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JIO and PTA conceived and designed the study. JMO and YME did literature review. All the authors were involved in the write-up, laboratory experiments, and statistical analysis; JIO and MNI revised the paper.

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Anti-bacterial Activity of *Moringa oleifera* Seeds against Selected Bacterial Pathogens

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Abstract:

Moringa oleifera seeds were collected and analysed for phytochemicals using ethanol and aqueous solvents in order to determine the antibacterial efficacy of *M. oleifera* seeds against certain bacterial infections. The agar well diffusion method was used to further investigate the antibacterial activity of two extracts (ethanol and aqueous) on a selection of test organisms at various concentrations. Additionally, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal (MBC) of the extracts were determined. The presence of alkaloids, tannins, flavonoids, saponin, cardiac glycoside, and phenol was confirmed by phytochemical examination. The ethanolic extract exhibited higher antibacterial activity in comparison to the aqueous extract. Our results revealed a significant difference ($p<0.05$) in the zones of inhibition at varying doses between the ethanol and the aqueous extract of the seed. *Escherichia coli* showed the highest zone of inhibition (21.50±7.07 mm), followed by *Staphylococcus aureus* (20.00±0.01 mm) while *Pseudomonas aeruginosa* (16.00±0.00 mm) showed the lowest zone of inhibition against different concentrations of the ethanol seed extract. *S. aureus* showed the highest zone of inhibition (24.00±1.41 mm) followed by *P. aeruginosa* (22.50±0.00 mm) and *E. coli* showed the lowest zone of inhibition (19.50±8.07 mm) against different concentrations of the aqueous seed extract. The minimum inhibitory concentration (MIC) for all test organisms using ethanol extract was 50% while for aqueous extract both *E. coli* and *S. aureus* showed 40%, and *P. aeruginosa* 25%. In the ethanol extract, the MBC values for *E. coli* were 30%, while *S. aureus* and *P. aeruginosa* showed 25%. However, in the aqueous extract, the MBC values for *S. aureus* were 30%, *E. coli* were 25%, and there was no discernible MBC for *P. aeruginosa*. Antimicrobial activity against the test species were demonstrated by the ethanolic and aqueous extracts at varying doses. It is feasible to use the extracts, especially the ethanol extract, as an antibacterial agent to treat infectious pathogenic disorders since it had the greatest impact of all the extracts. Drugs for the treatment of many illnesses and disorders can be made using *M. oleifera* in the pharmaceutical industry.

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INTRODUCTION

The need for novel antimicrobial drugs is intimately related to the issue of strains becoming resistant to the majority of synthetic antibiotics emerging (Edrees and Anbar, 2021; Iqbal and Ashraf, 2018a; Iqbal and Ashraf, 2020a; Shawish *et al.*, 2020). Bacterial infectious illnesses have emerged as the leading cause of mortality globally, accounting for around 50% of deaths in tropical nations (Asowata *et al.*, 2013). Because of the widespread use of antibiotics, most antimicrobial medicines on the market today are ineffective at treating some bacterial illnesses (Viera *et al.*, 2010). It appears that bacteria have changed to the point where some antibacterial medications can no longer treat bacterial illnesses and have negative side effects in humans (Iqbal and Iqbal, 2020; Iqbal and Ashraf, 2020b; Ishiwu *et al.*, 2014). There is growing evidence that suggests using medicinal herbs as a substitute for conventional treatments for mild instances of infectious illnesses may be appropriate (Iqbal and Ashraf, 2019; Iqbal and Ashraf, 2021). They could also be a source of newly developed, reasonably priced antibiotics that pathogenic strains cannot resist. Plants are often used to treat infectious disorders, and this practice has scientific validation according to several studies (Kitula, 2007). Medicinal plants are the best source of a wide range of medications (Ashraf *et al.*, 2020a; Bello and Jamiu, 2017; Iqbal and Ashraf, 2018b; Shahzad *et al.*, 2017). Therefore, these plants have been studied for a better understanding of their medicinal properties (Al-Mahweety, 2016).

Plants can be used medicinally by administering their roots, barks, stems, leaves, and seeds, as well as by using their extracts and decoctions (Ashraf and Iqbal, 2022; Ogbulie *et al.*, 2007; Shahzad *et al.*, 2017). Phenolic compounds, nitrogen compounds, vitamins, terpenoids, and endogenous metabolites produced by plants act as defense mechanisms for plants against herbivores, bacteria, and insects (Boncan *et al.*, 2020; Takshak and Agrawal, 2019; War *et al.*, 2012). Since harmful bacteria are becoming more resistant to common antibiotics, attention must be paid to alternate antibiotic sources (Ashraf *et al.*, 2020b; Bello and Echevarría, 2022; Saleem *et al.*, 2020). Herbal medicine can serve as a substitute for some prescription medications (Ashraf and Iqbal, 2022; Asowata *et al.*, 2013; Farooq *et al.*, 2022).

There are several known culinary and healthcare benefits associated with *Moringa oleifera* (Ashraf and Iqbal, 2021; Ibiene *et al.*, 2021; Osarugue *et al.*, 2020). *M. oleifera* seeds are considered to be antipyretic, acrid, and bitter (Oliveira *et al.*, 1999) and reported to have some antimicrobial activity. *M. oleifera* possesses antibacterial and antifungal, anti-helminthic, antiviral, antifibrotic, anti-inflammatory, anti-hyperglycemic, anti-tumor, anti-cancer, anti-clastogenic, analgesic, antipyretic, antihypertensive and anti-fertility properties (Farooq *et al.*, 2012). The problem of rising antibiotic resistance, together with the variety of adverse effects resulting from current medicines and the rise of illnesses for which there is now no cure, makes the hunt for novel antimicrobial drugs a very pertinent and significant area of study. The purpose of this study was to evaluate the antibacterial activity of *M. oleifera* seed extracts against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*.

MATERIALS AND METHODS

Sample collection

Already characterized and identified pure clinical isolates of *S. aureus*, *E. coli* and *P. aeruginosa* was obtained from the Department of Microbiology, Jospeh Sarwuan Tarka University Makurdi, Benue State, Nigeria. The isolates were kept at 4°C on nutrient agar slants until they were required for use. Fresh sample of *Moringa oleifera* seeds was collected and identified at the Department of Botany.

Plant sample preparation

The seeds were sorted, cleaned, and allowed to air dry in the laboratory for two weeks at room

temperature. The dried seeds were ground into powder using mortar and pestle which was then filtered through a sieve. After that, the powder was kept for later extraction in an airtight container.

Preparation of crude extract

The seeds were dried under shade for 7 days and pulverized using a clean sterile electric blender into powdered form. About 40g of the powdered seed were soaked in 300ml of each solvents namely, ethanol and water. Each conical flask were covered with cotton wool wrapped with aluminum foil and were left in a rotary shaker for two days. The seed extract was filtered via Whattman No. 1 sterile filter paper. The filtrates were dried by evaporating them in a water bath set at 70°C for two days. A sterile spatula was used to scrape the extract into a sterile sample vial (Stohs and Hartman, 2015).

Phytochemical screening of the extracts

All the phytochemicals were screened using standard methods as described previously (Al-Hakami *et al.*, 2022; Mubarak *et al.*, 2021). After mixing 2 ml of distilled water with 2.0 ml of aqueous extract, a few drops of $FeCl_3$ solution were added. The presence of tannins was demonstrated by the production of a green precipitate (Auwal *et al.*, 2014; Okafor, 1999). In a test tube, 5 ml of aqueous extract and 5 ml of distilled water were shaken vigorously before being heated. The presence of saponins was demonstrated by the production of stable foam (Auwal *et al.*, 2014). 1 ml of 10% lead acetate solution was combined with 1 ml of aqueous extract. The presence of flavonoids was shown by the production of a yellow precipitate (Mohamed *et al.*, 2023). A steam bath was used to mix 3 ml of 1% HCl with 3 ml of aqueous extract. Reagents from Mayer and Wagner were added to the mixture. The presence of alkaloids was indicated by the precipitate's turbidity (Etangetuk and Idung, 2023). Each extract was diluted in 2.0 ml of chloroform, to which 2 ml of sulphuric acid were added and gently agitated. The existence of a steroidal ring was indicated by a reddish-brown color (George *et al.*, 2010).

Reconstitution of extracts

Two (2) g of each of the two extracts was dissolved discretely in l0mls of distilled water to give concentration of 200mg/ml, lower concentrations was obtained by diluting the extracts to obtain concentrations of l00mg/ml, 50mg/ml and 25mg/ml. The tubes with the different concentrations were labeled and used promptly (Joe *et al.*, 2009).

Antimicrobial activity of plant extracts

The disc diffusion approach, which was previously revealed, was used to accomplish the antimicrobial activity of plant extracts. Sterilized Petri plates were filled with Mueller Hinton agar medium, which was then let to harden. On the solidified agar, 10 ml of the 0.5 McFarland standards of test organisms were added and evenly distributed throughout the surface. Using sterile forceps, the soaked discs were removed and placed onto the agar's surface. The plates were incubated at 37°C for duration of 24 hours. A meter rule was used to measure the clearance zone (Iqbal *et al.*, 2016; Iqbal *et al.*, 2015; Saleem *et al.*, 2018a; Saleem *et al.*, 2018b). Discs soaked in ethanol and water, respectively, without any plant extract were used as controls (Joe *et al.*, 2009).

Determination of MIC of plant extracts

The minimum inhibitory concentration of the plant extracts was accessed using Broth dilution technique (Mogana *et al.*, 2020). In separate test tubes, one millilitre (1.0) of the diluted extracts at concentrations of 250 mg/ml, 200 mg/ml, 150 mg/ml, 50 mg/ml, and 25 mg/ml was combined with 9 ml of Mueller Hinton broth. After properly mixing the contents, 0.1 ml of the test isolates' 0.5 McFarland standards were added to the tubes for inoculation. After a day of being incubated at 37 °C, the tubes were checked for turbidity. In each instance, the minimum inhibitory concentration (MIC) was determined by determining the lowest concentration of the extract that prevented the inoculated test isolates from exhibiting turbidity in the broth medium. As controls, test tubes that had been

injected with the test isolates but not the extracts were used (Sah *et al.*, 2020).

Determination of MBC of the extracts

Mueller-Hinton agar plates were inoculated with extract concentrations that did not exhibit noticeable growth after 24 hours of incubation at 37°C. The minimal bactericidal concentration was determined by taking the lowest concentration of the extract that, after 24 hours, did not cause any growth on the plate (Joe *et al.*, 2009; Loo *et al.*, 2018).

Statistical analysis

The mean and standard deviation of the data were determined using a statistical package service solution (SPSS) version 21. ANOVA was used to test for significant differences in the parameter at $p<0.05$.

RESULTS

Results for the phytochemical composition of *Moringa oleifera* seed extracts are presented in Table 1. The presence of alkaloids, tannins, flavonoids, saponin, cardiac glycoside, and phenol was confirmed in both aqueous and ethanolic seed extracts.

Table 1. Phytochemical screening of the aqueous and ethanol seed extract of *M. oleifera*.

Phytochemical constituent	Aqueous extract	Ethanolic extract
Alkaloid	+	+
Flavonoids	+	+
Phenols	+	+
Saponins	+	+
Cardiac glycoside	+	+
Tannins	+	+

Key: Present (+)

The data regarding zones of inhibition in (mm) of the ethanol seed extract of *M. oleifera* is shown in Table 2. *E. coli* showed the highest zone of inhibition (21.50±7.07 mm) across the different concentrations, followed by *S. aureus* (20.00±0.01 mm) while *P. aeruginosa* (16.00±0.00 mm) showed the lowest zone of inhibition against different concentrations of the ethanol seed extract.

The data regarding zones of inhibition in (mm) of the aqueous seed extract of *M. oleifera* is shown in Table 3. *S. aureus* showed the highest zone of inhibition (24.00±1.41 mm) followed by *P. aeruginosa* (22.50±0.00 mm) and *E. coli* showed the lowest zone of inhibition (19.50±8.07 mm) against different concentrations of the aqueous seed extract.

The minimum inhibitory concentration (MIC) for all test organisms using ethanol seed extract of *M. oleifera* was 50% while for aqueous extract both *E. coli* and *S. aureus* showed 40%, and *P. aeruginosa* 25% (Table 4).

In the ethanol seed extract of *M. oleifera*, the Minimum Bactericidal Concentration (MBC) values for *E. coli* were 30%, while *S. aureus* and *P. aeruginosa* showed 25%. However, in the aqueous extract, the MBC values for *S. aureus* were 30%, *E. coli* were 25%, and there was no discernible MBC for *P. aeruginosa* (Table 5).

Table 2. Zones of inhibition (mm) of the ethanol extract of *M. oleifera* seeds on selected test organisms.

Test organisms	Zones of inhibition (mm) at different concentrations (mg/ml) of extracts					
	250 (mg/ml)	200 (mg/ml)	150 (mg/ml)	50 (mg/ml)	25 (mg/ml)	Control (Fluconazole)
E. coli	21.50±7.07	19.50±3.54	19.00±1.414	16.00±0.00	6.50±9.12	23.00±8.9
S. aureus	20.00±0.01	18.50±7.074	17.00±1.42	15.00±1.41	-	-
P. aeruginosa	16.00±0.00	14.50±7.07	14±0.00	-	-	-

Data are expressed as mean plus standard deviation of replicate plates (P-value < 0.05).

Table 3. Zone of inhibition (mm) of the aqueous seed extract of M. oleifera on selected test organisms.

Test organisms	Zones of Inhibition (mm) at different concentrations (mg/ml) of extracts					
	250 (mg/ml)	200 (mg/ml)	150 (mg/ml)	50 (mg/ml)	25 (mg/ml)	Control (Fluconazole)
E. coli	19.50±8.07	14.50±1.87	10.00±2.12	6.00±1.41	-	20.50±17.00
S. aureus	24.00±1.41	22.50±7.07	19.00±1.41	18.00±1.41	-	31,00±20.50
P. aeruginosa	22.50±0.00	19.50±2.12	17.50±7.07	-	0.00	-

Data are expressed as mean plus or plus standard deviation of replicate plates (P-value < 0.05).

Table 4. Minimum inhibitory concentrations (MIC) of ethanol and aqueous seed extracts of *M. oleifera* on selected test organisms.

Test organisms	Minimum inhibitory concentrations (MIC)	
	Ethanol	Aqueous
	MIC	MIC
E. coli	50	40
S. aureus	50	40
P. aeruginosa	50	25

Table 5. Minimum bactericidal concentration (MBC) of the ethanol and aqueous seed extracts of *M. oleifera* on the selected test organisms.

Test organisms	Minimum bactericidal concentration (MBC) (%age)	
	Ethanol	Aqueous
	MBC	MBC
E. coli	30	25
S. aureus	25	30
P. aeruginosa	25	-

DISCUSSION

This study evaluated the antibacterial activity of ethanol and aqueous seed extracts of *Moringa oleifera* on some selected bacteria. The extracts showed antibacterial effects on the test organisms at different concentration, this effect is due to the different phytochemical constituents of the plants, because the phytochemicals have certain chemicals that inhibit the growth of certain bacteria.

The phytochemical components of the aqueous and ethanol seed extracts of the plants comprised alkaloid, steroid, phenols, saponins, tannin, and flavonoid. This result agrees with the result of a previous study that reported the same phytochemicals in Moringa (Nouman *et al.*, 2014).

Phytochemicals can be employed as agents for chemoprevention, which is the use of substances to stop, reverse, or delay

tumorigensis, or as chemotherapeutic drugs. The documented use of alkaloids for medical purposes dates back about 5000 years. Alkaloids have anti-malarial, anti-cancer, anti-asthma, and antibacterial pharmacological constituents for humans (Ogbulie *et al.*, 2007). The antibacterial effects of plant extract on bacterial strains can take several forms, including destroying or inactivating genetic material and damaging enzymes responsible for cellular energy generation and structural component creation. According to the ANOVA, the ethanol and aqueous extracts at various concentrations showed a significant difference in the zones of inhibition with a $P<0.005$.

It was observed that ethanol extracts have the same Minimum Inhibitory Concentration (MIC) of 50% for each test organism while MIC for aqueous extracts was 40% for *E. coli* and both *S. aureus* and *P. aeruginosa* showed 25%.

From the findings of this study, it agrees with the previous work that reported some antimicrobial activity of *M. oleifera* seed on *E. coli, P. aeruginosa* and *S. aureus* (Farooq *et al.*, 2012; Oliveira *et al.*, 1999; Osarugue *et al.*, 2020). The work disagreed with another study that evaluated the antimicrobial activity of *M. oleifera* seed extracts against four gram-positive bacteria and two gram-negative bacteria (Pal *et al.*, 1995).

CONCLUSION

The phytochemicals included in *M. oleifera* seed extracts, including tannin, saponin, alkaloids, steroids, phenol, and flavonoids, provide the plant with its therapeutic qualities. At varying doses, the aqueous and ethanolic extracts demonstrated antibacterial activity against the test pathogens. Since the ethanol extract had the greatest impact among the extracts, it may be used as an antibacterial agent to treat infectious pathogenic disorders. *M. oleifera* can also be used in the pharmaceutical industry to make medications that are used to treat a range of illnesses. The extracts can be used as an alternative to the Fluconazole antibiotics for treatment.

RECOMMENDATION

- It is advised that the ethanol extract be used to treat infectious disorders as it exhibited the strongest antibacterial activity across all test species at various doses.
- Further studies should be carried out to determine the minimal concentration or the acceptable dosage in which *M. oleifera* can be used for chemotherapeutic purposes.
- As ethanol and aqueous extracts of the *M. oleifera* have been shown to have antibacterial properties, it can be rationally recommended that further work needs to be carried out to identify and investigate the relationship between the phytochemical constituents and the efficacy of the plant extracts.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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