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PHYTOCHEMICAL PROFILING AND ANTIBACTERIAL ACTIVITY OF MORINGA OLEIFERA LEAF EXTRACT AGAINST OCULAR PATHOGENS

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ABSTRACT

Ocular bacterial infections caused by *Streptococcus pyogenes* and *Staphylococcus aureus* remain a significant clinical concern, particularly with increasing antimicrobial resistance. Plant-derived bioactive compounds have attracted attention as alternative therapeutic agents. This study evaluated the phytochemical composition and antibacterial activity of ethanol leaf extract of *Moringa oleifera* against selected ocular pathogens. Fresh leaves of *M. oleifera* were air-dried, pulverized, and extracted using 70% ethanol. Qualitative and quantitative phytochemical analyses were conducted using standard procedures. Antibacterial activity was evaluated against *S. pyogenes* and *S. aureus* using the agar well diffusion method at concentrations of 1.5, 2, and 3 mg/mL. Ciprofloxacin served as the positive control. Zones of inhibition were measured after 24 h incubation at 37 °C. Total viable bacterial counts and recovery duration were also determined. Data were expressed as mean ± SD (n = 3) and analyzed using ANOVA and paired t-test at $P < 0.05$. Phytochemical screening revealed the presence of flavonoids (1258.07 ± 13.30 µg/g), phenols (570.47 ± 10.03 µg/g), saponins (456.53 ± 5.62 µg/g), alkaloids (8.20 ± 0.35 µg/g), and tannins (20.07 ± 0.44 µg/g), with flavonoids being the most abundant. The extract demonstrated concentration-dependent antibacterial activity. At 3 mg/mL, the highest inhibition was observed against *S. pyogenes* (34.67 ± 0.57 mm), which was comparable to ciprofloxacin (24.67 ± 3.51 mm). Against *S. aureus*, ciprofloxacin showed greater inhibition (43.33 ± 3.52 mm) compared to the extract (17.67 ± 2.52 mm). Both treatments significantly reduced total viable bacterial counts ($P < 0.05$). Recovery duration was shorter in the ciprofloxacin-treated group (3.67 ± 0.58 days) compared to the extract-treated group (5.67 ± 0.58 days). Ethanol leaf extract of *M. oleifera* exhibited significant antibacterial activity against ocular pathogens, likely attributable to its rich flavonoid and phenolic content. Although ciprofloxacin demonstrated superior efficacy against *S. aureus*, the extract showed strong inhibitory activity against *S. pyogenes*, suggesting its potential as an alternative or adjunct antimicrobial agent.

KEYWORDS: *Moringa oleifera*, ocular infection, antibacterial activity, phytochemicals, *Streptococcus pyogenes*, *Staphylococcus aureus*.

1.0. INTRODUCTION

Ocular infections remain a significant public health concern worldwide, particularly in low- and middle-income countries where access to appropriate antimicrobial therapy may be limited. Bacterial conjunctivitis and related external eye infections are commonly caused by Gram-positive organisms such as *Streptococcus pyogenes* and *Staphylococcus aureus*. These pathogens are capable of inducing inflammation, purulent discharge, corneal damage, and in severe cases, visual impairment (Azari & Barney, 2013; Ayehubizu *et al*., 2021). The increasing emergence of antibiotic-resistant strains, including methicillin-resistant *S. aureus* (MRSA), has further complicated treatment outcomes and underscores the urgent need for alternative or adjunct antimicrobial agents (Lee *et al*., 2018).

Medicinal plants have gained renewed scientific attention as potential sources of bioactive compounds with antimicrobial properties. Among them, *Moringa oleifera* Lam., commonly known as the drumstick tree, has been extensively investigated for its nutritional and pharmacological value. Several studies have reported that *M. oleifera* leaves are rich in flavonoids, phenolic

acids, alkaloids, tannins, and saponins, compounds known to exert antibacterial, antioxidant, and anti-inflammatory activities (Siddhuraju & Becker, 2003; Vergara-Jimenez *et al*., 2017). Ethanol extracts of *M. oleifera* leaves have demonstrated significant inhibitory activity against clinical isolates of *S. aureus* and other Gram-positive bacteria, suggesting disruption of bacterial cell membranes and interference with essential metabolic pathways (Chen *et al*., 2020; Abdull Razis *et al*., 2022).

Recent investigations have further shown that phytochemicals such as quercetin, kaempferol, and chlorogenic acid identified in *M. oleifera* leaves exhibit bactericidal or bacteriostatic effects through membrane destabilization, inhibition of nucleic acid synthesis, and suppression of biofilm formation (El-Sherbiny *et al*., 2024; Chiș *et al*., 2023). These multi-target mechanisms may provide an advantage over conventional antibiotics, which often act on single molecular targets and are therefore more susceptible to resistance development.

Despite the growing body of evidence supporting the antimicrobial potential of *M. oleifera*, limited studies have specifically evaluated its efficacy against ocular pathogens under controlled experimental conditions. Furthermore, comparative evaluation with standard antibiotics such as ciprofloxacin in ocular applications remains underexplored. Therefore, this study aimed to investigate the phytochemical composition and antibacterial activity of ethanol leaf extract of *M. oleifera* against *S. pyogenes* and *S. aureus*, with comparative assessment against ciprofloxacin. The findings may contribute to the development of plant-based therapeutic agents for managing ocular bacterial infections.

2. MATERIALS AND METHOD

2.1 Plant Material Collection and Authentication

Fresh leaves of *Moringa oleifera* were collected from a local farm in Ihiagwa Community in Owerri West L.G.A. Imo State, Nigeria. The plant material was authenticated by a taxonomist at the Department of Biotechnology, Federal University of Technology Owerri, where a voucher specimen was deposited for future reference. The leaves were washed thoroughly with distilled water to remove debris and air-dried at room temperature (25–28 °C) for two weeks until constant weight was achieved, following standard phytochemical preparation procedures (Siddhuraju & Becker, 2003). The dried leaves were pulverized using a laboratory blender and stored in airtight containers until extraction.

2.2 Preparation of Ethanol Extract

Two hundred grams (200 g) of powdered leaf material were macerated in 1 L of 70% ethanol for 72 hours at room temperature with intermittent shaking, following the extraction protocol. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40 °C. The concentrated extract was further dried in a water bath to obtain a crude extract, which was stored at 4 °C until use. Stock solutions of 1.5 mg/mL, 2 mg/mL, and 3 mg/mL were prepared using sterile distilled water.

2.3 Phytochemical Screening

2.3.1 Qualitative Phytochemical Analysis

Qualitative screening for alkaloids, flavonoids, tannins, saponins, and phenols was conducted using standard phytochemical methods as described by Harborne (1998) and Sofowora (2008). Wagner's reagent was used for alkaloids, ferric chloride test for phenols and tannins, frothing test for saponins, and alkaline reagent test for flavonoids.

2.3.2 Quantitative Phytochemical Determination

Total phenolic content was determined using the Folin–Ciocalteu method (Singleton *et al*., 1999). Flavonoid content was estimated using the aluminum chloride colorimetric assay (Chang *et al*., 2002). Saponins and alkaloids were quantified using gravimetric methods as described by Obadoni and Ochuko (2002). All measurements were performed in triplicate.

2.4 Test Microorganisms

Clinical isolates of *Streptococcus pyogenes* and *Staphylococcus aureus* were obtained from the Microbiology Laboratory of Federal Medical Centre Owerri. The isolates were confirmed using Gram staining and standard biochemical tests according to Cheesbrough (2005). Bacterial suspensions were adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) prior to use.

2.5 Antibacterial Susceptibility Testing

The antibacterial activity of the extract was evaluated using the agar well diffusion method as described by the Clinical and Laboratory Standards Institute (Wayne, 2010). Mueller–Hinton agar plates were inoculated with standardized bacterial suspensions using sterile swabs. Wells of 6 mm diameter were bored into the agar, and 0.1 mL of each extract concentration (1.5, 2, and 3 mg/mL) was introduced into respective wells. Ciprofloxacin (1.5, 2, and 3 mg/mL) served as the positive control. Plates were incubated at 37 °C for 24 hours. Zones of inhibition were measured in millimeters using a calibrated ruler. All experiments were conducted in triplicate.

2.6 Determination of Total Viable Bacterial Count

Total viable counts were determined using the serial dilution and plate count method described by Cappuccino and Welsh (2018). After treatment, ocular samples were serially diluted in sterile normal saline and plated on nutrient agar. Plates were incubated at 37 °C for 24 hours, and colonies were counted and expressed as colony-forming units per milliliter (CFU/mL).

2.7 Statistical Analysis

Data were expressed as mean ± standard deviation (SD) of triplicate determinations. Statistical analysis was performed using SPSS version 25. One-way analysis of variance (ANOVA) followed by post hoc Tukey test was used to determine significant differences between groups. Paired sample t-test was applied where appropriate. Differences were considered statistically significant at $P < 0.05$.

3.0. RESULTS

3.1 Phytochemical Composition of Moringa oleifera Leaf Extract

The qualitative and quantitative phytochemical composition of *Moringa oleifera* leaf ethanol extract is presented in Table 1.

Table 1: Phytochemical Composition of *Moringa oleifera* Leaf Extract.

Phytochemicals	Qualitative Presence	Quantitative Availability (µg/g)
Alkaloids	+	8.20 ± 0.35
Phenols	++	570.47 ± 10.03
Saponins	++	456.53 ± 5.62
Flavonoids	+++	1258.07 ± 13.30
Tannins	+	20.07 ± 0.44

Values are presented as mean ± standard deviation (n = 3).

Key:
+++ = Present in high amount
++ = Present in moderate amount
= Present in little amount

Flavonoids were the most abundant phytochemical constituent (1258.07 ± 13.30 µg/g), followed by phenols (570.47 ± 10.03 µg/g) and saponins (456.53 ± 5.62 µg/g). Alkaloids and tannins were present in comparatively lower concentrations.

3.2 Antibacterial Activity of Moringa oleifera Leaf Extract

The antibacterial activity of the ethanol extract against *Streptococcus pyogenes* and *Staphylococcus aureus* was evaluated using the agar diffusion method. Results are shown in Table 2.

Table 2: Zones of Inhibition (mm) of *Moringa oleifera* Extract and Ciprofloxacin.

Treatment	*S. pyogenes* (mm)	*S. aureus* (mm)
MO extract 3 mg/ml	34.67 ± 0.57^{e}	17.67 ± 2.52^{b}
MO extract 2 mg/ml	27.33 ± 1.16^{d}	18.00 ± 1.00^{b}
MO extract 1.5 mg/ml	16.33 ± 0.58^{a}	9.67 ± 1.53^{a}
Ciprofloxacin 3 mg/ml	24.67 ± 3.51^{cd}	43.33 ± 3.52^{e}
Ciprofloxacin 2 mg/ml	22.67 ± 0.58^{bc}	32.33 ± 0.58^{d}
Ciprofloxacin 1.5 mg/ml	20.00 ± 1.00^{b}	27.67 ± 2.52^{c}

Values are presented as mean ± SD (n = 3). Different superscript letters within the same column indicate significant difference ($P < 0.05$).

The extract demonstrated concentration-dependent antibacterial activity. At 3 mg/ml, the extract exhibited significantly higher inhibition against *S. pyogenes* (34.67 ± 0.57 mm) compared to ciprofloxacin at the same concentration (24.67 ± 3.51 mm). However, ciprofloxacin showed superior activity against *S. aureus*, particularly at 3 mg/ml (43.33 ± 3.52 mm).

3.3 Recovery Duration Following Ocular Application

The recovery duration (days) following ocular infection treatment is presented in Table 3.

Table 3: Recovery Duration (Days).

Test Agent (3 mg/ml)	*S. pyogenes*	*S. aureus*
MO ethanol extract	5.67 ± 0.58	6.67 ± 0.58
Ciprofloxacin	3.67 ± 0.58	5.33 ± 0.58

Paired sample analysis showed a significant difference in recovery duration ($t = -5$, $df = 2$, $P = 0.038$). Ciprofloxacin resulted in faster recovery compared to the extract.

3.4 Total Viable Bacterial Count on Day 5

The total viable bacterial counts following ocular application are presented in Table 4..

Table 4: Total Viable Bacterial Count (CFU/ml) on Day 5.

Test Agent	*S. pyogenes* (CFU/ml)	*S. aureus* (CFU/ml)
MO ethanol extract	$1.52 \times 10^2 \pm 0.27$	$2.15 \times 10^2 \pm 0.12$
Ciprofloxacin	$1.57 \times 10^2 \pm 0.15$	$2.74 \times 10^2 \pm 0.49$

Values are presented as mean ± SD (n = 3).

Statistical analysis revealed a significant difference in bacterial counts following treatment ($P < 0.05$). Therefore, the null hypothesis was rejected, indicating that both treatments significantly reduced bacterial load, with observable differences between test agents.

4.0. DISCUSSION

The present study demonstrated that *Moringa oleifera* leaf ethanol extract exhibits significant antibacterial activity against *Streptococcus pyogenes* and *Staphylococcus aureus*, with effectiveness increasing in a concentration-dependent manner. This antibacterial trend corresponds with several recent studies establishing *M. oleifera* as a potential source of natural antimicrobial agents, particularly against Gram-positive pathogens (*S. aureus* and drug-resistant strains) and other clinically relevant bacteria (Apenteng-Takyiako *et al*., 2025; Royani *et al*., 2023).

The phytochemical analysis showed a high abundance of flavonoids, phenols, and saponins. These compounds have been widely reported to contribute to antibacterial activity through multiple mechanisms including cell wall disruption, inhibition of nucleic acid synthesis, and interference with bacterial enzymes (Royani *et al*., 2023; Koné *et al*., 2025). Flavonoids and phenolic compounds can destabilize bacterial membranes and form complexes with extracellular proteins, which reduces bacterial viability (Fontana *et al*., 2023). The dominance of flavonoids in the extract aligns with the significant inhibition zones observed, particularly against *S. pyogenes*, which are comparable to or larger than some standard antibiotic effects at equivalent concentrations.

Recent research has emphasized the activity of *M. oleifera* against antibiotic-resistant clinical isolates of *S. aureus* and other pathogens. As in this case, aqueous and organic solvent extracts of *M. oleifera* leaves were effective against Methicillin-Resistant *S. aureus* (MRSA) and other resistant strains, with inhibition zones ranging between 20 mm and 32.5 mm (Apenteng-Takyiako *et al*., 2025). Although the present study used ethanol extract rather than aqueous or mixed solvents, the high inhibitory activity supports the notion that *M. oleifera* contains broad-spectrum antibacterial constituents capable of overcoming some resistance mechanisms.

The recovery duration observed in this study indicates that ciprofloxacin yielded faster recovery than *M. oleifera* extract at equivalent concentrations. This difference is likely because synthetic antibiotics are specifically engineered for rapid bactericidal action, whereas crude plant extracts deliver a complex mixture of compounds that may exhibit bacteriostatic effects before bactericidal effects become evident (Royani *et al*., 2023). However, the extract still significantly reduced infection durations compared to untreated controls, indicating its therapeutic potential especially where antibiotic resistance is a concern.

Total viable counts of both bacteria were reduced significantly by both treatments, further confirming antibacterial potency. These findings mirror other studies where *M. oleifera* extracts significantly lowered bacterial counts compared to untreated cultures (Moyo *et al*., 2012). Such antimicrobial effects could be partly attributed to the synergistic actions of multiple bioactive compounds within the extract. Flavonoids, tannins, and saponins often act in concert to enhance antibacterial efficacy by targeting multiple bacterial structures and metabolic pathways (Royani *et al*., 2023; Fontana *et al*., 2023).

Importantly, antibacterial effectiveness is influenced by the nature of bacterial cell walls. Gram-positive bacteria, such as *S. aureus* and *S. pyogenes*, possess a thick peptidoglycan layer which can interact differently with plant phytochemicals compared to Gram-negative bacteria. Studies have shown that the lipophilic nature of certain flavonoids and phenols allows them to penetrate Gram-positive cell walls more readily, causing membrane disruption and subsequent inhibition (Fontana *et al*., 2023; Souleymane *et al*., 2025). This may explain the relatively high zones of inhibition observed in the present work.

Mechanistic insights from recent molecular docking studies suggest that compounds such as quercetin present in *M. oleifera* bind effectively to key bacterial proteins associated with cell wall synthesis, DNA replication, and metabolic pathways (Buru, 2025). These findings indicate that antibacterial activity does not solely rely on membrane disruption but also on the inhibition of critical intracellular processes, making *M. oleifera* extracts promising candidates for multi-target antibacterial agents. Despite the promising antibacterial results, it should be acknowledged that crude extracts exhibit variability in compound concentration and bioavailability compared to purified phytochemicals or conventional antibiotics.

5.0. CONCLUSION

In summary, the antibacterial activity demonstrated by *M. oleifera* leaf ethanol extract against ocular pathogens is supported by its rich phytochemical composition. This activity aligns with recent evidence of *M. oleifera*'s potential as a natural antibacterial agent, particularly in the context of increasing antibiotic resistance. The

findings highlight the relevance of plant-based extracts in the search for alternative or adjunct antimicrobial therapies.

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