Ugochi Anyanwueze*

Department of Biology/Biological, Southeast Missouri State University, Cape Girardeau, MO, USA

*Corresponding author

ABSTRACT

Keywords

Moringa oleifera,
antimicrobial
activity,
Bacteria,
Fungi.

Article Info

Received:
25 February 2026
Accepted:
31 March 2026
Available Online:
10 April 2026

The present study was designed to investigate the antibacterial and antifungal activity of aqueous, methanolic, and ethanolic leaf extracts of *Moringa oleifera*. The extracts were screened using the disc diffusion method against selected bacterial and fungal pathogens. The leaf extracts exhibited broad-spectrum antimicrobial activity. For antibacterial activity at a concentration of 30 mg/mL, zones of inhibition ranged from (10.0–17.0 mm) for the aqueous extract, (9.0–19.0 mm) for the methanolic extract, and (0–10.0 mm) for the ethanolic extract. The methanolic extract showed the highest antibacterial activity against the tested enteropathogenic bacteria. For antifungal activity, the ethanolic extract demonstrated the greatest inhibition, with zones ranging from (16–20 mm), while the aqueous extract showed the least activity (7.0–10.0 mm). The extracts showed varying degrees of effectiveness against bacterial strains such as *Staphylococcus aureus*, *Klebsiella species*, *Shigella dysenteriae*, *Bacillus cereus*, *Escherichia coli*, *Proteus species*, *Vibrio cholerae*, *Salmonella typhi*, *Enterococcus sp.*, *Providencia stuartii* and fungal isolates including *Fusarium sp.*, *Alternaria sp.*, *Pullarium sp.*, *Candida albicans*, and *Curvularia sp.* suggesting that *Moringa oleifera* leaf extracts possess significant antimicrobial potential, with activity influenced by the extraction solvent.

Introduction

Moringa oleifera is a globally recognized plant that has been extensively studied for its remarkable nutritional value and medicinal benefits.

The *Moringa* tree is cultivated in many regions around the world to serve both as a source of food and for its medicinal properties. Its widespread cultivation is attributed to the plant's versatility and usefulness in

supporting nutrition and health. (Olson, 2002). In addition to its nutritional value, *Moringa oleifera* is frequently utilized in traditional practices for the prevention and management of various health conditions.

The plant's dual role in nutrition and medicine indicates its importance in communities where it is grown and consumed. (Olson, 2002, Oluduro 2012). *Moringa oleifera* is one of fourteen species belonging to the family Marantaceae, it is a plant species with recognized

medicinal value, and the chemical compounds it contains have attracted considerable interest in Africa. The Moringa tree was introduced to Africa from India in the early twentieth century, where it was initially used as a nutritional and health supplement. (Muluvi *et al.*, 1999).

The leaves of *Moringa oleifera* Lam. are widely consumed in several African countries, including Ghana, Ethiopia, Nigeria, regions of East Africa, Malawi and America. All parts of the Moringa tree are edible and have long been used as a food source by humans (Fuglie, 2000). *M. oleifera* leaves has been reported to possess a wide range of biological activities.

They are described as natural anthelmintic, antibiotic, detoxifying, and immune-boosting agents, and are used in some regions to manage conditions such as malnutrition and malaria (Thilza *et al.*, 2010).

Moringa oleifera has been widely used in the treatment of various diseases, largely due to its antibacterial properties. It contains bioactive compounds such as rhamnase-containing molecules, as well as distinctive groups of compounds including glucosinolates and isothiocyanates. (Fahey 2001, Bennett 2003).

In addition, they exhibit antihypertensive, cholesterol-lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial, and antifungal properties (Nikkon *et al.*, 1988), and are widely applied in traditional medicine for the treatment of various ailments (Mughal *et al.*, 1999).

Different parts of the plant, including the leaves, roots, seeds, bark, fruits, flowers, and immature pods, have been reported to stimulate cardiac and circulatory function and exhibit anti-tumor properties (Guevara AP *et al.*, 1999), antipyretic, anti-inflammatory, antiulcer (Pal SK *et al.*, 1995, Sayeed *et al.*, 2012), antispasmodic, diuretic, (Caceres A *et al.*, 1992, Morton 1991), *Moringa* seed oil (yield 30-40% by weight), also known as Ben oil, is a sweet non-sticking, non-drying oil that resists rancidity (Tsaknis *et al.*, 1999).

Due to its availability in the United States and its established medicinal properties, *Moringa oleifera* was selected for this study. This study investigates the antimicrobial and antifungal activity of sequential leaf extracts while highlighting the relevance of plant-based therapeutics in the United States and worldwide, especially for addressing antimicrobial resistance for extracts of *Moringa oleifera* (Lam.) leaves.

Materials and Methods

Collection of Plant Material

Fresh leaves of *Moringa oleifera* were collected from the greenhouse at Southeast Missouri State University, Cape Girardeau, Missouri, USA. The plant material was identified and authenticated at the Department of Biology, Botany Unit, Southeast Missouri State University. The collected leaves were washed with distilled water to remove dirt and debris.

Plant extraction

One hundred and fifty grams of fresh *Moringa oleifera* leaves were shade-dried at room temperature (30–37 °C) for five days until a constant weight was obtained. The dried leaves were then ground into a fine powder using a mortar and pestle. A 30 g portion of the powdered sample was extracted separately in 500 mL conical flasks containing 100 mL of distilled water (aqueous extraction), 60% methanol (methanolic extraction), and 90% ethanol (ethanolic extraction).

The flasks were sealed with rubber corks, shaken at 120 rpm for 25 minutes, and then left at room temperature for five days with intermittent manual agitation every 24 hours using a sterile glass rod. The extracts were filtered using sterile Whatman No. 1 filter paper to obtain clear filtrations. The filtrates were subsequently concentrated using an evaporator and lyophilized to obtain dry extracts.

Microorganisms

A pure culture of microorganisms used for the evaluation of the antimicrobial potential of the leaves extracts include enteropathogenic *Staphylococcus aureus*, *Klebsiella species*, *Shigella dysenteriae*, *Bacillus cereus*, *Escherichia coli*, *Proteus species*, *Vibrio cholerae*, *Salmonella typhi*, *Enterococcus sp.*, *Providencia stuartii* and fungal isolates which include *Fusarium sp.*, *Alternaria sp.*, *Pullarium sp.*, *Candida albicans*, and *Curvularia sp.* The enteropathogenic bacterial isolates were all locally isolated pure cultures (LIO) obtained from the culture collection unit of Southeast Missouri State University, Cape Girardeau United States. The fungal isolates were isolated from the air of Kelso site, golf cot Cape Girardeau, United States, on Potato dextrose agar. All fungal isolates were maintained on Potato dextrose agar at temperature of 6 °C.

Antimicrobial activity assay (*in vitro*)

Antimicrobial activity of the aqueous, ethanolic and methanolic extracts of *Moringa oleifera* leaves was carried out using a disc diffusion method in accordance with Clinical and Laboratory Standards Institute guidelines (CLSI, 2020), with slight modifications (Oluma *et al.*, 1984; Doughari *et al.*, 2007; Oluduro, 2012). The concentrated leaf extracts were dissolved in 5% dimethyl sulfoxide (DMSO) and sterile discs (6mm, Hi-media, india) were each impregnated with 30µl of 30mg/ml of each extract. The discs were carefully and firmly placed on the Mueller-Hinton Agar (MHA) plates earlier seeded with standardized bacterial suspensions (0.5 McFarland (~10⁸ CFU/mL). The plates were then incubated at 37 °C for 24 h. Antibacterial activity was determined by measurement of zone of inhibition around each paper disc. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

Phytochemical Screening

Chemical tests were carried out using aqueous extract for qualitative detection of Alkaloids, tannin, saponin, flavonoids and Phlobatannins were performed on the extracts as follows:

Test for the presence of alkaloids

5 ml aqueous extract was stirred with 5 ml of 1% HCl on steam bath. Meyer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Meyer's reagent) or reddish-brown precipitate (with Dragendorff's reagent) was seen as positive for the presence of alkaloid. (Trease and Evans, 1983).

Test for the presence of Tannins: About 3 ml of the aqueous extract was stirred with 3 ml of distilled water and few drops of ferric chloride reagent added (FeCl₃) Solution were added. Formation of blue-black green precipitate was indication of presence of tannins. (Trease and Evans, 1978).

Test for presence of Saponins: 5 ml of aqueous extract was properly mixed with 5 ml of distilled water in a test tube and warmed up. The formation of a foaming that was continues indicated the presence of saponins.

Test for presence of Flavonoids: 2 ml of aqueous extract, 2ml of 10% lead acetate solution was added. Yellow precipitate was seen and was taken as a positive test for flavonoids.

Test for presence Phlobatannins: About 3 ml of aqueous extract was added to 3ml of 1% HCl and the mixture was boiled. Deposits of red precipitates were seen and taken as evidence for the presence of phlobatannins.

Results and Discussion

In the present investigation, the antimicrobial and antifungal activity of the leaves extract of *Moringa oleifera* were assayed against ten enteropathogenic *Staphylococcus aureus*, *Klebsiella species*, *Shigella dysenteriae*, *Bacillus cereus*, *Escherichia coli*, *Proteus species*, *Vibrio cholerae*, *Salmonella typhi*, *Enterococcus sp.*, *Providencia stuartii* and fungal isolates which include sven pathogenic fungi *Fusarium sp.*, *Alternaria sp.*, *Pullarium sp.*, *Candida albicans*, and *Curvularia sp.*

At a concentration of 30 mg/mL, *Moringa oleifera* leaf extracts showed broad-spectrum antimicrobial activity against the tested organisms. For antibacterial activity, zones of inhibition ranged from 10.0–17.0 mm for the aqueous extract, 9.0–19.0 mm for the methanolic extract, and 0–10.0 mm for the ethanolic extract. The methanolic extract exhibited the strongest antibacterial effect, indicating that methanol was the most effective solvent for extracting active antimicrobial compounds from the leaves.

Antifungal activity was highest with the ethanolic extract, which produced zones of inhibition ranging from 17.0–20.0 mm, indicating strong activity. The aqueous extract showed the weakest antifungal effect, with inhibition zones between 7.0–10.0 mm. The results of this study differ from those reported by Doughari *et al.*, (2007), who investigated the antibacterial activity of aqueous, acetone, and ethanolic extracts of *Moringa oleifera* leaves.

In their study, the ethanolic extract exhibited the maximum antibacterial activity, whereas the aqueous extract showed the minimum activity at a concentration of 100 mg/mL. Different solvents vary in their ability to extract compounds, as they dissolve different types of phytochemicals depending on their chemical properties. (Majorie, 1999; Srinivasan *et al.*, 2001).

Table.1 In-vitro antimicrobial activity of the leaves of *moringa oleifera* lam

Organism Source	Name of Bacteria	Diameter of zone of inhibition (mm)		
		Aqueous extract (30mg/ml)	Methanol extract 30mg/ml)	Ethanollic Extract (30mg/ml)
Enteropathogens	<i>Escherichia coli</i>	11.00	19.00	0.50
	<i>Klebsiella species</i>	10.00	18.00	0.20
	<i>Shigella dysenteriae</i>	12.00	19.00	0.30
	<i>Bacillus cereus</i>	14.00	16.00	2.00
	<i>Staphylococcus aureus</i>	12.00	9.00	0.90
	<i>Proteus species</i>	10.00	12.00	0.10
	<i>Vibrio cholerae</i>	12.00	17.00	1.00
	<i>Salmonella typhi</i>	14.00	16.00	1.85
	<i>Enterococcus sp.</i>	15.00	17.00	0.90
	<i>Providencia stuartii</i>	17.00	13.00	10.00

Table.2 In-vitro Antifungal activity of the leaves of *moringa oleifera* lam

Name of fungi isolates	Diameter of zone of inhibition (mm)		
	Aqueous extract (30mg/ml)	Methanolic extract (30mg/ml)	Ethanollic extract (30mg/ml)
<i>Fusarium sp.</i>	8.0	14.0	18.0
<i>Alternaria sp.</i>	7.0	2.0	17.0
<i>Pullarium sp.</i>	9.0	1.0	19.0
<i>Candida albicans</i>	10.0	16.0	20.0
<i>Curvularia sp.</i>	6.0	12.0	16.0

Previous studies have shown that constituents of some flowering plants possess antimicrobial activity; however, a key limitation of this line of research is the lack of standardized methods used to assess such activity. This inconsistency has led to conflicting results across different studies, and even within the same study when different methods are applied to the same sample (Alade and Irobi, 1993; Iwu, 2000). To address this issue, a review of the experimental methods used to evaluate the antimicrobial activity of plant extracts and essential oils indicates that diffusion methods are suitable for testing polar compounds of small to medium molecular size and for determining antimicrobial spectrum, as they allow multiple compounds to be tested against a single microorganism.

On the other hand, the solid dilution method is more appropriate for assessing both polar and non-polar substances, including complex extracts (Rios *et al.*, 1998). This method is particularly useful for comparing the relative potency of extracts or essential oils and for

defining their antimicrobial spectrum, since it allows multiple strains to be tested against the extracts on the same plate. (Oluduro 2012).

These findings point to a promising avenue for identifying potent antimicrobial agents from *Moringa oleifera* leaves. The results indicate that the plant contains bioactive compounds with potential for development into phytomedicines for the treatment of infections. Systematic screening of natural compounds and identification of active constituents remain essential, as early recognition of lead molecules can significantly improve the efficiency of drug development. Overall, the extracts demonstrated inhibitory activity against Gram-positive and Gram-negative bacteria, as well as fungal species.

Author Contributions

Bridget Ugochi Anyanwueze: Investigation, formal analysis, writing—original draft.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

- Bauer AW, Kibry WM, Sherris JC, Turek M. Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Pathol* 1966; 45:493-496. https://doi.org/10.1093/ajcp/45.4_ts.493
- Barry AL. Procedure for testing antimicrobial agent in agar media. In V. Lorian (ed) *Antibiotica in laboratory medicines*. Willims and Wilkins Co, Baltimore, 1980, 1-23.
- Bennett RN, Mellon FA, Foidl N *et al.*, Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L. (Horseradish tree) and *Moringa stenopetala* L. *J Agric Food Chem* 2003;51: 3546–3553. <https://doi.org/10.1021/jf0211480>
- Caceres A, Saravia A, Rizzo S, Zabala L, Leon ED, Nave F. Pharmacologic properties of *Moringa oleifera*: 2: Screening for antispasmodic, anti-inflammatory and diuretic activity. *J Ethnopharmacol* 1992; 36:233–237. [https://doi.org/10.1016/0378-8741\(92\)90049-C](https://doi.org/10.1016/0378-8741(92)90049-C)
- Doughari, J. H., Pukuma, M. S. and De, N. (2007). Antibacterial effects of *Balanites aegyptiaca* L. Drel. and *Moringa oleifera* Lam. on *Salmonella typhi*. *African Journal of Biotechnology* 6(19): 2212-2215. <https://doi.org/10.5897/AJB2007.000-2351>
- Evans, William Charles. *Trease and Evans' pharmacognosy*. Elsevier Health Sciences, 2009.
- Fuglie, L. J. (2000). New Uses of *Moringa* Studied in Nicaragua. ECHO Development Notes #68, June 2000. Available from: <http://www.echotech.org/network/modules.php?name=News&file=article&sid=194>.
- Fahey JW, Zalcmann AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 2001; 56: 5–51. [https://doi.org/10.1016/S0031-9422\(00\)00316-2](https://doi.org/10.1016/S0031-9422(00)00316-2)
- G. F. and Evans, W. C. (1978). *A Textbook on Pharmacognosy*. Eleventh Edition, Baillere Trindal London. pp. 22-40.
- Guevara AP, Vargas C, Sakurai H. An antitumor promoter from *Moringa oleifera* Lam. *Mutat Res* 1999; 440: 181–188. [https://doi.org/10.1016/S1383-5718\(98\)00292-0](https://doi.org/10.1016/S1383-5718(98)00292-0)
- Iwu, M. M. (2000). *Empirical Investigation of Dietary Plants used in Igbo Ethnomedicine: Vol 2*, Cesta Press Enugu; Nigeria. pp. 4-5.
- Alade, P. I. and Irobi, O. N. (1993). Antimicrobial Activities of Crude Leaf Extracts of *Acalypha wilkesiana*. *Journal of Ethnopharmacology* 39: 171-174. [https://doi.org/10.1016/0378-8741\(93\)90048-2](https://doi.org/10.1016/0378-8741(93)90048-2)
- Islam, M. A., *et al.*, "Determination of minimum inhibitory concentration (MIC) of cloxacillin for selected isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) with their antibiogram." *Bangladesh Journal of Veterinary Medicine* 6.1 (2008): 121-126.
- J. H., Pukuma, M. S. and De, N. (2007). Antibacterial effects of *Balanites aegyptiaca* L. Drel. and *Moringa oleifera* Lam. on *Salmonella typhi*. *African Journal of Biotechnology* 6(19): 2212-2215.
- Kavit, Mehta, B. Patel, and B. Jain. "Phytochemical analysis of leaf extract of *Phyllanthus fraternus*." *Research Journal of Recent Sciences ISSN 2277* (2013): 2502.
- Morton JF. The horseradish tree, *Moringa pterygosperma* [Moringaceae], A boon to arid lands. *Econ Bo* 1991; 45:318–333.
- Muluvi G.M.; Sprent J.I.; Soranzo N.; Provan J.; Odee D.; Folkard G.; McNicol J.W.; and Powell W. Amplified fragment length polymorphism (AFLP) analysis of genetic variation in *M. oleifera* Lam. *J. of Mol. Ecol.*, 1999; 8: 463-470.
- Majorie, M. C. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Review* 12(4): 564-582.
- Mughal MH, Ali G, Srivastava PS, Iqbal M. Improvement of drumstick [*Moringa*

- pterygosperma* Gaertn.] – a unique source of food and medicine through tissue culture. *Hamdard Med* 1999; 42:37–42.
- Miliauskas, G.; Venskutonis, P.; Van Beek, T. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.* 2004, 85, 231–237.
<https://doi.org/10.1016/j.foodchem.2003.05.007>
- Marrufo, Tatiana, *et al.*, "Chemical characterization and determination of antioxidant and antimicrobial activities of the leaves of *Moringa oleifera*." *International Network Environmental Management Conflicts* 2.1 (2013): 1-15.
- Nikkon F, Saud ZA, Rehman MH, Haque ME. *In vitro* antimicrobial activity of the compound isolated from chloroform extract of *Moringa oleifera* Lam. *Pak J Biol Sci* 2003; 22:1888–1890.
<https://doi.org/10.3923/pjbs.2003.1888.1890>
- Olson, M. E. (2002). Amplified fragment length polymorphism (AFLP) analysis of genetic variation in *Moringa oleifera* Lam. *Molecular Ecology* 8(3): 463-470.
- Oluma, H. O., Umeh, E. U., Onekutu, A. and Okolo, J. (2004). Antibacterial potentials of eight medicinal plants from the lower Benue valley of Nigeria. *Nigerian Journal of Botany* 17: 1-11.
- Oluduro, Anthonia Olufunke. "Evaluation of antimicrobial properties and nutritional potentials of *Moringa oleifera* Lam. leaf in South-Western Nigeria." *Malaysian Journal of Microbiology* 8.2 (2012): 59-67.
- Pal SK, Mukherjee PK, Saha BP. Studies on the antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models in rats. *Phytother Res* 1995; 9:463–465.
<https://doi.org/10.1002/ptr.2650090607>
- Srinivasan, D., Perumalsamy, L. P., Nathan, S. and Sures, T. (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *Journal of Ethnopharmacology* 94: 217-222.
[https://doi.org/10.1016/S0378-8741\(01\)00250-0](https://doi.org/10.1016/S0378-8741(01)00250-0)
- Sayeed, Mohammed Abu, *et al.*, "In vitro antimicrobial activity of methanolic extract of *Moringa oleifera* Lam. fruits." *Journal of Pharmacognosy and Phytochemistry* 1.4 (2012): 94.
- Tsaknis, J., S. Lalas, V. Gergis, V. Douroglou and V. Spiliotis, 1999, Characterization of *Moringa oleifera* variety Mbololo seed oil of Kenya, *Journal of Agricultural and Food Chemistry*, 47: 4495-4499. <https://doi.org/10.1021/jf9904214>
- Thilza, L. B., Sanni, S., Zakari, A. I., Sanni, F. S., Talle, M. And Joseph, B. M. (2010). *In vitro* Antimicrobial activity of water extract of *Moringa oleifera* leaf stalk on bacteria normally implicated in eye diseases. *Academia arena* 2(6): 80-82.

How to cite this article:

Bridget Ugochi Anyanwueze. 2026. Evaluation of Antimicrobial Activity of Aqueous, Methanolic, and Ethanolic Extracts of *Moringa oleifera* Leaves (Lam.) in Cape Girardeau, Missouri, USA. *Int.J.Curr.Microbiol.App.Sci.* 15(4): 190-195. doi: <https://doi.org/10.20546/ijcmas.2026.1504.023>