



## PHYTOCHEMICAL COMPOSITION, TRACE ELEMENTS CONTENT, AND ANTIMICROBIAL ACTIVITY OF *Moringa oleifera* LEAVES CULTIVATED IN JOS, NIGERIA

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### ABSTRACT

This study evaluated the phytochemical composition, trace elements content, and the antimicrobial activity of *Moringa oleifera* leaves cultivated in Jos, Nigeria. The plant leaves were harvested directly from the farm, taken to the laboratory and extracted via maceration with 80% methanol after thorough washing under running water. Qualitative phytochemical screening of the plant leaves was carried out, trace metal content was determined in triplicates using AAS, and antimicrobial activity was done to determine its activity on *S. aureus*, *B. subtilis*, *S. pneumoniae* and *E. coli* organisms. Qualitative phytochemical screening revealed high concentrations of flavonoids and carbohydrates (+++), moderate levels of tannins and alkaloids (++) , low levels of steroidal terpenes and cardiac glycosides (+), while saponins and anthraquinones were absent. The extract exhibited concentration-dependent inhibition against *S. aureus* (23.5 mm at 500 mg/mL), *B. subtilis* (25.5 mm at 500 mg/mL), and *E. coli* (21 mm at 500 mg/mL), but showed no activity against *S. pneumoniae*. The minimum inhibitory concentration (MIC) was 250 mg/mL for *S. aureus* and *B. subtilis*, and 31.25 mg/mL for *E. coli*. Elemental analysis detected iron (23.16 mg/kg), sodium (193.75 mg/kg), calcium (28.76 mg/kg), and zinc (31.14 mg/kg) within recommended dietary ranges, with no toxic metals detected. The study finally suggests further studies on this plant using different solvents and methods to explore other parts of the plant in order to ascertain and utilize the potential bioactive components of the plant.

**Keywords:** Phytochemicals, *Moringa oleifera*, Trace Elements, Antimicrobial Activity and Nutritional Value

### INTRODUCTION

*Moringa oleifera* is a member of the family *Moringaceae*, the plant has been reported to be widely cultivated because of its nutritional, medicinal, and therapeutic importance (Klimek-Szczykutowicz *et al.*, 2024). *M. oleifera* is well grown in tropical and subtropical regions. In Nigeria, the plant is traditionally used in the management of various ailments (Mwami *et al.*, 2024), and its leaves are valued due to their rich composition of bioactive compounds. The presence of these bioactive components make the plant a subject of increasing scientific interest for pharmaceutical and nutraceutical applications (Mahaveerchand and Abdul-Salam, 2024).

Phytochemical investigations have revealed that *M. oleifera* leaves contain diverse secondary metabolites such as flavonoids, phenolics, alkaloids, saponins, terpenoids, and steroids, which are responsible for its antioxidant and antimicrobial properties (El-Sherbiny *et al.*, 2024; Abhang *et al.*, 2024). In addition, the plant is known to accumulate essential trace elements including iron, zinc, copper, and manganese, which contribute to its nutritional value and biological activity (Sakuntala *et al.*, 2023). The composition and concentration of these phytochemicals and trace elements are influenced by environmental factors such as soil type, climate, and geographical location, leading to variation in the medicinal potential of *M. oleifera* grown in different regions (Sukmawaty *et al.*, 2024; Omwango *et al.*, 2024).

Trace metals are mineral elements that occur in small quantities in plants and soils but play essential roles in biological systems. In medicinal plants such as *M. oleifera*, trace metals like iron, zinc, copper, and manganese contribute to enzymatic functions, antioxidant activity, and overall therapeutic potential (Musa *et al.*, 2023). However, their concentration largely depends on environmental factors such as soil composition, agricultural practices, and pollution

levels. While essential trace metals are beneficial at low levels, excessive accumulation may pose health risks due to toxicity (Kaur *et al.*, 2023).

Studies have demonstrated the antimicrobial efficacy of *M. oleifera* leaf extracts against pathogenic microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa* and others, suggesting its potential as a natural antimicrobial agent (Nkamkeu *et al.*, 2025, Ezeagwula and Oji, 2023; Danjuma *et al.*, 2025, Ojewumi *et al.*, 2025). Studies have demonstrated the antimicrobial efficacy of *M. oleifera* leaf extracts against pathogenic microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa* and others, suggesting its potential as a natural antimicrobial agent (Nkamkeu *et al.*, 2025, Ezeagwula and Oji, 2023; Danjuma *et al.*, 2025, Ojewumi *et al.*, 2025). However, variations in extraction methods, geographical location, and environmental conditions may significantly influence the antimicrobial potency and spectrum of activity of the plant extract. Despite extensive research on *M. oleifera* in various regions, there is limited location-specific information on the phytochemical composition, trace element content, and antimicrobial activity of the leaves cultivated in Jos, Nigeria. Most previous studies have examined these parameters separately rather than providing an integrated analysis that links phytochemicals and trace metals to antimicrobial efficacy. Furthermore, there is insufficient evidence correlating the biochemical profile of *M. oleifera* grown in Jos with its activity against selected pathogens, creating a clear gap that this study seeks to address.

Therefore, this study aimed to: (i) determine the phytochemical composition *M. oleifera* leaves methanolic extract cultivated in Jos, (ii) quantify essential trace elements of the plants leave extract and (iii) evaluate antimicrobial activity against selected pathogens of *S. aureus*, *B. subtilis*, *S.*

*pneumoniae* and *E. coli*. Furthermore, the research addressed questions such as: (i) what are the phytochemical composition of *M. oleifera* leaves cultivated in Jos, (ii) how much of essential trace elements are present in the leave extract and finally (iii) what is the level of antimicrobial activity of the plants extract against some pathogens.

## MATERIALS AND METHODS

### Sample Collection and Preparations

Sampled *Moringa oleifera* leave was collected directly from the tree at old Legislative Quarters Angwan Rukuba Jos which was given a voucher number FHJ: 35121 and was authenticated by Mr. J. J. Azila at Federal College of Forestry Jos in Forestry herbarium Jos. The leaves were properly wrapped in a polyethylene bag and taken to University of Jos

Chemistry department's laboratory for washing under running water and air dried at ambient temperature for 12 days. The dried sample was crushed into powdered form for further extraction.

### Sample Extraction (Maceration Method)

About 150 g of the powdered leaves was macerated in 1 L of 80% methanol (methanol: water, 80:20 v/v) for 48 hours at room temperature with occasional stirring. The macerated sample was filtered using a muslin cloth and the filtrate was concentrated using a rotary evaporator at a controlled temperature of 60 °C and a drying cabinet was finally used to complete the drying at 30°C. The obtained extract was stored in a well tight glass bottle at ambient temperature.



Figure 1: *Moringa oleifera* Extract

### Percentage Yield

The percentage yield was calculated using the formula below:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of crude powder}} \times 100$$

### Qualitative Phytochemical Screening of *Moringa Oleifera* Leave Extract

The qualitative phytochemical screening was carried out using procedures described by Prabhavathi *et al.* (2016). All qualitative phytochemical screening were done in triplicates.

#### Test for Alkaloids (Wagner's test)

A few drops of Wagner's reagent (Picric solution) was added to 0.5 g of the extract in a test tube. A reddish- Brown precipitate confirms the presence of alkaloids.

#### Test for Saponins

The extracts 0.5 g was placed in a test tube and 10 mL of distilled water was added. The suspension was shaken in a graduated cylinder for 15 minutes. A two layer of foam observed indicates the presence of saponins

#### Test for Cardiac Glycosides

The plant extract 0.1 g was dissolved in 1 mL glacial acetic acid and two drops of ferric chloride solution was added. 1ml of conc. H<sub>2</sub>SO<sub>4</sub> was added to the solutions. Brown ring coloration indicated the presence of cardiac glycosides.

#### Test for Flavonoids

To a 5 mL of the sample solution, 5 mL of 20% NaOH was added, yellow solution indicates the presence of flavonoid.

#### Test for Anthraquinone

The sample extract 0.5 g was placed in a test tube of the chloroform was added and shaken for 5 minutes, the extract was filtered and the filtrate shack with equal volume of concentrated ammonia solution. A violet or red color was indicated the presence of free anthraquinone.

#### Test for Tannins

The plant extract 0.5 g was dissolved in 1ml of distill water and filtered, few drops of ferric chloride was added to the filtrate a blur-black, or green or blue-green precipitate confirms its presence.

#### Test for Steroids and Terpenes

The plant extract 0.1 g was dissolved in 1ml chloroform, 1 ml of acetic and hydride and two drops concentrated H<sub>2</sub>SO<sub>4</sub> was added, a pink color which changes to bluish green indicate the presence of steroids and terpenes.

#### Test for Carbohydrates

The plant extract 0.1 g was dissolved in 1 ml Of concentrated H<sub>2</sub>SO<sub>4</sub> acid then heated. A blackening effervescence indicated the presence of carbohydrate (Sofowora, 2008).

### Determination of Trace Elements

#### Dry Ashing

Air dried plant material (leaves) were pulverized into powder, transferred into crucible dish and put to muffle furnace, heated at 500 °C for 30minutes. It was then removed and allowed in a desiccator to cooled and dried, as described by Ashiq *et al.*, (2013).

**Digestion of Sample**

The ashed sample (5 g) was transferred into 250 mL beaker. 10 mL Hydrochloric acid (HCl) was added and covered with watch glass and heated for 15 minutes, removed and cooled. 5 mL of concentrated Nitric acid (HNO<sub>3</sub>) was added and heated to dryness and dehydrated. 1 mL of 6M of Hydrochloric acid (HCl) was added again. 10 mL of distilled water was added and heated to redissolved, cooled and filtered with a Whatman No. 541 into a 100 mL volumetric flask up to the mark levelled. It was then transferred into a polythene bottle for elements analysis, as described by AOAC, (1990).

**Sample Analysis**

The method applied in the evaluations of some trace elements of the plant material after ashing and digestion was the use of Atomic Absorption Spectrophotometric (AAS) techniques as described by AOAC, (2005). All analyses were performed in triplicate. Standard reference materials were used for calibration and quality assurance

**Antimicrobial Activity of *Moringa Oleifera* leaf extract****Bacterial Strain**

The antimicrobial activity of the plant extract was evaluated using four bacterial strains. Two strains of Gram negative (*Escherichia coli*, *Pseudomas aeruginosa*) and two strain of Gram positive (*Bacillus subtilis*, *Staphylococcus aureus*). The bacterial strain was provided from the culture collection of Microbiology section of faculty of Pharmacy University of Jos, Plateau State.

**Standardization of Inoculum**

Each bacterial isolate was sub cultured from nutrient agar slant into nutrient broth and incubated for 6 h. the inoculum was sub cultured onto nutrient agar plate and incubated for 24 h at 37 °C. Pure culture of each organism was selected. Sterile wire loop was used to pick 2 to 3 colonies of the organism and emulsified in a tube containing 5 ml of physiological saline. The tube was inserted into a sensititre-nephelometer (TREK Diagnostic system, UK) after calibration adjustment was made with extra inoculum or diluents, until 0.5 McFarland standard (1.5 x 10<sup>8</sup> cfu/ml).

**Bacterial Susceptibility Testing**

Agar diffusion method was carried out as described by Palladini *et al.* (2023) with slide modifications. Mueller-Hinton agar (MHA) plates were prepared and they were incubated for sterility check at 37 °C for 24 h. the plates were flooded with one thousand microliter (1000 µl) of the standardized organism separately. Excess was drained off and allowed to remain on the bench for 10 minutes. A sterile cork borer of 5 mm diameter was used to make 5 wells on each plate. The wells were sealed using molten MHA and labeled. One hundred microliter (100 µl) of the various extract concentrations (500, 250, 125 and 62.5 mg/ml) were dispensed into each well and into the remaining well, Ciprofloxacin (30 mg/ml) was dispensed as positive control. The inoculated plates were left on the bench for 10 minutes to allow the extract to diffuse into the agar. The plates were incubated aerobically at 37 °C for 24 h. The presence of inhibition zones were measured by meter a meter rule, recorded and considered as indication for antimicrobial activity.

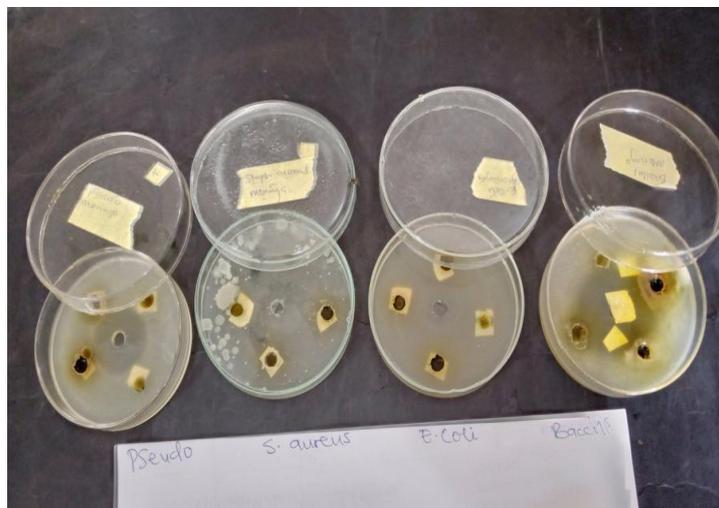


Figure 2: Antimicrobial Activity of *Moringa oleifera* Leaf Extract

**Determination of Minimum Inhibitory Concentration (MIC)**

The Minimum Inhibitory Concentration (MIC) was determined using a modified tube dilution method as described by Barotti *et al.* (2025). A series of two-fold serial dilutions of the methanolic leaf extract were prepared in sterile nutrient broth to obtain concentrations of 500, 250, 125, 62.5, 31.25, and 15.63 mg/mL. Each test tube contained an equal volume of nutrient broth and the respective extract concentration. Thereafter, 20 µL of standardized bacterial

inoculum (adjusted to 0.5 McFarland standard, approximately 1.5 × 10<sup>8</sup> CFU/mL) was aseptically added to each tube. A tube containing broth and inoculum without extract served as the growth control, while a tube containing broth only served as the sterility control. All tubes were incubated at 37°C for 24 hours under aerobic conditions. After incubation, the tubes were examined for turbidity (visible growth). The MIC was defined as the lowest concentration of the extract that showed no visible turbidity, indicating complete inhibition of bacterial growth.



Figure 3: Minimum Inhibition Concentration (MIC) of *Moringa oleifera* Leave Extract

**Determination of Minimum Bactericidal Concentration (MBC)**

The MBC was determined by subculturing samples from the tubes used in the MIC test that showed no visible bacterial growth. A small volume from each of these tubes was streaked

onto sterile Mueller-Hinton Agar (MHA) plates, which were then incubated at 37 °C for 24 hours. The MBC was recorded as the lowest concentration of the extract at which no bacterial colonies grew on the agar, indicating complete killing of the test organism.

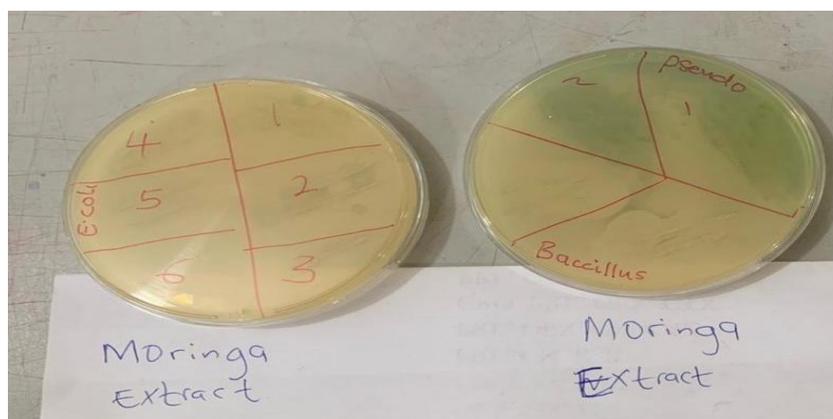


Figure 4: Minimum Bactericidal Concentration (MBC) of *Moringa oleifera* Leave Extract. Water was used as Negative Control for *S. aureus*, *B. subtilis*, *S. pneumoniae* and *E. coli* Organisms

**Statistical Analysis**

All experiments were performed in triplicate, and results are expressed as mean ± standard deviation (SD). Data were analyzed using statistical package for the social science (SPSS) version 30.0

**RESULTS AND DISCUSSION**

**Results**

**Percentage Yield**

$$\begin{aligned} \% \text{ Yield} &= \frac{\text{Weight of extract}}{\text{Weight of crude powder}} \times 100 \\ &= \frac{87.60}{150} \times 100 \\ &= 58.40\% \end{aligned}$$

**Phytochemical Screening of *Moringa oleifera* Leave Extract**

**Table 1: Qualitative Phytochemical Screening of *Moringa oleifera* Leave Extract**

Phytochemicals	Result
Saponins	-
Tannins	++
Alkaloids	++
Flavonoids	+++
Steroid terpenes	+
Cardiac Glycosides	+
Anthraquinones	-
Carbohydrates	+++

+: present, ++: moderately present, +++ highly present, - absent. All tests were performed in triplicate with consistent results

**Antimicrobial Evaluation of *Moringa oleifera* Leaf Extract**

**Table 2: Antimicrobial activity of *Moringa oleifera* Leaf Extract**

Organism	Concentration of extract (mg/mL)/ diameter of zones of inhibition (mm)				Positive control Ciprofloxacin (30mg/ml)
	500	250	125	62.5	
SA	23.50 ± 2.10	19.00 ± 1.85	0.00 ± 0.00	0.00 ± 0.00	38.00 ± 3.20
BS	25.50 ± 2.45	21.00 ± 2.00	15.00 ± 1.75	12.00 ± 1.60	35.00 ± 2.95
SP	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	27.00 ± 2.50
EC	21.00 ± 1.95	18.00 ± 1.80	12.00 ± 1.50	10.00 ± 1.35	10.25 ± 1.20

Key: SA = *Staphylococcus aureus*, BS = *Bacillus subtilis*, SP = *Streptococcus pneumoniae*, EC = *Escherichia coli*. All analysis was done in triplicates. The extraction solvent (negative control) showed no inhibition against any test organism

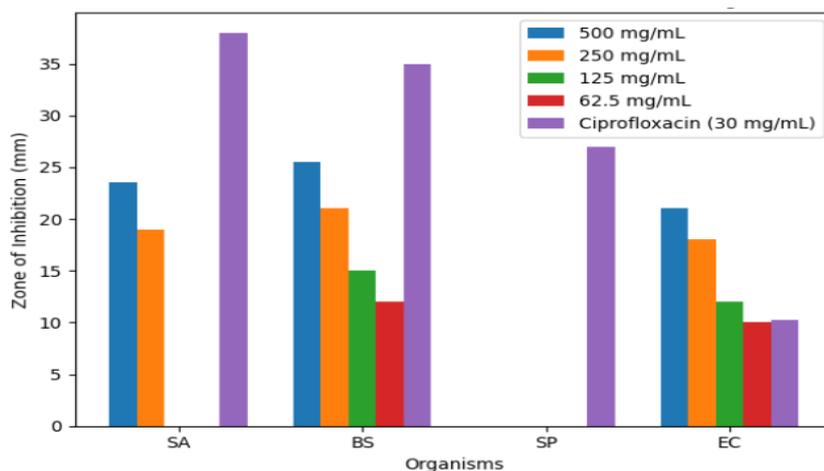


Figure 5: Effects of Extract Concentrations and Standard Drug on Test Organisms

**Table 3: Minimum Inhibitory Concentration (MIC), of the *Moringa Oleifera* Leaves Extract**

Organism	Concentration of extract (mg/ml)						MIC (mg/ml)
	500	250	125	62.5	31.25	15.63	
SA	-	-	+	+	+	+	500
BS	-	-	+	+	+	+	250
SP	-	-	-	-	-	-	125
EC	-	-	-	-	-	+	31.25

+: present turbidity, -: no turbidity

MIC values were determined as the lowest concentration showing no visible growth in duplicate tubes

**Table 4: Minimum Bactericidal Concentration (MBC) Sample**

Organism	500mg/mL	250mg/mL	125mg/mL	62.5mg/mL	31.25 mg/mL	15.63mg/mL	MBC(mg/mL)
SA	0	210.40 ± 5.20	108.35 ± 3.10	54.80 ± 2.45	28.60 ± 1.20	14.20 ± 0.85	500
BS	430.25 ± 6.50	220.10 ± 4.35	112.75 ± 2.80	58.40 ± 2.10	29.75 ± 1.05	15.10 ± 0.70	250
SP	0	0	0	0	0	0	0
EC	410.60 ± 5.90	205.45 ± 4.10	118.20 ± 3.25	60.35 ± 1.95	30.10 ± 0.95	15.40 ± 0.60	62.5

**Table 5: MBC/MIC Ratio Table**

Organism	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC Ratio	Interpretation
SA	500	500	1.0	Bactericidal
BS	250	250	1.0	Bactericidal
SP	125	0	-	Bacteriostatic
EC	31.25	62.5	2.0	Bactericidal

The MBC/MIC ratio indicated bacteriostatic (ratio >4) or bactericidal (ratio ≤4) activity

**Atomic Absorption Spectroscopy (AAS) Analysis on the Leave of *Moringa oleifera***

**Table 6: Essential Mineral Content of the Leave of *Moringa oleifera* Cultivated in Jos with the Recommended Dietary Allowance (RDA)**

Elements	Concentration (mg/kg)	Recommended dietary Allowance (RDA) (mg/d)
Na	193.75 ± 13.56	120-420
Ca	28.76 ± 2.01	1000-3000
Fe	23.16 ± 1.62	0.5-27

Elements	Concentration (mg/kg)	Recommended dietary Allowance (RDA) (mg/d)
Ni	N.D	-
Cu	4.65 ± 0.33	0.2-10
Zn	31.14 ± 2.18	2-13

Note: N.D means not detected. - : not analyzed

### Discussion

The phytochemical screening of *Moringa oleifera* leaves from Jos in table 1 showed high levels of flavonoids and carbohydrates (+++), moderate tannins and alkaloids (++), low steroid terpenes and cardiac glycosides (+), with saponins and anthraquinones absent. These findings agree with earlier studies reporting flavonoids, tannins, and alkaloids as dominant compounds in *M. oleifera* leaves (Khalid et al., 2023), contributing to their medicinal value (Shafiq et al., 2024; El-Sherbiny et al., 2024). Minor variations, such as the absence of saponins and anthraquinones. Saponins and anthraquinones were reported to be present in a study conducted by Nweze and Nwafor (2014) from South-Eastern part of Nigeria. Oluduro (2012) reported absence of steroids, terpenoids and cardiac glycoside while Bamishaiye et al. (2011) reported absence terpenoids and cardiac glycoside in the leaf extract. The absence of saponins and anthraquinones in this study may be due to differences in extraction methods, environmental conditions or the polarity of the solvent that was used. While high flavonoid content suggests strong antioxidant and antimicrobial potential, as flavonoids can scavenge free radicals and inhibit microbial growth. The absence of saponins may reduce foaming and membrane-permeabilizing activity, potentially affecting some antimicrobial and pharmacological properties

The antimicrobial activity of *Moringa oleifera* leaf extract presented in table 2 demonstrated clear concentration-dependent effects, with the largest inhibition zones observed at 500 mg/mL and progressively smaller zones at lower concentrations, particularly against *Staphylococcus aureus* (23.50 ± 2.10 mm at 500 mg/mL) and *Bacillus subtilis* (25.50 ± 2.45 mm at 500 mg/mL). These findings are consistent with previous studies reporting increased antibacterial efficacy with rising extract concentrations Saqib et al. (2019), reflecting higher availability of bioactive phytochemicals for bacterial inhibition (Sweet et al., 2024; Arya et al., 2025). The extract showed different susceptibility between Gram-positive and Gram-negative bacteria, with Gram-positive organisms generally more sensitive, likely due to the more accessible peptidoglycan layer compared to the protective outer membrane of Gram-negative bacteria, which limits penetration of phytochemicals (Sadah Al-Halfi et al., 2024). Notably, *Streptococcus pneumoniae* exhibited complete resistance across all tested concentrations, a phenomenon that may be attributed to its unique capsular structure and potential efflux mechanisms that reduce intracellular accumulation of antimicrobial agents (Zahari et al., 2023). When compared to the standard drug, Ciprofloxacin (30 mg/mL), which produced larger inhibition zones (35–38 mm), the crude extract showed moderate potency, highlighting the potential for further purification to enhance efficacy. The negative control (water) showed no inhibition, confirming that the observed effects were due to the bioactive compounds in the extract rather than experimental artifacts. While the extract displayed measurable activity, the high effective concentrations (up to 500 mg/mL) indicate moderate potency. In a clinical context, such doses may be impractical for systemic use, underscoring the need for purification or enrichment of active compounds to achieve therapeutic relevance. These results shows the

antimicrobial potential of *M. oleifera* leaves, particularly against Gram-positive pathogens, while highlighting limitations related to bacterial resistance and extract potency. Table 3 shows that the *Moringa oleifera* leaf extract exhibited varying inhibitory effects against the tested organisms, with Minimum Inhibitory Concentration (MIC) values determined as the lowest concentrations showing no visible growth. The extract showed the highest MIC against *Staphylococcus aureus* (500 mg/ml) and *Bacillus subtilis* (250 mg/ml), indicating lower susceptibility, while *Salmonella paratyphi* had an MIC of 125 mg/ml. *Escherichia coli* demonstrated the greatest sensitivity with the lowest MIC value of 31.25 mg/ml, confirming the antibacterial potential of *M. oleifera* leaves as reported in previous studies (Royani et al., 2023; Segwatibe et al., 2023).

The essential mineral analysis of *Moringa oleifera* leaves cultivated in Jos (Table 5) revealed good concentrations of sodium (193.75 ± 13.56 mg/kg), calcium (28.76 ± 2.01 mg/kg), iron (23.16 ± 1.62 mg/kg), copper (4.65 ± 0.33 mg/kg), and zinc (31.14 ± 2.18 mg/kg), while nickel was not detected, indicating the nutritional potential of the leaves. The sodium value (193.75 ± 13.56 mg/kg) falls within acceptable dietary ranges, whereas the iron content (23.16 ± 1.62 mg/kg) and zinc content (31.14 ± 2.18 mg/kg) suggests that the leaves could serve as a good source of essential trace elements. Higher iron content (~107.48 ppm) was reported by Mawouma et al., (2024). The iron concentration in the present study is lower, possibly due to variations in soil composition and environmental conditions (Saleem et al., 2023). Studies have reported comparable calcium and zinc contents in *M. oleifera* leaves (Masitlha et al., 2024; de Oliveira et al., 2023), confirming that the plant may contain macro or micro minerals mineral content across different agro-ecological zones

### CONCLUSION

This study provided an integrated assessment of the phytochemical composition, trace element content, and antimicrobial activity of *Moringa oleifera* leaves cultivated in Jos, Nigeria. The qualitative phytochemical screening revealed high presence of flavonoids and carbohydrates, moderate presence of tannins and alkaloids, and low steroid terpenes and cardiac glycosides. Saponins and anthraquinones were absent, suggesting environmental or solvent-related influences on phytochemicals. The extract demonstrated concentration-dependent antimicrobial activity, showing bactericidal effects against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*, and bacteriostatic activity against *Streptococcus pneumoniae*, although relatively high concentrations were required, indicating moderate potency of the crude extract. Atomic Absorption Spectroscopy confirmed the presence of essential minerals, including sodium (193.75 ± 13.56 mg/kg), iron (23.16 ± 1.62 mg/kg), zinc (31.14 ± 2.18 mg/kg), copper (4.65 ± 0.33 mg/kg), and calcium (28.76 ± 2.01 mg/kg), while nickel was not detected, highlighting the nutritional relevance of the leaves, particularly as a potential source of iron and zinc. However, the study was limited to methanolic crude extract and only four bacterial strains, without isolation of active compounds or in vivo validation. Therefore, future studies should focus on purification and

characterization of bioactive constituents, toxicity evaluation, broader antimicrobial screening, and correlation of soil composition with mineral uptake.

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