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Antibacterial Activities of Methanol and Ethanol Extracts of *Moringa oleifera* (Lam) Leaves

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Abstract

Moringa oleifera is known for its therapeutic potential, particularly in treating bacterial infections. This study aimed to determine the Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) of *Moringa oleifera* extracts (ethanol and methanol) on two bacterial strains: *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive). The extracts were tested at concentrations of 200, 150, 100, 50, and 25 mg/ml. Phytochemical analysis was also conducted. Results showed that *Moringa* extracts exhibited significant antibacterial activity, with the methanol extract showing an MIC and MBC of 200 mg/ml, and the ethanol extract showing an MIC and MBC of 150 mg/ml. The zones of inhibition were 19 mm (*E. coli)* and 18 mm (*S. aureus*) for methanol extract, and 18 mm (*E. coli*) and 17 mm (*S. aureus*) for ethanol extract. These results were similar to those of Ciprofloxacin (a standard antibiotic), indicating that *Moringa* extracts have strong antimicrobial properties. The study suggests that *Moringa oleifera* could be used as an alternative or adjunct to conventional antibiotics. Future studies should explore the synergistic effects of combining *Moringa* extracts with existing antibiotics to enhance their effectiveness and combat antibiotic resistance.

Keywords: Antibacterial activity; drug-resistance; *Escherichia coli*; *Staphylococcus aureus*; phytochemicals.

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Introduction

The amazing therapeutic properties of *Moringa oleifera* plant as praised by many societies and cultures, are becoming more and more supported by data and studies from science (Suleiman and Salihu, 2023). Naturally occurring secondary bioactive substances with a broad range of bioactivity that support health are called phytochemicals and they are produced from plant sources (Abdurrashid and Sharhabil, 2021). It has been established that plants and their parts are fully packed with phytochemicals (Misra *et al.*, 2024). Phytochemicals are one of the

most significant classes of bioactive compounds found occurring naturally in plants and they include but are not limited to flavonoids, tannins, phenolic acid, polyphenolic amides, etc. These phytochemicals are responsible for mitigating the adverse effect of reactive oxygen species known to aggravate inflammation and other degenerative human ailments (Jomova *et al*., 2023; Shin *et al*., 2020). Several studies have been done on the phytochemical constituent of *Moringa oleifera* plants (Adekanmi *et al*., 2020; Fahal, 2018).

The increasing prevalence of antibiotic-resistant bacteria is a significant global health threat. Antibiotic resistance occurs when bacteria evolve to withstand the effects of drugs that previously killed them or inhibited their growth (WHO, 2019). This resistance leads to treatment failures, longer hospital stays, more intensive care, and higher medical costs (Naghavi *et al*., 2019). Many bacterial infections that were once easily treatable are now becoming more difficult, if not impossible, to cure with existing antibiotics. As antibiotics become less effective, there is a growing need for new antimicrobial agents. Natural products, particularly plant-based extracts, have been used for centuries to treat infections and are a promising source for discovering new antibiotics. Plants like *Moringa oleifera* have shown potential antimicrobial properties, making them candidates for alternative or complementary treatments. Incorporating plant-based solutions could help address the urgent need for novel antibiotics, reduce reliance on synthetic drugs, and combat antibiotic resistance. Therefore, this research aimed to offer a plant-based option for treating human pathogenic infections like *Staphylococcus aureus* and *Escherichia coli* in environments with low resources, like Nigeria.

Materials and method

The *Moringa oleifera* leaves were collected behind multi laboratory in Kwara State University, Malete, Nigeria and transported to the central research laboratory in Tanke, (beside MTN telecommunication office) Ilorin Kwara State Nigeria for further laboratory procedures.

The leaf parts of the plant were washed under running tap water and-air dried at ambient temperature for 7 days. The air-dried samples were pulverized into powders and kept in a dry bottle until further use. The pulverized sample (300 g) was macerated at room temperature with occasional shaken in 95% methanol or ethanol in a ratio 1:5 (v/v) for 24 hours (Edeoga *et al*., 2005). The extract was filtered and concentrated over a reduced pressure at 40°C using rotary evaporator model: Labtech (AN ISO 13485:2003). The concentrated extract was stored in an air-tight bottle and kept in refrigerator at 4ºC until it was used for the antioxidant test.

Phytochemical analysis of plant extract.

The phytochemicals such as saponins, terpenoids, alkaloid, steroids, tannin, flavonoids, glycosides and free glycoside-bound anthraquinones were tested according to the method described by Edeoga *et al*. (2005)

Test for Saponin: Saponin was tested by making a 5.0 ml aliquot of extract in 20 ml of deionized water and shaken vigorously. The presence of persistent foaming indicates the presence of saponins.

Test for Tannins: In testing for tannins, 1 mL of extract was mixed with 10 mL deionized water, and then 3 drops of ferric chloride were added. Tannins were detected in the form of a greenish-brown precipitate.

Test for Phenolics: 2 drops of 5% $FeCl_3$ was added to 1mlof the extracts in a test tube. A greenish precipitate indicates the presence of phenolics. The chemical equation is represented as:

$$\mathbf{6C_6H_5OH + FeCl_3 \rightarrow [Fe(C_6H_5O)_6]^{3-}\ (violet\ colour\ complex) + 3HCl + 3H^+}$$

Test for glycosides: To test for glycosides, 1ml of the extract was mixed with 5 ml of dilute sulphuric acid in a test-tube and heated for 15 min in a water bath, then cooled and neutralized with a 20% KOH solution. A mixture of equal volumes of Fehling's solution A and B was added to 10 ml of water and heated for five minutes. The presence of glycoside was identified by a more dense brick red precipitate.

Test for Steroids: Steroids was tested by extracting a known quantity of the extract in chloroform and filtered. The filtrate was then carefully mixed with 2 ml of conc. H_2SO_4 to form a lower layer of sulphuric acid. The presence of a steroidal ring was indicated by a reddish-brown color in the interphase.

Test for Terpenoids: 5ml of aqueous extract of the sample was mixed with 2ml of $CHCl_3$ in a test tube and 3ml of conc. H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

Test for Triterpenes Five (5) drops of acetic anhydride was added to 1ml of the extracts. A drop of concentrated H_2SO_4 was then added and the mixture was steamed for 1 hour, neutralized with NaOH followed by the addition of chloroform. A blue green colour indicates the presence of triterpenes.

$C_{30}H_{48}OH+(CH_3CO)2O \rightarrow C_{30}H_{48}OCOCH_3+CH_3COOH$ & $C_{30}H_{48}OCOCH_3+H_2SO_4 \rightarrow$ Colored Complex.

Test for Flavonoids: Test for flavonoids was done heating a portion of extract for 3 minutes in ethylacetate (10 ml), then the solution was filtered and chilled. After that, 1 mL of dilute ammonia solution was added to the filtrate (4 ml), and flavonoids were indicated by intense yellow colorations.

Test for amino acid: Two drops of ninhydrin solution (10mg of ninhydrin in 200ml of acetone) were added to 2 ml of aqueous filtrate. A characteristic purple colour indicates the presence of amino acids.

Test for Phlobatannins: 1ml of the extracts was added to 1% HCl. A red precipitate indicates the presence of phlobatannins.

Preparation of Bacteria Media

Nutrient Agar (NA) was utilized in accordance with the manufacturer's instructions to cultivate the bacteria: Using a Sensitive Mettler weighing scale, 7.5g of nutrient agar (NA) was weighed and transferred into a sterile 250ml conical flask. In order to properly homogenize the contents, about 250 milliliters of distilled water were added to the flask and it was maintained in a water bath at 100 degrees Celsius. After that, it spent 15 minutes autoclaved at 121°C.

Collection of organism

Staphylococcus aureus and *Escherichia coli* were obtained from Microbiology Department of the Institute of Agricultural research and Training, Moor Plantation, Apata Ibadan, Nigeria

Infusion of the extract and inoculation of the two strains of bacteria

After sterilizing and cooling to a temperature of between 40 and 45 degrees Celsius of the media, an aseptic addition of *Moringa oleifera* methanol/ethanol extract was made to the Nutrient Agar. Five (5) holes were then drilled using a 5 mm flame-sterilized cork borerand the plate was left to stand for 24 hrs full day. After either *Staphylococcus aureus* or *Escherichia coli* was left out of the incubation for 24 hours, the *Staphylococcus aureus* and *Escherichia coli* were employed to replace the hole with a sterile corkscrew with the same diameter. There are distinct species in every hole. (one species per two holes). Zone of inhibition were measured after 24 hours using a ruler and expressed in centimeters.

Determination of minimum inhibitory concentration (MIC) of methanol and ethanol extract of *Moringa oleifera*

The method of Eucast (2003) with little modifications was adopted. A dilution method was employed in the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). In test tubes containing sterile nutritional broth, ciprofloxacin was diluted into several concentrations: 200, 150. 100, 50 and 25 mg/ml. Inoculation of either *Staphylococcus aureus* or *Escherichia coli* into the test tube was done according to the method described by Eucast, 2003. In this method, 10 µl of either *E. coli* culture or *S. aureus* methanol or ethanol extract of *Moringa oleifera* with 0.5 McFarland standard was added into the nutritional broth. An addition of 1 ml of the various ciprofloxacin concentrations was also added into the test tube. After 18 to 24 hours of incubation at 37°C, the tubes were checked for growth or turbidity. A loopful of broth from every test tube that did not exhibit growth was then added to a nutrient agar plate as an inoculant. Unaided eye examination of agar plates was performed to check for growth or

turbidity as done to indicate MIC (CLSI, 2012).

Determination of minimum bactericidal concentration (MBC) of methanol/ethanol extract of *Moringa oleifera*

Minimum bactericidal concentration (MBC) for the two strains of the bacteria was determined using the subculture from MIC. After 48 hours of incubation at 37°C, a volume of 0.1 mL was extracted from the wells in the microtiter plates and inoculated onto the surface of Tryptic soy agar plates and MBC was determined to be the lowest concentration of the etxract of *Moringa oleifera* at which colonies did not form under these conditions, and they were cultured for 48 hours at 37°C.

RESULTS

Results of qualitative and quantitative phytochemical composition of the Methanol leaf extract of *Moringa oleifera*

Table 1 provides a comprehensive overview of the qualitative phytochemical composition of both methanol and ethanol extracts of *Moringa oleifera* leaves. The analysis reveals that both extracts contain saponins, phenolics, glycosides, terpenoids, coumarins, flavonoids, and alkaloids, indicating a rich array of bioactive compounds. Notably, the methanol extract exhibits a broader spectrum of phytochemicals, including tannins and steroids, which are absent in the ethanol extract. Conversely, the ethanol extract lacks triterpenes, anthocyanins, amino acids, and phlobatanins. This variation suggests that methanol is a more effective solvent for extracting certain phytochemicals from *Moringa oleifera* leaves. Figure 1 presents the quantitative phytochemical composition of the methanol extract of *Moringa oleifera* leaves, detailing the concentrations of various compounds measured in mg/g. Similarly, Figure 2 illustrates the quantitative phytochemical composition of the ethanol extract. Both figures provide essential insights into the relative abundances of specific phytochemicals present in each extract. By comparing these quantitative data with the qualitative findings from Table 1, it becomes evident how different extraction methods can influence both the presence and concentration of bioactive compounds in *Moringa oleifera*. This information is vital for understanding the potential therapeutic applications of these extracts in antimicrobial research and other medicinal uses.

Table 1: Qualitative phytochemical composition of Methanol and Ethanol extracts of *Moringa oleifera* leaves

Phytochemicals	Methanol extract	Ethanol extract
Saponin	+	+
Tannin	+	-
Phenolics	+	+
Glycosides	+	+
Steroids	+	-
Terpenoids	+	+
Triterpenes	-	-
Coumarins	+	+
Flavonoids	+	+
Anthocyanine	-	-
Amino acid	-	-
Phlobatanin	-	-
Alkaloids	+	+

Key: + = Present: - = Absent

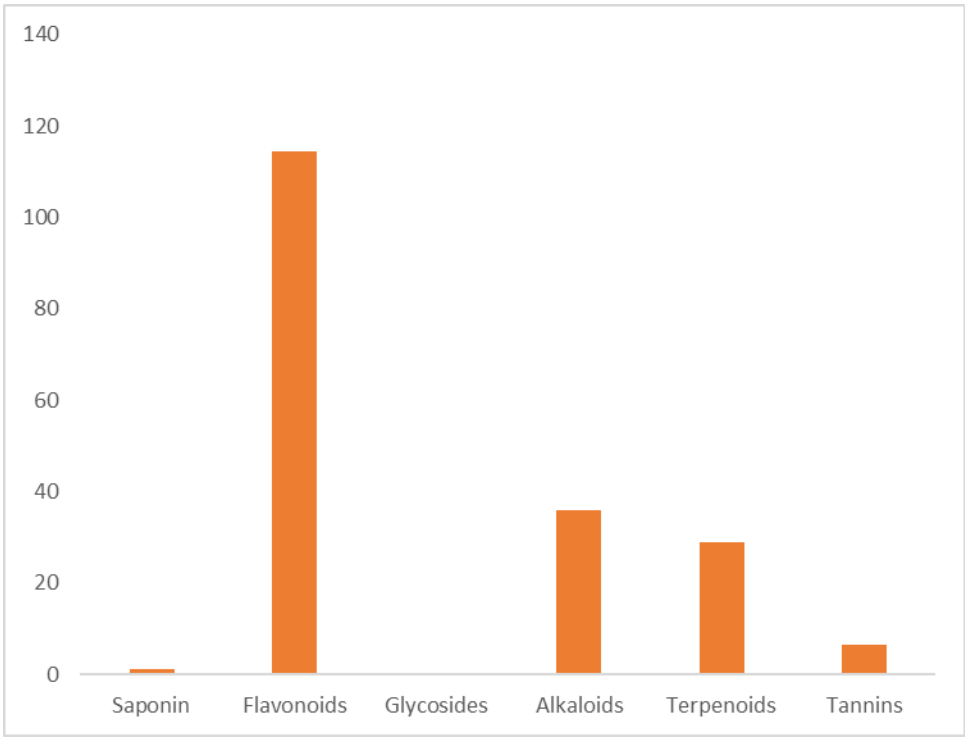

Figure 1: Quantitative Phytochemical composition (mg/g) of the Methanol extract of *Moringa oleifera* leaves (mg/g)

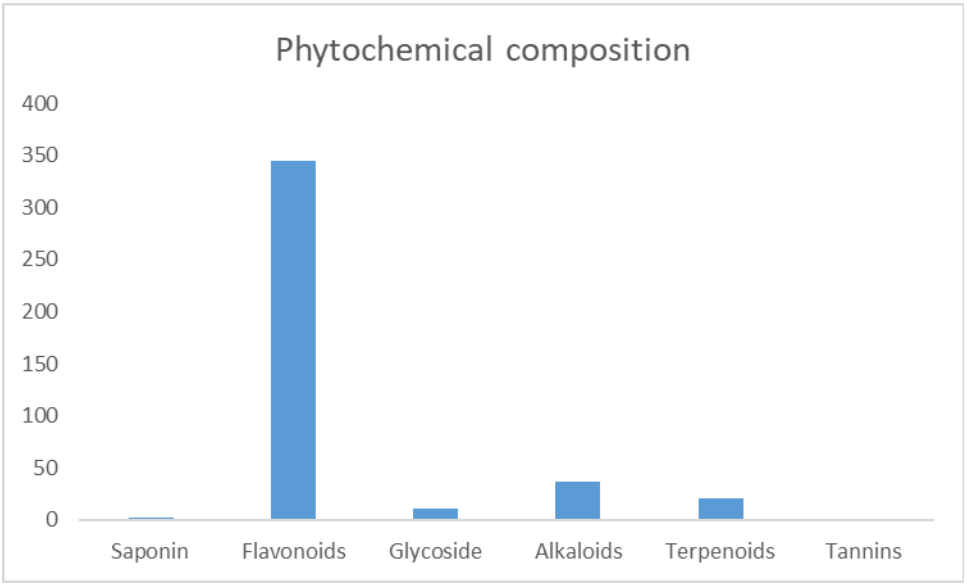

Figure 2: Quantitative Phytochemical composition (mg/g) of the Ethanol extract of *Moringa oleifera* leaves (mg/g)

Antibacterial Sensitivity Test

The results from the antimicrobial assays of *Moringa oleifera* extracts, as presented in Tables 2, 3, 4, and 5, provide valuable insights into the antibacterial properties of both methanol and ethanol extracts against common pathogens, specifically *E. coli* and *S. aureus*. In Table 2, the methanol extract demonstrates a clear concentration-dependent effect on bacterial inhibition. At the highest concentration of 200 mg/ml, the zone of inhibition measured 19 mm for *E. coli* and 18 mm for *S. aureus*. As the concentration decreases to 25 mg/ml, the zones of inhibition also diminish to 10 mm and 11 mm respectively. Notably, the positive control (Ciprofloxacin) showed significantly larger zones of inhibition (22 mm for *E. coli* and 21 mm for *S. aureus*), indicating that while Moringa extracts exhibit antibacterial activity, they are less potent than standard antibiotics. Table 3 presents similar findings for the ethanol extract of *Moringa oleifera*. Here, the maximum zones of inhibition at 200 mg/ml are slightly lower than those observed with the methanol extract, measuring 18 mm for *E. coli* and 17 mm for *S. aureus*. This suggests that while both extracts possess antimicrobial properties, the methanol extract may be more effective overall. The diminishing effect with lower concentrations is consistent across both extracts, reinforcing the idea that higher concentrations yield better antibacterial activity. The negative control (sterile water) showed no inhibitory effect, further validating the efficacy of both extracts.

Table 2: Zone of inhibition of antimicrobial Assay of Methanol extract of *M. oleifera*

Concentration of the extract (mg/ml)	Zone of Inhibition (mm) of the ethanol extract of *Moringa oleifera on the organisms used*	
	E. coli	*S.aureus*
200	19	18
150	15	17
100	12	15
50	11	13
25	10	11
Control (-ve)	19	20
Control (+ve)	22	21

Key: +ve = Negative control (Sterile water): -ve = Positive control (Ciprofloxacin)

Table 3: Zone of inhibition of antimicrobial Assay of Ethanol extract of *M. oleifera*

Concentration of the extract (mg/ml)	Zone of Inhibition (mm) of the ethanol extract of *Moringa oleifera*	
	E. coli	*S. aureus*
200	18	17
150	15	17
100	12	13
50	10	12
25	10	11
Control (-ve)	20	18
Control (+ve)	24	20

Key:+ve = Negative control (Sterile water); ve = Positive control (Ciprofloxacin)

Results of minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) of the Methanol leaf extract of *Moringa oleifera*

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) results in Tables 4 and 5 provide additional context regarding the effectiveness of the methanol extract. Both tables indicate an MIC and MBC of 200 mg/ml for both *E. coli* and *S. aureus*, where growth was inhibited at this concentration but not at lower levels (150 mg/ml down to 25 mg/ml). This suggests that while *Moringa oleifera* extracts can inhibit bacterial growth at high concentrations, they may not be suitable for therapeutic use at lower concentrations without further enhancement or formulation adjustments. The findings highlight the potential of *Moringa oleifera* as a natural antimicrobial agent but also emphasize the need for further research to enhance its efficacy against pathogenic bacteria in practical applications.

Table 4: Minimum Inhibitory Concentration (MIC) of methanol extracts of *Moringa oleifera*

Minimum inhibitory concentration (MIC) of the Methanol extract of *Moringa oleifera* (mg/ml)	Microorganisms	
	E. coli	*S. aureus*
200	NG	NG
150	G	G
100	G	G
50	G	G
25	G	G

Key: NG – Growth inhibited; G - Growth; MIC= 200mg/ml

Table 5: Minimum Bactericidal Concentration (MBC) of methanol extracts of *Moringa oleifera*

Minimum Bactericidal concentration (MBC) of the methanol extract of *Moringa oleifera* (mg/ml)	Microorganisms	
	E. coli	*S. aureus*
200	NG	NG
150	G	G
100	G	G
50	G	G
25	G	G

Key: G=Growth; NG= Growth inhibited; MBC = 200mg/ml

Results of minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) of the ethanol leaf extract of *Moringa oleifera*

The results presented in Tables 6 and 7 indicate that the ethanol extract of *Moringa oleifera* exhibits significant antibacterial activity against *E. coli and S. aureus*, with both the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determined to be 150 mg/ml. At this concentration, growth of both bacterial strains is completely inhibited, as indicated by the "NG" (No Growth) results. However, at lower concentrations (100 mg/ml and below), bacterial growth resumes, as evidenced by the "G" (Growth) notation, suggesting that the ethanol extract loses its efficacy at these

levels. These findings highlight the potential of *Moringa oleifera*'s ethanol extract as an effective antimicrobial agent, particularly at higher concentrations. The consistent MIC and MBC values reinforce the idea that a concentration of 150 mg/ml is necessary to achieve both inhibition and bactericidal effects against these pathogens. This suggests that while *Moringa oleifera* may serve as a natural alternative for combating bacterial infections, further research is needed to explore its practical applications in therapeutic contexts, particularly at concentrations that are safe and feasible for use in clinical settings.

Table 6: Minimum Inhibitory Concentration (MIC) of ethanol extracts of *Moringa oleifera*

Minimum inhibitory concentration (MIC) of the ethanol extract of *Moringa oleifera* (mg/ml)	Microorganisms	
	E. coli	*S.aureus*
200	NG	NG
150	NG	NG
100	G	G
50	G	G
25	G	G

Key: NG = Growth inhibited: G = Growth: MIC = 150mg/ml

Table 7: Minimum bactericidal concentration (MBC) of ethanol extracts of *Moringa oleifera*

Minimum Bactericidal concentration (MBC) of the ethanol extract of *Moringa oleifera* (mg/ml)	Microorganisms	
	E. coli	*S.aureus*
200	NG	NG
150	NG	NG
100	G	G
50	G	G
25	G	G

Key: NG =Growth inhibited; G = Growth; MBC = 150mg/ml

Discussion

The outcome of the qualitative Phytochemical composition of the Methanol leaf extract of *Moringa oleifera* indicated the presence of saponin, tannins, phenolics, glycosides, steroids, terpenoids, coumarin, flavonoids and alkaloids. The qualitative composition of *Moringa oleifera* extracted with ethanol shows that it contains the same phytochemicals as that extracted with methanol, although it lacks tannins and steroids. Phytochemicals, which are beneficial compounds found in plants, tend to be polar. Ethanol and methanol are both polar solvents, they are both good at dissolving these phytochemicals. As a result, the types and amounts of phytochemicals extracted from plants using ethanol will be very similar to those extracted using methanol which is related to the report given by Bitwell *et al*. (2023).

Some renowned authors have posited that the extract of *Moringa oleifera* are fully packed with phytochemical compounds of phenol, flavonoids, steroids, β-carotenes, vitamin A, C, D, etc (Metsopkeng *et al*., 2019; 2020). The results of the ethanol extract devoid of steroids is in tandem with the earlier reports of Idris and Abubakar, (2016) and the argument put forward was that the functional group in the phytoconstituents/phytochemicals may have been hydrolyzed by acid, which could have depended on the steroid structure and

conjugation site hence the absence of steroids.

The result of the quantitative phytochemical constituent of both the methanol and ethanol extract of *Moringa oleifera* leaves presented in Fig 1 and 2 is the quantitative result of the Methanol extract and ethanol extract respectively. This findings agrees with the research of Royani *et al*. (2023) where different composition of the phytochemicals were reported in *Moringa oleifera* extract.

It is therefore evident from Table 1, Figures 1 and 2 that *Moringa oleifera* leaves extract of both methanol as well as ethanol origin are fully packed with phytochemical compounds which tend to reduce oxidative stress and perhaps stop or slow down oxidation (Chaudhary *et al*., 2023; Jomova *et al*., 2023). This result corroborate with the findings of Royani *et al*. (2022).in a research where *Moringa oleifera* leaf extract was analysed for antimicrobial agent.

Antibacterial activities of the Methanol leaf extract of *Moringa oleifera*

Table 3 and 4 shows the minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) of methanol extracts of *Moringa oleifera* on the activities of *Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S. aureus*). The minimum bactericidal concentration also known as MBC is not to be confused with the minimum inhibitory concentration (MIC). This is because the lowest concentration at which an antimicrobial drug can prevent observable bacterial growth is called MIC while the minimum concentration required to destroy the bacteria is indicated by the MBC. It is worthy of note therefore to say that the MIC represents the lowest concentration required to stop the bacterium from growing. For this research, the MIC and MBC against the bacteria were 200mg/ml for the methanol extract of *Moringa oleifera* leaves (Table 3 and 4). These results indicated that both extracts of *M. oleifera* exhibited high antibacterial activities against *E. coli* and *S. aureus*, as shown in Table 2 and 3. This result agrees with the findings of Obrohet *et al*., 2021 where the efficacy of *Moringa oleifera* leaf extract was reported on some strains of Gram-positive and Gram-negative bacteria.

Furthermore, the zone of inhibition reported in this research is quite large. For example in 200mg/ml of the methanol extract 19mm was recorded for *E. coli*, 18mm for *S.aureus*, and for 25mg/ml, 10mm was recorded for *E.coli* and 11mm for *S. aureus* (Table 2). Similar trends was seen with the ethanol extract of *Moringa oleifera* (Table 3). This result contradicts that of Obrohet *et al*. 2021 in comparison to the zone of inhibition because smaller zone of inhibition was reported in their research. The reason for difference in results could be because of the synergistic use of *Moringa oleifera* leaf extract with the extract of *Gongronema latifolium.*

The ability of *Moringa oleifera* to impede the growth of the two strains of bacteria examined in this research could be associated with the presence of valuable antioxidant such flavonoids (Table 1) which has been reported to impede the growth of several other bacteria strains (Al-Khalasi *et al*., 2021; Ribeiro *et al*., 2022).

In addition, one important phytochemical confirmed to be present in the leaf extract of *M. oleifera* is phenols. Meanwhile some notable authors have submitted that phenol is involved in cell membrane activities responsible for the release of functional enzymes, which could command the Deoxyriblonucleic acid of the cell to either impede or enhance the growth of microorganisms (Silva *et al*., 2020; Ribeiro *et al*., 2022).

Table 5 shows the minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) of ethanol extracts of *Moringa oleifera* on the activities of It can be deduced from the table that with 150 and 200mg/ml of the ethanol extract of *M. oleifera* there was no growth in the two organisms incubated while with the application of 100, 50, 25 mg/ml of the extract there were bacterial growth observed. This indicates that the MIC and MCB for ethanol extracts of

Moringa oleifera was 150 mg/ml (Table 5). The growth of the two strains of bacterial examined revealed that there was higher activity in the zone of inhibition. It becomes imperative therefore to say that low concentration of the extract encourages the growth of both bacteria but reverse was the case at high dosage for both extracts. This result agrees with the result documented by Metsopkeng *et al*. (2022) where it was concluded that the leaf of *Moringa oleifera* is bacteriostatic in nature. The term "bacteriostatic" describes a substance that stops bacteria from growing, or maintains them in the stationary phase of growth. Traditional remedies made from *Moringa oleifera* may be exploited to create more affordable, safe, and effective medicinal agents according to Naeem *et al*. (2022).

The mode of action of *M. olefeira* generally target important cellular functions like transcription, replication, protein synthesis, and cell wall formation as the case in this research (Smith *et al*., 2020). Mostafa *et al*. (2018) further posited that terpenoids, alkaloids, and phenolic compounds could alter the functionality of the cellular membrane of the microbes which could cause a flux of protons to scatter towards the outside of the cell thereby interfering with the synthesis of amino acid needed for growth by the microbes and thus cell aptosis. Additional research by Adeyemi *et al*. (2021) and Unuigbe *et al*. (2014) demonstrated the application of *Moringa oleifera*'s various parts to have strong in vivo and in vitro antioxidant activity.

Conclusion

Conclusively, methanol extracted tannins and steroids which was not extracted by ethanol and this is one of the few researches to report this on *Moringa oleifera*. Furthermore, this study found a wide zone of inhibition of the microorganisms (*Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S. aureus*) in contrast to prior studies that found a narrow zone of inhibition.

References

Abdurrashid, H. and Sharhabil, M.Y. (2021). Recent advances in the chemistry of bioactive compounds from plants and soil microbes. *Chemistry Africa,* 4(2):231-248

Adekanmi A.A., Adekanmi S.A. and Adekan O.S. (2020). Qualitative and Quantitative Phytochemical Constituents of Moringa Leaf. *International Journal of Engineering and Information Systems* (IJEAIS), 4: 10-17.

Adeyemi, S., Larayetan, R., Onoja, A.D., Ajayi, A., Yahaya, A., Ogunmola, O.O, Adeyi, A.O, and Chijioke, O. (2021). Anti-hemorrhagic activity of ethanol extracts of *Moringa oleifera* leaf on envenomed albino rats. *Sci African*, 12:1–10.

Ajayi, A.O, Fadeyi T.E. (2015). Antimicrobial activities and phytochemical analysis of *Moringa oleifera* leaves on *Staphylococcus aureus* and *Streptococcus* species. *American Journal of Phytomedical Clinical Therapy*, 3(10):643–653

Al-Khalasi, S., Al-Ghafri, A., Al-saqri, S., and Al-Khatri, M (2023). Antibacterial activity of *Moringa oleifera* extracts in comparison with ciprofloxacin antibiotic against *staphylococcus aureus. European Journal of Theoretical and Applied Sciences,* 1: (5) 85

Blair, J., Webber, M., Baylay, A., Ogbolu, D. and Piddock, L. (2015). Molecular mechanisms of antibiotic resistance *Nat. Rev. Microbiol*., 13: 42-51

Bitwell, C., Indra, S.S., Luke, C., Kakoma, M.S. (2023). Areview of modern and conventional extraction techniques and their applications for extracting phytochemicals from plant. *Scientific African*, 19 (01585)

Chaudhary, P., Janmeda, P., Docea, A.O., Yeskaliyeva, B., Abdull Razis, A.F., Modu, B., Calina, D. and Sharifi-Rad, J. (2023). Oxidative stress, free radicals and antioxidants; potential crosstalk in the pathophysiology of human diseases,

Sec. Medicinal and Pharmaceutical Chemistry, 11 (1158198).

Campbell, W.R., Li, P., Whitman, T.J., Blyt h D.M., Schnaubelt, E.R. and Mende, K. (2017). Multi-Drug-Resistant Gram-Negative Infections in Deployment-Related Trauma Patients. *Surg Infect*. 18:357–67

Clinical and Laboratory Standards Institute (CLSI). (2012). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard, 9th ed., CLSI document M07-A9. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA

Clinical and Laboratory Standards Institute (CLSI). (2019). M100 Performance Standards for Antimicrobial Susceptibility Testing, 29th ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA.

Debelo, H., Li, M. and Ferruzzi, M.G. (2020). Processing influences on food polyphenol profiles and biological activity. *Current Opinion on Food Science*, 32: 90–102.

Dzotam, J.K., Touani, F.K. and Kuete, V. (2015): Antibacterial and antibiotic-modifying activities of three food plants (*Xanthosoma mafaffa* Lam., *Moringa oleifera* (L.) Schott and *Passiflora edulis* Sims) against multidrug-resistant (MDR) Gram-negative bacteria. *BMC Complementary and Alternative Medicine*, 16: 9.

Edeoga, HO., Okwu, D.E., and Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian Medicinal plants. *African Journal of Biotechnology*, 4: 685-688.

Eucast, (2003). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. *Clinical Microbiolological Infection*, 6(9):509-515.

Fahal, E., Majeedkutty, B.R.A., Aklakur, m.d, Chanu, T.I. (2018): Qualitative and Quantitative phytochemical analysis of *Moringa oleifera* (Lam) pods. *International Journal of Current Microbiology and Applied* Sciences, 7(05):657-665

Hotez, F. (2019): DR Congo and Nigeria: New neglected tropical disease threats and solutions for the bottom 40%. *PLos Negl Trop Dis*, 13 (8): 0007145.

Idris, A. and Abubakar S. (2016): Phytochemical and antibacterial investigations of moringa (*Moringa oleifera*) leaf extract on selected bacterial pathogens. *Journal of Microbiology and Antimicrobials*, 8(5): 28-33.

Ilanko, P., McDonnell, P. A. and Vuuren, S. V. (2019). "Interactive antibacterial profile of

Moringa oleifera Lam. extracts and conventional antibiotics against bacterial triggers of some autoimmune inflammatory diseases," *South African Journal of Botany*, 124:420–435.

Jomova, K., Renata, R., Suliman, Y. A., Saleh, H.A., Eugenie, N., Kamil, K., and Marian, V. (2023). Reactive oxygen species, toxicity, oxidative stress and antioxidants; chronic diseases and aging, *Archives of Technology,* 97: 2499-2574.

Royani, A., Hanafi, M. and Manaf, A. (2022). "Prospect of plant extracts as eco-friendly biocides for microbiologically influenced corrosion: a review," *International Journal of Corrosion and Scale Inhibition*, 11(3):862–888.

Royani, A., Hanafi, H., Lotulng, P.D., Julistiono, H. (2023): Analysis of the Antibacterial Activity and the Total Phenolic and Flavonoid Contents of the *Moringa oleifera* Leaf Extract as an Antimicrobial Agent against *Pseudomonas aeruginosa*. *Scientifica*, 5782063:1-13.

Metsopkeng, C. S., Lontsi Djimeli, C., Noah Ewoti, O. V., Moungang, L. M., Nana, P. A., Tamsa A. A., Ngo B. P., Bricheux, G., Sime-Ngando, T., and Nola, M. (2019). *Moringa oleifera* seeds extract activity on Enteropathogenic *Escherichia coli* and *Aeromonas hydrophila* cells in aquatic microcosm. *Journal of Applied Biotechnology*, 7(2), 13-30.

Metsopkeng, C. S., Nougang, M. E., Nana, P. A., Tamsa Arfao, A., Ngo Bahebeck, P., Lontsi Djimeli, C., Eheth, J. S., Noah Ewoti, O. V., Moungang, L. M., Agbor, G. A., Perrière, F., Sime-Ngando, T., and Nola, M. (2020). Comparative study of *Moringa stenopetala* root and leaf extracts against the bacteria *Staphyloccocus aureus* strain from aquatic environment. *Scientific African*, 10, e00549.

Misra, R.C., Thimmappa, R., and Bonfill, M. (2024).Editorial: Advances in discoveries of plant phytochemicals, *Front. Plant Sci*., Sec. *Plant Metabolism and Chemodiversity*, 15 (1414150)

Mikore, D. and Mulugeta E. (2017). Determination of proximate and mineral compositions of *Moringa oleifera* and *Moringa stenopetala* leaves cultivated in Arbaminch Zuria and Konso. *Ethiopia Afr J Biotechnol* 16(15):808–818.

Mostafa, A.A, Al-Askar, A.A., Almaary K.S., Dawoud, T.M., Sholkamy E.N. and Bakri, M.M. (2018) Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi J Biol Sci*. 25(2): 361-6

Naghavi, M., Bielicki, J., Shionogi, S., Carvalheiro, C., Scott,J., Walsh, T., Ashley, E., Krapp, F., Khorana, M., Boonkasidecha, S., Peleg, A., *et al.* (2019). Global burden of bacterial antimicrobial resistance in: a systematic analysis. *Lancet*; 399: 629–55.

Naeem, U.B, Iftikar, W., Fafiq, M., and Muhammad B. K. (2022). Antibacterial and cytotoxic evaluation of sequential extract of *Moringa oleifera* leaves. *Article / Investigación*, 7(1):15

Obroh, A.A., Oshim, I.O., Odeyemi, O., Urama, E.U and Olise, N.A. (2021). Demonstration of the minimum inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC) of both *Moringa oleifera* and *Gongronema latifolium* extract mixture against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*. *Journal of advances Medical and Pharmaceutical Sciences*, 23(1): 46-52.

Ribeiro, J., Silva, V., Aires, A., Carvalho, R., Barros, L., Gaivao, I., Igrejas, G., & Poeta, P. (2022). *Plantanus hybrida's* phenolic profile, antioxidant power, and antibacterial activity against methicillin-resistant Staphylococcus aureus (MRSA). *Horticulturae*, 8: 243.

Silva, V., Falco, V., Dias, M. I., Barros, L., Silva, A., Capita, R., Allonso-calleja, C., Amaral, J. S., Igrejas, G., Ferreira, I. C. F. R., & Poeta, P. (2020). Evaluation of the phenolic profile of *Castanea sativa* M/ill. By-products and their antioxidant and antimicrobial activity against multiresistant bacteria. *Antioxidants,* 9(1): 87.

Smith, B.E., Orders, T., Slate, J., Bauldry, S., Emrani, J. I. J., Ahmed, M., Cecile, J., Bailey, C.S., Nguyen, N.A. and Mowa, C. (2020): Proteomics analysis reveal that *Moringa oleifera* kills *Escherichia coli* by altering multiple biological processes. *South African Journal of Botany*, 129: 366-378.

Shin, S.A., Joo, B.J., Lee, J.S., Ryu, G., Han, M., Kim, W.Y., Park, H.H., Lee, J.H., and Lee, C.S (2020). Phytochemicals as anti-inflammatory agents in animal models of prevalent inflammatory diseases. *Molecules* 25(24):5932.

Suleiman, A. and Salihu, M.M. (2023): Phytochemical Analysis and Antimicrobial Activities of Different *Moringa oleifera* Extract. Available at SSRN: https://ssrn.com/abstract=4578519 or http://dx.doi.org/10.2139/ssrn.4578519

Unuigbe, C.A., Okeri, H.A., Erharuyi, O., Oghenero, E.E., Obamedo, D.A (2014): Phytochemical and antioxidant evaluation of *Moringa oleifera* (Moringaceae) leaf and seed. *J Pharmacol Bioresour* 11(2):51–57

WHO (2019). Antimicrobial Resistance Collaborators. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*; 399(10325): P629-655.