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Antimicrobial Screening of Silver Nanoparticles and Moringa Oleifera Leaf Extract on Salmonella Typhi

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Abstract: Salmonella typhi (S. typhi) is a gram-negative bacteria that affects the liver and intestine and also the causative agent of typhoid fever, a ravaging disease of underdeveloped and developing countries leading to over 110,000 deaths per year globally. S. typhi infection is characterized by watery stool, excessive sweating, prolonged fever and abdominal discomfort. Due to antimicrobial resistance (AMR), most of the conventional antibiotics are ineffective against the bacteria which make the discovery of alternate treatment imperative. Moring oleifera (M. oleifera) is a very common plant in Africa and Asia known as "the miracle tree" due its traditional use in the treatment of so many diseases. Silver nanoparticles were synthesized using M. oleifera leaves extracts and both the leaf extract and combination (nan0-moringa) were used in an invitro lab to screen against S. typhi. Ciprofloxacin, a conventional antibiotic was also screened and a zone of inhibition of 25mm was obtained at 0.01mg/ml while moringa extract and nano-moringa extract exhibited zones of inhibition of 11mm at 125mg/ml minimum inhibitoy concetration (MIC) and 8mm at 6.25mg/ml MIC respectively. The minimum bactericidal concentration (MBC) for was obtained as 250mg/ml and 12.5mg/ml for moringa and nano-moringa extracts respectively. Out of 10 antibiotics used, S. typhi was only susceptible to ciprofloxacin. M. oleifera leaves and synthesized silver nanoparticles showed extraordinary synergy and possess anti S. typhi properties. However, further studies are needed to outright conclude that they can substitute the conventional treatment been used.

Keyword: Moringa Oleifera, Nano-Moringa, Silver Nanoparticles, Salmonella Typhi, Typhoid.

INTRODUCTION

Typhoid fever, a severe systemic illness caused by the bacterium Salmonella enterica serovar Typhi (S. typhi), remains a formidable threat to global public health. Characterized by high prolonged fever, abdominal discomfort, malaise, and systemic dissemination through the reticuloendothelial system, typhoid fever primarily affects low- and middle-income regions where access to clean water and adequate sanitation infrastructure is limited (Almuzaini, 2023; Orole et al., 2024).

The global disease burden of S. typhi infection is substantial. According to estimates from the World Health Organization (WHO), typhoid fever accounts globally for an estimated 9 to 11 million illnesses and approximately 110,000 deaths annually, with the highest morbidity occurring among children and young adults in endemic regions such as Sub-Saharan Africa and South Asia. Beyond its mortality and physical morbidity, S. typhi imposes a profound financial burden on individuals, families, and healthcare infrastructure. Direct medical costs—encompassing diagnostic evaluations, hospitalization, and antibiotic regimens—are compounded by indirect economic losses resulting from decreased productivity, loss of income, and extended caregiving requirements. In low-resource settings, these expenditures often lead to catastrophic household health costs, exacerbating socioeconomic inequities (Ikhimiukor et al., 2022; Carey et al., 2023; Akinduti et al, 2026).

Taxonomically, S. typhi belongs to the domain Bacteria, phylum Pseudomonadota (formerly Proteobacteria), class Gammaproteobacteria, order Enterobacterales, and family Enterobacteriaceae. Under the Kauffmann–White classification scheme, it is classified as a

specific human-restricted serovar within the species Salmonella enterica subspecies enterica. As a Gram-negative, facultatively anaerobic rod-shaped bacterium, S. typhi possesses specific surface antigens including the somatic O antigen, flagellar H antigen, and capsular Vi polysaccharide antigen that mediate host tissue colonization, intracellular survival, and evasion of host immune responses. (Ikhimiukor et al., 2022; Saranraj & Manigandan, 2025).

The management of typhoid fever has been increasingly compromised by the rapid rise and spread of antimicrobial resistance (AMR) in S. typhi. Strains demonstrating resistance to traditional first-line therapeutics, such as ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole, led to the development of multidrug-resistant (MDR) typhoid. Subsequent reliance on fluoroquinolones (e.g., ciprofloxacin) and third-generation cephalosporins has led to the emergence of extended-spectrum beta-lactamase (ESBL)-producing and extensively drug-resistant (XDR) S. typhi strains. These drug-resistant variants render standard antibiotic treatments progressively ineffective, escalating treatment failures, lengthening hospital stays, increasing healthcare costs, and elevating mortality risks. Consequently, there is an urgent imperative to investigate novel, environmentally friendly, and broad-spectrum therapeutic modalities (Jahan et al., 2022; Nkamkeu et al., 2025).

Botanical resources offer a rich, bio-sustainable reservoir for alternative antimicrobial remedies. Among these, Moringa oleifera Lam. (family Moringaceae), commonly referred to as the "miracle tree", has gained significant attention. M. oleifera leaves are highly valued for their rich nutritional profile, containing proteins, essential amino acids, dietary minerals (such as calcium, iron, potassium, and zinc), and essential vitamins (including vitamins A, C, and E) (Gomes et al., 2024). Phytochemically, M. oleifera tissues are abundant in bioactive secondary metabolites, including flavonoids (e.g., quercetin, kaempferol), phenolic acids (e.g., chlorogenic acid), alkaloids, tannins, saponins, and isothiocyanates. These compounds possess potent intrinsically broad-spectrum antibacterial, antioxidant, anti-inflammatory, and immunomodulatory properties (Huang et al., 2024; Rodríguez-Negrete et al., 2024; Okpara & Orkuma, 2025). In vivo and in vitro evaluations demonstrate that M. oleifera leaf extracts effectively inhibit Salmonella species by disrupting bacterial cell wall integrity, inhibiting DNA gyrase, altering membrane permeability, suppressing biofilm formation, reducing systemic tissue damage, and enhancing host immune clearance mechanisms (Jahan et al., 2022; Nkamkeu et al., 2025; Saranraj & Manigandan, 2025; Kusuma et al., 2026).

In modern biological and therapeutic applications, the integration of plant extracts with nanotechnology presents a promising strategy to overcome bacterial resistance. Green synthesis of metallic nanoparticles—specifically silver nanoparticles (AgNPs) mediated by biological materials offers key advantages over traditional chemical and physical synthesis methods. Green synthesis eliminates the need for toxic reducing agents, hazardous solvents, and high energy inputs, yielding biocompatible, eco-friendly, and cost-effective nanomaterials with minimal environmental footprint (Kundu et al., 2019; Khalifa et al., 2025) When M. oleifera leaf extracts are employed in the biogenic synthesis of silver nanoparticles, the plant's naturally occurring polyphenols, flavonoids, and proteins simultaneously act as reducing agents to convert metal ions to elemental nanoparticles and as capping agents to prevent nanoparticle aggregation. The resulting bio-functionalized silver nanoparticles possess high surface-area-to-volume ratios, exhibiting potent synergistic bactericidal mechanisms such as cell membrane disruption, reactive oxygen species (ROS) generation, and intracellular component damage making them potent candidates for combating resistant S. typhi infections (Khalifa et al., 2025; Singh et al., 2025).

To address the growing threat of drug-resistant S. Typhi infections, this study aims to synthesize silver nanoparticles using M. oleifera leaf extract as an eco-friendly reducing and capping agent and to establish the potential of the combination's potential as an alternative therapeutic strategy against S. typhi.

MATERIALS AND METHOD

Sample Collection and Preparation

Fresh leaves of the plant Moringa oleifera were collected and identified at the Federal University of Lafia herbarium. The leaves were air-dried for one week after which was grinded using mortar and pestle and then sieved.

The powdered plant was used for cold extraction of active compounds. The sample (85g) was macerated in 850ml distilled water allowed to stand for 72hrs with periodic shaking. As described by Ugwu et al., 2011 and Kundu et al., 2019. Whatman filter paper no. 1 was used to filter and then transferred to a conical flask. It was then placed into a water bath at 45 oC until it was concentrated.

Test Bacteria

A bacterial stock was obtained from Bafawat lab, Lafia and the sample was cultured on selective media and was gram stained at Federal University of Lafia Microbiology lab where it was incubated within 2 to 3 h of collection at 37°C for 24 h. Further biochemical tests were carried out which confirmed the bacterial culture as S. typhi. The colonies from the plate were taken and diluted to 1.5 × 108 colony-forming units/ mL (CFU/ml) in normal saline according to McFarland turbidity standard N° 0.5.

Qualitative Phytochemicals Screening

The plant extract was subjected to phytochemical screening as follows:

Test for Flavonoids

A 2 ml volume of the extract was mixed with 1 ml of 10% ammonia which changed the appearance to yellow color. Diluted HCL was until the solution changed to colorless which indicated a positive test.

Test for Saponins

A 1ml volume of crude extract with 1ml of distilled water in a test tube was shaken vigorously. The formation of stable foam indicated the presence of saponins.

Test for Steroids

Liebermann-Burchad Test: A small quantity of the extract was dissolved in chloroform and filtered. A 3ml volume of filtrate and 2ml of acetic acid was taken and cooled in ice. Few drops of concentrated H2SO4 was carefully added down the side of the test tube. A color change from violet to blue or bluish green indicated a positive test.

Test for Tannins

Ferric Chloride Test: 2g of sample extract was added to 10ml of distilled water and heated in a water bath. The mixture filtered and to it, few drops of concentrated H2SO4 and 5% ferric chloride were added. Formation of blue black, green or blue-green precipitate indicated a positive test.

Test for Alkaloids

Mayer reagent: 3ml of sample extract and 5ml of 10% aqueous HCL were mixed placed on water bath and filtered. One (1) ml of the filtrate was taken and into it few drops of Mayer's reagent was added. A yellow precipitate formation indicated a positive test.

Green Synthesis of Silver Nanoparticles

Silver nitrate (AgNO3) solution (0.001M) was prepared in distilled water. 1ml of plant extract was added drop-wise into 100ml of the AgNO3 solution and kept on a magnetic stirrer for 15 minutes at 65°C. The mixture was covered and left overnight in a dark chamber to facilitate the reduction of AgNO3 to its corresponding nanoparticles. A colour change to reddish-brown signified the successful reduction of AgNO3 to silver nanoparticles. The resulting extract is represented further in this study as nano-moringa.

In Vitro Sensitivity Test

2.8 g of nutrient agar powder was added to 100 ml of distilled water. The mixture was

sterilized in an autoclave at 121°C for 15 mins and was then allowed to cool to 45°C before pouring it in petri dishes. Each petri dish contained the cultured S. typhi and was rotated anticlockwise. The medium was evenly distributed and allowed to solidify. The sensitivity disks (Mast Diagnostics, UK) containing single concentrations of each antimicrobial agent (Pefloxacin, Gentamycin, Ampiciox, Zinnacef, Amoxacillin, Rocephin, Ciprofloxacin, Aithromycin, Levofloxacin, Erythromycin) was placed onto the surface of the inoculum and incubated for 24 h at 37°C. The clear zone of inhibition was measured in millimeter (mm) using a straight-line ruler.

Moringa and nano-moringa were weighed from which 10g and 0.1g were taken respectively and each added in separate test tubes containing 10ml of peptone; the resulting concentrations were 1000 milligram/millimeter (mg/ml) for moringa A serial dilution was performed by transferring 1ml of the stock solution into the first tube. Then, 1ml from this mixture was transferred into the second tube containing 1ml of peptone water. This process was repeated sequentially across all the tubes, ensuring that the concentration decreased progressively with each dilution and the resulting concentrations were 500, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, and 1.9mg/ml for moringa and 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, and 0.19mg/ml for nano-moringa. Subsequently, 0.05ml of bacterial suspension, equivalent to 0.5 McFarland standards, was added to each tube, and the tubes were incubated at 37°C for 18–24 hours and the zones of inhibition were also measured.

Determination of Minimum Inhibitory Concentration (MIC)

The antibacterial activity was determined by MIC micro titer test tube method (20-23). McFarland turbidity standard to adjust densities of bacteria suspension 24h growth was prepared.

Preparation of 1mg/ml neem stock extract: Peptone water was serially diluted in a 10 well test tube and inoculated as follows; Lane 1 received 1ml of the neem extract concentration. Two fold dilutions were accomplished resulting in concentration of 1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, and 1.9mg/ml. Lane 2 received 1ml of nano-neem extract concentration. Two fold dilutions were accomplished resulting in concentration of 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, and 0.19mg/ml. To the serially diluted extract of each concentration was inoculated with 0.50µl of Escherichia coli test organism, after 18 hours of incubation at 37ºC, the test tubes were observed for turbidity. The least concentration where no turbidity was observed was determined and noted as the Minimum inhibitory concentration (MIC) value.

The procedure was the same for both aqueous and ethanol extracts.

Determination of Minimum Bacterial Concentration (MBC)

MBC was determined from the broth dilution resulting from the MIC tubes by sub-culturing to antimicrobial free agar as Described by (Usman et al., 2017). In this technique, the contents of the test tubes resulting from MIC was streaked using a Sterile wire loop on agar plate free of bacteria and incubated at 37ºC for 18 hours. The lowest concentration of the extract which showed no bacterial growth was noted and recorded as the MBC.

RESULT AND DISCUSSION

Table 1: Qualitative phytochemical Screening

S/N	Parameters	Observation Qualitative
1.	Tanins	+++
2	Flavanoids	++
3	Steroids	++
4	Alkanoids	+
5	Saponins	+

+++: Highly Present,++: Moderately Present,+: Present

From table 1, it is shown that tannins were the most abundant phytochemicals in M. oleifera followed by flavonoids, then steroids, alkaloids and finally saponins. Flavonoids are

established strong antioxidant and free-radical scavenger. Saponins naturally increase activity of antioxidants by inhibiting Nitric oxide (NO), Tumor necrotic factor- alpha (TNF-α) and subsequently, inhibiting Nuclear factor kappa B (NF-κB) which are all oxidative stress markers in the blood.

Tannins prevent the activity of some enzymes and inhibit nucleic acids metabolism, preventing microbes from multiplying and leading to cell death. Their ability to generally boost the immune system makes them the ideal them the ideal first line of defense phytochemicals, as such, their high presence in M. oleifera supports the traditional claim of the plant been a universal healer of all diseases (Huang et al., 2024).

Table 2: Sensitivity disc test

Clinical Isolate	Antibiotics zone of inhibition									
	CPX	AM	LEV	PEF	APX	R	AZ	GN	Z	E
E coli	S	R	R	R	R	R	R	R	R	R

R: Resistant, I: Intermediate, S: Sentitive

From table 2, it is clearly seen that S. typhi strain used for this study is resistance to all the commonly over the counter antibiotics with the exception of Ciprofloxacin (CPX). This is a testament of the adamancy of the strain present in Lafia and its surroundings and a strong justification for finding new treatment strategies for typhoid fever and other S. typhi related diseases.

Table 3: Agar well diffusion of E. coli clinical isolate by zone of inhibition

	Moringa	Nano-moringa	Ciprofloxacin
Concentration	125	6.25	0.01
S. typhi	11.00	8.00	25.00

The results from the zone of inhibition (ZOI) test on table 3 shows that nano-moringa had a zone of inhibition of 8mg/ml at 6.25mg/ml on S. typhi isolate while the moringa extract had ZOI of 11mm at 125mg/ml. This inhibition of both extracts against S. typhi could be attributed to the ability of M. oleifera to prevent DNA replication by binding to a very important replication enzyme called DNA gyrase. The abundance of tannins is also responsible for the inhibition as tannins inhibit nucleotide synthesis, also preventing DNA replication. Nano-moringa exhibited a higher zone of inhibition because silver nanoparticles possess higher penetrating power than pure M. oleifera extracts due to their greater surface area and smaller size.

Ciprofloxacin exhibited a wider zone of inhibition (25mm) at 0.01mg/ml compared to moringa and nano-moringa extracts. The lower inhibitory zones attributed to both extracts could be attributed to the fact that ciprofloxacin is in its pure form and can easily bind to the nucleic acids and enzymes of the cells while the plant extracts are a complex mix of phytochemicals, proteins, lipids etc.

Table 4: Minimum Inhibitory concentration (MIC) in mg/ml

Test isolate	Moringa	Nano-moringa
E. coli	125	6.25

Table 4 shows the MIC of both extracts which was obtained as 125mg/ml and 6.25mg/ml for moringa and nano-moringa respectively.

Table 5: Minimum Bactericidal concentration (MBC) in mg/ml

Test isolate	Moringa	Nano-moringa
E. coli	250	12.5

Table 5 shows a record of MBC as 250mg/ml for moringa and 12.5mg/ml for nano-moringa. Moringa had an MBC of 250mg/ml after the second serial dilution signifying that the constituents of Moringa oleifera leaves have great bactericidal properties. Constituents such as flavonoids, alkaloids and saponins which excellent antioxidants prevent the accumulation of S. typhi in the body by upregulating the activity of SOD, MDA and glutathione. These antioxidants also help in boosting the immune system, they help in the release of pro-inflammatory cytokines in cases of bacterial invasion, hence, destroying the bacteria. Tannins and saponins have the ability to bind to and destroy the cell membrane/wall of gram negative bacteria. Tannins prevents formation of bacterial cell wall by inhibiting reverse transcriptase and DNA topoisomerase while saponins can Penetrate the outer membrane and disrupt the cytoplasmic membrane thereby inducing cytoplasmic leakage leading to bacterial cell death (Weil et al., 2019).

CONCLUSION AND RECOMMENDATION

The phytochemicals present in the leaves of M. oleifera extract exhibited some kind of synergy with silver nanoparticles thereby making nano-neem a potential antimicrobial against typhoid fever and other S. typhi related diseases. This study proved that M. oleifera leaves does possess inhibitory and bactericidal properties against S. typhi emphasizing its use in the treatment of typhoid fever. The study also shows the antimicrobial properties of silver nanoparticles just as shown in literature. Upon broader invitro studies, further preclinical and clinical studies, the combination could become a substitute for conventional over the counter drus, thus, combating the wide-spread antimicrobial resistance.

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CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

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