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Antimicrobial activity of Moringa oleifera leaf extracts on multiple drug resistant bacterial isolates from urine samples in Benin City

*Eremwanarue, O. A.[1,2] and Shittu, H. O.[1]

[1]Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria
[2]Lahor Research Laboratories and Diagnostics Centre, 121, Old Benin-Agbor Road, Benin City, Nigeria

Abstract

The aim of this study was to examine the antibacterial effect of *Moringa oleifera* leaf extracts on selected multiple drug resistant (MDR) bacterial isolates. *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were isolated urine samples from patients attending Lahor Research Medical Centre. Multiple drug resistant bacteria were generated from the isolates by carrying out antibiotic susceptibility testing using the Kirby-Bauer disc diffusion technique. Three isolates each from the multidrug resistant bacteria were selected and molecular characterization was performed for confirming microbial identity. The antibacterial activity of methanol, chloroform and aqueous leaf extracts of *M. oleifera* at different concentrations (6.25, 12.5, 25, 50 and 100 mg/ml) were analyzed on the selected MDR bacteria using agar disc diffusion method. M. oleifera leaf extracts were observed to inhibit the growth of multidrug resistant bacteria. The highest antibacterial activity 9.32±1.45 mm was observed with the chloroform extracts, while the lowest value of 0.27±0.27 mm was obtained for the aqueous leaf extract. The antibacterial activity examined in this study showed that chloroform and methanol *M. oleifera* leaf extracts are capable of exerting inhibitory effect on multidrug resistant bacteria. The results obtained in this study indicated that *M. oleifera* can be a potential source for the treatment of different infections caused by multiple drug resistant bacteria.

Keywords: Urine, Multidrug resistant bacteria, polymerase chain reaction, *Moringa oleifera*, antimicrobial

Correspondence author: eremwanarueaibuedefe@yahoo.com, **Phone:** 08037945407

Introduction

In Africa and other continents of the world, phytomedicine has been in use from time immemorial to take care of various diseases long before the introduction of modern medicine. Herbal medicine is still widely used in many parts of the world especially in areas where people do not have access to modern medicine (Ajibade et al., 2005). Plants have been reported to contain large varieties of chemical substances that possess important defensive and remedial therapies (Nascimento et al., 2000). About 80 % of individuals from developed countries use conventional medicine, which have compounds derived from medicinal plants (Igbinosa et al., 2009). Regardless of the presence of different approaches to drug discovery, plants still remain the key reservoir of natural medicine (Mahomed and Ojewole, 2006).

Consideration in plants with antimicrobial properties has been rejuvenated as a result of resistance to routine antibiotics. Multiple drug resistance can simply be defined as the ability of a microorganisms to resist the effect of more

than one antibiotic or groups of antibiotics to which it was formally susceptible to either by the production of enzymes that alter the structure of the antibiotics, by the modification of the antibiotics, by bypassing certain pathways or by acquisition of resistant plasmids from other bacteria strain. Resistance can either be plasmid, chromosome mediated or both plasmid and chromosome mediated. This resistance could be ascribed to indiscriminate use of commercial drugs or not taking an antibiotic prescription according to instruction (Aliero and Afolayan, 2006). In addition, certain antibiotics present undesirable side effects such as nausea, depression of bone marrow leading to the emergence of previously uncommon diseases (Poole, 2002). This has given scientists the drive to search for newer and alternative antimicrobial compounds from medicinal plants (Aliero and Afolayan, 2006). Besides, the high cost of conventional drugs, particularly in resource inadequate communities were access to good health is costly for the common citizens has led to increased use of plants as alternative for treatment of some diseases. In recent years, interest has grown in the utilization of what has come to be known as "multipurpose" plants; one of such plants is *Moringa oleifera* Lam, the most widely cultivated species of a monogeneric family Moringaceae (Shittu et al., 2017). All parts of *Moringa* tree are edible and have been consumed by humans for its remarkable array of medicinal uses and high nutritional values (Mohammad et al., 2012). The study was aimed at investigating the antimicrobial effect of aqueous, ethanol and chloroform leaf extracts of *Moringa oleifera* on selected multiple-drug resistant bacteria isolates.

Materials and Methods

Clinical sample collection

A total of 70 urine specimens were randomly collected from patients attending Lahor Research Medical Centre, 121, Old Benin-Agbor Road, Benin City. Urine specimens were collected in sterile universal bottles.

Plant sample collection

Fresh leaves of *Moringa oleifera* were collected from a garden at Egor Local Government Area, Benin City, Edo State, Nigeria. The leaves were identified at the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Nigeria.

Ethical clearance

Approval was obtained from the Medical Directors of the hospitals whose patients participated in this study and the patients gave their consent after being informed of the objectives of the study.

Bacteriological procedures/identification of isolates

Specimens were aseptically inoculated onto MacConkey, Blood and Nutrient agars and incubated aerobically at 37°C for 24 hours and observed for colonial growth. All isolates were identified based on their colonial appearances on MacConkey agar and nutrient agar plates. Isolates were identified using the morphological and biochemical test (Cheesbrough, 2000) and confirmation was done using polymerase chain reaction technique.

Antibiotic susceptibility testing

Susceptibility to antibiotics was assessed using the Kirby-Bauer disc diffusion method, and clear area around the antibiotic discs referred to as zones of inhibition were read using the Clinical and Laboratory Standards Institute's guidelines (CLSI, 2010).

DNA extraction from multiple drug resistant Bacterial isolates

Multiple drug resistant bacteria isolates were sub-cultured overnight in Luria-Bertani broth (Merck, Germany) and genomic DNA was extracted from typical colonies of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* and positive control strains obtained from Sigma-Aldrick (United Kingdom) using a DNA extraction kit (Zymo research fungi/bacterial DNA MiniPrep), Single colony from Nutrient agar plate was inoculated into 3 ml of Laura broth and incubated at 37 °C overnight. Aliquot of 1ml of the overnight culture was transferred into a microcentrifuge tube. The overnight culture was centrifuged at 13000 rpm for 3 minutes and the supernatant was discarded. The pellet was resuspended in 200 µl

of nucleases free water and was then transferred into a ZR BashingBead lysis tube where Lysis solution (750 µl) was added. ZR BashingBead lysis tube was then centrifuged in a microcentrifuge at 10,000 x g for 1 minute. Aliquot of 400 µl of the supernatant was pipetted into a Zymo-spin IV spin filter in a collection tube and centrifuge at 7,000 rpm for 1 minute. Fungal/Bacterial DNA binding buffer (1,200 µl) was added to the filtrate in the collection tube. The mixture was centrifuged at 10,000 x g for 1 minute. Pre-wash buffer (200 µl) was added and centrifuge at 10,000 x g for 1 minute. Aliquot of 500 µl of the fungal/bacterial DNA wash buffer was added to the Zymo-spin IIC column and Centrifuged at 10,000 x g for 1 minute. The Zymo-spin IIC column was transferred to a clean 1.5 ml microcentrifuge tube and 80 µl of DNA elution buffer was added directly to the column matrix and centrifuge at 10,000 x g for 30 seconds to elute the DNA

Polymerase Chain Reaction for the detection of Bacterial isolates

Amplification of bacteria species-specific genes were carried out using Polymerase Chain Reaction (PCR) technique. The details of the primers used in the study are given in Table 1. Quick load One Taq one step PCR master mix (2X) was purchased from lnqaba Biotech, Hartfield, South Africa Incorporated and used according to the manufacturer's instruction. The PCR was performed in a 25 µl reaction mixture containing 12.5 µl Quick load One Taq one- step PCR master mix (2x), 1.25 µl of each species-specific forward primer (20 µM), 1.25 µl of each species-specific reverse primer (20 µM), 5 µl of nuclease free water and 5 µl of DNA template was added last. The PCR was conducted under the following conditions: Initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing temperature for the different primers are shown in Table 1 for 30 seconds, extension at 72°C for 1min and a final extension at 72°C for 15 minutes and final holding at 4°C. Ten microliters (10 µl) of the amplified PCR products of test isolates and positive control strain were fractionated on a 1.0% agarose gel containing ethidium bromide in Tris/Borate/EDTA (TBE) Buffer along side negative control containing nuclear free water and DNA loading dye. Electrophoresis was performed at 90 volts for 60 minutes. Products were visualized by Wealth Dolphin Doc UV transilluminator and photographed. Amplicons molecular weights were calculated using molecular weight standard maker.

Preparation of Moringa oleifera leaf extracts

Leaves of M. oleifera were washed with running tap water and then air dried at room temperature for 15 days (Shobayo et al., 2014). Ten gram (10 g) of the powdered leaves was separately extracted in 500ml conical flasks with 100ml of deionised distilled water (aqueous extraction), 100ml of 99% methanol (methanolic extraction) and 100ml of 99% chloroform (chloroform extraction). The conical flasks were plugged with rubber corks and allowed to stand at room temperature for two weeks with intermittent shaking twice a day (Bukar et al., 2010). The extracts were separately filtered using sterile Whatman No. 1 filter paper. The resulting filtrate obtained were then stored in a refrigerator at 4°C (Akueshi et al., 2002) which were used as experimental drug for the present study.

Standardization of inoculums

The standard was prepared from the stock cultures, maintained on nutrient agar slant at 4°C and subcultured onto a nutrient broth using a sterilized wire loop. The density of the suspension inoculated onto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (Cheesbrough, 2002).

Screening of Moringa oleifera leaf extracts for antibacterial activity

Moringa oleifera aqueous, methanol and chloroform extracts were tested against the MDR bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*) by agar well diffusion method, as described by Makanjuola et al. (2013). Then, 25 ml sterile-molten Mueller Hinton Agar (Watin-Biolife, KSA) was loaded on the Petri-dish and left at room temperature to solidify. After that, wells were punched into the agar with a sterile cork borer (6 mm in diameter). Aliquot of 0.1 ml of the aqueous extract with different concentrations of

6.25, 12.50, 25, 50, 100 mg/ml was loaded into the wells and same procedure was applied for the methanol and chloroform extract. 100 µl abolute ethanol and chloroform were used as a positive control. All plates were kept in the incubator for 24 hours at 37°C. Tests were repeated three times and the mean zone of inhibition was calculated.

Statistical Analysis

Experiments were performed in triplicate and results were presented as mean ± standard error. The data obtained were subjected to parametric and descriptive statistics using the Statistical Package for the Social Sciences (SPSS) version 20 software (Chicago, IL, USA). Means were separated by the Tukey's multiple range test when Anova was significant ($P<0.05$).

Table 1: Summary of the primers used for the identification of multiple drug-resistant bacteria

Name of organism	Primer code	Primer sequence 5`-3´	Annealing temperature (°C)	Expected amplicon size (bp)
E. coli	URF -301- URR-432-	TGTTACGTCCTGTAGAAAGCCC AAAACTGCCTGGCACAGCAATT	55	154
P. aeruginosa	Pa16S-F- Pa16S-R-	GGGGGATCTTCGGACCTCA TCCTTAGAGTGCCCACCCG	59	956
S. aureus	nuc-1- nuc-2-	TCAGCAAATGCATCACAAACAG CGTAAATGCACTTGCTTCAGG	53	225

Results and Discussion

In a total of 70 bacterial cells isolated from urine samples obtained from patients attending Lahor Research Medical Centre, 13 (18.6%) isolates were *Escherichia coli* *Escherichia coli* which produces pink colonies on MacConkey agar and *E. coli* appeared colorless on nutrient agar; 10 (14.3%) were *Pseudomonas aeruginosa*, which were rod shape, motile, catalase positive, gram negative, produced pale colored colonies on MacConkey agar and greenish colonies on nutrient agar and 8 (11.4%) were *Staphylococcus aureus* isolates that were found to be non-motile, catalase negative and gram positive cocci, produce golden yellow color on mannitol salt agar plate as shown in Figure 1.

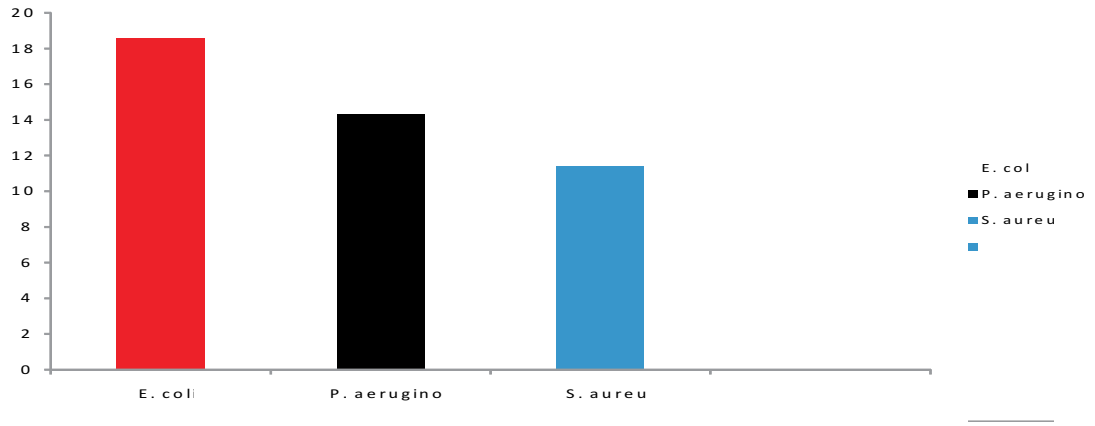

Figure 1: Percentage Bacterial isolation from urine samples

The use of conventional antibiotic discs indicated high rate of MDR bacterial isolates. Three isolates among those found to be resistant to multiple antibiotics for each organism were selected and designated as EC1, EC5, EC7 for *Escherichia coli*, PA2, PA3, PA8 for Pseudomonas aeruginosa and SA1, SA4 and SA6 for Staphylococcus aureus (Table 2).

Table 2: Antibiotic sensitivity testing of *Escherichia coli* and *Pseudomonas aeruginosa*

Isolates	Antibiotic zone of inhibition (mm)							
	AUG	OFL	CXM	GEN	CRX	CAZ	CPR	NIT
EC1	0.00±0.00	0.00 ± 0.00	0.00±0.00	0.00 ± 0.00	0.00±0.00	0.00 ± 0.00	0.00 ± 0.00	14.00±0.95
EC5	0.00±0.00	0.00 ±0.00	0.00±0.00	0.00 ±0.00	0.00±0.00	0.00 ±0.00	0.00 ±0.00	26.10±0.22
EC7	0.00±0.00	0.00 ±0.00	0.00±0.00	0.00 ±0.00	0.00±0.00	0.00 ±0.00	0.00 ±0.00	18.20±0.56
PA2	0.00±0.00	20.10±0.34	0.00±0.00	0.15±0.45	0.00±0.00	15.08±0.40	25.10±0.20	0.00±0.00
PA3	0.00±0.00	18.20±0.33	0.00±0.00	0.12±0.25	0.00±0.00	0.00±0.00	23.32±0.11	0.00±0.00
PA8	0.00±0.00	14.12±0.30	0.00±0.00	18.20±0.41	0.00±0.00	0.00±0.00	0.11±0.18	0.00 ± 0.00

Mean ± standard error of means, AUG: Augmentin, OFL: Ofloxacin, CXM: Cefixime, GEN: Gentamycin, CRX: Cefuroxime, CAZ: Ceftazidime, CPR: Ciprofloxacin, NIT: Nitrofurantion, EC1: *Escherichia coli* isolate 1, EC2: *Escherichia coli* isolate 2, EC3: *Escherichia coli* isolate 3, PA2: *Pseudomonas aeruginosa* isolate 2, PA3: *Pseudomonas aeruginosa* isolate 3, PA8: *Pseudomonas aeruginosa* isolate 8

Multiple drug resistant *Escherichia coli* (EC1, EC5 and EC7) were resistant against seven antibiotics used and only sensitive to Nitrofurantion with EC5 having the highest zone of inhibition of 26.10±0.22 mm and 14.00±0.95 mm was recorded for EC1. All *P. aeruginosa* isolates showed resistant against five antibiotics (AUG, CXM, CRX, CAZ, NIT) and sensitive to OFL, GEN, CPR. Highest zone of inhibition of 25.10±0.20 mm for ciprofloxacin were observed in PA2 and the lowest were seen in gentamycin for PA2 and ofloxacin for PA8 with 10.15±0.45 mm and 14.12±0.30 mm. In a study carried out by Indu et al. (2014), it was reported that 36.2% (21/58) *P. aeruginosa* strains were MDR and they were categorized into antibiotypes 1, 3 and 5.

It was observed from the result that SA1 were resistant against six antibiotic discs (CAZ, CRX, CTR, ERY, CXC, AUG) used and was sensitive to GEN and OFL (Table 3).

Table 3: Antibiotic sensitivity testin of *Staphylococcus aureus*

Isolates	Antibiotic zone of inhibition (mm)							
	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG
SA1	0.00±0.00	0.00±0.00	19.21±0.44	0.00 ±0.00	0.00 ±0.00	0.00±0.00	14.17±0.35	0.00±0.00
SA4	0.00±0.00	0.00±0.00	10.20±0.48	14.15±0.87	0.00±0.00	0.00±0.00	14.18±0.76	0.00±0.00
SA6	0.00±0.00	0.00±0.00	15.14±0.18	0.00 ±0.00	14.43±0.00	0.00±0.00	24.25±0.00	0.00±0.00

Mean ± standard error of means, CAZ: Ceftazidime, CRX: Cefuroxime, GEN: Gentamycin, CTR: Ceftriaxone, ERY: Erythromycin, CXC: Cloxacillin, OFL: Ofloxacin, AUG: Augmentin, SA1: *Staphylococcus aureus* isolate 1,

SA4 were resistant against five antibiotic discs which include CAZ, CRX, ERY, CXC and AUG, while SA6 were resistant against five antibiotics discs which are CAZ, CRX, CTR, CXC, AUG and sensitive to GEN, ERY, OFL. Among all the three *S. aureus* isolates, the highest zone of inhibition were recorded for SA6 ofloxacin with 24.25±0.00 mm and the lowest zone for gentamycin with 10.20±0.48 mm were recorded for SA4. Karthy et al. (2009) reported that all 12 *Staphylococcus aureus* isolates studied were multidrug resistant strains; resistant to at least 6, out of 12 antibiotics. Lister et al. (2009) reported that indiscriminate use of antibacterials without *in vitro* sensitivity testing of organisms resulted in multidrug resistant bacteria.

Molecular identification of MDR bacteria using PCR showed that EC1, EC5 and EC7 were positive for *Escherichia coli* with 154 base pair (bp) fragment size alongside positive control (Plate 1). Bej et al. (1991) reported the same result in the detection of low levels of

microorganisms in environmental samples by using PCR. Successful PCR amplifications were achieved for *Pseudomonas aeruginosa* (PA2, PA3 and PA8) with amplicon size of 956bp as same were recorded for the positive control (Plate 2) using specific set of primers (PA-SS) according to Neha et al. (2011). Isolates SA1, SA4 and SA6 were *Staphylococcus aureus* with amplicon size of 225bp. Thus, all the isolates were confirmed as *Staphylococcus aureus* with appropriate positive control (Plate 3). This was in line with the report of Poulsen et al. (2003).

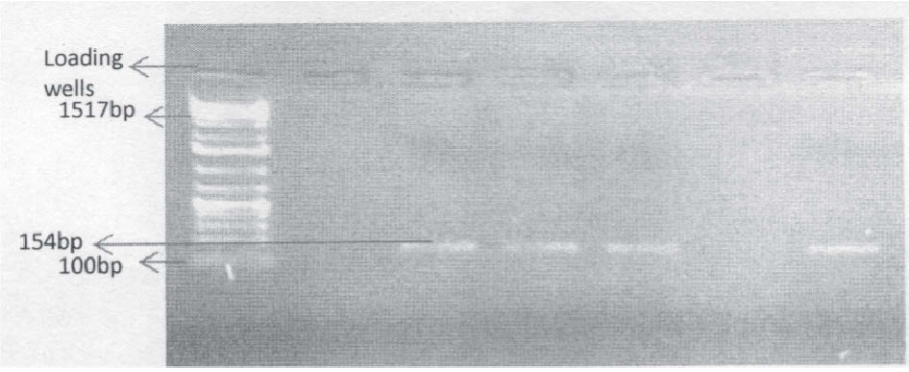

Plate 1: Molecular identification of *Escherichia coli* using polymerase chain reaction technique. L: 100-1517bp DNA ladder; NC: Negative control; PC: Positive *Escherichia coli* control strain with American type culture collection number 25295.

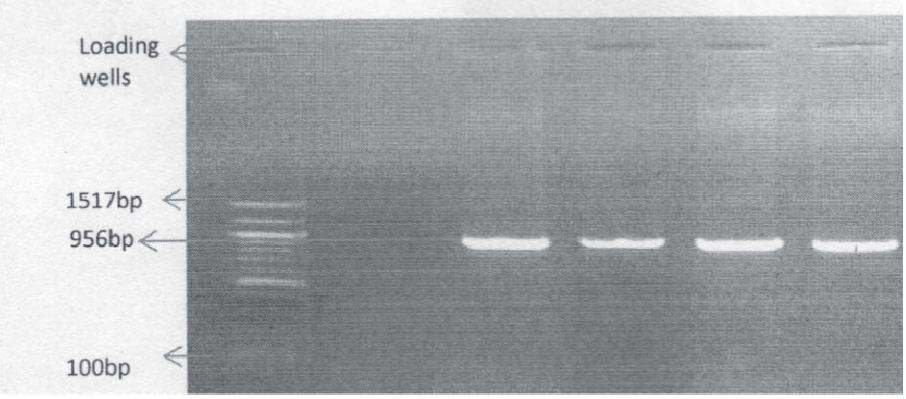

Plate 2: Molecular identification of *Pseudomonas aeruginosa* using polymerase chain reaction technique. L: 100-1517bp DNA ladder; NC: Negative control; PC: Positive *Pseudomonas aeruginosa* control strain with American type culture collection number **27852**.

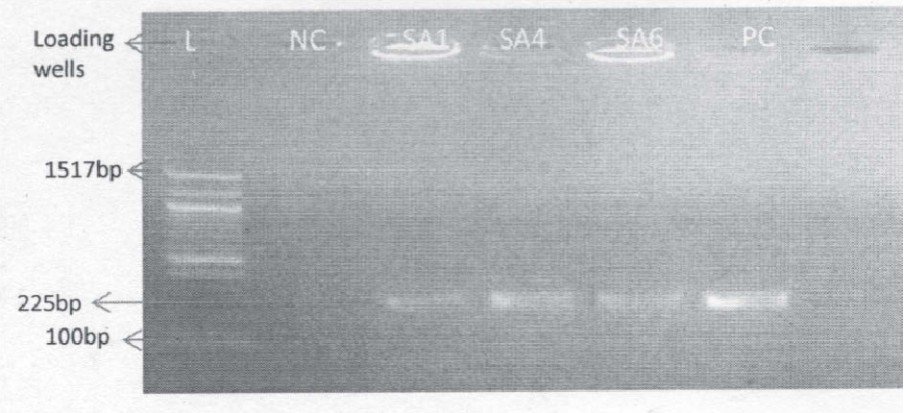

Plate 3: Molecular identification of *Staphylococcus aureus* using polymerase chain reaction technique. Isolates L: 100-1517bp DNA ladder; NC: Negative control;y PC: positive *Staphylococcus aureus* control strain with American type culture collection number **29212**.

The result of the antibacterial activity of *M. oleifera* leaf extracts against three isolates of MDR *E. coli* using aqueous, methanol and chloroform at different concentrations (Table 4) exhibited different degrees of antibacterial activities when compared with the control. For Chloroform extract, there was considerable effect at 6.25 and 12.50 mg/ml with 1.80±0.50 and 3.48±0.86 mm; significant effect was seen at 25 and 50 mg/ml which were higher when compared with the control. Highest mean zone of inhibition of 9.32±1.45 mm was recorded at 100 mg/ml. Methanol extract at concentrations of 6.25, 12.50 and 25 mg/ml, no zones of inhibitions were observed but at 50 mg/ml, the effect were same as the control followed by 100 mg/ml with 7.09±0.50 mm zone of inhibition. It was clear that there were no effect observed at 6.25, 12.50 and 25, 50 mg/ml for aqueous extract meanwhile, mean zone of inhibition of 1.37±0.61 were recorded at 100 mg/ml as shown in Plate 4. Akhtar et al. (2012) reported that *M. oleifera* ethanol leaf extract showed moderate effect against all of the isolates and the highest inhibition zone (20.07 ± 0.5 mm) was found against *E. coli* at 30 mg/ml concentration, while petroleum ether, acetone and chloroform extracts did not exhibit any inhibitory activity.

Table 4: Antibacterial activity of methanol, chloroform and aqueous leaf extracts of *M. Oleifera* against *Escherichia coli*

	Treatment		
Dosage	Chloroform Extract	Methanol Extract	Aqueous Extract
6.25 mg/ml	1.80±0.50^{C} (0.00-4.18)	0.00-0.00^{C} (0.00-0.00)	0.00±0.00^{B} (0.00-0.00)
12.5 mg/ml	3.48±0.86^{BC} (2.12-10.13)	0.00-0.00^{C} (0.00-0.00)	0.00±0.00^{B} (0.00-0.00)
25 mg/ml	4.92±0.98^{B} (3.19-11.44)	0.72-0.36^{C} (0.00-2.18)	0.00±0.00^{B} (0.00-0.00)
50 mg/ml	6.90±1.29^{B} (4.05-14.10)	2.38-0.11^{B} (2.11-3.16)	0.00±0.00^{B} (0.00-0.00)
100 mg/ml	9.32±1.45^{A} (5.19-16.21)	7.09-0.50^{A} (5.15-10.32)	1.37±0.61^{A} (0.00-5.11)
Control	2.46±0.16^{C} (2.04-3.32)	2.46-0.16^{B} (2.11-3.21)	0.00±0.00^{B} (0.00-0.00)
P-value	0.000	0.000	0.001

Similar letters indicate means that are not significantly different (P> 0.05), P< 0.01 -Highly significantly different, P<0.001 - Very highly significantly different. Values are mean ± SEM. Figures in parenthesis represent lowest and highest zones of inhibition. Reading of significance is in column.

The result obtained for *Pseudomonas aeruginosa* revealed that chloroform extracts at 6.25, 12.50 and 25 mg/ml was observed to have same effect as the control followed by 50 mg/ml with 3.30±0.48 (Table 5). Highest mean zone of inhibition of 7.32±1.17 mm were recorded at 100 mg/ml. Methanol extract zones of inhibition at 6.25, 12.5 mg/ml were seen to have lesser effect than the control but there were no significant difference at 50 mg/ml effect when compared with the control, methanol extract at 100mg/ml recorded mean zone of inhibition of 7.15±1.08 mm. Aqueous extract at 6.25, 12.5, 25 and 50 mg/ml were not significantly different from the control and aqueous extract at 100 mg/ml were observed to show considerable antibacterial activity when compared with the control. Meanwhile, aqueous extract showed lowest antibacterial effect among the three extracts used as shown in Plate 5. Emad (2016) shown that at a concentration of 200 mg/ml, ethyl acetate extract revealed the highest antibacterial activity against *Staphylococcus epidermidis* (16.0±0.5 mm), *Staphylococcus aureus* (13.6±0.3 mm), *Pseudomonas aeruginosa* (13.3±0.3 mm). The results of this investigation was in harmony with numerous previous studies on *M. oleifera* leaves with different extraction methods and solvents such as aqueous, ethanol, methanol, chloroform and many more reported interesting antibacterial activity against wide spectrum microorganisms, from both gram positive and gram negative bacteria (Abalaka et al, 2012; Arun and Rao, 2011).

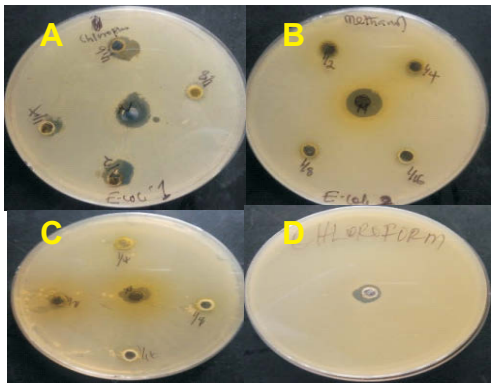

Plate 4: The antibacterial activity of Moringa oleifera **leaf extracts using different solvent against** multiple drug resistant Escherichia coliA: Chloroform, B: Methanol, C: Water (aqueous leaf extracts), D: Absolute chloroform (control)

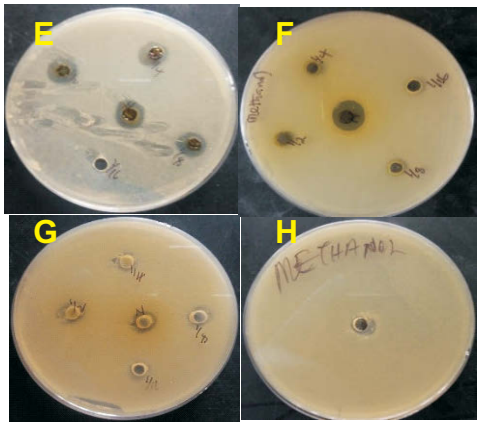

Plate 5: The antibacterial activity of Moringa oleifera **leaf extracts using different solvent against** multiple drug resistant Pseudomonas aeruginosa E: Chloroform, F: Methanol, G: Water (aqueous leaf extract), H: Absolute methanol (control)

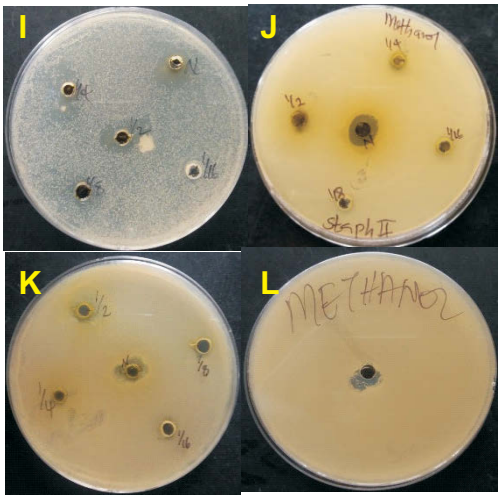

Plate 6: The antibacterial activity of Moringa oleifera **leaf extracts using different solvent against** multiple drug resistant S. aureus. I: Chloroform, J: Methanol, K: Water (aqueous leaf extracts), L= Absolute methanol (control)

Antibacterial activity of M. oleifera leaf extracts on MDR *Staphylococcus aureus* indicated that chloroform, methanol and aqueous extracts exhibited different levels of antibacterial activity when compared with the control as shown in Table 6 and Plate 6. From the three extracts, chloroform performs better hen compared to methanol and aqueous extract. Chloroform extract at 6.25 and 12.50 mg/ml recorded same effect as the control while 25 and 50 mg/ml were observed to be significantly the same; mean zone of inhibition of 7.08±0.92 mm were recorded at 100 mg/ml. methanol extract at 6.25, 12.50 and 25 mg/ml has less effect than the control, at 50 mg/ml, the effect (2.64±2.31 mm) were not significantly different when compared with the control and 100 mg/ml were seen to have highest zone of inhibition with 4.83±0.75 mm. Aqueous extract at 6.25, 12.50 and 25 mg/ml, there were no zone of inhibition recorded just as the control. Zones of inhibition were recorded at 50 and 100 mg/ml (1.70±0.48 and 4.17±0.46 mm). Jabeen et al. (2008) reported that crude extract of *M. oleifera* had strong activity against *Fusarium solani, Bacillus subtillis* and *Staphylococcus aureus*. The relatively high potency of the ethanol extract may be attributed to the dissolving power of alcohols (Majorie, 1999). Emad (2016) reported that Butanol extract was active only against *Staphylococcus epidermidis* (14.0±0.0 mm) and *Staphylococcus aureus* (10.3±0.3 mm). Water extract was active only against *Staphylococcus epidermidis* (12.3±0.6 mm). Chloroform extract showed antibacterial activity against *Staphylococcus aureus* (11.0±0.5 mm). This activity against both gram negative and gram positive bacteria may be attributed to presence of some broad-spectrum antibacterial compounds (Vinoth et al., 2012).

Table 5: Antibacterial activity of methanol and aqueous leaf extracts of *M. oleifera* against *Pseudomonas aeruginosa*

	Treatment		
Dosage	Chloroform Extract	Methanol Extract	Aqueous Extract
6.25 mg/ml	1.09±0.55^{C} (0.00-4.00)	0.00±0.00^{C} (0.00-0.00)	0.00±0.00^{B} (0.00-0.00)
12.5 mg/ml	1.71±0.66^{C} (0.00-4.00)	0.49±0.33^{C} (0.00-2.41)	0.00±0.00^{B} (0.00-0.00)
25 mg/ml	2.32±0.62^{C} (0.00-4.00)	0.87±0.45^{C} (0.00-3.28)	0.00±0.00^{B} (0.00-0.00)
50 mg/ml	3.30±0.48^{B} (2.0-5.00)	2.05±0.62^{B} (0.00-5.25)	0.27±0.27^{B} (.00-2.39)
100 mg/ml	7.32±1.17^{A} (2.0-14.00)	7.15±1.08^{A} (5.18-15.21)	3.10±0.44^{A} (2.11-5.35)
Control	2.65±0.22^{C} (2.00-4.00)	1.94±0.25^{B} (1.00-3.21)	0.00±0.00^{B} (0.00-0.00)
P-value	0.000	0.000	0.000

Similar letters indicate means that are not significantly different (P>0.05), P<0.01 -Highly significantly different, P<0.001 - Very highly significantly different. Values are mean ± SEM. Figures in parenthesis represent lowest and highest zones of inhibition. Reading of significance is in column.

Table 6: Antibacterial activity of methanol and aqueous leaf extracts of *M. oleifera* against *Staphylococcus aureus*

	Treatment		
Dosage	Chloroform Extract	Methanol Extract	Aqueous Extract
6.25 mg/ml	0.28±0.28^{C} (0-2.51)	0.00 ±0.00^{C} (0-0)	0.00 ±0.00^{C} (0-0)
12.5 mg/ml	0.73±0.35^{C} (0-2.24)	0.00 ±0.00^{C} (0-0)	0.00 ±0.00^{C} (0-0)
25 mg/ml	2.68±0.35^{B} (1.05-4.35)	0.72±0.51^{C} (0-4.3)	0.00 ±0.00^{C} (0-0)
50 mg/ml	3.74±0.58^{B} (2.01-6.71)	2.64±2.31^{B} (1.25-2.11)	1.70±0.48^{B} (0-4.31)
100 mg/ml	7.08±0.92^{A} (5.11-12.08)	4.83±0.75^{A} (3.15-9.17)	4.17±0.46^{A} (01-7.04)
Control	1.8922±0.1334^{C} (1.32-2.32)	2.3422±0.1080^{B} (2.01-3.01)	0.0000 ±0.0000^{C} (0-0)
P-value	0.000	0.326	0.000

Similar letters indicate means that are not significantly different (P>0.05), P<0.01 -Highly significantly different, P<0.001 - Very highly significantly different. Values are mean ± SEM. Figures in parenthesis represent lowest and highest zones of inhibition. Reading of significance is in column.

Conclusion

The current investigation has showed the potential of *M. oleifera* leaves as antibacterial agent against the tested multiple drug resistant Gram positive and Gram negative bacteria pathogens.

The end result of this current study reinforced the previous works on the effectiveness of the plant and it thus stands an opportunity for animal studies as an alternate treatment for bacteria pathogens infection. Whereas we have been competent in assessing the therapeutic potential of this plant extract *in vitro*, the molecular mechanism behind their antibacterial activities would need more investigation, and also the clinical expertise of health care practitioners are crucial for evaluating their effectiveness. Since many plant antimicrobials contain totally different purposeful elements in their structure, their antimicrobial activity is attributed to multiple mechanisms. Therefore, not like antibiotics, the potential for pathogens to develop resistance to plant antimicrobials is comparatively smaller. The utilization of those alternatives within the management of infections would assist to avoid riotous bacteria drug resistance evoked by the recurrent use of antibiotics.

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