Original Research Article

Moringa oleifera Lam. Leaf Extract Fractions Against Multidrug-Resistant *Pseudomonas aeruginosa* Strains from Surgical wound infections

ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogen with the potential to cause severe surgical wound infections and remains a major dilemma especially in developing countries like Nigeria. This problem has led to investigating antimicrobial efficacy of *Moringa oleifera* leaf extract fractions on multidrug resistant (MDR) *Pseudomonas aeruginosa* strains. Strains of *Pseudomonas aeruginosa* were isolated from post-surgical wound in the two locations (Central Hospital, Benin and University of Benin Teaching Hospital) used for the study, antibiotics susceptibility testing to identify MDR isolates were performed, qualitative phytochemical screening of *Moringa oleifera* leaf extract fractions were carried out using standard procedures followed by antibacterial testing of the different *M. oleifera* leaf extracts on selected multidrug-resistant isolates. The results showed that 99 (35%) of patients studied had wound infection out of the total 284 samples collected. Thirty-four (54.8%) *Pseudomonas aeruginosa* strains were found to show multidrug-resistant ability from both locations studied. Qualitative analysis of phytochemical revealed the presence of flavonoid, phenol, saponin, steriod, tannin and terpenoids. Antibacterial assay results indicated that *Moringa oleifera* leaf ethyl acetate extract exhibited highest antibacterial activity against *Pseudomonas aeruginosa* strain Iraq.PA -9 followed by dichloromethane all at a concentration of 100 mg/ml. The varying effect could be ascribed to the presence of phytochemicals in the different *Moringa oleifera* leaf extracts. The result of this study has shown the potentials of *Moringa oleifera* leaf extracts as antibacterial agent by inhibiting the growth of the test organisms isolated from post-surgical wound infection.

Keywords: Multiple drug resistance, *Moringa oleifera*, Leaf fraction, Surgical wound infection

1. INTRODUCTION

Surgical wound infections caused by isolates of multi-drug resistant bacteria pose a serious challenge to the treatment of such infections worldwide [1]. Surgical wound sites with elevated microorganism contaminants represent a significant drawback within the hospital particularly in surgical procedures where clean operations will become contaminated and then infected. The extent to which surface wounds are infected by nearby bacteria contaminants became clinically necessary [2]. The risk of infection is commonly based on the openness of surgical wounds to microorganism contamination. Clean surgery carries a 1 to 5 percent risk of after operation wound infection, and in unclean procedures that are considerably more liable to endogenous contamination, a 27% risk of infection has been projected [3]. Minimizing the incidence of post-operative wound infection depends on adequate sterility, preservation, and protection of the host's local defense [3]. Asepsis involves the use of effective infection management procedures to reduce exogenous microbial contamination throughout the surgery. Antisepsis involves the utilization of skin antiseptics on the operative site and in the case of dirty surgical procedures, administration of prophylactic antibiotics at a time before surgery to guarantee adequate tissue levels of antibiotics throughout the surgery. The exposed skin following thermal injury is vulnerable to infection and may be contaminated with resistant organisms serving as a supply of prolonged infection touching different burn patients [4].

Pseudomonas spp. is one of the major bacterial isolates that cause post-surgical wound infections in different parts of the world. Other bacteria isolates usually also incriminated in wound infections include but not limited to *Staphylococcus, Klebsiella, Proteus* species, *Escherichia coli, Acinetobacter baumannii, Enterococcus* spp. in addition to anaerobes such as *Clostridium, Bacteroides, Peptostreptococcus* spp. *and Propionibacterium acnes*. It has been revealed that *Pseudomonas* spp., *Staphylococcus* and *Klebsiella* are the foremost normally isolated pathogens in wounds of patients attending the Ogun State Teaching Hospital, Nigeria [5]. *Pseudomonas aeruginosa* is often isolated from infected wounds after surgery due to their intrinsic ability to stay in unfavourable environment [6]. These pathogens have gained fame in wound site infection because of their accrued resistance to routinely used antibiotic drugs [6]. The dilemma of bacteria resistance to contemporary antimicrobial drugs has led to the wide use of conventional medicine, and many plant extracts with antimicrobial activities have provided a scientific basis for their use in the treatment of several diseases and infections with promising results [7].

The need for new antimicrobial agents is closely linked with the problem of emergence of strains that are resistant to most synthetic antibiotics. The exploration for remedies in plants is not new. The restricted effective life span of current antibiotics, the shortage of compliance of patients, the unmonitored use in agriculture, and also the slow rate in releasing new antimicrobial agents have led to a fearsome increase in antimicrobial resistance. *Moringa oleifera* Lam. has been used extensively in conventional medication for the treatment of many ailments. It is ordinarily well-known by totally different regional names like Drumstick trees, Horse radish, Morango [8]. In Nigeria, it's referred to as Zugale within the northern region and commonly named a miracle tree plant. *Moringa oleifera* Lam. belongs to the Moringaceae family and genus *Moringa.* The tree is native to Arabia and India where it is commonly planted in compounds. It is now extensively disseminated in the tropics and West Africa [9]. In the genus *Moringa* of the family Moringaceae, *Moringa oleifera* is best known among all the species [10]. *Moringa oleifera* Lam. is a multipurpose plant amazingly medicinal and nutritious, a vegetable tree with many possible benefits. The antimicrobial machinery of *Moringa* has been validated after the detection of inhibitory action against several microorganisms. Bacteria are ranked first among the

microorganisms causing opportunistic diseases [11]. Various antibacterial agents are presently used in treating bacterial infections. Nevertheless, the extensive and haphazard use of antibacterial agents resulted in the development of drug resistance among many pathogenic bacteria species [12]. Many of the currently used antibacterial agents are connected with undesirable effects such as toxicity, hypersensitivity and tissue residues posing a public health hazard. Furthermore, the newer broad-spectrum antibiotics are exorbitant and are not within the reach of poor citizens. These disadvantages demoralize the therapeutic usefulness of the currently obtainable antibacterial and thus necessitating the need for finding other remedies for the cure of bacterial diseases. Therefore, the possibility of using plants that is inexpensive and effortlessly available such as *Moringa oleifera* to treat MDR *Pseudomonas aeruginosa* surgical wound infections is inevitable especially in developing country like Nigeria.

2. MATERIALS AND METHODS

2.1 Collection, authentication and processing of plant materials

Moringa oleifera fresh leaf was collected from Doctor's Quarters, University of Benin Teaching Hospital, Egor Local Government Area, Edo State, Nigeria. The plant materials were identified and authenticated by a Botanist at the Department of Plant Biology and Biotechnology, University of Benin, Nigeria. Confirmation of plant taxonomic identity was done by comparison with voucher specimens kept at the Herbarium of the Department of Plant Biology and Biotechnology, University of Benin. The plant materials were air-dried in the laboratory at room temperature for 15 days [13]. The dried leaves were grounded into powdered form, using a mortar and pestle, and stored for future use.

2.2 Preparation of *Moringa oleifera* leaf extracts

The powdered plant material of five hundred grams (500 g) was macerated in two point five liters of methanol (2.5 L) at room temperature for three days [14]. The percolates were filtered with Whatman's No 1 filter paper. The extract was concentrated to dryness using a rotary evaporator at reduced pressure. The dried extract was weighed and the percentage yield calculated. The extract was stored in an air-tight container and kept in the refrigerator at 4 ^{o}C until further experiment [15].

2.3 Solvent-Solvent Extraction

Pre-Fractionation/Partitioning of the crude methanol extract (25 g) was dissolved in 100 mL of methanol-water (4:1) and extracted successively with dichloromethane [16]. Briefly, 500 mL of dichloromethane was added to the methanol extract in a separating funnel. The mixture was slightly agitated and the pressure accumulated in the funnel was released by opening the tap. The mixture was allowed to stand for a few minutes and the dichloromethane layer was collected. The recovered methanol extract was again extracted with 500 mL of dichloromethane and separated. This procedure was repeated until a total of 2 L of dichloromethane was used. The dichloromethane portions were combined and evaporated to dryness. The ethyl acetate fraction was also concentrated to dryness, weighed and the percentage yields calculated.

2.4 Phytochemical Analysis

Phytochemical screening was performed to identify phytochemicals in ethyl acetate and dichloromethane extract of *Moringa oleifera* leaf used in this study. The phytochemicals were

detected by colour tests. Each extract was tested for the presence of alkaloids, anthroquinines, flavonoids, glycosides, phenols, saponins, sterols and tannins using different known methods [17-24]. The tests were performed in triplicates to ensure accurate results.

2.5 Screening of *Moringa oleifera* Leaf Extracts for Antimicrobial Activity

Antibacterial activity of *Moringa oleifera* ethyl acetate and dichloromethane extracts fractions were tested against the multidrug resistant *Pseudomonas aeruginosa* strains using the agar well diffusion method [25].

2.6 Test organism sample collection

Random swab sampling of 284 post-operative surgical wound patients was performed using both outpatient and inpatient in Central Hospital Benin (CHB) and University of Benin Teaching Hospital (UBTH), Benin City.

2.7 Bacteriological procedures/identification of isolates

Swab samples were aseptically inoculated onto MacConkey, Blood and Nutrient agar and incubated aerobically at 37 ^{o}C for 24 hours and checked for colony growth. Isolates were screened for *Pseudomonas aeruginosa*. All isolates were identified using conventional techniques [26].

2.8 Antibiotic susceptibility testing

The susceptibility of bacterial isolates to commonly used antibiotic was determined by the Kirby-Bauer disk diffusion method for *in vitro* antibiotic sensitivity as described by [27] against the following antibiotics for Gram negative bacteria include: Augmentin (AUG, 30µg), Ofloxacin (OFL 5µg), Cefixime (CXM 5µg), Gentamy-cin (GEN 30µg), Cefuroxime (CRX 30µg), Ceftazidime (CAZ 30µg), Ciprofloxacin (CPR 5µg), Nitrofurantion (NIT 300µg). The concentrations of antibiotics susceptibility and explanation of zones of inhibition were in accordance to Performance Standards for antimicrobial disk susceptibility tests of Clinical and Laboratory Standards Institute.

2.9 Standardization of Inoculum

The inocula were prepared from the stock cultures, which were maintained on nutrient agar slant at 4 ^{o}C and subcultured onto nutrient broth using a sterilized wire loop. The density of suspension inoculated onto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of Barium sulphate solution [26].

2.10 Screening of *Moringa oleifera* leaf extracts for antimicrobial activity

Antibacterial activity of *Moringa oleifera* leaf extracts fractions were tested against the multiple drug-resistant *P. aeruginosa* strains using the agar diffusion method. Mueller-Hinton agar medium was prepared, sterilized, cooled and poured into sterile petri-dishes to a depth of 4 mm about 20 ml/plate and was allowed to solidify. Overnight cultures of bacterial isolate were diluted with sterile normal saline to give inoculum size of 106 cfu/ml was used to flood the surface of the Mueller-Hinton agar media, discarded and allowed to dry. Five wells of 6 mm in diameter each were aseptically bored using a sterile cork borer on each agar plate. The base of each well was filled with molten agar to seal the bottom and allowed to gel. Aliquot of 0.2 ml of the extract with different concentrations (100.00**,** 50.00, 25.00, 12.50 and 6.25 mg/ml) was added

to the different wells. The same procedure was applied to all extract fractions. The plates were left to allow for diffusion of extract before incubation at 37 ^{o}C for 24 hours. The zones of inhibition (clearance) produced around the wells after incubation were observed, measured and recorded.

2.11 Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentrations of the extracts of Mo*ringa oleifera* leaf was determined by two-fold serial dilution method as described by [28].

2.12 Statistical analysis

The data from the experiment were analyzed with SPSS version 20.0 using one way analysis of variance (ANOVA) where there was significant difference, Duncan Multiple Range test was used to separate the mean. Chi-square was also used to test for significant difference. The results were expressed as mean ± SEM (Standard error of mean).

3. RESULTS

In this study, two hundred and eighty-four (284) post-operative surgical wound swabs specimens from in and out patients were analyzed. Ninety-nine (35%) of patients studied had wound infection. Sixty-two (62.6%) *P. aeruginosa* were isolated from surgical wound infections and screened with eight commonly used antibiotics to identify multiple drug antibiotic resistant strains. Thirty-four (54.8%) isolates (18 isolates from UBTH and 16 strains from CHB) showed multiple drug resistance ability. Based on the antibiotics susceptibility results obtained, most of the isolates screened showed high resistance to Ceftazidime, Augmentin, Cefixime and gentamicin (54.8%) as is shown in Table 1.

Table 1. Susceptibility profile of *Pseudomonas aeruginosa* isolates to tested antibiotics

Classof Antibiotics	Type of antibiotics	CHB No. tested =27 (%)		UBTH No. tested = 35 (%)	
		R	S	R	S
Penicillin	Augmentin (30 µg)	17(63)	10(37)	22(62.9)	13(37.1)
Aminoglycoside	Gentamycin (30 µg)	19(70)	8(30)	24(68.9)	11(31.1)
Cephalosporin	Ceftazidime (30 µg)	23(85.2)	4(14.8)	21(60)	14(40)
	Cefuroxime (30 µg)	19(70)	8(30)	20(57.1)	15(42.9)
	Cefixime (5 µg)	22(81.5)	5(18.5)	20(57.1)	15(42.9)
Nitrofuran	Nitrofuration (300 µg)	18(66.7)	9(33.3)	27(77.1)	8(22.9)
Quinolones	Ofloxacin (5 µg)	16(59.3)	11(40.7)	18(51.4)	17(48.6)
	Ciprofloxacin(5 µg)	13(48.1)	14(51.9)	13(37.1)	22(62.9)

Moringa oleifera powered leaf (500 g) were extracted with two point five liters (2.5 L) of methanol and then evaporated to dryness. Fractionation of the crude methanol extract was carried out using ethyl acetate and dichloromethane which was also evaporated to dryness (Table 2). Qualitative analysis of extracts fractions revealed the presence of six (6) phytochemical constituents (flavonoid, phenol, saponin, steriods, tannin and terpenoid) out of the nine tested in ethyl acetate and Dichloromethane showed 3 (phenol, steriods and terpenoid) as shown in Table 3.

Table 2. Dried extract yield of *Moringa oleifera* leaf

***Moringa oleifera* leaf extract**	**Extract dried yield (grams)**
Dichloromethane	3.8
Ethyl acetate	2.5

Table 3. Phytochemical screening of *Moringa oleifera* leaf extracts

Phytochemicals	Ethyl acetate fraction	Dichloromethane fraction
Alkaloid	–	–
Anthraquinone	–	–
Flavonoid	+	–
Glycoside	–	–
Phenol	+	+
Saponin	+	–
Steriods	+	+
Tannin	+	–
Terpenoid	+	+

Keys:
+: Presence of phytochemicals
–: Absence of phytochemicals

The antibacterial property of *M. oleifera* ethyl acetate fraction of leaf extract on different MDR *Pseudomonas aeruginosa* strains from CHB indicated that ethyl acetate fraction had an inhibitory effect of diverse degrees when compared with the solvent used as control. There was significant difference in the mean zones of inhibition at the different concentrations. MDR *Pseudomonas aeruginosa* strains S2H16 recorded the highest mean zone of inhibition of 35.33± 2.67 mm at 100 mg/ml while MDR *P. aeruginosa* strains D2 showed the lowest acceptable mean zone of inhibition of 10.67 ± 0.67 mm at 12.5 mg/ml as shown in Table 4.

Moringa oleifera ethyl acetate leaf fraction antibacterial activity on MDR *P. aeruginosa* strains from UBTH revealed that the fraction exhibited different levels of activity. It was observed that there was significant difference in the mean zones of inhibition at the different concentrations. The highest mean zone of inhibition of 36.33 ± 0.88 mm was observed for MDR *P. aeruginosa* strains Iraq. PA-9 and 10.33 ± 0.33 mm lowest acceptance zone of inhibition at 6.25 mg/ml was recorded for MDR *P. aeruginosa* strain AR442 (Table 5).

Table 4. Antibacterial activity of *M. oleifera* ethyl acetate leaf extracts against different MDR *Pseudomonas aeruginosa* strains from CHB

Isolates	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	P-value
P. a strain PS2	$34.67^{a} \pm 0.67$	$28.67^{b} \pm 0.67$	$23.33^{c} \pm 0.33$	$6.67^{d} \pm 0.67$	$1.00^{e} \pm 0.00$	P<0.01
P. a strain NAPCC-1	$26.67^{a} \pm 0.67$	$15.67^{b} \pm 0.33$	$11.33^{c} \pm 1.33$	$1.00^{d} \pm 0.00$	$1.00^{d} \pm 0.00$	P<0.01
P. a strain DHS01	$34.67^{a} \pm 0.67$	$21.33^{b} \pm 0.67$	$10.67^{c} \pm 0.67$	$2.67^{d} \pm 0.67$	$1.00^{d} \pm 0.00$	P<0.01
P. a strain AR442	$20.67^{a} \pm 0.67$	$4.67^{b} \pm 0.67$	$3.33^{b} \pm 0.67$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain R7-520-1	$22.67^{a} \pm 1.33$	$17.00^{b} \pm 3.51$	$4.67^{c} \pm 0.67$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain H25883	$30.67^{a} \pm 5.21$	$25.67^{a} \pm 0.33$	$19.67^{b} \pm 0.33$	$12.00^{c} \pm 1.16$	$4.67^{c} \pm 0.67$	P<0.01
P. a strain PA-VAP-2	$21.33^{a} \pm 0.67$	$12.67^{b} \pm 0.67$	$4.67^{c} \pm 0.33$	$2.00^{d} \pm 0.00$	$1.67^{d} \pm 0.33$	P<0.01
P. a strain R7-583	$26.33^{a} \pm 0.88$	$16.33^{b} \pm 0.88$	$10.67^{c} \pm 0.67$	$2.67^{d} \pm 0.67$	$1.00^{d} \pm 0.00$	P<0.01
P. a strain PA006	$25.67^{a} \pm 0.33$	$18.67^{b} \pm 0.67$	$4.33^{c} \pm 0.33$	$1.00^{d} \pm 0.00$	$1.00^{d} \pm 0000$	P<0.01
P. a strain S2H16	$35.33^{a} \pm 2.67$	$30.67^{b} \pm 0.67$	$25.67^{c} \pm 0.33$	$20.33^{d} \pm 0.33$	$15.67^{e} \pm 0.33$	P<0.01
P. a strain KAR21	$25.67^{a} \pm 0.33$	$16.00^{b} \pm 0.58$	$10.67^{c} \pm 0.67$	$2.67^{d} \pm 0.67$	$2.00^{d} \pm 0.00$	P<0.01
P. a strain D2	$27.67^{a} \pm 0.33$	$15.0^{b} \pm 0.58$	$10.67^{c} \pm 0.67$	$10.67^{c} \pm 0.67$	$3.33^{d} \pm 0.67$	P<0.01

Similar letters indicate means that are not significantly different, P<0.05 were considered significant, values are mean ± SEM, reading of significance is in row, CHB: Central Hospital, Benin

Table 5. Antibacterial activity of *M. oleifera* ethyl acetate leaf extracts against different MDR *Pseudomonas aeruginosa* strains from UBTH

Isolates	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	P-value
P. a strain SWD	$20.67^{a} \pm 0.67$	$10.67^{b} \pm 0.67$	$3.33^{c} \pm 0.67$	$2.67^{c} \pm 0.67$	$2.00^{c} \pm 0.00$	P<0.01
P. a strain Exo25	$20.00^{a} \pm 0.00$	$10.67^{b} \pm 0.67$	$10.00^{b} \pm 0.00$	$9.00^{b} \pm 0.58$	$8.67^{b} \pm 1.33$	P<0.01
P. a strain R8-768	$30.67^{a} \pm 0.67$	$17.67^{b} \pm 0.33$	$10.67^{c} \pm 0.67$	$2.67^{d} \pm 0.67$	$2.33^{d} \pm 0.88$	P<0.01
P. a strain YPAB1	$16.33^{a} \pm 0.88$	$6.67^{b} \pm 0.67$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain VITMS7	$27.33^{a} \pm 0.33$	$16.67^{b} \pm 0.67$	$10.67^{c} \pm 0.67$	$6.67^{d} \pm 0.67$	$2.00^{e} \pm 0.00$	P<0.01
P. a strain AR442	$28.67^{a} \pm 0.67$	$21.00^{b} \pm 0.58$	$10.67^{c} \pm 0.67$	$11.33^{c} \pm 0.67$	$10.33^{c} \pm 0.33$	P<0.01
P. a strain AS23	$21.33^{a} \pm 0.67$	$15.00^{b} \pm 0.58$	$10.33^{c} \pm 0.33$	$10.00^{c} \pm 0.00$	$7.33^{d} \pm 1.33$	P<0.01
P. a strain DKH-3	$20.67^{a} \pm 0.67$	$4.67^{b} \pm 0.67$	$2.00^{c} \pm 0.00$	$1.67^{c} \pm 0.33$	$1.33^{c} \pm 0.33$	P<0.01
P. a strain H25883	$11.33^{a} \pm 0.67$	$3.33^{b} \pm 0.33$	$2.67^{b} \pm 0.67$	$2.00^{b} \pm 0.00$	$2.00^{b} \pm 0.00$	P<0.01
P. a strain Y15	$25.33^{a} \pm 0.33$	$19.33^{b} \pm 0.67$	$10.67^{c} \pm 0.67$	$3.33^{d} \pm 0.67$	$2.00^{d} \pm 0.00$	P<0.01
P. a strain PA016	$16.33^{a} \pm 0.88$	$12.00^{b} \pm 1.16$	$4.67^{c} \pm 0.67$	$2.67^{d} \pm 0.67$	$2.00^{d} \pm 0.00$	P<0.01
P. a strain R8- 768-1	$29.67^{a} \pm 0.33$	$26.00^{b} \pm 1.00$	$21.00^{c} \pm 0.58$	$19.33^{c} \pm 0.67$	$15.33^{d} \pm 0.33$	P<0.01
P. a strain KAR21	$27.33^{a} \pm 0.33$	$20.67^{b} \pm 0.67$	$14.67^{c} \pm 0.67$	$8.67^{d} \pm 0.67$	$6.67^{e} \pm 0.67$	P<0.01
P. a strain Iraq.PA -9	$36.33^{a} \pm 0.88$	$10.67^{b} \pm 0.67$	$8.67^{b} \pm 1.33$	$2.67^{c} \pm 0.67$	$2.67^{c} \pm 0.67$	P<0.01

Similar letters indicate means that are not significantly different, P<0.05 were considered significant, values are mean ± SEM, reading of significance is in row, UBTH: University of Benin Teaching Hospital

Dichloromethane fraction of *M. oleifera* crude methanol leaf extract on different MDR *P. aeruginosa* strains from CHB indicted that dichloromethane fraction had inhibitory effect at the different concentrations (Table 6). There was significant difference in the mean zones of inhibition at the different concentrations tested. MDR *P. aeruginosa* strains PS2 and *P. aeruginosa* strains DHS01 was observed to have recorded the highest mean zone of inhibition of 30.33 ± 0.88 mm at a concentration of 100 mg/ml while MDR *P. aeruginosa* strains NAPCC-1 and *P. aeruginosa* strains PA-VAP-2 showed the lowest acceptable mean zone of inhibition of 10.67 ± 0.67 mm at a concentration of 50 mg/ml, *P. aeruginosa* strains DHS01 also recorded zone of inhibition of 12.00 ± 0.58 mm at a concentration of 25 mg/ml.

Moringa oleifera dichloromethane fraction of crude methanol leaf extract testing on the different MDR *Pseudomonas aeruginosa* strains from UBTH showed that the fraction had an inhibitory effect at the different concentrations employed. There was significant difference in the mean zones of inhibition observed for the different concentration used (Table 7). Multiple drug-resistant *P. aeruginosa* strains Iraq.PA-9 recorded the highest mean zone of inhibition of 36.00 ± 0.58 mm at a concentration of 100 mg/ml and the lowest acceptable mean zone of inhibition of 15.67 ± 0.33 mm at a concentration of 25 mg/ml for *P. aeruginosa* strain Iraq.PA –9.

Table 6. Antibacterial activity of *M. oleifera* dichloromethane leaf extracts against different MDR *Pseudomonas aeruginosa* strains from CHB

Isolates	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	P-value
P. a strain PS2	$30.33^{a} \pm 0.88$	$20.33^{b} \pm 0.33$	$15.00^{c} \pm 0.58$	$1.00^{d} \pm 0.00$	$1.00^{d} \pm 0.00$	P<0.01
P. a strain NAPCC-1	$28.33^{a} \pm 0.88$	$10.67^{b} \pm 0.67$	$2.33^{c} \pm 0.88$	$1.33^{c} \pm 0.33$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain DHS01	$30.33^{a} \pm 0.33$	$15.67^{b} \pm 0.33$	$12.00^{c} \pm 0.58$	$4.33^{d} \pm 0.33$	$1.00^{e} \pm 0.00$	P<0.01
P. a strain AR442	$21.67^{a} \pm 0.33$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	P<0.01
P. a strain R7-520-1	$21.00^{a} \pm 0.58$	$11.67^{b} \pm 0.88$	$4.33^{c} \pm 0.33$	$1.00^{d} \pm 0.00$	$1.00^{d} \pm 0.00$	P<0.01
P. a strain H25883	$18.33^{a} \pm 0.88$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	P<0.01
P. a strain PA-VAP-2	$21.00^{a} \pm 0.58$	$10.67^{b} \pm 0.67$	$2.33^{c} \pm 0.33$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain R7-583	$18.67^{a} \pm 0.67$	$4.33^{b} \pm 0.33$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain PA006	$17.00^{a} \pm 0.58$	$12.67^{b} \pm 0.67$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain S2H16	$21.00^{a} \pm 0.58$	$9.33^{b} \pm 0.67$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain KAR21	$21.67^{a} \pm 1.67$	$3.00^{b} \pm 1.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	P<0.01
P. a strain D2	$25.67^{a} \pm 0.33$	$4.67^{b} \pm 0.67$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01

Similar letters indicate means that are not significantly different, P<0.05 were considered significant, values are mean ± SEM, reading of significance is in row, CHB: Central Hospital, Benin

Table 7. Antibacterial activity of *M. oleifera* dichloromethane leaf extracts against different MDR *Pseudomonas aeruginosa* strains from UBTH

Isolates	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	P-value
P. a strain SWD	$22.33^{a} \pm 0.33$	$16.67^{b} \pm 0.67$	$2.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain Exo25	$31.00^{a} \pm 0.58$	$10.33^{b} \pm 0.33$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain R8-768	$20.33^{a} \pm 0.33$	$9.33^{b} \pm 0.67$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain YPAB1	$15.00^{a} \pm 0.58$	$2.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	P<0.01
P. a strain VITMS7	$23.67^{a} \pm 0.33$	$15.67^{b} \pm 0.33$	$8.67^{c} \pm 0.67$	$1.00^{d} \pm 0.00$	$1.00^{d} \pm 0.00$	P<0.01
P. a strain AR442	$26.00^{a} \pm 0.58$	$12.67^{b} \pm 0.67$	$4.67^{c} \pm 0.67$	$1.00^{d} \pm 0.00$	$1.00^{d} \pm 0.00$	P<0.01
P. a strain AS23	$26.00^{a} \pm 1.00$	$11.67^{b} \pm 0.33$	$3.00^{c} \pm 1.00$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain DKH-3	$15.67^{a} \pm 0.33$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	P<0.01
P. a strain H25883	$16.33^{a} \pm 0.88$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	P<0.01
P. a strain Y15	$26.67^{a} \pm 0.67$	$15.67^{b} \pm 0.33$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain PA016	$25.33^{a} \pm 0.33$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	P<0.01
P. a strain R8- 768-1	$24.67^{a} \pm 0.67$	$15.33^{b} \pm 0.33$	$2.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain KAR21	$21.00^{a} \pm 0.58$	$9.33^{b} \pm 0.67$	$6.67^{c} \pm 0.67$	$1.00^{d} \pm 0.00$	$1.00^{d} \pm 0.00$	P<0.01
P. a strain Iraq. PA -9	$36.00^{a} \pm 0.58$	$22.33^{b} \pm 0.33$	$15.67^{c} \pm 0.33$	$1.00^{d} \pm 0.00$	$1.00^{d} \pm 0.00$	P<0.01

Similar letters indicate means that are not significantly different, $P<0.05$ were considered significant, values are mean ± SEM, reading of significance is in row, UBTH: University of Benin Teaching Hospital

4.0 DISCUSSION

Medicinal plants are gaining more recognition even among educated folks in cities, possibly due to the rising inefficacy of many modern-day drugs used for the control of various infections as well as the increase in resistance by numerous bacteria to different routinely used antibiotics and the growing cost of prescription drugs, for the maintenance of individual's health [29]. The use of antibiotics, increased prevalence of multiple drug-resistant strains of several pathogenic bacteria has revitalized the interest in plants with antimicrobial properties [30]. This has led to the screening of *Moringa oleifera* leaf extract using different solvents. Antimicrobial properties of *Moringa oleifera* have been ascribed to the diverse parts of the plant, such as the leaves, seeds, pods and stems [31] which are recognized for their antibacterial action and are counted as the rich basis of antimicrobial agents [32]. In this study, fractionation of *Moringa oleifera* crude methanol leaf extract was carried out using dichloromethane and ethyl acetate and the yield obtained were 3.8 and 2.5 grams respectively. Qualitative phytochemical screening was used in revealing the secondary metabolites of the extract. The result showed the presence of flavonoid, phenol, saponin, steroid, tannin and terpenoid (Table 2). Steroid, terpenoid and phenol were present in both ethyl acetate and dichloromethane. Flavonoids, saponins and tannins were present only in ethyl acetate extracts. The result obtained from this work was in line with the earlier work described by other researchers that investigated the phytochemical constituents of *Moringa oleifera* as a medicinal plant [33-34]. In a study by [35], phytochemical analysis of *Moringa oleifera* leaf extracts showed the presence of flavonoids, saponins, sterols and tannins in both aqueous and ethanol extracts. The existence of flavonoids, saponins, steroids, terpenoids and tannins in *Moringa oleifera* leaf extract have been reported [36]. It has been reported that various solvents used for extraction have different extraction capabilities and spectrum of solubility for

the phytochemical constituents and the findings from this study also correspond with the documented report by [37].

The antibacterial activity of ethyl acetate and dichloromethane extracts of dried *Moringa oleifera* leaf were determined using the different strains of MDR *Pseudomonas aeruginosa* isolated from post-surgical wound swabs in the two government-owned hospitals in Benin City, Nigeria. All *M. oleifera* leaf extracts fractions tested had different inhibitory effects on the various MDR *P. aeruginosa* strains at different concentrations. There was a significant antimicrobial activity demonstrated by the ethyl acetate fraction for *P. aeruginosa* strains S2H16 isolated from surgical wound patients in the CHB being the most susceptible organism in this study (Table 3) followed by dichloromethane for *P. aeruginosa* strains DHS01 and *P. aeruginosa* strains PS2 all at a concentration of 100 mg/ml. The least considerable zone of inhibition (10.33 ± 0.33) was observed in ethyl acetate fraction for *P. aeruginosa* strains P2S at a concentration of 6.25 mg/ml making it the minimum inhibitory concentration for this fraction. On the other hand, amongst all *P. aeruginosa* strains isolated from the UBTH, ethyl acetate and dichloromethane showed high susceptibility for *P. aeruginosa* strains Iraq.PA-9 and *P. aeruginosa* strain KAR21 (Tables 5 and 6) at a concentration of 100 mg/ml. However, minimum inhibitory concentration was observed in ethyl acetate fraction (10.33 ± 0.33 mm) at 12.5 mg/ml followed by dichloromethane (10.33 ± 0.33) at 50 mg/ml. The findings from this work conform to other reports on the antibacterial activity of *Moringa oleifera* extracts [38-40]. The activity of *Moringa oleifera* extracts against the tested strains of *P. aeruginosa* could be ascribed to the occurrence of some broad-spectrum antibacterial compounds [41]. More so, it has been reported that purified methanol and dichloromethane extracts from *M. oleifera* had antibacterial effects on both Gram-positive and negative bacteria [42]. The findings from this work revealed that *Moringa* leaf extract had both bactericidal activities on the different *P. aeruginosa* strains tested. This is an indication that the leaf extracts may be used in the treatment of post-surgical wound infection caused by multiple drug-resistant *P. aeruginosa* strains. Antimicrobial phytochemicals especially tannins work by binding with the cell walls and inactivate the enzymes [43]. *Moringa oleifera* leaf rich in tannins has proved to be efficient in the treatment of infection and healing of wounds [44]. It has been reported that the presence of terpenoids and saponins may cause hemolysis [45]. In another study, flavonoids have been reported to inhibit nucleic acid synthesis, alteration in cytoplasmic membrane function, energy metabolism inhibition, decrease in cell attachment and varying the membrane permeability [46]. The worldwide appearance of MDR *P. aeruginosa* strains is increasing, therefore limiting the efficacy of some routinely used drugs and resulting in treatment failure. Novel move towards the prevention of antibiotic resistance of pathogenic organisms by employing new compounds that are not based on existing synthetic antimicrobial agents is the way to go in tackling the menace of MDR.

6.0 CONCLUSION

The two extract fractions showed varying antibacterial activity, ethyl acetate *M. oleifera* leaf extract fraction showed highest degrees of antimicrobial activity on the microorganisms tested. The activity of *M. oleifera* indicated a powerful source of new antimicrobial substitute. However, further work is needed to isolate the secondary metabolites from the extract in order to test for specific antimicrobial activity. This *in vitro* study demonstrated that folk medicine can be as effective as modem medicine to combat pathogenic microorganisms. According to World Health Organization, microbial resistance to routine antibiotics is on the rise and medicinal plants offer

a good source of alternative. *Moringa oleifera* represents an economic and safe option to treat infections in addition to several other uses.

COVER STATEMENTS

This is to indicate that, this article is the fruits of our original Research and that this article has not been submitted elsewhere for publication. Where ever the information has been adapted, the author has provided adequate citation.

ETHICAL CLEARANCE

Approval was obtained from the University of Benin Teaching Hospital and Central Hospital, ethical committee and all patients gave their support after being educated of the objectives of study.

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