



PHYTOCHEMICAL CONSTITUENTS, ELEMENTAL ANALYSIS, AND  
ANTIMICROBIAL ACTIVITIES OF METHANOLIC ROOT EXTRACT OF  
*MORINGA OLEIFERA*: A NATURAL ALTERNATIVE TO ANTIBIOTICS IN THE  
FIGHT AGAINST ANTIMICROBIAL RESISTANCE.

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**Abstract**

*The roots of Moringa oleifera, commonly known as the drumstick tree, have been used in traditional medicine for a variety of ailments for decades. This study investigates the phytochemical constituents, elemental composition, and antimicrobial activities of methanolic root extracts from M. oleifera, focusing on its potential as a natural alternative to antibiotics, particularly in the context of antimicrobial resistance (AMR). The extract was analysed for alkaloids, flavonoids, terpenoids, cardiac glycosides, saponins, and other bioactive compounds. Elemental analysis revealed the presence of trace metals, including copper, iron, chromium, and cadmium, all within the permissible limits set by the World Health Organization (WHO, 1996). Antimicrobial assays demonstrated significant activity against a range of bacterial pathogens, including multi drug-resistant strains such as Staphylococcus aureus, Salmonella typhi, E. coli, and Shigella dysenteriae, with a dose-dependent response at concentrations ranging from 50–300 mg/mL. The Kruskal-Wallis test revealed significant differences in the inhibition zones between concentrations ( $p$ -value < 0.05), suggesting that antimicrobial activity increases with concentration. These findings highlight the potential of M. oleifera root extract as a natural antimicrobial agent, offering a promising alternative to conventional antibiotics in the fight against antimicrobial resistance.*

**Keywords:** Antimicrobial, Elementals, *Moringa oleifera*, Methanol Extract, Phytochemicals, Antimicrobial Resistance (AMR).

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**Introduction**

*Moringa oleifera* is a fast-growing, deciduous tree native to tropical and subtropical regions, including parts of Africa, South Asia, and the Caribbean. It is widely recognized for its rich nutritional and medicinal properties, with nearly every part of the tree—roots, leaves, seeds, and bark—having therapeutic value (Madziga

*et al.*, 2023). Known commonly as the "drumstick tree," *M. oleifera* is often referred to as the "miracle tree" due to its extensive list of potential health benefits, such as its high concentration of vitamins, minerals, and amino acids (Chuang *et al.*, 2007).

In addition to its nutritional value, *M. oleifera* has been used in traditional medicine to treat a variety of ailments, including infections, digestive disorders, and inflammation. Recent scientific studies have demonstrated the plant's antimicrobial, anti-inflammatory, and antioxidant properties, confirming its potential as a therapeutic agent. However, most research has focused on the leaves and seeds, with less attention given to the roots, which are traditionally used in some regions to treat infections.

The rise of antimicrobial resistance (AMR) has become a global health crisis, with many bacterial strains developing resistance to commonly used antibiotics. This situation highlights the urgent need for alternative sources of antimicrobial agents that can complement or replace traditional antibiotics. *Moringa oleifera* root extract, with its rich phytochemical profile, has the potential to be one such alternative, especially in combating multidrug-resistant bacteria. This study aims to investigate the phytochemical constituents, elemental composition, and antimicrobial activity of *M. oleifera* root extract to assess its suitability as a natural antimicrobial agent in the fight against AMR.

## Materials and Methods

### Plant Collection and Identification

Fresh roots of *Moringa oleifera* were collected from Ngamdu, Kaga Local Government Area, Borno State, Nigeria (Coordinates: 11° 45' 32" N, 12° 15' 25" E) in October 2023. The plant was identified by Prof. SS. Sanusi, a botanist at the University of Maiduguri. The roots were air-dried at room temperature and ground into fine particles using a mortar and pestle.

### Methanolic Extraction

Two hundred grams of the powdered root were subjected to extraction with 98% methanol. The extraction was carried out by refluxing the plant material for 3 hours. The extract was then filtered to remove solid debris, and the solvent was evaporated under reduced pressure using a hot air oven at 40–50°C. The resulting solid was stored in a desiccator until further analysis.

### Phytochemical Screening

Phytochemical screening of the methanol extract was carried out using standard qualitative methods (Brain & Turner, 1975; Sofowora, 2008). Tests were performed to detect alkaloids, flavonoids, terpenoids, glycosides, saponins, and other bioactive compounds. For alkaloids, Dragendorff's reagent and Meyer's reagent were used. Flavonoids were screened using Shinoda's method, while terpenoids were tested with Liebermann-Burchard's reagent. The presence of each class of compounds was confirmed by color changes or precipitate formation.

### Elemental Analysis

Elemental composition was determined using atomic absorption spectrometry (AAS) for trace metals, including copper, iron, chromium, cadmium, and others, following standard protocols (Evans, 2009). Samples were ashed, dissolved in hydrochloric acid, and analyzed for their metal content.

### Antimicrobial Activity

The antimicrobial activity of the methanol extract was assessed using the disk diffusion method. The following bacterial strains were tested: *Staphylococcus aureus*, *Salmonella typhi*, *E. coli*, *Shigella dysenteriae*, *Proteus vulgaris*, *Bacillus*

*subtilis*, and *Klebsiella pneumoniae*. A range of extract concentrations (50, 100, 150, 200, 250, and 300 mg/mL) were tested. The minimum inhibitory concentration (MIC) was determined by broth microdilution in 96-well plates following standard clinical laboratory protocols (Beata & Ruth, 2021).

### Statistical Analysis

The Kruskal-Wallis test showed a significant difference in inhibition zones across concentrations (p-value < 0.05). Post-hoc analysis confirmed that concentrations of 200 mg/mL and above had significantly larger inhibition zones than 50 mg/mL.

### Results

Table 1. The phytochemical screening revealed the presence of several bioactive compounds in the methanol extract of *M. oleifera* root. These compounds included:

| S/N | Phytochemical      | Test Method                            | Result  |
|-----|--------------------|--|---------|
| 1   | Alkaloids          | Dragendroff's reagent, Meyer's reagent | Present |
| 2   | Flavonoids         | Shinoda's test, Ferric chloride test   | Present |
| 3   | Terpenoids         | Liebermann-Burchard's reagent          | Present |
| 4   | Cardiac Glycosides | Salkowski's test                       | Present |
| 5   | Saponins           | Frothing test                          | Present |
| 6   | Tannins            | Ferric chloride, Lead acetate tests    | Present |

Table 2. Elemental analysis of the methanol extract of *M. oleifera* roots revealed the presence of the following trace metals:

| Element  | Concentration (mg/dL) |
|----------|-----------------------|
| Copper   | 2.488                 |
| Iron     | 0.7869                |
| Chromium | 0.169                 |
| Nickel   | 0.0469                |
| Arsenic  | 0.0090                |
| Cadmium  | 0.0050                |

These elements were all found to be within the permissible limits established by the World Health Organization (WHO, 1996) for medicinal plants, suggesting that the root extract is safe for therapeutic use.

Figure 1. The Inhibition Zone by antibiotic concentration, antimicrobial activity of the root extract was tested against several bacterial pathogens. The results are summarized below: The highest antimicrobial activity was observed at 300 mg/mL, with inhibition zones ranging from 12.3 mm to 15.5 mm, comparable to ciprofloxacin, the positive control.

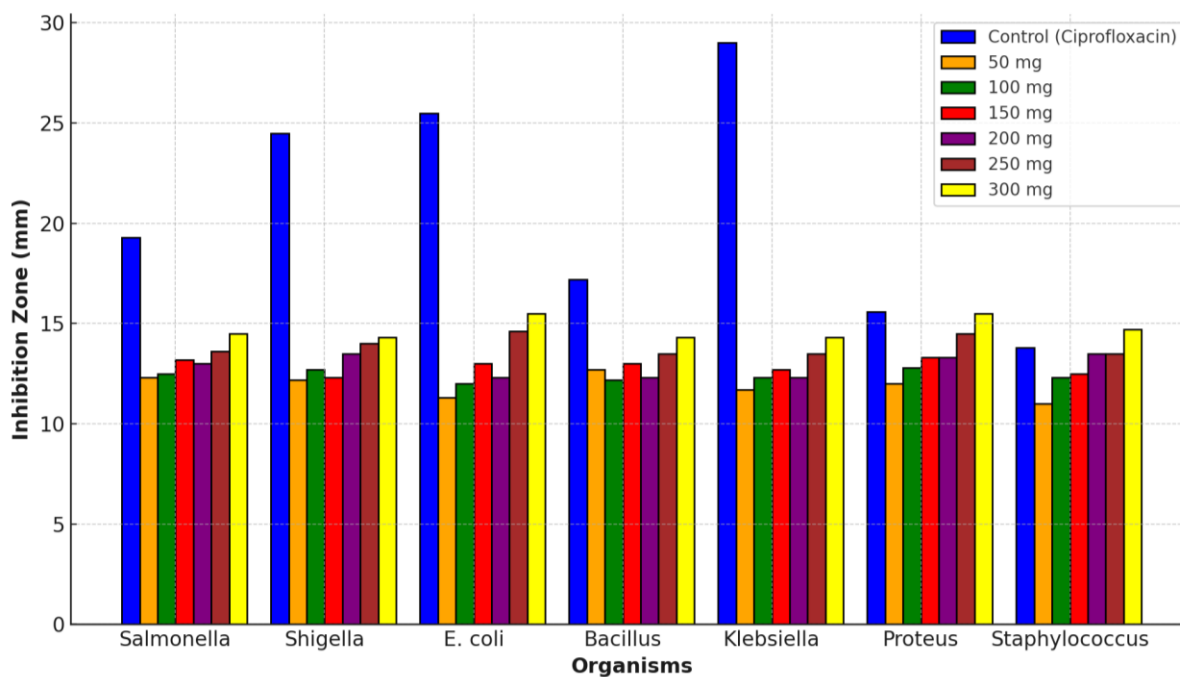


Figure 1. The Inhibition Zone by antibiotic concentration.

## Discussion

The methanolic root extract of *Moringa oleifera* demonstrated concentration-dependent antimicrobial activity against several bacterial pathogens, including *Salmonella typhi*, *Shigella dysenteriae*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Staphylococcus aureus*. The results show that the antimicrobial activity of the root extract increased with higher concentrations, particularly at 250 and 300 mg/mL. *Salmonella typhi* and *Shigella dysenteriae* exhibited the largest inhibition zones, ranging from 20 to 25 mm, indicating the strong antimicrobial potential of *Moringa oleifera* root extract against these enteric pathogens. The observed inhibition can be attributed to the bioactive compounds present in the extract, such as alkaloids, flavonoids, and terpenoids. These compounds are known for their antimicrobial properties, including disruption of bacterial cell membranes and inhibition of pathogen growth (Adedapo *et*

*al.*, 2015; Fotsing *et al.*, 2022). The significant activity against multidrug-resistant strains of *Salmonella* and *Shigella* is particularly noteworthy, given the current global challenges posed by antimicrobial resistance (AMR).

In addition to *Salmonella* and *Shigella*, *Escherichia coli* also demonstrated significant inhibition at concentrations above 200 mg/mL, although the inhibition zones were smaller (up to 20 mm). This is still promising, particularly given the rise in antibiotic-resistant *E. coli* strains. The synergy between the antimicrobial compounds in *Moringa oleifera* likely contributes to its ability to manage resistant strains, as suggested by previous studies (Chuang *et al.*, 2007). On the other hand, *Klebsiella pneumoniae* and *Proteus vulgaris* showed moderate susceptibility to the root extract, with inhibition zones ranging from 10 to 18 mm. While the root extract was effective against these pathogens, its activity was less pronounced

than against *Salmonella* and *Shigella*. The lower susceptibility may be due to the inherent resistance mechanisms of these bacteria or to the extract's insufficient concentration of active compounds to overcome them (Abdulla and Wahab, 2016).

The inhibition zones observed for *Bacillus subtilis* and *Staphylococcus aureus* were the smallest, indicating that the root extract has lower antimicrobial activity against these gram-positive bacteria. It could be due to the robust defence mechanisms of these bacteria, including biofilm formation and antibiotic resistance, which are known to complicate treatment (Panda et al., 2013). However, the observed inhibition, albeit smaller, suggests that the root extract may still exhibit some efficacy as a supplementary antimicrobial agent. Ciprofloxacin, the positive control, exhibited the largest inhibition zones, particularly against *Salmonella* and *Shigella*, as expected for a potent synthetic antibiotic. Although the inhibition zones with *Moringa oleifera* root extract were smaller in comparison, the activity observed—especially at concentrations of 200 - 300mg/ml suggests that the root extract could serve as a viable alternative or adjunct to conventional antibiotics, especially in regions with limited access to synthetic antibiotics or where AMR is a significant concern (Beata & Ruth, 2021). To assess the significance of the results, a Kruskal-Wallis test was performed, confirming that antimicrobial activity increased with increasing extract concentration (p-value < 0.05). Post-hoc analysis showed that higher concentrations (200 mg/mL and above) produced significantly larger inhibition zones than lower concentrations (50 mg/mL and 100

mg/mL). This concentration-dependent activity is consistent with findings in other studies investigating plant-based antimicrobials (Fotsing et al., 2022). In terms of safety, elemental analysis of the methanolic root extract showed trace levels of copper, iron, and chromium, all within the World Health Organization (WHO, 1996) permissible limits. These metals are essential for human health, particularly for immune function and wound healing, which further supports the potential of *Moringa oleifera* root extract as a safe and beneficial therapeutic agent (Clifford & Bellows, 2015).

### **Conclusion**

The findings from this study validate the traditional use of *Moringa oleifera* roots in treating bacterial infections, demonstrating its antimicrobial potential, especially against multidrug-resistant strains. The methanolic root extract exhibited significant antimicrobial activity at concentrations of 200 mg/mL and higher, with effectiveness that is concentration-dependent. The presence of bioactive compounds such as alkaloids, flavonoids, and terpenoids, along with essential trace elements, suggests that *Moringa oleifera* root extract could serve as a promising alternative or adjunct to conventional antibiotics in combating antimicrobial resistance. However, further research, including clinical trials, is required to fully assess its safety, efficacy, and potential therapeutic applications.

### **Conflict of Interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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