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Action of Fractionated *Moringa oleifera* Lam Leaf Extracts on Multidrug Resistant *Pseudomonas aeruginosa* Strains

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen with the potential to cause serious surgical wound infections and remains a major problem especially in developing countries of the world. This has led to investigating antimicrobial actions of fractionated *Moringa oleifera* leaf extract on multidrug *Pseudomonas aeruginosa* strains. Isolation of *P. aeruginosa* strains from post-surgical wound in two locations used for the study, antibiotics susceptibility testing, qualitative and quantitative phytochemical screening 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%) *P. aeruginosa* strains were found to show multidrug resistant ability from both locations studied. Qualitative analysis of phytoconstituents revealed the presence of flavonoid, phenol, saponins, steriods, tannin and terpenoids. Quantitative analysis showed that the different phytochemical recorded varying amount. Antibacterial assay results revealed that *M. oleifera* leaf n-hexane extract exhibited highest antibacterial activity against *P. aeruginosa* strain NAPCC-1 followed by methanol and aqueous methanol all at a concentration of 100 mg/ml while aqueous methanol best inhibited *P. aeruginosa* strain KAR12 growth followed by methanol then n-hexane extract. The varying effect could be attributed to the presence of phytochemicals in the different *M. oleifera* leaf extracts. The result of this study has shown the potentials of *M. oleifera* extracts as antibacterial agent by inhibiting the growth of the test organisms isolated from post-surgical wound infection.

Keywords

Pseudomonas aeruginosa, *Moringa oleifera,* wound swabs, Nosocomial infections

1. Introduction

Phytomedicine in Africa and other continents of the world has been utilized from time immemorial to treat various diseases way back before the beginning of modern medicine. The use of medicinal plants is still broadly em-

ployed in many parts of the world particularly in areas where the populace does not have access to modern medicine [1]. Generally, plants have been reported to enclose large varieties of chemical substances that hold vital defensive and remedial therapies [2, 3]. About 80% of the population from urbanized countries uses conventional medicine, which have compounds derived from medicinal plants [4]. Despite the presence of diverse approaches to drug breakthrough, plants still remain the key pool of natural medicine [5, 6].

Moringa oleifera is a vastly cherished plant and dispersed in many countries of the tropics and subtropics, well-known for its wide range of medicinal properties. It has a remarkable variety of medicinal uses with high nutritional value. Different parts of this plant enclose a profile of vital minerals, and are excellent source of protein, vitamins, β-carotene, amino acids and different phenolics [7].

Moringa plant provides a rich and uncommon mixture of zeatin, quercetin, kaempferol and many other phytochemicals. Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods are utilized as cardiac, circulatory stimulants and well possess anti-tumour, anti-pyretic, anti-epileptic, anti-inflammatory, anti-ulcer properties [8]. Other important medicinal properties of the plant include antihypertensive, cholesterol lowering [9], antioxidant, anti-diabetic, antibacterial and antifungal activities [10]. The crushed seeds were conventionally utilized for water purification purpose by flocculating Gram-positive and Gram-negative bacterial cells [11, 12], used as a less expensive bio absorbent for the elimination of heavy metals [13]. Extracts of roots and seeds have shown to have antimicrobial property and juices from the leaves have been employed in treating eye infections and other bacterial infections due to its phytochemical composition.

Pseudomonas aeruginosa is an opportunistic pathogen causing severe healthcare-related infections particularly in immune-compromised post-surgical wound patients [14]. Generally, surgical wounds through the repression of body defense system become appropriate spot for microbial reproduction. Hence, *P. aeruginosa* infection in post-surgical wound patients is common and is seen as one of the leading serious life-threatening situations in surgical operation units [15]. Furthermore, as a result of the resistance of these microbes to a vast collection of regularly used antibiotics in recent years, handling infections caused by them has been dreary and has resulted in enhanced mortality [16]. The difficulty in treating *P. aeruginosa* infection is because of its acquired and intrinsic resistance to diverse antibiotics caused by many mechanisms such as low outer membrane permeableness, over expression of efflux pump and protein modifications has led to increased use of plants as substitute for treatment of some bacterial infections [17]. 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 [18]. Despite the array of uses *Moringa* tree are employed, scanty literature is available on the antibacterial action of fractionated *Moringa oleifera* leaf extract against multiple drug resistant *Pseudomonas aeruginosa* strains isolated from post-surgical wound infections.

2. Materials and Methods

2.1 Collection, authentication and processing of plant materials

The fresh leaf of *M. oleifera* 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 [19]. 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 (3) days [8]. 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 [20].

2.2.1 Solvent-Solvent Extraction

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 n-Hexane [21]. Briefly, 500 mL of n-Hexane was added to the methanol extract in a separating funnel. The mixture was slightly agitated and the pressure accumu-

lated in the funnel was released by opening the tap. The mixture was allowed to stand for a few minutes and the hexane layer was collected. The recovered methanol extract was again extracted with 500 mL of n-hexane and separated. This procedure was repeated until a total of 2 L of n-hexane was used and the hexane layer became clear. The n-hexane portions were combined and evaporated to dryness. The various fractions were concentrated to dryness, weighed and the percentage yields calculated.

2.2.2 Phytochemical Analysis

Phytochemical screening was performed to identify phytochemicals in the Methanol, n-hexane and aqueous methanolic 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. The tests were performed in triplicates to ensure accurate results.

2.2.3 Detection of alkaloids

Three methods were used for the detection of alkaloids with some modifications.

i. Dragendorff's test

There drops of Dragendorff's reagent was added in 1ml of extract filtrate. Formation of orange-brown precipitate indicates the occurrence of alkaloids [22].

ii. Mayer's test

To a 1ml of test extract filtrate in a watch glass, two few drops of Mayer's reagent were added. Formation of cream-colored precipitate shows the presence of alkaloids [23].

iii. Wager's test

The occurrence of alkaloids was tested using method reported by [23]. 10 ml of the extract were evaporated to dryness. Two ml of 2% HCL acid were added to the dry residue. Two drops of Wagner's reagent were added to the solution. Reddish brown precipitate indicates the presence of alkaloids.

2.2.4 Detection of flavonoids

The test was based on method described by [24]. Few drops of NaOH were added to two ml of the extract and intense yellow color appeared. Few drops of dilute HCL were added and the solution turned to colorless as indicator of presence of flavonoids.

2.2.5 Detection of saponins

Five milliners of distilled water was added to 1 ml of *Moringa oleifera* leaf extract in a test tube. The solution was then shaken vigorously and stable persistent froth indicates the presence of saponins [25].

2.2.6 Detection of phenols

Two drops of ferric chloride solution was added to 1 ml of extract. Formation of bluish black colour indicates the presence of phenol [25].

2.2.7 Detection of steroids

The test was performed based on method described by [26]. Two ml concentrated sulphuric acid were added to 1 ml of the extract. Formation of red precipitate indicates the presence of sterols.

2.2.8 Detection of tannins

Two drops of 10% lead acetate were added to 2ml of each extract. The appearance of white precipitate indicates the presence of tannins as described by [27].

2.2.9 Detection of terpenoids

Two milliners of chloroform and 1 ml concentrated tetraoxosulphate (VI) acid was added to 1 ml of extract to form a layer. The presence of a reddish brown colouration at the interface shows the occurrence of terpenoids [28].

2.2.10 Detection of Glycosides

Few drops of glacial acetic acid and ferric chloride was added to 1ml of each extract, 3 drops of concentration sulphuric acid were added. The appearance of blue-green colour indicates the presence of glycosides [27].

2.2.11 Detection of anthraquinones

One milliliter of extracts was boiled in 10% HCl for 5 mins and the filtrate was allowed to cool. The filtrate were treated with equal volume of $CHCl_3$ with few drops of 10% ammonia was added to the 2ml filtrate. The formation of rose-pink colour implies the presence of anthraquinones [29].

2.3 Quantitative phytochemical analysis of *Moringa oleifera* extract

2.3.1 Determination of total phenol

Total phenol contents in the extracts were determined by the method described by [30]. The extract solution (0.5 mL) with a concentration of 1,000 µg/mL was added to 4.5 mL of deionized distilled water and 0.5 mL of Folin Ciocalteu's reagent (previously diluted with water 1:10, v/v) which was then added to the solution. After mixing the tubes, they were maintained at room temperature for 5 minutes followed by the addition of 5 mL of 7% sodium carbonate and 2 mL of deionized distilled water. After mixing the samples, the samples were incubated for 90 minutes at room temperature. The absorbance was measured by spectrophotometer at 750 nm. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract). The standard curve was prepared by gallic acid in six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/L).

2.3.2 Determination of total flavonoid

Total flavonoid contents were estimated using the method described by [31]. Briefly, 0.5 mL of extract sample (1 mg/mL) was mixed with 1.5 mL of methanol and then, 0.1 mL of 10% aluminium chloride was added, followed by 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured by a spectrophotometer at 415 nm. The results were expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g extract). The standard curve was prepared by quercetin in six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/L).

2.3.3 Determination of total tannin content

The tannins were determined by Folin-Ciocalteu method as previously described by [31] with slight modification. About 0.5 ml of the sample extract (1 mg/mL) was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu phenol reagent, 1 mL of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. a set of reference standard solutions of tannic acid (20, 40, 60, 80, 100 µg/ mL) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/ Visible spectrophotometer. The estimation of the tannin content was carried out in triplicate. The tannin content was expressed in terms of mg of tannic acid equivalents/g of dried sample.

2.3.4 Determination of total saponin content

The saponin content of the samples was determined by double extraction gravimetric method described by [32]. A measured weight (0.5 g) of the powdered sample was mixed with 5 mL of 20% aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 minutes at 55ºC; it was then filtered through Whatman filter paper (No 42). The extract was transferred to a separating funnel where 20 mL of diethyl ether was added and shaken vigorously. Re-extraction by partitioning was repeated twice (each with 20 mL diethyl ether) until the aqueous layer become clear in colour. The ether layer was discarded and the saponins in the aqueous layer were extracted 3 x (each with 20 mL) of normal butanol. The combined butanol extracts were washed with 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. It was dried at 60ºC in the oven and reweighed after cooling in a dessicator. The process was repeated one more time to get an average. Saponin content was determined by difference and calculated as a percentage of the original sample thus: Percentage saponin = W2 – W1/weight of sample x 100

Where: W1 = Weight of evaporating dish, W2 = Weight of evaporating dish + sample

2.3.5 Determination of total terpenoid content

This protocol describes a rapid, small-scale, high-throughput assay for approximating the total terpenoids content in plant tissue using a monoterpeneas standard reagent with conc. sulfuric acid. All estimation has been done spectroscopically at 538 nm. In brief, one point five microliters of chloroform was added to 200µl of sample solution (10mg/mL). The sample mixture was vortexed thoroughly and allowed to stand for 3 minutes then 100µl Conc. Sulfuric acid (H2SO4) was added to each 2 ml microcentrifuge tube. The assay tube was incubated at room temperature for 1.5h in the dark but for standard solution, (Thymol) incubation was not more than 5 minutes. At the end of incubation, a reddish-brown precipitation was formed in each assay microcentrifuge tube which was carefully and gently decanted without disturbing the precipitation. 1.5 ml of 95% (Vol/Vol) methanol was added to the mixture and vortex thoroughly until all the precipitation was dissolved in the methanol completely. The sample was then transferred from assay tube to Colorimetric cuvette. Methanol [95% (Vol/Vol)] was used as blank] and absorbance was read at 538 nm. Total terpenoids concentration of unknown plant sample was calculated as Thymol

equivalents using the regression equation of thymol standard curve.

2.3.6 Estimation of total Steroid content

One milliliter of test extract of steroid solution (1 mg/mL) was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70°C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank. The quantity in milligram of the steroid in the 10 mL aliquot of the test solution is given by the expression; At / As X Cs

Where At = absorbance of the test solution; As = absorbance of the standard solution; Cs = quantity, in milligram, of the reference substance in the 10 ml aliquot of the standard solution. The quantity of the steroid in the substance was calculated on the basis of the aliquot taken for the determination and from the declared content of the steroid in the appropriate reference substance. Cholesterol was used as the reference substance.

2.3.7 Test organism sample collection

A total of 284 random swab sampling of post-operative surgical wound patients was collected from both outpatient and inpatient in University of Benin Teaching Hospital (UBTH) and Central Hospital Benin (CHB), Benin City.

2.3.8 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.

2.3.9 Bacteriological procedures/identification of isolates

Swab samples were aseptically inoculated onto MacConkey, Blood and Nutrient agar and incubated aerobically at 37°C for 24 hours and checked for colony growth. Isolates were screened for *Pseudomonas aeruginosa*. All specimens were processed at Lahor research Laboratories, Benin City, Nigeria using standard microbiological methods. All isolates were identified using conventional techniques [33].

2.3.10 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 CLSI (2011) against the following antibiotics for Gram negative bacteria include: Augmentin (AUG, 30µg), Ofloxacin (OFL 5µg), Cefixime (CXM 5µg), Gentamycin (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.

2.3.11 Standardization of Inoculum

The inocula were prepared from the stock cultures, which were maintained on nutrient agar slant at 4°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 [33].

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

Antibacterial activity of *Moringa oleifera* methanolic extracts and the different fractions were tested against the multidrug resistant *Pseudomonas 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 10^{6}cfu/ml which was used to flood the surface of the Mueller-Hinton agar media. It was then 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 6.25mg/ml) was added to the different wells. The same procedure was applied to all extracts. The plates were left to allow for diffusion of extract before incubation at 37°C for 24 hours. The zones of inhibition (clearance) produced around the wells after incubation were observed, measured and recorded.

2.5 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 [34]. The stock concentration of 100.00 mg/ml of the extracts was double diluted separately to achieve different concentrations of 50.00, 25.00, 12.50 and 6.25 mg/ml. Aliquot of 2 ml of the crude extract in the desired concentration was introduced into tube one containing 2 ml of distilled water and mixed thoroughly. The content (2 ml) from tube one was transferred into tube two, it was also mixed thoroughly and 2 ml of the content of tube two was also transferred into test tube three. The procedure was repeated for the remaining test tubes. Pre-diffusion period of 15 minutes was allowed before incubation, and the result recorded after the incubation period at 37°C for 24 hours.

2.6 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. Graphical representation was done using Microsoft word. The results were expressed as mean ± SEM (Standard error of mean).

3. Results

In the present 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 routinely used antibiotics to identify multiple drug antibiotic resistant strains. Thirty-four (54.8%) isolates 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 Figure 1.

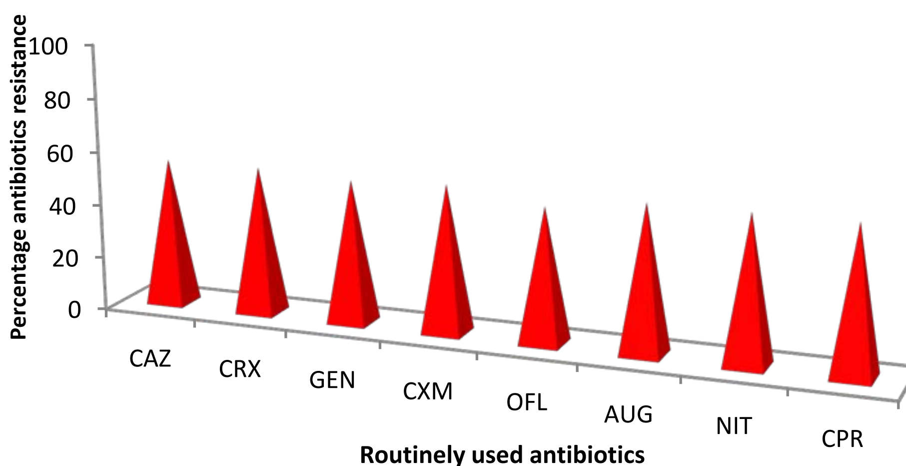

Figure 1. Antibiotics resistance pattern of *P. aeruginosa* strains. CAZ: Ceftazidime. CRX: Cefuroxime, GEN: Gentamicin, CXM: Cefixime, OFL: Ofloxacin, AUG: Augmentin, NIT: Nitrofuration, CPR: Ciprofloxacin.

Five hundred grams (500 g) of powered leaf of *M. oleifera* were extracted with two point five liters (2.5 L) of methanol and then evaporated to dryness. The extract yielded 26.5 g crude extract of methanol. Fractionation of crude methanol extract were carried out using n-Hexane and yielded 7.0 g, the aqueous methanol extract were also evaporated to dryness and yielded 2.5 g (Table 1). Qualitative analysis of all extracts revealed the presence of six (6) phytochemical constituents out of the nine tested in methanol, 5 phytochemical present in aqueous methanol and n-hexane showed 2 phytochemicals as shown in Table 2. Quantitative analysis of the phtytoconstituent present revealed varying quantities of the phytochemicals screened (Table 3).

Table 1. Dried extract yield of *Moringa oleifera* leaf

Moringa oleifera leaf Extract	Extract dried yield (grams)
Methanol	26.5
n-Hexane fraction	7.0 g,
Aqueous methanol	2.2 g

Table 2. Phytochemical screening of *Moringa oleifera* methanol, aqueous methanol and n-hexane leaf extract fractions

Phytochemicals	Methanolic extracts	Aqueous methanolic fraction	n-Hexane fraction
Alkaloids	–	–	–
Anthraquinone	–	–	–
Flavonoid	+	+	–
Glycoside	–	–	–
Phenol	+	+	–
Saponins	+	+	–
Steriods	+	+	+
Tannin	+	–	–
Terpenoids	+	+	+

Where +: indicate the presence of phytochemicals, –: indicate the absence of phytochemicals

Table 3. Quantitative phytochemical analysis of *Moringa oleifera* methanol leaf extracts

Phytochemical	Standard	Total phytochemical Content
Flavonoid	Quercetin	202.845 ± 53.88 mg/g
Phenol	Gallic Acid	46.027 ± 2.871mg/g
Saponins*	–	105.9 ± 7.140 mg/g
Steriods	Cholesterol	2.9 ± 2.950 mg/ml
Tannin	Tannic acid	69.473 ± 4.686 mg/g
Terpenoids	Thymol	33.83 ± 29.796 mg/ml

*: Gravimetric Method; Values are mean ± SEM

Testing of antibacterial activity of *M. oleifera* methanol leaf extracts on different strains of MDR *Pseudomonas aeruginosa* from Central Hospital Benin (CHB) showed that methanol extracts had inhibitory effect of various levels when compared with the control (standard reference) as shown in Table 4. Statistical analysis revealed that there was a high significant difference in the mean zone of inhibition at the different concentrations. The highest mean zone of inhibition of 22.67 ± 1.45 millimeters were seen in *Pseudomonas aeruginosa* strains R7-583 at 100 mg/ml and the lowest acceptable mean zone of inhibition of 9.67 ± 0.88 millimeters at 25 mg/ml were observed in *Pseudomonas aeruginosa* strains PS2.

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

Isolates	100mg/ml $\bar{X} \pm SE$	50mg/ml $\bar{X} \pm SE$	25mg/ml $\bar{X} \pm SE$	12.5mg/ml $\bar{X} \pm SE$	6.25mg/ml $\bar{X} \pm SE$	P-value
P. a strain PS2	$21.33^{a} \pm 0.67$	$12.0^{b} \pm 1.16$	$9.67^{c} \pm 0.88$	$1.00^{d} \pm 0.00$	$1.00^{d} \pm 0.00$	P<0.01
P. a strain NAPCC-1	$18.33^{a} \pm 1.67$	$10.0^{b} \pm 1.16$	$8.00^{b} \pm 1.16$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain DHS01	$19.33^{a} \pm 0.67$	$4.67^{b} \pm 1.45$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain AR442	$10.0^{a} \pm 2.89$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	P<0.01
P. a strain R7-520-1	$24.67^{a} \pm 0.88$	$12.33^{b} \pm 1.45$	$12.0^{b} \pm 1.16$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain H25883	$21.67^{a} \pm 1.67$	$6.33^{b} \pm 0.88$	$6.00^{b} \pm 1.16$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain PA-VAP-2	$13.67^{a} \pm 2.03$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	P<0.01
P. a strain R7-583	$22.67^{a} \pm 1.45$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	P<0.01
P. a strain PA006	$10.00^{a} \pm 2.89$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	P<0.01
P. a strain S2H16	$15.0^{a} \pm 2.89$	$3.67^{b} \pm 0.88$	$3.67^{b} \pm 0.88$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<0.01
P. a strain KAR21	$10.0^{a} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	P<0.01
P. a strain D2	$20.67^{a} \pm 1.76$	$7.33^{b} \pm 1.20$	$3.67^{c} \pm 0.88$	$1.00^{c} \pm 0.00$	$1.00^{c} \pm 0.00$	P<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. Reading of significance is row.

Antibacterial activity of *M. oleifera* methanol leaf extracts on different strains of MDR *Pseudomonas aeruginosa* from University of Benin Teaching Hospital (UBTH) revealed that methanol extracts had inhibitory effect of various levels when compared with the control as shown in Table 5. Statistical analysis showed that there was a high significant difference in the mean zone of inhibition at the different concentrations with the highest mean zone of inhibition of 32.33 ± 1.45 millimeters were seen in *Pseudomonas aeruginosa* strains Iraq.PA-9 at 100 mg/ml and the lowest acceptable mean zone of inhibition of 12.00 ± 1.67 millimeters at 25 mg/ml were observed in *P. aeruginosa* strains R8-768-1 followed by *P. aeruginosa* strains KAR21 and *P. aeruginosa* strains Iraq.PA-9 with 12.33 ± 1.20 millimeters respectively.

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

Isolates	100mg/ml $\bar{X} \pm SE$	50mg/ml $\bar{X} \pm SE$	25mg/ml $\bar{X} \pm SE$	12.5mg/ml $\bar{X} \pm SE$	6.25mg/ml $\bar{X} \pm SE$	P-value
P. a strain SWD	$12.00^a \pm 1.16$	$6.33^b \pm 0.88$	$3.67^c \pm 0.88$	$1.33^d \pm 0.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain Exo25	$16.33^a \pm 0.88$	$6.33^b \pm 0.88$	$6.00^b \pm 1.16$	$5.00^b \pm 0.58$	$1.00^c \pm 0.00$	P<0.01
P. a strain R8-768	$16.00^a \pm 0.58$	$6.67^b \pm 0.88$	$1.00^c \pm 0.00$	$2.00^c \pm 0.58$	$1.00^c \pm 0.00$	P<0.01
P. a strain YPAB1	$22.67^a \pm 1.45$	$13.33^b \pm 0.88$	$4.00^c \pm 1.16$	$2.00^c \pm 0.58$	$1.00^c \pm 0.00$	P<0.01
P. a strain VITMS7	$22.00^a \pm 1.16$	$15.67^b \pm 0.88$	$6.00^c \pm 1.16$	$2.00^d \pm 0.58$	$1.00^d \pm 0.00$	P<0.01
P. a strain AR442	$12.00^a \pm 1.16$	$3.67^b \pm 0.88$	$3.33^b \pm 0.67$	$2.33^c \pm 0.67$	$1.00^c \pm 0.00$	P<0.01
P. a strain AS23	$16.33^a \pm 0.88$	$3.33^b \pm 0.88$	$2.67^b \pm 0.88$	$2.00^b \pm 0.58$	$1.00^b \pm 0.00$	P<0.01
P. a strain DKH-3	$23.00^a \pm 1.16$	$16.67^b \pm 0.88$	$3.00^c \pm 0.58$	$4.00^c \pm 1.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain H25883	$22.0^a \pm 1.16$	$16.33^b \pm 0.88$	$14.00^b \pm 0.58$	$6.33^c \pm 0.88$	$1.00^d \pm 0.00$	P<0.01
P. a strain Y15	$20.0^a \pm 1.16$	$12.67^b \pm 1.45$	$6.33^c \pm 0.88$	$4.00^d \pm 1.16$	$1.00^d \pm 0.00$	P<0.01
P. a strain PA016	$24.67^a \pm 0.88$	$6.67^b \pm 0.88$	$5.33^b \pm 0.67$	$1.67^c \pm 0.33$	$1.00^c \pm 0.00$	P<0.01
P. a strain R8- 768-1	$25.00^a \pm 1.16$	$16.00^b \pm 1.16$	$12.00^c \pm 1.16$	$1.67^d \pm 0.33$	$1.00^d \pm 0.00$	P<0.01
P. a strain KAR21	$31.67^a \pm 0.88$	$15.00^b \pm 1.16$	$12.33^b \pm 1.20$	$1.33^c \pm 0.33$	$1.00^c \pm 0.00$	P<0.01
P. a strain Iraq.PA -9	$32.33^a \pm 1.45$	$22.00^b \pm 1.16$	$12.33^c \pm 1.45$	$6.67^d \pm 0.67$	$3.33^d \pm 0.67$	P<0.01

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. Reading of significance is row.

The antibacterial activity of *M. oleifera* aqueous methanol fraction of crude methanol leaf extracts on MDR *Pseudomonas aeruginosa* strains from surgical wound swab in Central Hospital Benin indicated that aqueous methanol fractions exhibited different levels of antibacterial activity when compared with the control as shown in Table 6. It was showed that there was a high significant difference in the mean zone of inhibition at the different concentrations. Highest mean zone of inhibition of 24.67 ± 0.88 millimeters was recorded for MDR *Pseudomonas aeruginosa* strains S2H16 and 9.67 ± 0.33 millimeters lowest acceptance zone of inhibition at 25 mg/ml was recorded for MDR *P. aeruginosa* strains D2 and *P. aeruginosa* strains H25883 respectively.

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

Isolates	100mg/ml $\bar{X} \pm SE$	50mg/ml $\bar{X} \pm SE$	25mg/ml $\bar{X} \pm SE$	12.5mg/ml $\bar{X} \pm SE$	6.25mg/ml $\bar{X} \pm SE$	P-value
P. a strain PS2	$19.67^a \pm 0.33$	$10.67^b \pm 0.67$	$4.33^c \pm 0.33$	$1.00^d \pm 0.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain NAPCC-1	$20.33^a \pm 0.33$	$12.33^b \pm 0.33$	$4.67^c \pm 0.33$	$1.00^d \pm 0.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain DHS01	$19.00^a \pm 0.58$	$10.33^b \pm 0.33$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	P<0.01
P. a strain AR442	$19.67^a \pm 0.33$	$10.67^b \pm 0.33$	$6.00^c \pm 1.00$	$1.00^d \pm 0.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain R7-520-1	$23.33^a \pm 1.20$	$14.67^b \pm 0.33$	$7.67^c \pm 0.33$	$1.00^d \pm 0.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain H25883	$23.00^a \pm 1.53$	$14.33^b \pm 0.33$	$9.67^c \pm 0.33$	$1.00^d \pm 0.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain PA-VAP-2	$21.00^a \pm 0.58$	$15.33^b \pm 0.33$	$4.67^c \pm 0.67$	$1.00^d \pm 0.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain R7-583	$19.67^a \pm 0.33$	$9.67^b \pm 0.33$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	P<0.01
P. a strain PA006	$19.67^a \pm 0.33$	$11.67^b \pm 0.33$	$4.67^c \pm 0.33$	$1.00^d \pm 0.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain S2H16	$24.67^a \pm 0.88$	$14.67^b \pm 0.33$	$9.67^c \pm 0.33$	$4.67^d \pm 0.33$	$1.00^e \pm 0.00$	P<0.01
P. a strain KAR21	$23.67^a \pm 0.88$	$11.33^b \pm 0.33$	$7.33^c \pm 0.33$	$1.00^d \pm 0.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain D2	$15.00^a \pm 0.58$	$5.33^b \pm 0.33$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	P<0.01

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. Reading of significance is row.

The antibacterial activity of residual aqueous methanol fraction of *M. oleifera* leaf crude methanol extracts on MDR *Pseudomonas aeruginosa* strains from UBTH indicated that different concentrations of the fraction exhibited antibacterial activity against tested isolates as shown in Table 7. Statistical analysis of data obtained revealed that there was a high significant difference in the mean zone of inhibition at the different concentrations. Highest mean zone of inhibition of 34.00 ± 0.58 millimeters and lowest acceptance zone of inhibition of 10.33 ± 0.33 millimeters at 12.5 mg/ml recorded for MDR *P. aeruginosa* strains KAR21.

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

Isolates	100mg/ml $\bar{X} \pm SE$	50mg/ml $\bar{X} \pm SE$	25mg/ml $\bar{X} \pm SE$	12.5mg/ml $\bar{X} \pm SE$	6.25mg/ml $\bar{X} \pm SE$	P-value
P. a strain SWD	$19.67^a \pm 0.33$	$11.33^b \pm 0.33$	$9.67^c \pm 0.33$	$4.33^d \pm 0.33$	$1.00^d \pm 0.00$	P<0.01
P. a strain Exo25	$20.67^a \pm 0.33$	$10.33^b \pm 0.33$	$4.33^c \pm 0.33$	$1.00^d \pm 0.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain R8-768	$18.33^a \pm 0.33$	$10.33^b \pm 0.33$	$5.67^c \pm 0.33$	$1.00^d \pm 0.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain YPAB1	$20.33^a \pm 0.33$	$9.67^b \pm 0.00$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	P<0.01
P. a strain VITMS7	$15.00^a \pm 1.73$	$10.67^b \pm 0.33$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	P<0.01
P. a strain AR442	$19.67^a \pm 0.33$	$12.33^b \pm 0.33$	$4.33^c \pm 0.33$	$1.00^d \pm 0.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain AS23	$20.00^a \pm 0.58$	$15.67^b \pm 0.67$	$10.33^c \pm 0.33$	$1.00^d \pm 0.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain DKH-3	$20.33^a \pm 0.33$	$11.00^b \pm 0.58$	$10.67^b \pm 0.67$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	P<0.05
P. a strain H25883	$17.67^a \pm 0.88$	$11.33^b \pm 0.88$	$4.33^c \pm 0.33$	$1.00^d \pm 0.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain Y15	$24.67^a \pm 0.33$	$12.67^b \pm 0.67$	$9.33^c \pm 0.33$	$4.67^d \pm 0.33$	$1.00^e \pm 0.00$	P<0.01
P. a strain PA016	$15.33^a \pm 0.33$	$8.67^b \pm 0.67$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	P<0.01
P. a strain R8- 768-1	$15.33^a \pm 0.33$	$11.00^b \pm 0.58$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	P<0.01
P. a strain KAR21	$34.00^a \pm 0.58$	$24.67^b \pm 0.33$	$18.33^c \pm 0.33$	$10.33^d \pm 0.33$	$7.67^e \pm 0.33$	P<0.01
P. a strain Iraq.PA -9	$15.33^a \pm 0.33$	$1.00^b \pm 0.00$	$1.00^b \pm 0.00$	$1.00^b \pm 0.00$	$1.00^b \pm 0.00$	P<0.01

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. Reading of significance is row.

The antibacterial property of n-hexane fraction of crude methanol *M. oleifera* leaf extracts on MDR *Pseudomonas aeruginosa* strains indicated that n-hexane fractions from Central Hospital Benin showed different levels of antibacterial activity when compared with the control as shown in Table 8. It was showed that there was a high significant difference in the mean zone of inhibition at the different concentrations of the extract. The highest mean zone of inhibition of 40.33 ± 0.33 and 40.00 ± 0.58 millimeters was recorded for MDR *P. aeruginosa* strains NAPCC-1 and MDR *P. aeruginosa* strains R7-520-1 respectively. Meanwhile, lowest acceptable zone of inhibition of 9.33 ± 0.67 millimeters at 6.25 mg/ml was recorded for MDR *P. aeruginosa* strain PA-VAP-2.

Table 8. Antibacterial activity of *M. oleifera* n-Hexane leaf extracts against different MDR *Pseudomonas aeruginosa strains* from CHB

Isolates	100mg/ml $\bar{X} \pm SE$	50mg/ml $\bar{X} \pm SE$	25mg/ml $\bar{X} \pm SE$	12.5mg/ml $\bar{X} \pm SE$	6.25mg/ml $\bar{X} \pm SE$	P-value
P. a strain PS2	$36.00^a \pm 0.58$	$24.67^b \pm 0.33$	$20.67^c \pm 0.33$	$15.33^d \pm 0.00$	$10.33^e \pm 0.33$	P<0.01
P. a strain NAPCC-1	$40.33^a \pm 0.33$	$21.67^b \pm 0.33$	$20.33^b \pm 0.33$	$13.00^c \pm 1.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain DHS01	$30.33^a \pm 0.33$	$20.33^b \pm 0.33$	$16.00^c \pm 0.58$	$10.67^d \pm 0.67$	$8.67^e \pm 0.67$	P<0.01
P. a strain AR442	$25.00^a \pm 0.58$	$21.00^b \pm 0.58$	$15.33^c \pm 0.33$	$13.00^d \pm 0.58$	$10.67^e \pm 0.67$	P<0.01
P. a strain R7-520-1	$40.00^a \pm 0.58$	$20.33^b \pm 0.33$	$10.33^c \pm 0.33$	$4.67^d \pm 0.67$	$2.00^e \pm 0.00$	P<0.01
P. a strain H25883	$31.00^a \pm 0.58$	$20.33^b \pm 0.33$	$21.00^b \pm 0.58$	$11.33^c \pm 0.67$	$1.67^d \pm 0.33$	P<0.01
P. a strain PA-VAP-2	$22.33^a \pm 0.33$	$14.67^b \pm 0.33$	$13.67^b \pm 0.88$	$12.67^b \pm 0.67$	$9.33^c \pm 0.67$	P<0.01
P. a strain R7-583	$30.33^a \pm 0.33$	$25.33^b \pm 0.33$	$20.33^c \pm 0.33$	$17.00^d \pm 0.58$	$11.00^e \pm 0.58$	P<0.01
P. a strain PA006	$30.00^a \pm 0.58$	$21.00^b \pm 0.58$	$15.33^c \pm 0.33$	$9.00^d \pm 0.58$	$5.67^e \pm 0.67$	P<0.01
P. a strain S2H16	$29.00^a \pm 0.58$	$18.33^b \pm 0.33$	$10.33^c \pm 0.33$	$5.33^d \pm 0.33$	$2.00^e \pm 0.00$	P<0.01
P. a strain KAR21	$20.67^a \pm 0.33$	$15.33^b \pm 0.33$	$10.33^c \pm 0.33$	$5.00^d \pm 0.58$	$2.00^e \pm 0.00$	P<0.01
P. a strain D2	$39.67^a \pm 0.88$	$35.33^b \pm 0.33$	$21.00^c \pm 0.58$	$11.33^d \pm 0.67$	$11.00^d \pm 0.58$	P<0.01

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. Reading of significance is row.

The antibacterial effect of n-hexane fraction of crude methanol *M. oleifera* leaf extracts on MDR *Pseudomonas*

aeruginosa strains from UBTH indicated that n-hexane fractions revealed that different concentrations of antibacterial activity as shown in Table 9. High significant difference in the mean zone of inhibition at the different concentrations of the extract was observed. The highest mean zone of inhibition of 31.00 ± 0.58 millimeters was recorded for MDR *P. aeruginosa* strains Y15 and lowest acceptable zone of inhibition of 10.33 ± 1.16 millimeters at 12.5 mg/ml was recorded for MDR *P. aeruginosa* strains R8-768-1.

Table 9. Antibacterial activity of *M. oleifera* n-hexane leaf extracts against different MDR *Pseudomonas aeruginosa strains* from UBTH

Isolates	100mg/ml $\bar{X} \pm SE$	50mg/ml $\bar{X} \pm SE$	25mg/ml $\bar{X} \pm SE$	12.5mg/ml $\bar{X} \pm SE$	6.25mg/ml $\bar{X} \pm SE$	P-value
P. a strain SWD	$30.67^a \pm 0.67$	$21.00^b \pm 0.58$	$11.33^c \pm 0.67$	$2.67^d \pm 0.67$	$1.00^d \pm 0.00$	P<0.01
P. a strain Exo25	$25.33^a \pm 0.33$	$20.67^b \pm 0.67$	$12.67^c \pm 0.67$	$9.00^d \pm 0.58$	$4.67^e \pm 0.67$	P<0.01
P. a strain R8-768	$18.67^a \pm 0.67$	$10.67^b \pm 0.67$	$8.00^c \pm 1.16$	$2.67^d \pm 0.67$	$1.67^d \pm 0.33$	P<0.05
P. a strain YPAB1	$29.33^a \pm 0.67$	$18.67^b \pm 0.67$	$11.33^c \pm 0.67$	$3.33^d \pm 0.67$	$2.00^d \pm 0.00$	P<0.01
P. a strain VITMS7	$15.67^a \pm 0.33$	$10.67^b \pm 0.67$	$8.00^c \pm 1.16$	$4.67^d \pm 0.67$	$1.00^e \pm 0.00$	P<0.01
P. a strain AR442	$19.67^a \pm 0.88$	$17.33^b \pm 0.67$	$11.00^c \pm 0.58$	$3.00^d \pm 0.58$	$1.00^e \pm 0.00$	P<0.01
P. a strain AS23	$19.33^a \pm 0.67$	$15.00^b \pm 0.58$	$10.67^c \pm 0.67$	$2.00^d \pm 0.00$	$2.00^d \pm 0.00$	P<0.01
P. a strain DKH-3	$19.00^a \pm 0.58$	$10.67^b \pm 0.67$	$5.33^c \pm 0.33$	$2.00^d \pm 0.00$	$2.00^d \pm 0.00$	P<0.01
P. a strain H25883	$25.33^a \pm 0.33$	$21.00^b \pm 0.58$	$4.67^c \pm 0.67$	$2.00^d \pm 0.00$	$2.00^d \pm 0.00$	P<0.01
P. a strain Y15	$31.00^a \pm 0.58$	$22.33^b \pm 0.33$	$16.00^c \pm 0.58$	$1.00^d \pm 0.00$	$1.00^d \pm 0.00$	P<0.01
P. a strain PA016	$16.33^a \pm 0.88$	$12.33^b \pm 1.45$	$9.33^c \pm 0.67$	$2.00^d \pm 0.00$	$2.00^d \pm 0.00$	P<0.05
P. a strain R8- 768-1	$19.00^a \pm 0.58$	$21.00^a \pm 0.58$	$15.67^b \pm 0.33$	$10.00^c \pm 1.16$	$8.00^c \pm 2.00$	P<0.05
P. a strain KAR21	$21.67^a \pm 0.88$	$19.00^a \pm 0.58$	$13.00^b \pm 0.58$	$11.33^b \pm 0.67$	$6.67^c \pm 1.76$	P<0.05
P. a strain Iraq.PA -9	$21.00^a \pm 0.58$	$13.00^b \pm 0.58$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	$1.00^c \pm 0.00$	P<0.01

Similar letters indicate means that are not significantly different (P>0.05), P<0.01 -Highly significantly different, P<0.001 - Very highly significantly different. Values are mean ± SEM. Reading of significance is row.

4. Discussion

Notwithstanding the several conventional medications in use for the treatment and management of various pathophysiological conditions, observable limitations ranging from relatively high cost to somewhat unavailability has been documented [3, 35]. Owing to this, it has become necessary for a switch to a readily available and cheaper alternative in the form of phyto/herbal medicine. Accordingly, the World Health Organization recommends the use of medicinal plants as a therapeutic source for the management of various pathophysiological conditions, and also encouraged the expansion of the frontiers of scientific evaluation on properties of varied plant species [3]. Hence, the present study examined the action of fractionated *Moringa oleifera* lam leaf extracts on multidrug resistant *Pseudomonas aeruginosa* strains. The result showed that two hundred and eighty-four post-operative surgical wound swabs specimens from patients (one hundred and forty-two from each location) were analyzed. Ninety-nine (35%) of patients studied had wound infection. Although this is lower than 39.9% previously reported [36] but higher than 9.6% reported by [37]. However, World Health Organization gave a prevalence of 5%-34% of surgical site infection and this is in agreement with the result of this study. The distribution of etiologic agents of surgical wound infections showed that *P. aeruginosa* (62.6%) was found to be more predominant. This is in conformity with the study of Agbonlahor *et al.* (2018) who also found that *P. aeruginosa* were more prevalent in surgical wound infections [38]. Thirty-four (54.8%) strains were found to be multidrug resistant *Pseudomonas aeruginosa* isolates. The broad spread of antibiotic resistant genes among bacteria as well as *P. aeruginosa* strains is a rising worry in the cure of post-surgical wound infections. In this study, the antibiotic sensitivity testing revealed that most of our isolates showed resistance (>50.0%) to commonly used antibiotics. [39], reported high resistance of bacterial isolates from clinical samples to first, second and third generation antibiotics. [40] reported that bacterial isolated from surgical wound infections are mostly resistant to Ceftazidime and Augmentin. This is most likely to be due to the presence of enzymes such as Cephalosporinase and Penicillinase which can prevent the activity of Beta-lactam ring of the antibiotics [38]. Current problems linked with the use of antibiotics, increased prevalence of multiple drug resistant (MDR) strains of a number of pathogenic bacteria has revitalized the interest in plants with antimicrobial properties [41]. The antimicrobial properties of *Moringa oleifera* have been ascribed to the diverse parts of the plant, such as the leaves, seeds, pods and stems [42] which are recognized for their antibacterial action and are counted as rich basis of antimicrobial agents [43]. In this study, phytochemical screening (qualitative analysis) was

used to reveal the secondary metabolites of the *Moringa oleifera* leaf extract. *M. oleifera* revealed the presence of flavonoids, phenol, saponins, steroids, tannin and terpenoids (Table 2). Flavonoids, phenol and saponins were present in methanol and aqueous methanol extracts. Tannins were present in only methanol extract and absent in aqueous methanol and n-Hexane fractions. Steriods and terpenoids were present in methanol, aqueous methanol and n-Hexane fractions while flavonoids, phenol and saponins were absent in n-Hexane fractions. In the study of [44], phytochemical analysis of *Moringa oleifera* leaf extracts shows the presence of flavonoids, saponins, sterols and tannins in both aqueous and ethanolic extracts. Occurrence of flavonoids, saponins, sterols and tannins in Guava (*Psidium guajava* L.) aqueous, ethanol and chloroform leaf extracts was reported by [45]. [46] reported the presence of flavonoids, saponins, steroids, terpenoids and tannins in *Moringa oleifera* leaf extract. These findings also correspond with the documented report by [47]. Nevertheless, [48] reported absence of steroids, terpenoids and cardiac glycoside while [49] reported absence of terpenoids and cardiac glycoside in *Moringa oleifera* leaf extract. It has been documented that various solvents used for extraction have different extraction capabilities and spectrum of solubility for the phytochemical constituents [50]. Quantitative analysis of secondary metabolites can help to disclose the chemical composition of the plant extract and to identify which phytochemical dominate over the other. This analysis can as well be used to find bioactive constituents for development of products that may enclose medicinal values [51]. The quantitative analysis results of the secondary metabolites in *Moringa oleifera* revealed the amount of flavonoids in crude methanol leaf extract was (202.845 ± 53.88 mg/g), amount of phenol was (46.027 ± 2.871mg/g), amount of saponins was (105.9 ± 7140mg/g), amount of sterols was (2.9 ± 2.950 mg/ml), amount of tannins was (69.473 ± 4.868mg/g) and amount of terpenoids was (33.83 ± 29.796 mg/ml) using different standards based on the phytochemical. In another study, quantitative analysis *M. oleifera* extract showed various amount of phytochemical constituents including terpenoids (4.84 ± 0.05), tannins (9.36 ± 0.04), flavonoid (3.56 ± 0.03) and saponins (1.46 ± 0.03) all in g/100g which were lower when compared to the result in our study but steroids (3.21 ± 0.00) was higher when compared with our result [46]. In *Emblica officinalis* pod extract, the quantity of flavonoids obtained was (0.04%), amount of saponins was (0.55%), and quantity of tannins was (1.10%), [52]. Fruit extract of *Tribulus terrestris* also revealed that the quantity of flavonoids was (1.10%), amount of saponins was (0.85%), and amount of tannins was (1.25%) [53]. In some plant parts, the amount of these phytoconstituents is comparatively higher than others as reported by [54].

The antibacterial activity of methanol, aqueous methanol and n-Hexane extracts of dried *Moringa oleifera* leaf was determined using different strains of *Pseudomonas aeruginosa* isolated from post-surgical wound patients in two government owned hospitals in Benin City, Nigeria. All three *M. oleifera* leaf extracts tested had different inhibitory effect on the various *Pseudomonas aeruginosa* strains at the different concentrations. There was significant antimicrobial activity demonstrated by the n-Hexane fractions for *P. aeruginosa* strains NAPCC-1 isolated from surgical wound patients in Central Hospital Benin (CHB) being the most susceptible organism followed by aqueous methanol and methanol extract for *P. aeruginosa* strains S2H16 and *P. aeruginosa* strains R7-520-1 respectively all at a concentration of 100 mg/ml. The least considerable zone of inhibition (10.33 ± 0.33) was observed in n-Hexane fractions for *P. aeruginosa* strains P2S at a concentration of 6.25 mg/ml followed by aqueous methanol and methanol extract at a concentration of 25mg/ml. On the other hand, aqueous methanol extract was observed to be more susceptible for *P. aeruginosa* strains KAR21 followed by methanol extract best for *P. aeruginosa* strains Iraq. PA-9 then n-Hexane for *P. aeruginosa* strains Y15 isolated from University of Benin Teaching Hospital (UBTH) all at a concentration of 100mg/ml. Moreso, the least active concentration was observed in aqueous methanol and n-Hexane fractions (10.33 ± 0.33, 10.00 ± 1.16 respectively) at 12.5mg/ml followed by methanol at 25mg/ml concentration. Our findings conform to other reports on the antibacterial activity of *Moringa oleifera* extracts [55, 56, 57].

[48] employed methanol and aqueous extracts of the *Moringa* seeds and reported considerable inhibitory action against bacterial isolated from wound infections, including *E. coli.* They as well affirmed the broad spectrum activity of *M. oleifera* extracts. [58] had also reported that methanolic and purified dichloromethane *M. oleifera* extracts had antimicrobial action against both Gram positive and negative organisms. In another study by [59] reported that both methanol and n-hexane extracts of *Moringa oleifera* displayed antibacterial activity against *S. typhi.* Findings from this work reveal that *Moringa* leaf extract had both bactericidal and bacteriostatic activity on the different *P. aeruginosa* strains tested. This is an indication that the leaf extracts may possibly 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 [60]. *M. oleifera* leaf rich in tannins have prove why it is used in treatment of infection and healing of wounds [61]. It has been reported that the presence of terpenoids and saponins may cause hemolysis [62], flavonoids have been reported to inhibit nucleic

acid synthesis, alteration in cytoplasmic membrane function, energy metabolism inhibition, decrease in cell attachment and varying of the membrane permeability [63]. The worldwide emergence of multiple drug resistant *P. aeruginosa* strains is increasing, limiting the efficacy of recent drugs and treatment failure of infections. 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 this menace. Based on the outcome of this study, it could be suggested that extracts of this plant should be further analyzed to isolate the specific antibacterial compounds. Clinical trials should be encouraged to investigate the pharmaceutical potential of this medicinal plant in the treatment of bacterial infections.

5. Conclusion and Recommendation

Although all extracts showed varying antibacterial effect, n-Hexane *M. oleifera* leaf extract showed highest degrees of antimicrobial activity on the microorganisms tested. n-Hexane extract exhibited a higher degree of antimicrobial activity when compared with other extracts. 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. There is also the need for more work to be carried out to establish the conflicting findings from various laboratories on the efficacy of the *M. oleifera* extracts. 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.

Conflict of Interests

The authors hereby declare that there is no conflict of interests over this article as we did not received support from any individual or organization.

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