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ANTI-BACTERIAL AND PHYTOCHEMICAL POTENTIAL OF *MORINGA OLEIFERA LEAF* EXTRACTS ON SOME WOUND AND ENTERIC PATHOGENIC BACTERIA

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ABSTRACT: *Majority of Africans today depend either totally or partially on medicinal plants for the healing of their ailments which was 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. As part of the efforts to ascertain the healing capability credited to Moringa oleifera by the general public and some traditional practitioners, this work aimed at determining the antibacterial potentials and phyto-chemical constituents of M. oleifera was embarked on. Aqueous and ethanol extracts of fresh and dried leaf of Moringa oleifera (FMLE, FMLDW and DMLE, DMLDW) were obtained using a standard method (1). The antibacterial efficacy of aqueous and ethanol extracts of fresh and dried leaves of Moringa oleifera was tested against Staphylococcus aureus , Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi, isolated from wound and feaces respectively, to ascertain its effectiveness in the treatment of wound infection and typhoid fever using Agar diffusion by punch method. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and phyto-chemistry of the extracts were also evaluated. The mean values of zones of inhibition obtained were statistically analyzed using ANOVA. The least significant difference was determined according to LSD test at $P \leq 0.05$. Results obtained showed that FMLE at 500mg/ml has the highest zone of inhibition of 22.00^b against S. aureus, E. coli and lowest 15.00^b against S.typhi, compared with DMLE with the highest zone of inhibition of 20.00^b against S. pyogenes and lowest of 10.00^b against S. aureus. FMLDW presented the highest inhibitory activity 28.00^b against S. pyogenes and no activity against S. aureus and P. aeruginosa while DMLDW recorded 20.00^b P. earuginosa and 10.00^b against S. pyogenes. Both the aqueous and the ethanol extracts of Moringa oleifera leave exhibited appreciable level of inhibition against the test bacteria, but the aqueous extracts were not as effective as the ethanolic extracts. Phyto-chemical analysis of aqueous and ethanol extracts of fresh and dried leaf of Moringa oleifera revealed the presence of alkaloids, saponin, flavonoids and tannins. The findings from this work could be of interest and suggest the need for further investigations in terms of toxicological studies and purification of active components with a view to using the plant in novel drug development.*

KEY WORDS: Moringa oliefera, Leaves, Aqueous, Ethanol Extracts, Pathogenic Bacteria, Phytochemicals, Toxicology.

INTRODUCTION

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

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Moringa oleifera (Quattrocchi, 2000). 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 (Somali *et al*., 1984; Mughal *et al.,* 1999). 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 (Rebecca *et al.,* 2006). 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 (Janick and Robert, 2008). *M. oleifera* has enormous medicinal potentials which have been long recognized in the Ayuvedic and Unani system (Mughal *et al.,* 1999).

In fact, Indian ancient tradition of ayurveda says that *Moringa* leaves prevent 300 diseases (Francis, 2011). Nearly every part of this plant has been used for various ailments in the indigenous medicine (Odebiyi and Sofowora, 1999). The Moringa plant provides a rich and rare combination of zeatin, quercetin, Kaempferom and many other phytochemicals, which are very important for its medicinal value. Various parts of Moringa plants possess anti-inflamatory, anti-asthmatic and analgesic properties (Farooq *et al.*, 2012). Consequent upon these reasons and the use of *Moringa oleifera* in traditional medicine, this research aims at evaluating the antibacterial activity and the phytochemical constituents of aqueous and ethanolic leaf extracts of *M. oleifera* against some wound and enteric bacterial pathogens in order to authenticate the claims about the efficacy of this plant by the dealers and the traditional healers and to proffer better counseling to users.

MATERIALS AND METHODS

Plant Collection

The fresh leaves of *M. oleifera* were collected from Aba South Local Government Area, Abia State, Nigeria. These were authenticated by Dr. C. Duru, Department of Plant Science and Biotechnology Imo State University, Owerri, Nigeria. The leaves were dried at room temperature for two weeks and ground into powdered form using a blender. The powder was packaged into clean polythene bags, labeled accordingly and stored for future use. The other part of the fresh leaves was ground and used fresh.

Extraction

The extraction from the leaves was done using 98% ethanol and distilled water solvents (Fatope *et al*., 1993). Fifty (50) grams of the ground fresh and dried leaves were weighed and dissolved in 500ml of the appropriate extracting solvents in one-liter conical flasks, stoppered and kept for ten days with intermittent shaking.

The resultant mixtures were filtered through Whatman's No.1filter paper. The ethanol extracts were concentrated at 40^{o}C under reduced pressure using rotary evaporator (R100). On the other hand, the distilled water aqueous extracts were concentrated in hot air oven at 40^{o}C

The concentrated extracts were collected in sterile screw capped bottles and labeled FMLE (Fresh *moringa* leaf ethanolic extract), DMLE (Dried *moringa* leaf ethanol extract), FMLDW (Fresh *moringa* leaf distilled water extract), DMLDW (Dried moringa leaf distilled water extract).

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Phytochemical Screening

This was carried out by using a modified method of Lajubutu *et al.*, (1995). The leaves were tested for the presence of: Alkaloids, Tannins, Saponin and Flavonoids.

Alkaloids: 0.5g of the extract was stirred in 5ml of 1% aqueous hydrochloric acid in a steam bath and filtered. I ml of the filtrate was mixed with 5 drops of Potassium bismuth iodide solution (Dragendorff's reagent). Turbidity or precipitation with this reagent indicates the presence of alkaloids.

Saponins: 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. Then 4ml of the filtrate was shaken with 1ml of 1% aluminium chloride solution.

Turbidity or precipitation indicated the presence of flavonoids

Tannins: 0.5mg of extract was dissolved in distilled water and 10ml of brome water added. Decolourization of bromine water indicated the presence of tannins (Ogunjiobi and Ogunjiobi, 2011).

Antibacterial Assay

This was carried out using the agar diffusion technique (punch method) as described by Osadebe and Ukwueze, (2004).

0.1ml of 24 -hr broth culture of the test organisms was aseptically inoculated on sterile dried Nutrient Agar (NA) plates by spread method.

Seven wells (5.0mm diameter) were made in the plates using a sterile cork borer. Fifth and the Sixth wells were for negative controls, seventh well was for positive control.

Sterile distilled water and ethanol were used as negative control, while ciprofloxacin served as positive control.

Double dilutions of the extracts were made to different concentrations (500mg/ml, 250mg/ml, 125mg/ml and 63mg/ml) used for the antibacterial assay.

Five tubes in a row in 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 transferred and dissolved in the first tube containing 2ml of peptone water. 1ml was transfered 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 wells 1-4 were sealed with one drop of sterile nutrient agar to prevent diffusion of the extracts under the agar. 0.1ml of the four different dilution concentrations of the extracts were transferred into the wells 1-4 using a sterile Pastuer pipette.

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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 (Esimone *et al.*, 1988) and then incubated at 37ºC 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 (Abayomi, 1982; Junaid *et al*., 2006).

Minimum Inhibitory Concentration (MIC) and minimum Bactericidal Concentration (mbc).

The MIC and MBC of the potent extracts were determined by Macro Broth Dilution Techniques (Boron and Fingold, 1990). Double fold dilution was made to get 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 37ºc 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 without visible growth (no turbidity) in MIC determination was sub-cultured onto fresh extract free NA plates and incubated for 24 hours at 37ºc. The least concentration with no visible growth was noted as the MBC.

RESULT

Table 1: Phytochemical Components of the leaves of *Moringa oleifera*.

MO Sample	Phytochemical Components			
	Alkaloids	Saponin	Flavonoid	Tannins
FMLE	+	+	+	+
DMLE	+	+	+	+
FMLDW	+	+	+	+
DMLDW	+	+	+	+

KEY: FMLE= Fresh *moringa* leaf ethanoicl extract, DMLE= Dried *moringa* leaf ethanol extract, FMLDW= Fresh *moringa* leaf distilled water extract, DMLDW= Dried *moringa* leaf distilled water extract + = present, - = Absent.

Table 2: Mean* diameter (mm) of zone of inhibition of ethanolic extract of fresh Moringa leaves (FMLE) and the control treatments on the test microorganisms.

EXTRACTS CONCENTRATION	**MICRO ORGANISMS**				
	S. aureus	*S. pyogenes*	*E. coli*	*S.Typhi*	*P. aeruginosa*
FMLE 500mg/ml	22.00[b]	18.00[b]	22.00[b]	15.00[b]	16.00[b]

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FMLE 250mg/ml	6.00[c]	0.00[c]	7.00[c]	8.00[c]	10.00[c]
FMLE 125mg/ml	0.00[c]	0.00[c]	0.00[d]	0.00[d]	0.00[d]
FMLE 63mg/ml	0.00[c]	0.00[c]	0.00[d]	0.00[d]	0.00[d]
Ethanol	0.00[c]	0.00[c]	0.00[d]	0.00[d]	0.00[d]
Ciproxin	30.00[a]	30.00[a]	24.00[a]	40.00[a]	29.00[a]
LSD	**6.55**	**6.64**	**0.95**	**6.55**	**0.95**

* Means in the same column having the same letter are not significantly different at P≤ 0.05, according to LSD test.

Table 3: Mean* diameter (mm) of zone of inhibition of dried Moringa leaf ethanolic extract (DMLE) and the controls on the test micro-organisms

EXTRACTS CONCENTRATION	MICROORGANISMS				
	S. aureus	***S. pyogenes***	***E. coli***	***S. Typhi***	***P. aeruginosa***
DMLE 500mg/ml	10.00[b]	20.00[b]	12.00[b]	12.00[b]	18.00[b]
DMLE 250 mg/ml	0.00[c]	0.00[c]	0.00[c]	0.00[c]	0.00[c]
DMLE 125 mg/ml	0.00[c]	0.00[c]	0.00[c]	0.00[c]	0.00[c]
DMLE 63mg/ml	0.00[c]	0.00[c]	0.00[c]	0.00[c]	0.00[c]
Ethanol	0.00[c]	0.00[c]	0.00[c]	0.00[c]	0.0 0[c]
Ciproxin	25.00[a]	30.00[a]	30.00[a]	35.00[a]	32.00[a]
LSD	**0.86**	**8.68**	**6.64**	**0.86**	**0.86**

*Means in the same column having the same letter are not significantly different at P≤0.05, according to LSD test.

Table 4: Mean*diameter (mm) of zone of inhibition of fresh *Moringa* leaf distilled water extract (FMLDW) and the controls on the test micro –organisms

EXTRACTS CONCENTRATION	MICROORGANISMS				
	S. aureus	***S. pyogenes***	***E. coli***	***S. typhi***	***P. aeruginosa***
FMLDW 500mg/ml	0.00[b]	28.00[a]	10.00[b]	20.00[b]	0.00[b]
FMLDW 250mg/ml	0.00[b]	10.00[b]	0.00[c]	0 .00[c]	0.00[b]
FMLDW 125mg/ml	0.00[b]	0.00[c]	0.00[c]	0.00[c]	0.00[b]
FMLDW 63mg/ml	0.00[b]	0.00[c]	0.00[c]	0.00[c]	0.00[b]
Distilled water	0.00[b]	0.00[c]	0.00[c]	0.00[c]	0.00[b]
Ethanol	0.00[b]	0.00[c]	0.00[c]	0.00[c]	0.00[b]
Ciproxin	20.00[a]	30.00[a]	28.00[a]	30.00[a]	35.00[a]
LSD	**6.72**	**6.55**	**0.86**	**8.68**	**0.67**

* Means in the same column having the same letter are not significantly different at P≤0.05, according to LSD test.

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Table 5: Mean* diameter (mm) of zone of inhibition of dried *Moringa* leaf distilled water extract (DMLDW) and the controls on the test bacteria.

Extracts Concentration	Microorganisms				
	S. aureus	***S. pyogenes***	***E. coli***	***S. typhi***	***P. aeruginosa***
DMLDW 500mg/ml	0.000^{b}	10.00^{b}	0.00^{b}	0.00^{b}	20.00^{b}
DMLDW 250 mg/ml	0.000^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{C}
DMLDW 125mg/ml	0.000^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{C}
DMLDW 63mg/ml	0.000^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{C}
Distilled water	0.000^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{C}
Ethanol	0.000^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{c}
Ciproxin	20.00^{a}	21.00^{a}	20.00^{a}	32.00^{a}	30.00^{a}
LSD	**6.72**	**0.86**	**6.72**	**0.67**	**8.68**

* Means in the same column having the same letter are not significantly different at P≤0.05, according to LSD test.

Table 6: Mean* diameter (mm) of zone of inhibition of test bacteria based on the extraction solvent.

Methods of Extraction & Concentration	Micro-organisms				
	S. aureus	***S. pyogenes***	***E. coli***	***S. typhi***	***P. aeruginosa***
FMLE 500mg/ml	22.00^{c}	18.00^{b}	22.00^{a}	15.00^{ab}	16.00^{a}
DMLE 500mg/ml	10.00^{e}	20.00^{b}	12.00^{c}	12.00^{b}	18.00^{a}
DMLDW 500mg/ml	0.00^{f}	10.00^{cd}	0.00^{e}	0.00^{c}	20.00^{a}
FMLDW 500mg/ml	0.00^{f}	28.00^{a}	10.00^{d}	20.00^{a}	0.00^{b}
LSD	**0.90**	**5.57**	**0.81**	**5.69**	**7.60**

* Means in the same column having the same letter are not significantly different at P<0.05, according to LSD test.

Table7a: The minimum inhibitory concentration (MIC) of fresh *moringa* leaf ethanolic extract (FMLE) on the test bacteria.

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

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Table 7b: The minimum bactericidal concentration (MBC) of fresh *moringa* leaf ethanolic extract (FMLE) on the test organisms.

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

Table 8a: The minimum inhibitory concentration (MIC) of dried *moringa* leaf ethanolic extract (DMLE) on the test organisms.

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

Table 8b: The minimum bactericidal concentration (MBC) of dried *moringa* leaf ethanolic extract (DMLE) on the test organisms.

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

Table 9a: The minimum inhibitory concentration (MIC) of fresh *moringa* leaf distilled water extract (FMLDW) on the test organisms.

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

Table 9b: 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	Negative Distilled, Water	Ethanol,	Positive Ciproxin
S. aureus	+	+	+	+	+	+	-
S. pyogenes	-	+	+	+	+	+	-
E. coli	-	+	+	+	+	+	-
S. typhi	-	+	+	+	+	+	-
P. aeruginosa	+	+	+	+	+	+	-

Table 10a: 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	Negative Distilled , Water	Ethanol	Positive Ciproxin
S. aureus	+	+	+	+	+	+	-
S. pyogenes	-	-	+	+	+	+	-
E. coli	+	+	+	+	+	+	-
S. typhi	+	+	+	+	+	+	-
P. aeruginosa	-	+	+	+	+	+	-

Table 10b: The minimum bactericidal concentration (MBC) of dried *moringa* leaf distilled water extract (DMLDW) on the test organisms.

Test	DMLDW Concentrations	Controls

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Organism	500mg /ml	250mg /ml	125mg /ml	63mg / ml	Negative Distilled, Water	Ethanol	Positive Ciproxin
S. aureus	+	+	+	+	+	+	-
S. pyogenes	+	+	+	+	+	+	-
E. coli	+	+	+	+	+	+	-
S. typhi	+	+	+	+	+	+	-
P. aeruginosa	+	+	+	+	+	+	-

DISCUSSION AND CONCLUSION

The phytochemical screening results of the aqueous and ethanolic extracts of fresh and dried *Moringa oleifera* leaves (Table1) revealed the presence of alkaloids, tannins, saponins, flavonoids. This agrees with the report of Kaufman *et al.*,(1989) and Vinoth *et al*., (2012) and may be responsible for all the medical activities of this plant (Dutta,1993). The phytochemical result of the ethanolic extracts of *Moringa oleifera* leaves in this study corroborates the reports by Bukar *et al.,* (2010). However, alkaloids and tannins reported in the present study were not determined by him. This may acount for the higher inhibitory activities recorded in this work.

The results of this research indicated that both the aqueous and ethanolic extracts of *Moringa oleifera* leaves exhibited antibacterial effect against all the test organisms, although with different levels of sensitivities to the extracts. The antibacterial properties of the leaf extracts of *M. oleifera* as revealed in this research agrees with the reports by Aktar *et al.,* (2006) and Foidl *et al.,* (2001) who reported that *M. oleifera* leaves possess antibacterial properties. This work showed that the ethanolic extracts of FMLE and DMLE had inhibitory effect against *S. aureus, E. coli, S. pyogenes, S. typhi* and *P. aeruginosa* which supports study by Nepolean *et al.,* (2009).

Generally and as in this study, the ethanolic extracts of the leaves of *M. oleifera* were more effective than the aqueous extracts, indicating that ethanol is a better solvent than water (Dutta ,1993; Ogunjobi and Nnadozie ,2004 and Ezeifeka *et al.,* 2004). Comparing the antibacterial activity of fresh and dried *Moringa* leaf it was discovered that the fresh leaf extracts was more effective than the dried one. It was also observed that virtually all the extracts showed appreciable inhibition against *P. aeruginosa* and *Salmonella typhi.*

The result of this research has demonstrated that *M. oleifera* leaf extracts have potential antibacterial effects on wound and enteric bacteria pathogens. Inhibition of both Gram-positive and Gram-negative organisms by this plant extract depicts that it can serve as a source of broad spectrum antibiotics, which justified the traditional use of this plant for therapeutic purposes.

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