

Antibacterial Efficacy of *Moringa oleifera* Leaf Extracts on Some Selected Pathogenic Bacteria

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ABSTRACT

The global escalation of antimicrobial resistance (AMR) demands urgent exploration of plant-based alternatives with broad-spectrum antibacterial properties. This study evaluated the phytochemical composition and antibacterial activity of aqueous and ethanolic leaf extracts of *Moringa oleifera* against six pathogenic bacterial isolates. Qualitative phytochemical screening revealed that the aqueous extract contained tannins, flavonoids, saponins, alkaloids, and steroids, whereas the ethanolic extract contained only tannins and flavonoids. The antibiotic susceptibility profiles showed that *Salmonella typhi* was the most sensitive to conventional antibiotics, with no significant difference between the antibiotics tested for this isolate ($\chi^2 = 0.284$, $df = 9$, $p = 1.000$), while *Clostridium botulinum* exhibited the highest resistance with significant difference in relation to the antibiotics tested against it ($\chi^2 = 60.364$, $df = 9$, $p < 0.001$). Other isolates, including *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus aureus*, and *Bacillus subtilis*, demonstrated variable resistance patterns with highly significant differences in their responses across antibiotics ($p < 0.001$). At the level of individual antibiotics, significant variation in bacterial responses was also observed ($p < 0.001$); ciprofloxacin, though more consistent, showed a statistically significant difference ($\chi^2 = 12.548$, $df = 5$, $p = 0.028$). In contrast, both aqueous and ethanolic *Moringa oleifera* extracts exhibited insignificant antibacterial effects across all concentrations against all test organisms. Ciprofloxacin, used as the positive control, showed significant inhibition only for *S. typhi* and *S. aureus*, with no measurable effect against the remaining isolates. These findings suggest that while *Moringa oleifera* contains bioactive compounds, its antibacterial efficacy in the tested forms and concentrations is limited. Further research is recommended to optimize extraction methods and assess potential synergistic applications in combating AMR.

Keyword: *Moringa oleifera*; Bacteria; Pathogens; Antimicrobial Resistance; Gram Negative Bacteria; Gram Positive Bacteria; Aqueous and Ethanolic Leaf Extracts; Plant-Based Alternatives

Abbreviations: SD: Standard Deviation; R: Resistant; I: Intermediate; S: Susceptible; SXT: Cotrimoxazole; CH: Chloramphenicol; TET: Tetracycline; CPX: Ciprofloxacin; AM: Amoxicillin; AU: Augmentin; CN: Gentamicin; ERY: Erythromycin; CEF: Cefazidime/Clavulanic Acid; CF: Cefazolin

Introduction

Antimicrobial resistance (AMR), which occurs when parasites, fungi, viruses, and bacteria adapt to resist the effects of medications, is a serious danger to global health. The hazard to human health increases as a result of this resistance, which makes traditional therapies useless. Antibiotics, antivirals, antifungals, antibacterials, and

antiparasitic medications are examples of antimicrobial agents. In the battle against infectious diseases, these medications are essential. Drug resistance has emerged as a result of microorganisms' evolutionary reaction to the use of antimicrobial medicines to treat and prevent illnesses [1,2]. This happens when bacterial pathogens lose their ability to respond to deadly antibiotic dosages or become insensitive to high concentrations of several antibiotic types. The efficacy

of antibiotics in treating infectious diseases is seriously threatened by this type of resistance, which is referred to as multidrug resistance (MDR) [3]. According to statistics, multidrug resistance (MDR) and antimicrobial resistance (AMR) are serious global health issues that have a big impact on public health. About 7.7 million people die from bacterial illnesses every year; 4.95 million of these deaths are linked to drug-resistant diseases, and 1.27 million are directly caused by germs that are resistant to the medicines that are currently on the market [4].

Antibiotic resistance has a significant financial impact on Europe, with hospital-related expenses accounting for almost EUR 900 million of the expected minimum EUR 1.5 billion in costs. AMR's yearly economic effect in the United States is estimated by the Centers for Disease Control and Prevention (CDC) to be over USD 55 billion. This comprises direct healthcare expenses of USD 20 billion and societal expenditures of USD 35 billion as a result of lost productivity. With hospital charges in Europe and direct healthcare costs in the U.S. being key contributors, these numbers demonstrate the substantial financial burden that antimicrobial resistance (AMR) places on healthcare systems and society at large. Moreover, this economic difficulty is made worse by the decrease in productivity brought on by AMR-related health problems [5,6]. Phytomedicine has long been used to treat a variety of illnesses in Africa and other parts of the world, even before modern medicine was invented. In many regions of the world, particularly those without access to contemporary medical care, herbal medicine is still commonly utilized [7]. Numerous chemical compounds with significant defense and restorative therapeutic properties have been found in plants [8-11]. Conventional medicine, which contains substances derived from medicinal plants, is used by about 80% of people in affluent nations [12]. Plants continue to be the primary source of natural medicine, even in the face of various drug discovery methodologies. The drumstick tree, or *Moringa oleifera*, is a member of the Moringaceae family, specifically the genus *Moringa* [13]. Worldwide, there are numerous varieties of *Moringa* that are well-known for their variety of uses. *Moringa oleifera* is considered one of the mystical plants due to its numerous medicinal uses [14]. Many microorganisms have been treated with the antibacterial components of *Moringa oleifera*. *Moringa oleifera* aqueous extracts have demonstrated antibacterial activity against a range of pathogenic microorganisms, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* [15,16].

Sayeed et al. [17] demonstrated that *Moringa oleifera* fruit extract had broad-spectrum antimicrobial activity. Flavonoids isolated from *Moringa oleifera* seeds demonstrated antibacterial and antibiofilm properties against *Candida albicans*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, which generate biofilms [18,19]. Both *P. aeruginosa* and MRSA-infected lesions healed after using a formulation of *Moringa oleifera* extract [20]. Among the many phytochemicals found in *Moringa* species are flavonoids, which are well-known for

their anti-inflammatory and antioxidant qualities; terpenoids, which have been demonstrated to possess anti-cancer and anti-microbial qualities; tannins, which effectively reduce inflammation and combat infections; anthocyanins, which are well-known for their anti-inflammatory and antioxidant qualities; and proanthocyanidins, which have strong antioxidant qualities and support cardiovascular health [14]. Because of these compounds, *Moringa* has strong antioxidant, antigenotoxic (protective of DNA), and immunostimulant (immune-boosting) properties, which make it a valuable plant for promoting health and preventing disease [21-23]. The current investigation was therefore conducted with the express purpose of examining the possible antibacterial activity of *M. oleifera* leaf aqueous and ethanol extracts against several pathogenic microorganisms.

Materials and Methods

Plant Materials

Collection and Identification: The leaves of *M. oleifera* were collected at Faya, a local market in Langtang South Local Government Area, Plateau State, and were identified by Adeniji Adewale (Herbarium Department, Federal College of Forestry, Bauch Ring Road, 930105 Jos, Plateau State, Nigeria).

Processing of Plant Material: Following their separation, cleaning, and washing in sterile distilled water, the fresh *M. oleifera* leaves were allowed to air dry for two weeks before being finely ground into powder using a pestle and mortar [24]. Until it was time for examination, the powdered form was kept out of direct sunlight in an airtight glass container.

Preparation of Ethanol and Aqueous Leaf Extracts of *Moringa Oleifera*: The Yudistian et al. [25] suggested procedure was used to manufacture the extract. A hundred grams (100 g) of dried *M. oleifera* leaves were macerated in 500 milliliters of ethanol (Hi Media Laboratories Pvt. Ltd., Thane, MH, India) or distilled water for twenty-four hours at 32°C, with periodic shaking with a magnetic stirrer. The macerate was filtered using Whatman no. 1 filter paper after being centrifuged for 20 minutes at 3000 rpm. In order to make the filtrate entirely dry, it was concentrated under low pressure at 45 degrees celsius in a rotary evaporator (Napco model 630, Portland, OR, USA).

Qualitative Phytochemical Screening of the Plant Extract: To find secondary metabolites such flavonoids, saponins, tannins, and alkaloids, the extracts were put through phytochemical assays using standard techniques [26].

Test for Alkaloids (Mayer's test): Six drops of Mayer's reagent were applied to one milliliter of leaf extract. Alkaloids were present because a yellowish precipitate formed [27].

Test for Saponins (Foam test): One ml of leaf extracts was mixed with 5 ml of distilled water. The contents were heated in a boiling water bath. Frothing indicated the presence of saponins [28].

Test for Tannins (Braymer’s test): One ml of the leaf extract was mixed with 2 ml of water. To these, 2 drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate indicated the presence of tannins [26].

Test for Steroids (Salkowski test): To 2 ml of the leaf extract, 2 ml of chloroform was added followed by concentrated sulphuric acid. Formation of reddish-brown ring at the junction showed the presence of steroids [27].

Test for Flavonoids: One ml of the leaf extracts was added with 1ml of sulphuric acid. Orange color formation confirmed the presence of flavonoids [26].

Test Organism

The National Veterinary Research Institute Vom in Plateau State, Nigeria, provided stock cultures of *Shigella dysenteriae*, *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis*, *Clostridium botulinum*, and *Staphylococcus aureus* on a tryptone soy agar (TSA) slant. Using Salmonella–Shigella agar (SSA) for *Shigella* and *Salmonella* species, MacConkey agar for *E. coli*, Tryptone yeast agar for *Bacillus* species, Tryptic soy agar for *Staphylococcus aureus*, and Botulism selective medium for *Clostridium* sp., discrete colonies of the bacterium were isolated and incubated at 37°C for eighteen hours before being identified by biochemical tests [29].

Preliminary Susceptibility Testing

All bacterial isolates were tested for antibiotic susceptibility using Kirby-Bauer disk diffusion method and the results was interpreted as sensitive (S), intermediate (I) or resistant (R) according the standards of the Clinical and Laboratory Standard Institute [30]. The antibiotic discs containing the following antibiotics was used: Beta-lactam groups: amoxicillin (20/10 µg), augmentin (30 µg); sulfonamides: cotrimoxazole (25 µg); Tetracycline: Tetracycline (30 µg); Cephalosporin: Cefazolin (30 µg), ceftazidime/clavulanic acid (30 µg); macrolides: Erythromycin (15 µg); chloramphenicol: chloramphenicol (30 µg); aminoglycoside: gentamicin (10 µg); fluoroquinolones: ciprofloxacin (5 µg). The choice of antimicrobials was based on their importance both in veterinary medicine, due to their frequent use, and in human medicine, as treatment options [30]. The discs were aseptically placed on the surface of Mueller-Hinton agar (MHA) plates that has already been seeded with 0.5 McFarland standards of the test isolates and will be incubated at 37°C for 18-24 hrs. After incubation, diameters of zone of inhibitions were observed and measured in millimeters accordingly. Isolates exhibiting resistance to three or more antibiotic classes was define as multi-drug resistance (MDR).

Antibacterial Testing

The antimicrobial activity of ethanolic and aqueous leaf extracts was evaluated using a cup-plate agar diffusion assay as outlined by Kirvaitienė et al [31]. Briefly, 100 µL of fresh Mueller–Hinton broth

culture (approximately 106 CFU/mL) was spread uniformly on sterile Mueller–Hinton agar plates and allowed to air-dry. After this, 6 mm wells were made in the Mueller–Hinton agar (MHA) plates using sterilized cork borer, and the base was sealed with melted MHA. Exactly 0.2 ml of 50.0–400.0 mg/mL concentrations of the extract was prepared in 10% (w/v) dimethyl sulfoxide corresponding to 50.0 mg, 100.0 mg, 200.0 mg, and 400.0 mg of the extract, which was dispensed into the wells. The plates were allowed to stand for 1 h at 32°C for pre-diffusion and placed in an incubator at 37°C for 24 h. The diameter of zones of inhibition against the test bacteria was measured and recorded. Ciprofloxacin at 20 mg was used as the positive control.

Data Analysis

Data was analyzed using R Console version 4.4.1. Data were presented in tables and figures for clarity. Chi-square test was use to analyze the antibiotic susceptibility profile of bacterial isolates. Probability values less than 0.05 were considered statistically significant.

Results

Phytochemical Properties of Aqueous and Ethanolic Leaf Extracts of Moringa Oleifera

In the qualitative phytochemical analysis of aqueous and ethanolic leaf extracts of *Moringa oleifera*, tannins and flavonoids were all present in both aqueous and ethanolic extracts of the leaf. Saponins, Alkaloids and Steroids were all absent in the ethanolic extract but present in the aqueous leaf extract (Table 1). Overall, the aqueous extract contained more phytochemical constituents than the ethanolic extract.

Table 1: Phytochemical Constituents of Aqueous and Ethanolic Leaf Extracts of Moringa oleifera.

Phytochemicals	Aqueous	Ethanolic
Tannins	+	+
Saponins	+	-
Alkaloids	+	-
Flavonoids	+	+
Steroids	+	-

Note: Present (+); Absent (-)

Antibiotic Susceptibility Profile of Bacterial Isolates

Table 2 presents the antibiotic susceptibility profiles of six bacterial isolates tested against ten different antibiotics. Across the isolates, *Salmonella typhi* demonstrated the highest overall sensitivity, exhibiting large and consistent inhibition zones ranging from 28 mm to 31 mm for all antibiotics except for ciprofloxacin (29 mm, intermediate) and showing no resistance. However, there was no significant difference ($\chi^2 = 0.284$, $df = 9$, $p = 1.000$) among the antibiotics tested for this isolate. In contrast, *Shigella dysenteriae* displayed mixed re-

sponses: it was sensitive to sulfamethoxazole-trimethoprim (22 mm) and chloramphenicol (38 mm) but resistant to most other antibiotics tested, with inhibition zones at or near zero. Therefore, there was a

very high significant difference ($\chi^2 = 175.05$, $df = 9$, $p < 0.001$) in its response across antibiotics.

Table 2: Antibiotic Susceptibility Profile of Bacterial Isolates.

Isolates	Diameter of Zone of Inhibition (mm) (Mean $\pm$ S.D) of the Antimicrobials										χ^2	DF	P-VALUE
	SXT	CH	TET	CPX	AM	AU	CN	ERY	CEF	CF			
<i>Shigella dysenteriae</i>	22 $\pm$ 0.00 ^S	38 $\pm$ 0.71 ^S	0 $\pm$ 0.00 ^R	14 $\pm$ 0.00 ^R	0 $\pm$ 0.00 ^R	0 $\pm$ 0.00 ^R	0 $\pm$ 0.00 ^R	0 $\pm$ 0.00 ^R	6 $\pm$ 1.41 ^R	4 $\pm$ 0.00 ^R	175.05	9	<0.001
<i>Salmonella typhi</i>	28 $\pm$ 0.00 ^S	30 $\pm$ 0.00 ^S	30 $\pm$ 0.71 ^S	29 $\pm$ 1.41 ^I	30 $\pm$ 0.00 ^S	31 $\pm$ 1.41 ^S	28 $\pm$ 0.71 ^S	30 $\pm$ 0.00 ^S	30 $\pm$ 0.71 ^S	30 $\pm$ 0.00 ^S	0.28378	9	1.000
<i>Escherichia coli</i>	31 $\pm$ 1.41 ^S	30 $\pm$ 0.71 ^S	18 $\pm$ 0.00 ^S	22 $\pm$ 0.00 ^I	0 $\pm$ 0.00 ^R	0 $\pm$ 0.00 ^R	24 $\pm$ 0.00 ^S	17 $\pm$ 1.41 ^R	18 $\pm$ 0.00 ^S	0 $\pm$ 0.00 ^R	81.125	9	<0.001
<i>Staphylococcus aureus</i>	18 $\pm$ 0.00 ^S	29 $\pm$ 1.41 ^S	21 $\pm$ 1.41 ^S	32 $\pm$ 0.00 ^S	10 $\pm$ 0.71 ^R	8 $\pm$ 0.71 ^R	14 $\pm$ 0.00 ^I	20 $\pm$ 0.00 ^R	22 $\pm$ 0.00 ^S	14 $\pm$ 0.71 ^I	28.489	9	0.0007898
<i>Clostridium botulinum</i>	0 $\pm$ 0.00 ^R	0 $\pm$ 0.00 ^R	14 $\pm$ 0.00 ^I	17 $\pm$ 1.41 ^I	0 $\pm$ 0.00 ^R	0 $\pm$ 0.00 ^R	13 $\pm$ 1.41 ^I	8 $\pm$ 0.00 ^R	10 $\pm$ 0.71 ^R	4 $\pm$ 0.00 ^R	60.364	9	<0.001
<i>Bacillus subtilis</i>	18 $\pm$ 0.00 ^S	29 $\pm$ 1.41 ^S	21 $\pm$ 1.41 ^S	32 $\pm$ 0.00 ^S	10 $\pm$ 0.71 ^R	8 $\pm$ 0.71 ^R	14 $\pm$ 0.00 ^I	20 $\pm$ 0.00 ^R	22 $\pm$ 0.00 ^S	14 $\pm$ 0.71 ^I	28.489	9	0.0007898
χ^2	30.538	33.462	28.808	12.548	82.00	92.021	30.935	34.663	21.333	54.364			

Note: SD: Standard Deviation; R: Resistant; I: Intermediate; S: Susceptible; SXT: Cotrimoxazole; CH: Chloramphenicol; TET: Tetracycline; CPX: Ciprofloxacin; AM: Amoxicillin; AU: Augmentin; CN: Gentamicin; ERY: Erythromycin; CEF: Ceftazidime/clavulanic acid; CF: Cefazolin

Escherichia coli showed strong sensitivity to sulfamethoxazole-trimethoprim (31 mm), chloramphenicol (30 mm), tetracycline (18 mm), gentamycin (24 mm), and ceftriaxone (18 mm) but complete resistance to ampicillin, augmentin, and ciprofloxacin, with some intermediate effects observed for a few agents. Consequently, there was a very high significant variation ($\chi^2 = 81.125$, $df = 9$, $p < 0.001$) in the isolates response across the antibiotics studied. *Staphylococcus aureus* and *Bacillus subtilis* showed comparable patterns, each sensitive to chloramphenicol, tetracycline, ciprofloxacin, and ceftriaxone but resistant or intermediate to other antibiotics, especially beta-lactams and macrolides. Both isolates had very high statistically significant ($\chi^2 = 28.489$, $df = 9$, $p < 0.001$) variation in the iso-

lates response across the antibiotics studied. *Clostridium botulinum* exhibited the highest resistance overall, with zero inhibition zones for sulfamethoxazole-trimethoprim and chloramphenicol and only intermediate effects for tetracycline, ciprofloxacin, gentamycin, and ceftriaxone. Therefore, the isolates response across the antibiotics studied showed a very high significant difference ($\chi^2 = 60.364$, $df = 9$, $p < 0.001$).

At the level of individual antibiotics, chi-square tests revealed significant differences in bacterial responses for nearly all agents ($p < 0.001$); ciprofloxacin also showed a statistically significant difference, though at a lower level ($\chi^2 = 12.548$, $df = 5$, $p = 0.028$).

Antibacterial Activity of Aqueous Extract of *Moringa Oleifera*

The antibacterial activity of aqueous extracts of *Moringa oleifera* against the bacterial isolates, as shown in Figure 1, revealed insignifi-

cant effects across all concentrations. Ciprofloxacin (control) showed significant inhibition zones of 28 mm, 31 mm, 30 mm for *Shigella dysenteriae*, *Salmonella typhi* and *Staphylococcus aureus*, respectively, and insignificant inhibition zones of 0.0 mm each for *Escherichia coli*, *Clostridium botulinum*, and *Bacillus subtilis*.

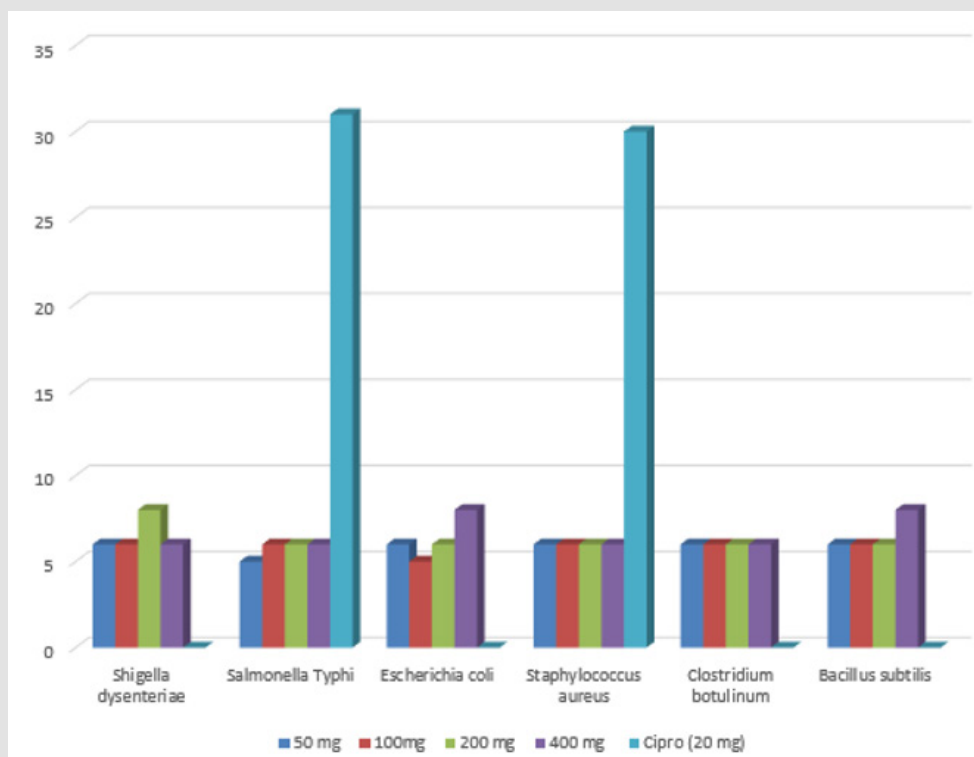


Figure 1: Antibacterial activity of *Moringa oleifera* aqueous leaf extract against bacterial isolates.

Antibacterial Activity of Ethanol Extract of *Moringa Oleifera*

The ethanolic extracts of *Moringa oleifera* demonstrated insignificant antibacterial activity against bacterial isolates at all concen-

trations (Figure 2). Ciprofloxacin (control) again showed inhibition zones of 31 mm for *S. typhi* and 30 mm for *Staphylococcus aureus*, and insignificant inhibition zones of 0.0 mm each for *Shigella dysenteriae*, *Escherichia coli*, *Clostridium botulinum* and *Bacillus subtilis*.

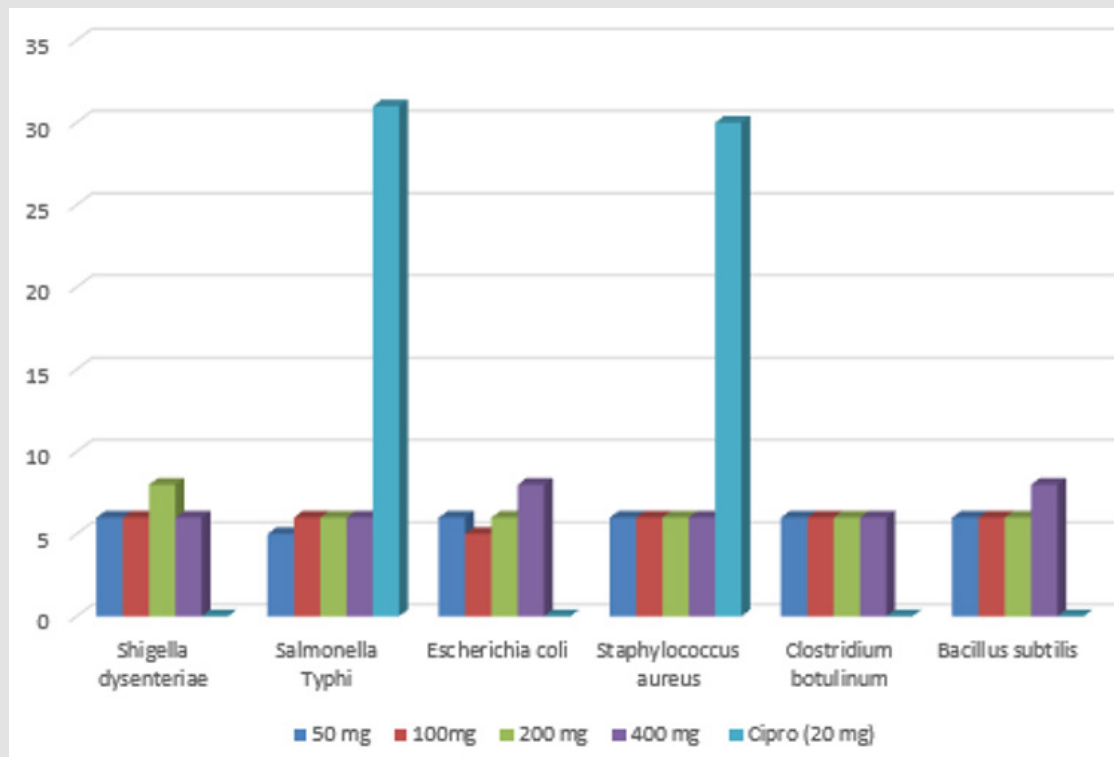


Figure 2: Antibacterial activity of *Moringa oleifera* ethanolic leaf extract against bacterial isolates.

Discussion

The phytochemical screening of *Moringa oleifera* leaf extracts in this study confirmed the presence of key bioactive compounds such as alkaloids, tannins, saponins, flavonoids, and steroids in appreciable amounts, especially in the aqueous extract. This finding aligns with Kashyap et al. [32], who emphasized the consistent extraction of diverse phytochemicals using aqueous methods for *M. oleifera*. Comparatively, the ethanolic extract yielded fewer bioactive constituents, supporting Kossonou et al. [33] who observed similar solvent-dependent variations. However, this result contradicts earlier reports suggesting that alcoholic solvents like ethanol and methanol are generally more effective for extracting secondary metabolites than water [34-37]. This apparent contradiction highlights the complex interplay between solvent polarity, compound solubility, and extraction yield [38,39]. Specifically, highly polar solvents such as water (Snyder Polarity Index 9.0) efficiently extract polar compounds, which dominate *M. oleifera* leaves [40]. The abundant presence of these metabolites underpins the plant's traditional use as a medicinal agent [41-45].

This study assessed the antibiotic susceptibility profiles of selected pathogenic bacteria and revealed varying degrees of resistance

and sensitivity, highlighting significant public health implications. *Salmonella typhi* demonstrated high susceptibility to nearly all tested antibiotics, with consistently large inhibition zones ranging from 28 mm to 31 mm and no detectable resistance. This aligns with reports by Eze and Iheanacho [46], Yusuff et al. [47], and Omotayo et al. [48], which noted that *S. typhi* strains remain relatively sensitive to multiple antibiotic classes in some regions, although emerging resistance has been documented elsewhere due to misuse and incomplete treatment regimens. Conversely, *Shigella dysenteriae*, *Escherichia coli*, and *Clostridium botulinum* exhibited substantial resistance, particularly to beta-lactams, sulphonamides, and macrolides. The observed complete resistance of *Shigella dysenteriae* to eight out of ten tested antibiotics is alarming and corroborates previous studies by Egwu et al. [49] and Ibiam et al. [50], who highlighted the rapid development of multidrug resistance in enteric pathogens due to selective pressure from inappropriate antibiotic use. Similarly, *E. coli* showed a mixed pattern: sensitivity to chloramphenicol and gentamicin but clear resistance to ampicillin and augmentin, echoing findings by Yusuff et al. [47] and Omotayo et al. [48], who linked such resistance to efflux pump mechanisms and biofilm formation, which protect bacteria from antimicrobial agents.

Clostridium botulinum demonstrated the most extensive resistance overall, with minimal inhibition zones for most antibiotics, except for intermediate effects seen with tetracycline and gentamicin. This resistance pattern raises concerns about potential treatment failure, especially for infections caused by toxigenic *Clostridium* species. The observed heterogeneity in susceptibility was statistically significant for most isolates, reinforcing the variability in resistance mechanisms within and across bacterial species [40]. Interestingly, *Staphylococcus aureus* and *Bacillus subtilis* displayed relatively moderate resistance patterns, with good susceptibility to tetracycline, chloramphenicol, and ceftriaxone but resistance to beta-lactams and augmentin. This trend aligns with global reports that methicillin-resistant *S. aureus* (MRSA) and penicillin-resistant *Bacillus* species remain prevalent in community and hospital settings [42,44]. These findings underscore the critical threat posed by multidrug-resistant bacteria, particularly among gram-negative pathogens. The prevalence of resistance to multiple antibiotic classes signals the diminishing efficacy of commonly used therapeutics and highlights the urgent need for alternative approaches, such as the development of plant-derived antimicrobials or novel synthetic agents [51].

Moreover, they emphasize the necessity of implementing strict antimicrobial stewardship, promoting rational drug use, and enhancing public awareness to curb the spread of resistant strains [43]. Despite the confirmed phytochemical richness, the antibacterial assays demonstrated limited inhibitory activity for both aqueous and ethanolic extracts. Zones of inhibition were absent against all gram-negative bacteria tested except when ciprofloxacin, the positive control, was applied, yielding robust zones (28–31 mm) for *S. dysenteriae*, *S. typhi*, and *S. aureus*. These observations align with El-Sherbiny et al. [52], and Soto et al. [53], who acknowledged *M. oleifera*'s potential but noted variable efficacy against different pathogens under different extraction protocols.

Other botanicals like *Erythrina senegalensis* have demonstrated broader antimicrobial effects, including antifungal activities [51,54], highlighting that extraction technique, plant part used, and pathogen structure all influence outcomes. The outer membrane of gram-negative bacteria like *E. coli* acts as a formidable barrier, limiting phytochemical penetration. Moreover, virulence factors, secretion systems, and structural complexity may explain the resistance seen here, despite the documented antimicrobial constituents in *M. oleifera* leaves.

Conclusion

This study demonstrates that aqueous extraction of *Moringa oleifera* leaves yields a higher quantity and diversity of phytochemicals compared to ethanolic extraction, supporting traditional water-based preparations. However, despite the confirmed presence of bioactive compounds, both aqueous and ethanolic leaf extracts exhibited limited antibacterial activity against multidrug-resistant gram-negative pathogens such as *E. coli* and *Shigella dysenteriae*. In contrast, ciprofloxacin remained highly effective, highlighting the limitations of crude plant extracts as standalone therapeutic agents in managing resistant infections. These findings underscore the need for further refinement to fully harness *Moringa oleifera*'s antibacterial potential.

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Recommendation

Future research should prioritize the isolation and characterization of specific bioactive fractions from *M. oleifera* leaves using advanced fractionation and purification methods. Exploring the use of alternative solvents, solvent combinations, or green extraction techniques may yield extracts with enhanced potency. Furthermore, evaluating other plant parts such as roots, seeds, or bark, and investigating synergistic interactions between purified compounds and standard antibiotics may offer a viable strategy to combat multidrug-resistant bacterial infections. Comprehensive *in vivo* studies and clinical trials will also be crucial to translate these laboratory findings into practical, evidence-based applications in public health and clinical practice.

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