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Comparison of antityphoidal efficacy of *Moringa oleifera* seed oil to antibiotics against *Salmonella typhi*

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Abstract

Background: Typhoid fever, a severe febrile illness prevalent in tropical regions, remains a significant cause of mortality. Drug-resistant pathogens have spurred research into medicinal plants as alternative treatments. While previous studies have focused on the inhibitory effects of *Moringa oleifera* leaf extract, limited attention has been given to *M. oleifera* seed oil and its effects on Gram-negative bacteria. This study aimed to evaluate the anti-typhoidal activity of *M. oleifera* seed oil against both typed and clinical *Salmonella typhi* isolates.

Methods: The anti-typhoidal activity of *M. oleifera* seed oil was assessed using agar diffusion and disk diffusion methods. Phytochemical screening of the seed oil was also conducted to identify its constituents.

Results: *M. oleifera* seed oil exhibited negligible or weak inhibitory effects on clinical and typed *S. typhi* strains ($p>0.05$), in contrast to the substantial inhibition observed with commercial antibiotics. Ciprofloxacin demonstrated the highest zones of inhibition (≥30 mm) against *S. typhi*, while tetracycline showed the least pronounced inhibition (≤16 mm). Phytochemical screening of *M. oleifera* seed oil identified terpenoids, cardiac glycosides, saponins, tannins, and flavonoids, with terpenoids being the most abundant and flavonoids the least abundant.

Conclusion: *M. oleifera* seed oil lacks significant therapeutic potential for the treatment of typhoid fever. Its antibacterial constituents are minimal or absent, resulting in insignificant inhibition against *S. typhi*.

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Introduction

Throughout history, typhoid fever has presented a significant challenge to human populations and remains an ongoing public health concern, causing approximately 200,000 deaths annually (1). This disease is endemic in areas characterized by inadequate sanitation standards (2). Outbreaks often occur due to the contamination of water supplies through various means, such as the cross-connection of human and polluted water sources, faecal contamination of wells, or the consumption of contaminated poultry products. *Salmonella typhi* has been identified as the primary causative agent of typhoid fever (1). This Gram-negative, flagellated, non-encapsulated, facultative anaerobic bacillus differentiates itself from other *Salmonella* infections by manifesting as a life-threatening systemic disease rather than the self-limiting gastroenteritis commonly associated with most other *Salmonella* strains. Moreover, *S. typhi* exclusively infects humans, unlike other *Salmonella* strains that can infect a wide range of hosts (1,2). The clinical presentation of typhoid fever resulting from *S. typhi* infection includes symptoms such as malaise, diffuse abdominal pain, and constipation (3). Untreated typhoid fever is a debilitating illness that can progress to delirium, obtundation, intestinal haemorrhage, bowel perforation, and ultimately death within one month of symptom onset (3). Over 90% of typhoid patients can be effectively managed with oral antibiotics, along with reliable care and close medical monitoring to identify and address complications or inadequate responses to treatment (4). However, the issue of relapse after antibiotic treatment and the growing resistance of *S. typhi* to conventional drugs have led to investigations into the potential use of medicinal plants as alternative therapeutic options (5).

The use of herbal medicine, including *Moringa oleifera*, has been documented in Nigeria and other countries due to its cultural acceptance, affordability, accessibility, and the presence of bioactive compounds capable of combating diseases caused by microorganisms, viruses, insects, and parasites (5). The secondary metabolites present in the seed extract of *M. oleifera*, such as alkaloids, tannins, saponins, phenols, and flavonoids, contribute to the extract's antibacterial activity (6). Consequently, the aim of this study is to evaluate the in-vitro anti-typhoidal activity of *M. oleifera* seed oil.

Methods

Seed collection

The seeds of *M. oleifera* were obtained from a farmland in Bolorunduro (Coordinates 7.4209°N, 5.00114°E), Ifedore LGA, Ondo State. The seeds were then authenticated at the Department of Crop, Soil, and Pest (CSP) Management, School of Agricultural Technology, The Federal University of Technology, Akure, Ondo State, Nigeria.

Preparation of plant extract

Dehulled and cleaned seeds were sun-dried until they reached a constant weight of 2 kg. The dried seeds were then crushed and finely pulverized using a Philip blender (Model HR2102). The oil from *M. oleifera* seeds was extracted using 240 mL of n-hexane in a Soxhlet extractor arrangement. The n-hexane was evaporated from the obtained oil using a rotary evaporator, and the remaining oil was quantified and stored in a glass bottle for later use (7).

Seed oil dosage preparation

The seed oil of *M. oleifera* was reconstituted with Tween 20 and sterile distilled water to prepare various concentrations: 1000, 500, 250, 125, and 62.5 mg/mL (7) for the anti-typhoidal assay. The dosages of the seed oil administered to the typed and clinical *S. typhi* strains were prepared by dissolving 1.0 g of the seed oil in a sterile universal bottle containing 8 mL of distilled water and 2 mL of Tween 20 to obtain a 1000 mg/mL dosage. The dosages of 500, 250, 125, and 62.5 mg/mL were obtained through successive two-fold dilutions from the 1000 mg/mL concentration.

Source and preservation of bacteria

Clinical and typed *S. typhi* strains were obtained from the Microbiology Laboratory, University of Ibadan Teaching Hospital, Ibadan, Nigeria, and transferred to the Department of Microbiology, Federal University of Akure (FUTA). The standard *S. typhi* used in this study was *S. typhi* ATCC 6539. This standard strain is a Gram-negative, flagellated, non-encapsulated, facultative anaerobic bacillus. Standard inocula of both clinical and typed *S. typhi* strains were used in the study, and the bacterial cell density was quantified using the method described by Cheesbrough (8).

Preparation of standard inocula of *Salmonella typhi*

The method employed for preparing the standard inocula of *S. typhi for in vitro* assay was based on the procedure described by Akinyemi and Dada (3). Overnight cultures of the isolates were transferred to tubes containing sterile saline. The bacterial suspension was compared to 0.5 McFarland standards using black lines drawn on a white sheet of paper. The density of the bacterial suspension was adjusted to match the McFarland 0.5 standard by adding sterile saline or additional bacterial growth. The bacterial suspension was then diluted to obtain a concentration of 106 colony-forming units per millilitre (CFU/mL).

Antityphoidal sensitivity testing of the extract on clinical and typed isolates

The procedure described by Muhammed et al. (8) was adopted for this step. Using a sterile pipette, 0.5 mL of the bacterial suspension was drawn and aseptically introduced into sterile Petri dishes. Mueller-Hinton Agar (MHA), cooled to approximately 45°C, was poured into the Petri dishes containing the *S. typhi* suspension. Each Petri dish was swirled gently in a clockwise direction to ensure the homogeneous distribution of bacteria within the MHA. After allowing the plates to stand for 40 minutes, four wells of approximately 6 mm were aseptically bored into each agar plate using a sterile cork borer, with a distance of 30 mm between adjacent wells and between the wells and the edge of the Petri dish. A 0.1 mL volume of the different concentrations of the extract was then introduced into each well using a sterile syringe. A control well at the centre contained 0.1 mL of the extracting solvent (n-hexane, Sigma-Aldrich). The plates were labelled and incubated at 37°C for 24, 48, and 72 hours. The resulting zones of inhibition were measured using a calliper, and the average of three readings was taken as the zone of inhibition for the bacterial isolate at that specific concentration and contact time.

Antibiotics assay

The effectiveness of standard antibiotics from Oxoid, such as gentamicin (30 µg), chloramphenicol (30 µg), ciprofloxacin (10 µg), tetracycline (30 µg), amoxicillin (30 µg), and nalidixic acid (30 µg), was compared with the seed oil of *M. oleifera* against clinical and typed *S. typhi* isolates. Using a sterile pipette, 0.1 mL of the bacterial suspension was drawn and aseptically introduced into sterile Petri dishes. Mueller–Hinton Agar (MHA), cooled to about 45°C, was poured into the Petri dishes containing the *S. typhi* suspension. Each Petri dish was gently swirled in a clockwise direction to ensure even distribution of the bacteria. The plates were allowed to stand for 40 minutes to enable the bacteria to establish in the medium. The standard discs were placed aseptically on the plates using sterile forceps. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition around the discs were observed. The diameters of the clear zones were measured in millimetres (mm) using a calliper, and the average of three readings was taken as the zone of inhibition for the bacterial isolate at that specific concentration and contact time (2).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts were determined using the broth dilution technique. Dilutions of the extract in Mueller-Hinton broth were prepared in tubes. The tubes containing the different concentrations of seed oil in Mueller-Hinton broth were then inoculated with 0.5 mL of the standardized culture. The cultures were incubated at 37°C for 48 hours, and the lowest concentration that inhibited growth was considered the MIC. Visual observation was performed to detect turbidity in the test tubes (3).

Phytochemical screening of seed oil of *Moringa oleifera*

The seed oil of *M. oleifera* was subjected to quantitative and qualitative phytochemical screening using standard procedures described by Ijarotimi et al. (9) and Donkor et al. (10).

Ethical considerations

This study used clinical isolates obtained from the Microbiology Laboratory, University of Ibadan Teaching Hospital, Ibadan, Nigeria. The use of these isolates was approved by the Ethical Review Committee of the University of Ibadan Teaching Hospital (Approval number: UI/EC/22/0143).

Results

Antityphoidal activity of seed oil of *Moringa oleifera* and antibiotics assay

The evaluation of the antityphoidal activity of the seed oil of *M. oleifera* against typed and clinical isolates of *S. typhi* is presented in Table 1. The results indicate that *S. typhi* exhibited weak or no susceptibility to the seed oil of *M. oleifera*. The zones of inhibition observed for typed and clinical *S. typhi* at a concentration of 1000 mg/mL of *M. oleifera* seed oil were 0.21 ± 0.04 mm and 0.23 ± 0.03 mm, respectively. At a concentration of 500 mg/mL, the zones of inhibition were 0.20 ± 0.03 mm and 0.19 ± 0.01 mm for typed and clinical *S. typhi*, respectively. The MIC for both typed and clinical *S. typhi* were found to be 500 mg/mL, while the MBC could not be determined, as there was no evidence of bacterial death at a concentration of 1000 mg/mL.

Table 1. Antityphoidal activity of seed oil of *Moringa oleifera* against typed and clinical *Salmonella typhi*

Moringa oleifera seed oil dosage (mg/mL)	Typed *S. typhi*	Clinical *S. typhi*
1000	0.21 ± 0.04^b	0.23 ± 0.03^a
500	0.20 ± 0.03^a	0.19 ± 0.01^b
250	0.00 ± 0.00	0.00 ± 0.00
125	0.00 ± 0.00	0.00 ± 0.00
62.5	0.00 ± 0.00	0.00 ± 0.00

Values are mean ± S.E (n = 3). Means with different superscripts in the same row show significant ($P \leq 0.05$).

The susceptibility of both typed and clinical isolates of *S. typhi* to commercially available antibiotics was assessed. Typed *S. typhi* showed the highest zones of inhibition against amikacin, ciprofloxacin, and chloramphenicol, with a maximum zone of inhibition of 30 mm. Ciprofloxacin exhibited a zone of inhibition of 35.45 ± 0.40 mm against clinical isolates of *S. typhi*. Tetracycline resulted in the lowest zones of inhibition for both typed and clinical *S. typhi*, with recorded values of 14.75 ± 0.25 mm and 16.30 ± 0.42 mm, respectively. Figure 1 depicts a comparison of the treatment of *S. typhi* with the seed oil of *M. oleifera* and the use of antibiotics, indicating that the efficacy of the seed oil was not significant when compared to antibiotics. The enhanced antimicrobial effects of commercial antibiotics compared to crude extracts can be attributed to their higher purity levels and their ability to diffuse in the culture media (11-14).

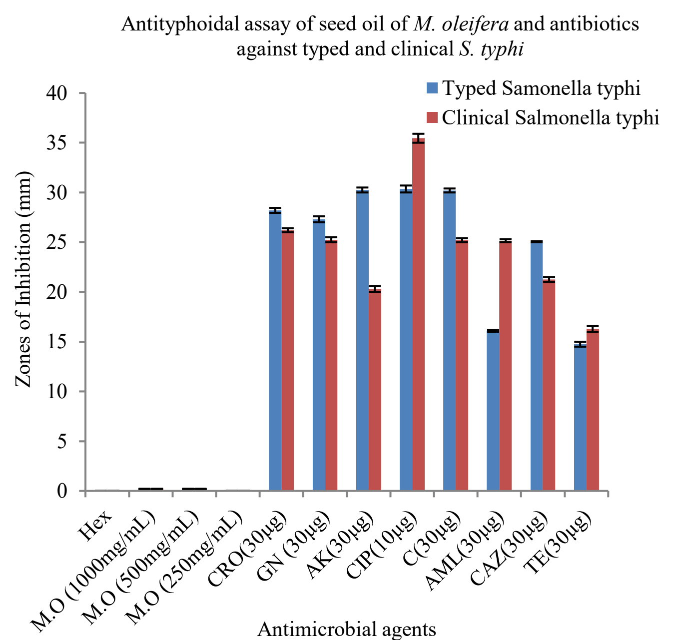

Figure 1. Comparative zones of inhibition of standard antibiotics and seed oil of *Moringa oleifera* against clinical and typed *Salmonella typhi* (Abbreviations: Hex, n-hexane; M.O, seed oil of *M. oleifera*; CRO, Ceftriaxone; GN, Gentamicin; AK, Amikacin; CIP, Ciprofloxacin; C, Chloramphenicol; AML, Amoxicillin; CAZ, Ceftazidime; TE, Tetracycline).

Phytochemical constituents of seed oil of *Moringa oleifera*

Table 2 presents the phytochemical constituents of the seed oil of *M. oleifera*. The analysis reveals the presence of various qualitative and quantitative phytochemical bioactives in the seed oil of *M. oleifera*. The secondary metabolites identified include terpenoid, cardiac glycosides, saponin, tannin, and flavonoid, with respective amounts of 5.52 ± 0.04 mg/100 g, 3.84 ± 0.05 mg/100 g, 1.55 ± 0.27 mg/100 g, 1.01 ± 0.01 mg/100 g, and 0.45 ± 0.01 mg/100 g. However, phytochemicals such as steroid, phlobatannin, alkaloid, and anthraquinone were found to be absent in the seed oil of *M. oleifera*. The phytochemical composition in this study is consistent with the findings of Makanjuola et al. (14). However, this contrasts with the study by Akintelu et al. (6), which reports the presence of phenols, alkaloids, and other similar components in the seed extract of *M. oleifera* using methanol as a solvent. The diverse phytochemical constituents in *M. oleifera* contribute to its pharmacological uses, medicinal potency, and disease protection (7,13,15).

Table 2. Phytochemical constituents of seed oil of *Moringa oleifera*

Phytochemicals	Qualitative	Quantitative (mg/100g)
Terpenoid	+	5.52 ± 0.04^{a}
Cardiac glycosides	+	3.84 ± 0.05^{b}
Saponin	+	1.55 ± 0.27^{c}
Tannin	+	1.01 ± 0.01^{d}
Flavonoid	+	0.45 ± 0.01^{e}
Steroid	-	0.00 ± 0.00^{f}
Phlobatannin	-	0.00 ± 0.00^{f}
Alkaloid	-	0.00 ± 0.00^{f}
Anthraquinone	-	0.00 ± 0.00^{f}

Values are mean ± S.E (n = 3). Means with different superscripts in the same column show significant ($P \leq 0.05$). KEYS: + = Present; - = Absent

Discussion

The evaluation of the anti-typhoidal activity of *M. oleifera* seed oil on typed and clinical *S. typhi* strains indicates limited or negligible inhibitory effects at dosages of 1000 and 500 mg. Several factors contribute to this observation, including the presence of lipopolysaccharide (LPS) in *S. typhi* (11), inadequate or absent specific phytochemicals (6,12), and the choice of solvent used for seed oil extraction (6) from *M. oleifera*. LPS shields the bacteria from bactericidal agents by preventing the entry of hydrophobic molecules, while the constant alteration of the bacteria's outer membrane structure aids in their defense against such agents (11). These findings align with studies reporting weak or no inhibitory effects of *M. oleifera* seed oil on tested Gram-negative bacterial strains, possibly due to the strains' resistance to the constituents of *M. oleifera* (12).

Phenolic compounds are identified as the primary contributors to the antimicrobial efficacy (12,13) of essential oils against Gram-negative bacteria, which are lacking in this study. The current research addresses the limitations of previous studies (12), such as the unknown extraction source and undetermined phytochemical constituents of the *M. oleifera* seed oil used. Furthermore, although flavonoids exhibit antimicrobial properties (13), their abundance in this study is minimal. Additionally, other studies demonstrate the antibacterial potential of bioactive compounds such as phenols, alkaloids, and flavonoids present in *M. oleifera* seed extract obtained using methanol as a solvent, suggesting their potential as antibiotic ingredients against Gram-positive and Gram-negative bacterial infections (6,12).

Comparison with previous studies

The findings of this study, which indicate the ineffectiveness of *M. oleifera* seed oil against *S. typhi*, are consistent with some previous research. For instance, Abu El-Wafa and Abd El-All (12) reported weak or no inhibitory effects of *M. oleifera* seed oil on certain Gram-negative bacteria. This similarity could be attributed to factors such as the presence of LPS in Gram-negative bacteria, which hinders the penetration of hydrophobic compounds, and the absence of key antimicrobial phytochemicals such as phenols in the oil extract, as observed in our study.

However, our results contrast with studies that have demonstrated the antibacterial potential of *M. oleifera* seed extracts. Akintelu et al. (6) found that methanol extracts of *M. oleifera* seeds exhibited antibacterial activity, likely due to the presence of compounds such as phenols and alkaloids. This discrepancy highlights the importance of the extraction solvent, as different solvents can extract different phytochemicals, thereby influencing the bioactivity of the extract.

Implications for treatment

The results of this study have significant implications for the potential use of *M. oleifera* seed oil in treating typhoid fever. The observed lack of significant anti-typhoidal activity suggests that this particular preparation of *M. oleifera* seed oil is not a suitable alternative to conventional antibiotic treatments. It reinforces the importance of relying on established antibiotic therapies for the management of typhoid fever.

Conclusion

In conclusion, while *M. oleifera* seed oil may possess the capability to treat other diseases, it proves ineffective against typhoid fever. The concentration of phytochemical constituents in the seed oil of *M. oleifera* is insufficient to cause death or meaningful inhibition of *S. typhi* growth.

Limitations and future research

This study has some limitations that should be considered. First, the study focused solely on the *in vitro* activity of *M. oleifera* seed oil. Further research is needed to evaluate its efficacy in *in vivo* models, which can better mimic the complex interactions within a living organism. Second, while the phytochemical composition of the seed oil was analyzed, a more detailed investigation into the specific compounds responsible (Or the lack thereof) for the observed antibacterial activity could provide deeper insights.

Future research directions could include:

- Evaluating the synergistic effects of combining *M. oleifera* seed oil with antibiotics.
- Investigating the potential of other extraction methods to obtain *M. oleifera* seed oil with enhanced anti-typhoidal activity.
- Identifying the specific compounds in *M. oleifera* seed oil that may have weak antibacterial effects and exploring ways to enhance their activity.

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Not applicable

Ethical Statement

This study utilized clinical *S. typhi* isolates obtained from the Microbiology Laboratory, University of Ibadan Teaching Hospital, Ibadan, Nigeria. The use of these isolates was approved by the Ethical Review Committee of the University of Ibadan Teaching Hospital (UI/EC/22/0143). As this research involved the use of existing clinical isolates and did not involve direct interaction with patients, individual patient consent was not required. This study was conducted in accordance with all relevant guidelines and regulations.

Conflicts of Interest

The author declares no conflicts of interest. The author is solely responsible for the content and writing of this article.

Author Contributions

Abdulahi S.K. designed the study, conducted the experiments, analyzed and interpreted the data, wrote the manuscript, revised it critically, and approved the final version.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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