BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 1, April 2025 | Pages: 351-355 | DOI: 10.14421/biomedich.2025.141.351-355 ISSN 2540-9328 (online)

Antibacterial Effect of *Moringa oleifera* Leaves on *Staphylococcus aureus* Based on Different Concentration and Harvest Time

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Manuscript received: 22 April, 2025. Revision accepted: 27 June, 2025. Published: 04 July, 2025.

Abstract

This study investigates the antibacterial activity of *Moringa oleifera* leaf extract against *Staphylococcus aureus* ATCC 25923, focusing on the effects of harvest time and extract concentration. Leaves were harvested in the morning (08:00–10:00) and afternoon (15:00–17:00), then extracted with 96% ethanol. The antibacterial efficacy was evaluated using the Kirby-Bauer disc diffusion method at 60%, 70%, and 80%. The largest inhibition zone was observed at 80% concentration from the afternoon harvest (10.38 ± 0.43 mm), while the smallest was recorded at 60% from the morning harvest (8.25 ± 0.20 mm). Statistical analysis confirmed a significant concentration-dependent antibacterial effect ($p < 0.001$, $\eta^2 = 88.6\%$). However, harvest time did not significantly affect inhibition zone size ($p = 0.882$). These results suggest that *M. oleifera* is an effective natural antimicrobial agent against *S. aureus*, with concentration being the key determinant of antibacterial activity. Further research is recommended to explore its potential combined with antibiotics and its full therapeutic applicability.

Keywords: antibacterial activity; harvest time; *Moringa oleifera*, *Staphylococcus aureus*.

INTRODUCTION

Staphylococcus aureus is a gram-positive bacterium that is the leading cause of community-acquired and healthcare-associated infections globally. It is responsible for a broad spectrum of diseases, ranging from superficial skin infections to severe systemic conditions such as pneumonia, osteomyelitis, endocarditis, and sepsis. The growing prevalence of methicillin-resistant *S. aureus* (MRSA) has significantly reduced the effectiveness of conventional antibiotics, leading to treatment failures, increased healthcare costs, and higher mortality rates (Abebe & Birhanu, 2023). Given the rising threat of antimicrobial resistance (AMR), the search for alternative antibacterial agents, particularly those derived from natural bioactive compounds, has become an urgent priority.

Moringa oleifera—the drumstick tree—has bioactive compounds that exhibit antimicrobial, anti-inflammatory, and antioxidant activities. The plant is rich in bioactive secondary metabolites such as flavonoids, phenolic acids, carotenoids, and tocopherols, which have been reported to exhibit antibacterial activity against various pathogenic bacteria, including *S. Aureus* (Pareek et al., 2023. Among these, flavonoids such as galangin, kaempferide, and kaempferide-3-O-glucoside have been shown to disrupt bacterial cell membranes, inhibit DNA synthesis, and interfere with metabolic pathways (Górniak et al., 2019). Given its extensive bioactive profile, *M. oleifera* has gained attention as a potential natural antibacterial agent, particularly in increasing antibiotic resistance.

Despite the well-documented antibacterial potential of *M. oleifera*, several factors influencing its efficacy remain poorly understood. Harvest time is a crucial but underexplored factor affecting the biosynthesis and accumulation of secondary metabolites in medicinal plants (Persic et al., 2018). The production of flavonoids and phenolic compounds is susceptible to environmental conditions such as light exposure, temperature, and humidity, which fluctuate throughout the day. Although environmental factors may affect *M. oleifera*'s antibacterial efficacy, few studies have examined the role of harvest time against S. aureus (Ioannou et al., 2020). Most existing studies on *M. oleifera* have focused on optimizing extraction methods and determining antibacterial activity at a single time point, without systematically comparing leaves harvested at different times of the day. Additionally, there is a lack of data on the optimal extract concentration required for maximum bacterial inhibition. Understanding these variables is essential for standardizing *M. oleifera* as a natural antimicrobial agent with consistent efficacy.

This study aims to evaluate the antibacterial efficacy of *M. oleifera* leaf extract against *S. aureus*, focusing on the influence of harvest time and extract concentration on bacterial inhibition. Specifically, this study seeks to determine whether leaves harvested in the morning and afternoon exhibit differential antibacterial activity and to identify the optimal extract concentration for maximum inhibition. By addressing these knowledge gaps, this study provides novel insights into the role of harvest timing in modulating *M. oleifera*'s antibacterial properties, contributing to its potential standardization as a natural antimicrobial agent.

MATERIALS AND METHODS

Study Design and Ethical Approval

This study employed a posttest-only control group design to evaluate the antibacterial activity of *M. oleifera* leaf extract against *S. aureus*. The research was conducted at the Microbiology Laboratory, Prof. Chairuddin P. Lubis Hospital, Medan, Indonesia. Ethical approval was obtained from the Universitas Sumatera Utara Ethics Committee (Approval No. 842/KEPK/USU/2022), ensuring compliance with institutional and international ethical standards.

Plant Material and Extract Preparation

Fresh *M. oleifera* leaves were collected from Tanjung Morawa District, Deli Serdang Regency, North Sumatra, Indonesia. Botanical identification and authentication of the plant material were conducted at the Organic Chemistry Laboratory, Universitas Sumatera Utara. To evaluate the effect of harvest time, leaves were collected in the morning (08:00–10:00 AM) and afternoon (03:00–05:00 PM). Leaves were selected based on visual inspection, including only fully developed, fresh, undamaged green leaves from the third to sixth position from the shoot. While yellow, perforated, moldy, or wilted leaves were excluded to ensure uniformity in plant quality. Leaves were washed with distilled water, oven-dried at 40 °C for 24 h, and ground into a fine powder. The powdered leaves were subjected to ethanol maceration using 96% ethanol at a 1:10 (w/v) ratio for 24 hours with continuous stirring. The mixture was filtered using Whatman No. 1 filter paper, and the remaining plant material underwent three additional extraction cycles to maximize bioactive compound yield. The pooled filtrates were concentrated using a rotary evaporator at 40°C under reduced pressure until a thick extract was obtained (Munira et al., 2021). The extract was stored at 4°C in an amber glass bottle to prevent degradation. Phytochemical screening was performed to confirm the presence of flavonoids, saponins, and tannins, which are known to contribute to the antibacterial properties of *M. oleifera*.

Figure 1. *Moringa oleifera* during the washing and drying stages.

Bacterial Strain and Culture Preparation

A pure strain of *S. aureus* was obtained from the Microbiology Laboratory, Universitas Sumatera Utara. A bacterial suspension was prepared in Mueller-Hinton broth and adjusted to a 0.5 McFarland standard (1.5×10^8 CFU/mL) using a spectrophotometer at 600 nm to ensure uniform bacterial concentration across experiments.

Antibacterial Activity Assay

The antibacterial activity of *M. oleifera* leaf extract was assessed using the Kirby-Bauer disc diffusion method, following Clinical and Laboratory Standards Institute (CLSI) guidelines. Mueller-Hinton agar plates were inoculated with 100 µL of bacterial suspension and evenly spread using a sterile cotton swab. Sterile 6 mm filter paper discs (Whatman No. 1) were impregnated with 20 µL of extract at concentrations of 60%, 70%, and 80% and air-dried before application. Using sterile forceps, the extract-impregnated discs were aseptically placed onto inoculated Mueller-Hinton agar plates, ensuring uniform spacing to prevent inhibition zone overlap. A negative control (sterile distilled water) and a positive control (commercial antibiotic disc) were included in each assay. Plates were incubated at 37°C for 24 hours under aerobic conditions (Munira et al. 2021). Following incubation, the diameter of the inhibition zones (clear zones around the discs) was measured using a digital caliper in two perpendicular directions, and the mean values were recorded. The experiment was performed in quadruplicate to ensure statistical reliability.

Statistical Analysis

All statistical analyses were conducted using SPSS version 25 (IBM Corp., USA), with $p < 0.05$ considered statistically significant. Data were analyzed for normality (Shapiro-Wilk test) and homogeneity (Levene's test). As both assumptions were met, Two-Way ANOVA was applied to assess the effects of extract concentration and harvest time, including their interaction. Post hoc analysis (Tukey's test) was conducted for pairwise

comparisons. Effect size (η^2 and Cohen's f^2) was calculated to quantify the strength of effects. Linear regression analysis evaluated the dose-response relationship between concentration and inhibition zone diameter.

RESULTS AND DISCUSSION

Phytochemical Characterization of *Moringa oleifera* Extract

Phytochemical screening confirmed the presence of flavonoids, saponins, and tannins in *M. oleifera* leaf extracts from both morning and afternoon harvests (Table 1). These bioactive compounds have been reported to contribute to antibacterial activity, particularly against *S. aureus*.

Table 1. Phytochemical screening.

Secondary Metabolite	Test Reagent	Morning Harvest	Afternoon Harvest
Flavonoids	Mg Powder + HCl	+	+
Saponins	Distilled Water + HCl	+	+
Tannins	$FeCl_3$	+	+

Legend: (+) Present, (-) Absent

Antibacterial Activity of Moringa oleifera Extract

The antibacterial activity of *M. Oleifera* extract was evaluated by measuring inhibition zone diameters (Table 2). The highest inhibition was observed in the 80% extract concentration from the afternoon harvest (S80, 10.38 ± 0.43 mm), while the lowest was recorded in the 60% morning harvest group (P60, 8.25 ± 0.20 mm). However, inhibition zones between morning and afternoon harvests at the same concentration did not differ significantly, suggesting that harvest time did not substantially influence antibacterial efficacy.

Table 2. Mean Inhibition Zone Diameters.

Treatment Group	Mean Inhibition Zone (mm) ± SD
P60	8.25 ± 0.20
P70	9.19 ± 0.24
P80	10.31 ± 0.24
S60	8.50 ± 0.20
S70	8.81 ± 0.55
S80	10.38 ± 0.43

Statistical Analysis of Antibacterial Activity

The normality assumption was confirmed using the Shapiro-Wilk test ($p > 0.05$), and homogeneity of variance was verified using Levene's test ($p > 0.05$). Therefore, Two-Way ANOVA was performed to evaluate the effects of extract concentration and harvest time, including their interaction. Extract concentration significantly influenced antibacterial activity ($p < 0.001$), confirming that higher concentrations resulted in larger inhibition zones. Harvest time did not have a statistically significant effect ($p = 0.882$), indicating that variations in time of collection did not alter antibacterial efficacy. No significant interaction was observed between extract concentration and harvest time ($p = 0.197$), suggesting that concentration effects were independent of harvest conditions. Post-hoc analysis using Tukey's HSD test confirmed that inhibition zones differed significantly between extract concentration groups ($p < 0.05$), while no significant differences were observed between morning and afternoon harvests at the same concentration level. Effect size analysis confirmed that extract concentration strongly influenced inhibition zone diameter ($\eta^2 = 88.6\%$, $f^2 = 7.77$), explaining nearly 89% of the observed variation. In contrast, harvest time had a negligible effect ($\eta^2 = 0.1\%$, $f^2 = 0.001$). A linear regression analysis ($R^2 = 0.83$) confirmed a strong dose-response relationship between extract concentration and inhibition zone diameter.

Influence of Extract Concentration on Antibacterial Activity

This study demonstrated a significant dose-dependent relationship between *M. oleifera* extract concentration and antibacterial activity against *S. aureus* ($p < 0.001$, $R^2 = 0.83$). Higher extract concentrations exhibited larger inhibition zones, consistent with previous studies reporting that flavonoids, tannins, and saponins contribute to bacterial growth inhibition through membrane disruption, enzyme inactivation, and DNA synthesis interference (Jahan et al. 2022; van den Berg and Kuipers 2022; Yan et al. 2024; Huang et al. 2024). The observed inhibition zone values align with prior research, where *M. oleifera* extracts at 50–100 µg/mL demonstrated inhibition zones of 10–16 mm against *S. Aureus* (El-Sherbiny et al., 2024). Although a clear concentration-dependent effect was observed, we did not determine Minimum Inhibitory Concentration (MIC) or Minimum Bactericidal Concentration (MBC). Without these assays, it is difficult to distinguish between bacteriostatic and bactericidal actions of *M. oleifera.*

Impact of Harvest Time on Antibacterial Efficacy

Contrary to expectations, harvest time did not significantly affect antibacterial activity ($p = 0.882$). This contrasts with findings in other medicinal plants, where secondary metabolite accumulation varies throughout the day due to diurnal regulation of biosynthetic pathways (Neugart et al., 2021). Possible explanations for this include: genetic factors, prior studies indicate that metabolite composition in *M. oleifera* varies more significantly between cultivars than within a single plant species due to daily fluctuations (Hamada et al. 2024). Secondary metabolite stability in *M. oleifera, u*nlike plants where flavonoids accumulate in response to sunlight, *M. oleifera* may exhibit more stable metabolite profiles regardless of harvest time (Rahmawati et al., 2024). Future research should quantify flavonoid and phenolic content at multiple harvest times (e.g., early

morning, midday, and evening) and across different seasons to further assess potential variations.

Study Limitations and Future Directions

The limitation of this study is the absence of MIC and MBC assays, which are critical for determining whether *M. oleifera* exhibits bacteriostatic or bactericidal effects. Future studies should employ broth microdilution techniques to establish precise antimicrobial thresholds. Additionally, while phytochemical screening confirmed the presence of flavonoids, tannins, and saponins, their quantitative concentrations were not measured. Future research should incorporate high-performance liquid chromatography (HPLC) or mass spectrometry analysis for detailed metabolite profiling. Further investigation is also needed to evaluate the synergistic potential of *M. oleifera* with conventional antibiotics, which could enhance treatment efficacy while reducing the risk of resistance development.

CONCLUSIONS

This study demonstrated that *M. oleifera* leaf extract exhibits significant antibacterial activity against *S. aureus*, with a strong dose-response relationship between extract concentration and inhibition zone size. However, harvest time did not significantly influence antibacterial efficacy, suggesting that *M. oleifera* may maintain a stable bioactive profile independent of short-term environmental variations. These findings support the potential application of *M. oleifera* as a natural antimicrobial agent, particularly in the context of antibiotic resistance. Future studies should focus on MIC/MBC determination, quantitative phytochemical analysis, and synergy testing with conventional antibiotics to further validate its therapeutic potential.

Acknowledgements: We would like to express our gratitude to Microbiology Laboratory, Prof. Chairuddin P. Lubis Hospital for facilitating the laboratory analyses conducted in this research. We also extend our appreciation to the Organic Chemistry Laboratory, Universitas Sumatera Utara, for their valuable assistance in the identification and authentication of the plant materials used in this study.

Authors' Contributions: Conceptualization: JAP, RLK, YY, ATL. Data Collection: JAP,RLK. Data Extraction: JAP. Methodology: JAP, YY. Supervision: RLK, YY, ATL. Writing Manuscript: JAP. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

Funding: This research is not financially supported by any Organization or Individuals.

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