

Phytochemical Analysis and Antibacterial Activity of *Moringa oleifera* Leaves Extracts against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*

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Authors' contributions

This work was carried out in collaboration among all authors. Author SBI carry out the Bioassay, some preliminary data analysis, and wrote the first draft of the manuscript. Author MM make the final proof reading for corrections and observe the appropriate set standards and protocols. Finally, author IR managed the data analyses of the study, carryout an extensive literature search and review, observed a strict adherence to relevant protocols, proof read the manuscript and make insertions where applicable, and format the manuscript according to the journal set guidelines. All authors read and approved the final manuscript.

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ABSTRACT

This study tests the antibacterial activities of *Moringa oleifera* leaf extract against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, obtained from Microbiology laboratory, Al-Hikmah University Ilorin. Phytochemical analysis reveals the presence of tannins in all the three extracts (Aqueous, ethanolic and N-hexane), while flavonoids, alkaloids and Saponins were only present in the Aqueous and ethanolic extracts. The leaves extracts were screened for antibacterial

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activity by agar well diffusion method, employing five different extracts concentrations (100mg/ml, 80mg/ml, 60mg/ml, 40mg/ml and 20mg/ml). The aqueous extracts had a mean activity of 11.50±0.70mm, 7.50±0.70mm and 8.5±0.70mm for *S. aureus*, *E. coli* and *P. aeruginosa* respectively. The ethanol extracts had a mean activity of 12.00±1.41mm, 10.00±1.41mm and 8.00±1.21mm for *S. aureus*, *E. coli* and *P. aeruginosa* respectively, while the N-hexane extracts exert no any activity. The antibiotics discs exert more inhibitory effect compared to the extract. The MIC for the aqueous extracts are at a concentrations of 60mg/ml for *S. aureus*, 80mg/ml for *P. aeruginosa* and 80mg/ml for *E. coli*. While that of the ethanol extract of the leaves are at a concentration of 60mg/ml for *S. aureus*, 80mg/ml for *E. coli* and 100mg/ml for *P. aeruginosa*. The MBC for the aqueous Extract of the leaves are at a concentration of 80mg/ml for *S. aureus*, 100mg/ml for *P. aeruginosa* and 100mg/ml for *E. coli*, while that of the ethanol extract are at a concentration of 80mg/ml for *S. aureus* and 100mg/ml for *E. coli*, while no any MBC was recorded for *P. aeruginosa*. The antibacterial activity tests indicate that the ethanol extract was more active. Among the three isolates, *S. aureus* is more sensitive to the aqueous and ethanol extracts. The activity exhibited by the extracts may be related to the presence of a number of Phytoconstituents.

Keywords: *Moringa oleifera*; antibacterial; *Ilorin s*; *aureus e*; *coli* and *p*; *aeruginosa*.

1. INTRODUCTION

Nigeria has a wide range of plant diversity, with great potential of identifying plants of pharmacological relevance. Herbal preparations represent one of the important traditional medicine therapies and it is still the mainstay of about 80% of the world populations, mainly in the developing countries for primary health care. It has been estimated that 25% of the modern medicines are made from plants first used traditionally. Among several factors contributing towards the potential use of phyto-medicine are safety, lack of adverse reactions and side effects which have been mostly found to particularly influence the use of such medicines in developed countries. In rural areas there are additional cultural factors that encourage the use of herbal preparations, people believe that where an area give rise to a particular disease it will also support plants that can be used to cure it [1,2].

The plant *Moringa oleifera* was reported to possess various biological activities and have been an important source of natural products for human health [1]. *Moringa oleifera* is a source of food, shelter and traditional medicine for many people in developing countries. It is an exceptional source of vitamins, minerals, amino acids, lipids, carotenoids, flavonoids, sterols and phenolics. Owing to its diverse nutritional and phytochemical composition, it is widely consumed and used in therapeutics [3]. Many of the existing synthetic drugs are known to cause various side effects, such as intoxication, nausea, and other allergies [2]. By implication, herbal medicine is now forming an alternative therapy that has become the mainstream

throughout the world due to the growing resistance of pathogens to conventional antibiotics [4].

Moringa plant provides a rich source of zeatin, quercetin, kaempferol and many other phytochemicals [5]. The leaves are outstanding as source of vitamins like beta-carotene of Vitamin A, vitamin B such as folic acid, pyridoxine and nicotinic acid, vitamin C, D and E [6]. The leaves also possess minerals like iron, calcium, copper, potassium, zinc and magnesium [7,8]. The *Moringa oleifera* has been used extensively in traditional medicine for the treatment of several ailments; promote digestion, skin disease, diarrhoea, as stimulant in paralytic afflictions, epilepsy and hysteria [2]. The roots, stem bark, seeds and leaves of the plant have been reported to possess some antibacterial, anti-cancer [9,10] and anti-inflammatory activities diverse biological activities, including hypocholesterolemic, antidiabetic, hypertensive agent [8].

Moringa oleifera is medium sized tree, about 10m height, found in the sub-Himalayan tract, which belongs to family Moringaceae, native to, Africa, Arabia, South Asia, South America, Himalaya region, India, Pakistan, the pacific and Caribbean Islands. *Moringa oleifera* has been naturalized in many tropic and subtropics regions worldwide, the plant is referred to number of names such as horseradish tree, drumstick tree, oil tree, miracle tree, and "Mothers best friend". [8].

The continuous search for valuable Medicinal plants, in order to combat the surge of antimicrobial resistance has proven promising. *Moringa oleifera* which is native to South Asia and now found throughout the tropics continue to plays a major role in the production of novel drugs [11].

This study aimed to investigate and update the antibacterial activities and efficacy of aqueous and ethanol extract of *Moringa oleifera* leaves acting separately on *S. aureus*, *E. coli* and *P. aeruginosa* along with their minimum inhibitory and bactericidal concentration.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

Fresh *Moringa oleifera* leaves were obtained at the biological garden, University of Ilorin, and authenticated at Herbarium Section, Department of Plant Biology, University of Ilorin where a reference voucher specimen number (UILH/001/559) was obtained. The leaves were washed under running tap water and shade dried at a room temperature. The dried leaves were ground to powder for further analysis [4].

2.2 Collection and Maintenance of Bacterial Isolates

Clinical isolates of *S. aureus*, *P. aeruginosa* and *E. coli*, were obtained from Microbiology laboratory at Al-Hikmah University Ilorin, Kwara State, and were maintained as stock cultures at 4°C in the refrigerator.

2.3 Preparation of Leaves Extracts

Three solvents were used, Two polar (Aqueous, Ethanol) and one non-polar (N-Hexane) solvent. About 70g of the powdered leaves of *Moringa oleifera* was weighed in triplicate into 3 different bottles containing 300ml of distilled water, 95% ethanol and N-hexane, for aqueous, ethanol and N-hexane extracts respectively. The solutions were shaken, covered and left on the mechanical shaker to extract at 130RPM for 48hrs. The extract was filtered and evaporated to dryness using water bath at 45°C [12].

2.4 Phytochemical Screening

Phytochemical screening was carried out for the aqueous, ethanolic and N-hexane extracts of the

leaves using the methods as described by Abdulfatai et al. and Sofowara [4,13].

2.4.1 Test for terpenes

About 0.5g of each of the aqueous, ethanol and N-hexane extracts were dissolved separately in 5ml of water and 2-3 drops of 10% of ferric chloride solution was added. Violet precipitate indicates the presence of Terpenes.

2.4.2 Test for phenols and tannins

This test was intensified by weighing 0.5grams of each of the aqueous, ethanol and N-hexane extracts were mixed separately with 2ml of 2% solution of ferric chloride ($FeCl_3$). Blue green or black colouration indicates the presence of Phenols and Tannins [11].

2.4.3 Test for flavonoids (alkali reagent test)

This test was intensified by weighing 0.5grams of each of the aqueous, ethanol and N-hexane extracts were mixed separately with 2ml of 2% solution of NaOH. The formation of an intense yellow colour which turned colourless upon the addition of few drops of dilute acid indicates the presence of flavonoids [11,14].

2.4.4 Test for alkaloids

This test was intensified by weighing 0.5grams of each of the aqueous, ethanol and N-hexane extracts separately and mixed with 1% hydrochloric acid and shaken for two minutes. The mixture was filtered and drops of Mayers reagent was added to each different mixture. Formation of a yellow cream precipitate indicates the presence of alkaloids [11,15].

2.4.5 Test for saponins

This test was intensified by weighing 0.5grams of each of the aqueous, ethanol and N-hexane extracts separately and mixed with 5ml of distilled water in a test tube and was vigorously shaken. The formation of stable foam is an indication of the presence of Saponins [11,16].

2.4.6 Test for terpenoids

This test was intensified by weighing 0.5grams of each of the aqueous, ethanol and N-hexane extracts separately and mixed with 2ml of chloroform followed by the addition of 3ml concentrated H_2SO_4 to each, to form a layer. The

formation of reddish brown colour in the interphase indicates the presence of Terpenoids [11,17].

2.5 Concentration Procedure

Different concentration of the extracts (aqueous, ethanol and N-hexane) were obtained in the following projection;

Solution A: 1g of the extract + 10ml of diluent (distilled water) = 100mg/ml.

Solution B: 4ml of solution A + 1ml of diluent i.e. $4/5 \times 100 = 80\text{mg/ml}$.

Solution C: 3ml of solution A + 2ml of diluent i.e. $3/5 \times 100 = 60\text{mg/ml}$.

Solution D: 2ml of solution A + 3ml of diluent i.e. $2/5 \times 100 = 40\text{mg/ml}$.

Solution E: 1ml of solvent + 4ml of diluent i.e. $1/5 \times 100 = 20\text{mg/ml}$.

2.6 Sterility Test of Leave Extract

A drop of each of the extracts was place on sterile Muller Hinton agar plate and incubated at 37°C for 24hours. Absence of growth on the plates confirm the sterility of the extracts [11,18].

2.7 Determination of Antibacterial Activity of *Moringa oleifera*

The aqueous, ethanolic and N-hexane extracts of leaves of *M. oleifera* were screened for antibacterial activity by agar well diffusion method. The turbidity was adjusted to 0.5% McFarland standard to give a suspension containing approximately 10^6 Cfu/ml [4,11,19].

The standardized inocula were inoculated in an already prepared Mueller Hinton agar. Using a sterile cork borer, five wells of a diameter of 5mm were bored on the agar surface, and 0.1ml of the various extracts concentration (100mg/ml, 80mg/ml, 60mg/ml, 40mg/ml and 20mg/ml) were dispensed into each well. The plates were allowed to stand for one hour for the pre-diffusion of the extracts to occur before incubating for 24 hours at 37°C. The plates were observed for the presence of zone of inhibition and measured accordingly. All tests were carried out in duplicate and their mean values were recorded [12].

2.8 Determination of Antibacterial Activity for Commercially Prepared Antibiotics

Each of the bacterial inoculum was streaked on an already prepared Mueller Hinton agar surface and the plates were left to stand for 15 minutes, after which the antibiotics discs (Ofloxacin 'OFX 5µg' and Ciprofloxacin 'CIP 5µg') were placed on the surface of the inoculated plate. The discs were pressed down firmly with the aid of sterile forceps to ensure proper contact and the plates were incubated at 37°C for 24hours [11].

This serves as the positive control. All tests were carried out in duplicate and their mean values were recorded [12].

2.9 Determination of Minimum Inhibitory Concentration (MIC)

Various extracts (Aqueous, Ethanol and N-hexane) concentrations were prepared by dilution using distilled water to obtain different concentrations of 100mg/ml, 80mg/ml, 60mg/ml, 40mg/ml and 20mg/ml. one (1) ml of each extract concentrations and that of Mueller Hinton broth was mixed, and 0.1ml of standardized inoculum (1.5×10^6 CFU/ml) was added to each of the test tubes above. The tubes were incubated at 37°C for 24 hours. Tubes containing broth and leaf extracts were used as positive control while tubes containing broth and inocula were used as negative controls. The tubes were observed after 24 hours of incubation. All tests were carried out in duplicate and their mean values were recorded [13,20].

2.10 Determination of Minimum Bactericidal Concentration (MBC)

Sterile Mueller Hinton agar plates were separately inoculated with culture from each of the MIC tubes that showed no evidence of turbidity. The plates were incubated at 37°C for 24 hours. The MBC was determined as the highest dilution that yielded no single bacterial colony on the agar surface. All tests were carried out in duplicate and their mean values was recorded [13,20].

2.11 Statistical Analysis

The tests were carried out in duplicate, and data generated were analysed using descriptive statistics [21].

Statistical values that were calculated include mean and standard deviation of SPSS software version 16. For easy and fast comprehension, graphical presentation of data was also employed where necessary, using Microsoft word Excel 2016.

3. RESULTS AND DISCUSSION

The sterility test carried out on all the different extracts (aqueous, ethanol and N-hexane), revealed the absence of any contaminant (Table 1). The antibacterial activity of all the extracts against *S. aureus*, *E. coli* and *P. aeruginosa* indicates that all the extracts except N-hexane showed significant activity against all the isolates.

Table 1. Extract sterility test

S/N	Extract Used	Result of Sterility
1.	Aqueous	-
2.	Ethanol	-
3.	N-hexane	-

Keys: (-) absence of contaminant, an indication for sterility

The phytochemical screening of the various extracts reveals the presence of various bioactive compounds (Table 2). The presence of bioactive compounds in plants is an indication of the presence of compounds which can be inhibitory against clinical isolates. The results of these findings can be compared with the work of

Farooq et al. [2] Amabye et al. [8] and Amal and Nashwa [22] with some minor variations in the presence of Tannins and Saponins.

In the aqueous extract, *S. aureus* has the highest zone of inhibition with a mean activity of 11.50 ± 0.70 mm followed by *P. aeruginosa* and *E. coli* having $(8.50 \pm 0.70$ mm) and $(7.50 \pm 0.70$ mm), all at 100mg/ml concentration respectively, as presented in (Table 3).

Values represented in the table are means of the duplicate readings and standard error of the zone of inhibition measured in millimetre as analysed using SPSS software version 16.

In the ethanolic extract, *S. aureus* has the highest inhibition with a mean activity of $(12.00 \pm 1.41$ mm), followed by *E. coli* $(10.00 \pm 1.4$ mm) and *P. aeruginosa* $(8.00 \pm 1.21$ mm) at 100g/ml (Table 4). While the N-hexane extract, exerts no any activity against all the isolates in all concentrations.

All the extract showed highest zones of inhibition at 100mg/ml concentration. The results of these findings is similar with the findings of Kiran and Tafida, [23] who reported a significant activity of the leaves extracts against *P. aeruginosa* and *E. coli*. However, a slight variation was observed when compared with the findings of Amabye and Tadesse, [8] who reported a lower inhibitory activity of the leaves extract on *E. coli*, *S. aureus* and *P. aeruginosa*.

Table 2. Phytochemical constituents of *moringa oleifera* aqueous, ethanolic and n-hexane leaf extracts

Phytoconstituents	Aqueous extracts	Ethanolic extracts	N-hexane extracts
Tarpenes	-	+	+
Phenols	-	-	-
Tannins	+	+	+
Flavonoids	+	+	-
Alkaloids	+	+	-
Saponins	+	+	-
Tarpenoids	-	-	+

Keys: (+) = Positive, (-) = Negative

Table 3. Zones of inhibition in (mm) for different concentrations of aqueous extracts of *moringa oleifera* leaves

Extracts Concentration in mg/ml		20	40	60	80	100
S/N	Test isolates					
1.	<i>E. coli</i>	5.00 ± 0.00	5.00 ± 0.00	5.50 ± 0.70	6.00 ± 0.00	7.50 ± 0.70
2.	<i>P. aeruginosa</i>	5.00 ± 0.00	5.50 ± 0.70	5.50 ± 0.70	6.50 ± 0.70	8.50 ± 0.70
3.	<i>S. aureus</i>	5.00 ± 0.00	5.50 ± 0.70	6.00 ± 1.41	7.00 ± 1.73	11.50 ± 0.70

However, *S. aureus* is more sensitive to both the aqueous and ethanol extract of *Moringa oleifera* leaves than *E. coli* and *P. aeruginosa*. The commercial antibiotics (Ofloxacin '5µg' and Ciprofloxacin '5µg') used on the test isolate were more effective than the *Moringa oleifera* plant extract, with Ofloxacin having the highest activity on all the test isolates. Oflatoxin is reported to be effective against *P. aeruginosa* and other Gram negative bacteria and it has low level of bacterial resistance recorded [24,25].

The antibacterial activity tests results indicate that ethanol extracts of the plant were more active than the aqueous extracts, while the N-hexane extract exert no any effect on the isolates (Table 5). The activity exhibited by the aqueous and ethanolic extracts may be related to the presence of a number of Phytoconstituents, especially flavonoids, alkaloids and Saponins which were all present in the active extracts. In particular, flavonoids were reported to be responsible for antibacterial activity associated

with some ethno medicinal plants [9]. Phytoconstituents are reported to be responsible for antimicrobial properties of some ethno-medicinal plants and these Phytoconstituents varies with the solvent of extraction and method used [4,26].

Values represented in the table are means of the duplicate readings and standard error of the zone of inhibition measured in millimetre, as analysed using SPSS software version 16.

The MIC for the aqueous extract are at a concentrations of 60mg/ml for *S. aureus*, 80mg/ml for *P. aeruginosa* and 80mg/ml for *E. coli*. While that of the ethanol extract are at a concentration of 60mg/ml for *S. aureus*, 80mg/ml for *E. coli* and 100mg/ml for *P. aeruginosa*.

Table 6 present the results of these findings. This is similar with the findings of Kiran and Tafida [23] who reported a higher activity among the ethanol and aqueous *Moringa* leaves extracts.

Table 4. Zones of inhibition in (mm) for different concentrations of ethanol extracts of *moringa oleifera* leaves

Extracts Concentration in mg/ml						
S/N	Test isolates	20	40	60	80	100
1.	<i>E. coli</i>	5.00±0.00	5.00±0.00	5.50±0.00	8.00±1.41	10.00±1.41
2.	<i>P. aeruginosa</i>	5.00±0.00	5.25±0.50	5.75±0.95	6.00±1.73	8.00±1.21
3.	<i>S. aureus</i>	5.00±0.00	5.55±1.00	6.00±1.73	10.33±1.15	12.00±1.41

Table 5. Zones of inhibition in (mm) for different concentrations of N-hexane extracts of *Moringa oleifera* leaves

Extracts Concentration in mg/ml						
S/N	Test isolates	100	80	60	40	20
1.	<i>E. coli</i>	0.00	0.00	0.00	0.00	0.00
2.	<i>P. aeruginosa</i>	0.00	0.00	0.00	0.00	0.00
3.	<i>S. aureus</i>	0.00	0.00	0.00	0.00	0.00

Table 6. Minimum inhibitory concentration (mic) of *moringa oleifera* aqueous and ethanolic extract against the test isolates

Extracts Concentration in mg/ml						
Extracts	Test isolates	100	80	60	40	20
Aqueous	<i>E. coli</i>	-	-	+	+	+
	<i>P. aeruginosa</i>	-	-	+	+	+
	<i>S. aureus</i>	-	-	-	+	+
Ethanolic	<i>E. coli</i>	-	-	+	+	+
	<i>P. aeruginosa</i>	-	+	+	+	+
	<i>S. aureus</i>	-	-	-	+	+

Keys: (+) = Indicate Turbid (growth). (-) = Indicate not Turbid (no growth)

who reported a higher activity among the ethanol and aqueous Moringa leaves extracts.

The MBC for the aqueous Extract is at a concentration of 80mg/ml for *S. aureus*, 100mg/ml for *P. aeruginosa* and 100mg/ml for *E. coli*, while that of the ethanol extract are at a concentration of 80mg/ml for *S. aureus* and 100mg/ml for *E. coli*, while no any MBC was recorded for *P. aeruginosa*. Table 7 presents these findings.

The MIC and MBC results obtained in this study, varies with the findings of Gustavo et al. [27] and Pal et al. [26] who reported a lower value. However, the disparity may be due to the variation in the plant Phytoconstituents recorded in this study [3,28]. Phytoconstituents of a plant, varies with the geographical location in which the plant is been collected [4]. The MIC results recorded in this study, varies with the findings of Amal and Nashwa [22]. Who reported a lower value of 10mg/L⁻¹ with an MBC value of 30 mg/L⁻¹ mg.

Table 7. Minimum bactericidal concentration (MBC) of *moringa oleifera* aqueous and ethanolic extract against the test isolates

Extracts Concentrations in mg/ml		100	80	60	40	20	MBC
Aqueous	<i>E.coli</i>	–	+	+	+	+	100
	<i>P.aeruginosa</i>	–	+	+	+	+	100
	<i>S.aureus</i>	–	–	+	+	+	80
Ethanolic	<i>E.coli</i>	–	+	+	+	+	100
	<i>P.aeruginosa</i>	+	+	+	+	+	Nil
	<i>S.aureus</i>	–	–	+	+	+	80

Keys: (+) = Indicate growth.(-) = no growth

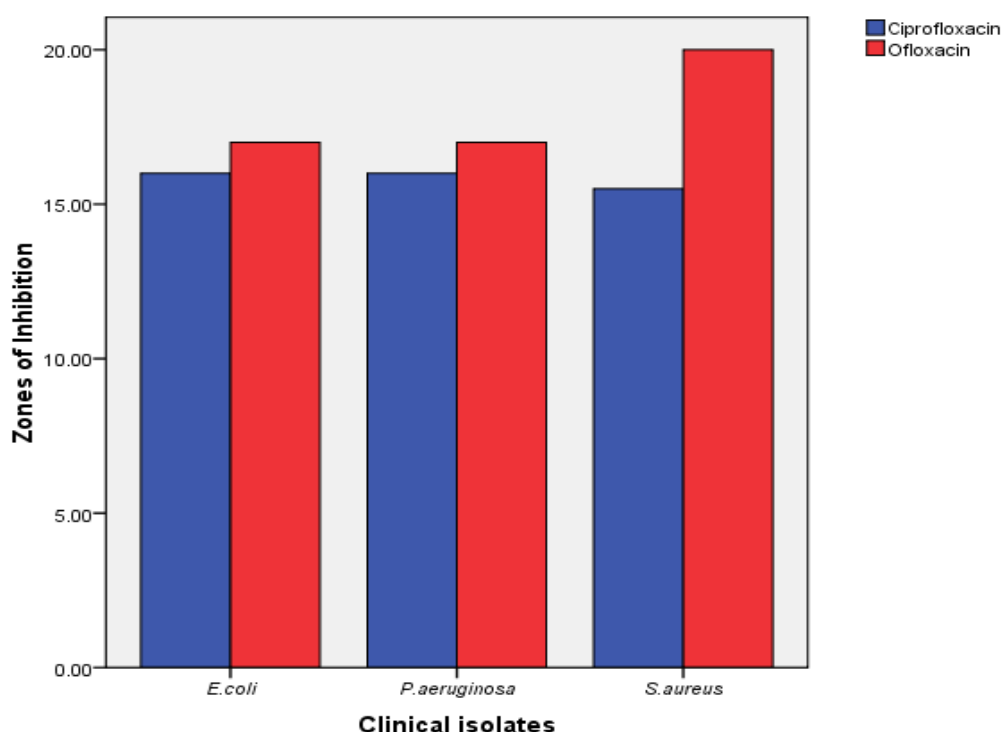


Fig. 1. Ranges of the zones of inhibition of standard commercially sold antibiotics used against the test isolates

There is continuous and urgent need for discovery of new Antimicrobial compounds with diverse chemical structures and novel mechanisms of action because of alarming increase in the incidence of new and re-emerging infectious diseases [11,21]. Antibiotics are utilized as growth promoters at sub-therapeutic levels and for treatment of diseases [29]. The beneficial effects of antibiotic in combating bacterial infections are well documented. However, there may be problems associated with usage of antibiotics such as drugs toxicity, residual effects and development of microbial resistance [21,30,31].

4. CONCLUSION

The results of this study indicated that, *S. aureus* is more sensitive to both the aqueous and ethanol extract of *Moringa oleifera* leave than the other isolates while N-hexane extract exerts no antibacterial property against the tested isolates. Also among all the extracts, the ethanol extracts possess more inhibitory effect on the test isolates. The aqueous extracts had a mean activity of 11.50 ± 0.70 mm, 7.50 ± 0.70 mm and 8.5 ± 0.70 mm for *S. aureus*, *E. coli* and *P. aeruginosa* respectively. The ethanol extracts had a mean activity of 12.00 ± 1.41 mm, 10.00 ± 1.41 mm and 8.00 ± 1.21 mm for *S. aureus*, *E. coli* and *P. aeruginosa* respectively, while the N-hexane extracts exert no any activity. The antibiotics discs exert more inhibitory effect compared to the extract. The MIC for the aqueous extracts are at a concentrations of 60mg/ml for *S. aureus*, 80mg/ml for *P. aeruginosa* and 80mg/ml for *E. coli*. While that of the ethanol extract of the leaves are at a concentration of 60mg/ml for *S. aureus*, 80mg/ml for *E. coli* and 100mg/ml for *P. aeruginosa*. The MBC for the aqueous Extract of the leaves are at a concentration of 80mg/ml for *S. aureus*, 100mg/ml for *P. aeruginosa* and 100mg/ml for *E. coli*, while that of the ethanol extract are at a concentration of 80mg/ml for *S. aureus* and 100mg/ml for *E. coli*, while no any MBC was recorded for *P. aeruginosa*. The commercial antibiotics (Ofloxacin '5µg' and Ciprofloxacin '5µg') used on the test isolate were more effective than the *Moringa oleifera* leaves extract, with Ofloxacin having the highest activity on all the test isolates. The inactivity of the N-hexane extracts could probably be due to their difference in polarity, which results in poor extraction of the Phytoconstituents, which is directly responsible for the plant bioactivity. However, these extract could be a promising

reservoir for antibacterial agents with potential application in treating bacterial infections.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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