

Original Research Article

Phytochemical and Antibacterial profile of *Moringa oleifera lam* seed extracts on some wound and enteric bacterial pathogens

ABSTRACT

Drug resistance has become a serious challenge in the present day medical practice, and effort is now geared towards the production of new and potent antimicrobials. The medicinal properties of *Moringa oleifera* seeds were studied in this research to evaluate the phytochemical constituents and assess the antibacterial activities of the seed extracts against some wound and enteric bacterial pathogens. Aqueous and ethanolic extracts of fresh and dried *Moringa oleifera* seeds were obtained using standard method. The phytochemical constituents of the extracts were evaluated using standard methods, while the antibacterial efficacy of extracts (FMSE, FMSA and DMSE, DMSA) were tested against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* isolated from wound and faeces of typhoid fever patients using agar diffusion technique by punch method. The Minimum Inhibitory Concentration (MIC) was determined using Two-fold tube dilution method and Minimum Bactericidal Concentration (MBC) was ascertained. The means of zones of inhibition obtained were subjected to statistical analysis using ANOVA. The least significant difference was determined according to LSD test at $P \leq 0.05$. Phytochemical analysis of aqueous and ethanol extracts of fresh and dried seeds of *Moringa oleifera* revealed the presence of alkaloids, saponin, flavonoids and tannins. Both the aqueous and ethanolic extracts exhibited antibacterial effects against all the test organisms. DMSE at 500mg/ml had the highest zone of inhibition of 38.00^a against *S. aureus* and lowest of 10.00^b against *E. coli* and *S. typhi*, compared with FMSE with the highest zone of inhibition of 20.00^b against *S. typhi* and *P. aeruginosa* and lowest of 14.00^b against *E. coli*. This research revealed that *Moringa oleifera* seed extracts have potential antibacterial effects. Ethanolic extracts were effective than the aqueous extracts, meaning that the potency is solvent dependent. Dried seed extracts were more effective than fresh extracts. Inhibition of Gram-positive and Gram-negative organisms portrays this plant as a potential source of broad spectrum antibiotics. The findings from this work suggest further purification of the active components with a view to using the plant in novel drug development.

Keywords: Phytochemical, Antibacterial, Typhoid fever, Extracts, Plant.

1.0. INTRODUCTION

The existence of infectious diseases in this terrestrial ball is as old as man. The morbidity and mortality rates of these plagues are as embarrassing as shocking especially in developing countries. This is not unconnected to malnutrition, poor sanitation, high level of illiteracy and poverty associated with these areas [1]. Homosapiens in a bid to contend, contain and eradicate the myriad of sicknesses that pose a great challenge to her health and survival has resorted to the use of various medicinal plants found in her local environment.

Medicinal plants and plants in general have been of immense importance to man. The medicinal use of herbs and shrubs in the treatment of various diseases both physiologically and otherwise is an important break-through in the pharmacognosy and is a great contribution to the development of modern pharmacotherapeutics in Africa [2]. Majority of Africans today depend either totally or partially on healing of their ailments with medicinal plants, being the method used by their ancestors. This form of treatment, which is referred to as ethno medicine is sometimes the only kind of health care available to the rural populations.

Traditional medicine is widespread throughout the world and it can be described as the total combination of knowledge and practices, whether explicable or not, used in diagnosing, preventing or eliminating a physical, mental or social disease and which may rely exclusively on past experience and observation handed down from generation, verbally or written [3].

Medical plant has been defined by world health organization (WHO) consultative group as any plant which in one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [1].

For many years medicine depended exclusively on leaves, flowers and bark of plants, only recently have synthetic drugs come into use and in many instances, they are carbon copies of chemicals identified in plants. In orthodox medicine, a plant may be subjected to several chemical processes before its active ingredient is extracted, refined and made ready for consumption, while in the traditional medicine a plant is simply eaten raw, cooked or infused in water or native wine or even prepared as food [2].

One of the medicinal plants that has enjoyed wide use in folk medicine is *Moringa*. *Moringa* is the sole genus in the flowering plant family Moringaceae. The name-*Moringa* is derived from the Tamil word murungai or Malayalam word Muringa, both of which refer to *Moringa oleifera* [4]. It contains 13 species from tropical and subtropical climates that range in size from tiny herbs to massive trees.

Moringa oleifera (MO) is native to the western Asia-minor, Africa and Arabia [5]. It is the most widely cultivated specie of the genus *Moringa*. English common names

include Moringa, and drumstick tree, from the appearance of the long slender, triangular seed pods, horseradish tree, from the taste of the roots which resembles horseradish, or ben oil tree, from the oil derived from the seeds. The tree itself is rather slender, with drooping branches that grow to approximately 10 meters in height. In cultivation, it is often cut back annually to 1-2 meters and allowed to regrow so the pods and leaves remain within arm's reach.

The Moringa tree is cultivated and used as vegetable (leaves, green pods, flowers, roasted seeds), for spice (mainly roots), for cooking and cosmetic oil (seeds), and as a medicinal plant [6].

Moringa grows quickly in many types of environments. Much of the plant is edible by human or by farm animals. It has a high nutritional value and contains carbohydrate, fat and protein. The leaves are rich in vitamin A, vitamin B, vitamin C, and minerals [7].

M. oleifera has enormous medicinal potentials which have long been recognized in the Ayurvedic and Unani system. In fact, Indian ancient tradition of ayurveda says that the leaves of the Moringa tree prevent 300 diseases [8]. Nearly every part of this plant, including the root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine [9].

The Moringa plant provides a rich and rare combination of zeatin, quercetin, Kaempferol and many other phytochemicals, which are very important for its medicinal value. Various parts of Moringa plants such as leaves, roots, seeds, bark, fruits, flowers and immature pods possess anti-inflammatory, anti-asthmatic and analgesic properties [10].

The issue of drug resistance has become a serious challenge in the present day medical practice, and effort is now geared towards the production of new and potent antimicrobials. Consequent upon the afore-mentioned medicinal properties of *Moringa oleifera* and its use in traditional medicine, this research thus intends to evaluate the phytochemical constituents and antibacterial activity of aqueous and ethanol seed and leaf extracts of *M. oleifera* against some wound and enteric bacterial pathogens. This will help to authenticate the claims about the efficacy of this plant by the dealers and the traditional healers, as well as provide alternative for the treatment

of wound and typhoid fever infections which have become resistant to most synthetic antibiotics.

2.0. MATERIALS AND METHODS

Plant Collection

The fresh leaf and seed of *M. oleifera* were collected and authenticated at the Department of Plant Science and Biotechnology Imo State University, Owerri. The leaf and seed were dried at room temperature for two weeks and then ground into powdered form using a mortar and pestle. They were thereafter packed separately into clean polythene bags, labeled accordingly and stored for future use. The other part of the leaf and seed used fresh were collected, ground and used immediately without drying.

Extraction

The extraction of the leaf and seed were carried out using 98% ethanol and distilled water as extracting solvents. Fifty (50) grams of the ground fresh and dried leaf and seed were weighed and dissolved in 500ml of the extracting solvents inside one liter capacity conical flasks, stoppered and kept for ten days with intermittent shaking.

The resultant mixtures were then filtered with Whatman's No.1 filter paper. The ethanol extracts were concentrated at 40°C under reduced pressure using rotary evaporator (R100). On the other hand, the distilled water aqueous extracts were concentrated in hot oven at 40°C [11]. The concentrated extracts were then collected in sterile screw capped bottles and labeled FMSE (Fresh *moringa* seed ethanol extract), DMSE (Dried *moringa* seed ethanol extract), FMLE (Fresh *moringa* leaf ethanol extract), DMLE (Dried *moringa* leaf ethanol extract), FMSDW (Fresh *moringa* seed distilled water extract), DMSDW (Dried *moringa* seed distilled water extract), FMLDW (Fresh *moringa* leaf distilled water extract), DMLDW (Dried *moringa* leaf distilled water extract).

Phytochemical Screening

This was carried out by using a modified method of Lajubutu *et al.*, [12], and the following were tested for, Alkaloids, Tannins, Saponin and Flavonoids.

Alkaloids

To test for alkaloids, 0.5g of the extract was stirred in 5ml of 1% aqueous hydrochloric acid in a steam bath. Five drops of Dragendorff's reagent (Potassium bismuth iodide solution) were mixed with 1 ml of the filtrate. Turbidity or precipitation with this reagent was taken as evidence of the presence of alkaloids.

Saponins

Exactly 0.5g of the extract was dissolved in distilled water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for saponins

Flavonoids

The presence of flavonoids was determined by dissolving 0.5g of the extract in 10ml of ethyl acetate solution. 4ml of the filtrate was shaken with 1ml of 1% aluminium chloride solution. Turbidity or precipitation indicated the presence of flavonoids

Tannins

The presence of tannins was determined by dissolving 0.5g of the extract in distilled water and about 10ml of bromine water added. Decolourization of bromine water indicated the presence of tannins [13].

Test Bacteria

The bacteria used for this research were *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. These were isolated from wound swabs obtained from accident and maternity wards of Abia State University Teaching Hospital Aba, Abia State, Nigeria. The swabs were collected after submitting a letter to the chief medical Director and obtaining the approval of the medical and ethics committee of the hospital. *Salmonella typhi* was isolated from stool samples. Cultural and morphological identification besides biochemical characterization of isolates were carried out. *S. typhi* was biochemically differentiated from other *Salmonellae* by being citrate negative, not producing gas and forming only small amount of hydrogen sulphide. Serologically it was differentiated by the presence of vi antigen [14]. Pure culture of isolates were maintained in appropriate media for future use.

Antibacterial Assay

This was carried out using the agar gel diffusion technique (punch method) as described by Osadebe and Ukwueze, [15]. In this method, broth culture of the test isolates (0.1ml) was aseptically inoculated by spreading evenly onto the surface of

Nutrient Agar (NA) plates-using a sterile bent glass rod. Seven wells (5.0mm diameter) were then made in the plates using a sterile cork borer. The fifth and the sixth wells served as negative control, while the seventh well served as positive control. Sterile distilled water and ethanol were used as negative control, while ciprofloxacin was used as positive control. Double dilution of the extracts were done to get the various concentrations (500mg/ml, 250mg/ml, 125mg/ml and 63mg/ml) used for the antibacterial assay. This was done by setting up five tubes in a row using a test tube rack. 2ml of peptone water was added to the first tube while 1ml was added to the other four tubes. 1gram of the extract was converted into 1000miligrams by multiplying it by 1000. This 1000mg was now dissolved in the first tube containing 2ml of peptone water. 1ml was taken from the first tube into the second tube containing 1ml of peptone water to get 500mg/ml. To get 250mg/ml 1ml was transferred from the second tube into the third tube. The same process was repeated to get 125mg/ml and 63mg/ml. The bottoms of the wells1-4 were sealed with one drop of sterile nutrient agar to pre vent diffusion of the extracts under the agar. Fixed volumes (0.1ml) of the four different concentrations of the extracts were transferred into the wells 1-4 using a sterile Pastuer pipette. The control wells were filled with 0.1ml of distilled water, ethanol and ciprofloxacin respectively. The plates were left on the bench for 40 minutes for pre-diffusion of the extracts [16] and then incubated at 37°C for 24hours. Antibacterial activity of the extracts was determined by measurement of the resulting zone diameters of inhibition (mm) against each test bacteria using a ruler. The experiment was carried out in triplicates and the mean values of the result were taken as antibacterial activity [17, 18].

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC of the potent extracts were determined according to the macro broth dilution techniques [19]. Double dilution was also done here to get the four different concentrations of the extracts. Standardized suspensions of the test organisms were inoculated into a series of sterile tubes of peptone water containing dilutions (500, 250, 125 and 63mg/ml) of seed and leaf extracts and incubated at 37°C for 24 hours. The MICs were read as least concentration that inhibited any visible growth (absence of turbidity) of the test organisms. For MBC determination, a loopful

of broth from each of the tubes that did not show any visible growth (no turbidity) during MIC determination was sub cultured onto fresh extract free NA plates and further incubated for 24 hours at 37°C. The least concentration, at which no visible growth was observed, was noted as the MBC.

3.0 RESULTS

The results obtained in this research is shown in tables. Table 1 shows the phytochemical constituents of the fresh and dried seed and leaf of *Moringa oleifera*. Tables 2 to 9 show the inhibitory effect of the fresh and dried seed and leaf of *Moringa oleifera* on the test organisms. Tables 10a to 17b display the minimum inhibitory concentration and minimum bactericidal concentration of the fresh and dried seed and leaf of *Moringa oleifera* on the test organisms.

Table 1: Phytochemical Components of the seed and leaf of *Moringa oleifera*.

MO	Phytochemical Components			
	Alkaloids	Saponin	Flavonoid	Tannins
Sample				
FMSE	+	+	+	+
DMSE	+	+	+	+
FMLE	+	+	+	+
DMLE	+	+	+	+
FMSDW	+	+	+	+
DMSDW	+	+	+	+
FMLDW	+	+	+	+
DMLDW	+	+	+	+

Table 1 above shows the result of the Phytochemical analysis carried out on the eight extracts:- FMSE, DMSE, FMLE, DMLE, FMSDW, DMSDW, FMLDW and DMLDW. From the table, the four phytochemical components tested for viz, alkaloids, saponin, flavonoid and tannins were found to be present in each of the extracts. Their presence were indicated with a “plus” (+) sign.

KEY:

FMSE- Fresh *moringa* seed ethanol extract
 DMSE- Dried *moringa* seed ethanol extract
 FMLE- Fresh *moringa* leaf ethanol extract
 DMLE- Dried *moringa* leaf ethanol extract
 FMSDW- Fresh *moringa* seed distilled water extract
 DMSDW- Dried *moringa* seed distilled water extract
 FMLDW- Fresh *moringa* leaf distilled water extract
 DMLDW- Dried *moringa* leaf distilled water extract

Table 3 above shows the zone of inhibition produced by DMSE extract. This extract showed inhibitory effect against all the test organisms at the concentration of 500mg/ml. The most inhibited organism is *S. aureus* with a zone diameter of 38mm, while the least is *E. coli* and *S. typhi* with zone diameters of 10mm respectively. DMSE extract also inhibited the growth of *S. aureus* at the concentration of 250mg/ml producing a zone diameter of 14mm. At other concentrations DMSE was unable to inhibit the growth of any of the test organisms. The negative controls displayed no inhibitory effect against any of the test organisms, whereas the positive control inhibited the growth of all the test organisms with different zone diameters of inhibition.

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<i>S. pyogenes</i>	20mm	0 mm	0 mm	0 mm	0 mm	0 mm	30mm
<i>E. coli</i>	12mm	0 mm	0 mm	0 mm	0 mm	0 mm	30mm
<i>S. typhi</i>	12mm	0 mm	0 mm	0 mm	0 mm	0 mm	35mm
<i>P. aeruginosa</i>	18mm	0 mm	0 mm	0 mm	0 mm	0 mm	32mm

From the table above DMLE inhibited the growth of all the test organisms only at the concentration of 500mg/ml. The highest zone diameter of inhibition (20mm) was recorded against *S. pyogenes* while the least (10mm) was recorded against *S. aureus*. At other concentrations no inhibitory effect was recorded against any of the test organisms. Distilled water and ethanol did not exhibit inhibitory action against any of the test organisms. Ciproxin on the other hand inhibited all the test organisms.

Table 6: The diameter of zone of inhibition of fresh *Moringa* seed distilled water extract (FMSDW) on the test organisms.

Test Organism	FMSDW Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	0mm	0 mm	0 mm	0 mm	0 mm	0 mm	20mm
<i>S. pyogenes</i>	6mm	0 mm	0 mm	0 mm	0 mm	0 mm	30mm
<i>E. coli</i>	0mm	0 mm	0 mm	0 mm	0 mm	0 mm	26mm
<i>S. typhi</i>	10mm	0 mm	0 mm	0 mm	0 mm	0 mm	35mm
<i>P.aerugino</i> <i>sa</i>	0mm	0 mm	0 mm	0 mm	0 mm	0 mm	36mm

The inhibitory effect of FMSDW on *S. pyogenes* and *S. typhi* was only shown at the concentration of 500mg/ml with zone diameters of 6mm and 10mm respectively. It was unable to inhibit the growth of the organisms at other concentrations. Distilled water and ethanol exhibited no inhibitory effect against the test organisms. Ciproxin on the other hand inhibited the growth of all the test organisms producing different zone diameters of inhibition.

Table 7: The diameter of zone of inhibition of dried *Moringa* seed distilled water extract (DMSDW) on the test organisms.

Test Organism	DMSDW Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	26mm	0 mm	0 mm	0 mm	0 mm	0 mm	32mm
<i>S. pyogenes</i>	12mm	0 mm	0 mm	0 mm	0 mm	0 mm	26mm
<i>E. coli</i>	10mm	0 mm	0 mm	0 mm	0 mm	0 mm	18mm
<i>S. typhi</i>	14mm	0 mm	0 mm	0 mm	0 mm	0 mm	30mm
<i>P. aeruginosa</i>	20mm	0 mm	0 mm	0 mm	0 mm	0 mm	32mm

DMSDW exhibited inhibitory effect against all the test organisms at the concentration of 500mg/ml. The most inhibited organism is *S. aureus* with a zone diameter of 26mm, while the least is *E. coli* with a zone diameter of 10mm. Just like the negative controls, it was unable to inhibit the growth of the test organisms at other concentrations. The positive control however inhibited the growth of all the test organisms.

Table 8: The diameter of zone of inhibition of fresh *moringa* leaf distilled water extract (FMLDW) on the test organisms.

Test Organism	FMLDW Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	0mm	0 mm	0 mm	0 mm	0 mm	0 mm	20mm
<i>S. pyogenes</i>	28mm	10mm	0 mm	0 mm	0 mm	0 mm	30mm
<i>E. coli</i>	10mm	0 mm	0 mm	0 mm	0 mm	0 mm	28mm
<i>S. typhi</i>	20mm	0 mm	0 mm	0 mm	0 mm	0 mm	30mm
<i>P. aeruginosa</i>	0mm	0 mm	0 mm	0 mm	0 mm	0 mm	35mm

Table 8 above shows the zone of inhibition produced by FMLDW extract against the test organisms. At the concentration of 500mg/ml it inhibited the growth of *S. pyogenes*, *E. coli* and *S. typhi*. The highest zone diameter of inhibition was recorded against *S. pyogenes*, while the least was recorded against *E. coli*. *S. aureus* and *P. aeruginosa* were resistant to the extract. At other concentrations the extract failed to inhibit the growth of any of the test organisms just like the negative controls. Ciproxin inhibited the growth of all the test organisms.

Table 9: The diameter of zone of inhibition of dried *moringa* leaf distilled water extract (DMLDW) on the test organisms.

Test Organism	DMLDW Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin

<i>S. aureus</i>	0mm	0 mm	0 mm	0 mm	0 mm	0 mm	20mm
<i>S. pyogenes</i>	10mm	0 mm	0 mm	0 mm	0 mm	0 mm	21mm
<i>E. coli</i>	0mm	0 mm	0 mm	0 mm	0 mm	0 mm	20mm
<i>S. typhi</i>	0mm	0 mm	0 mm	0 mm	0 mm	0 mm	32mm
<i>P. aeruginosa</i>	20mm	0 mm	0 mm	0 mm	0 mm	0 mm	30mm

As shown in table 9 above DMLDW extract inhibited the growth of only two of the test organisms at the concentration of 500mg/ml. The highest zone diameter of inhibition (20mm) was recorded against *P. aeruginosa* while the least (10mm) was recorded against *S. pyogenes*. *S. aureus*, *E. coli* and *S. typhi* were resistant to DMLDW at this concentration. At other concentrations no inhibitory action was exhibited by the extract against the test organisms just like the negative controls. The positive control on the other hand inhibited the growth of all the test organisms.

Table 10a: The minimum inhibitory concentration (MIC) of Fresh *moringa* seed ethanol extract (FMSE) on the test organisms.

Test Organism	FMSE Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	-	-	-	+	+	+	-
<i>S. pyogenes</i>	-	-	+	+	+	+	-
<i>E. coli</i>	-	-	+	+	+	+	-
<i>S. typhi</i>	-	-	-	+	+	+	-
<i>P. aeruginosa</i>	-	-	-	+	+	+	-

- = Inhibition (no growth),

+ = No inhibition (growth).

From the table above, FMSE inhibited the growth of *S. aureus*, *S. typhi* and *P. aeruginosa* at the concentrations of 500mg/ml, 250mg/ml and 125mg/ml respectively.

At the concentration of 63mg/ml no inhibition was produced against these organisms. The MIC of FMSE for *S. aureus*, *S. typhi* and *P. aeruginosa* is 125mg/ml. Inhibition of *S. pyogenes* and *E. coli* by the extract was reported at the concentrations of 500mg/ml and 250mg/ml respectively. It failed to inhibit the growth of these organisms at other concentrations. Therefore, the MIC of the extract for *S. pyogenes* and *E. coli* is 250mg/ml. Distilled water and ethanol did not exhibit inhibitory effect against any of the test organisms, while ciproxin inhibited the growth of all the test organisms.

Table 10b: The minimum bactericidal concentration (MBC) of Fresh *moringa* seed ethanol extract (FMSE) on the test organisms.

Test Organism	FMSE Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	-	-	+	+	+	+	-
<i>S. pyogenes</i>	-	-	+	+	+	+	-
<i>E. coli</i>	-	-	+	+	+	+	-
<i>S. typhi</i>	-	-	+	+	+	+	-
<i>P. aeruginosa</i>	-	-	+	+	+	+	-

As depicted in the above table FMSE exhibited bactericidal effect on all the test organisms at the concentrations of 500mg/ml and 250mg/ml. Thus, the MBC of the extract for *S. aureus*, *S. pyogenes*, *E. coli*, *S. typhi* and *P. aeruginosa* is 250mg/ml. The positive control exhibited bactericidal effect against all the test organisms, while the negative control did not. From table 10a above, the inhibitory action of FMSE against *S. aureus*, *S. typhi* and *P. aeruginosa* at the concentration of 125mg/ml was bacteriostatic in nature.

Table 11a: The minimum inhibitory concentration (MIC) of dried *moringa* seed ethanol extract (DMSE) on the test organisms.

Test Organism	DMSE Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	-	-	+	+	+	+	-
<i>S. pyogenes</i>	-	+	+	+	+	+	-
<i>E. coli</i>	-	+	+	+	+	+	-
<i>S. typhi</i>	-	+	+	+	+	+	-
<i>P.aeruginosa</i>	-	+	+	+	+	+	-

As shown in the table above DMSE inhibited the growth of *S. aureus* at the concentrations of 500mg/ml and 250mg/ml respectively. However, no inhibitory action was shown at other concentrations. The MIC of the extract for *S. aureus* is 250mg/ml. At the concentration of 500mg/ml, DMSE exhibited inhibitory effect against *S. pyogenes*, *E.coli*, *S. typhi* and *P. aeruginosa*. No inhibitory action was produced by the extract against these organisms at other concentrations. The MIC of the extract for these test organisms is 500mg/ml. Whereas the negative controls did not inhibit the growth of any of the test organisms, the positive control inhibited all of them.

Table 11b: The minimum bactericidal concentration (MBC) of dried *moringa* seed ethanol extract (DMSE) on the test organisms.

Test Organism	DMSE Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	-	-	+	+	+	+	-
<i>S. pyogenes</i>	+	+	+	+	+	+	-
<i>E. coli</i>	-	+	+	+	+	+	-

<i>S. typhi</i>	-	+	+	+	+	+	-
<i>P. aeruginosa</i>	+	+	+	+	+	+	-

Bactericidal effect was produced by DMSE against *S. aureus* only at the concentrations of 500mg/ml and 250mg/ml. The MBC of the extract for *S. aureus* is 250mg/ml. No bactericidal effect was exhibited by the extract against *S. pyogenes* and *P. aeruginosa* at all the concentrations. At the concentration of 500mg/ml, DMSE showed bactericidal effect against *E. coli* and *S. typhi*. Thus, the MBC of the extract for these organisms is 500mg/ml. The positive control produced bactericidal effect against all the test organisms, while the negative controls did not show bactericidal effect against any of the test organisms. From table 11a above, the inhibitory effect of DMSE on *S. pyogenes* and *P. aeruginosa* at the concentration of 500mg/ml was bacteriostatic in nature.

Table12a: The minimum inhibitory concentration (MIC) of fresh *moringa* leaf ethanol extract (FMLE) on the test organisms.

Test Organism	FMLE Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Positive Ciproxin
<i>S. aureus</i>	-	-	+	+	+	+	-
<i>S. pyogenes</i>	-	+	+	+	+	+	-
<i>E. coli</i>	-	-	+	+	+	+	-
<i>S. typhi</i>	-	-	+	+	+	+	-
<i>P. aeruginosa</i>	-	-	+	+	+	+	-

From the table above, FMLE inhibited the growth of *S. aureus*, *E. coli*, *S. typhi* and *P. aeruginosa* at the concentrations of 500mg/ml and 250mg/ml respectively. At other concentrations, the extract did not show any inhibition against these organisms. Distilled water and ethanol were unable to inhibit the growth of these organisms while ciproxin inhibited their growth. Therefore, the MIC of FMLE for *S. aureus*, *E. coli*, *S. typhi* and *P. aeruginosa* is 250mg/ml. The inhibition of *S. pyogenes* by the extract was recorded at the concentration of 500mg/ml. At other concentrations no inhibition was produced by the extract. This was the same for the negative controls. However, the

positive control inhibited the growth of *S. pyogenes*. The MIC of FMLE for *S. pyogenes* is 500mg/ml.

Table 12b: The minimum bactericidal concentration (MBC) of fresh *moringa* leaf ethanol extract (FMLE) on the test organisms.

Test Organism	FMLE Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	-	+	+	+	+	+	-
<i>S. pyogenes</i>	+	+	+	+	+	+	-
<i>E. coli</i>	-	+	+	+	+	+	-
<i>S. typhi</i>	-	+	+	+	+	+	-
<i>P. aeruginosa</i>	-	+	+	+	+	+	-

At the concentration of 500mg/ml FMLE exhibited bactericidal effect against *S. aureus*, *E. coli*, *S. typhi* and *P. aeruginosa*. The negative controls did not show any bactericidal effect against these organisms. On the other hand, the positive control showed bactericidal effect against the aforementioned organisms. The MBC of FMLE for *S. aureus*, *E. coli*, *S. typhi* and *P. aeruginosa* is 500mg/ml. Both the extract and the negative controls did not show any bactericidal effect against *S. pyogenes* compared with the positive control.

From table 12a above, the nature of the inhibitory action produced by FMLE against *S. aureus*, *E. coli*, *S. typhi* and *P. aeruginosa* at the concentration of 250mg/ml and against *S. pyogenes* at the concentration of 500mg/ml was bacteriostatic.

Table 13a: The minimum inhibitory concentration (MIC) of dried *moringa* leaf ethanol extract (DMLE) on the test organisms.

Test Organism	DMLE Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	-	-	+	+	+	+	-
<i>S. pyogenes</i>	-	-	+	+	+	+	-
<i>E. coli</i>	-	+	+	+	+	+	-
<i>S. typhi</i>	-	+	+	+	+	+	-
<i>P. aeruginosa</i>	-	+	+	+	+	+	-

As indicated in the table above, DMLE inhibited the growth of *S. aureus* and *S. pyogenes* at the concentrations of 500mg/ml and 250mg/ml respectively. At other concentrations no inhibitory effect was exhibited by this extract against the organisms. Distilled water and ethanol did not show any inhibition, while ciproxin inhibited the growth of these organisms. The MIC of DMLE for *S. aureus* and *S. pyogenes* is 250mg/ml. For *E. coli*, *S. typhi* and *P. aeruginosa* inhibition by the extract was only produced at the concentration of 500mg/ml. It failed to inhibit the growth of these organisms at other concentrations just like the negative controls. The positive control inhibited the growth of these organisms. The MIC of DMLE for these organisms is 500mg/ml.

Table 13b: The minimum bactericidal concentration (MBC) of dried *moringa* leaf ethanol extract (DMLE) on the test organisms.

Test Organism	DMLE Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	-	+	+	+	+	+	-
<i>S. pyogenes</i>	-	+	+	+	+	+	-
<i>E. coli</i>	+	+	+	+	+	+	-

<i>S. typhi</i>	+	+	+	+	+	+	-
<i>P. aeruginosa</i>	+	+	+	+	+	+	-

DMLE exhibited bactericidal effect against *S. aureus* and *S. pyogenes* at the concentration of 500mg/ml. Distilled water and ethanol did not show any bactericidal effect against these organisms. However, the positive control exhibited bactericidal effect against these organisms. Thus, the MBC of DMLE for *S. aureus* and *S. pyogenes* is 500mg/ml. The extract did not exhibit any bactericidal effect against *E. coli*, *S. typhi* and *P. aeruginosa*. Also, the negative controls did not show any bactericidal effect against these organisms compared with the positive control. From table 13a above, the inhibitory effect of DMLE against *S. aureus* and *S. pyogenes* at the concentration of 250mg/ml, and *E. coli*, *S. typhi* and *P. aeruginosa* at the concentration of 500mg/ml was bacteriostatic in nature.

Table 14a: The minimum inhibitory concentration (MIC) of fresh *moringa* seed distilled water extract (FMSDW) on the test organisms.

Test Organism	FMSDW Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	+	+	+	+	+	+	-
<i>S. pyogenes</i>	-	+	+	+	+	+	-
<i>E. coli</i>	+	+	+	+	+	+	-
<i>S. typhi</i>	-	+	+	+	+	+	-
<i>P. aeruginosa</i>	+	+	+	+	+	+	-

In the table above, FMSDW did not exhibit any inhibitory effect against *S. aureus*, *E. coli* and *P. aeruginosa* at all the concentrations. Unlike the positive control, the negative controls also were unable to inhibit the growth of these organisms. At the concentration of 500mg/ml, FMSDW inhibited the growth of *S. pyogenes* and *S. typhi*, but failed to inhibit them at other concentrations. The positive control inhibited the growth of these organisms, while the negative controls did not. The MIC of the extract for *S. pyogenes* and *S. typhi* is 500mg/ml.

Table 14b: The minimum bactericidal concentration (MBC) of fresh *moringa* seed distilled water extract (FMSDW) on the test organisms.

Test Organism	FMSDW Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	+	+	+	+	+	+	-
<i>S. pyogenes</i>	+	+	+	+	+	+	-
<i>E. coli</i>	+	+	+	+	+	+	-
<i>S. typhi</i>	+	+	+	+	+	+	-
<i>P. aeruginosa</i>	+	+	+	+	+	+	-

As shown in the table above, FMSDW did not exhibit bactericidal effect against any of the test organisms. This was the same for the negative controls. On the other hand, bactericidal effect was exhibited by the positive control against all the test organisms. From table 14a above, the inhibitory effect produced by the extract against *S. pyogenes* and *S. typhi* at the concentration of 500mg/ml was bacteriostatic.

Table 15a: The minimum inhibitory concentration (MIC) of dried *moringa* seed distilled water extract (DMSDW) on the test organisms.

Test Organism	DMSDW Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	-	+	+	+	+	+	-
<i>S. pyogenes</i>	-	+	+	+	+	+	-
<i>E. coli</i>	-	+	+	+	+	+	-
<i>S. typhi</i>	-	+	+	+	+	+	-

<i>P. aeruginosa</i>	-	+	+	+	+	+	-
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At the concentration of 500mg/ml, DMSDW inhibited the growth of the five test organism, but did not show any inhibition at other concentrations. Distilled water and ethanol did not show any inhibition against the test organisms, while ciproxin inhibited the growth of each of the test organisms. The MIC of DMSDW for the test organisms is 500mg/ml.

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Table 15b: The minimum bactericidal concentration (MBC) of dried *moringa* seed distilled water extract (DMSDW) on the test organisms.

Test Organism	DMSDW Concentrations				Controls		
	500mg/ Ml	250mg/ Ml	125mg/ Ml	63mg/ ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	+	+	+	+	+	+	-
<i>S. pyogenes</i>	+	+	+	+	+	+	-
<i>E. coli</i>	+	+	+	+	+	+	-
<i>S. typhi</i>	+	+	+	+	+	+	-
<i>P. aeruginosa</i>	+	+	+	+	+	+	-

Neither DMSDW nor the negative controls exhibited any bactericidal effect against any of the test organisms. Bactericidal effect was shown by the positive control against each of the test organisms. From table 15a above, the inhibitory action of DMSDW against *S. aureus*, *S. pyogenes*, *E. coli*, *S. typhi* and *P. aeruginosa* at the concentration of 500mg/ml was bacteriostatic in nature.

Table 16a: The minimum inhibitory concentration (MIC) of fresh *moringa* leaf distilled water extract (FMLDW) on the test organisms

Test Organism	FMLDW Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	+	+	+	+	+	+	-
<i>S. pyogenes</i>	-	-	+	+	+	+	-
<i>E. coli</i>	-	+	+	+	+	+	-
<i>S. typhi</i>	-	+	+	+	+	+	-
<i>P. aeruginosa</i>	+	+	+	+	+	+	-

No inhibitory effect was shown by FMLDW against *S. aureus* and *P. aeruginosa* at all the concentrations. At the concentrations of 500mg/ml and 250mg/ml, the extract inhibited the growth of *S. pyogenes*. However, it failed to inhibit it at other concentrations. For *E. coli* and *S. typhi*, inhibition by the extract was only recorded at the concentration of 500mg/ml. The negative controls were unable to inhibit the growth of any of the five test organisms compared with the positive control. The MIC of the extract for *S. pyogenes* is 250mg/ml, while for *E. coli* and *S. typhi* it is 500mg/ml.

Table 16b: The minimum bactericidal concentration (MBC) of fresh *moringa* leaf distilled water extract (FMLDW) on the test organisms.

Test Organism	FMLDW Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	+	+	+	+	+	+	-
<i>S. pyogenes</i>	-	+	+	+	+	+	-
<i>E. coli</i>	-	+	+	+	+	+	-

<i>S. typhi</i>	+	+	+	+	+	+	-
<i>P. aeruginosa</i>	+	+	+	+	+	+	-

From the table above, FMLDW did not exhibit any bactericidal effect against *S. aureus*, *S. typhi* and *P. aeruginosa*. Bactericidal effect was however exhibited against *S. pyogenes* and *E. coli* by the extract at the concentration of 500mg/ml. While the negative controls did not show any bactericidal effect against any of the test organisms, the positive control exhibited bactericidal effect against each of the test organisms. The MBC of the extract for *S. pyogenes* and *E. coli* is 500mg/ml. From Table 16a above, the inhibition of *S. pyogenes* by FMLDW at the concentration of 250mg/ml and *S. typhi* at 500mg/ml was bacteriostatic in nature.

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Table 17a: The minimum inhibitory concentration (MIC) of dried *moringa* leaf distilled water extract (DMLDW) on the test organisms.

Test Organism	DMLDW Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	+	+	+	+	+	+	-
<i>S. pyogenes</i>	-	-	+	+	+	+	-
<i>E. coli</i>	+	+	+	+	+	+	-
<i>S. typhi</i>	+	+	+	+	+	+	-
<i>P. aeruginosa</i>	-	+	+	+	+	+	-

As indicated in the table above, DMLDW exhibited no inhibitory effect against *S. aureus*, *E. coli* and *S. typhi* at all the concentrations. Inhibitory action was however, recorded at the concentrations of 500mg/ml and 250mg/ml respectively for *S. pyogenes* by the extract. DMLDW also inhibited the growth of *P. aeruginosa* at the concentration of 500mg/ml only. The negative controls did not inhibit the growth of any of the test organisms compared with the positive control which inhibited the growth of all the test organisms. The MIC of the extract for *S. Pyogenes* is 250mg/ml, while 500mg/ml for *P. aeruginosa*.

Table 17b: The minimum bactericidal concentration (MBC) of dried *moringa* leaf distilled water extract (DMLDW) on the test organisms.

Test Organism	DMLDW Concentrations				Controls		
	500mg/ml	250mg/ml	125mg/ml	63mg/ml	Distilled Water	Ethanol	Ciproxin
<i>S. aureus</i>	+	+	+	+	+	+	-
<i>S. pyogenes</i>	+	+	+	+	+	+	-
<i>E. coli</i>	+	+	+	+	+	+	-
<i>S. typhi</i>	+	+	+	+	+	+	-
<i>P. aeruginosa</i>	+	+	+	+	+	+	-

As depicted in the above table, DMLDW did not exhibit bactericidal action against any of the test organisms. The positive control showed bactericidal effect against each of the test organisms, while the negative controls did not. The nature of the inhibition produced by the extract against *S. pyogenes* at the concentrations of 500mg/ml and 250mg/ml and *P. aeruginosa* at 500mg/ml in table 17a above was bacteriostatic.

4.0. DISCUSSION

Undoubtedly medicinal plants and plants in general have been of immense importance to man. Sofowora [20] stated that medicinal use of herbs and shrubs in the treatment of so many diseases both physiological and otherwise is an important breakthrough in pharmacognosy and is a great contribution to the development of modern pharmacotherapeutics in Africa. This forms the basis of this research.

Secondary compounds like alkaloids, tannins, saponins, flavonoids etc. were reported to be present in higher plants by Kaufman *et al.*, [21]. The preliminary phytochemical screening of the aqueous and ethanolic extracts of fresh and dried *Moringa* leaf and seed (Table 1) revealed the presence of alkaloids, tannins, saponins, flavonoids which agrees with this report. These phytochemicals confer antibacterial potentials to any plant in which they are found. These compounds were reported by Kaufman *et al.*, [21] and Dutta [22] to be an indication of the potential medicinal value of the plants in which they appear.

The phytochemical result of the ethanolic leaf and seed extracts of *Moringa* corroborates the earlier reports by Bukar *et al.*, [23]. However, alkaloids and tannins were reported in the present study which were not determined by Bukar *et al.*, [23]. This may be responsible for the higher rate of inhibition recorded in this work. Farooq *et al.*, [24] reported that plants occur in varying habitats, thus, a great magnitude of variation in the concentration and composition of photochemical ingredients in the different parts of such plant is expected. Moreover, Walter and Nowacki [25] reported that phytochemicals are produced in response to perceived threats by the plants, therefore variation exist in the production of these phytochemicals depending on the type and amount of threat encountered by the plant.

Besides *Moringa* contains pterygospermin (originally found in *Moringa pterygosperma*) which has powerful antibacterial and fungicidal effects Rao *et al.*, [26]. Several other specific components of *Moringa* have been reported with antibacterial activity including 4-(4-O-acetyl- α -L-rhamnopyranosyloxy) benzyl isothiocyanate, 4-(α -L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin, benzyl isothiocyanate and 4-(α -L-rhamnopyranosyloxy) benzyl glucosinolate [27]. Other bioactive compounds, such as spirochin and Anthonine are found in root and are active against several bacteria. Anthonine has potent inhibitory activity against *Vibrio cholerae* [28].

The result obtained in this study indicated that both the aqueous and ethanol extracts of leaf and seed of *Moringa oleifera* exhibited antibacterial effect against all the test organisms. The test organisms showed different level of sensitivities to the extracts ranging from sensitive, intermediate to resistant. The antibacterial properties of the leaf and seed of *M. oleifera* as revealed in the present research agrees with the earlier reports by Aktar *et al.*, [29] and Foidl *et al.*, [30] who reported that *M. oleifera* seed and leaf possess antibacterial properties.

This work showed that the four ethanolic extracts (FMSE, DMSE, FMLE and DMLE) had inhibitory effect against *S. aureus*, *E. coli*, *S. pyogenes*, *S. typhi* and *P. aeruginosa* respectively. This corroborates a similar study by Nepolean *et al.*, [31] who reported that ethanolic extracts of leaves, seeds and flowers of *M. oleifera* showed antimicrobial activity against *E. coli*, *K. pneumonia*, *Enterobacter* species, *P. mirabilis*, *P. aeruginosa*, *S. aureus*, *S. typhi*, *Streptococcus* and *Candida albicans*.

In general, the ethanol extracts of the seed and leaf of *M. oleifera* are obviously more effective than the aqueous extracts. Dutta [22] reported that different solvents dissolve different active ingredients from the same plant and this is presumed to have direct bearing to the type of microorganism affected. It is likely that the active constituents of the plants were better extracted with ethanol than with water indicating that ethanol is a better solvent than water. This observation is similar to the report of Ogunjobi and Nnadozie [32] and Ezeifeka *et al.*, [33] that reported the higher antimicrobial activities of ethanol extracts of plant parts than the aqueous extracts.

Unlike in previous studies, the antibacterial efficacy of fresh and dried *Moringa* seed and leaf were compared in this work. It was shown that dried *Moringa* seed produced more inhibitory activity than the fresh seed. As shown in table 3 above, DMSE inhibited the growth of *S. aureus* with a zone diameter of 38mm making it the most sensitive organism in this work, as against the highest zone diameter of 20mm produced by FMSE against *S. typhi* and *P. aeruginosa* respectively in table 2. This shows that the dried seed is more effective than the fresh seed. This may be due to the reduction in water content of the dry seed thereby making the antibacterial agent to be more concentrated. The antibacterial activity of *M. oleifera* seed has been highlighted by many authors; such as Olsen, [34]; Madsen *et al.*, [35]; Kawo, [36]. The antimicrobial activity of *M. oleifera* seed is due to the presence of an array of phytochemicals, but most importantly due to the activity of a short polypeptide named

4(d-L-rhamnosyloxy) benzyl-isothiocyanate [37, 38]. The peptide may act directly on microorganisms and result in growth inhibition by disrupting cell membrane synthesis or synthesis of essential enzymes [39, 40].

Besides, a comparison of the antibacterial activity of fresh and dried *Moringa* leaf showed that the fresh leaf is more effective than the dried one. As shown in table 4 above, FMLE extract inhibited the growth of both *S. aureus* and *E. coli* with zone diameters of 22mm respectively, while the highest zone diameter of inhibition produced by DMLE extract in table 5 was 20mm against *S. pyogenes*.

The aqueous extracts of *M. Oleifera* leaf and seed also showed appreciable level of inhibition against the test organisms. In fact each of the extracts inhibited at least two of the test organisms. DMSDW showed inhibitory action against *S. aureus*, *S. pyogenes*, *E. coli*, *S. typhi*, and *P. aeruginosa*. This is in agreement with a similar study carried out by Saadabi and Abu [41], who reported that aqueous extract of *M. oleifera* was found to be inhibitory against many pathogenic bacteria including *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*. The inhibitory action produced by each of the eight extracts used in this research agrees with the report of [9], who reported that nearly every part *M. oleifera* plant, including root, bark, gum, leaf, fruit, flowers, seed and seed oil have been used for the treatment of various ailments in the indigenous medicine.

In this research it was also observed that virtually all the extracts showed appreciable level of inhibition against *P. aeruginosa* and the commonest etiologic agent of enteric fever *Salmonella typhi*. Brooks *et al.*, [42], reported that enteric fever had mortality rate of 10-15% in developing countries of which Nigeria is one. The successful inhibition of these organisms by the extract is a welcome development, especially when considering the level of multi-resistance these bacteria have developed against conventional antibiotics over the years. This shows that the extracts can be used as therapy for the treatment of typhoid fever and other infections caused by these organisms.

CONCLUSION

The result of this research has demonstrated that *M. oleifera* leaf and seed extracts have potential antibacterial effects which can be explored. It also showed that the dried seed is more effective than the fresh seed. The successful inhibition of both Gram-positive organisms (*S. aureus*, *S. pyogenes*) and Gram-negative organisms (*E.*

coli, *S. typhi* and *P. aeruginosa*) by the plant extract depicts that it is a potential source for the production of broad spectrum antibiotics, which can be used to treat some of the infections that have constituted a nightmare in the present day medical practice, owing to their resistance to most conventional antibiotics. This study has justified the traditional usage of this plant for therapeutic purposes.

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