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Evaluation of Antibacterial Activity and Preliminary Phytochemical Screening of *Moringa oleifera* on Pathogenic Bacteria

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Abstract

The use of higher plants and their extracts to treat infections is an old practice in traditional African medicine. However, scientific research has shown that bioactive compounds in plants are valuable medically in the treatment of infections caused by pathogenic microorganisms. This research work is aimed to evaluate the antibacterial potential of *Moringa oleifera* extracts on standard microorganisms strains as well as multi-drug resistant strains of medical importance. Acetone, aqueous, ethanol and chloroform extracts of bark, leaves and seeds of *Moringa oleifera* were investigated for antibacterial activity against *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Methicillin resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa* and *Proteus mirabilis.* The preliminary phytochemical screening and antibacterial assay were carried out using chemicals and agar well diffusion method respectively. The results of phytochemicals analysis revealed differences in the presence of alkaloids, reducing sugars, saponins and volatile oil in all the extracts. Tannins were present in the extract of leaves while terpenes were present in the extract of bark and leaves. Phlobatannins and flavonoids were absent in all the extracts. The antibacterial assay results showed that *M. oleifera* extracts exhibited broad spectrum activity against four to six bacteria isolates as indicated by the zone of inhibition ranging from 10 to 36mm with variation in the percentage sensitivity of < 100%, = 100% and >100% depending on the plant part and solvent used. The minimum inhibitory concentration (MIC) and bactericidal concentration (MBC) ranged from 100mg/ml to 450mg/ml and 250µg/ml to 500mg/ml respectively against the isolates used. Standard antibiotic disc (Ofloxacin- 5µµg) inhibited the growth of all the tested bacteria isolates except *P. mirabilis*. The results of this research work showed that *M. oleifera* has great potential as antibacterial compounds against Gram positive and Gram negative bacteria.
Keywords: Bioactive compounds, Acetone, ethanol, chloroform and aqueous plant extracts, Bacteria isolates, inhibition

1. Introduction

The use of higher plants and their extracts to treat infections is an old practice in traditional African medicine. Numerous plants or herbs are used all over Nigeria as an alternative medicine by traditional medicine practitioners in the treatment of infections or diseases caused by pathogens. About 70 to 80% of Africans depend on these for the treatment of some diseases as concoctions [1, 2]. However, most of the 21st Century therapeutic drugs used in the treatment of specific health problems were isolated and purified by scientists from the bioactive components of many plants. For example, vincrictine (an antitumor drug), digitalis (a heart regulator), and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants. However, some plant's secondary metabolites such as alkaloids, phenols, tannins, glycosides, terpenoids, saponins, flavonoids and steroids have also been implicated in their ability to inhibit the formation of pro-inflammatory signaling molecules such as prostaglandin or leukotrienes and antibacterial activity [3].

Moringa oleifera commonly referred to as "drumstick" or "horseradish" tree in English, "*Zogale or Bagaruwar maka*" in Hausa "*Ewe' Igbale or Ewe'Ile*" in Yoruba, "*Okwe oyibo"* in Igbo and "*Okoji- owowo"* (the whole tree) or '*ufu owowo'* (leaves) by Igede people of Benue State. It is the most widely cultivated species of the genus *Moringa,* which is the only genus in the family *Moringaceae.* It is an exceptionally nutritious vegetable tree with a variety of potential uses. Several researches have shown the bioactive compounds of *M. oleifera* to be nutritious, antihelmintic, antibacterial, antitumor, antipyretic, antiepileptic, ant-inflammatory, antiulcer, antispasmodic, anti-diuretic, antihypertensive, anti-cholesterol antioxidant, antidiabetic, hepato-protective and antifungal activities [4, 5]. This research work was aimed to

evaluate the antibacterial potential of *Moringa oleifera* extracts (leaves, stem-bark, and seeds) on standard microorganisms' strains as well as multi-drug resistant strains of medical importance.

2. Materials and Methods

2.1 Sample collection and preparation

The plant leaves, stem-bark and seeds were collected from the Botanical garden of Obafemi Awolowo University, Ile Ife, Osun State, Nigeria, and were brought to the Microbiology laboratory of the Federal Polytechnic, Ede, Osun State, Nigeria for analysis, where they were air dried for weeks, then crushed with mortar and pestle to smaller particles. After which they were grounded with electric blender to powder. Pure clinical isolates of *Pseudomonas aeruginosa, Proteus mirabilis, Bacillus cereus* and *Klebsiella pneumoniae, Methicilin resistant Staphylococuss aureus* (*MRSA*) and *Escherichia coli* were collected from the Department of Microbiology and Parasitology, Obafemi Awolowo University Teaching Hospital Complex, Ile Ife (O.A.U.T.H.C), Nigeria and were sub-cultured and maintained in the Microbiology Laboratory of the Federal Polytechnic, Ede prior to use.

Then 10g of the powdered leaves, stem-bark and seeds of the plant were percolated in 100ml of hot water, 99.8/100% acetone, ethanol and chloroform in 250ml conical flasks, stoppered with cotton wool, aluminium foil and sealed with paper tape respectively. The percolated samples were allowed to stand for 48 hours at room temperature. The percolates were filtered with filter paper. The extracts were concentrated by evaporation in rotary evaporator at 40˚C and later transferred to Bijou bottles where they were weighed accordingly. The extracts were stored at 4˚C in the refrigerator until when they were ready to be used.

2.2 Qualitative phytochemical analysis of the extracts

Phytochemical analysis for qualitative detection of alkaloids, flavonoids, saponins, tannins, phlobatannins, terpenes, volatile oils, reducing sugars in plant aqueous extract were carried out using the method described by [6].

2.3 Antibacterial activity of *Moringa oleifera* extracts on the bacterial isolates

The antibacterial activity of the plant extracts were assayed using Agar well diffusion technique [7]. The inocula were prepared from the stock culture, which were maintained on nutrient agar slant at 4˚C and sub cultured into peptone water using sterilized wire loop. To standardized the inocula, the density of the bacterial suspension were determined by comparison with 0.5 McFarland standard using colorimeter at 540nm (modified). This suspension was approximately 1.0 $x10^7$ CFU/ml [7, 8].

0.1ml of inocula were introduced into the sterile Mueller Hinton agar (Biotec) plates and were spread evenly with sterile bend glass rod; 8mm cork-borer was used to bore holes and 0.1ml of plant crude extracts were introduced into the holes accordingly, the inoculated plates were allowed to stand for one hour for proper diffusion of the extracts into the medium and were incubated at 37˚C for 24 hours. The inoculated plates were examined for zone of inhibition. Each zone of inhibition was measured in millimeter with a ruler at 90˚ to each other and the mean of the two readings were then calculated. Solvents used for the extraction of the plant materials and standard antibiotic disc (Ofloxacin 5µg) were used as control.

2.4 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts against bacterial isolates

The minimum inhibitory concentrations (MIC) of the extracts were determined using tube dilution technique [9]. Stock solution of known weight (1.6 to 5.0g) of each extract in 1ml of sterile distilled water were serially double diluted to achieve various concentrations in µg/ml. 5ml of sterile peptone water was dispensed aseptically in to sterile test tubes, 0.1ml of each inoculum and extract was introduced. The inoculated tubes were incubated at 37˚C for 24 hours. The tubes were checked for visible turbidity which was read with colorimeter at 540nm. Minimum Bactericidal Concentration (MBC) was determined by sub culturing from the tubes with no significant growth into sterile molten Mueller Hinton agar plates and was incubated at 37˚C for 24 hours. The plate with no significant growth was taken as minimum bactericidal concentration.

3. Results

Preliminary phytochemicals screening of the extracts revealed the presence of alkaloids, reducing sugars, saponins and volatile oils in all the extract of leaves, stem-bark, and seeds; tannins were present in the leaves, terpenes were present in the extract of bark and leaves while phlobatannins and flavonoids were absent in all the plant extracts (table 1).

The results of the zone of inhibition in millimeter (mm) of antibacterial activity of *M. oleifera* extracts on clinical bacteria isolates presented (table 2). The percentage potency or sensitivity of the extracts compared with the standard ofloxacin antibiotic disc (5µg) were calculated (table 3), [10]. The results showed that *M. oleifera* bark crude extracts had

the broadest spectrum activity against the tested bacteria isolates. It showed that, it had an activity against four to six bacteria isolates; the next extract with broad spectrum activity was the leaves extracts which had activity against two to six isolates while the seeds extracts had the least broad spectrum activity against one to five bacteria isolates.

The results of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts against the bacteria isolates were determined. The results showed significant reduction or inhibition at minimum concentration ranging from 100 to 450µg/ml and bactericidal effect at concentration ranging from 250 to 500µg/ml on the isolates investigated.

Table 1: Preliminary phytochemicals analyses of *Moringa oleifera* aqueous extracts

Phytochemicals	Bark extract	Leave extract	Seed extract
Alkaloids	+	+	+
Flavonoids	-	-	-
Phlobatannins	-	-	-
Reducing sugar	+	+	+
Saponins	+	+	+
Tannins	-	+	-
Tarpenes	+	+	-
Volatile oil	+	+	+

Key: +: Present and -: Absent

Table 2: Antibacterial activity of crude extracts of *Moringa oleifera* on the bacteria isolates

		Diameter of Zone of Inhibition (mm)					
Solvent used	**Plant parts**	***B. cereus***	***E. coli***	***K. pneumoniae***	***MRSA***	***Ps. aeruginosa***	***P. mirabilis***
Acetone	Control	-	-	11	12	09	-
	Bark	22	-	16	15	17	-
	Leaves	18	-	15	15	-	-
	Seeds	-	-	19	-	-	-
Ethanol	Control	23	26	-	25	13	14
	Bark	30	23	12	21	21	22
	Leaves	29	27	16	20	24	25
	Seeds	18	21	29	25	29	-
Chloroform	Control	18	12	14	10	16	-
	Bark	22	13	-	14	12	-
	Leaves	25	20	-	16	10	-
	Seeds	23	17	24	17	15	-
Aqueous	Control	-	-	-	-	-	-
	Bark	15	12	-	11	12	-
	Leaves	16	-	-	19	-	-
	Seeds	36	-	-	34	-	-
Standard Antibiotic	Ofloxacin (5µg)	15	23	28	28	11	-

Key: Zone of inhibition included 8mm cork-borer, -: No inhibition, *MRSA = Methicillin Resistant Staphylococcus aureus.*

Table 3: Percentage sensitivity (potency) of the extracts on the bacterial isolates

Solvent Used	**Plant Parts**	% sensitivity of the extracts against isolates					
		B. cereus	***E. coli***	***K. pneumoniae***	***MRSA***	***Ps. aeruginosa***	****P. mirabilis***
Acetone	Control	0.0	0.0	39.3	42.9	81.8	0.0
	Bark	146.7	0.0	57.1	53.6	154.6	0.0
	Leaves	120	0.0	53.6	53.6	0.0	0.0
	Seeds	0.0	0.0	67.9	0.0	0.0	0.0
Ethanol	Control	153.3	113.0	0.0	89.3	118.2	ND
	Bark	200.0	100.0	42.9	75.0	190.9	ND
	Leaves	193.3	117.4	57.1	71.4	218.2	ND
	Seeds	120.0	91.3	103.6	89.3	263.6	0.0
Chloroform	Control	120.0	53.2	50.0	35.2	145.5	0.0
	Bark	146.7	56.5	0.0	50.0	90.9	0.0
	Leaves	166.7	87.0	0.0	57.1	145.5	0.0
	Seeds	153.3	73.9	85.7	60.7	136.4	0.0
Aqueous	Control	0.0	0.0	0.0	0.0	0.0	0.0
	Bark	100.0	52.2	0.0	39.3	109.1	0.0
	Leaves	106.7	0.0	0.0	67.9	0.0	0.0
	Seeds	156.5	0.0	0.0	121.4	0.0	0.0

Key: * Resistant to standard ofloxacin antibiotic and most extracts, < 100% less potent, 100% equipotent, >100% most potent and ND = Not determined

$$\% \text{ sensitivity} = \frac{\text{Diameter of zone of inhibition of extracts}}{\text{Diameter of zone of inhibition of standard antibiotic disc}} \times 100 \quad [9].$$

4. Discussion

Moringa oleifera qualitative phytochemical screening revealed the presence of alkaloids, reducing sugars, saponins and volatile oils in all the extract of leaves, stem-bark, seeds and pods in agreement with the report by [4]. Tannins was present in the leaves in agreement with the report by [4], terpenes was present in the extract of bark and leaves. Phlobatannins (this was not determined by [4] and flavonoids were absent in all the extracts against the report by [4]. The disparity that occurred between the present study and the report of [4] could be as a result of variation in the habitat and threat encountered by the plant, as supported by [11] which reported that plants occurred in varying habitat with a great magnitude of variation in the concentration and composition of phytochemicals in different parts of the plant. However, it has been reported that phytochemicals are produced in response to perceived threats by the plants; hence variation could exist in the production of these phytochemicals or bioactive compounds depending on the nature and amount of threat encountered by the plants. It is documented that phytochemicals in plants based-foods can improve glucose metabolism as well as enhance the overall health of diabetic patients by improving lipid metabolism, antioxidant status, improving capillary function and lowering blood pressure and cholesterol [12].

The results of antibacterial activity showed the zone of inhibition in millimeter (mm) and percentage sensitivity or potency of *M. oleifera* extracts on pathogenic bacteria isolates were calculated [10]. The sensitivity of <100%, 100% and >100% observed in the present study indicated the potency of the extracts to be less, equipotent and more potent respectively when compared with the standard ofloxacin antibiotic disc used. *Moringa oleifera* bark crude extract had the broad spectrum activity against the tested bacteria isolates with zone of inhibition ranging from 12 to 30mm against four to six bacteria isolates. Acetone bark crude extract (ABE) inhibited the growth of *Bacillus cereus* 22mm(146.7%), *Klebsiella pneumoniae* 16(57.1%), *Pseudomonas aeruginosa* 17(154.6%) and *methicillin resistant Staphylococcus aureus MRSA* 15(53.6%), while *E. coli and Proteus mirabilis* were not susceptible to the acetone bark crude extract used. Chloroform crude extract of bark (CBE) inhibited *B. cereus* 22(146.7%), *E. coli* 13(56.5%), *MRSA* 14(50.0%) and *Ps. aeruginosa* 12(109.1%) while *K. pneumoniae* and *P. mirabilis* were not susceptible to the extract. *M. oleifera* ethanol bark crude extract (EBE) inhibited the growth of all the bacteria isolates: *B. cereus* 30(200.0%), *E. coli* 23(100%), *K. pneumoniae* 12(42.9%), *Ps. aeruginosa* 21(190.9%), *P. mirabilis* 21(ND) and *MRSA* 22(75.0%). Aqueous crude extract of bark (WBE) inhibited the growth of *B. cereus* 15(100%), *E. coli* 12(52.2%), *MRSA* 11(39.3%) *and Ps. aeruginosa* 12(109.1%) while *K. pneumoniae and P. mirabilis* were not susceptible to the extract.

M. oleifera acetone leaves extract (ALE) showed activity against *B. cereus* 18(120%), *K. pneumoniae* 15(53.6%), *MRSA* 15(53.6%) but was unable to inhibit the growth of *E. coli, Ps. aeruginosa and P. mirabilis*.. *M. oleifera* ethanol leaves crude extract (ELE) showed the broad spectrum activity against *B. cereus* 29(193.3%), *E. coli* 27(117.4%), *K. pneumoniae* 16(57.1%), *Ps. aeruginosa* 20(71.4%), *P. mirabilis* 24(ND) and *MRSA* 25(218.2%). [4][13] Also reported *Ps. aeruginosa, E. coli* and *S. aureus* to be susceptible to *M. oleifera* ethanol leaves extract. Chloroform leaves crude extract (CLE) inhibited the growth of *B. cereus* 25(166.7%), *E. coli* 20(87.0%), *MRSA* 16(50.0%) and *Ps. aeruginosa* 10(90.9%), while *K. pneumoniae and P. mirabilis* were not susceptible to chloroform leaves crude extract (CLE). This is supported by the report of [4][14] who reported that *Moringa oleifera* leaves ethanol and chloroform extracts showed activity against *E. coli, Ps. aeruginosa* and *S. aureus*. Aqueous leaves crude extract (WLE) was active against *B. cereus* 16(106.7%) and *MRSA* 19(67.9%)*; E. coli, K. pneumoniae, Ps. aeruginosa* and *P. mirabilis* were not susceptible to aqueous leaves crude extract (WLE). [15] Also reported that aqueous leaves extract of *M. oleifera* inhibited the growth of *Ps. aeruginosa and S. aureus;* however the strain of the *S. aureus* was not stated.

M. oleifera acetone seeds crude extract (ASE) inhibited the growth of only *K. pneumoniae* 19(67.9%) with MIC of 150µg/ml. Chloroform seeds crude extract (CSE) was active against five bacteria isolates: *B. cereus* 23(153.3%), *E. coli* 17(73.9%), *MRSA* 17(60.7%), *K. pneumoniae* 15(85.7%) and *Ps. aeruginosa* 24(136.4%) while *P. mirabilis* was not susceptible to the extract used. [4] Reported that *E. coli* was susceptible to chloroform seed extract (CSE) while *Ps. aeruginosa* was resistance to it. However, the present study showed *Ps. aeruginosa* to be susceptible to CSE of *M. oleifera,* the disparity observed here could be due to the source of the isolate, incubation temperature, the medium used and bioactive compounds in the extracts [4]. Ethanol seeds crude extract (ESE) of *M. oleifera* was active against five bacteria isolates: *K. pneumoniae* 29(103.6%), *Ps. aeruginosa* 29(263.6%), *E. coli* 21(91.3%), *B.* cereus 18(120%) and *MRSA* 25(89.3%) however, *P. mirabilis* was not susceptible to the extract. Aqueous seeds extract of *M. oleifera* (WSE) was active on *B. cereus* 36(156.5%) and *MRSA* 34(121.4%) While *E. coli, K. pneumoniae, Ps. aeruginosa* and *P. mirabilis* were resistance to the extract. This supports the earlier report by [16] that aqueous seeds extract of *M. oleifera* inhibited the growth of *Ps. aeruginosa and S. aureus.* However, there is disparity with the findings of [16] in the present study where *Ps. aeruginosa* was not inhibited. The variation that occurred in this research with that of [16] may be attributed to some factor such as the source of the isolate, incubation condition, the medium and potency of the extract used.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts showed activity against most of the isolates ranging from 100 to 450μg/ml and 250 to 500μg/ml respectively. High MIC and MBC observed against most bacteria isolates may be an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds since the extracts were not purified. However, further purification could enhance its antibacterial activity at low concentration.

The results of the antibacterial activity showed that *M. oleifera* bark extract had high broad spectrum activity and potency against the bacteria isolates; this could be attributed to the presence of large number of phytochemicals which could also act as an antagonist to each other since the extracts were not purified and isolated. Seed extracts had least broad spectrum activity against the bacteria isolates and high potency against some specific isolates as indicated by the percentage sensitivity (table 3). The inability of seeds extracts to inhibit wider number of bacteria isolates might be linked to its phytochemicals contents as few were present. However, the high potency of these extracts could be that they were rich in active ingredients. The standard antibiotic (Ofloxacin 5μg) used as control inhibited the growth of all the bacteria isolates with zone of inhibition ranging from 11 to 28mm except *P. mirabilis.* The ability of ethanol bark and leaves crude extracts to inhibit *P. mirabilis* could be as a result of the large number of phytochemicals, possibly terpenes which was absent in the seeds and the ability of ethanol to extract the active ingredients from the plant more than the other solvents.

The antibacterial properties of *M. oleifera* extracts as shown in the present study corroborate the claims by [4, 17, 18, 19]. The antibacterial activity of *M. oleifera* seeds was suggested to be due to the presence of an array of phytochemicals such as short polypeptide, named 4 -(α-L- rhamnopyranosyloxy) benzyl-isothiocyanate [20] as reported by [4]. The peptide may act directly on microorganisms and result in growth inhibition by disrupting cell membrane synthesis or synthesis of essential enzymes [4, 21, 22].

This research work also highlighted that the organic solvent extracts exhibited greater antibacterial activity than aqueous extracts because the antimicrobial principles may be either polar or non-polar which were extracted through the organic solvent and aqueous medium [23]. In addition, water may have a low penetration and extraction ability to extract high amount of active ingredients from the plant compared to organic solvents. The present observation suggests that the organic solvents and aqueous extraction were suitable to verify the antimicrobial properties of medicinal plants and this was supported by many investigators [24- 26] as reported by [27]. The fact that the *M. oleifera* extracts were active against both Gram positive and Gram negative bacteria tested may indicate its broad spectrum therapeutic activity.

5. Conclusion

The results of this research work showed that *Moringa oleifera* has great potential as antibacterial compounds against both Gram positive and Gram negative bacteria. Hence, it is a potential source for production of drugs with a broad spectrum activity for the treatment of gastroenteritis, urethritis, otitis media, wound infections, bronchopneumonia, septicemia, osteomyelitis, peritonitis and diarrhoea caused by these pathogenic bacteria. The resistance of some of these organisms in this work to some extracts may be due to the fact that the phytochemicals present in the extracts had little or no effect on those bacteria isolates. Therefore, further pharmacological evaluations, purification, modification, toxicological studies and possible isolation of the therapeutic antibacterial from this plant should be done to enhance its ability to inhibit or destroy the growth of microorganisms at low concentration.

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