



● ORIGINAL ARTICLE

Phytochemical Screening and Antibacterial Activity of Yemeni *Moringa oleifera* Leaves Extract

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Abstract

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BACKGROUND

Moringa oleifera is a valuable medicinal plant widely cultivated in tropical and subtropical regions. Its leaves are abundant in vitamins, minerals, and essential phytochemicals with documented pharmacological activities. Previous studies have reported diverse therapeutic properties, including antibacterial, anti-diabetic, anti-inflammatory, antioxidant, cardiovascular, anti-ulcer, anti-allergic, and wound-healing properties. This study specifically focuses on evaluating the phytochemical composition and antibacterial potential of the methanolic extract of *Moringa oleifera* leaves cultivated in the Fuwah area of Al-Mukalla, Yemen, addressing a regional knowledge gap.

METHODS

Leaves were collected from Al-Mukalla, Yemen, authenticated, dried, and extracted by methanol using cold maceration. Phytochemical screening was conducted following standard protocols. *In vitro* antibacterial activity was assessed using the agar well diffusion method against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Klebsiella* spp., *Proteus* spp., *Escherichia coli*) bacterial strains.

RESULTS

Phytochemical analysis confirmed the presence of saponins, tannins, alkaloids, flavonoids, and phenols. The methanolic extract exhibited clear concentration-dependent antibacterial activity against *Staphylococcus aureus*, with inhibition zones of 13 mm, 17 mm, and 22 mm at 125, 250, and 500 mg/mL, respectively. No inhibitory effect was observed against any of the tested Gram-negative bacteria.

CONCLUSION

The methanolic extract of Yemeni *Moringa oleifera* leaves contains diverse bioactive compounds and demonstrates potent antibacterial activity against Gram-positive *S. aureus* with no clear effect on the tested Gram-negative strains.

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KEYWORDS

Antibacterial activity · *Moringa oleifera* · Phytochemical screening · Gram-positive bacteria · Gram-negative bacteria



Introduction

Medicinal plants have historically served and continue to serve as primary sources of drugs used in traditional, community, and modern medicines.¹ *Moringa oleifera*, a significant health-promoting plant within the *Moringaceae* family, is widely cultivated globally and has been recognized for its extensive nutritional and commercial uses.^{2,3} Traditionally, *Moringa oleifera* leaves have been used to address various health issues, including asthma, diabetes, hypertension, diarrhea, fever, cough, spasms, hepatomegaly and splenomegaly, infections, ulcers, inflammation, and wound healing.^{4,5}

A study on *Moringa oleifera* leaves, cultivated in Eastern Libya in 2021, which also employed methanol extraction, found significant antibacterial activity, markedly against Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and marked effects against Gram-negative bacteria (*Escherichia coli*).² The phytochemical screening in that study detected carbohydrates, alkaloids, and phenols. Similarly, fresh *Moringa oleifera* leaves were examined using methanol extraction and demonstrated inhibitory activity against Gram-positive bacteria and marked activity against Gram-negative bacteria (*Escherichia coli*), with phytochemical screening identifying carbohydrates, alkaloids, and phenols.¹ These studies provide a baseline for comparison with our methanolic extract from Yemeni origin.

Furthermore, a study on extraction and comparative evaluation conducted in Yemen investigated the phytochemical properties of *Moringa oleifera* seed oil and identified dominant fatty acids, notably lauric acid, myristic acid, and oleic acid.⁶ However, investigations specifically addressing the antibacterial properties of Yemeni *Moringa oleifera* leaves remain limited.

Antimicrobial resistance is a major, expanding worldwide issue that requires urgent attention. There are rising rates of multidrug-resistant infections globally, and specifically in Yemen where healthcare infrastructure faces significant challenges.²⁻⁷ Fighting dangerous bacteria, particularly drug-resistant pathogens, necessitates the development of novel, potent antibacterial therapies, including botanical medicines.²

According to searches in Google Scholar and PubMed databases, few previous studies have investigated the

phytochemical screening and antibacterial activity of *Moringa oleifera* leaves in Yemen. Therefore, the current study aims to identify the phytochemical composition of the methanolic extract of *Moringa oleifera* leaves obtained by maceration and to assess its antibacterial activity using the agar well diffusion method, in accordance with previously published research.²⁻⁶

Materials and Methods

This is an experimental laboratory-based study that assessed the phytochemical composition of the methanolic extract of *Moringa oleifera* leaves obtained by maceration and collected from Al-Mukalla, Yemen.

Plant Material

Fresh leaves of *Moringa oleifera* were collected from the Fuwah area in Al-Mukalla, Yemen (Figure 1). Identification and authentication were performed by a senior botanist at the Pharmacy Department, College of Medicine and Health Sciences, Hadhramout University. The leaves were then washed, air-dried at room temperature, ground into a fine powder using a mortar and pestle, and stored in 100 g aliquots.

Figure 1. Fresh *Moringa oleifera* leaves at the collection site, Fuwah area, Al-Mukalla, Yemen.



Chemicals, Reagents, Equipment, and Instruments

Equipment and Instruments

The equipment and instruments used in this study included an autoclave, water bath, Buchner funnel, sensitive balance, incubator, Petri dishes, filter papers, micro-tips, beakers, conical flasks, flame, test tubes, measuring cylinder, micropipette, round-bottom flask, wire loop, containers, gloves, sterile cotton swab, mortar, and pestle.

Chemicals and Reagents

The chemicals and reagents used in this study included methanol, distilled water, sodium hydroxide (NaOH), ferric chloride (FeCl_3), Dragendorff's reagent, and Mueller–Hinton agar (MHA).

Microorganisms

Clinical bacterial isolates were obtained from the maintained microorganism collection at Ibn Sina General Hospital, Al-Mukalla, Hadhramout, Yemen. The clinical bacterial isolates included the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli*, *Klebsiella* spp., and *Proteus* spp.

The isolates were subcultured on Mueller–Hinton agar and maintained at 4 °C until use. The identities of the isolate strains were confirmed using standard biochemical tests. Before antibacterial testing, each strain was standardized to 0.5 McFarland turbidity ($\approx 1.5 \times 10^8$ CFU/mL) to ensure reproducible inoculum density.

Processing and Extraction of Plant Material

Around 100 g of the powdered samples were cold-macerated in 1 L of analytical-grade methanol for five days.⁸ The resulting extract was dried and stored in sterile, airtight containers at 4 °C until further analysis. Extraction yield was calculated as the percentage of dried extract relative to the initial dry weight.

Phytochemical Screening

Qualitative phytochemical screening was performed according to standard protocols to detect the presence of major classes of phytoconstituents such as saponins, tannins, alkaloids, flavonoids, and phenolic compounds in the *Moringa oleifera* leaf extract.⁹

Test for Saponins (Froth Test)

One gram of the extract was weighed into a conical flask, 10 mL of distilled water was added, and the mixture was boiled for five minutes and then filtered. Subsequently, 2.5 mL of the filtrate was transferred to a test tube

containing 10 mL of distilled water. The mixture was shaken vigorously for 30 seconds and allowed to stand for 30 minutes. The formation of stable honeycomb froth (≥ 1 cm height) indicated the presence of saponins.⁹

Test for Tannins (Ferric Chloride Test)

Three to four drops of 10% ferric chloride solution were added to a portion of the extract diluted with distilled water. The appearance of a green color indicated the presence of tannins.

Test for Alkaloids (Dragendorff's Test)

One milliliter of Dragendorff's reagent was added to 1 mL of extract in a test tube. The formation of a cloudy orange or reddish-brown precipitate indicated the presence of alkaloids.¹⁰

Test for Flavonoids (Alkaline Reagent Test)

One milliliter of extract was added to 1 mL of NaOH solution and gently shaken. The appearance of yellow or red coloration indicated the presence of flavonoids.¹⁰

Test for Phenols (Ferric Chloride Test)

One milliliter of distilled water was mixed with 2 mL of extract and warmed to 45–50 °C. Then, 2 mL of 3% ferric chloride solution (FeCl_3) was added. The development of dark green or bluish-black coloration indicated the presence of phenolic compounds.¹¹ All tests were performed in triplicate to ensure consistency of observations.

In Vitro Antibacterial Activity Assay

The antibacterial activity of the methanolic leaf extract of *Moringa oleifera* was evaluated using the agar well diffusion method following CLSI-adapted procedures.^{1,12} The bacterial strains isolated were sub-cultured on Mueller–Hinton agar (MHA) and incubated at 37 °C for 18–24 hours to obtain fresh cultures.

Agar Well Diffusion Method

Mueller–Hinton agar (MHA) was prepared by dissolving 38 g of agar powder in 1 L of distilled water. The mixture was heated with continuous stirring over a flame until completely dissolved. It was then autoclaved at 121 °C for 15 minutes, followed by a 15-minute warming period inside the autoclave. The medium was allowed to cool for several minutes, poured into sterile Petri dishes, and left to dry.

Inoculation and Preparation

A sterile cotton swab was used to inoculate the bacterial suspension onto the surface of the agar. Using a sterile test tube, four 10 mm diameter cups were cut into each

plate, and the agar discs were removed. Using an automatic microliter pipette, two alternate cups were filled with 0.2 mL of the extract sample at different concentrations (500, 250, and 125 mg/mL). The remaining two cups received the respective solvent as a negative control and the standard antibiotic ceftriaxone 400 µg/mL as a positive control.

Incubation and Measurement

The plates were allowed to stand at room temperature for two hours to facilitate diffusion. They were then incubated in an upright position at 37 °C for 18–24 hours. Two replicates were carried out for every extract concentration against each test organism. Following incubation, the diameters of growth inhibition zones were measured in millimeters using a digital caliper, averaged, and recorded as mean values.^{1,12}

Data Analysis

Antibacterial activity was interpreted based on inhibition zone diameters. Extracts producing inhibition zones ≥ 10 mm were considered active. Results were summarized descriptively due to the qualitative nature of phytochemical screening and the limited number of replicates.

Results

Extraction Yield

Fresh leaves of *Moringa oleifera* equivalent to 100 g dry weight yielded 13.15 g of crude methanolic extract, corresponding to an extraction yield of 13.15%. The extract was dark green, viscous, and readily soluble in methanol.

Phytochemical Screening

Qualitative phytochemical analysis of the methanolic extract revealed the presence of all five tested phytochemical groups. Table 1 summarizes the results, with relative intensities indicated according to the

strength of the observed reactions. As shown in Table 1, the methanolic extract of Yemeni *Moringa oleifera* leaves contains saponins, tannins, alkaloids, flavonoids, and phenolic compounds, with tannins, alkaloids, and flavonoids showing strong positive reactions. These findings indicate a diverse phytochemical profile with multiple classes of bioactive compounds.

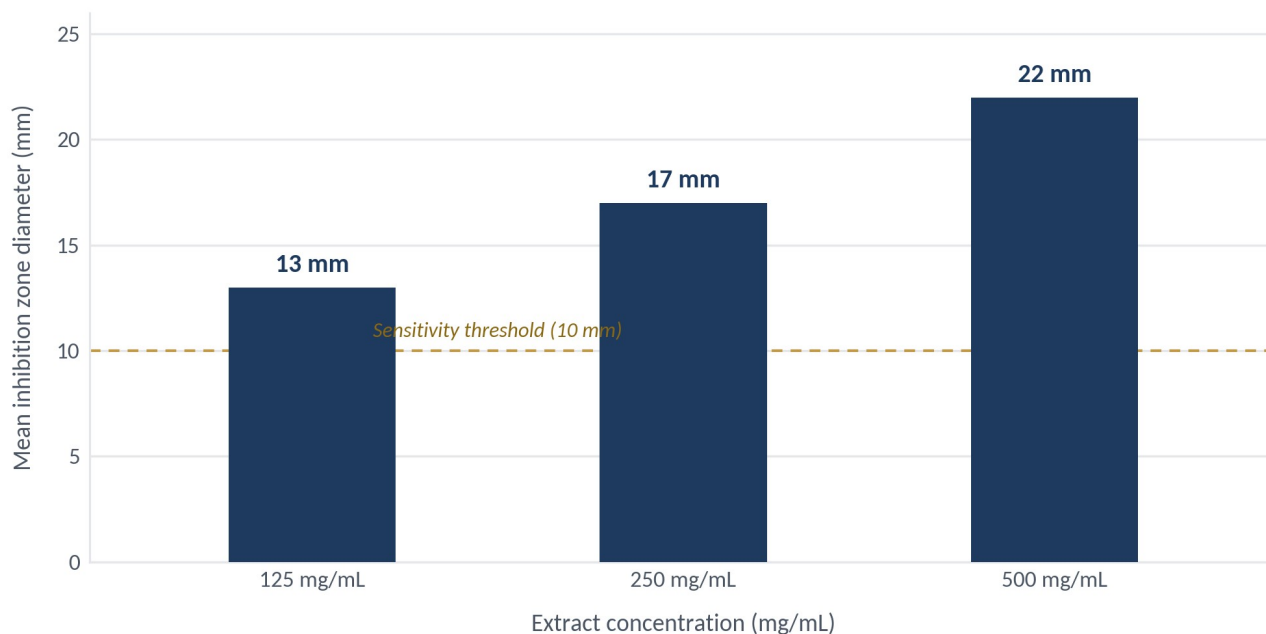
Table 1. Qualitative phytochemical composition of the methanolic extract of *Moringa oleifera* leaves.

Phytochemical class	Test performed	Intensity*
Saponins	Froth test	+
Tannins	Ferric chloride test	++
Alkaloids	Dragendorff's test	++
Flavonoids	Alkaline reagent test	++
Phenolic compounds	Ferric chloride test	+

* Intensity was based on the observed strength of each reaction: + = weak to moderate presence; ++ = strong presence.

Antibacterial Activity

The antibacterial activity of the methanolic extract of *Moringa oleifera* leaves at concentrations of 125, 250, and 500 mg/mL was evaluated against the Gram-positive bacterium *Staphylococcus aureus* and three Gram-negative bacterial strains: *Escherichia coli*, *Klebsiella* spp., and *Proteus* spp. Methanol was used as a negative control, while ceftriaxone (400 µg/mL) was used as the positive control. Results are expressed as mean inhibition zone diameters (mm) obtained from duplicate experiments. The results demonstrated that the methanolic extract exhibited clear, concentration-dependent antibacterial activity exclusively against *Staphylococcus aureus*. The inhibition zone diameters increased progressively with increasing extract concentration (Figure 2). All tested concentrations (125–500 mg/mL) produced inhibition zones exceeding the 10 mm sensitivity threshold, indicating susceptibility. Mean inhibition zone diameters increased progressively with extract concentration: **125 mg/mL**: 13 mm, **250 mg/mL**: 17 mm, and **500 mg/mL**: 22 mm. This pattern demonstrates a consistent dose-response relationship.

Figure 2. Mean inhibition zone diameters of *Moringa oleifera* methanolic leaf extract against *Staphylococcus aureus* (125–500 mg/mL).

Values are means of duplicate experiments. The dashed line marks the 10 mm sensitivity threshold applied in this study.

In contrast, no inhibitory effect was observed against any of the Gram-negative bacterial strains tested (*E. coli*, *Klebsiella* spp., *Proteus* spp.), even at the highest concentration of 500 mg/mL. The positive control ceftriaxone produced expected inhibition zones against all strains. The negative control, methanol, showed no inhibition, confirming that the observed antibacterial activity was attributable to the extract components rather than to the solvent.

Discussion

This study investigated the phytochemical composition and antibacterial activity of the methanolic extract from *Moringa oleifera* leaves cultivated in Fuwah, Al-Mukalla, Yemen. The methanolic extract had a sticky, dark-green appearance and an extraction yield of 13.15%. The extraction yield obtained in this study is comparable to the 14.68% yield reported in a recent Nepali study (2023) using a similar methodology.¹³

Phytochemical Composition

Phytochemical screening confirmed the presence of saponins, tannins, alkaloids, flavonoids, and phenolic compounds in the methanolic extract. These findings are consistent with previous studies, although some variations in the detected phytochemical constituents have been reported across different geographical regions.^{1,2,13}

The Libyan study published in 2021 using methanol extraction revealed the presence of alkaloids, phenols, and flavonoids but did not detect tannins or saponins.² Similarly, a study published in 2020 identified alkaloids, tannins, and phenols but did not report the presence of flavonoids.¹ In contrast, the more recent Nepali study (2023) and the present study both detected all five phytochemical classes. These findings suggest that extraction methodology, plant maturity, or geographical factors may influence the phytochemical profiles of *Moringa oleifera*.¹³ The consistency between the present findings and those of the 2023 Nepali study reinforces the reproducibility of phytochemical profiles and suggests that *Moringa oleifera* from different geographical regions may share several core phytochemical characteristics.

The presence of these phytochemical compounds is noteworthy due to their well-documented therapeutic properties. Saponins exhibit antimicrobial and immunomodulatory activities, primarily through membrane permeabilization.¹⁴ Tannins exhibit astringent and antimicrobial effects through multiple mechanisms, including protein precipitation and enzyme inhibition.¹⁵ Alkaloids display a range of pharmacological activities, including the ability to intercalate with bacterial DNA.^{16,17} Flavonoids function as potent antioxidants and possess anti-inflammatory and antimicrobial properties through mechanisms such as membrane disruption and enzyme inhibition.^{18,19}

Phenols act as effective antimicrobial agents by disrupting bacterial cell membranes and denaturing proteins.²⁰ The identification of these phytochemical constituents in Yemeni *Moringa oleifera* leaves supports the potential biological activity of the plant and provides a basis for further investigation of its possible pharmaceutical applications.

Antibacterial Activity

The evaluation of antibacterial activity revealed that the methanolic extract of Yemeni *Moringa oleifera* leaves

exhibited strong, concentration-dependent antibacterial activity against *Staphylococcus aureus*, with inhibition zones ranging from 13 to 22 mm. Notably, even the lowest concentration tested (125 mg/mL) produced an inhibition zone exceeding the 10 mm sensitivity criterion used in this study. This activity can be compared with findings from previous studies, as summarized in Table 2.

Table 2. Comparative antibacterial activity of *Moringa oleifera* extracts against *S. aureus*.

Study	Extract type	Concentration (mg/mL)	Mean inhibition zone (mm)
Current study	Methanol	125	13
		250	17
		500	22
Gupta et al. 2023 ¹³	Ethanol	200	9
		400	15
		600	18
		800	22
Bagheri et al. 2020 ¹	Methanol	50	9

Under the conditions tested, the methanolic extract in the present study produced greater inhibition than that reported in the 2023 Nepali study using ethanolic extraction.¹³ At 125 mg/mL, the present study produced a 13 mm inhibition zone, whereas the previous study reported only 9 mm at 200 mg/mL. At 250 mg/mL, the present extract produced a 17 mm inhibition zone compared with 15 mm at 400 mg/mL in the previous study. At 500 mg/mL, the present extract produced a 22 mm inhibition zone, which was greater than the 18 mm zone reported at 600 mg/mL and comparable to the 22 mm zone reported at 800 mg/mL.

The complete absence of antibacterial activity against the Gram-negative bacteria tested (*E. coli*, *Klebsiella* spp., *Proteus* spp.) is consistent with previous studies demonstrating that *Moringa oleifera* extracts exhibit greater antibacterial activity against Gram-positive bacteria.^{1,2,13} This difference in susceptibility may be related, at least in part, to structural differences between the cell envelopes of Gram-positive and Gram-negative bacteria.

Conclusion

The present study demonstrated that the methanolic extract of *Moringa oleifera* leaves cultivated in Yemen contains a wide range of active phytochemical compounds, including saponins, tannins, alkaloids,

flavonoids, and phenolic compounds. These constituents may contribute to the plant's biological properties, particularly its antibacterial activity. The extract exhibited significant, concentration-dependent inhibitory effects against *Staphylococcus aureus*, with inhibition zones ranging from 13–22 mm at concentrations of 125–500 mg/mL. However, no activity was observed against the Gram-negative bacteria tested (*Escherichia coli*, *Klebsiella* spp., and *Proteus* spp.). The observed difference in susceptibility may be attributed to structural differences in bacterial cell walls. This study contributes to the growing body of evidence supporting the antibacterial potential of *Moringa oleifera* and provides preliminary phytochemical and antibacterial characterization of *Moringa oleifera* leaves cultivated in Yemen. The findings address a regional knowledge gap and provide a foundation for further investigations into the chemical composition, mechanisms of action, and therapeutic potential of Yemeni *Moringa oleifera*.

Limitations of the Study

A major limitation of the present study is that the phytochemical analysis was limited to qualitative screening of major classes of phytochemicals. While this approach provides preliminary evidence of the presence of potentially bioactive constituents in the *Moringa*

oleifera leaf extract, it does not allow for the identification or quantification of individual compounds or their concentrations.

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Conflict of Interest

The authors declare that they have no conflicts of interest related to this study.

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Authors' Contributions

Mohammed Hassan supervised the study and **Abubakr A. Hammad** co-supervised it. **Aseela Altaffi** assisted with the microbiological laboratory work. **Abdullah Khanbri** coordinated the student research group and prepared the abstract. **Ahmed Baothman** prepared the introduction. **Mohammad Al-Sadi, Qusai Barajeh** and **Saleh Mgower** prepared the materials and methods. **Fatima Al-Jaberi** and **Hania Al-Sebaili** prepared the processing and extraction section. **Abrar Al-Hoson** and **Nojoud Bin Makhshin** prepared the results. **Somia Bashan** and **Somia Bajry** prepared the discussion. **Mohammad Al-Maqdi, Taha Al-Habshi** and **Saleh Basleb** prepared the conclusion, data analysis and limitations. **Al-Anood Al-Shamasi** and **Salem Saeed** wrote, organized and formatted the manuscript. All authors reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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