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# *In vitro Study of Moringa oleifera* Lam. Leaf Extract Fractions against Multidrug-resistant *Pseudomonas aeruginosa* Strains from Surgical Wound Infections

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author EAO designed the study, wrote the protocol and performed the experiment and statistical analysis as well as writing the first draft of the manuscript. Authors EMNI and IOE managed the analyses of the study. Author IOE managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

*Pseudomonas aeruginosa* is an opportunistic pathogen that can cause serious surgical site infections and remains a major dilemma, especially in developing country like Nigeria. This issue has led to investigation of the antibacterial activity of *Moringa oleifera* leaf extract against multidrug-resistant (MDR) *Pseudomonas aeruginosa* strains. *Pseudomonas aeruginosa* strains were isolated from postoperative wounds at the two sites used in the study (Central Hospital, Benin and University of Benin Teaching Hospital) and antibiotic susceptibility testing was performed to identify MDR isolates. A qualitative phytochemical screening of leaves was performed using standard methods, followed by antibacterial testing of various *M. oleifera* leaf extracts against selected

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multidrug-resistant isolates. Results showed that 99 (35%) of the patients examined had wound infections, out of a total of 284 specimens collected. Thirty-four (54.8%) *P. aeruginosa* strains showed multidrug-resistance capacity from both learning sites. Qualitative analysis of phytochemicals revealed the presence of flavonoids, phenols, saponins, steroids, tannins and terpenoids. *In vitro* results of antibacterial tests showed that ethyl acetate extract from leaves of *Moringa oleifera* had the highest antibacterial activity against *Pseudomonas aeruginosa* strain Iraq.PA-9, followed by dichloromethane at a concentration of 100 mg/ml. The different effects may be attributed to secondary plant substances contained in different leaf extracts of *Moringa oleifera*. The results of this study demonstrated the potential of *Moringa oleifera* leaf extract as an antibacterial agent by inhibiting the growth of test organisms isolated from postoperative wound infections.

*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 generally due to the vulnerability of surgical wounds to microbial contamination. Clean surgery has a 1–5% risk of postoperative wound infection, while dirty surgery, which is significantly more susceptible to endogenous contamination, is predicted to have a 27% risk of infection [3]. Minimizing the incidence of postoperative wound infections relies on proper sterility, maintenance, and protection of local host defenses [3]. Aseptic procedure includes using effective infection control procedures to reduce exogenous microbial contamination during surgery. Disinfection includes the use of skin antiseptics at the surgical site. Also, in cases of messy surgery, this includes administering prophylactic antibiotics prior to surgery to ensure adequate levels of antibiotics in the tissue during 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 spp, Klebsiella spp, Proteus spp, Escherichia spp, Acinetobacter spp, Enterococcus spp.* in addition to anaerobes such as *Clostridium spp*, *Bacteroides spp, Peptostreptococcus spp*. *and Propionibacterium spp*. It has been revealed that *Pseudomonas* spp., *Staphylococcus spp* and *Klebsiella spp* 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 antibacterial agents is closely related to the problem of emergence of strains resistant to most synthetic antibiotics. The study of medicinal substances in plants is not new. Due to the limited effective life of current antibiotics, poor patient compliance, uncontrolled agricultural use, and the slow release of new antimicrobials, antimicrobial resistance has risen to a worrying level. *Moringa oleifera* has been widely used in conventional pharmacotherapy to treat 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]. *Moringa oleifera* is the best known of all species in the *Moringa* genus [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 number one among microbes that cause opportunistic diseases [11,12]. Many of the antimicrobial agents currently in use are associated with undesirable effects such as toxicity, hypersensitivity, and tissue debris that pose public health risks. Moreover, new broad-spectrum antibiotics are prohibitively expensive and out of reach for poor citizens. These shortcomings reduce the therapeutic utility of currently available antimicrobial agents, thus necessitating the need to find other means of treating bacterial diseases. Therefore, the possibility of using inexpensive and readily available plants such as *Moringa oleifera* to treat multidrug-resistant *Pseudomonas aeruginosa* surgical wound infections is inevitable, especially in developing countries like Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Collection, Authentication and Processing of Plant Materials

Fresh leaves of *Moringa oleifera* were collected from the clinic of University of Benin Teaching Hospital, Egor Local Government Region, Edo State, Nigeria. Plant material was identified and authenticated by botanists from the Department of Plant Biology and Biotechnology, University of Benin, Nigeria. Taxonomic identity of plants was confirmed by comparison with herbarium specimens from the Department of Plant Biology and Biotechnology, University of Benin. Plant material was air-dried for 15 days at room temperature in the laboratory [13]. The dried leaves were ground into a powder with a mortar and pestle and saved for later use.

### 2.2 Preparation of *Moringa oleifera* Leaf Extracts

Five hundred grams (500 g) of powdered plant material was soaked in 2.5 liters of methanol (2.5 L) for 3 days at room temperature [14]. “The permeate was filtered through Whatman No 1 filter paper with a pore size of 11 micrometers. The extract was concentrated to dryness using a vacuum rotary evaporator (model number SARET43). The dry extract was weighed and the percent yield was calculated. The extract was stored in an airtight container and kept in a refrigerator at 4 °C until further experiments” [15].

### 2.3 Solvent-solvent Extraction

“A pre-fractionation/partition of the crude methanol extract (25 g) was dissolved in 100 ml methanol-water (4:1) and successively extracted with dichloromethane” [16]. Briefly, 500 mL of dichloromethane was added to the methanol extract via a separatory funnel. The mixture was gently agitated and the stopper was opened to release the pressure built up in the funnel. The mixture was allowed to stand for several minutes and the dichloromethane layer collected. The collected methanol extract was re-extracted with 500 mL of dichloromethane and separated. This procedure was repeated until a total of 2 L of dichloromethane was consumed. The dichloromethane portions were combined and evaporated to dryness. The ethyl acetate fraction was also concentrated to dryness and weighed on a Thermofisher electronic balance (model 4200) to calculate percent yield.

### 2.4 Phytochemical Analysis

“A phytochemical screen was performed to identify phytochemicals in the ethyl acetate and dichloromethane extracts of *Moringa oleifera* leaves used in this study. Phytochemicals were detected by color testing. Each extract was tested for the presence of alkaloids, anthraquinones, flavonoids, glycosides, phenols, saponins, sterols, and tannins using various known methods” [17–24]. The test was run three times to ensure accurate results.

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

Antibacterial activities of *Moringa oleifera* ethyl acetate and dichloromethane extracts was tested employing agar wells Diffusion method against multidrug-resistant *Pseudomonas aeruginosa* strains [25].

### 2.6 Test Organism Sample Collection

Random swab collection from 284 postoperative patients with surgical wounds was performed in both outpatient and inpatient settings at Central Hospital, Benin City (CHB) and University of Benin Teaching Hospital (UBTH).

## 2.7 Bacteriological Procedures / Identification of Isolates

Swab specimens were aseptically inoculated onto MacConkey agar, blood agar and nutrient agar and incubated aerobically for 24 hours at 37 °C to check for colony growth. All bacterial isolates were screened using conventional methods to identify *Pseudomonas aeruginosa* [26].

## 2.8 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing of bacteria was performed using the following antibiotics against Gram-negative bacteria including Augmentin (AUG, 30 µg), Ofloxacin (OFL 5 µg), cefixime (CXM 5 µg), gentamicin (GEN 30 µg), cefuroxime (CRX 30 µg), ceftazidime (CAZ 30 µg), ciprofloxacin (CPR 5 µg), nitrofurantoin (NIT 300 µg) *in vitro* as described in [27]. It was determined using the substance-sensitive Kirby-Bauer disc diffusion method. The declaration of antimicrobial susceptibility levels and zones of inhibition complied with the Institute of Clinical and Laboratory Standards performance standards for antimicrobial susceptibility testing of discs.

## 2.9 Standardization of Inoculum

"Inoculum was prepared from stock cultures maintained at 4 $^{o}$C on nutrient agar slants and subcultured onto nutrient broth using sterile wire loops. The density of suspensions seeded in media for susceptibility testing was determined by comparison with a 0.5 McFarland standard in barium sulfate solution" [26].

## 2.10 Screening of *Moringa oleifera* Leaf Extracts for Antimicrobial Activity

The antibacterial activity of *Moringa oleifera* leaf extract was tested against multidrug-resistant *Pseudomonas aeruginosa* strains using the agar diffusion method. Mueller-Hinton agar was prepared, sterilized, cooled, poured into sterile Petri-dishes 4 mm deep, approximately 20 ml/plate, and allowed to solidify. Overnight cultures of bacterial isolates were diluted in sterile saline to an inoculum size of $10^6$ cfu/ml and used to flood the surface of Mueller Hinton agar, discarded and dried. Five 6 mm diameter wells were aseptically drilled on each agar plate using a sterile cork borer. The bottom of each well was filled with melted agar to seal and gel the bottom. 0.2 ml aliquots of different concentrations (100.00, 50.00, 25.00, 12.50 and 6.25 mg/ml) of extract were added to different wells. The same procedure was applied to all extracted fractions. Plates were left at 37 °C before 24 hours of incubation to allow the extract to diffuse. The zone of inhibition (clearance) generated around the wells after incubation was observed, measured and recorded.

## 2.11 Determination of Minimum Inhibitory Concentration

"The minimum inhibitory concentration of *Moringa oleifera* leaf extract was determined by the two-fold serial dilution method described in" [28].

## 2.12 Statistical Analysis

Data from experiments were analyzed with SPSS version 20.0 using one-way analysis of variance (ANOVA). Where there were significant differences, Duncan's multiple range test was used to separate the means. Chi-square was also used to test for significance. Results were expressed as mean ± SEM (standard error of the mean).

# 3. RESULTS

This study analyzed 284 postoperative surgical wound swab specimens from inpatients and outpatients. Ninety-nine (35%) of these patients studied had bacterial associated wound infections. Sixty-two (62.6%) *P. aeruginosa* isolates from surgical site infections were screened with eight commonly used antibiotics, and multiple antibiotic-resistant strains were identified. Thirty-four (54.8%) isolates (18 from UBTH and 16 from CHB) showed multidrug-resistance capacity. Based on the antibiotic susceptibility results obtained, most of the screened isolates were highly resistant to ceftazidime, augmentin, cefixime, and gentamicin (54.8 %), as shown in Table 1.

**Table 1. Antibiotics susceptibility profiles of *Pseudomonas aeruginosa* isolates**

| 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) |
| **Fluoroquinolones** | 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) |

**Table 2. *Moringa oleifera* dried leaf extract yield**

| ***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*

**Table 4. Antimicrobial activity of *Moringa oleifera* ethyl acetate leaf extract 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 |

*Relative letters indicate means without significant differences. P<0.05 was considered significant, values are mean ± SEM, interpretation of significance is in row, CHB: Central Hospital, Benin*

**Table 5. Antimicrobial activity of *Moringa oleifera* ethyl acetate leaf extract 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 |

*Relative letters indicate means without significant differences. P<0.05 was considered significant, values are mean ± SEM, interpretation of significance is in row, UBTH: Benin University Teaching Hospital*

**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 |

*Relative letters indicate means without significant differences. P<0.05 was considered significant, values are mean ± SEM, interpretation of significance is in row, CHB: Central Hospital, Benin*

**Table 7. *Moringa 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 |

*Relative letters indicate means without significant differences. P<0.05 was considered significant, values are mean ± SEM, interpretation of significance is in row, UBTH: Benin University Teaching Hospital*

*Moringa oleifera* rich leaves (500 g) were extracted with 2.5 liters (2.5 L) of methanol and evaporated to dryness. Fractionation of the crude methanol extract was done using ethyl acetate and dichloromethane, which was also evaporated to dryness (Table 2). Qualitative analysis of the extract fractions revealed the presence of 6 of the 9 phytochemical components (flavonoids, phenols, saponins, steroids, tannins, terpenoids) tested in ethyl acetate and dichloromethane indicated three (phenols, steroids, and terpenoids) as shown in Table 3.

Antibacterial properties of the ethyl acetate fraction of *M. oleifera* from leaf extracts against different MDR strains of *Pseudomonas aeruginosa* from CHB showed a different magnitude of inhibitory effect compared to the solvent (ethyl acetate) used as a control. There was a significant difference in mean zone of inhibition at different concentrations. MDR *Pseudomonas aeruginosa* strain S2H16 recorded the highest mean zone of inhibition of 35.33 ± 2.67 mm at 100 mg/mL, whereas MDR *P. aeruginosa* strain D2 had the lowest acceptable mean zone of inhibition of 10.67 ± 0.67 mm at 12.5 mg/ml (Table 4). Antibacterial activity of ethyl acetate *Moringa oleifera* leaf fractions against MDR *Pseudomonas aeruginosa* strains of UBTH showed that the fractions had different levels of activity. Significant differences in mean zones of inhibition were observed at different concentrations. The highest mean zone of inhibition of 36.33±0.88 mm was observed with MDR *Pseudomonas aeruginosa* strain in Iraq. The lowest receptive zone of inhibition at PA-9 and 10.33 ± 0.33 mm 6.25 mg/ml was recorded for the MDR *Pseudomonas aeruginosa* AR442 strain (Table 5).

The dichloromethane fraction of crude methanolic leaf extract of *M. oleifera* showed that the dichloromethane fraction had an inhibitory effect at different concentrations (Table 6). There was a significant difference in the mean zones of inhibition at the different concentrations tested. MDR *Pseudomonas aeruginosa* strain PS2 and *Pseudomonas aeruginosa* strain DHS01 showed the highest mean zone of inhibition of 30.33 ± 0.88 mm at a concentration of 100 mg/ml, whereas MDR *Pseudomonas aeruginosa* strain NAPCC-1 and *Pseudomonas aeruginosa* strain PA-VAP-2 showed minimum acceptable mean zone of inhibition of 10.67 ± 0.67 mm at a concentration of 50 mg/ml, *P. aeruginosa* strain DHS01 also showed a zone of inhibition of 12.00 ± 0.58 mm at a concentration of 25 mg/ml. Susceptibility testing using dichloromethane fraction on various MDR strains of *Pseudomonas aeruginosa* from UBTH showed that this fraction had an inhibitory effect at the various concentrations used. There was a significant difference in the average zones of inhibition observed at the different concentrations tested (Table 7). Several drug-resistant *Pseudomonas aeruginosa* strains Iraq.PA-9 recorded the highest (36.00 ± 0.58 mm) and lowest (15.67 ± 0.33 mm) mean zone of inhibition at a concentration of 100 mg/mL and 25.00 mg/mL respectively.

## 4. DISCUSSION

Medicinal plants are also gaining more and more recognition among urban educated populations. This is probably due to the increasingly ineffectiveness of many modern drugs to fight various infections, and the growing resistance of many bacteria to a wide variety of routinely used antibiotics and the cost of prescribing them is increasing [29]. The use of antibiotics and the increasing prevalence of multiple drug-resistant strains of multiple pathogenic bacteria have revived interest in plants with antimicrobial properties [30]. This led to the screening of *Moringa oleifera* leaf extracts using different solvents. The antibacterial properties of *Moringa oleifera* are attributed to different parts of the plant, such as leaves, seeds, pods, and stems [31], which are known for their antibacterial activity and are believed to be a rich base of antibacterial agents [32]. In this study, fractionation of crude methanolic leaf extracts of *Moringa oleifera* was performed using dichloromethane and ethyl acetate with yields of 3.8 and 2.5 grams, respectively. A qualitative phytochemical screen was used to reveal secondary metabolites in the extract. The results showed the presence of flavonoids, phenols, saponins, steroids, tannins and terpenoids (Table 2). Steroids, terpenoids and phenols were present in both ethyl acetate and dichloromethane. Flavonoids, saponins and tannins were present only in the ethyl acetate extract. The results of this work were consistent with previous work by other researchers studying the phytochemical constituents of *Moringa oleifera* as a medicinal plant [33,34]. In a study by [35], phytochemical analysis of *Moringa oleifera* leaf extracts revealed the presence of flavonoids, saponins, sterols and tannins in both aqueous and ethanolic extracts. *Moringa oleifera* leaf extracts have been reported to contain

flavonoids, saponins, steroids, terpenoids, and tannins [36]. Different solvents used for extraction have been reported to result in different extractability and solubility spectra of phytochemicals, and the results of this study are also consistent with the documented report by [37].

The antibacterial activity of ethyl acetate and dichloromethane extracts of dried *Moringa oleifera* leaves was tested in two government hospitals in Benin City, Nigeria, using different strains of MDR *Pseudomonas aeruginosa* isolated from postoperative wound swabs. *Moringa oleifera* leaf tested at different concentrations showed different inhibitory effects against different MDR *Pseudomonas aeruginosa* strains. There was significant antibacterial activity provided by the ethyl acetate fraction against *Pseudomonas aeruginosa* strain S2H16, isolated from a patient with a surgical wound from CHB, which was the most susceptible organism in this study (Table 3), followed by *Pseudomonas aeruginosa* strain DHS01 and *Pseudomonas aeruginosa* strain PS2 all treated with 100 mg/ml dichloromethane. A minimal zone of inhibition (10.33 ± 0.33) was observed in the ethyl acetate fraction on *Pseudomonas aeruginosa* strain P2S at a concentration of 6.25 mg/ml, the lowest inhibitory concentration for this fraction. On the other hand, among all *P. aeruginosa* strains isolated from UBTH, ethyl acetate and dichloromethane showed high susceptibility to *P. aeruginosa* strain Iraq.PA-9 and *P. aeruginosa* strain KAR21 at 100 mg/ml concentration ( Tables 5 and 6). However, the lowest inhibitory concentration was observed at 12.5 mg/ml in the ethyl acetate fraction (10.33 ± 0.33 mm) followed by 50 mg/ml in dichloromethane (10.33 ± 0.33). The results of this study are consistent with other reports of the antibacterial activity of *Moringa oleifera* extract [38-40]. The activity of *Moringa oleifera* extract against tested strains of *Pseudomonas aeruginosa* may be attributed to the presence of several broad-spectrum antimicrobial compounds [41]. Additionally, purified methanol and dichloromethane extracts from *M. oleifera* has been reported to have antibacterial effects against both Gram-positive and Gram-negative bacteria [42]. The results of this study showed that *Moringa* leaf extract had bactericidal effects against the various strains of *Pseudomonas aeruginosa* tested. This indicates that leaf extracts can be used to treat post-surgical wound infections caused by multiple drug-resistant *Pseudomonas aeruginosa* strains. Antimicrobial phytochemicals, especially tannins, work by binding to cell walls and inactivating enzymes [43]. The tannin constituent of *Moringa oleifera* leaves have been shown to be effective in treating infections and healing wounds [44]. It has been reported that the presence of terpenoids and saponins can cause hemolysis [45]. Another study reported that flavonoids inhibit nucleic acid synthesis, alter cytoplasmic membrane function, inhibit energy metabolism, decrease cell adhesion, and alter membrane permeability [46]. The global incidence of MDR *P. aeruginosa* strains is increasing, limiting the efficacy of some routinely used drugs and causing treatment failure. A new step to prevent antibiotic resistance in pathogenic organisms by using new compounds not based on existing synthetic antimicro-bials is the right way to combat the threat of MDR.

## 5. CONCLUSION

The two extract fractions showed different antibacterial activities, with the ethyl acetate *M. oleifera* leaf extract fraction showing the highest level of antibacterial activity against the microorganisms tested. The activity of *M. oleifera* makes it a potent source of new antimicrobial alternatives. However, further work is needed to isolate secondary metabolites from extracts and test them for specific antimicrobial activity. This *in vitro* study showed that folk remedies can be as effective as modern medicine in combating pathogenic microbes. According to the World Health Organization, resistance of microbes to routine antibiotics is on the rise, medicinal plants offer an excellent alternative. *Moringa oleifera* represents, among several other uses, an economical and safe option for treating infections.

## ETHICAL CLEARANCE

After being informed of the purpose of the study, approval was given by the University of Benin Teaching and Central Hospital, the Ethics Committee, and all patients.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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