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Original article

In vitro Antibacterial Activity and Antibiotic-Modulating Effects of a Methanol–Water *Moringa oleifera* Leaf Extract against Selected Bacterial Isolates from Libya

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Abstract
Antimicrobial resistance has increased interest in plant-derived antimicrobial candidates. This laboratory-based in vitro study evaluated a methanol–water extract of locally collected *Moringa oleifera* leaves against selected isolates of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Antibacterial activity was assessed by agar well diffusion, while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth dilution. Antibiotic susceptibility was evaluated by disk diffusion, and a modified disk-soaking procedure was used to screen for changes in antibiotic inhibition-zone diameters after exposure to the extract. The extract inhibited all tested isolates, with the largest inhibition zone against *S. aureus* (20.0 ± 1.2 mm) and the smallest against *P. aeruginosa* (12.0 ± 0.8 mm). Reported MIC/MBC values ranged from 10/20 mg/mL for *S. aureus* to 40/50 mg/mL for *P. aeruginosa*. The extract was associated with increased inhibition zones for selected antibiotic–isolate combinations, including amoxicillin–clavulanic acid against *S. aureus* and meropenem against *P. aeruginosa*. These findings provide laboratory evidence that locally cultivated *M. oleifera* leaves contain antibacterial activity and identify promising antibiotic–extract combinations for confirmation with standardized interaction assays, broader isolate collections, and subsequent translational studies.
Keywords. *Moringa oleifera*, In Vitro Antibacterial Activity, Antibiotic Modulation, Medicinal Plants, Antimicrobial Resistance.

Introduction

Antimicrobial resistance (AMR) is a major global public-health challenge that reduces the effectiveness of conventional antimicrobial therapy and contributes to increased morbidity, mortality, and healthcare costs. The World Health Organization has emphasized the continuing emergence and spread of resistant bacterial pathogens and the need for new antimicrobial approaches [1]. Medicinal plants contain diverse secondary metabolites and remain valuable candidates for antimicrobial discovery. Their constituents may affect multiple cellular processes and may complement conventional antimicrobial research. Plant extracts have also been investigated for their capacity to modify the measured activity of conventional antibiotics. *Moringa oleifera* Lam. (family Moringaceae) is cultivated widely in tropical and subtropical regions. Its leaves contain flavonoids, phenolic compounds, tannins, alkaloids, glucosinolates, and isothiocyanates that have been associated with biological activity [2,3]. Previous reports describe antibacterial activity of *M. oleifera* leaf extracts against selected bacterial organisms, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [4,5]. Reported activity varies with plant source, extraction method, concentration, organism, and assay design; therefore, comparisons across studies require caution. Plant extracts may influence the measured activity of conventional antibiotics when tested in combination. Such effects can include increased or reduced inhibition zones, depending on the extract, antibiotic, organism, and assay conditions. Promising observations should be confirmed with validated interaction methods, including checkerboard testing with fractional inhibitory concentration indices or time-kill analysis [7,8]. The present work contributes localized laboratory evidence on *M. oleifera* cultivated in Libya. Because plant origin and environmental conditions can influence phytochemical composition and biological activity, these findings provide a useful basis for future comparative and translational investigations. The primary objective was to evaluate the in vitro antibacterial activity of a methanol–water (3:1, v/v) leaf extract of locally cultivated *Moringa oleifera* against selected Gram-positive and Gram-negative bacterial isolates. Secondary objectives were to determine MIC and MBC values and to assess changes in antibiotic inhibition-zone diameters after exposure to the extract. The results are intended to identify candidates for expanded mechanistic, pharmacological, and clinical-development research.

Materials and Methods

Study Design

This laboratory-based in vitro study evaluated the antibacterial activity of a *Moringa oleifera* leaf methanol–water (3:1, v/v) extract against selected bacterial isolates and assessed its effects on measured antibiotic inhibition zones. All assays were performed in triplicate under standardized laboratory conditions. CLSI guidance was applied to standard antibiotic disk-diffusion procedures where applicable [9], while the crude-extract agar well-diffusion and disk-soaking procedures were used as comparative research assays.

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Plant Material Collection and Botanical Authentication

Fresh, mature leaves of *Moringa oleifera* Lam. (Family: Moringaceae) were collected in January 2025 from a cultivated farm in Sabratha City, Libya. The plant material was taxonomically identified and authenticated by specialists in the Department of Botany, Faculty of Science, University of Sabratha, before experimental work. Future expanded studies will include voucher-specimen deposition and detailed geographic documentation to support wider reproducibility. Leaves were rinsed with distilled water to remove surface contaminants, shade-dried at ambient laboratory temperature (25–28°C) for approximately two weeks to constant weight, and ground to a fine powder using a sterile electric grinder. The powder was stored in sterile, airtight amber glass containers at room temperature, protected from light and moisture until extraction and phytochemical analysis.

Chemicals and Reagents

All chemicals and reagents were analytical grade. Methanol (≥99.8%), ferric chloride ($FeCl_3$), hydrochloric acid (HCl), sodium hydroxide (NaOH), and dimethyl sulfoxide (DMSO, ≥99.9%) were obtained from certified suppliers in Germany. Mueller–Hinton Agar (MHA) and Mueller–Hinton Broth (MHB) were purchased from HiMedia Laboratories (supplied through authorized distributors in Turkey). Normal saline (0.85% NaCl) and sterile distilled water were prepared under aseptic conditions; distilled water was produced using a laboratory water purification system. Commercial antibiotic discs for antimicrobial susceptibility testing were obtained from certified manufacturers in China and supplied through authorized local distributors. Reagents and media were prepared and used according to manufacturers' instructions.

Preparation of* Moringa oleifera *Leaf Extract

The methanol–water (3:1, v/v) extract of *Moringa oleifera* leaves was prepared by a modified cold maceration method. Twenty-five grams of dried leaf powder were mixed with 200 mL of methanol–water (3:1, v/v) in a sterile amber glass flask and macerated at room temperature (25–28°C) for 48 h with intermittent manual shaking. The mixture was filtered through Whatman No. 1 filter paper and centrifuged at 4,000 rpm for 15 min to remove residual particulates. The clear supernatant was concentrated in a laboratory oven at 40–45°C until a semi-solid crude extract was obtained and stored in sterile amber glass bottles at 4°C until testing. Before assays, the extract was dissolved in 5% (v/v) DMSO to prepare the working solutions. Future optimization studies will further standardize extraction yield, extract concentration, stability, and sterility verification.

Preliminary Phytochemical Screening

Qualitative phytochemical screening was performed according to Harborne [6] for flavonoids, phenolic compounds, alkaloids, tannins, and saponins, using characteristic color changes or precipitate formation. This analysis provided an initial chemical profile that supports future quantitative characterization of the constituents associated with antibacterial activity.

Bacterial Strains

Four bacterial isolates were obtained from the Microbiology Laboratory, Department of Medical Laboratory Sciences, Faculty of Medical Technology, University of Sabratha, Libya: two Gram-positive species (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and two Gram-negative species (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The isolates were identified by conventional microbiological and biochemical methods and maintained under appropriate laboratory conditions until use. The selected organisms provided a clinically relevant panel for comparing extract activity under the study conditions.

Preparation of Bacterial Inoculum

Fresh colonies were suspended in sterile physiological saline (0.85% NaCl) and adjusted to the turbidity of a 0.5 McFarland standard (~1.5 × 10^8 CFU/mL) before inoculation.

Agar Well Diffusion Assay

We assessed the extract's antibacterial activity by the agar well-diffusion method as a comparative in vitro screening assay [12]. Mueller–Hinton agar plates were inoculated uniformly with standardized bacterial suspension. Wells of 6 mm diameter were produced aseptically with a sterile cork borer, and 100 µL of extract solution was dispensed into each well. Plates were allowed to stand at room temperature for 30 min to permit diffusion, then incubated at 37°C for 24 h. Inhibition-zone diameters were measured in millimeters using a ruler or digital caliper. Experiments were performed in triplicate, and mean inhibition-zone diameters were recorded.

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk-diffusion method on Mueller–Hinton agar according to CLSI guidance [9]. Inhibition-zone diameters were measured after incubation and interpreted using the applicable CLSI criteria for the selected organism–antibiotic combinations.

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Evaluation of the Combined Effect of Moringa oleifera *Extract and Antibiotics*

The extract's effect on selected antibiotic discs was evaluated using a modified disk-diffusion soaking procedure. Commercial antibiotic discs were immersed in the plant extract for 30 min under sterile conditions, allowed to air-dry aseptically, and then placed on Mueller–Hinton agar plates inoculated with the test organisms. After incubation at 37°C for 24 h, inhibition zones were measured and compared with antibiotic discs alone. Changes in zone diameter were reported as apparent increases, apparent reductions, or no detectable change. This screening approach identifies combinations that merit confirmation using standardized checkerboard/FICI, time-kill, or related interaction assays.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 23. All experiments were carried out in triplicate, and results are presented as mean ± standard deviation (SD). Differences among measurements were explored by one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple-comparison test, with $p < 0.05$ considered statistically significant. Expanded studies using larger isolate collections will allow more detailed assessment of isolate-level and population-level variability.

Quality Assurance

All microbiological procedures were performed under sterile conditions. Culture media and reagents underwent quality-control procedures before use, and laboratory equipment was calibrated periodically according to institutional standard operating procedures. The study provides a platform for future work incorporating expanded quality-control panels, detailed acceptance ranges, and harmonized interlaboratory validation.

Ethical Considerations

This study did not involve human participants or experimental animals. Investigations used previously identified and preserved bacterial isolates obtained from the Microbiology Unit, Department of Medical Laboratory Sciences, Faculty of Medical Technology, University of Sabratha, Libya, maintained for academic and research purposes. All procedures complied with institutional biosafety regulations and laboratory safety guidelines of the Faculty of Medical Technology, University of Sabratha.

Results

Antibacterial Activity of Moringa oleifera *Leaf Extract*

The methanol–water (3:1, v/v) leaf extract of *Moringa oleifera* exhibited antibacterial activity against all tested bacterial isolates, with inhibition-zone diameters varying by species (Table 1). *Staphylococcus aureus* showed the largest mean inhibition zone (20.0 ± 1.2 mm), followed by *Klebsiella pneumoniae* (18.0 ± 1.3 mm) and *Staphylococcus epidermidis* (15.0 ± 1.0 mm). *Pseudomonas aeruginosa* showed the smallest mean inhibition zone (12.0 ± 0.8 mm). Qualitative phytochemical screening detected flavonoids, phenolic compounds, tannins, alkaloids, and saponins, supporting the value of subsequent quantitative chemical characterization.

Table 1. Antibacterial activity of the methanol–water (3:1, v/v) extract of* Moringa oleifera *leaves against the selected bacterial isolates

Bacterial isolate	Gram reaction	Inhibition zone (mm), Mean ± SD
Staphylococcus aureus	Positive	20.0 ± 1.2
Staphylococcus epidermidis	Positive	15.0 ± 1.0
Klebsiella pneumoniae	Negative	18.0 ± 1.3
Pseudomonas aeruginosa	Negative	12.0 ± 0.8

Antibiotic Susceptibility Profile

Antimicrobial susceptibility results for the tested isolates against the four antibiotics are summarized in Table 2. The observed profiles provide a useful antibiotic-response context for interpreting the extract and extract–antibiotic findings under the study conditions.

Table 2. Antibiotic susceptibility profiles of the selected bacterial isolates

Bacterial isolate	AMC	CTR	MEM	CAZ
Staphylococcus aureus	S	S	S	I
Staphylococcus epidermidis	S	S	S	I
Klebsiella pneumoniae	I	R	S	I
Pseudomonas aeruginosa	R	R	S	R

Abbreviations: AMC, amoxicillin–clavulanic acid; CTR, ceftriaxone; MEM, meropenem; CAZ, ceftazidime; S, susceptible; I, intermediate; R, resistant.

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Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC values for the methanol–water (3:1, v/v) *Moringa oleifera* leaf extract are shown in (Table 3). *S. aureus* had the lowest MIC/MBC values (10/20 mg/mL), whereas *P. aeruginosa* required the highest values (40/50 mg/mL). The concentration-dependent pattern supports further work to optimize extract preparation, define active constituents, and evaluate activity across broader bacterial collections.

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the methanol–water (3:1, v/v)* Moringa oleifera *leaf extract against the selected bacterial isolates.

Bacterial isolate	MIC (mg/mL)	MBC (mg/mL)
Staphylococcus aureus	10	20
Staphylococcus epidermidis	15	25
Klebsiella pneumoniae	20	30
Pseudomonas aeruginosa	40	50

Modulatory Effect of* Moringa oleifera *Leaf Extract on Antibiotic Activity

The methanol–water (3:1, v/v) leaf extract was associated with changes in the activity of selected antibiotics that varied by bacterial isolate and antibiotic (Table 4). For *S. aureus*, increased inhibition zones were observed after exposure to the extract for all evaluated antibiotics; the largest change was observed with amoxicillin–clavulanic acid, where the inhibition zone increased from 15 to 20 mm. These results identify combinations suitable for further confirmation using standardized interaction assays. In *S. epidermidis*, an apparent increase was observed with amoxicillin–clavulanic acid, while apparent reductions were observed with meropenem and ceftazidime; no detectable change was observed with ceftriaxone. For *K. pneumoniae*, apparent increases were observed with amoxicillin–clavulanic acid, meropenem, and ceftazidime, whereas the ceftriaxone zone was reduced from 15 mm to no zone (NZ; recorded as 6 mm) after soaking. For *P. aeruginosa*, an increased zone diameter was observed with meropenem, reduced zone diameters (to NZ; 6 mm) were observed with ceftazidime and ceftriaxone, and no detectable change was observed with amoxicillin–clavulanic acid (NZ before and after soaking). These isolate-specific patterns support systematic investigation of the underlying interaction mechanisms. The observed changes in zone diameter demonstrate that *M. oleifera* extract can influence antibiotic performance under the tested in vitro conditions. Standardized checkerboard/FICI and time-kill studies will clarify which of the promising combinations represent reproducible pharmacological interactions.

Overall Comparison of Antibacterial Performance

Across the selected isolates, antibacterial response varied by organism and antibiotic. This pattern highlights the value of extending the work to a broader collection of bacterial isolates and resistant phenotypes to define the range of activity and determinants of response.

Table 4. Changes in inhibition-zone diameters after exposure of selected antibiotics to the methanol–water (3:1, v/v)* Moringa oleifera *leaf extract using the modified disk-soaking procedure

Bacterial isolate	Antibiotic	Before soaking (mm)	After soaking (mm)	Observed change
S. aureus	AMC	15	20	Increased zone diameter
	MEM	24	28	Increased zone diameter
	CAZ	13	15	Increased zone diameter
	CTR	21	22	Increased zone diameter
S. epidermidis	AMC	21	23	Increased zone diameter
	MEM	26	24	Reduced zone diameter
	CAZ	19	17	Reduced zone diameter
	CTR	24	24	No detectable change
K. pneumoniae	AMC	16	18	Increased zone diameter
	MEM	20	21	Increased zone diameter
	CAZ	10	12	Increased zone diameter
	CTR	15	NZ (6)	Reduced zone diameter (NZ)
P. aeruginosa	AMC	NZ (6)	NZ (6)	No detectable change
	MEM	20	25	Increased zone diameter
	CAZ	16	NZ (6)	Reduced zone diameter (NZ)
	CTR	15	NZ (6)	Reduced zone diameter (NZ)

Abbreviations: AMC, amoxicillin–clavulanic acid; MEM, meropenem; CAZ, ceftazidime; CTR, ceftriaxone; NZ, no zone of inhibition, recorded as 6 mm (paper-disk diameter). Changes are descriptive observations from a qualitative disk-soaking screen and do not indicate synergy or antagonism.

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Discussion

The present study evaluated the antibacterial activity of a methanol–water (3:1, v/v) *Moringa oleifera* leaf extract against four clinically relevant bacterial isolates and assessed its influence on selected conventional antibiotics. The extract exhibited antibacterial activity against all tested organisms, with susceptibility varying by species. The greater observed activity against *S. aureus* and the lower response of *P. aeruginosa* may be associated with differences in cell-envelope architecture, including the Gram-negative outer membrane, which can impede entry of bioactive phytochemicals. Isolate-specific factors and crude-extract composition may also contribute to the observed pattern [2,4,5]. The qualitative detection of flavonoids, phenolic compounds, tannins, alkaloids, and saponins provides a plausible chemical basis for the observed antibacterial activity. These metabolite classes have documented antimicrobial properties and may affect membrane integrity, permeability, enzymes, and macromolecular synthesis [2,6]. Quantitative profiling will strengthen the linkage between extract composition and biological activity. The MIC and MBC findings support concentration-dependent antibacterial activity of the extract. The lower values observed for *S. aureus* and higher values for *P. aeruginosa* identify a useful pattern for future studies using standardized broth microdilution, active-fraction testing, and wider isolate panels [11]. The extract–antibiotic observations varied with both the bacterial isolate and the antibiotic. Increased zones with amoxicillin–clavulanic acid against *S. aureus* and meropenem against *P. aeruginosa* are particularly promising candidates for mechanistic follow-up. The disk-soaking procedure provides an exploratory first screen; validation with checkerboard/FICI or time-kill methods will determine the nature and reproducibility of the interaction [7,8]. The combined inhibition-zone, MIC/MBC, and antibiotic-modulation findings identify *M. oleifera* leaf extract as a promising source of antibacterial candidates and provide a rational foundation for further development. The results support continued work on active-fraction isolation, standardized formulation, and evaluation against clinically relevant bacterial panels. The controlled evaluation of selected clinically relevant isolates enabled direct comparison of extract activity under uniform laboratory conditions. Future research can build on this foundation by incorporating multiple independent clinical and reference isolates, standardized extract characterization, and validated interaction designs, thereby extending the findings toward translational and clinical relevance. Future work should include chromatographic characterization and quantification of active phytochemicals, testing against larger collections of clinically relevant and antimicrobial-resistant isolates, evaluation of antibiofilm activity, cytotoxicity assessment, formulation studies, and in vivo models. These steps will help define the safety, mechanisms, and therapeutic potential of *M. oleifera* leaf extracts as plant-derived antimicrobial candidates.

Conclusion

The methanol–water (3:1, v/v) leaf extract of *Moringa oleifera* demonstrated in vitro antibacterial activity against the selected Gram-positive and Gram-negative bacterial isolates. Activity was strongest against *Staphylococcus aureus*, whereas *Pseudomonas aeruginosa* showed the lowest response and required higher MIC/MBC values. The extract was also associated with changes in the inhibition-zone diameters of selected conventional antibiotics, including increased zones for amoxicillin–clavulanic acid against *S. aureus* and meropenem against *P. aeruginosa*. These findings identify promising antibiotic–extract combinations for confirmatory interaction testing. The detected phytochemical classes provide a biologically plausible basis for the observed antibacterial activity and support quantitative characterization of the active constituents. Overall, the study provides a useful laboratory foundation for the development of expanded research on *Moringa oleifera* as a plant-derived antimicrobial candidate. Standardized interaction testing, broader isolate collections, cytotoxicity studies, formulation development, and in vivo evaluation will be important next steps toward assessing future therapeutic and clinical applicability.

Conflict of interest. Nil

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