UJMR, Vol. 10 No. 1, June, 2025, pp. 217 - 229 E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

https://doi.org/10.47430/ujmr/25101.022

Received: 06/01/2025 Accepted: 12/06/2025

# Exploring the Antibacterial Potential of *Vitex doniana* and *Jatropha curcas* Leaf Extracts against Multidrug-Resistant *Pseudomonas aeruginosa* from Clinical and Environmental Sources

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***Abstract***

***Multidrug-resistant Gram-negative bacteria remain of significant global health and economic concern, affecting both developed and developing nations, including Nigeria. Among these, Pseudomonas aeruginosa is one of the most challenging to manage due to its extreme resistance to multiple antibiotics and its association with a high-rate nosocomial infections, thus creating a significant burden in the clinical world. These gaps necessitate the search for new and more effective treatment options for multidrug-resistant Pseudomonas aeruginosa. This study evaluates the antibacterial properties of Vitex doniana and Jatropha curcas leaf extracts against clinical and environmental multidrug-resistant P. aeruginosa strains. Chloroform was used for extraction, and subsequent qualitative and quantitative phytochemical screening assays were performed. One hundred and fifty clinical and environmental samples were collected for the isolation of P. aeruginosa. Resistance levels and the antibiotic susceptibility patterns were tested using the standard disc diffusion bioassay. The expression levels of multidrug resistance genes AcrA, blaOXA-23, and blaVIM were analyzed to identify strains resistant to multiple drugs. An in vitro bioassay was employed to determine the antimicrobial potential of the extracts, and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined. Phytochemical screening revealed the presence of tannins, saponins, alkaloids, flavonoids, phenols, terpenoids, quinones, phytosterols, and phlobatannins in the extracts with an unusually high concentration of phenolic compounds. Eleven highly multidrug-resistant P. aeruginosa strains were successfully grown, primarily from wound swab cultures and clinical samples. All strains carried at least two of the examined resistance genes (blaTEM, blaSHV, and CTX-M). The in vitro antimicrobial activity bioassay confirmed that Jatropha curcas extract was more potent than the Vitex doniana extract, which was weaker against some strains. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Jatropha curcas extract were as low as 0.125 mg/ml. Overall, the findings suggest that Jatropha curcas extract exhibits potent antibacterial activity and is a potential therapeutic agent for treating multidrug-resistant P. aeruginosa infections.***

***Keywords: Bioassay, Extreme Multidrug Resistance, Jatropha curcas, Pseudomonas aeruginosa, Resistance Genes, Vitex doniana***

## INTRODUCTION

*Pseudomonas aeruginosa* infection has recently become a dire threat to the health of human beings as a result of the constant emergence of multidrug-resistant forms of the bacterium (Poursina *et al.*, 2022), which is majorly responsible for producing chronic infections that These include the capacity to develop biofilm-forming colonies, the induction of drug efflux are not easily curable, such as gastroenteritis, meningitis, skin infection, toxicoses, toxic shock syndrome, and food poisoning (Abdel-hamid *et al.*, 2020).

Therapeutic resistance in *Pseudomonas aeruginosa* is driven primarily by a combination of naturally occurring resistance mechanisms. systems (Hassan *et al.*, 2021), and the development of multidrug-resistant persister

cells. Additionally, cellular components such as resistance genes (AcrA, blaOXA-23, and blaVIM) and antibiotic-inactivating enzymes, including beta-lactamases, contribute to its resistance (Sadek *et al.*, 2021). This organism can rapidly evolve other antibiotic resistance mechanisms to all forms of broad-spectrum antibiotics (Ruiz-Roldan *et al.*, 2021). Numerous studies have also highlighted the high ability of *Pseudomonas aeruginosa* to rapidly acquire or evolve differ`rent genetic resistance mechanisms because of its high genetic plasticity.

This adaptability enables the bacterium to resist harmful antibiotic molecules (Ndukwe *et al.*, 2014), chemical agents, and antimicrobial peptides, thereby conferring advanced and intimidating resistance levels (Djordjevic *et al.*, 2017). *Pseudomonas aeruginosa* antimicrobial resistance is, therefore, a contributing result of mechanisms of resistance that include intrinsic, acquired, and adaptive antibiotic resistance (Tacconelli *et al.*, 2021). *Pseudomonas aeruginosa* possesses inherent resistance and virulence factors, including secreted products such as siderophores, exopolysaccharides (Han et al., 2021), toxins, and enzymes like proteases. However, they assist the bacterium in biofilm formation, Immune system evasion, and bacterial adhesion (Gao *et al.*, 2023). Medicinal plants possess potential medical agents and compounds known as phytometabolites.

These are traditionally accessed and used to manage all forms of diseases (Hassan *et al.*, 2020). All these kinds of plants have been researched and were found to contain such biochemistry such as phenolics (flavonoids, tannins), Saponins, alkaloids and terpernoids, which all have been reported to proven (Hassan *et al.*, 2021) to be responsible for so many kinds of antimicrobial activities, against so many kinds of pathogenic microorganisms (Oke, 2006). Most research focuses on single multidrug-resistant strains, primarily from clinical sources, and does not address the genomic characteristics and resistance mechanisms of such pathogens, especially in areas such as northern Nigeria.

On the other hand, this work examined the behavior of highly drug-resistant bacteria, their nucleotide sequences, the distinct resistance genes present in them, and the antibacterial activity of extracts from the Vitex doniana and Jatropha curcas plants against them in greater depth. The study aimed to isolate and characterize Pseudomonas aeruginosa strains with ultra-extreme antibiogram profiles from clinical and environmental samples collected within the Kaduna State metropolis, and to evaluate the antibacterial activity of *V. doniana* and *J. curcas* extracts against these highly antibiotic-resistant nosocomial pathogens.

## MATERIALS AND METHODS

### Isolation, Preparation and Storage of the Plant's Material

Leaves of *Vitex doniana* and *Jatropha curcas* were collected from Malumfashi, Katsina State, Nigeria. The leaves were identified, processed, and stored following the procedure described by Hassan *et al.* (2020).

### Extraction of Plant Material

Five hundred grams of the plant material were extracted using a chloroform maceration protocol for a period of seven days, with periodic shaking. The extract was subsequently dried using a rotary evaporator and stored at 4°C until required for use (Abu *et al.*, 2020).

2.3 Phytochemical Screening

The individual plant materials were separately subjected to qualitative and quantitative phytometabolic analysis, as expounded by Foyet et al. (2017), Ishola et al. (2014), Sofowara et al. (1993), and Lakache et al. (2016).

### Preparation of Extracts Stock Solution

A 500 mg/mL stock solution of each plant extract was prepared using 5 g in 5% dimethyl sulfoxide (Kossah *et al.*, 2013). After carrying out an extract clean-up as a pre-measure for further sterilization of the extract.

2.5 Isolation of *Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* was isolated from various general hospitals in Kaduna City, Nigeria, on different types of clinical samples, including wound swabs, ear swabs, urine, pus, and sputum. Further confirmatory tests (microbiologically, as described by Ejaz et al. (2021) and biochemically, as reported by Ehlers *et al.* (2009) were also carried out to confirm the isolates' species and genus specificity, as explained by Shikov et al. (2022), Dahiru and Thgriki (2016), and Breidenstein et al. (2011).

### Antibiotic Susceptibility Bioassay

Kirby-Bauer susceptibility testing was performed using the 0.5 McFarland standard, Mueller-Hinton agar medium, and a standard Gram-negative antibiotic disc. The plates were incubated and read after 24 h, and the results were recorded in millimeters (Sarwat *et al.*, 2015).

### Molecular Identification of *Pseudomonas aeruginosa* Isolates

Genomic DNA extraction was performed on the selected individual isolates (Shaebth et al., 2018). Additionally, polymerase chain reaction (PCR) and agarose gel electrophoresis with photo documentation (Shikov et al., 2022) were also conducted.

Ultimately, 16S ribosomal RNA gene sequencing was performed to determine the nucleotide sequences of the isolates and their genus-species identity (Najafi, 2015).

**Confirmatory Analysis of Resistance Gene**

Multiplex polymerase chain reaction analysis was performed on the extracted DNA of the bacteria (Shaebth *et al.*, 2018), using the following primers of resistance genes:

BLA VIM F- TTTGGTCGCATATCGCAACG
R- CCATTCAGCCAGATCGGCAT
BLA OXA-23 F- GATCGGATTGGAGAACCAGA
R- ATTTCTGACCGCATTTCCAT
acrA F- CTCTCAGGCAGCTTAGCCCTAA
R- TGCAGAGGTTCAGTTTTGACTGTT

The products were electrophoresed using standard procedures as described by Ehlers et al. (2009).

**Inoculum Preparation**

An equivalent 0.5 McFarland inoculum was prepared in normal saline, as described by Cheesbrough et al. (2000).

**Antimicrobial Bioassay of the Plant Extracts against the Highly Resistance Isolates**

The antibacterial activity of the plant materials was carried out by the disc diffusion assay (Black and Black, 2018). Sterile filter paper discs were impregnated with 100 µL of a stock solution of each extract (10 mg/mL) using Mueller-Hinton agar.

**Minimum Inhibitory Concentration of *Jatropha curcas* Extract**

The minimum inhibitory concentration (MIC) of the bioactive extract was determined through serial dilution using distilled water to obtain five different extract concentrations. Two (2 mL) of Mueller-Hinton broth were dispensed into sterilized test tubes. Exactly 0.1 ml of standardized inoculums (3.3 x 106 cfu/ml) was put into each of the above test tubes. The tubes with broth and plant extract, but without inocula, served as the positive control, and those with broth and inocula as the negative control (Cheesbrough *et al.*, 2000).

**Determination of the Minimum Bactericidal Concentration of *Jatropha curcas* Extract**

Nutrient agar plates were separately inoculated using culture tubes showing negative turbidity (MIC) and incubated at 35°C for 24 hours. The highest dilution without visible growth was taken as the MBC (Sofowora, 1993).

**Biostatistical Analyses**

Thus, the obtained results were analyzed using the measurement of dispersion analysis ANOVA (SPSS) on the basis of a P-value of less than or equal to 0.01 at a 98% confidence level.

## RESULTS

**Plant Extraction Result**

Table 1 presents the physical properties of Vitex doniana and Jatropha curcas leaf chloroform extracts, including individual physical properties and percentage yield.

**Table 1**: *Vitex doniana* and *Jatropha curcas* extract physical properties

| Plant Name | Sample Weight (g) | Yield (g) | Yield (%) | Colour | Texture |
|---|---|---|---|---|---|
| *Vitex doniana* | 495 | 37.80 | 7.60 | Greenish | Gummy |
| *Jatropha curcas* | 500 | 52.00 | 10.41 | Brownish | Crystalline |

**Phytochemical Analyses of Plants Extracts**

*Vitex doniana* and *Jatropha curcas* leaf extracts undergo phytochemical screening, revealing that the plants contain phytochemicals such as saponins, alkaloids, flavonoids, phenols, tannins, terpenoids, quinones, and phytosterols. The analysis also shows that both extracts have a high percentage concentration of phenolic compounds (Tables 2 & 3).

**Table** 2: Phytochemical Content of *Vitex doniana* leaf extract

| N | Phyto-metabolite | Concentration (mg/g dry wt) | |
|---|---|---|---|
| | | Qualitative | Quantitative |
| 1 | Flavonoid | + | 2.20 ± 1.13 |
| 2 | Alkaloid | + | 3.05 ± 1.23 |
| 3 | Saponins | + | 1.94 ± 1.22 |
| 4 | Phytosterols | + | 1.46 ± 2.05 |
| 5 | Phenols | + | 8.35 ± 2.17 |
| 6 | Terpenoids | + | 1.46 ± 0.49 |
| 8 | Triterpenoids | + | |
| 9 | Tannins | + | 5.50 ± 2.18 |
| 10 | Cardiac glycoside | + | 1.83 ± 0.55 |
| 11 | Anthraquinones | + | 2.05 ± 1.80 |
| 12 | Anthocyanins | _ | |
| 13 | Phlobatannins | + | |
| 14 | Flavonols/flavones | + | |
| 15 | Coumarins | + | |
| 16 | Quinones | + | |
| 17 | Resins | + | |
| 18 | Amino acids | _ | |
| 19 | Chalcones | + | |
| 20 | Vitamin A | + | |
| 21 | Vitamin D | + | |
| 22 | Acidic compound | _ | |

Key:
+ = Presence - = Absence
Results are presented as mean ± standard deviation.

**Table** 3: Phytochemical Content of *Jatropha curcas* Leaf Extract

| N | Phyto-metabolite | Concentration (mg/g dry wt) | |
|---|---|---|---|
| | | Qualitative | Quantitative |
| 1 | Flavonoid | + | 1.05 ± 1.28 |
| 2 | Alkaloid | + | 9.27 ± 0.70 |
| 3 | Saponins | + | 2.90 ± 2.92 |
| 4 | Phytosterols | + | 0.63 ± 0.73 |
| 5 | Phenols | + | 4.55 ± 2.35 |
| 6 | Terpenoids | + | 1.68 ± 0.94 |
| 8 | Triterpenoids | + | |
| 9 | Tannins | + | 2.53 ± 1.36 |
| 10 | Cardiac glycoside | + | 0.18 ± 0.18 |
| 11 | Anthraquinones | + | 0.59 ± 1.77 |
| 12 | Anthocyanins | _ | |
| 13 | Phlobatannins | + | |
| 14 | Flavonols/flavones | + | |
| 15 | Coumarins | _ | |
| 16 | Quinones | + | |
| 17 | Resins | + | |
| 18 | Amino acids | _ | |
| 19 | Chalcones | _ | |
| 20 | Vitamin A | _ | |
| 21 | Vitamin D | _ | |
| 22 | Acidic compound | + | |

Key:
+ = Presence - = Absence (mean ± standard deviation)

**Isolation of clinical and environmental isolates of *Pseudomonas aeruginosa***

One hundred and fifty samples were collected for the individual isolation of extreme multidrug resistant strains of *P. aeruginosa*. A total of 108 and 48 collected samples are of clinical and environmental origin, respectively (Figures 1 and 2).

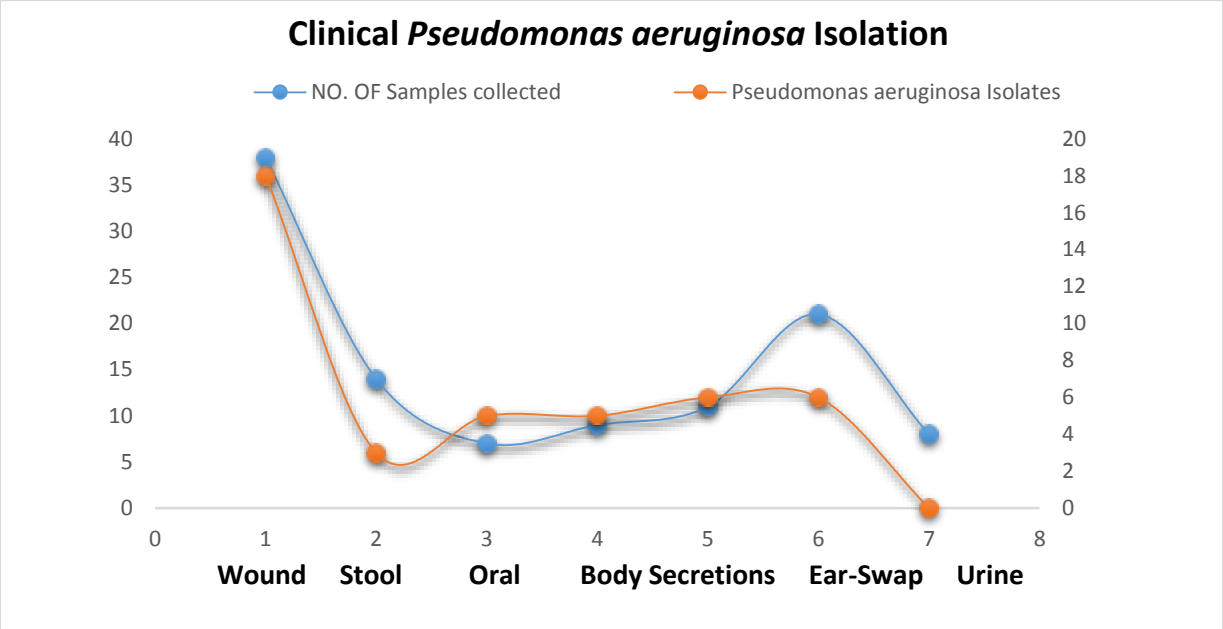


**Figure 1**: *Pseudomonas aeruginosa* Isolation from Clinical Samples

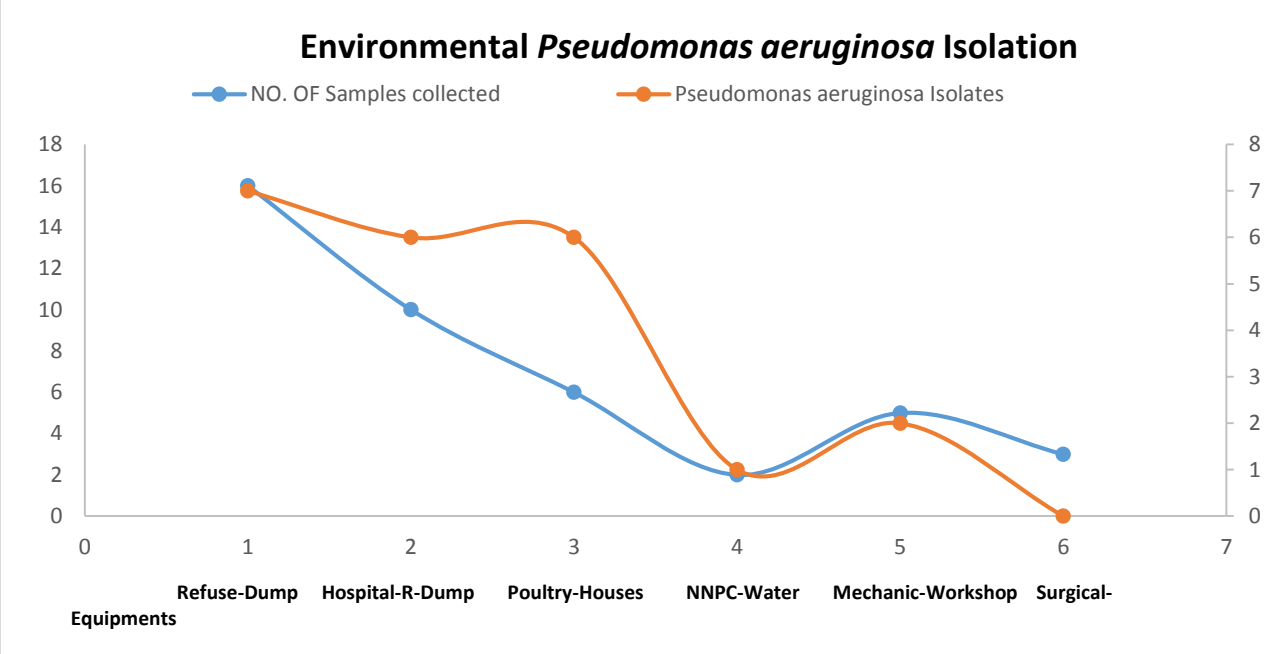


**Figure 2**: *Pseudomonas aeruginosa* Isolation from Different Environmental Samples

**Antibiotic Sensitivity Assay of *Pseudomonas aeruginosa* Isolates**

All identified *P. aeruginosa* isolates were subjected to a multiple antibiotic sensitivity assay to identify multidrug-resistant strains, if present, as shown in a heat map representing the sensitivity pattern of all the tested isolates (Figure 3). Results from the analyses reveal that eleven of the one hundred and fifty clinical and environmental isolates tested are extremely multidrug-resistant, that is, they were able to resist five or more of the seven classes of antibiotics (Table 4).

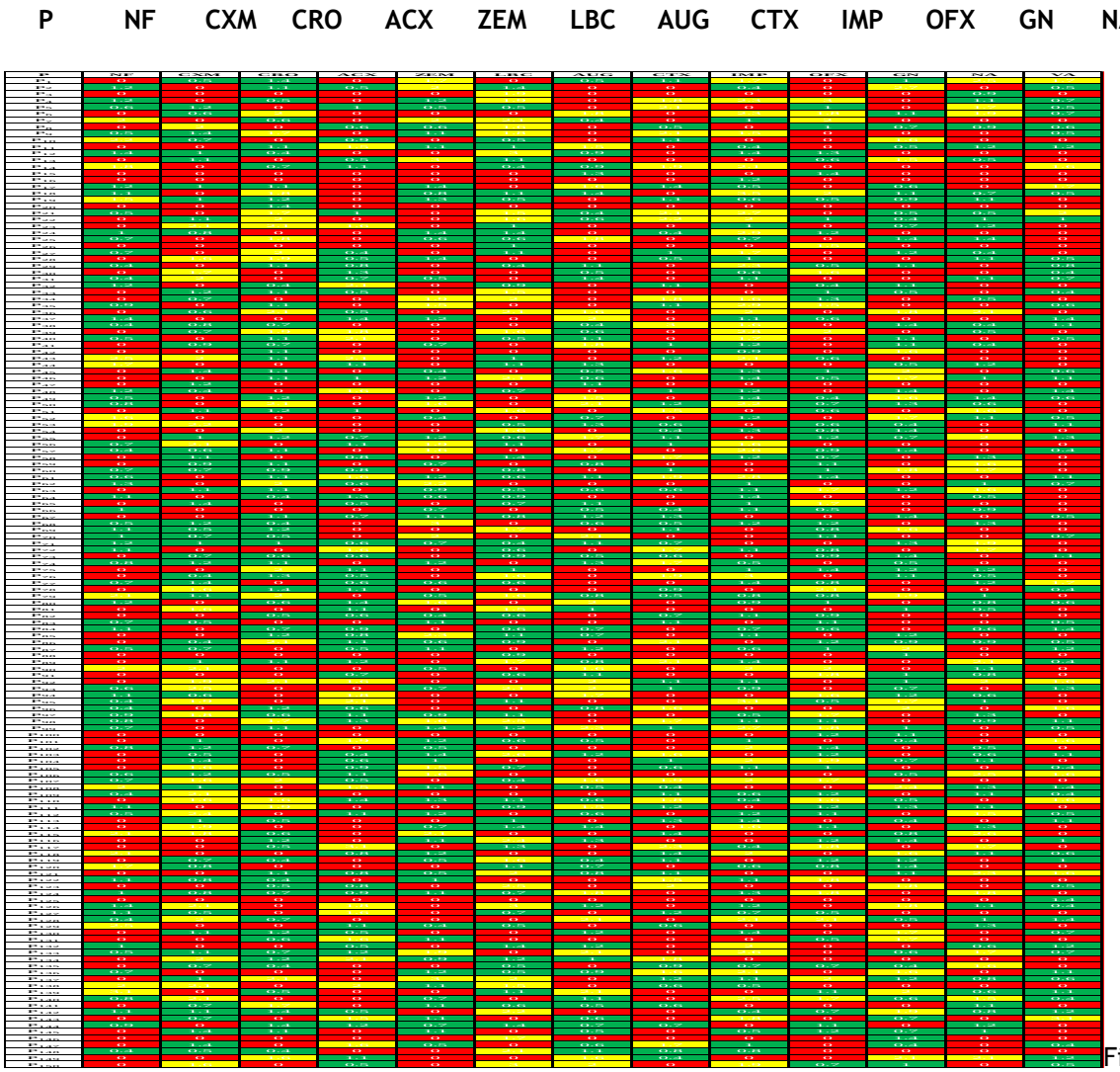


Figure 3: Heat-Map of the Antibiotic bioassay for the isolated strains of *Pseudomonas aeruginosa* (Yellow: Sensitive, Green: Intermediate & Red: Resistant, P: *Pseudomonas aeruginosa*)

**Table** 4: Extreme resistant *Pseudomonas aeruginosa* sensitivity assay

| **Resistant strain** | **Antibiotics (Zones in cm)** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **NO** | **CXM** | **CRO** | **ACX** | **CEF** | **LC** | **AUG** | **CTX** | **IMP** | **OFX** | **GE** | **N** | **VA** |
| **$P_1$** | # | # | # | # | # | S | # | # | # | # | # | I | # |
| **$P_2$** | # | # | # | # | # | # | I | # | # | I | # | # | # |
| **$P_3$** | # | # | # | # | # | # | # | # | I | # | # | # | # |
| **$P_4$** | # | # | I | # | # | # | # | # | # | # | # | # | # |
| **$P_5$** | # | # | # | # | # | I | # | # | # | S | # | # | # |
| **$P_6$** | # | # | # | # | # | # | # | # | I | # | S | # | # |
| **$P_7$** | # | I | # | # | # | # | I | # | # | # | # | # | # |
| **$P_8$** | # | # | # | # | # | I | # | # | # | # | I | # | # |
| **$P_9$** | # | # | # | # | # | # | # | # | # | I | I | # | # |
| **$P_{10}$** | # | # | # | # | # | # | # | # | # | # | # | # | I |
| **$P_{11}$** | # | # | # | # | # | S | # | # | # | # | I | # | # |

Key: I- Intermediate 0.5 – 1.4, #- Resistance ≤ 0.4, S- Sensitive ≥ 1.5

CXM – Cefuroxime, AUG - Amoxicilin Clavulanate, CRO – Ceftriaxone Sulbactarm, CEF - Cefexime, IMP - Imipenem, OFX - Ofloxacin, GE – Gentamycin, CTX – Cefotaxime, N - Nalidixic Acid, VA – Vancomycin, NO - Nitrofurantoin, LC – Levofloxacin and ACX - Ampiclox

**Antibiotic Susceptibility Pattern of *Pseudomonas aeruginosa* Strains against Different kinds of Antibiotics**

The antibiotic sensitivity assay of all the isolated strains of *P. aeruginosa* reveals dynamic resistance/susceptibility patterns against various broad-spectrum antibiotics, with many P. aeruginosa isolates being sensitive to Imipenem and Cilastatin (Carbapenems) (Table 5).

**Table 5**: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa*

| No. of *P. aeruginosa* Strains | Antibiotics | Concentration | Susceptible (%) | Resistant (%) |
|---|---|---|---|---|
| N= 150 Strains | Nitrofurantoin (NF) | 300µg | 81 (54) | 69 (46) |
| | Cefuroxime (CXM) | 30µg | 92 (61.33) | 58 (38.67) |
| | Ceftriaxone Sulbactarm (CRO) | 45µg | 93 (62) | 57 (38) |
| | Cefexime (ZEM) | 5µg | 82 (54.67) | 68 (45.33) |
| | Cefotaxime (CTX) | 25µg | 80 (53.33) | 70 (46.67) |
| | Ampiclox (ACX) | 10µg | 85 (56.67) | 65 (43.33) |
| | Amoxicilin Clavulanate (AUG) | 30µg | 86 (57.33) | 64 (42.67) |
| | Levofloxacin (LBC) | 5µg | 96 (64) | 54 (36) |
| | Ofloxacin (OFX) | 5µg | 88 (58.67) | 62 (41.33) |
| | Nalidixic Acid (NA) | 30µg | 83 (55.33) | 67 (44.67) |
| | Imipenem (IMP) | 10µg | 101 (67.33) | 49 (32.67) |
| | Cilastatin (CLN) | 10µg | 101 (67.33) | 49 (32.67) |
| | Gentamycin (GN) | 10µg | 88 (58.67) | 62 (41.33) |
| | Vancomycin (VA) | 30µg | 77 (51.33) | 73 (48.67) |

**Molecular Characterization of Resistant *Pseudomonas aeruginosa***

The extreme antibiotic-resistant isolates of P. aeruginosa were subjected to 16S ribosomal RNA sequencing to reconfirm their identity (Plate 1). All the strains were identified as P. aeruginosa strains, with a percentage identity high enough to molecularly characterize their genus-species identity, and individual accession numbers were assigned to the eleven multi-drug-resistant strains (Table 6).

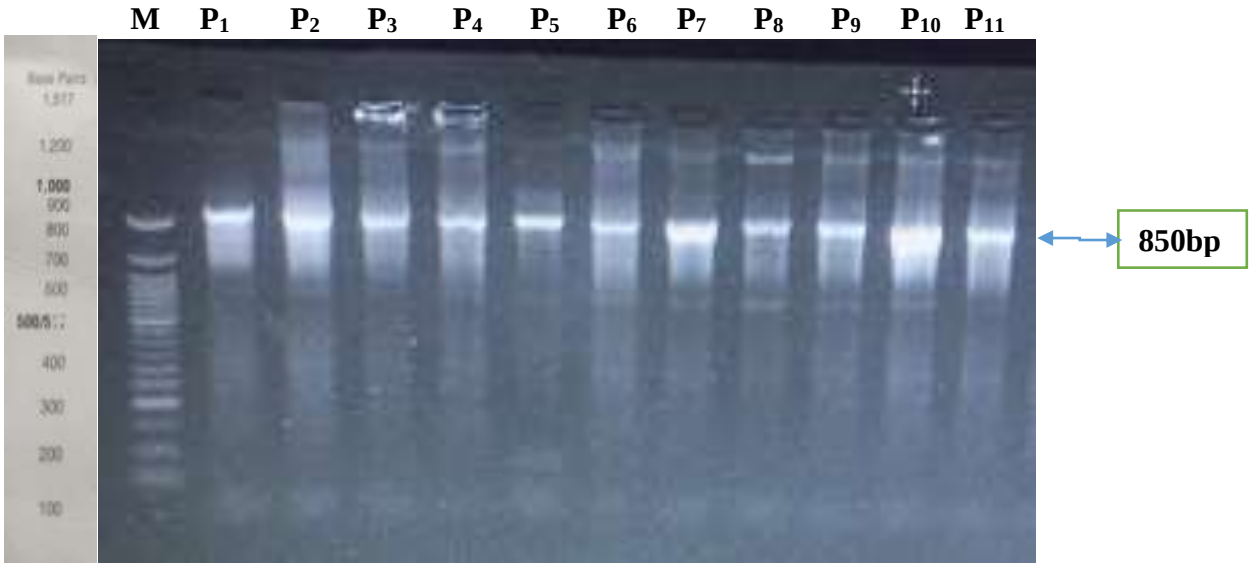


Plate 1: Gel electrophoresis plate of the 16S ribosomal RNA PCR product of the multidrug resistance bacterial strains

Table 6: Result for the molecular NCBI nucleotide blast of the extreme multidrug resistance strains sequence

| Strain | Percentage Identity (%) | E-value | Query Cover (%) | NCBI Accession No |
|---|---|---|---|---|
| $P_1$ | *Pseudomonas aeruginosa:* 100 | 0.0 | 100 | OR732111 |
| $P_2$ | *Pseudomonas aeruginosa* 100 | 0.0 | 100 | OR730952 |
| $P_3$ | *Pseudomonas aeruginosa* 100 | 0.0 | 100 | OR731940 |
| $P_4$ | *Pseudomonas aeruginosa* 81.2 | 0.0 | 100 | PP340917 |
| $P_5$ | *Pseudomonas aeruginosa* 100 | 0.0 | 100 | OR732109 |
| $P_6$ | *Pseudomonas aeruginosa* 99 | 0.0 | 98 | OR731711 |
| $P_7$ | *Pseudomonas aeruginosa* 100 | 0.0 | 98 | OR732373 |
| $P_8$ | *Pseudomonas aeruginosa* 100 | 0.0 | 100 | OR731827 |
| $P_9$ | *Pseudomonas aeruginosa* 99.44 | 0.0 | 91 | OR733250 |
| $P_{10}$ | *Pseudomonas aeruginosa* 100 | 0.0 | 100 | OR731870 |
| $P_{11}$ | *Pseudomonas aeruginosa* 100 | 0.0 | 100 | OR731583 |

**Results for the Molecular Identification of Resistance Genes**

Result for the genomic DNA analyses for the identification of Acra A, *bla* OXA-23 and *bla* VIM resistance genes. These genes confer resistance to beta-lactams antibiotics among other. They codes for the production of extended-spectrum beta-lactamases. The analyses showed the presence of numerous resistance genes within the resistant isolates genome (Plate 2).

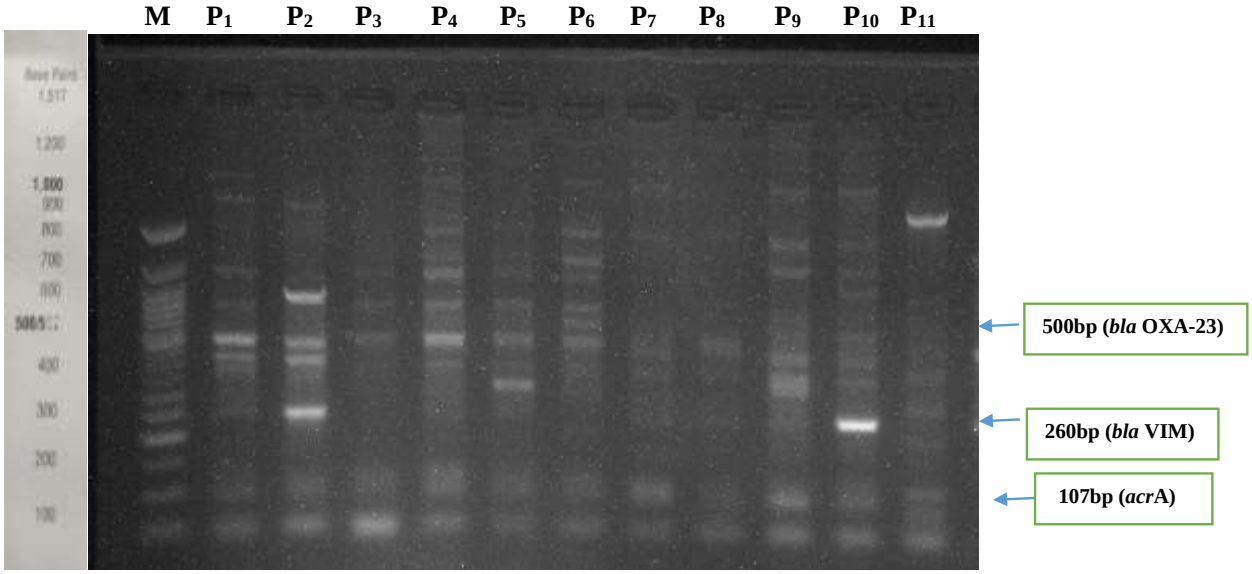


**Plate 2**: Agarose gel electrophoresis of acrA, *bla* OXA-23 and *bla* VIM

**Bioassay Result of *Vitex doniana* and *Jatropha curcas* Leaf Extracts**

The bioassay analyses reveal that both extracts have a significant activity against all the isolated resistant isolates. However, the *J. curcas* extract was seen to have the highest antimicrobial activity against resistant strains (Table 7).

Table 7: Bioassay results

| S/N | Resistant Isolates | Sample Source | Extract Inhibitory Activity (cm) | | Control (cm) |
|---|---|---|---|---|---|
| | | | *Vitex doniana* | *Jatropha curcas* | Gentamycin |
| 1 | $P_1$ | Clinical | 0.5 ± 2.11 | 1.3 ± 2.04 | R |
| 2 | $P_2$ | Clinical | 1.1 ± 0.51 | 1.2 ± 1.18 | R |
| 3 | $P_3$ | Clinical | R | 1.5 ± 0.21 | R |
| 4 | $P_4$ | Env. | R | 0.6 ± 1.05 | R |
| 5 | $P_5$ | Clinical | 1.0 ± 0.32 | 2.0 ± 0.44 | 0.5 |
| 6 | $P_6$ | Clinical | R | 1.6 ± 1.10 | 1.4 |
| 7 | $P_7$ | Clinical | 0.7 ± 0.32 | 1.8 ± 0.04 | R |
| 8 | $P_8$ | Clinical | R | 1.4 ± 1.62 | 0.8 |
| 9 | $P_9$ | Clinical | 2.0 ± 1.53 | 2.3 ± 1.03 | 1.1 |
| 10 | $P_{10}$ | Env. | 1.2 ± 1.22 | 1.4 ± 0.74 | R |
| 11 | $P_{11}$ | Clinical | R | 2.0 ± 1.02 | 1.3 |

Key:
R: Resistant
±: Standard Deviation
Env.: Environmental
P: *Pseudomonas aeruginosa*

**Result for the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of *Jatropha curcas***

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assay was performed on the J. curcas leaf extract to determine the extract's bacterial inhibitory MIC/MBC value against eleven extremely multidrug-resistant *Pseudomonas aeruginosa* isolates (Table 8 & 9).

Table 8: Minimum Inhibitory Concentration of the Chloroform leaf extract of *J. curcas*

| Test Organisms | Turbidity at various concentrations of *J. curcas* Extracts mg/ml | | | | |
|---|---|---|---|---|---|
| | 1.0 mg/ml | 0.5 mg/ml | 0.25 mg/ml | 0.125 mg/ml | 0.0625 mg/ml |
| $P_1$ | * | * | # | ## | ## |
| $P_2$ | * | # | ### | ### | ### |
| $P_3$ | * | * | * | ## | ### |
| $P_4$ | * | * | # | ### | ### |
| $P_5$ | * | * | * | # | ## |
| $P_6$ | * | * | # | # | ## |
| $P_7$ | * | * | * | ## | ### |
| $P_8$ | * | * | ## | ## | ## |
| $P_9$ | * | * | * | * | # |
| $P_{10}$ | * | * | * | * | ## |
| $P_{11}$ | * | * | * | # | ### |

Key: (#) slight turbidity, (##) moderate turbidity, (###) very turbid, (*) no growth

Table 9: Minimum bactericidal concentration (MBC) of *Jatropha curcas* plant extract

| Test Organism | *Jatropha curcas* |
|---|---|
| | MBC (mg/ml) |
| $P_1$ | 0.5 |
| $P_2$ | >1.0 |
| $P_3$ | 0.25 |
| $P_4$ | 1.0 |
| $P_5$ | 0.25 |
| $P_6$ | 1.0 |
| $P_7$ | 0.5 |
| $P_8$ | 0.5 |
| $P_9$ | 0.125 |
| $P_{10}$ | 0.25 |
| $P_{11}$ | 0.5 |

## DISCUSSION

The prevalence of epidemic multidrug-resistant isolates of various pathogenic bacteria types to standard antibiotics and therapeutics globally has become one of the most alarming and deleterious public health concerns, especially in developing countries like Nigeria, where it has become a leading serious medical, economic, and social issue (Braide *et al.*, 2018). Pathogens like those that cause nosocomial infections are some of the most dangerous types of pathogens due to their association with the hospital population, where immunocompromised patients are readily infected by certain nosocomial pathogens, such as *Pseudomonas aeruginosa* strains (Ibtihaj *et al.*, 2021). The issue of multidrug resistance in Gram-negative bacteria, especially the nosocomial pathogens such as Pseudomonas aeruginosa, is a newly emerging and one of the most important areas of study in modern medicine (Oluwatosin *et al.*, 2019). This calls for the quest for new antimicrobial agents, especially those derived from natural sources such as ethnomedicinal plants, which exhibit low pathogenic resistance and minimal therapeutic side effects. In the present work, the antibiogram profile of Pseudomonas aeruginosa isolates in Kaduna State metropolis was described, and the antimicrobial properties of Vitex doniana and Jatropha curcas plant extracts were examined against extreme multidrug-resistant strains. The leaves of *Vitex doniana* and *Jatropha curcas* were collected, authenticated, and prepared, with chloroform used to extract the plant materials. The extraction results of the plants show that the Vitex doniana chloroform extract is dark greenish in color and gummy in nature, while the Jatropha curcas extract is greenish-brown in color and crystalline in nature, with differing individual percentage yields. This is consistent with the work done by Hassan *et al.* (2020) on the activity of Ficus sycomorus phenolic extracts against certain chosen snake venoms and also the work done by Prithviraj *et al.* (2023) and Alagbe *et al.* (2021). Qualitative and quantitative phytochemical analysis was conducted on the two plant extracts, revealing that both contain phytosterols, saponins, tannins, flavonoids, phlobatannins, phenols, terpenoids, alkaloids, and quinones. This suggests that both plants contain high levels of phytometabolites, especially phenolics like flavonoids and tannins, which have been reported to exhibit different types of antibacterial activities (Hassan et al., 2023; Cyentanociv *et al.*, 2021). Quantitative phytochemical screening of the plant extracts suggests that both extracts contain phenolic phytometabolites with the highest concentration percentage in them. This finding is in line with the outcomes of studies by Rivas et al. (2021), Basel et al. (2017), and Adedeji et al. (2007). Of the 150 P. aeruginosa isolates obtained, forty three of the isolates obtained from samples were biochemically identified as P. aeruginosa strains (39.81 %) and 21 out of 42 environmental isolates were identified as P. aeruginosa strains.

Refuse dump and wound exchange samples supplied the greatest variety of P. aeruginosa isolates from environmental and clinical samples accordingly. These generated a similar research outcome to that carried out by Adekunle et al. (2022). A standard antibiotic sensitivity test was performed on all the bacterial isolates found, wherein a higher percentage of the isolates were multi drug resistance to more than three of the seven classes of the standard antibiotics (Cephalosporins, Penicillins, Quinolones, Carbapenems, Aminoglycosides, Glycopeptides and Nitrofurantoin), whereas eleven were highly multi drug resistance (resisted six or seven of the 7 class of standard antibiotics). About 81.8% of the eleven highly multidrug-resistant *P. aeruginosa* isolates are clinical isolates, of which more than half are of wound swab origin. Part of the possible reasons for this finding may be the successful ability of *P. aeruginosa* strains to form biofilms, which exposure of wound matrix cells to tissue facilitates, thereby developing conditions conducive to the pathogens' formation. The bacteria can grow favorably on the wet wound surface, which also favors the development of effective efflux systems within the bacterial matrix. These findings align with those reported by Pandukur et al. (2020) on the prevalence of bacterial pathogens among diabetic outpatients at Plateau Specialist Hospital, Jos, Nigeria. And that of Chaimae *et al.* (2019), regarding the antibiotic resistance and virulence genes of *Pseudomonas aeruginosa* isolated from patients in Northwestern Morocco. The same conclusion was reached by Rubina *et al.* (2014) regarding the antibiogram of *Pseudomonas aeruginosa* and Methicillin-resistant Staphylococcus aureus in diabetic patients. Molecular characterization, including 16S RNA sequencing of all eleven extreme multidrug resistance isolates, reveals and confirms that all eleven isolates are strains of *Pseudomonas aeruginosa*. These sequences are deposited and published on the Protein Data Bank (PDB), with individual accession numbers assigned to them. Multidrug resistance gene identification tests have been performed, showing that all extreme resistance strains contain two or three acrA, bla OXA-23, and bla VIM resistance genes targeted in their respective genomes.

The same research results were reported by Nitz et al. (2021) for the molecular detection of drug-resistance genes blaOXA-23-blaOXA-51 and mcr-1 in clinical isolates of *Pseudomonas aeruginosa*. An antimicrobial bioassay conducted on Vitex doniana and Jatropha curcas leaf extracts against high multidrug-resistant Pseudomonas aeruginosa isolates shows that Jatropha curcas extracts have good antibacterial activity against all the resistance strains, in contrast to the extracts of Vitex doniana, whose some strains that are even fully resistant to. This indicates that the phytometabolites of the *Jatropha curcas* plant can be utilized and developed as therapeutic compounds to combat multidrug-resistant strains of *Pseudomonas aeruginosa*.

Another identical research finding was reported by Abu et al. (2020), who found that the plant extract was strongly active even against multidrug-resistant bacteria. Henik *et al*. (2023) also reported a study finding on *Jatropha curcas* plant extracts with good anti-fungal activity against *Fusarium oxysporum* and *Alternaria solani*. The MIC and MBC tests carried out for the *Jatropha curcas* extract against the 11 extreme multidrug-resistant extracts show that the extract has good MIC and MBC values as low as 0.125 mg/mL against some of the resistance strains. Similar results were also noted by Arekemase *et al*. (2011) in their study, which examined the antimicrobial potential and phytochemical screening of Jatropha curcas against selected microorganisms. The Jatropha curcas extract was shown to possess a very good MIC/MBC value against certain bacterial pathogens, such as *Pseudomonas aeruginosa* strains.

## CONCLUSION

The *Jatropha curcas* extract has the potential to serve as a drug agent or an ingredient in drug formulations for the treatment of diseases caused by multidrug-resistant *Pseudomonas aeruginosa*, and hence further studies are needed in that direction.

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