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Research Article

Antibacterial Activity of *Moringa Oleifera* Leaf Extract Against Clinically Significant Bacteria

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Abstract

The global rise of multidrug-resistant (MDR) bacteria calls for different alternative antimicrobial strategies. *Moringa oleifera*, which is found to be rich in secondary metabolites compounds, has shown some antibacterial potential. This study assesses the ethanol-extracted antibacterial activity of *M. oleifera* leaves against different clinically significant bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis* and *Klebsiella pneumonia.* A laboratory-based experimental study was done using the disc diffusion method at two extract of different concentrations (500 mg/ml and 1000 mg/ml). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests were performed to observe bacteriostatic and bactericidal effects. The study identified the presence of alkaloids, flavonoids, tannins, saponins and terpenoids, highlighting the diverse phytochemical composition of the analyzed sample. The extract of different concentrations demonstrated the zone of inhibition for *S. aureus* (12 mm at both concentrations) and *P. aeruginosa* (17 mm at 500 mg/ml, 20 mm at 1000 mg/ml). *E. coli* only exhibited the inhibition (11 mm) at 1000 mg/ml. Likewise, *E. faecalis* and *K. pneumoniae* showed inhibition (16 mm and 12 mm) at 1000 mg/ml. However, MIC and MBC tests were found negative, where the regrowth of the bacteria was observed, which further suggested that the liquid culture has only limited efficacy. The selective secondary metabolites, particularly against *P. aeruginosa* advocates potential external applications or adjunctive use with antibiotics rather than the standalone. However, the lack of bactericidal activity in liquid culture shows the need for further research on higher concentrations, active compound purification, and synergistic effects with conventional antimicrobials.

Keywords: Antibacterial activities, *Moringa oleifera*, Multidrug-resistant bacteria, Secondary metabolites

Introduction

The rise of multidrug-resistant (MDR) bacteria has become a global health crisis due to the overuse and misuse of antibiotics. The World Health Organization (WHO) identifies antibiotic resistance as a major threat to public health, food security and development. Different pathogens such as

methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* are deeply linked to increased morbidity, mortality and healthcare costs. Despite the urgent need for antibiotics, the discovery has not kept pace with the emergence of resistant strains (Muteeb et al., 2023), entailing the exploration of alternative antimicrobial agents, particularly from natural sources (Newman & Cragg, 2020).

Moringa oleifera, known as the drumstick or miracle tree, has gained some attention for its different medicinal properties and nutritional benefits (Kashyap et al., 2022). Its leaves are found rich in essential nutrients and different bioactive compounds, such as flavonoids, phenolic acids, alkaloids, saponins, and tannins (Nepolean et al., 2009). These secondary metabolites show diverse pharmacological activities, including antimicrobial effects, through their mechanisms such as bacterial cell membrane disruption and inhibition of essential bacterial processes (Donadio et al., 2021). Previous studies have shown that *M. oleifera* extracts show antibacterial activity against both Gram-positive and Gram-negative pathogens, including *E. coli*, *S. aureus* and *Pseudomonas aeruginosa* (Lar et al., 2011; Abalaka et al., 2012).

Despite its promising antimicrobial properties, the rate of research on *M. oleifera* where the use of ethanol as an extracting solvent remains low. Polar solvents are commonly used to extract polyphenols from plant matrices, with ethanol-based aqueous solutions being among the most effective options (Sultana et al., 2009). Particularly in screening its secondary metabolites and exploring potential synergies with conventional antibiotics. As a widely and locally available as well as easily cultivable plant, *M. oleifera* offers a sustainable and accessible approach to managing bacterial infections, especially in resource-limited settings where the burden of infectious disease is high. The WHO encourages integrating traditional medicine into healthcare systems, particularly in regions with limited access to conventional treatments.

The aim of this study is to evaluate the antimicrobial efficacy of *M. oleifera* leaf extract against clinically significant bacteria, assess its secondary metabolites components and also to explore its potential antibacterial activities. Given the increasing threat of antibiotic resistance, investigating *M. oleifera* as a potential antimicrobial agent is timely and may contribute to developing sustainable and effective healthcare solutions. The findings of this study could support evidence-based applications of *M. oleifera* in modern medicine, addressing the urgent need for alternative antimicrobial strategies.

Materials and Methods

Collection of plant materials

One kilogram of fresh *Moringa oleifera* leaves were collected from Narayani riverside, Bharatpur, Nepal (altitude: 252 m, latitude: 27.6765°N, longitude: 84.4357°E), ensuring they were free from disease, pests and contaminants. The leaves were washed with distilled water, shade-dried at room temperature (30°C) for 10 days, and finely ground into powder.

Extract preparation

About 50 g of powder was soaked in ethanol at a 1:10 (w/v) ratio. Soxhlet extraction was performed using a cellulose extraction thimble. The solvent was heated to its boiling point, evaporated, condensed, and repeatedly siphoned through the plant material until the solvent became colorless (Abubakar & Haque, 2020). The extract was concentrated using a water bath at 45°C, dried, weighed, and stored at 4°C in an airtight container.

Phytochemical screening

The presence of secondary metabolites was confirmed using standard qualitative tests conducted at the Microbiology Laboratory of Balkumari College, Narayangarh, Chitwan.

Alkaloids detection (Dragendorff's test & Wagner's test)

About 1 ml of extract was treated with a few drops of Dragendorff's reagent. The formation of a reddish-brown precipitate indicated the presence of alkaloids (Patel et al., 2014). For further confirmation, Wagner's reagent was added to another 1 ml of the extract. The appearance of a reddish-brown precipitate confirmed the presence of alkaloids (Santhi & Sengottuvel, 2016).

Flavonoids detection (Alkaline reagent test)

A few drops of sodium hydroxide (NaOH) were added to 1 ml of extract. A yellow coloration indicated flavonoids, which disappeared upon the addition of dilute hydrochloric acid (Sudha et al., 2021).

Phenolics and Tannins detection (Ferric chloride test and Lead acetate test)

A few drops of 5% ferric chloride solution were added to 1 ml of extract. The formation of a dark green or blue-black colour indicated phenolics and tannins. In the lead acetate test, 1 ml of extract was treated with 1% lead acetate solution. A yellow precipitate confirmed tannins.

Saponins detection (Foam test)

In the foam test, 1 ml of extract was vigorously shaken with 5 ml of distilled water. Persistent frothing for more than 10 minutes confirmed saponins (Santhi & Sengottuvel, 2016).

Terpenoids detection (Salkowski test)

For the Salkowski test, 1 ml of extract was mixed with 2 ml of chloroform then concentrated sulfuric acid was added on filtrated. A golden yellow colour confirmed terpenoids (Sudha et al., 2021).

Biochemical test of bacteria

The bacterial strains were isolated from clinical samples (urine and pus) at the Microbiology Laboratory of Narayani Samudayik Hospital, Bharatpur, Bagmati Province, Nepal. The pure cultures at hospital were transported to Balkumari College, where biochemical test was performed.

Antibacterial activity

Mueller-Hinton agar (MHA) medium was prepared, and bacterial suspensions were standardized to 0.5 McFarland (~10^8 CFU/mL). The obtaining of 0.5 McFarland suspension is controlled by the use of a spectrophotometer. The agar well diffusion method was used to measure the zone of inhibition (ZOI) against selected bacterial strains to determine the antimicrobial activity. The bacterial suspension was spread onto MHA plates. Wells (6 mm) were created, and the extract was added at two concentrations (500 mg/ml and 1000 mg/ml). The plates were incubated at 37°C for 24 hours, and the ZOI was measured.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

Stock extract solution (1000 mg/ml) was serially diluted (2-fold). Bacterial suspensions were adjusted to 0.5 McFarland and inoculated. MIC was determined as the lowest concentration inhibiting growth. MBC was determined by subculturing MIC-negative tubes. MBC/MIC ratio $\leq$ 4 indicated bactericidal activity, while >4 suggested bacteriostatic effects (Kowalska-Krochmal & Dudek-Wicher, 2021; Ishak et al., 2024). This ratio serves as a key parameter for determining whether an antibacterial agent is bactericidal or bacteriostatic.

Results and Discussion

Qualitative phytochemicals screening

The leaves were specifically chosen for this study due to several proven results from previous studies. *Moringa* leaves contain high concentrations of bioactive secondary metabolites such as phenols, flavonoids, tannins and alkaloids (Table 1), which contribute to their potent antibacterial properties (Azra et al., 2024; Jhones, 2022). Previous studies have extensively documented the antimicrobial effects of *Moringa* leaves, making them a well-established choice for further investigation (El-Sherbiny et al., 2024).

Qualitative phytochemical screening of *Moringa oleifera* leaf extract revealed various secondary metabolites. Alkaloids transitioned from green to radish brown, indicating their presence, which is associated with antimicrobial properties (Yan et al., 2021). Flavonoids conformed after became green to colourless, can be considered as their antioxidant significance (Dias et al., 2021). Tannins deepened in green colour, supporting their antimicrobial role. Foam formation confirmed saponins, which contribute to immune modulation and antimicrobial activity. Terpenoids changed from green to radish brown, confirming their therapeutic potential (Villanueva et al., 2023). These findings support the traditional medicinal use and pharmacological potential of *Moringa oleifera*. These findings

highlight the rich phytochemicals composition of *Moringa oleifera*, further supporting its potential as a natural source of bioactive compounds with therapeutic applications, and the results are supported by other studies too (Onyekwere, 2014). 2014).

Table 1: Qualitative tests of phytochemicals.

Phytochemical	Initial colour	Final colour	Remarks
Alkaloids	Green	Radish brown	Positive
Flavonoids	Green	Colourless	Positive
Tannins	Green	Dark green	Positive
Saponins	No foam	Foam formation	Positive
Terpenoids	Green	Radish brown	Positive

Biochemical test of bacteria

Biochemical tests characterized five bacterial strains. *S. aureus* and *E. faecalis* were Gram-positive cocci, while *P. aeruginosa*, *E. coli*, and *K. pneumoniae* were Gram-negative rods. Catalase positivity varied among strains. Coagulase positivity in *S. aureus* indicated its pathogenicity. Oxidase was positive only for *P. aeruginosa*. Indole and MR tests confirmed *E. coli*'s fermentative capabilities, while *K. pneumoniae* tested VP positive, signifying butanediol fermentation. Hemolysis patterns varied, highlighting strain-specific metabolic traits (Table 2).

Antibacterial activity

Moringa oleifera extract at 500 mg/ml exhibited antibacterial activity against *S. aureus* (12 mm ZOI) and *P. aeruginosa* (17 mm ZOI), Similar results are reported in previous studies (Khayra et al., 2020). No inhibition was observed against *E. coli*, *E. faecalis* and *K. pneumoniae*, suggesting resistance at this concentration (Figure 1). The absence of inhibition at 500 mg/ml could be due to the low concentration of active compounds in the extract may also be insufficient to exert an antibacterial effect. Furthermore, several crucial factors must be considered, including the selection of the medium, pH level, agar depth, moisture content and incubation conditions, along with maintaining accurate inoculum density. The size of the inhibition zone can be influenced by the tested substance's solubility, diffusion capacity and evaporation rate. Additionally, if water-insoluble substances precipitate on the disc, they can obstruct the diffusion of antimicrobial agents into the agar (Bubonja-Šonje et al., 2020).

At 1000 mg/ml, *Moringa oleifera* extract demonstrated broader antibacterial efficacy. *P. aeruginosa* had the highest ZOI (20 mm), similar ZOI was observed in previous studies (Abalaka et al., 2012) using chloroform as a extracting solvent. *E. coli* and *S. aureus* showed inhibition zone of 11 mm and 12 mm, consistent with previous report (Garba et al., 2021) highlighted an inhibitory effect at high concentrations of *M. oleifera* extract, supporting the concentration-dependent activity. *E. faecalis* and *K. pneumoniae* showed inhibition zone of 16 mm and 12 mm respectively indicating concentration-dependent activity (Garba et al., 2021). However, *S. aureus* showed no increase in ZOI compared to 500 mg/ml, suggesting its susceptibility plateaued. Average result reported in previous studies is consistence with our study (Pal et al., 1995), however, a significant increase with the concentration that could be due the variations in strain resistance (Garba et al., 2021). Our findings from the research do align with previous studies that highlighted the bioactive secondary metabolites in *M. oleifera*, which exhibit antimicrobial effects against a range of pathogenic bacteria (Kasolo et al., 2010; El-Sherbiny et al., 2024; Soto et al., 2025).

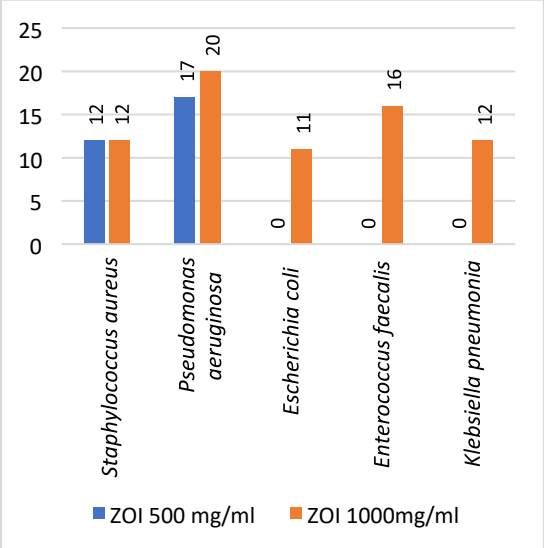

Figure 1: Comparative ZOI at 500 mg/ml and 1000 mg/ml concentrations.

Table 2: Biochemical test for bacteria.

Test	*S. aureus*	*P. aeruginosa*	*E. coli*	*E. faecalis*	*K. pneumonia*
Gram staining	+, cocci	-, rod	-, rod	+, cocci	-, rod
Catalase	+	+	+	-	+
Coagulase	+	-	-	-	-
Oxidase	-	+	-	-	-
Indole	-	-	+	-	-
MR	+	-	+	-	-
VP	+	-	-	+	+
Citrate	+	+	-	-	+
Urease	+	-	-	-	+
Hemolysis Beta	None	None	None	Alpha	None

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were performed on *Moringa oleifera* extract yielded negative results (Table 3). That could be due to the selection of appropriate methods is largely determined by the characteristics of the secondary metabolites, including its solubility and molecular weight. These factors affect how the compound interacts with the medium, influencing its diffusion, stability and overall effectiveness in experimental applications (Bubonja-Šonje et al., 2020). However, the minimum bactericidal concentration (MBC) revealed that there was the presence of more than 200 colonies. MBC/MIC ratio $\leq$ 4 indicating a bacteriostatic rather than a bactericidal effect at the tested concentrations. This finding suggests that *M. oleifera* may inhibit bacterial growth but may not completely eradicate the bacterial population at these specific concentrations. Compared to conventional antibiotics, the antibacterial efficacy of *M. oleifera* is modest, reinforcing its potential as an adjunct rather than a standalone antimicrobial agent. This supports previous findings that plant-based extracts can be effective against multidrug-resistant (MDR) bacteria (Al Alsheikh et al., 2020.; Košćak et al., 2023). However, it might require optimization in concentration or formulation for enhanced efficacy. This research suggests that further studies should focus on fractionating the extract to isolate specific bioactive compounds that are responsible for antibacterial action and then evaluate their synergistic effects with standard antibiotics.

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of different concentrations in bacterial strains.

Bacterial Strains	MIC (mg/ml)	MBC (mg/ml)	MBC Result at 500 mg/ml	MBC Result at 1000 mg/ml
E. coli	>1000	>1000	>200 colonies (No effect)	>200 colonies (No effect)
E. faecalis	>1000	>1000	>200 colonies (No effect)	>200 colonies (No effect)
K. pneumoniae	>1000	>1000	>200 colonies (No effect)	>200 colonies (No effect)
S. aureus	>1000	>1000	>200 colonies (No effect)	>200 colonies (No effect)
P. aeruginosa	>1000	>1000	>200 colonies (No effect)	>200 colonies (No effect)

Additionally, the study was limited to ethanol extracts which suggests the exploring of different solvents which could provide a broader understanding of the extract's full antimicrobial potential (Arora & Onsare, 2014). Due to resource constraints, the experiment could not be conducted at multiple concentrations to determine the precise MIC and MBC values. Therefore, the exact MIC/MBC ratio remains uncertain, and further dilution testing is required for a more accurate analysis.

Conclusion

Moringa oleifera leaf extract exhibits promising antibacterial activity against certain pathogens, particularly *Pseudomonas aeruginosa* and *Staphylococcus aureus*, but its efficacy is limited in liquid conditions, as indicated by the negative MIC and MBC results. Further research should explore higher extract concentrations, purification techniques, and possible synergistic effects with conventional antibiotics to enhance its potential as an alternative antimicrobial agent.

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