

In-vitro Antibacterial Activity of *Moringa oleifera* Leaf Extract Against *Staphylococcus aureus* and *Escherichia coli* Using Disc Diffusion Method

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Abstract

Original Research Article

Infectious diseases caused by pathogenic microorganisms remain an important public health concern, while the misuse and overuse of antibiotics contribute to antimicrobial resistance. Medicinal plants contain various phytochemicals and have traditionally been used for the management of infectious conditions. The present study evaluated the in-vitro antibacterial activity of *Moringa oleifera* leaf extract against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method. Leaves of *M. oleifera* were collected from the Botanical Garden of Lydia College of Pharmacy, East Godavari District, Andhra Pradesh, authenticated, dried, powdered and extracted with ethanol. Preliminary phytochemical screening was performed, and the antibacterial activity of the extract was evaluated at concentrations of 100, 200, 300, 400 and 500 mg/mL using 6-mm filter-paper discs containing 20 µL of test solution on Mueller–Hinton agar. *S. aureus* and *E. coli* were identified using morphological, staining and biochemical characteristics, and Ciprofloxacin was used for comparison. The extract showed measurable antibacterial activity against both organisms, with inhibition zones against *S. aureus* of 8, 10, 12, 14 and 16 mm, and against *E. coli* of 7, 9, 11, 13 and 15 mm at the respective concentrations. Ciprofloxacin produced inhibition zones of 16–24 mm against *S. aureus* and 15–23 mm against *E. coli*. Phytochemical screening indicated the presence of carbohydrates, proteins, alkaloids, glycosides, saponins, tannins, flavonoids and polyphenolic constituents. The findings demonstrate measurable in-vitro antibacterial activity of ethanolic *M. oleifera* leaf extract against both test organisms, with increasing inhibition-zone diameters at higher extract concentrations. Further studies are required to investigate the active antibacterial constituents and their potential applications.

Keywords: *Moringa oleifera*; antibacterial activity; *Staphylococcus aureus*; *Escherichia coli*; disc diffusion; phytochemicals; medicinal plants; Ciprofloxacin.

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1. INTRODUCTION

Infectious diseases are caused by pathogenic microorganisms such as bacteria, viruses, fungi and parasites. Bacterial infections remain an important cause of human illness, and antibiotics are widely used for their treatment. However, inappropriate and excessive use of antibiotics has contributed to the development of antimicrobial resistance. Therefore, there is increasing interest in identifying new antibacterial agents from natural sources.

Medicinal plants are important sources of biologically active compounds. They contain various phytochemicals, including alkaloids, flavonoids, tannins, phenolic compounds, saponins and glycosides, which

have been investigated for their antibacterial and other pharmacological properties. Scientific evaluation of medicinal plants, including preliminary phytochemical screening, can provide evidence for their traditional uses and help identify potentially active plant constituents.

Moringa oleifera Lam., belonging to the family Moringaceae, is commonly known as the drumstick tree or miracle tree and is widely cultivated in tropical and subtropical regions. Different parts of the plant, particularly the leaves, have traditionally been used for medicinal and nutritional purposes. The leaves contain several classes of phytochemicals, including alkaloids, flavonoids, tannins, saponins and glycosides, and

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previous studies have investigated the antimicrobial properties of *M. oleifera* extracts.

The present study evaluated the in-vitro antibacterial activity of ethanolic *M. oleifera* leaf extract against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method. *S. aureus* is a Gram-positive organism, whereas *E. coli* is a Gram-negative organism. Five concentrations of the leaf extract were tested, and the antibacterial activity was compared with Ciprofloxacin. The study also included preliminary phytochemical screening to identify the major classes of phytoconstituents present in the extract.

2. AIM AND OBJECTIVES

2.1 Aim

To evaluate the in-vitro antibacterial activity of *Moringa oleifera* leaf extract against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method.

2.2 Objectives

1. To collect and authenticate the leaves of *Moringa oleifera*.
2. To prepare the leaf extract using ethanol.
3. To perform preliminary phytochemical screening.
4. To isolate and identify *S. aureus* and *E. coli*.
5. To prepare different concentrations of the plant extract.
6. To evaluate antibacterial activity using the disc diffusion method.
7. To measure the zones of inhibition.
8. To compare the activity with Ciprofloxacin.
9. To analyze and interpret the experimental findings.
10. To provide preliminary evidence regarding the antibacterial potential of *M. oleifera*.

3. MATERIALS AND METHODS

3.1 Collection and Authentication of Plant Material

Moringa oleifera leaves were collected from the Botanical Garden of Lydia College of Pharmacy, East Godavari District, Andhra Pradesh, India. The plant material was identified by a taxonomist in the Botany Department at Andhra University.

The leaves were washed with distilled water and cool-dried for approximately two weeks. The dried leaves were powdered using a mortar and pestle, sieved and stored in a covered plastic container until extraction.

3.2 Preparation of Ethanolic Extract

The powdered leaves were extracted using ethanol as the solvent. The resulting extract was concentrated using a rotary evaporator under reduced pressure.

The exact starting weight of plant material, volume of ethanol, extraction duration and percentage extraction yield should be inserted from the original laboratory record before submission.

3.3 Preliminary Phytochemical Screening

Standard qualitative phytochemical tests were performed to determine the presence of major phytoconstituents. The investigation included tests for carbohydrates, proteins, alkaloids, glycosides, saponins, tannins, flavonoids and polyphenolic constituents.

The reactions were recorded as absent (-), present (+) or highly present (++)

3.4 Test Microorganisms

The organisms investigated were:

1. *Staphylococcus aureus* – Gram-positive bacterium
2. *Escherichia coli* – Gram-negative bacterium

According to the presentation, contaminated food and water samples were collected in sterile containers and transported to the microbiology laboratory. Portions of the samples were inoculated into nutrient broth and incubated at 37°C for 24 hours. Cultures were subsequently streaked onto nutrient agar and MacConkey agar for isolation.

3.5 Identification of *Staphylococcus aureus*

Identification was performed using colony morphology, Gram staining, catalase, coagulase, mannitol fermentation, oxidase, hemolysis and growth in 7.5% sodium chloride.

Purple cocci arranged in clusters on Gram staining, catalase positivity, coagulase positivity, mannitol fermentation and other supporting characteristics were used for identification of the presumptive *S. aureus* isolate.

3.6 Identification of *Escherichia coli*

The presumptive *E. coli* isolate was evaluated by colony morphology, MacConkey agar reaction, Gram staining, indole, methyl red, Voges-Proskauer, citrate and oxidase tests.

3.7 Maintenance of Cultures

Confirmed isolates were maintained on nutrient agar slants at 4°C. Fresh cultures were prepared in nutrient broth before antibacterial experimentation.

3.8 Culture Media

Nutrient broth, nutrient agar, MacConkey agar and Mueller-Hinton agar were used. Mueller-Hinton agar was used for the antibacterial disc diffusion assay. Media were prepared according to manufacturer instructions and sterilized by autoclaving at 121°C for 15 minutes.

3.9 Preparation of Extract Concentrations

Five concentrations of the ethanolic *M. oleifera* leaf extract were prepared:

- 100 mg/mL
- 200 mg/mL
- 300 mg/mL
- 400 mg/mL
- 500 mg/mL

3.10 Preparation of Extract Discs

Sterile 6-mm filter-paper discs were used. Twenty microlitres of each extract concentration was loaded onto individual discs under aseptic conditions. The discs were allowed to dry and were labelled according to concentration.

3.11 Preparation of Ciprofloxacin Discs

According to the presentation, Ciprofloxacin solutions were prepared at 100–500 mg/mL and 20 µL of each solution was loaded onto sterile 6-mm filter-paper discs.

Note: For publication, these should be described as laboratory-prepared Ciprofloxacin solution-impregnated discs rather than standardized commercial susceptibility discs unless commercial discs were actually used.

3.12 Inoculum Preparation

Bacterial suspensions were prepared in saline and adjusted to a 0.5 McFarland standard before inoculation.

3.13 Disc Diffusion Assay

Mueller–Hinton agar plates were inoculated with standardized bacterial suspensions using sterile swabs to obtain uniform bacterial lawns. Extract- and Ciprofloxacin-impregnated discs were placed onto the agar surface using sterile forceps.

Four experimental plates were described in the presentation:

- Plate 1: *S. aureus* + *M. oleifera* extract
- Plate 2: *S. aureus* + Ciprofloxacin
- Plate 3: *E. coli* + *M. oleifera* extract
- Plate 4: *E. coli* + Ciprofloxacin

3.14 Incubation

The inoculated plates were incubated at 37°C for 24 hours. Plates were incubated in an inverted position to minimize interference from condensation.

3.15 Measurement of Zone of Inhibition

After incubation, clear zones surrounding the discs were examined. The diameter of each inhibition zone was measured in millimetres using a transparent ruler or vernier caliper. Measurements included the disc diameter.

3.16 Statistical Presentation

The presentation states that experiments were performed in triplicate and results were expressed as mean ± standard deviation. The graphs in the presentation indicate an error range of approximately ±0.75–1.0 mm.

Because individual replicate measurements and exact SD values were not provided in the presentation, the numerical tables below reproduce the reported mean values and do not independently recalculate statistical significance.

4. RESULTS

4.1 Preliminary Phytochemical Screening

The qualitative phytochemical screening demonstrated the presence of several phytoconstituent classes in the ethanolic leaf extract of *M. oleifera*. The findings are summarized in Table 1.

Table 1: Preliminary phytochemical screening of ethanolic *Moringa oleifera* leaf extract

Phytoconstituent	Qualitative finding
Carbohydrates	Present (+)
Proteins	Present (+)
Alkaloids	Present (+)
Glycosides	Present (+)
Saponins	Highly present (++)
Tannins	Present (+)
Flavonoids	Present (+)
Polyphenols	Highly present (++)

Note: The presentation uses – for absent, + for present and ++ for highly present. Individual test reactions should be retained in the final manuscript if the original phytochemical-results sheet is available.

The results summarized in Table 1 indicate that the ethanolic extract contained multiple classes of phytoconstituents. The presence of flavonoids, tannins, saponins and polyphenolic constituents is particularly relevant because these classes have been investigated for biological activities including antimicrobial effects.

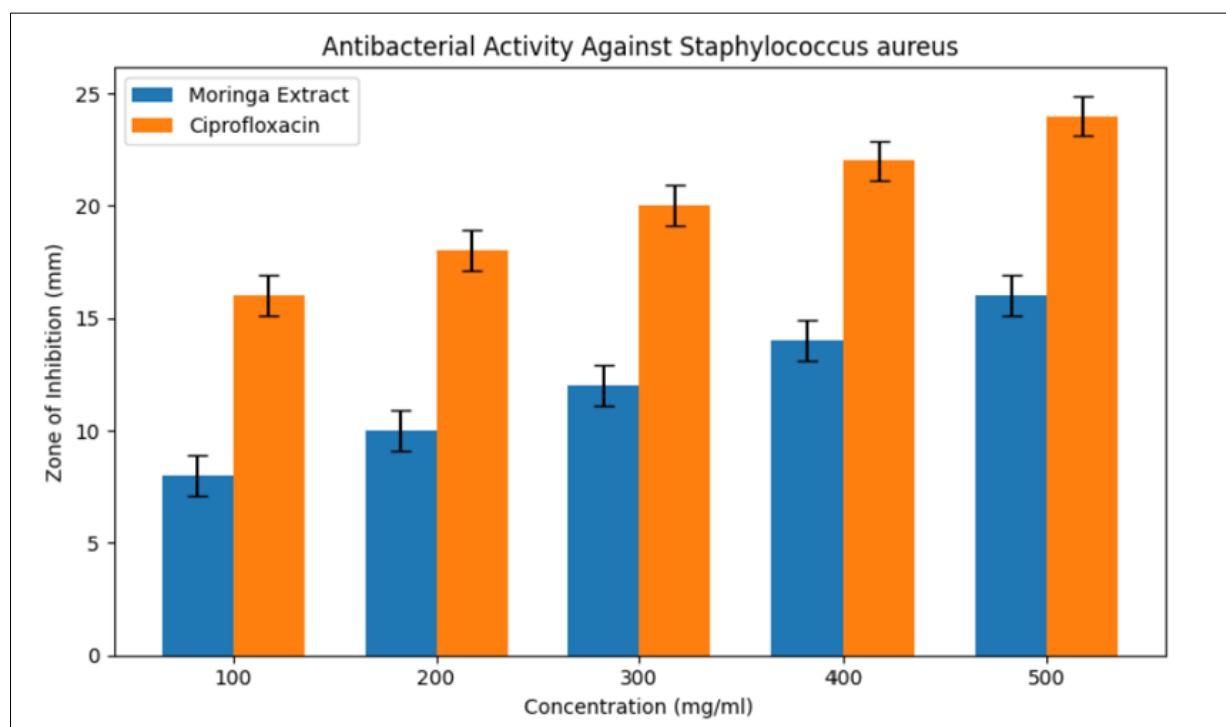
5. ANTIBACTERIAL ACTIVITY

5.1 Antibacterial Activity Against *Staphylococcus aureus*

The antibacterial activity of the ethanolic *M. oleifera* leaf extract against *S. aureus* is presented in

Table 2: Zone of inhibition produced by *Moringa oleifera* extract and Ciprofloxacin against *Staphylococcus aureus*

Concentration (mg/ml)	Zone of Inhibition (mm) – Moringa Extract	Zone of Inhibition (mm) – Ciprofloxacin
100	8	16
200	10	18
300	12	20
400	14	22
500	16	24



The concentration-dependent pattern shown in Table 2 is also illustrated graphically in Figure 1.

The values in Table 2 demonstrate that the inhibition zone produced by the *M. oleifera* extract increased from 8 mm at 100 mg/mL to 16 mm at 500 mg/mL. Thus, an 8-mm increase in inhibition-zone diameter was observed across the tested concentration range.

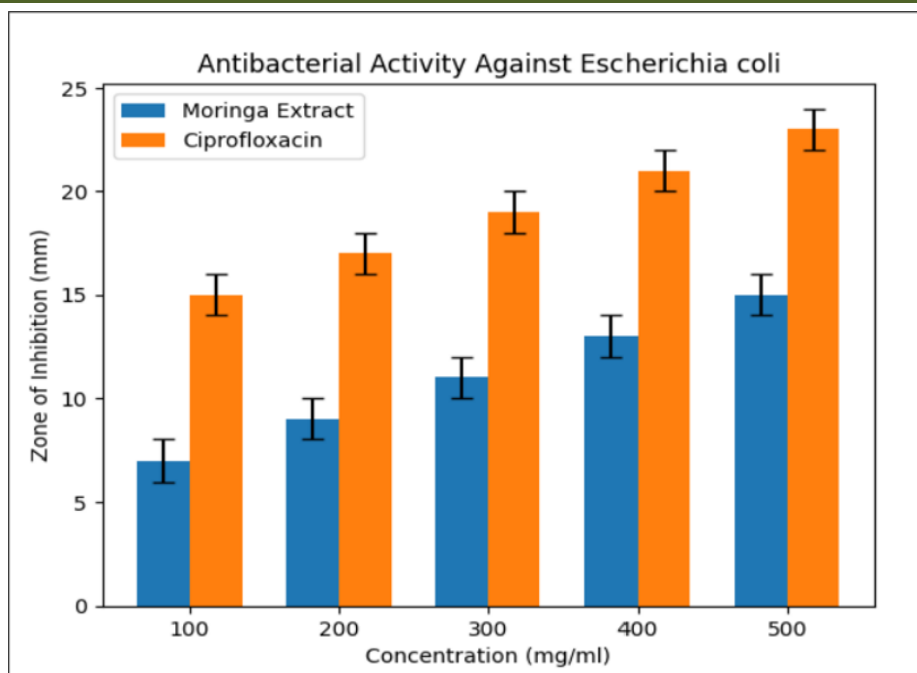
Ciprofloxacin produced inhibition zones of 16, 18, 20, 22 and 24 mm, respectively. The corresponding values were consistently 8 mm greater than those observed for the plant extract.

5.2 Antibacterial Activity Against *Escherichia coli*

The antibacterial activity against *E. coli* is summarized in

Table 3: Zone of inhibition produced by *Moringa oleifera* extract and Ciprofloxacin against *Escherichia coli*

Concentration (mg/ml)	Zone of Inhibition (mm) – Moringa Extract	Zone of Inhibition (mm) – Ciprofloxacin
100	7	15
200	9	17
300	11	19
400	13	21
500	15	23



According to Table 3, the *M. oleifera* extract produced inhibition zones of 7, 9, 11, 13 and 15 mm at 100, 200, 300, 400 and 500 mg/mL, respectively.

Ciprofloxacin produced corresponding inhibition zones of 15, 17, 19, 21 and 23 mm. Therefore, the difference between the reported Ciprofloxacin and extract values was 8 mm at each tested concentration. The concentration-associated increase in inhibition is illustrated in Figure 2.

6. COMPARATIVE INTERPRETATION OF THE ANTIBACTERIAL RESULTS

The numerical findings from Tables 2 and 3 show that the *M. oleifera* extract produced measurable inhibition against both test organisms at all concentrations.

At 100 mg/mL, the extract produced an inhibition zone of 8 mm against *S. aureus* and 7 mm against *E. coli*. At 500 mg/mL, these values increased to 16 mm and 15 mm, respectively.

Similarly, Ciprofloxacin produced zones of 16 and 15 mm against *S. aureus* and *E. coli*, respectively, at 100 mg/mL. At 500 mg/mL, the corresponding zones increased to 24 and 23 mm.

Therefore, the reported data show a consistent increase of 2 mm in inhibition-zone diameter for every 100 mg/mL increase in extract concentration for both organisms. The same 2-mm increment was observed for the reported Ciprofloxacin values.

The results also show that the *S. aureus* inhibition zone was 1 mm greater than the *E. coli* inhibition zone at every corresponding concentration of

the plant extract. This pattern is visible in Table 2, Table 3, Figure 1 and Figure 2.

7. DISCUSSION

The present study evaluated the in-vitro antibacterial activity of ethanolic *Moringa oleifera* leaf extract against *Staphylococcus aureus* and *Escherichia coli*. The phytochemical screening presented in Table 1 showed the presence of carbohydrates, proteins, alkaloids, glycosides, saponins, tannins, flavonoids and polyphenolic constituents. These phytochemical classes may contribute to the biological properties of the extract; however, qualitative screening alone cannot determine which constituent is responsible for the observed antibacterial activity. The antibacterial results in Tables 2 and 3 and Figures 1 and 2 showed a concentration-associated increase in inhibition-zone diameter. Against *S. aureus*, the extract produced zones from 8 mm at 100 mg/mL to 16 mm at 500 mg/mL, while against *E. coli*, the zones increased from 7 mm to 15 mm over the same concentration range. Thus, an overall increase of 8 mm was observed for both organisms. The extract consistently produced a 1-mm larger zone against *S. aureus* than *E. coli* at each concentration. This difference may be related to differences in the structural characteristics of Gram-positive and Gram-negative bacterial envelopes; however, the present study did not investigate the mechanism of action, so this explanation cannot be established from the experimental data.

The comparison with Ciprofloxacin, shown in Tables 2 and 3, demonstrated larger inhibition zones for Ciprofloxacin under the experimental conditions reported. Against *S. aureus*, Ciprofloxacin produced zones of 16–24 mm, compared with 8–16 mm for the extract, while against *E. coli*, it produced 15–23 mm, compared with 7–15 mm for the extract. The findings

therefore demonstrate measurable antibacterial activity of *M. oleifera* leaf extract and are consistent with the general rationale for investigating *M. oleifera* as a medicinal plant, as also reflected in previous studies cited in the presentation. However, inhibition-zone diameter represents an in-vitro screening outcome and should not be considered direct evidence of clinical effectiveness, because agar diffusion is influenced by factors such as concentration, loading volume, molecular characteristics and diffusion properties. Further studies should therefore determine minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and the active constituents responsible for the observed antibacterial activity.

8. CONCLUSION

The present study demonstrated that ethanolic *Moringa oleifera* leaf extract possesses measurable in-vitro antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Phytochemical screening showed the presence of carbohydrates, proteins, alkaloids, glycosides, saponins, tannins, flavonoids and polyphenols. The extract produced inhibition zones of 8–16 mm against *S. aureus* and 7–15 mm against *E. coli* at concentrations of 100–500 mg/mL, respectively, with inhibition increasing as the extract concentration increased. Ciprofloxacin produced larger inhibition zones under the experimental conditions reported.

13. Conflict of interest: The authors declare that there is no conflict of interest associated with the present study.

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