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# Antibacterial activity against multidrug-resistant *Salmonella*, toxicity and biochemical effects of *Moringa oleifera* leaf extracts in mice

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**Abstract.** *Ngemenya MN, Itoe LO, Awah LA, Asongana R, Ndip RA. 2024. Antibacterial activity against multidrug-resistant* Salmonella, *toxicity and biochemical effects of* Moringa oleifera *leaf extracts in mice. Asian J Nat Prod Biochem 22: 43-50.* The emergence of multidrug resistance has significantly compromised the treatment of *Salmonella* infections. The anti-*Salmonella* activity, toxicity, and biochemical effects of *Moringa oleifera* Lam. leaf crude extracts were studied by disc diffusion and microdilution against Multidrug-Resistant (MDR) strains. Cytotoxicity was investigated on monkey kidney epithelial cells (LLCMK2) and acute toxicity in BALB/c mice. Both hexane ($MO_{HEX}$) and methanol ($MO_{MET}$) extracts produced small zones of inhibition of 8mm at 1 mg and 2 mg per disc, indicating weak activity, but the minimum inhibitory concentration showed high (0.0625 mg/mL) to low activity (10 mg/mL) activity. No minimum bactericidal concentration was recorded at the concentrations tested. The 50% cytotoxic concentration ($CC_{50}$) for methanol and hexane extracts were 2524 µg/mL and 5004 µg/mL, respectively, indicating a low risk of toxicity. No mortality or adverse effects were recorded in the acute toxicity test. Both extracts had no significant effects ($p < 0.05$) on renal function and one liver enzyme (alanine aminotransferase), but $MO_{MET}$ significantly increased aspartate aminotransferase ($p = 0.04$), suggesting possible liver toxicity. The study shows that *M. oleifera* leaves possess bacteriostatic activity against multidrug-resistant *Salmonella* and are non-toxic; hence, it is a potential alternative treatment against multidrug-resistant *Salmonella*. Further studies of fractions and pure natural products of the extracts should be pursued.

**Keywords:** Antibacterial, biochemical, *Moringa oleifera*, multidrug-resistance, *Salmonella*, toxicity

## INTRODUCTION

The morbidity and mortality of *Salmonella* Lignieres, 1900 infections, including typhoidal and non-typhoidal, are persistently high, particularly in developing countries. The global burden of *Salmonella* infections has been reported to be very high, resulting in over 10% mortality of all cases (He et al. 2023). In situations of poor hygiene, food and water are easily contaminated with *Salmonell*a, leading to the contraction of the infection via the oral and fecal routes. Managing severe infection cases requires antibiotics, given that a suitable preventive vaccine is unavailable.

Antibiotics initially used to treat *Salmonella* infections included penicillins, sulphamethoxazole–trimethoprim, chloramphenicol, and some macrolides. Due to the emergence of multidrug resistance in *Salmonella* to these antibiotics, other antibiotics, including fluoroquinolones, third-generation cephalosporins, and azithromycin, are currently being used to treat infections caused by multidrug-resistant *Salmonella* strains. However, recently, there have been reports of increasing resistance to the currently used antibiotics (Tack et al. 2020; Ndip et al. 2022) in both typhoidal and non-typhoidal *Salmonella* (Matic et al. 2018; Marchello et al. 2020; Park et al. 2021). Multidrug-Resistant (MDR) *Salmonella* is increasing with high levels in Africa and Asia, where extensively resistant strains have been detected (Marchello et al. 2020). Meanwhile, there is a high burden of invasive non-typhoidal *Salmonella* infections in sub-Saharan Africa mainly due to *Salmonella enterica* serovar Typhimurium with emerging extensive and pan-drug resistance (Puyvelde et al. 2023). Pursuing the discovery and development of efficacious anti-*Salmonella* therapies is imperative in this context. The approaches employed include synthetic medicinal chemistry, drug repurposing, combination therapy, study of natural products, and synthetic biology (Vila et al. 2020; Ahmed et al. 2023a).

*Moringa oleifera* Lam. (Moringaceae) is a tropical tree indigenous to India but has spread to Africa, Asia, and Southern America (van den Berg and Kuipers 2022). It is used in Indian traditional medicine in poultices and ointments to treat wounds, infections, and abscesses, and its leaves are a rich source of proteins. It is also used to improve memory and alertness, treat respiratory system ailments (asthma, sore throat), fevers, urinary tract infections, and diarrhea, as a food preservative and water purification; hence, it is termed a miracle tree due to its numerous properties (van den Berg and Kuipers 2022). An ethnobotanical survey in South Benin revealed various medicinal uses for infectious and non-communicable diseases involving all plant parts, with the leaves being the most used (Agoyi et al. 2017). Several parts of the plant, particularly the leaves, are widely used as food and in food recipes across Africa due to their rich nutrient content

(Matic et al. 2018). Studies of the plant have demonstrated anti-cancer, anti-diabetic, plasma lipid-lowering, tissue-protective, anti-inflammatory, and antimicrobial effects. These effects are due to its rich phytochemistry and nutrient contents (van den Berg and Kuipers 2022). Several studies have reported wide-ranging antibacterial activity for various parts of *M. oleifera*. A review of its antibacterial activity by (van den Berg and Kuipers 2022) reported that the leaf extract showed weak to moderate activity against Gram-negative bacteria (*Klebsiella pneumoniae* (Schroeter, 1886) Trevisan, 1887, *Proteus vulgaris* B, *Escherichia coli* E, *Pseudomonas aeruginosa* A); however, the methanol extract showed the best activity among the three solvents analyzed. A study reported high antibacterial activity against pyogenic multidrug-resistant bacteria isolated from camel abscesses (Fouad et al. 2019). Significant inhibition of the growth of food-borne pathogens, including *S. enterica* serovar Typhimurium, in preserved food by an aqueous extract of the leaves has been reported (Abdallah et al. 2023). In other studies, the aqueous and ethanolic extracts of *M. oleifera* leaves showed high activity against control strains of *Salmonella typhi* (Schroeter, 1886) Warren & Scott, 1930 and *S. enterica* (Ahmed et al. 2023b), while moderate activity was recorded for the ethanol extract only against multidrug-resistant clinical *Salmonella* isolates (Enerijiof et al. 2021). However, reports of studies conducted on characterized clinical multidrug-resistant bacteria, including *Salmonella*, are very rare. Considering the well-documented use of *M. oleifera* in ethnomedicine against a wide range of infectious diseases, this study aimed to investigate the activity of *M. oleifera* leaf crude extracts against multidrug-resistant clinical strains of *Salmonella*, which has not been extensively investigated. In addition, the investigation of the toxicity of the extracts was among the objectives of the study.

## MATERIALS AND METHODS

### Plant collection and preparation of extracts

The leaves of *M. oleifera* were collected in Kumba in the South West region of Cameroon. A voucher specimen was taken to the Limbe Botanic Garden, where it was authenticated by a botanist using the voucher specimen number SCA 7706. The fresh leaves were dried under a shaded area for 4 weeks and ground to a fine powder. The powder was weighed, giving a mass of 700.9 g. It was macerated sequentially, first by submerging it in hexane and then kept for 48 hours with occasional stirring. Then, the mixture was filtered through Whatman filter paper No.1, and the residue was dried at room temperature to constant mass to remove the hexane solvent. The filtrate was concentrated at 69°C in a rotary evaporator (BUCHI Rotavapor R-200, Switzerland). The dried residue was similarly macerated in methanol and concentrated at 40°C. The two extracts were dried to constant mass at room temperature to remove residual solvent and then stored at -20°C. Amounts of extract required for use were weighed from the stored stock.

### Screening of phytochemical constituents

Standard chemical tests were done to detect the presence of phytochemicals (flavonoids, glycosides, saponins, tannins, steroids, phenols, alkaloids) in the extracts and their relative amounts, based on the color intensity of the test reaction mixture as described (Pant et al. 2017; Shaikh and Patil 2020).

### Antibacterial screening

Sixteen (16) multidrug-resistant clinical isolates of *Salmonella* and two (2) control strains were used in this research. The clinical strains were isolated and characterized using culture, biochemical, and molecular analyses and stored in 50% glycerol in Muller Hinton (MH) broth (Liofilchem, Italy) at -20°C (Ndip et al. 2022). All assays were conducted under sterile conditions.

The antibacterial screening was initially done using a disc diffusion test as described (Mbah et al. 2012), with some modifications. Briefly, 100 and 200 mg of each extract were dissolved in 1mL of 0.5% dimethyl sulfoxide (DMSO), giving 100 and 200 mg/mL solutions. Each *Salmonella* strain was first cultured on nutrient agar to obtain a pure culture. Paper discs (6 mm in diameter) were cut out from Whatman filter paper No. 1 and sterilized by autoclaving, then 10 µL of extract solution containing 1 and 2 mg of extract were transferred onto separate discs and kept to dry. A 0.5 McFarland bacterial suspension ($1.5 \times 10^8$ CFU/mL) was prepared by adding a pure colony to about 1 mL, then mixed and diluted to the required density with saline. Then, 100 µL of the suspension was spotted and spread on the surface of Mueller Hinton (MH) agar culture plates, followed by gently placing the extract discs on the bacterial spread. Positive control discs of gentamycin (10 µg) and ciprofloxacin (5 µg) and a negative control disc (10 µL of 0.5% DMSO) were included. The plate was incubated at 37°C (DHP-1952, England incubator) for 24 hours; the inhibition zone around each disc was first observed for the absence of any colonies, and the diameters of clear zones were measured using a millimeter scale.

The Minimum Inhibitory Concentration (MIC) was determined by microdilution (Ngemenya et al. 2022). Briefly, 40 mg of each extract was dissolved in 1 mL of 5% DMSO and then diluted to 20 mg/mL in MH broth. The extract solution was diluted to give double the required concentrations, then 75 µLof each solution was added into a 96-well microtitre plate in duplicate wells, followed by 75 µL of bacterial suspension ($1 \times 10^6$ CFU/mL) in broth to obtain final extract concentrations of 10, 8, 6, 4, 2, 1, 0.5, 0.25, 0.125 and 0.0625 mg/ mL and cell density of $5 \times 10^5$ CFU/mL. Gentamycin or ciprofloxacin (50 µg/mL) and DMSO (2.5%) were positive and negative controls, respectively. The plate was observed visually, and optical densities (ODs) were read at 595 nm (Emax microplate reader, Molecular Devices, USA). The plate was incubated at 37°C for 24 hours, and the ODs were recorded after 24 hours. The percentage inhibition was calculated from the change (Δ) in OD using the formula (Ngemenya et al. 2022):

$$\%\text{Inhibition} = \frac{\Delta\text{OD Negative control} - \Delta\text{OD extract}}{\Delta\text{OD Negative control}} \times 100$$

MIC was taken as the lowest concentration of extract that produced at least ≥ 50% inhibition of bacterial growth. Minimum Bactericidal Concentration (MBC) was determined by spotting 10 µL of inhibited MIC well contents on nutrient agar on culture plates and incubating them at 37°C for 24 hours. The lowest concentration of the corresponding well without growth was considered the MBC.

**Cytotoxicity test**

This was done on monkey kidney epithelial cells, LLCMK2 Original (ATCC® CCL7™), (Virginia, USA), by the method of Ngemenya et al. (2019). Briefly, a complete culture medium, RPMI-1640 medium (CCM), was prepared with the addition of $NaHCO_3$, 25 mM Herpes, 0.3 g γ-irradiated L-glutamine powder, 10% heat-inactivated newborn calf serum, 200 units/mL penicillin and 200 µg/mL streptomycin and 0.25 µg/mL amphotericin B, and pH adjusted to 7.4. An incomplete medium (ICM, no calf serum) was also prepared similarly. The extract solution (2 mg/mL) was prepared in 4% DMSO in CCM. The cells were cultured in a T-shaped flask in a Heracell 150i (USA) incubator under conditions of 5% $CO_2$ and humidified air at 37°C. When cells grew to confluence, the medium was decanted, and the flask was washed off with ICM two times. The cells were dislodged with trypsin and centrifuged at 1000 rpm for 10 min (Eppendorf 5810R, Germany). Then, they were re-suspended in ICM and counted using a microscope (Nikon Eclipse TS100, China). The cells were diluted with CCM such that 3,000 cells in 100 µL were added in duplicate in a 96-well flat bottom microtitre plate. This was followed by incubation under the same conditions for 3 days for the cell to reach confluence. The extract was tested at 1 mg/mL by adding 100 µL of test solution. Wells containing auranofin (30 µM) and 2% DMSO were included as positive and negative controls, respectively. The plates were incubated at the same conditions for 5 days while well contents were examined for dead cells using a microscope daily to check for toxicity. An extract that showed toxicity was diluted to 15-1000 µg/mL and re-tested to determine the $CC_{50}$. After incubation, the medium was discarded, ICM was added to all wells, and the plate was shaken (IKA Labortechnik KS125 basic shaker) at 600 rpm for 5 minutes to wash off any colored solution. Then, 100 µL the cytotoxicity test reagent MTT formazan (5 mg/mL in ICM) was added into the required wells and then incubated for 30 minutes. The formazan precipitate formed in the MTT reduction reaction was dissolved by adding 100 µL DMSO.

Well contents were gently mixed by shaking, ODs were read at 595 nm, and percentage inhibitions were calculated using the formula below (Ngemenya et al. 2019):

$$\text{Percent inhibition (\%)} = \frac{\text{OD of control} - \text{OD of treatment}}{\text{OD of control}} \times 100$$

**Acute toxicity**

A stock solution of hexane extract was prepared by dissolving 25 mg in 60 µL of acetone to dissolve partially, and 250 µL DMSO was added for complete dissolution, followed by 690 µL of distilled water to give 25 mg/mL. The stock solution of the methanol crude extract was prepared similarly by adding 25 mg/mL in 1 mL of 2% DMSO (SIGMA, USA) and vortexed for complete dissolution. Corresponding control solutions without extract were prepared similarly. All solutions were stored at 4°C. The University of Buea granted the study ethical approval by the Institutional Animal Care and Use Committee (No. UB–IACUC No 13/2021). The guidelines of the Organization for Economic Cooperation and Development version 423 (OECD 2002) were followed concerning the dosage and handling of animals during the acute toxicity test, which was conducted according to the procedure of (Ngemenya et al. 2019). Briefly, nine-week-old mice were placed in three groups of five animals (the control and two test groups, with three females per group); the mice were acclimatized to the study conditions for one week before the test.

One animal per test group was weighed, fasted overnight with access to water only, and administered 2,000 mg/kg body weight extract orally in a 1 mL/100 g body weight volume using an oral gavage needle with a 22G ball tip. The treated animals were fasted further for 2 hours and observed for 24 hours with access to food. Following the survival of treated animals, the others were treated as above, and control solutions were also administered to corresponding animals. All animals were observed for gross changes as outlined (Ngemenya et al. 2019) for 14 days with access to food and water. After that, animals were weighed, fasted overnight, anesthetized with ketamine/xylazine (90/10 mg/kg), and blood was collected by retro-orbital bleeding into a dry Eppendorf tube. The blood was kept for 30 minutes to solidify, centrifuged at 2,000 rpm for 15 minutes (Eppendorf centrifuge 5702R), and the serum was analyzed for liver enzymes (alanine aminotransferase, ALT, and aspartate aminotransferase, AST) using commercial test kits (CHRONOLAB, Spain), following manufacturer's instructions. Kidney function tests (urea and creatinine) were performed similarly using Biorex Diagnostics (United Kingdom) kits.

**Data and statistical analysis**

A plot of the percentage inhibition of growth of bacteria against extract concentration in the microdilution assay was done using GraphPad Prism 5.0 (GraphPad Prism INC. USA) software, where the MIC (lowest concentration corresponding to 50% inhibition) was interpolated. The $CC_{50}$ (cytotoxic concentration for 50% of cells) was determined using the same method as the MIC. Biochemical parameters of control and test animals were compared using GraphPad Prism 5.0 software, and an unpaired two-tailed t-test was used to check for any significant difference ($P < 0.05$).

## RESULTS AND DISCUSSION

**Yield and phytochemical composition of extracts**

The amount of the hexane extract (30.9 g) obtained from the powder of the dried *M. oleifera* leaves gave a

higher yield of 4.4% (a percentage of the 700.9 g of powder used for the extraction) compared to the methanol extract (8.2 g), which gave a yield of 1.17%. From the phytochemical screening, both extracts had a similar content of secondary metabolites, with relatively high amounts of saponins and steroids and small amounts of tannins; smaller amounts of flavonoids and phenols were detected in the methanol extract only.

**Antibacterial activity of extracts**

In the disc diffusion test, both extracts produced small zones with diameters of 8 mm to 11 mm at 1 and 2 mg, respectively, which indicated weak activity against the 16 MDR clinical and control *Salmonella* strains. On the contrary, the gentamycin and ciprofloxacin positive control produced large inhibition zones ranging from 25 to 35 mm and 29 to 35 mm, respectively.

The MIC values of the hexane extract ranged from 0.0625 to 10 mg/mL against different *Salmonella* strains. In comparison, MIC values of the methanol extract ranged from 0.125 to 10 mg/mL (Table 1), showing varied activity against the MDR strains. According to an activity scale by (Cos et al. 2006), the hexane extract ($MO_{HEX}$) showed high activity (MIC = 0.0625 to 2 mg/mL) against 8 out of 18 strains of *Salmonella*, while the methanol extract ($MO_{MET}$), showed high activity against 4 strains only (Table 1). Hence, the hexane extract was more active than the methanol extract. The content of the MIC wells for both crude extracts, which showed inhibition, also showed bacterial growth (CFUs) on solid nutrient agar. Hence, no MBC was recorded within the concentration range tested.

**Toxicity of extracts**

*Cytotoxicity*

The $CC_{50}$ on monkey kidney cells for both the hexane and methanol extracts of *M. oleifera* was 5004 and 2524 µg/mL, respectively, much higher than the cut–off value for lack of toxicity ($CC_{50}$ > 30 µg/mL) (Ogbole et al. 2017). The relative selectivity of the extracts based on the lowest MIC values and the $CC_{50}$ (determined from $CC_{50}$/MIC in µg/mL) is 80.0 and 40.3 for the hexane and methanol extracts, respectively.

*Acute toxicity*

Animals treated with methanol extract were sluggish and dizzy, whereas those treated with hexane extract had irritation of the eyes and nose within the first 2 hours post-treatment. No mortality was recorded on day 14, and food and water intake was similar to that of control mice. No adverse effects were observed. The average weights of mice increased in test and control groups for both extracts, with no significant difference for mice treated with methanol extract (p = 0.497) and hexane extract (p = 0.2838).

**Table 1.** Minimum inhibitory concentrations of *Moringa oleifera* leaf extracts against multidrug-resistant *Salmonella* isolates

| MIC Value (mg/mL) | No. of Isolates $MO_{HEX}$ | No. of Isolates $MO_{MET}$ | Activity |
|---|---|---|---|
| 0.0625 | 1 | 0 | High |
| 0.125 | 1 | 3 | |
| 0.25 | 1 | 0 | |
| 1 | 1 | 0 | |
| 2 | 4 | 1 | |
| 4 | 0 | 6 | Moderate |
| 6 | 3 | 5 | |
| 8 | 2 | 2 | Low |
| 10 | 5 | 1 | |
| Total | 18 | 18 | |

Note: Interpretation of MIC values (mg/mL): ≤ 2: High activity; > 2 and < 6: Moderate activity; > 6: low activity (Cos et al. 2006). Extracts: $MO_{HEX}$ and $MO_{MET}$: Hexane methanol extracts of *M. oleifera*. MICs: Minimum Inhibitory Concentrations

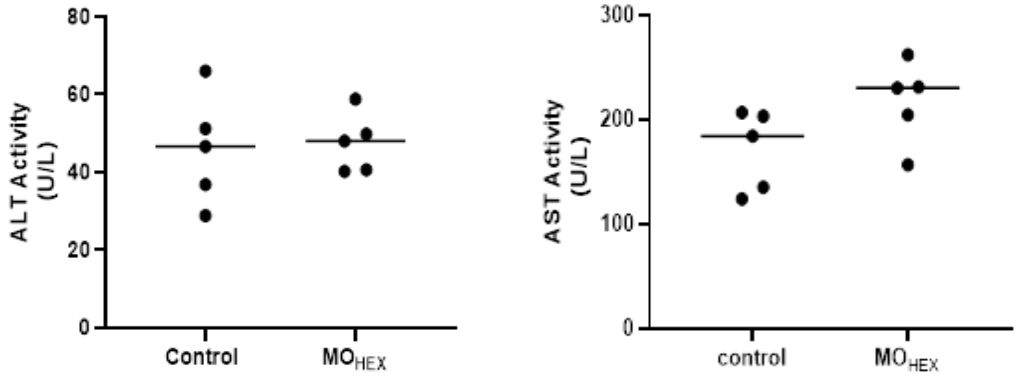


**Figure 1.** Effect of 2000 mg/kg hexane extract of *Moringa oleifera* ($MO_{HEX}$) on mouse liver enzyme activity in BALB/c ALT: Alanine aminotransferase (P = 0.831); AST: Aspartate aminotransferase (P = 0.0976)

**Effect of extracts on liver and renal functions**

The hexane extract ($MO_{HEX}$) did not affect alanine aminotransferase (ALT) but caused an increase in aspartate aminotransferase (AST). However, there was no significant difference in ALT and AST levels between the control mice and those treated with the hexane extract (p = 0.831 and 0.0976, respectively), as shown in Figure 1. The AST: ALT ratio of the mean enzyme activity for the test group was 0.22 (< 1), indicating no adverse effect in the liver. The methanol extract ($MO_{MET}$) produced the same pattern of effects on liver enzymes as $MO_{HEX}$. Still, there was also no significant difference in ALT levels between the control mice and the test group administered the methanol extract ($MO_{MET}$) (p = 0.824). However, there was a significant difference in AST level (p = 0.045) (Figure 2) and AST: ALT ratio of 5.7, which indicates adverse effects on the liver.

$MO_{HEX}$ caused a slight increase in urea and a decrease in creatinine. Still, the difference between treated mice and the control group was not significant (p = 0.639 and 0.051, respectively), as shown in Figure 3. Meanwhile, $MO_{MET}$ produced an increase in both urea and creatinine. Still, there was no significant difference in urea and creatinine levels between the treated ($MO_{MET}$) and a control group of mice, with p-values of 0.394 and 0.118, respectively (Figure 4).

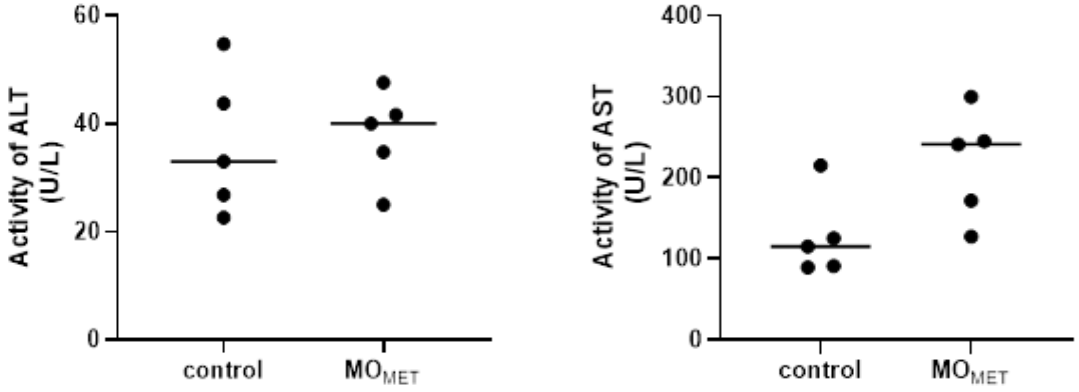


**Figure 2.** Effect of 2000 mg/kg methanol extract of *Moringa oleifera* ($MO_{MET}$) on mouse liver enzyme activity in BALB/c. ALT: Alanine aminotransferase (P = 0.824); Aspartate aminotransferase (P = 0.0456)

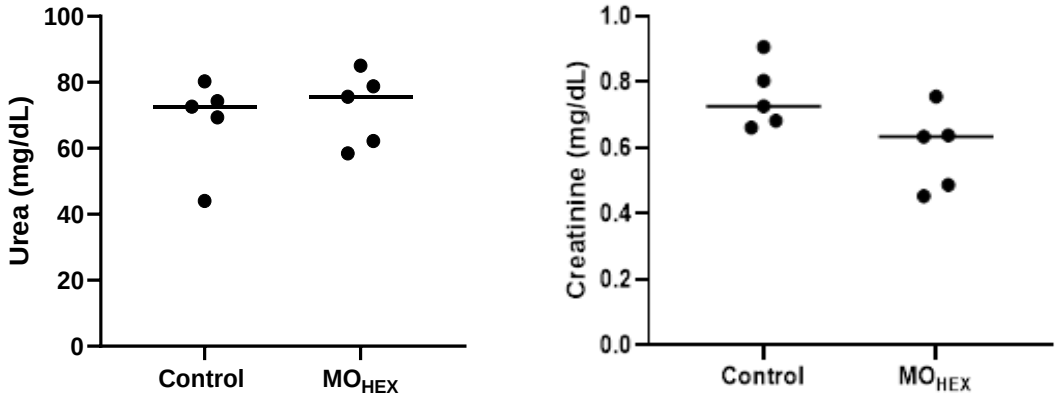


**Figure 3.** Effect of 2000 mg/kg hexane extract of *Moringa oleifera* ($MO_{HEX}$) on renal function in BALB/c mice. Urea (*P* = 0.639); Creatinine (*P* = 0.051)

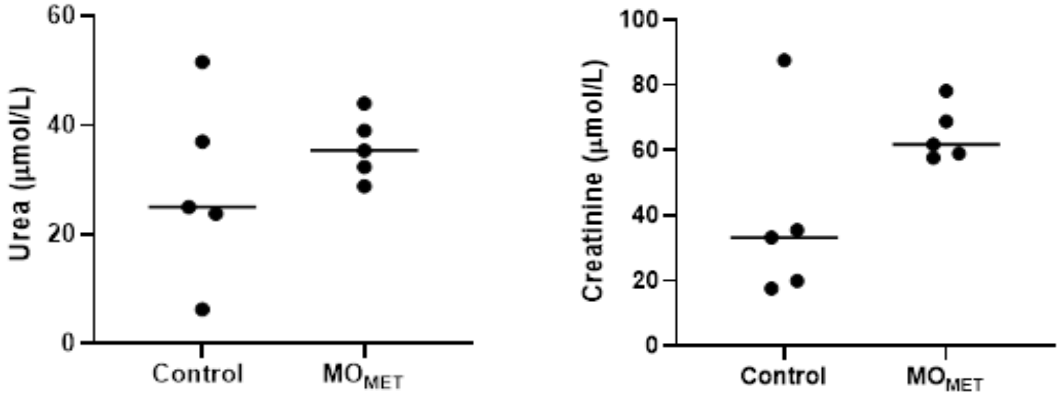


**Figure 4.** Effect of 2000 mg/kg methanol extract of *Moringa oleifera* ($MO_{MET}$) on renal function in BALB/c mice. Urea (P = 0.394); Creatinine (P = 0.118)

**Discussion**

As mentioned above, *M. oleifera* is widely used in traditional medicine to treat bacterial infections. Several reports on the plant's antibacterial activity support the traditional use. However, studies on its activity against MDR *Salmonella* are very rare. This study found high bacteriostatic activity against some clinical strains of MDR *Salmonella* and no adverse toxicity of the leaf extracts in mice. These findings constitute evidence to support the use of *M. oleifera* leaves in traditional medicine to treat infections caused by different bacteria. Furthermore, the result suggests that this plant's leaves are a potential alternative treatment to counter resistant *Salmonella* infections and, therefore, require further exploration. The hexane extract produced the highest activity against a higher number of MDR *Salmonella* isolates in the microdilution assay compared to the methanol extract; hence, the hexane extract carries a higher potential to counter resistance in *Salmonella*. This high activity is likely due to non-polar secondary metabolites present in the extract. Both extracts showed dose-dependent activity as more isolates were inhibited at higher MICs, and all 18 isolates were inhibited within the extract concentration range tested. No MBC was recorded within the concentration range tested, suggesting that the extracts are bacteriostatic; this points to the mechanism of action by inhibition of protein synthesis in the bacterial cell (Halawa et al. 2024). However, the actual mechanism of action needs to be investigated. The low activity recorded in the disc diffusion test could be due to several factors, including a low amount of bioactive secondary metabolites in the extract embedded in the disc or due to the well-known limitations of the disc test, such as poor diffusion of secondary metabolites in the agar medium among others (Bubonja-Šonje et al. 2020). The wide range in the MIC values is likely due to differences in the magnitude of resistance of the clinical *Salmonella* strains used; the strains were multidrug-resistant but had differences in their resistance gene as reported following molecular characterization (Ndip et al. 2022). Some studies have reported high antibacterial activity of *M. oleifera* leaf extract against *Salmonella*. In one study (Abdallah et al. 2023), an aqueous extract of the leaves significantly reduced the counts of spoilage bacteria in meatballs and other bacteria, including *S. typhimurium* inoculated in meatballs, making it a potentially natural, safer alternative preservative for meatballs as opposed to chemical preservatives. On the contrary (Anzano et al. 2022) reported that both polar and apolar extracts of the leaves and seeds showed weak activity against Gram-negative bacteria, *P. aeruginosa* and *S. enterica*, but the apolar extract of the seeds showed high activity against Gram-positive pathogens. However, unlike in this study, the two studies cited above did not specify the antibiotic susceptibility or resistance of the bacterial strains used. To explore the activity of M. oleifera fully, in vivo efficacy and further toxicity studies should be conducted to obtain more data that support its use. Studies in combination with other medications or treatments may reveal enhanced activity. To generalize these findings, the extracts should also be screened against strains of *Salmonella* found in other populations or regions.

Both extracts had very high selectivity index values, indicating that they are not likely to be toxic at relatively high doses required to inhibit the most resistant strains with the highest MIC values. The acute toxicity test did not show any adverse toxicity both macroscopically and in terms of biochemical changes, except for the significant increase in AST with a high AST:ALT ratio recorded for the methanol extract, which suggests severe liver damage. However, no mortality occurred in the mice treated with the methanol extract, suggesting any liver damage that occurred was reversible. Overall, the toxicity findings suggest that the leaves are not likely to produce adverse toxicity in humans when used in traditional medicine, although this depends on the dose administered. A work by (Asare et al. 2012) reported that the aqueous extract of the leaves was cytotoxic at a high dose of 20 mg/mL on human peripheral blood mononuclear cells, which is much higher than the higher $CC_{50}$ of 5 mg/mL and the highest MIC of

10 mg/mL recorded in this study. Interestingly, in this same study, an acute toxicity test of the extract did not cause liver or kidney toxicity and no hematological abnormalities at up to 3,000 mg/kg in Sprague-Dawley rats. Still, the leaf extract was genotoxic at this high dose. Also, de Barros et al. (2022) reported no abnormality in an acute toxicity test in Swiss female albino mice for *M. oleifera* leaf infusion or powder at 5,000 mg/kg. Still, they observed liver and kidney damage in a 28-day repeated dose toxicity test at a low dose of 500 mg/kg. The differences in the findings of other studies are likely due to the different cell lines, animal species used, doses and types of toxicity tests. However, similar to other studies, there is a likelihood of negligible toxicity at relatively low potent doses with a high selectivity index. Even though *M. oleifera* did not show adverse toxicity in the liver and kidney, the toxicity in other organs needs to be investigated.

In terms of strength, to the best of our knowledge, this study is likely the first to report on the antibacterial activity of the leaves of *M. oleifera* against MDR *Salmonella* strains. The findings suggest that *M. oleifera* could be used as an alternative treatment with the potential impact of decreasing the morbidity and mortality of *Salmonella* infections. However, the use of this plant as an alternative medicine should be controlled by experts in phytomedicine to avoid the emergence of resistance. As a limitation, other parts of the plant were not studied; this should be done by including factors that may affect its activity, such as environmental conditions, harvesting and processing techniques. Also, MDR strains of *Salmonella* found in other populations or regions, as well as those of other bacterial species, should be studied.

In conclusion, this study has shown considerable bacteriostatic anti-*Salmonella* activity of the leaves of *M. oleifera* against MDR strains and a low risk of toxicity at effective doses. Hence, the leaves are a potential alternative treatment for antibiotic-resistant infections of *Salmonella* and a possible source of an antibacterial lead. The findings support the use of this plant in the traditional treatment of bacterial infections; hence, further studies should be carried out on other resistant bacteria, and the anti-*Salmonella* activity should be investigated in an *in vivo* model.

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