

Phytochemical Screening and Antimicrobial Examination of *Moringa oleifera* Seeds and Leaves (Zogale) Extracts on Some Bacterial Isolates

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ABSTRACT

Moringa oleifera Lam. (Moringaceae), locally known as “zogale,” is widely used in traditional medicine across Northern Nigeria for the management of infections and other ailments. This study investigated the phytochemical composition, antimicrobial activity, and acute toxicological safety of *M. oleifera* seed and leaf extracts against five bacterial isolates. Following herbarium authentication (Accession Number BUKHAN 0011), extracts were prepared using water, ethanol at graded concentrations (30-100%), and methanol. Aqueous and 30% ethanol extraction gave the highest yields ($33.73 \pm 1.65\%$ and $33.89 \pm 0.40\%$, respectively), while 95% ethanol gave the lowest (10.7%); a one-way ANOVA confirmed a statistically significant, solvent-dependent effect on yield ($F(5,12) = 120.93$, $p < 0.001$). Qualitative screening of the aqueous and methanol extracts detected alkaloids, flavonoids, tannins, phenols, and anthraquinones in both, with saponins present only in the methanol extract and steroids absent from both. Quantitative

analysis of the active extract showed flavonoids as the most abundant constituent (26.29 ± 0.11 mg QE/g), followed by total phenols (2.87 ± 0.04 mg TAE/g) and tannins (2.83 ± 0.07 mg TAE/g). The extracts produced measurable zones of inhibition against all five isolates, with the widest recorded against MRSA (7–13 mm) and *Pseudomonas aeruginosa* (7–12 mm). Acute oral toxicity testing in adult Wistar rats (Lorke's method) produced no mortality at 1600 and 2900 mg/kg, variable mortality at 5000 mg/kg, and an estimated LD₅₀ of 1585–3808 mg/kg. Histopathology showed generally normal tissue architecture at lower doses, with mild-to-moderate hepatocellular necrosis and sinusoidal dilatation at higher doses, while the spleen was unaffected throughout. These findings indicate that *M. oleifera* seed and leaf extracts possess measurable antibacterial activity supported by a broad phytochemical profile, with a moderate acute toxicity margin that warrants dose-conscious use.

Keywords: *Moringa oleifera*; zogale; phytochemical screening; antimicrobial activity; acute toxicity; histopathology

INTRODUCTION

Moringa oleifera Lam. (family Moringaceae), locally known in Hausa as “zogale,” is a fast-growing, drought-tolerant tree widely distributed across the savannah belt of Nigeria and much of sub-Saharan Africa. Nearly every part of the plant — leaves, seeds, pods, flowers, roots, and bark — is put to nutritional, medicinal, or industrial use, and the leaves in particular are consumed as a vegetable in many Northern Nigerian households because of their high protein, vitamin, and mineral content (Divya et al., 2024). Beyond its nutritional value, *M. oleifera* has a long history of use in traditional medicine for the management of infections, inflammation, hypertension, diabetes, and malnutrition (Divya et al., 2024).

The therapeutic reputation of the plant is closely tied to its rich secondary metabolite profile: alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and phenolic compounds have all been isolated from various parts of the plant and are widely associated with antimicrobial, antioxidant, and anti-inflammatory effects (Divya et al., 2024; El-Sherbiny et al., 2024). At the same time, the growing global burden of antimicrobial resistance has renewed scientific interest in plant-derived antibacterial agents as alternative or adjunct therapeutic options (WHO, 2014). Reports elsewhere indicate that *M. oleifera* leaf and seed extracts inhibit both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, although the degree of inhibition varies considerably with extract type, solvent, and isolate tested (El-Sherbiny et al., 2024; Enerijiofi et al., 2021), underscoring the need for locally conducted, isolate-specific evaluation.

A therapeutic claim, however, is incomplete without a corresponding safety evaluation. Despite the widespread traditional use of *M. oleifera* leaves and seeds within Kano and its environs, there remains limited locally generated data comparing extraction yield across solvents, antimicrobial efficacy

against locally encountered isolates, and — most critically — the acute oral toxicity and organ-level safety of the extracts at commonly consumed doses (Lorke, 1983; OECD, 2002). This gap between long-standing traditional use and the evidence base needed to support it motivated the present study.

This study therefore aimed to evaluate the phytochemical composition, antimicrobial activity, and acute toxicological safety of *M. oleifera* seed and leaf extracts against selected bacterial isolates, through herbarium authentication of the plant specimen; multi-solvent extraction; qualitative and quantitative phytochemical screening; antimicrobial susceptibility testing with determination of the minimum inhibitory and bactericidal concentrations; and acute toxicity (LD50) and histopathological evaluation in adult Wistar rats.

Statement of the Problem

Despite the widespread traditional use of *Moringa oleifera* leaves and seeds for the management of infections and other ailments within Kano and its environs, there remains limited locally generated scientific data comparing the phytochemical yield of extracts obtained with different solvents, the antimicrobial efficacy of these extracts against specific bacterial isolates encountered in the study area, and — most critically — the safety of the extracts at the doses that are commonly consumed. Toxicological information, particularly acute oral toxicity (LD50) values and organ-level histopathological effects, is scarce for zogale preparations used within this locality, even though such data are considered essential before any plant-derived antimicrobial can be evaluated further (Lorke, 1983; OECD, 2002). This creates a gap between long-standing traditional use and the evidence base needed to support it, which this study seeks to help close.

Justification

This study is justified on scientific, public health, and practical grounds. Scientifically, it closes a documented evidence gap: although *Moringa oleifera*'s phytochemical richness and antimicrobial potential are well established internationally comparable multi-solvent, isolate-specific, and toxicity-linked data generated locally from Kano-grown zogale are lacking, and this study directly supplies that missing local evidence. On public health grounds, the work responds to the global burden of antimicrobial resistance (WHO, 2014), for which plant-derived antibacterial agents such as *Moringa oleifera* are increasingly investigated as alternative or adjunct therapeutic option, establishing which bacterial isolates the local extract is genuinely active against, and at what concentration, is a necessary precondition for any such agent to be considered further. On practical grounds, the study addresses a real safety gap in an already widespread practice: *Moringa oleifera* leaves and seeds are consumed daily as food and taken informally as a remedy in the study area with no standardised dosing (Divya et al., 2024), yet locally generated acute toxicity and organ-level safety data for this specific material did not previously exist to inform that use.

Taken together, the findings will contribute scientific evidence that either supports or qualifies the traditional antimicrobial use of *Moringa oleifera*, generate toxicological reference data — including an estimated LD50 and histopathological profile — useful for determining safe exposure levels, and provide baseline phytochemical data that can guide subsequent isolation and characterisation of the specific bioactive compounds responsible for any observed antimicrobial activity. The work is therefore justified both as a contribution to the scientific literature on *Moringa oleifera* and as locally relevant evidence for a plant already in widespread use within the study community.

Aim and Objectives

The Aim of this study is to determine phytochemical screenings and antimicrobial examination of *Moringa oleifera* leaf and seed Against some bacterial isolates.

This aim was achieved through the following specific objectives;

1. To authenticate and identify the plant specimen through herbarium certification.
2. To prepare seed and leaf extracts of *Moringa oleifera* using a range of solvents of increasing polarity.
3. To carry out qualitative phytochemical screening of the seed and leaf extracts.
4. To carry out quantitative phytochemical determination of the identified bioactive constituents.
5. To evaluate the antimicrobial activity of the extracts against selected bacterial isolates using standard susceptibility testing methods.
6. To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts showing activity.
7. To determine the acute toxicity and lethality profile (LD50) of the extracts in adult Wistar rats using Lorke's method.
8. To assess treatment-related organ changes through histopathological examination of the liver, kidney, and other vital organs.

MATERIALS AND METHODS

Study Area

This study was conducted at the Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Federal University of Science and Technology, Kabo, Kano State, Nigeria, with plant collection and identification carried out within Kano metropolis.

Plant Collection and Authentication

Fresh leaves and seeds of *Moringa oleifera* were collected within Kano metropolis, and the specimen was authenticated and certified at the Herbarium Unit, Department of Plant Science and Biotechnology, Bayero

University, Kano, where a voucher specimen was deposited (Accession Number BUKHAN 0011).

Bacterial Isolates

Five reference and clinical bacterial isolates — *Escherichia coli*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa* — were obtained from the stock culture collection of the Department of Pharmaceutical Microbiology, Bayero University, Kano, and identified by colonial morphology, Gram reaction, and standard biochemical tests (catalase, coagulase, oxidase, indole, citrate utilisation, sugar fermentation, and optochin susceptibility) (Cheesbrough, 2010; Ochei & Kolhatkar, 2008).

Preparation of Plant Extracts

Leaves and seeds were sorted, washed, air-dried under shade, pulverised, and sieved to a uniform particle size. The powdered material was extracted with distilled water, graded ethanol concentrations (30, 50, 70, 80, 95, and 100%), and methanol. Each extract was filtered (Whatman No. 1) and concentrated using a rotary evaporator under reduced pressure at 40–45°C, then stored at 4°C. Percentage yield was calculated as the weight of dried extract relative to the weight of powdered material used, following standard extraction practice (Sofowora, 1993; Harborne, 1998).

Qualitative Phytochemical Screening

Qualitative screening of the aqueous and methanol extracts was performed using standard colour and precipitation tests for alkaloids (Dragendorff's test), flavonoids (Shinoda test), tannins and phenols (ferric chloride test), saponins (frothing test), steroids (Liebermann–Burchard test), cardiac glycosides (Keller–Kiliani test), free anthraquinones (Borntrager's test), and terpenoids (Salkowski test), following established procedures (Sofowora, 1993; Harborne, 1998; Evans, 2009).

Quantitative Phytochemical Determination

Phytochemical classes detected on screening were quantified gravimetrically (alkaloids, flavonoids, saponins) or colorimetrically (tannins by the Folin–Denis method; total phenols by the Folin–Ciocalteu method), following Harborne (1998) and Evans (2009). Results are expressed as percentage yield (% w/w) or as milligrams of the relevant standard equivalent (quercetin equivalent, QE, for flavonoids; tannic acid equivalent, TAE, for tannins and phenols) per gram of dry extract.

Antimicrobial Susceptibility Testing

Antimicrobial activity was evaluated by the agar well diffusion method (Umar et al., 2020). Mueller-Hinton agar plates were seeded with a standardised inoculum ($\sim 1.5 \times 10^8$ CFU/mL, adjusted against a 0.5 McFarland standard) of each organism, and 8 mm wells were loaded with graded extract concentrations (100, 50, 25, and 12.5 mg/mL, reconstituted in 10% dimethyl sulfoxide, DMSO); 10% DMSO alone served as the negative control. Plates were incubated at 37°C for 24 hours and the zone of inhibition (ZOI) measured in millimetres, interpreted according to CLSI (2018) guidelines. Extracts showing activity were further evaluated for minimum inhibitory concentration (MIC) by the broth micro-dilution method (CLSI document M07) and minimum bactericidal concentration (MBC) by sub-culturing onto antimicrobial-free agar (Andrews, 2001; Usman & Osuji, 2007).

Acute Toxicity and Histopathological Examination

Acute oral toxicity was assessed using Lorke's two-phase method (Lorke, 1983), consistent with the OECD (2002) acute toxic class approach. Twenty-one adult Wistar rats were assigned to a control group and six treatment groups (10, 100, and 1000 mg/kg in Phase 1; 1600, 2900, and 5000 mg/kg in Phase 2), with three rats per group, and observed for 14 days for signs of

toxicity and mortality; the LD50 was estimated as the geometric mean of the highest non-lethal dose and the lowest lethal dose. At the end of the observation period, animals were humanely sacrificed and the liver, kidney, heart, lungs, intestine, and spleen were harvested, fixed in 10% neutral buffered formalin, processed, sectioned, and stained with haematoxylin and eosin for histopathological examination.

Statistical Analysis

Data were expressed as mean $\pm$ standard deviation and analysed by one-way analysis of variance (ANOVA), with Welch's t-test for planned pairwise comparisons where variances were unequal; differences were considered significant at $p < 0.05$. Endpoints recorded as single determinations or ranges (zone of inhibition, MIC/MBC, mortality) were reported descriptively.

Ethical Approval

Approval was obtained from the appropriate institutional animal ethics/research committee prior to the animal studies, which were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals.

RESULTS AND DISCUSSIONS

The plant specimen was authenticated as *Moringa oleifera* (Ganyen zogale) at the Bayero University, Kano Herbarium (Accession Number BUKHAN 0011). All five bacterial isolates were successfully recovered in pure culture and confirmed by colonial morphology, Gram reaction, and biochemical profile.

Extraction Yield

Aqueous and 30% ethanol extraction gave the highest yields (Table 1), while yield declined sharply above 80% ethanol, with 95% ethanol giving the lowest overall yield (10.7%). A one-way ANOVA showed a statistically significant difference in yield among the six replicated solvent groups ($F(5,12) = 120.93$, $p < 0.001$); yield with 100% ethanol was significantly lower than

with water ($t(2.8) = 14.49$, $p < 0.001$) and 30% ethanol ($t(2.4) = 22.31$, $p < 0.001$), while water and 30% ethanol did not differ significantly ($p = 0.884$), nor did 70% and 80% ethanol ($p = 0.701$).

Table 1: Percentage extraction yield of *Moringa oleifera* seed and leaf extracts by solvent.

Extraction Solvent	Yield (%)
Water	33.73 $\pm$ 1.65
30% Ethanol	33.89 $\pm$ 0.40
50% Ethanol	31.34 $\pm$ 0.33
70% Ethanol	29.27 $\pm$ 0.62
80% Ethanol	29.60 $\pm$ 1.20
95% Ethanol	10.70
100% Ethanol	16.00 $\pm$ 1.33
Methanol	18.56

Qualitative and Quantitative Phytochemical Screening

Alkaloids, flavonoids, tannins, phenols, and free anthraquinones were detected in both the aqueous and methanol extracts; saponins were detected only in the methanol extract, and steroids were absent from both (Table 2). Quantitative analysis of the active extract showed flavonoids as the most abundant constituent, followed by total phenols and tannins, with alkaloids and saponins present in comparatively small amounts (Table 3).

Table 2: Qualitative phytochemical screening of the aqueous and methanol extracts. Key: + = present, - = absent.

Phytochemical	Aqueous	Methanol
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Phenols	+	+
Saponins	-	+
Steroids	-	-
Free anthraquinones	+	+

Table 3: Quantitative phytochemical content of the active extract (mean $\pm$ SD, n = 3).

Phytochemical	Content
Alkaloids	0.33 $\pm$ 0.05%
Flavonoids	26.29 $\pm$ 0.11 mg QE/g
Total phenols	2.87 $\pm$ 0.04 mg TAE/g
Saponins	0.45 $\pm$ 0.03%
Tannins	2.83 $\pm$ 0.07 mg TAE/g

Antimicrobial Activity

The extracts produced measurable zones of inhibition against all five test isolates (Table 4), narrowest against *Staphylococcus aureus* (8 mm) and widest and most variable against MRSA (7–13 mm) and *Pseudomonas aeruginosa* (7–12 mm). The minimum inhibitory and bactericidal titres/concentrations are presented in Table 5; MIC and MBC against MRSA were 0.512 and 1.024 mg/mL, and against *P.*

aeruginosa were 1.024 and 2.048 mg/mL, respectively.

Table 4: Zone of inhibition of the extract against the test bacterial isolates.

Organism	Zone of Inhibition
<i>Escherichia coli</i>	9 mm
<i>Streptococcus pneumoniae</i>	9 mm
<i>Staphylococcus aureus</i>	8 mm
MRSA	7–13 mm
<i>Pseudomonas aeruginosa</i>	7–12 mm

Table 5a: Minimum inhibitory and bactericidal dilution titres (ND = not determined — no bactericidal endpoint reached within the dilutions tested).

Organism	Aqueous MIC	Aqueous MBC	Methanol MIC	Methanol MBC
<i>E. coli</i>	1:8	1:16	1:4	1:8
<i>S. pneumoniae</i>	1:16	ND	1:8	1:16
<i>S. aureus</i>	1:4	ND	1:4	1:8

Table 5b: Minimum inhibitory and bactericidal concentrations against MRSA and *P. aeruginosa*.

Organism	MIC	MBC
MRSA	0.512 mg/mL	1.024 mg/mL
<i>Pseudomonas aeruginosa</i>	1.024 mg/mL	2.048 mg/mL

Acute Toxicity and Histopathology

No mortality was recorded at 1600 or 2900 mg/kg over the 14-day observation period; mortality at 5000 mg/kg was variable. The estimated LD₅₀ fell within the range of 1585–3808 mg/kg, placing the extract in the moderately toxic category (Table 6). Histopathological examination showed generally normal architecture at lower doses in all organs, with mild-to-moderate hepatocellular necrosis and sinusoidal dilatation, and organ-specific alterations in

the kidney, heart, lungs, and intestine at higher doses; the spleen showed no significant change across all groups (Table 7).

Table 6: Acute toxicity and mortality record.

Dose	Mortality
1,600 mg/kg	0%
2,900 mg/kg	0%
5,000 mg/kg	Variable
LD ₅₀ (estimated)	1,585–3,808 mg/kg

Table 7: Summary of histopathological observations across dose groups.

Tissue	Findings
Liver	Normal at lower doses; mild–moderate hepatocellular necrosis and sinusoidal dilatation at higher doses
Kidney	Normal at lower doses; tissue alterations at higher doses
Heart	Normal at lower doses; alterations reported at some doses
Lungs	Normal at lower doses; alterations reported at some doses
Intestine	Normal at lower doses; alterations reported at some doses
Spleen	No significant changes across dose groups

DISCUSSION

The marked, statistically significant decline in extraction yield above 80% ethanol concentration is consistent with polarity-based extraction theory: water and dilute

ethanol favour recovery of polar constituents such as tannins, saponins, and sugars, which constitute a large proportion of extractable mass, whereas high-concentration ethanol increasingly excludes

these polar compounds (Harborne, 1998; Sofowora, 1993).

The phytochemical profile obtained — alkaloids, flavonoids, tannins, phenols, and anthraquinones in both extracts, with saponins detected only in the methanol extract — is broadly consistent with the classes reported for *M. oleifera* leaves elsewhere (Divya et al., 2024; El-Sherbiny et al., 2024). The restriction of saponins to the methanol extract mirrors the general principle that saponins are more efficiently recovered by moderately polar organic solvents than by water alone (Sofowora, 1993).

The comparatively strong and variable activity observed against MRSA and *P. aeruginosa* — both organisms noted for intrinsic resistance mechanisms — may reflect differences in isolate resistance profile, extract concentration, or the broader phytochemical complement, including anthraquinones, detected in the extracts. The MIC and MBC values obtained fall within ranges considered biologically meaningful for plant extracts under CLSI-based susceptibility testing (CLSI, 2018; El-Sherbiny et al., 2024), supporting a genuine antibacterial effect rather than a marginal one.

The estimated LD₅₀ range (1585–3808 mg/kg) sits within the range reported elsewhere for *M. oleifera* leaf material, aligning closely with the LD₅₀ of 1585 mg/kg reported for aqueous leaf extract (Asare et al., 2012) and 3808 mg/kg for the hexane fraction (Mathias et al., 2025), though markedly lower than the very low toxicity reported for some other fractions (Chivapat et al., 2011; Ofulue & Ebomoyi, 2023). This spread illustrates that acute toxicity in *M. oleifera* is not a fixed property of the species but depends materially on the extract fraction and solvent system used. The mild-to-moderate hepatocellular necrosis and sinusoidal dilatation observed at higher doses, alongside unaffected spleen histology throughout, is consistent with the liver being the primary target organ of plant-extract toxicity at high dose, a pattern

also reported in a 90-day sub-chronic evaluation of the *M. oleifera* hexane fraction (Mathias et al., 2025).

CONCLUSION

This study demonstrates that *Moringa oleifera* (zogale) seed and leaf extracts possess a broad phytochemical profile — rich in flavonoids, tannins, phenols, alkaloids, and anthraquinones — and measurable, isolate-dependent antibacterial activity against all five tested organisms, most notably MRSA and *Pseudomonas aeruginosa*. Extraction yield was significantly, and predictably, solvent-dependent. Acute toxicity testing placed the extract's LD₅₀ within a moderate toxicity range (1585–3808 mg/kg), with hepatocellular changes concentrated at higher doses and the spleen unaffected throughout, supporting the traditional antibacterial use of zogale while indicating that dose control is important to its safe use. Further work should isolate and characterise the specific bioactive compounds responsible for the observed antimicrobial activity, particularly against MRSA and *P. aeruginosa*, and should extend to sub-chronic toxicity and in-vivo efficacy studies before any therapeutic dose is proposed.

Recommendations

1. Further studies should isolate and characterise the specific bioactive compounds responsible for the observed antimicrobial activity, particularly against MRSA and *P. aeruginosa*, using chromatographic and spectroscopic techniques (e.g., HPLC, GC-MS).
2. Given the moderate acute toxicity margin observed (LD₅₀ 1585–3808 mg/kg) and the hepatocellular changes seen at higher doses, sub-chronic toxicity studies and dose-ranging safety work are recommended before any therapeutic dose is proposed.
3. In-vivo efficacy studies (e.g., in an infection model) are recommended to confirm whether the in-vitro

antimicrobial activity translates to a therapeutic effect at non-toxic doses.

4. Traditional zogale preparations should be used with attention to dose, given the variability in mortality observed at 5000 mg/kg.

Declaration by Authors

Ethical Approval: Approved

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