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# Antibacterial Activity and Phytochemical Screening of Ethanolic Extract from Moringa oleifera (Moringa) Leaves Cultivated in the Drylands of East Nusa Tenggara against Staphylococcus aureus

**Dinda Sari Utami[1], Nur Masyithah Zamruddin[2]*, & Najwi Hasani[3]**
[1]Universitas Muslim Nusantara Al Washliyah, Indonesia, [2]*Universitas Mulawarman, Indonesia,
[3]Universitas Muhammadiyah Banjarmasin, Indonesia
*Co e-mail: ita@farmasi.unmul.ac.id[2]

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**ABSTRACT**

*Moringa oleifera Lam. is widely cultivated in semi-arid regions such as East Nusa Tenggara (NTT), Indonesia, where dryland stress may enhance the accumulation of bioactive secondary metabolites. This study aimed to characterize the phytochemical profile and antibacterial activity of 96% ethanolic leaf extract of M. oleifera grown in NTT drylands against Staphylococcus aureus ATCC 25923. Leaves were extracted by maceration, followed by qualitative phytochemical screening and antibacterial testing using the Kirby-Bauer disc diffusion method at concentrations of 10–100% (w/v). The Minimum Inhibitory Concentration (MIC) was determined by broth microdilution. The extract contained alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolics. Antibacterial activity increased with extract concentration, reaching a mean inhibition zone of 21.4 ± 0.83 mm at 100% (w/v), classified as strong activity, with an MIC of 12.5% (w/v). These findings indicate that dryland-grown M. oleifera leaves from NTT contain diverse phytochemicals and exhibit strong antibacterial activity against S. aureus, supporting their potential as a natural antibacterial agent.*

***Keywords***: *Moringa Oleifera, Phytochemical Screening, Antibacterial Activity, Staphylococcus Aureus, East Nusa Tenggara, Ethanol Extract, Disc Diffusion*

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## INTRODUCTION

Bacterial infections continue to pose a major public health burden worldwide, especially across developing nations where healthcare access and antimicrobial supply are frequently constrained. *Staphylococcus aureus* stands out as one of the leading pathogens implicated in infections acquired both within hospitals and in the wider community. As a Gram-positive organism, it is capable of triggering diverse clinical manifestations ranging from skin and soft-tissue infections to pneumonia, bacteremia, endocarditis, and foodborne illness (Tong et al., 2015). The rise of methicillin-resistant *S. aureus* (MRSA) alongside other multidrug-resistant variants has made treatment considerably more difficult and has driven up illness and death rates globally (Lakhundi & Zhang, 2018). This resistance crisis has, in turn, pushed the search for novel antibacterial compounds particularly those derived from natural sources to the forefront of scientific inquiry (Alqaseer et al., 2023).

Plants used in traditional medicine have long served as an important reservoir of bioactive substances with wide-ranging pharmacological effects. The World Health Organization estimates that roughly 80% of people worldwide depend on plant-derived remedies as a core component of their primary healthcare (WHO, 2019). Over the past several decades, scientific attention toward confirming these traditional remedies and isolating their active ingredients for drug development has grown considerably. Indeed, natural products have historically been a cornerstone of drug discovery efforts, shaping the development of numerous modern therapeutics (Newman & Cragg, 2020).

Belonging to the family Moringaceae, *Moringa oleifera Lam.* is regarded as one of the most valuable plants in terms of both nutrition and pharmacology. Originally native to the sub-Himalayan belt of India, it is now grown broadly throughout tropical and subtropical areas, spanning Africa, Southeast Asia, and Pacific island nations (Anwar et al., 2007). Within Indonesia and particularly in East Nusa Tenggara (NTT) the species, locally called "kelor," has long held a place in everyday nutrition and traditional healing practices.

Nearly every part of *M. oleifera* leaves, seeds, flowers, pods, roots, and bark has been documented to display a broad spectrum of pharmacological effects, including antimicrobial, anti-inflammatory, antioxidant, antidiabetic, hepatoprotective, and anti-ulcer actions (Oyeyinka & Afolabi, 2021; Paikra et al., 2017). Leaves in particular have received the most research attention given their abundant secondary metabolite content. Phytochemical analyses indicate that the leaves harbor a range of compounds, among them glucosinolates, isothiocyanates, flavonoids (notably quercetin and kaempferol), phenolic acids, alkaloids, saponins, and tannins constituents that likely underlie many of the plant's reported biological effects (Vongsak et al., 2013; Dey et al., 2015).

The surrounding environment strongly shapes how medicinal plants biosynthesize and accumulate secondary metabolites. Stressors such as drought conditions, elevated temperatures, and scarce nutrients have been documented to trigger heightened production of protective phytochemicals as an adaptive plant response (Salehi et al., 2019). East Nusa Tenggara's climate fits this profile, marked by a semi-arid dryland pattern, an extended dry season of roughly seven to

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eight months annually, comparatively sparse rainfall, and intense solar exposure. Such conditions may impose oxidative stress on plants and consequently boost the synthesis of protective secondary metabolites, potentially making *M. oleifera* leaves grown here more pharmacologically potent than those raised in wetter climates (Frutos et al., 2022).

While a substantial body of literature has explored the phytochemical makeup and antimicrobial properties of *M. oleifera* leaves, studies focusing specifically on material grown in the dryland setting of East Nusa Tenggara remain scarce. Earlier work carried out in other geographic areas has shown meaningful antibacterial effects from *M. oleifera* leaf extracts, yet differences in growing conditions can substantially alter both the phytochemical makeup and biological performance of medicinal plants (Cheok et al., 2014). Examining leaves cultivated specifically in the NTT drylands is therefore scientifically warranted, offering insight into their distinctive phytochemical profile and antibacterial capacity.

Solvent selection is another factor with a strong bearing on bioactive compound recovery. Ethanol ranks among the most commonly chosen solvents for phytochemical extraction, owing to its capacity to dissolve both polar and moderately non-polar substances, its comparatively low toxicity, and its broad compatibility with diverse phytochemical classes (Omonijo et al., 2018). Prior research further indicates that ethanolic extracts from medicinal plants, *M. oleifera* included, tend to display stronger antimicrobial effects than extracts prepared with water or alternative solvents (Sultana et al., 2009).

Building on this background, the present study was designed to (1) carry out phytochemical screening of a 96% ethanolic extract from *M. oleifera* leaves grown in the NTT drylands, (2) assess its antibacterial capacity against *Staphylococcus aureus* ATCC 25923 via the disc diffusion technique, and (3) establish the Minimum Inhibitory Concentration (MIC) through broth microdilution. It is anticipated that these findings will contribute empirical support for developing *M. oleifera* into a natural antibacterial agent, drawing on the abundant botanical resources characteristic of East Nusa Tenggara.

## METHODS

Mature, fresh *M. oleifera* leaves were harvested in Kupang District, East Nusa Tenggara Province, Indonesia (10°10'S, 123°35'E; approximately 80 m above sea level) during the dry season of August 2024. Species identification and authentication were carried out by a botanist based at the Herbarium, Faculty of Sciences, Universitas Nusa Cendana (voucher specimen no. UNC-HRB-2024-087). Sample collection took place with the consent of local landowners and did not involve any protected or endangered species, in line with ethical standards governing medicinal plant research.

The leaves were rinsed with running water, sorted to discard damaged material, then oven-dried at 40°C for 48 hours under forced-air conditions until a constant weight was reached. The dried material was mechanically ground and passed through a 40-mesh sieve to produce a uniform powder, which was found to contain 7.2 ± 0.3% moisture.

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The maceration technique was used for extraction: 500 g of dried powder was immersed in 5,000 mL of 96% ethanol (technical grade, Bratachem, Indonesia) and left at room temperature for three consecutive 24-hour periods, with periodic stirring. The resulting mixture was passed through Whatman No. 1 filter paper, after which the residual material underwent two further extraction cycles using fresh solvent. The pooled filtrates were then concentrated at 40°C under reduced pressure using a Heidolph (Germany) rotary evaporator to produce a viscous extract, which was subsequently dried in a 50°C water bath until a semi-solid consistency was reached. Extraction yield was expressed as a percentage relative to dry weight.

$$\text{Yield (\%)} = (\text{Weight of dried extract} / \text{Weight of crude powder}) \times 100\%$$

Qualitative screening for alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, and phenolic compounds relied on standard colorimetric and precipitation techniques, adapted from the procedures of Harborne (1998) and Trease and Evans (2009), and further modified according to contemporary pharmaceutical practice (Akinmoladu et al., 2010; Trease & Evans, 2009):

a. **Alkaloids:** A 0.5 g portion of extract was dissolved in 1% HCl, filtered, and the resulting filtrate was split into three aliquots for testing with Mayer's reagent (indicated by a cream-colored precipitate), Dragendorff's reagent (orange-red precipitate), and Wagner's reagent (reddish-brown precipitate).
b. **Flavonoids:** Extract (0.5 g) was dissolved in methanol, followed by the addition of a few drops of concentrated HCl and a strip of magnesium ribbon; the appearance of an intense orange-red or pink color signaled a positive result for flavonoids.
c. **Tannins:** A 0.5 g sample of extract dissolved in distilled water was treated with 1% ferric chloride ($FeCl_3$) solution, with a blue-black or greenish-black color change taken as a positive tannin result.
d. **Saponins:** Extract (0.5 g) was shaken vigorously with 10 mL distilled water for two minutes; foam persisting for at least 15 minutes was interpreted as evidence of saponins.
e. **Terpenoids:** Terpenoid content was assessed using the Salkowski test, in which 0.5 g of extract dissolved in chloroform was layered with concentrated $H_2SO_4$; a reddish-brown ring at the interface confirmed the presence of terpenoids.
f. **Steroids:** Steroid detection followed the Liebermann-Burchard method using acetic anhydride together with concentrated $H_2SO_4$, where a green or blue-green color change would indicate steroids.
g. **Phenolics:** A 0.5 g extract sample dissolved in ethanol was mixed with two to three drops of 5% $FeCl_3$; a resulting dark blue, green, or black color indicated the presence of phenolic compounds.

The test organism, *Staphylococcus aureus* ATCC 25923, was sourced from the Microbiology Laboratory at the Faculty of Medicine, Universitas Nusa Cendana. Stock cultures were kept on Mueller-Hinton Agar (MHA; Oxoid, UK) and subcultured 24 hours prior to each test. Bacterial suspensions were prepared in sterile 0.9% NaCl and standardized to 0.5 McFarland turbidity (roughly $1.5 \times 10^8$ CFU/mL), verified spectrophotometrically at 625 nm (absorbance range 0.08–0.10).

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Antibacterial testing followed the disc diffusion approach in accordance with CLSI M02-A12 guidelines. Mueller-Hinton Agar plates were swabbed with the standardized bacterial suspension prior to disc application. Sterile 6 mm Whatman No. 1 filter paper discs were loaded with 10 µL of extract at each of six concentrations (10, 20, 40, 60, 80, and 100% w/v, dissolved in dimethyl sulfoxide [DMSO]). A ciprofloxacin disc (5 µg; Oxoid, UK) served as the positive control, while 100% DMSO served as the negative control.

Using sterile forceps, the discs were placed onto the inoculated agar surface, and plates were then incubated for 24 hours at 37°C. Inhibition zone diameters, measured with a digital caliper and inclusive of disc size, were recorded from triplicate trials. Resulting zones were categorized per the Davis and Stout scale, where ≤5 mm denotes inactivity, 5–10 mm weak activity, 11–20 mm moderate activity, and >20 mm strong activity.

MIC values were established through broth microdilution in 96-well microplates, following CLSI M07-A10 protocols. Two-fold serial dilutions of the extract, spanning 100% down to 0.098% (w/v), were prepared in Mueller-Hinton Broth (MHB; Oxoid, UK), and each well received *S. aureus* suspension to a final density of approximately $5 \times 10^5$ CFU/mL. After incubating plates at 37°C for 18–24 hours, bacterial growth was gauged visually by turbidity, with the MIC recorded as the lowest concentration showing no detectable turbidity.

Data are reported as mean ± SD across three independent replicates. Differences in inhibition zones across concentrations were analyzed using one-way ANOVA with Tukey's HSD post-hoc comparisons, with significance set at $p < 0.05$, using SPSS version 26.0 (IBM Corp., USA).

## RESULTS

### 1. Yield of Ethanol Extract

Maceration of 500 g of *M. oleifera* leaf powder in 96% ethanol produced 48.7 g of a semi-solid, dark green extract, equating to a 9.74% (w/w) yield. The extract's bitter taste, pronounced aroma, and dark green hue were consistent with the presence of chlorophyll pigments and various secondary metabolites.

### 2. Phytochemical Screening

Table 1 summarizes the qualitative phytochemical screening outcomes for the ethanolic extract of *M. oleifera* leaves sourced from NTT drylands.

**Table 1. Phytochemical Screening Results of Ethanol Extract of *M. oleifera* Leaves from East Nusa Tenggara Drylands**

| Phytochemical Compound | Reagent/Method | Observation | Result |
|---|---|---|---|
| **Alkaloids** | Mayer's, Dragendorff's, Wagner's | Cream ppt; Orange-red ppt; Reddish-brown ppt | + |

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| | | | |
|---|---|---|---|
| **Flavonoids** | Mg + HCl (Shinoda Test) | Intense orange-red coloration | + |
| **Tannins** | $FeCl_3$ 1% | Greenish-black coloration | + |
| **Saponins** | Foam Test | Persistent stable foam (>15 min) | + |
| **Terpenoids** | Salkowski Test | Reddish-brown coloration | + |
| **Steroids** | Liebermann-Burchard | No green/blue-green coloration | - |
| **Phenolic Compounds** | $FeCl_3$ 5% | Dark blue coloration | + |

*(+) = Present; (–) = Absent*

These results confirmed six major secondary metabolite classes in the extract — alkaloids (positive across all three test reagents), flavonoids, tannins, saponins, terpenoids, and phenolic compounds — while steroids were not detected.

### 3. Antibacterial Activity (Disc Diffusion)

Table 2 presents the antibacterial performance of the ethanolic *M. oleifera* leaf extract against *S. aureus* ATCC 25923 across the tested concentration range. Every concentration generated a measurable inhibition zone, whereas the DMSO negative control produced none, confirming that the observed inhibition stemmed from the extract itself rather than the solvent.

**Table 2. Inhibition Zones of Ethanol Extract of *M. oleifera* Leaves Against *S. aureus* ATCC 25923**

| Concentration (% w/v) | Inhibition Zone Rep-1 (mm) | Inhibition Zone Rep-2 (mm) | Inhibition Zone Rep-3 (mm) | Mean ± SD (mm) | Category |
|---|---|---|---|---|---|
| **10%** | 9.1 | 9.3 | 8.9 | $9.1 \pm 0.20^{a}$ | Weak |
| **20%** | 11.5 | 11.8 | 11.3 | $11.5 \pm 0.25^{b}$ | Moderate |
| **40%** | 14.2 | 14.5 | 13.9 | $14.2 \pm 0.30^{c}$ | Moderate |
| **60%** | 16.8 | 17.2 | 16.5 | $16.8 \pm 0.35^{d}$ | Moderate |
| **80%** | 19.3 | 19.6 | 18.9 | $19.3 \pm 0.35^{e}$ | Moderate |
| **100%** | 21.2 | 21.6 | 21.4 | $21.4 \pm 0.20^{f}$ | Strong |
| **Ciprofloxacin 5 µg (+)** | 31.5 | 31.8 | 31.2 | $31.5 \pm 0.30^{g}$ | Strong |
| **DMSO 100% (–)** | 0 | 0 | 0 | $0 \pm 0.00^{h}$ | Inactive |

*Values are expressed as mean ± SD (n=3). Different superscript letters indicate statistically significant differences ($p < 0.05$, Tukey's HSD test). Categories based on Davis and Stout criteria.*

A clear dose-response trend emerged, with inhibition zones growing in tandem with extract concentration. The 10% concentration yielded a mean zone of 9.1 ± 0.20 mm, falling into the weak-activity category, while concentrations from 20% to 80% produced zones ranging between 11.5 ±

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0.25 mm and 19.3 ± 0.35 mm, indicating moderate activity. The 100% concentration generated the largest zone at 21.4 ± 0.20 mm, meeting the criteria for strong antibacterial activity. One-way ANOVA indicated highly significant differences across the tested groups ($F = 2847.6$; $p < 0.001$), and subsequent Tukey's HSD comparisons confirmed statistically significant differences between all concentration pairs ($p < 0.05$), with variation only within replicates of the same concentration remaining non-significant.

**4. Minimum Inhibitory Concentration (MIC)**

Broth microdilution was used to establish the MIC of the ethanolic extract against *S. aureus* ATCC 25923, with results shown in Table 3.

**Table 3. Minimum Inhibitory Concentration (MIC) of Ethanol Extract of *M. oleifera* Leaves Against *S. aureus***

| Concentration (% w/v) | Turbidity | Bacterial Growth |
|---|---|---|
| **100** | Clear | Absent (−) |
| **50** | Clear | Absent (−) |
| **25** | Clear | Absent (−) |
| **12.5** | Clear | Absent (−) |
| **6.25** | Turbid | Present (+) |
| **3.125** | Turbid | Present (+) |
| **1.563** | Turbid | Present (+) |
| **0.781** | Turbid | Present (+) |
| **Positive Control (MHB + bacteria)** | Turbid | Present (+) |
| **Negative Control (MHB only)** | Clear | Absent (−) |

*MIC = 12.5% (w/v); (+) = Growth present; (−) = No growth*

The MIC was established at 12.5% (w/v), the lowest concentration at which no visible growth occurred. At concentrations of 6.25% and below, turbidity clearly indicated bacterial growth, whereas wells at 12.5% and higher remained clear, signifying complete growth inhibition.

## DISCUSSION

The findings of this study confirm that a 96% ethanolic extract of *M. oleifera* leaves grown under dryland conditions in East Nusa Tenggara contains a diverse range of phytochemicals and exhibits meaningful antibacterial activity against *Staphylococcus aureus* ATCC 25923. These outcomes align with, and add further depth to, the existing literature on the pharmacological characteristics of this species.

The 9.74% extraction yield recorded here falls within the range documented elsewhere. Vongsak et al. (2013), for instance, reported yields spanning 7.5% to 12.3% for *M. oleifera* leaf ethanol extracts, depending on the solvent used and the geographic source of plant material. The

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comparatively high yield obtained in the present work points to efficient recovery of soluble phytochemicals from dryland-grown *M. oleifera* in East Nusa Tenggara.

The screening confirmed alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds within the ethanolic extract, matching earlier reports by Anwar et al. (2007), Mahmood et al. (2010), and Oyeyinka and Oyeyinka (2018), all of whom identified comparable secondary metabolite classes in *M. oleifera* leaves from other regions. The absence of steroids in this study, by contrast, may stem from differences in extraction method, solvent polarity, leaf maturity, or environmental factors that shape secondary metabolite biosynthesis.

A clear concentration-dependent trend characterized the antibacterial results, with the 100% extract yielding a mean inhibition zone of 21.4 ± 0.20 mm against *S. aureus* classified as strong activity and exceeding values reported in earlier studies. Dey et al. (2015), for example, documented inhibition zones of 15–18 mm for ethanolic *M. oleifera* leaf extracts tested against the same organism. This stronger response observed here may reflect the particular environmental pressures of the East Nusa Tenggara dryland ecosystem, which could drive elevated production of bioactive secondary metabolites as a plant stress-adaptation strategy.

It is likely that the secondary metabolites detected work together synergistically to produce the observed antibacterial outcome. Flavonoids such as quercetin and kaempferol, the dominant flavonoid compounds in *M. oleifera* leaves, have been reported to hinder bacterial cell wall formation, compromise membrane integrity, and disrupt nucleic acid synthesis (Salehi et al., 2019). Tannins are understood to bind irreversibly with bacterial cell wall proteins, impairing membrane permeability and ultimately causing cell lysis (Frutos et al., 2022). Alkaloids like moringine and moringinine have been shown to interfere with bacterial DNA replication through inhibition of topoisomerase enzymes (Oyeyinka & Afolabi, 2021). Saponins primarily act by permeabilizing bacterial membranes, resulting in leakage of intracellular contents (Cheok et al., 2014), while phenolic constituents such as caffeic acid and chlorogenic acid derivatives appear to disrupt bacterial enzymatic activity and electron transport pathways (Vergara-Jimenez et al., 2017).

The 12.5% (w/v) MIC obtained here indicates that a fairly concentrated dose of the crude extract is needed to fully suppress bacterial growth an expected outcome for crude plant extracts, which contain not only active antimicrobial constituents but also inactive or even antagonistic substances that can dilute the effective potency of the active molecules. The hydrophilic character of certain phytochemicals may also restrict their ability to penetrate bacterial membranes within the liquid medium used for microdilution, in contrast to the more direct contact achieved on agar surfaces during disc diffusion testing (Sultana et al., 2009).

Relative to the ciprofloxacin positive control (31.5 ± 0.30 mm), the 100% plant extract's inhibition zone of 21.4 ± 0.20 mm equates to about 68% of the antibiotic's inhibitory strength under the study conditions. While a crude extract of this kind cannot substitute for standard antibiotics in clinical settings, the results point toward its potential as a complementary antibacterial agent. Additional research employing bioassay-guided fractionation would be valuable for isolating and characterizing the specific bioactive compounds driving this antibacterial effect.

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These results also carry meaningful public health implications for East Nusa Tenggara, a region confronting significant barriers to healthcare access and high medication costs. In such a setting, readily available medicinal plants like *M. oleifera* could serve as a valuable resource for community-level healthcare solutions. Given its widespread local cultivation and demonstrated antibacterial properties, the plant appears well suited for integration into traditional or community health practices particularly for treating skin infections and wound care, conditions in which *S. aureus* is a frequent causative agent.

Certain limitations of this work should nonetheless be noted. Testing was restricted to a single standard bacterial strain (*S. aureus* ATCC 25923), and future work should expand to clinical isolates including methicillin-resistant *S. aureus* (MRSA) as well as additional pathogenic species. This study also did not incorporate in vivo toxicity assessment or clinical validation. Subsequent research should prioritize bioassay-guided fractionation, structural characterization of active compounds via advanced spectroscopic tools such as NMR and mass spectrometry, in vivo antibacterial evaluation, toxicological testing, and the formulation of standardized extract products.

## CONCLUSIONS

This study found that the ethanolic extract of *M. oleifera* leaves grown in the drylands of East Nusa Tenggara, Indonesia, contains six classes of secondary metabolites alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds and exhibits significant, concentration-dependent antibacterial activity against *S. aureus* ATCC 25923, with the 100% (w/v) concentration achieving a strong inhibition zone of 21.4 ± 0.20 mm and an MIC of 12.5% (w/v). These findings lend scientific credibility to the traditional application of *M. oleifera* in NTT as an antimicrobial remedy and point to its potential as a basis for developing natural antibacterial therapeutics. Continued research through bioassay-guided fractionation, compound identification, and in vivo validation is recommended.

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