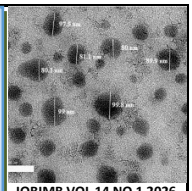
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In Vitro Activity of Ethanolic Extracts of *Vitex doniana* Against Multidrug-Resistant *Staphylococcus aureus* Isolated from Hospital Bed Rails

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Abstract

Hospital surfaces serve as reservoirs of pathogens, including multidrug-resistant *Staphylococcus aureus* (MDRSA), especially in resource-limited areas. This research aims to determine the *in vitro* activities of *Vitex doniana* leaf and root extracts on MDRSA isolated from bed rails. Eighty-eight (88) samples were collected from 113 hospital beds and grown on Mannitol Salt Agar at 37°C in 24 hours. Colonies formed were identified morphologically, by Gram-staining, and biochemical characterisation. Antibiotic susceptibility test was performed using Kirby-Bauer technique. The plant extracts were tested against the isolated bacteria through agar well diffusion method. The bacteria were found in 70 samples (79.55%), of which 54 (77.15%) were MDR. A higher percentage of MDRSA was found in the Female Medical and Surgical Wards as well as Male Surgical Wards having 10 (14.29% each), the theatre had the lowest, 1(1.43%). The bacteria were resistant to Ampiclox (69, 98.57%), cefuroxime (69, 98.57%), amoxicillin (65, 92.9%), and ceftriaxone (45, 64.29%). The MICs of the root and leaf extracts were 1.56 mg/mL, and the MBCs were 3.125 mg/mL and 1.56 mg/mL, respectively. Both extracts exhibited antimicrobial activity at 100 mg/mL, 70 mg/mL, and 50 mg/mL concentrations. The mean and standard deviations for 5 replicate assays of the leaf extract indicate that had higher activity (10.50 ± 0.10 - 28.50 ± 0.10 mm) than the root extract (8.50 ±0.10 - 17.50 ± 0.14 mm). High proportion of MDRSA calls for integrated management strategies, including the development and prudent use of potent antimicrobials and disinfection of hospital environments to prevent transmission.

INTRODUCTION

Staphylococcus aureus is among the WHO bacterial priority pathogen list 2024, from which medium- to highly resistant strains are categorized [1]. It is one of the human pathogens responsible for various diseases, including skin and respiratory tract infections) [2]. Antimicrobial resistance is identified as a major global health threat. The prevalence of antibiotic-resistant pathogens, including multidrug-resistant *Staphylococcus aureus* (MDRSA), has highlighted the need for alternative treatments [3]. Treatment is becoming more challenging due to the development of more resistant *S. aureus* to commonly used antibiotics, drastically decreasing the efficacy of traditional antimicrobial therapy. The rising prevalence of MDRSA presents significant challenges for public health systems. Community reservoirs complicate infection prevention strategies, as patients may enter healthcare facilities already colonised with resistant strains. This increases the risk of environmental contamination particularly the hospitals beds [4].

Vitex doniana, commonly known as black plum, is a deciduous tree that is widespread in tropical and subtropical Africa. The plant belongs to the Lamiaceae family and has been traditionally used in Nigeria to treat a variety of illnesses, including skin infections, respiratory conditions, and gastrointestinal diseases [5]. It is found in countries such as Nigeria, Ghana, and Cameroon; in Nigeria, it is called Dinya, Oori-nla, and Uchakoro in Hausa, Yoruba, and Igbo languages, respectively, and has been used to treat inflammation and bacterial diseases [6]. The phytochemical components of the

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plant demonstrated antibacterial, anti-inflammatory, and antioxidant capabilities and are responsible for its therapeutic qualities [7].

Hospital bed rails are the most frequently touched surfaces by patients, health workers, and patients' relatives, and one of the most important reservoirs of pathogenic microbes. Infections involving MDRSA are significant healthcare concerns, especially in resource-limited areas owing to limited effective antibiotics and the slow pace of new drug development, highlighting the urgent need for alternative therapeutic options [8]. The search for therapeutic alternatives has led to renewed interest in traditionally used plant extracts, including *V. doniana,* which could be considered a source of new antimicrobial agents. The study demonstrated novel evidence of the *in vitro* antibacterial activity of *V. doniana* leaf and root extracts against MDRSA isolated from hospital bed rails in Wukari Teaching Hospital, Taraba State, Nigeria. It offers comparative analysis of the antimicrobial efficacy of the extracts against MDRSA, highlighting their potential as alternative therapeutic agents.

MATERIALS AND METHODS

Sample collection

The hospital bed rails from Federal University Wukari Teaching Hospital, Taraba State, were swabbed using sterile swab sticks at a surface area of 100 cm^2 and immediately dipped in 0.5 mL of peptone water. The samples were placed in a cold box and transported to the laboratory within 2 hours, where they were immediately processed. The hospital consists of a total of 113 beds in 9 hospital wards, from which 88 samples were collected by proportionate sampling. The samples were collected in December 2026. The Slovin sample size formula described by Ryan [9] was used to calculate the sample size, as follows:

$$n = \frac{N}{1 + N(e)^2}$$

Where,
N is the number of beds examined (113).
The margin of error (e) was taken as 5% (0.05) at a 95% confidence interval.
The formula was employed because the study population is known and finite, and can prevent unnecessary sampling, and suitable for the study as a non-prevalent survey.

Media preparation

Mannitol Salt Agar (MSA), Mueller-Hinton agar (MHA), Simmons citrate agar, and tryptophan and nutrient broths were the media utilised; they were prepared in accordance with the manufacturer's instructions and warmed to 60°C to dissolve. They were then autoclaved for 15 minutes at 121°C and cooled to 50°C. Then 20 mL of MSA and MHA were respectively dispensed into Petri dishes and allowed to solidify as described by Cheesbrough [10].

Isolation of the Bacteria

Culture and microscopy were used to isolate and morphologically identify *S. aureus*. All swabs were aseptically streaked onto the surface of the MSA and incubated at 37°C for 24 hours in accordance with Cheesbrough, [10]. Grown colonies were observed physically for texture, elevation, colour, shape, and arrangement [11]. Distinct colonies were sub-cultured onto MSA by streaking a loopful of the surface of the medium. To make a bacterial suspension, a loopful of the colony was added to a drop of water placed on a clean glass slide and stirred. The slide was placed on the microscope stage and examined with 10x and 40x objective lenses for cocci [10].

Gram staining and biochemical tests

Gram staining and biochemical tests, including catalase, coagulase, oxidase, citrate, and indole tests, were carried out as described by Cheesbrough, [10].

Gram staining

A loopful of the pure colony was suspended in a drop of water on a clean, grease-free slide. The slide was air-dried and passed over a Bunsen flame. Crystal violet solution was added and allowed to act for one minute, then rinsed off with water. The slide was then flooded with iodine solution for one minute, then washed with water. It was then washed with 95% alcohol for 20 seconds and rinsed with water. Safranin was then added and allowed to act for 1 minute, then washed with water and air-dried. The stained slides were then examined with the oil immersion objective of the light microscope.

Catalase test

This was done by adding two drops of hydrogen peroxide (H_2O_2) onto a young culture, which was placed on a clean glass slide. The production of air bubbles due to the catalase enzyme converting H_2O_2 to water (H_2O) and oxygen (O_2) indicates a catalase reaction.

Coagulase test

A loopful of a distinct colony was added to a drop of water placed on a clean, grease-free glass slide and mixed to produce an even suspension, then a drop of plasma was added. Coarse clumps seen with the naked eye within 10 seconds were considered positive. Any reaction that takes more than 10 seconds to manifest or the absence of any discernible clumps was recorded as negative.

Oxidase test

A distinct colony was selected from a fresh bacterial culture using a wire loop and rubbed onto a piece of filter paper. Then, two drops of 1% Kovács reagent were added to observe colour changes. A colour change, within 10 seconds, to dark purple was considered positive. A colour change to purple within 1 minute was considered delayed oxidase-positive. No colour change or more than 2 minutes was considered a negative test.

Citrate

The isolates were inoculated onto Simmons citrate agar and incubated at 37 °C for 24 hours. A blue colouration was regarded as an indicator for a positive test for citrate utilisation and was recorded accordingly for the isolates tested.

Indole

Four millilitres (4 mL) of tryptone water were added into a sterile test tube. Then, a loop full of fresh bacterial culture was inoculated into the medium and incubated at 37°C for 24 hours. Kovac's reagent (0.5 mL) was added to the culture, and the tube was shaken and allowed to stand for a few minutes. The appearance of a red (cherry-red) ring in the reagent layer within seconds indicates a positive result.

Antibacterial susceptibility test

Antibiotic activity against *S. aureus* was evaluated using the Kirby-Bauer method according to CLSI M02. A bacterial suspension (100 µl) at a concentration of 10^5 was inoculated on the MHA. A sterile wire loop was used to streak the entire surface of MHA, ensuring even distribution of the inoculum. The plate was kept for 5 minutes at 4 °C, so that the inoculum could be

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properly absorbed. Sterile forceps were then used to pick up antibiotic discs (Oxoid, Thermo Fisher Scientific, UK) and placed at the centre of the plate. The antibiotic discs included amoxicillin, Ampiclox (ampicillin/cloxacillin), azithromycin, ciprofloxacin, erythromycin, gentamicin, levofloxacin, pefloxacin, ceftriaxone, and cefuroxime. The discs were lightly pressed with forceps to ensure good contact. The plates were incubated for 24 hours at 37 °C and the zones of inhibition were recorded in millimetres with a ruler and interpreted as susceptible (S), intermediate (I), and resistant (R), according to the Clinical and Laboratory Standards Institute (CLSI) breakpoint for *S. aureus* [12].

Collection and extraction of the plant material

Vitex doniana leaves and roots were collected from the Wukari metropolitan area and authenticated by a certified botanist at the Department of Biology, Faculty of Biosciences, Federal University Wukari (Authentication Code: NMP-195). Plant material extraction followed Mostafa et al. [13], with modifications. The roots and leaves of the plant were separately cleaned with distilled water, air-dried for two weeks at room temperature in the shade, and then ground using a mortar and pestle. The powder produced was passed through a 100 µm sieve to ensure a uniform particle size for extraction. A 100g of powder was mixed with 1,000 mL of 70% ethanol. After that, it was filtered through two layers of muslin, centrifuged for 10 minutes at 13,500 x g, and then filtered once more using Whatman filter paper No. 41. A rotary evaporator operating at 40 °C and low pressure (200 millibars) was used to dry the filtrate, and then stored in airtight light-protected containers at 4 °C until use. Each 100g of the powdered plant material yielded 16g of the extract.

Determination of antibacterial activity of the plant extract

The agar well diffusion technique was employed to assess the antibacterial activity of the plant extract against MDR *S. aureus*. A 0.5 McFarland standard inoculum (1.5 x 10^8 CFU/mL) was prepared from an overnight culture of MDR *S. aureus*. Fresh MHA was inoculated with the prepared inoculum by streaking uniformly to achieve an even distribution of the inoculum. Then, 6 mm diameter holes were punched using a cork borer to accommodate 5% (5 mg/100µl), 10% (1 mg/100 µl), 30% (3 mg/100 µl), 50% (5 mg/100 µl), 70% (7 mg/100 µl) and 100% (10 mg/100 µl) preparations. Ciprofloxacin (5 µg/disc) was used as a positive control, whereas a well containing 5% ethanol was used as a negative control. The *S. aureus* quality control strain used was ATCC25923 for each antibiotic. The plates were kept at 4 °C for 15 minutes, then incubated at 37 °C for 24 hours. The diameters of the zones of inhibition around wells were measured in millimetres using a ruler in accordance with CLSI M07 CLSI [12]. The assay was performed in 5 replicates and the means and standard deviations of the measurements was calculated.

Determination of minimum inhibitory concentration (MIC)

The broth microdilution method was employed to determine the MICs of the root and leaf extracts in accordance with the CLSI M07-Ed12, with modifications. A bacterial solution adjusted to a 0.5 McFarland standard (1.5 × 10^8 CFU/mL) was prepared, then diluted to 5 x 10^6 CFU/Ml. Two-fold dilutions of the extracts were made in sterile MHA broth to prepare 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.125 mg/mL, and 1.56 mg/mL concentrations. To each test tube, 1 mL of each of the concentrations of the extracts was added. Then 1 mL of bacterial suspension was added to a final concentration of 5 x 10^5 CFU/mL and a final volume of 2 mL. Negative controls were made with broth without the bacteria, and positive controls were the broth and bacterial inoculum without extract. The S. aureus ATCC 29213 was employed for quality control. The plates were incubated for 24 hours at 37°C, then checked for visible turbidity (bacterial growth). The lowest concentration of the extract in the test tube that shows no turbidity is considered the MIC. The plant extract itself had colour that could interfere with MIC reading; however, an extract-only blank solution that corresponded with the extract containing the bacteria was included. Then, the test tubes with the appropriate extract blanks were compared with the growth controls to determine the actual MIC [12].

Determination of minimum bactericidal concentration (MBC)

The MBC was assessed from the MIC obtained, where the suspensions from the tubes with no visible growth were gently mixed to resuspend the settled bacteria. The bacterial suspension (100 µl) was aliquoted from each of the clear tubes and inoculated into antimicrobial-free MSA. The inoculum was spread evenly onto the agar surface using a sterile wire loop. The plates were incubated at 37°C for 24 hours. The plates were observed for bacterial growth; the lowest extract concentration that produced no colonies on the MSA plates was known as the MBC [12].

Statistical analysis

The diameters of the zones of inhibition were recorded as the mean and standard deviation. Chis-squared test was employed to ascertain the significant difference between the isolated bacteria and the MDRSA Antibacterial activities were evaluated using two-way ANOVA to determine the associations between categories of antibiotics and effects of extract concentrations on the bacterial growth; $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

The grown colonies appear as circular, small, yellow, smooth, slightly raised, entire, and opaque. The organisms were microscopically clustered and Gram-positive. Biochemically, they were found to be positive for catalase and coagulase, and a change of MSA from pink to yellow. They showed negative tests for citrate, oxidase, and indole, presumably consistent with the profile of *S. aureus*, thereby supporting the presumptive identification of the organism. The bacteria were isolated from 70 samples (79.55%); the prevalence of MDRSA ranged from 1.43 to 14.29% across the hospital wards with overall value of 77.14%. The bacteria were resistant insensitive to multiple antibacterial classes. Antibacterial sensitivity test revealed that *V. doniana* leaf and root extracts were active against MDRSA at concentrations of 100 mg/mL, 70mg/mL, and 50 mg/mL. The root and leaf extract MICs were 1.56 mg/mL each, whereas the MBCs were 3.125 mg/mL and 1.56 mg/mL, respectively. The morphological and biochemical profiles of *S. aureus* in this study are consistent with those of hospital-associated isolates previously described by Muthoni [14]] and Omololu-Aso et al. [15]. *In vitro* antibacterial activity in this study demonstrates that the leaf extract was higher than root against MDRSA.

Table 1 shows the prevalence of *Staphylococcus aureus* isolated from bed rails in different sample collection units in the study area. Of the 88 samples collected, 70 (79.55%) were *S. aureus*-positive. The highest prevalence was in Female Medical and Surgical Wards (12, 13.64% each), followed by male medical and Paediatric Wards (10, 11.36% each); the lowest was in accident and emergency, psychiatry, and theatre (2, 2.27% each). No significant difference was found between the proportions of samples collected and the positive samples ($\chi^2 = 0$, $p=1$).

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Table 1. Prevalence of *Staphylococcus aureus* among the hospital wards in the study area.

Hospital wards	Number of Beds	Number of Samples collected (%)	Number of positive samples (%)
Accident and Emergency	3	2 (2.27)	2 (2.27)
Female Medical Ward	20	13 (14.77)	12 (13.64)
Female Surgical Ward	16	11 (12.50)	12 (13.64)
Male Medical Ward	20	13 (14.77)	10 (11.36)
Male Surgical Ward	17	11 (12.50)	11 (12.50)
Maternity Ward	15	10 (11.36)	9 (10.23)
Paediatric ward	20	13 (14.77)	10 (11.36)
Psychiatric ward	19	13 (14.77)	2 (2.27)
Theatre	3	2 (2.27)	2 (2.27)
Total	133	88 (100%)	70 (79.54)

Hospital surface contamination rates vary; according to recent research, most of which are lower than ours (42.5% by Vigny and Shu [16] in Cameroon, 42.4% by Al-Fawaresa et al. [17] in Jordan, and 14.3% by Yakubu et al. (2025) in selected hospitals of the Nigerian capital city. On the other hand, 85.25% of S. aureus was identified from Nigerian orthopaedic hospital surfaces by Udobi et al. [18], which is higher than our findings. Geographical location and hygiene behaviours might be the cause of the differences between the studies.

The higher positivity in the Female Surgical Ward and Male Surgical Ward is consistent with the fact that wards with high patient turnover and invasive procedures, such as Surgical Wards, frequently exhibit greater contamination as emphasized by Nandkishor et al. [19]. On the other hand, the low positive rate in the Psychiatric Ward could be because the rails in the wards of the mentally ill individuals are physically cleaner than those in acute medical/surgical settings since patients are mobile, continent, and not frequently touched by staff during medical care. This is consistent with research by Nadege et al. [20], which found that wards with fewer invasive procedures and less patient movement often had lower microbial presence.

The **S**ensitivity profiles of the tested antibiotics against the presumptive *S. aureus* isolates are shown in **Table 2**. Antibacterial sensitivity profiles revealed variability in the zones of inhibition produced by the tested antibiotics. The bacteria exhibited high resistance to some of the tested antibiotics, including Ampiclox, ceftriaxone, amoxicillin, and cefuroxime. The bacteria showed higher resistance to Ampiclox and cefuroxime with 69 (98.57%) prevalence each, amoxicillin with 65 (92.9%), and ceftriaxone with 45 (64.29%). However, the organism showed higher intermediate susceptibility to erythromycin (25, 35.71%) and pefloxacin (22, 31.42%). Levofloxacin (59, 84.29%), gentamicin (46, 65.71%), and azithromycin (48, 68.57%) were the most prevalent sensitive antibiotics. The chi-squared test indicates that there is a statistically significant association between the antibiotics and susceptibility categories ($\chi 2 = 512$, df =18, p<0.0001). Bonferroni correction revealed that a greater percentage of the antibiotic pairs differed significantly (p=<0.05).

The high resistance observed in β-lactam antibiotics (Ampiclox and amoxicillin) could be due to genes encoding an altered penicillin-binding protein 2a (PBP2a), which reduces the binding efficiency of the β-lactam ring [21]. A similar pattern was reported in previous studies where *S. aureus* isolates demonstrated reduced susceptibility to commonly used β-lactam antibiotics [22]. The finding of this study concerning the β-lactam antibiotics is higher than 70.00%, with a similar pattern regarding ciprofloxacin (30.00%), gentamicin (30.00%), and a higher percentage for erythromycin (50.00%), as found by Adeiza et al. [22], possibly due to the study design and geographic differences. The statistical analysis (p<0.05) implies that the antibacterial agents exhibit different levels of activity against the bacteria, with Levofloxacin and Gentamicin showing high sensitivity and lower resistance compared to others. The existence of resistant strains on hospital beds emphasises how the environment contributes to the spread of drug-resistant *S. aureus*. According to Gamuya et al. [23], hospital environments, including beds, medical equipment, and other frequently touched surfaces, can serve as reservoirs of resistant bacteria and contribute to the spread of healthcare-associated infections.

Table 2. **S**ensitivity profiles of the tested antibiotics against the *Staphylococcus aureus* isolates (n=70).

Antibiotic (µg)	Sensitive (%)	Resistant (%)	Intermediate (%)
Amoxicillin (25)	1(1.43)	65(92.9)	4(5.71)
Ampiclox* (10/5)	1(1.43)	69(98.57)	0(0.00)
Azithromycin (15)	48(68.57)	12(17.14)	10(14.29)
Ciprofloxacin (5)	27(38.57)	24(34.29)	19(27.14)
Erythromycin (15)	34(48.57)	11(15.71)	25(35.71)
Gentamicin (10)	46(65.71)	20(28.57)	4(5.71)
Levofloxacin (5)	59(84.29)	5(7.14)	6(8.57)
Pefloxacin (5)	27(38.57)	21(30.00)	22(31.42)
Rocephin (15)	21(30.00)	45(64.29)	4(5.71)
Zinacef (30)	1(1.43)	69(98.57)	0(0.00)

Note: Cefuroxime vs Ampiclox; Gentamicin vs Ampiclox; Levofloxacin vs Cefuroxime; Gentamicin vs Cefuroxime (p=0.0001)

All isolated bacteria (70, 100.00%) were resistant to at least 1 antibiotic; a substantial proportion (54, 77.14%) were multidrug-resistant, insensitive to multiple antibacterial classes, and were regarded as MDRSA. **Fig. 1** shows the proportions of the positive samples and MDRSA across the hospital sections. Female Medical and Surgical Wards had the highest incidence (12, 17.14%) each, followed by the Male Surgical Ward with 11 (15.70%). The lowest proportions were observed in the Accident and Emergency and Psychiatric Wards, and the theatre, with 2 (2.86%) each. Regarding MDRSA, a relatively higher proportion was found in the female medical and Surgical Wards as well as the Male Surgical Ward (10, 14.29%) each], Paediatric Ward followed with 9 (12.86%), with the lowest being the Theatre, with 1 (1.43%). Chi-squared test showed a statistically significant association between the sampling points and multidrug-resistant status for the bacteria isolated (χ^2 =14.37, df=8, p=0.073).

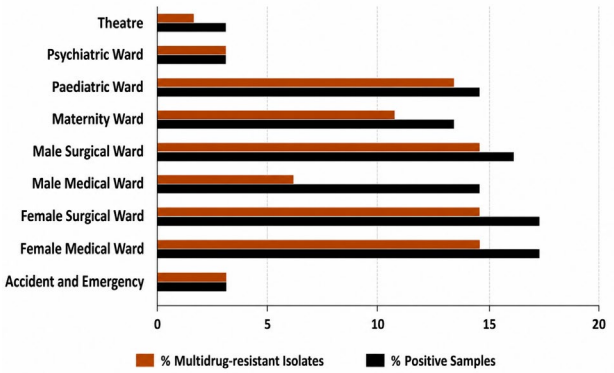


Fig. 1. Multidrug-resistant *S. aureus* among the bacteria isolated across the hospital units (n=70).

The occurrences of drug-resistant and MDRSA across the hospital wards provide important insight into the role of hospital bed rails as reservoirs of *S. aureus*. The high percentage of

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bacteria isolated could be due to the nature of the admitted subjects, frequent use of invasive devices, poor hand hygiene, overcrowding, sterilisation practices and sanitation practices. The bacteria were considered MDR, being non-susceptible to one or more agents in at least three different antimicrobial classes in accordance with the CLSI guidelines [12]. A high proportion of drug-resistant and MDRSA in the female Medical and Surgical Wards and Male Surgical Ward may be due to frequent human contact and inadequate disinfection of surfaces.

The proportion of MDRSA in this study is higher than the findings of Omololu-Aso et al. [15] in Ile-Ife (38.00%), Okojokwu et al. [24] in Jos (61.96%), Hamid et al. [25] in Islamabad, Pakistan (52.00%), and 47.80% by Agu et al. [26] in Ado-Ekiti. It also higher than 39.55% in East Africa Dagnaw et al. [27], Garba et al. [28] (46.70%) in Zaria, Kaduna State. It is lower than that of Adhikari et al. [29] (94.05%) in Himalaya, Nepal. The differences may be due to geographical and ethnic differences, population and sample sizes, and weather conditions of the study areas. They may also be due to sample source, the antibiotic treatment regimen used in different places, and differences in hospitalisation practices. Kampf et al. [30] reported that pathogenic bacteria can remain on fomites long enough to contribute significantly to nosocomial infections.

The ethanolic extracts of roots and leaves of *V. doniana* exhibit antibacterial activity against the isolated MDRSA as demonstrated in **Table 3**. At 100 mg/mL, the leaf extract inhibited the bacteria by 28.50 ± 0.10 mm. The inhibition zones decreased to 23.00 ± 0.18 mm in diameter at 70 mg/mL and 10.50 ± 0.10 at 50 mg/mL. The root extract had inhibition diameter of 17.50 ± 0.14 mm was recorded at 100 mg/mL, which declined to 12.50± 0.10 mm at 70 mg/mL and 8.50 ± 0.10 mm at 50 mg/mL. Two-way ANOVA showed significant effects of the two extract types (p=0.001) and their concentration (p=0.001) against *S. aureus*. There is also a significant difference between the concentrations of the extracts and extract types (p=0.001), which indicates that the antibacterial effect of the extracts differs in accordance with concentrations.

Table 3. Antimicrobial assay of *V. doniana* leaves and roots extract on MDRSA.

Extract Concentration (mg/mL)	Mean ± SD of the DZI (mm)	
	Leaf	Root
100.00	28.50 ± 0.10	17.50 ± 0.14
70.00	23.00 ± 0.18	12.50 ± 0.10
50.00	10.50 ± 0.10	8.50 ±0.10
30.00	0.00 ± 0.00	0.00 ± 0.00
10.00	0.00 ± 0.00	0.00 ± 0.00
5.00	0.00 ± 0.00	0.00 ± 0.00

Note: DZI = Diameter of Zones of Inhibition, Mean ± SD n=5.

Studies on the activity of *V. doniana* root extract are limited; however, Samuel et al. [31] found a 12.20 mm zone in the ethanolic extract at 6.75 mg/100ug (16.88%). Research carried out by Ali & Mudi [32] revealed that chloroform and hexane extracts (15 ug/mL each) of the plant leaf produced zones of inhibition of 6 mm each, with standard deviations of 0.22 and 0.21, respectively. The leaf extract generated noticeably larger zones of inhibition than the root extract. The impact of concentration on antibacterial activity varied between the leaf and root extracts across the concentrations (Mann-Whitney U, P<0.0079 for each concentration). None of the extracts showed activity at 30 mg/mL, 10 mg/mL, and 50 mg/mL. The leaf extract demonstrated greater activity against the tested bacteria.

The MICs of both extracts and their MBCs for the leaf and root indicate significant *in vitro* antibacterial activity. Bittner and Mu˘ [33] also reported moderate activity with an MIC of 20 mg/mL. The greater activity in this study may be due to differences in the solvents and extraction methodology. It may also be due to differences in the plant origins, maturity, and geographical locations. Phytochemical analyses have not been performed on the plant extracts. However, recent studies indicate that leaves composed of higher levels of secondary metabolites, including flavonoids (58.88 – 232.55mg/100 g), tannins (97.54 – 4200.67 mg/100g), saponins (31.66- 96.69 mg/100 g), alkaloids (173.33 – 194.75 mg/100 g), and phenolic compounds (11.06 – 66.15 mg/100 g) which are known for their antibacterial and anti-inflammatory properties [34].

Due to increased concentration of these phytoconstituents in foliar tissues, earlier studies on several medicinal plants found that leaf extracts had higher antibacterial activity than root extracts [33,35,36]. Additionally, leaves synthesise rich pools of phytochemicals and actively participate in photosynthesis. Large quantities of phenolic compounds and volatile oils are stored in the glandular hairs and vacuoles of leaves, which are the primary sources of their antibacterial properties [37]. Leaves had higher exposure to airborne bacteria and fungi, prompting the plant to concentrate its chemical defences there [38]; hence, leaves had higher metabolite concentrations, providing more bioactive molecules for bacterial inhibition.

LIMITATIONS OF THE STUDY

This study has some limitations that are collectively attributed to infrastructure and funding. First, the results may not be generalized to other healthcare settings and surfaces because the study was limited to hospital bed rails and conducted at a single tertiary institution. Secondly, molecular confirmation of *S. aureus* or vancomycin/methicillin-resistant genes could not be performed; bacterial identification relied on traditional culture and biochemical techniques. Additionally, the specific bioactive antibacterial in the extract was not identified; no investigations related to the pharmacodynamic, pharmacokinetic, cytotoxic effects or in vivo effectiveness were conducted. Lastly, the study did not examine the mechanism of antibacterial action.

CONCLUSION

The distribution pattern of MDRSA provides important insight into the role of hospital environments as reservoirs. The relatively higher proportions of presumably resistant and MDR in various sampling points suggest that contamination is influenced by frequent human contact. Ethanolic extracts of *V. doniana* leaves and roots have demonstrated antimicrobial activity against the MDRSA, with higher activity in the leaf extract. However, deeper pharmacokinetic and pharmacodynamics as of the refined products are needed so that the plant can be fully considered as a potential alternative therapy for *S. aureus* infections. There is a need for integrated strategies, including improved infection prevention and control, health education, routine environmental cleaning, prudent antibiotic therapy, antimicrobial stewardship programs, and surveillance of high-touch surfaces such as hospital beds and fomites to prevent the spread of MDRSA in healthcare facilities.

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AUTHORS' CONTRIBUTIONS

Surajo Adamu Wada, the first author, carried out the experimental process and drafted the first version of the manuscript. Formal analyses were done by Miracle Gomnati and Faith Akyara. The paper was reviewed and edited by Ibrahim Alhaji Sabo and Samuel Tamuno Brown, who also offered suggestions for improvement.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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