

***In vitro* Antibacterial Activity of *Terminalia avicennnioides* Extracts Against Multidrug Resistant *Staphylococcus aureus* Strains**

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Abstract: Infections caused by multidrug resistance bacteria are now alarming globally, and the increasing rates of antimicrobial resistance are resulting in fewer treatment options. The search for new phytochemicals that could be developed as useful drugs for treatment of infectious diseases consequently increased with medicinal plants extracts receiving greater attention. This study was carried out to determine *in vitro* antimicrobial activity of *Terminalia avicennnioides* extracts against multidrug resistant *Staphylococcus aureus* strains isolated from wound infections. Wound swab samples were collected from patients attending Barau Dikko Teaching Hospital Kaduna, Nigeria. Isolation and characterization of *Staphylococcus aureus* was carried out using standard phenotypic and genotypic identification methods. Antimicrobial susceptibility profile of *Staphylococcus aureus* isolates was carried out using standard procedures. Also, *Terminalia avicennnioides* extracts were prepared and their *in vitro* antimicrobial activities tested against multidrug resistant *Staphylococcus aureus* using standard procedures. The results of the susceptibility profile showed *Staphylococcus aureus* isolates to be resistant to a ranged of 8.18% to 100% conventional antibiotics used. However, the isolates were 100% sensitive to imipenem. Qualitative and quantitative phytochemical analysis of the extracts revealed the presence of tannin, alkaloids, flavonoids, cardiac glycoside, phenols, saponins and terpenoids and absent of anthraquinones in all extracts. Antimicrobial activity of *Terminalia avicennnioides* extracts against multidrug resistant *Staphylococcus aureus* isolates showed zones of growth inhibition ranged from 16.28±10.45 – 23.81±6.69 mm and showed significant difference ($P < 0.05$). Minimum inhibitory concentration (MIC) of the extracts ranged from 56.2500 ± 29.1241 – 31.2500 + 22.16013 gm/ml and showed no significant difference ($p > 0.05$). The minimum bactericidal concentration (MBC) of the extracts ranged from 175.000 ± 64.2910 – 68.7500 ± 45.8063 mg/ml and showed no significant difference ($p > 0.05$). Remarkably, the antimicrobial activity of the *Terminalia avicennnioides* extracts exhibit higher inhibitory effects against the multidrug resistant *Staphylococcus aureus* strains, hence, can further be study and developed for wound infection therapeutic purpose.

Keywords: *Staphylococcus aureus*, Multidrug Resistance, Wound, Antibacterial *Terminalia avicennnioides*

1. Introduction

Staphylococcus aureus is known to acquire resistance to new drugs and continues to defy attempts to control it. Infections caused by antibiotic resistant strains of *Staphylococcus aureus* have reached epidemic proportions globally and the increasing rates of antimicrobial resistance

are resulting in fewer treatment options [1].

The economic and health impact of multidrug resistant (MDR) infections on a global scale is enormous and dreadful and recently, it has been underestimated that worldwide, about 700,000 lives are lost annually due to antimicrobial-resistant infections [2-4]. The impact of MDR infections is worse in developing nations, including Nigeria, with unaccounted cost of

treatment of resistant infections and associated deaths. Currently, antimicrobial resistance infections is one of the risen concern to global health and its threats is being strengthened by some key factors such as global climatic change, globalization and change in demographics [3, 4]. It is also largely recognized that most of the currently available antibacterial, especially the synthetic ones have been misused and are ineffective [5-7]. Hence, the World Health Organization (WHO) recommended for a focus on discovery and development of new antibiotics specifically active against multidrugs and extensively drug-resistant bacteria; and development of new types of antibiotics that lacks cross- and co-resistance to the existing classes of antibiotics [3].

Interestingly, medicinal plants are considered potential sources of new antimicrobial molecules globally, and traditional herbal medicines have been used worldwide to treat various infectious diseases for thousands of years ago [8, 9]. Natural products, such as plants extracts, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries, hence, ethnopharmacology is now preferred due to the frequent development of adverse effects and microbial resistance by the chemically synthesized drugs [10, 11]. Traditional healing agents assume a central role in wound care due to their clinical efficacy, simplicity and affordability. These therapies represent a cost-effective alternative for the treatment of diverse difficult-healing wounds *e.g.* ulcers, burns, and infected wounds by providing a wide range of therapeutic effects that stimulate the healing process and improve the quality of the new skin [12]. This study focused on evaluating the *in vitro* antimicrobial activity of *Terminalia avicennioides* extracts against multidrug resistant *Staphylococcus aureus* isolated from wounds.

2. Materials and Methods

2.1. Ethical Consideration

Permission to collect patients' wound swab samples for isolation of *Staphylococcus aureus* was obtained from the research ethic committee (Reference number: HREC: 20-0004) of the Barau Dikko Teaching Hospital, Kaduna State University, Kaduna, Nigeria. Barau Dikko Teaching Hospital, Kaduna State University, Kaduna, Nigeria. Inform consent forms were administered to patients with wound infections for their consent before obtaining relevant data and wound swab samples. Prior to collection of wound swab from patients, the hospital ethical committee approval and appropriate intended research information were disseminated to the nurses of the selected hospital wards and units. A brief explanation of the aim and objectives of the research was done to enlighten the patients. Patients were also informed of their freedom to consent or decline participation. Guardian or parents of children with wound infection were requested to give assent for the children.

2.2. Collection of Wound Swabs and Isolation of *Staphylococcus aureus* from the Wound Swabs

A total of sixty wound swabs samples were collected from

in and out patients with wound at Barau Dikko Teaching Hospital Kaduna, Nigeria. Exudate or purulent or pus discharge were aseptically swabbed with sterile swab cotton tip and the cotton tip broke immediately into a sterile Brain Heart Infusion (BHI) broth in a universal bottle. The collection of the samples from the patients were carried out with the help of the hospital Nurses. The samples collected were then transported in ice packed thermo flasks to Kaduna State University Postgraduate Medical Microbiology Laboratory for isolation of *Staphylococcus aureus* isolates.

All media were prepared according to manufacturer's instructions. All clinical samples collected were cultured aerobically for isolation of *Staphylococcus aureus* in the laboratory as described by Vallis *et al.* [13] and Cheesbrough [14]. The swab samples were first cultured aerobically in an enrichment medium (Brain Heart Infusion (BHI) broth) at 37°C for 24 hours. The broth cultures from the BHI broth were then Manitol Salt agar (MSA) plates for selective isolation of *Staphylococcus aureus*. Pure culture colonies of presumptive *Staphylococcus aureus* on MSA plates were further subculture aerobically on Baird Parker agar plates at 37°C for 24 hours for morphological characteristics study of the isolates. Pure single colonies from this medium were subculture on nutrient agar slant and kept at 4°C for biochemical morphological and biochemical characterisation.

2.3. Morphological and Biochemical Characterisation of Presumptive *Staphylococcus aureus* Isolates

Biochemical characterisation of the pure isolates obtained was carried out as described by Aneja, Ochai and Kolhatkar, and Cheesbrough [14-16]. Motility, catalase, coagulase, hemolysis, citrate utilization, methyl red, Voges-Proskauer, indole, and sugars (lactose, mannitol and sucrose) fermentation test were carried out for identification of *Staphylococcus aureus* isolates.

2.4. Molecular Identification of *Staphylococcus aureus*

2.4.1. Chromosomal DNA Extraction

The DNA extraction was carried out using bioneer bacterial extraction kits (Genomic DNA extraction kits) protocols - "Bioneer accuprep genomic DNA extraction kit (K-3032).

Standard inoculum (a density of 1×10^8 cells/ml) of *Staphylococcus aureus* were prepared from 24 hours broth culture.

Two millilitre (2 ml) of the prepared standard inoculum was transferred to 5 ml sterile eppendorf tube and centrifuged for 5 min at 10,000 rpm. The supernatant was carefully discarded without disturbing the pellet. Another two millilitres (2 ml) of the standard inoculum added and centrifuged at 10,000 rpm for 5 min., followed by carefully discarding the supernatant, and repeated once again to obtain more quantity of DNA.

The pellets obtained was resuspended in 200 µl of phosphate buffer saline (PBS) in the eppendorf tube. Twenty microlitres (20 µl) of proteinase k was added to the tube

containing the pellet in PBS, followed by addition of 10 µl of RNase, then mixed thoroughly by vortexing and incubated at room temperature.

Two hundred microlitres (200 µl) of GB buffer (lysis buffer) was added to the sample and mixed by vortexing, followed by incubation at 60°C for 10 minutes using heating block.

Four hundred micro litres (400 µl) of absolute ethanol (Biological grade) was added and mixed well by pipetting, followed by careful transfer of the lysate into the upper reservoir of the binding or absorption column (fitted in the collection tube) without wetting the rim. The tube was closed and centrifuged at 8,000 rpm for 1 min. followed by discarding the solution from the collection tube and then reused the collection tube.

Five hundred micro litres (500 µl) of W₂ buffer was added without wetting the rim, followed by closing the tube and then centrifuged at 8,000 rpm for 1 minute. The solution from the collection tube was discarded and then reused the collection tube.

The sample was centrifuged once more at 13,000 rpm for 1 minute to completely removed ethanol, followed by checking to ensure that there were no droplets clinging to the bottom of the binding column tube. The binding column tube was transferred to new 1.5ml tube for elution and 100 µl of EA buffer (elution buffer) was added on to the binding column tube and then kept at room temperature (15-25°C) for 1 minute.

2.4.2. Polymerase Chain Reaction (PCR) - Accupower Hotstart PCR Premix (Bioneer)

Twenty microlitres (20 µl) reaction PCR set - up was prepared by adding; 16 µl dH₂O, 1 µl forward primer - GGACTACAGGGTATCTAAT 16S (RIBOSE-1), 1 µl reverse primer - AGAGTTTGATCCTGG 16S (RIBOSE-2), and 2 µl template DNA. PCR amplification reaction was performed using PTC 100 thermal cycler with Pre- denaturation at 95°C for 5 minutes, denaturation at 94°C for 1 minute, primer annealing at 54°C 1 minute, extension at 72°C 1 minute for 25 cycles, and final extension at 72°C 5 minutes. The PCR products were separated by electrophoresis in 1.5% agarose gel for 35 minutes at 125 volt and then visualized the gel DNA bands using UV lightbox/ gel imaging system (Biorad). Amplified PCR products were sequence and the nucleotides sequences of the 16S rRNA genes were searched for sequences similarities using online BLASTn.

2.5. Antimicrobial Susceptibility Tests Using Selected Conventional Antimicrobial Agents Used for Treatments of Wound Infections

Antimicrobial susceptibility test against *Staphylococcus aureus* isolates was carried out using Kirby-Bauer disc diffusion techniques described by Arora [17]. A loopful of 24 hours growth culture of each isolate in nutrient broth was suspended in 10ml sterile distilled water and then diluted in steps of 1:10 to give turbidity equivalent to the 0.5 McFarland standards (a density of 1x10⁸ cells/ml) before

inoculation. Sterile cotton wool swabs were dipped in the suspensions adjusted to 1x10⁸ cells/ml, the excess fluid was removed by pressing and rotating the swabs against the wall of the tubes, and then streaked on the surface of Muller Hinton agar plates. The inoculated plates were allowed to dry for about 5 minutes. Using disc dispenser, single disc Gram positive antibiotics (Oxoid); Gentamycin (10 µg), Amoxicillin- Clavulanic acid (30 µg), Nalidixic acid (30 µg), Kanamycin (30 µg), Ciprofloxacin (5 µg), Vancomycin (30 µg), Ampicillin (10 µg), Oxacillin (1 µg), Chloramphenicol (30 µg), Imipenem (10 µg), Cefoxitin (30 µg), and Sulphamethaxole (25 µg) were dispensed on inoculated plates of *Staphylococcus aureus*. After 30 minutes of applying the discs, the plates were then incubated aerobically at 37°C for 24 hours in an inverted position. Diameter of zone of growth inhibition were measured using a transparent metric ruler and the results were interpreted as either susceptible, intermediate, or resistant according to Clinical and Laboratory Standard Institute (CLSI) guidelines [18].

2.6. Collection and Authentication of Terminalia avicennioides Plant Materials

Fresh *Terminalia avicennioides* plant's parts was collected and transported for identification at the Herbarium Unit of Department of Biological Science, Faculty of Life Sciences, Ahmadu Bello University Zaria, Nigeria; where the voucher number of the plant was obtained (900239). Fresh *Terminalia avicennioides* plant's parts was collected after the authentication of the plant in large quantity and cut into small pieces and dried under shade at 30°C in a clean laboratory cabinet. The dried plant materials was first pounded in a mortar, followed by dry-milling with an electric blender and then sieved to obtained fine powder using 20 µm mesh size sieve.

2.7. Preparation of Plant Extracts

Water, acetone and ethanol were used as the extracting solvents. Twenty-five gram (25g) of the processed fine powder sample of plant was soaked in 250ml of ethanol in clean sterile 500ml conical flask and then covered the mouth of the flask with non-absorbent cotton wool followed by wrapping with aluminum foil paper. The flask was then agitated at 80 rpm for about 48 hours at 28±2°C using shaking incubator. The content was filtered first using clean muslin cloths, followed by Whatman's No. 1 filter paper. The filtrate was then evaporated using rotary evaporator to concentrate the extracts at 37°C. The same procedure was repeated with water and acetone as the extraction solvents.

2.8. Qualitative and Quantitative Phytochemical Screening

The extracts were subjected to qualitative phytochemical tests to determine the presence of saponins, tannins, phenolic compounds, anthraquinones, cardiac glycosides, alkaloids, and flavonoids, using standard procedures described by Trease and Evans, Harborne, and Sofowara [19-21]. The quantitative Phytochemical Test was also carried out for

detection of the amount of total Phenol, Flavonoids, Alkaloids, Saponins, Tannins, and terpenoids according to standard procedures described by; Harborne, AOAC, Chang *et al.*, Edeoga *et al.* and Oloyed [20, 22-24].

2.9. *In vitro* Determination of Antimicrobial Activity of the *Terminalia avicennioides* Extracts Against Multi Drug Resistant *Staphylococcus aureus* Isolates

2.9.1. Determination of Antimicrobial Potency

The antimicrobial potency of the plants extracts and AgNPs against all the multi drug resistant *Staphylococcus aureus* isolates was determined using a spread-plate and agar-well diffusion method according to Ochai and Kolhatkar [16], and Cheesbrough [14]. Zero-point eight grams of the extracts of *Terminalia avicennioides* was reconstituted in 2ml of 10% Dimethyl Sulfoxide (DMSO) in water to get a concentration of 400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml, and 25mg/ml concentrations were made from the initial concentration using a standard dilution method. Twenty millilitres (20 ml) of Sterile Muller-Hinton agar was poured into each of the petri plate and allowed to solidify on the bench. An overnight broth cultures of each pure isolate was prepared, and 0.1ml of the culture broth was added to 19.9ml sterilized distilled water, then adjusted by comparing with 0.5 Mcfarland turbidity standard (density of 1.0×10^8 cells/ml) against a light background. Sterilized cotton wool swab was dipped into the suspension, remove the excess fluid by pressing and rotating the swabs against the wall of the tubes and then streaked uniformly on the surface of Muller- Hinton culture plates. The inoculated plate was allowed to dry for 5minutes. Six millimetres (6mm) diameter cork borer was used to make wells on the inoculated culture plates and 0.2ml each of the reconstituted extracts concentrations was then loaded into the wells using sterile micropipettes. The plates were kept on the laboratory bench for 2 hours to allow the loaded extracts diffused into the culture medium. The plates were then incubated aerobically for 24 hours at 37°C. This was repeated using 1mg/ml of ciprofloxacin as positive control; and also 2% dimethyl sulphur oxide (DMSO) as negative controls. Zones of growth inhibition form around the wells were measured with a transparent meter rule and the results recorded in millimeter (mm). The antimicrobial activity was expressed as the average diameter of the zones of growth inhibition (mm).

2.9.2. Determination of Minimum Inhibitory Concentration (MIC)

The concentrations that showed antimicrobial activity from the potency test were selected for the determination of the minimum inhibitory concentrations of the solvents extracts against the multi drug resistant *Staphylococcus aureus* isolates. Zero-point eight grams of the extract of *Terminalia avicennioides* was reconstituted in 4 ml of 10% Dimethyl Sulfoxide (DMSO) in water to get a concentration of 200mg/ml. 100mg/ml, 50mg/ml, and 25mg/ml were prepared from the stock solution using Muller-Hinton broth as the diluent. An overnight broth cultures of each pure isolate was

prepared and 0.1ml of the broth culture was added to 19.9ml sterilized distilled water, then adjust by comparing with 0.5 Mcfarland turbidity standard (density of 1.0×10^8 cells/ml) in light background. Zero-point two millilitres each of the 10^8 cfu/ml isolate suspension was transferred to 2ml of each selected solvent extract concentration in tubes and gently mixed by shaking the tubes. The tubes were then incubated aerobically at 37°C for 24 hours. The lowest concentrations of the extracts which showed no visible growth was recorded as the minimum inhibitory concentrations of the extracts.

2.9.3. Determination of the Minimum Bactericidal Concentration (MBC)

For each of the test tubes in the MIC that showed no visible growth, a loopful of the broth cultures were collected from those tubes and streaked on sterile antibiotic free nutrient agar plates. The plates were incubated at 37°C for 24 hours. The concentrations at which no growth was observed were noted and recorded as the minimum bactericidal concentration (MBC) [25].

2.10. Data Analysis

Analysis of Variance (one way-ANOVA), Duncan multiple test, and independent T-test using SPSS version 23, were used for the data analyses.

3. Results

3.1. Morphological and Biochemical Characteristics of Presumptive *Staphylococcus aureus*

Presumptive *Staphylococcus aureus* colonies showed by table 1 appeared completely yellowish in colour with raised, circular and smooth edges on Manitol Salt agar (MSA). On Baird Parker agar, the colonies appeared black with shining characteristics and lytic edges. On blood agar, the colonies showed complete lysis of blood cells surrounding the colonies-characteristics of beta-hemolysis. Gram stains cell appeared purple/blueish in colour (Gram-positive characteristics) and cocci in shape, arranged in clusters (grape-like) under microscopic examination. The biochemical characteristics showed that the isolates are not motile, but catalase positive, coagulate positive, indole negative, methyl red positive, Voges-Proskauer positive, citrate utilization positive, beta-hemolytic, lactose utilization negative, mannitol utilization positive and sucrose utilization negative.

3.2. Molecular Characteristics of *Staphylococcus aureus* Isolates

Figure 1 showed the Gel electrophoresis of amplified PCR 16SrRNA genes bands of *Staphylococcus aureus* isolates respectively at 800bp of the 100 bp plus DNA marker. The sequences BLAST results (table 2) of the presumptive *Staphylococcus aureus* isolates; S1, S2 and S3 16SrRNA genes revealed the percentage identity and similarity of these isolates from the GeneBank database as 76.87%, 91.64% and 86.94% respectively, confirming the identity of these isolates

as *Staphylococcus aureus* strains.

Table 1. Morphological and Biochemical Characteristics of Presumptive *Staphylococcus aureus* Isolates.

Isolate Identification Code	Morphological Characteristics	Biochemical Characteristics	Probable Organism
	Colonial morphology on manitol salt agar (MSA) and baird parker agar and blood agar	Cellular/ microscopic morphology Gram reaction Motility Catalase coagulase Indole Methyl red Vogas-proskaver Citrat utilization Hemolysis lactose manitol Surcrose	
DR3, DR5, DR11, DR12, DR19, DR21, FSW1, FSW6, MSW2, MSW3, MSW4.	Complete yellow, raised, circular and smooth edges, and moderate colonies on MSA Black shining colonies with lysis at their edge	Cocci appeared in cluster (gape-like) or bundge with few in singles and pairs Gram positive - + + - - + ++ - + -	<i>Staphylococcus aureus</i>

Keys: + = positive, - = negative, DR = dressing room wound isolate, FSW = female surgical ward wound isolate, and MSW = male surgical ward isolate.

Table 2. BLAST Characteristics of *Staphylococcus aureus* Strains.

S/N	Sample Code	Organism	Sequence Searched Gene	Total Scores	Identity and Similarity (%)	E-Value	Query cover (%)	Sequence Searched Accession No
1.	S1	<i>Staphylococcus aureus</i>	16SrRNA	134	76.87	8e-29	44	LT6805131
2.	S2	<i>Staphylococcus aureus</i>	16SrRNA	878	91.64	0.0	99	LC429749.1
3.	S3	<i>Staphylococcus aureus</i>	16SrRNA	360	86.94	9e-94	43	LC57519.1

Key: S1 = DR₁₂, S2 = FSW₁, S3 = DR₁₁, DR = dressing room wound isolate, FSW = female surgical ward wound isolate, and MSW = male surgical ward isolate.

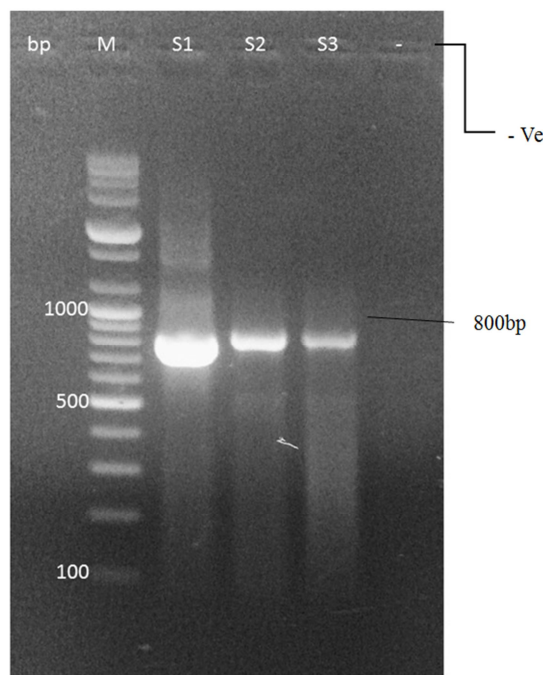


Figure 1. Gel electrophoresis of amplified PCR 16SrRNA genes bands of *Staphylococcus aureus* isolates at 800bp of the 100 bp plus DNA marker.

Key: M = 100bp DNA marker, S = *Staphylococcus aureus*, bp = base pair, - Ve = Negative Control
S1 = DR₁₂, S2 = FSW₁, S3 = DR₁₁

3.3. Antimicrobial Activity of Selected Conventional Antibiotics Against *Staphylococcus aureus* Strains

Tables 3 and 4 showed that all *Staphylococcus aureus* strains are multi-drug resistant isolates. Out of eleven *Staphylococcus aureus* isolates screened using twelve

selected conventional antibiotics, 2 (18.18%) were resistant to gentamycin, 3 (27.27%) resistant to kanamycin, 5 (45.45%) resistant to ciprofloxacin, 7 (63.64%) resistant to chloramphenicol and vancomycin, 10 (90.91%) resistant to amoxicillin-clavulanic acid and sulphamethoxazole, and 11 (100.00%) resistant to ceftazidime, ampicillin, oxacillin and cefoxitin. All 11 (100.00%) isolates were sensitive to

imipenem. The resistant pattern of *Staphylococcus aureus* isolates showed by table 4 indicated that four isolates (DR₁₉, DR₂₁, FSW₁ and FSW₆) were resistant each to 7 (58.33%) antibiotics used, five isolates (DR₃, DR₅, DR₁₁, MSW₃ and MSW₄) were resistant each to 8 (66.64%)

antibiotics used, and two isolates (DR₁₂, and MSW₂) were resistant each to 9 (75.00%) antibiotics. According to the results; imipenem, gentamycin and kanamycin were the most effective antibiotics against all the *Staphylococcus aureus* strains.

Table 3. Antimicrobial activity of Selected Conventional Antibiotics against *Staphylococcus aureus* strains.

Antibiotics	Strength	<i>Staphylococcus aureus</i> (n =11) n(%)		
		Sensitive	Intermediate	Resistant
Gentamycin	10 µg	9 (81.18)	0 (0.00)	2 (18.18)
Amoxicillin-Clavulanic acid	30 µg	1 (9.09)	0 (0.00)	10 (90.91)
Kanamycin	30 µg	7 (63.64)	1 (9.09)	10 (90.91)
Ciprofloxacin	5 µg	4 (36.36)	2 (18.18)	5 (45.45)
Vancomycin	30 µg	4 (36.36)	0 (0.00)	7 (63.64)
Ceftazidime	30 µg	0 (0.00)	0 (0.00)	11 (100.00)
Ampicillin	10 µg	0 (0.00)	0 (0.00)	11 (100.00)
Oxacillin	1 µg	0 (0.00)	0 (0.00)	11 (100.00)
Chloramphenicol	30 µg	4 (36.36)	0 (0.00)	7 (63.64)
Imipenem	10 µg	11 (0.00)	0 (0.00)	0 (0.00)
Cefoxitin	30 µg	0 (0.00)	0 (0.00)	11 (100.00)
Sulphamethoxazole	25 µg	1 (9.09)	0 (0.00)	10 (90.91)

Table 4. Susceptibility Profile of *Staphylococcus aureus* Strains against selected antibiotics.

Staphylococcus aureus	Conventional Antibiotics (n =12) n(%)		
	Sensitive	Intermediate	Resistant
DR3	4 (33.33)	0 (0.00)	8 (66.67)
DR5	4 (33.33)	0 (0.00)	8 (66.64)
DR11	3 (25.00)	1 (8.33)	8 (66.64)
DR 12	2 (16.67)	1 (8.33)	9 (75.00)
DR19	5 (41.67)	0 (0.00)	7 (58.33)
DR21	5 (41.67)	0 (0.00)	7 (58.33)
FSW1	4 (33.33)	1 (8.33)	7 (58.33)
FSW6	5 (41.67)	0 (0.00)	9 (75.00)
MSW2	3 (25.00)	0 (0.00)	9 (75.00)
MSW3	4 (33.33)	0 (0.00)	8 (66.64)
MSW4	4 (33.33)	0 (0.00)	8 (66.64)

Key:, DR = dressing room wound isolate, FSW = female surgical ward wound isolate, and MSW = male surgical ward isolate.

Table 5. Percentage Extracts Yield of *Terminalia avicennioides*.

S/N	Extract Category	Mean±SD Extract Yield (%)	P-value at $\alpha = 0.05$	Comment
1	Leave, Stem and Root Bark Extracts			
	Leaves	5.19 ±1.61 ^b	0.0052 (P< 0.05)	The percentage extracts yield based on plant extracts showed significant difference. Stem and root bark extract showed higher percentage yield compared to leave extracts
	Stem bark	15.98 ± 3.95 ^a		
	Root bark	13.28 ± 3.75 ^a		
2	Acetone, Ethanol and Aqueous Extracts			
	Acetone	11.95±6.90 ^a	0.5209 (P> 0.05)	Percentage extracts yield based on extracting solvents showed no significant difference.
	Ethanol	14.09 ± 6.42 ^a		
	Aqueous	8.40 ±3.66 ^a		

Table 6. Phytochemical Characteristics of Root Barks, Stem Barks and Leave Extracts of *Terminalia avicennioides*.

S/No	<i>Terminalia avicennioides</i> Plant Part	Type of Solvent Extract	Phytochemical Characteristics							
			Alkaloids	Flavonoids	Tannins	Saponins	Cardiac glycosides	Phenols	Anthraquinones	Terpenoids
1.	Root Barks	Ethanol	+	+	+	+	+	+	-	+
		Acetone	-	+	+	+	-	+	-	-
		Aqueous	-	+	+	+	+	+	-	-
2.	Stem Barks	Ethanol	+	+	+	+	+	+	-	-
		Acetone	+	+	+	+	+	+	-	+
		Aqueous	+	+	+	+	+	+	-	-
3.	Leaves	Ethanol	-	+	+	+	-	+	-	+
		Acetone	-	+	+	+	-	+	-	+
		Ethanol	-	+	+	+	+	+	-	+

Key: + = Positive; - = Negative.

3.4. Percentage Extract Yield of *Terminalia avicennoides*

The extracts from *Terminalia avicennoides* were obtained from dried processed powdered of stem bark, root bark and leaves using three extracting solvent; ethanol, acetone and water (Table 5). The percentage extracts yield based on plant parts showed significant difference ($P < 0.05$). The percentage extracts

yields ranged from 5.19 ± 1.61 – $15.98 \pm 3.95\%$, with stem bark extracts having high percentage yield ($15.98 \pm 3.94\%$). Based on extracting solvents, percentage extracts yield showed no significant difference ($P > 0.05$) and percentage extracts yield ranged from 8.40 ± 3.66 - $14.09 \pm 6.42\%$, with ethanol stem bark having high percentage yield ($14.09 \pm 6.42\%$).

Table 7. Quantitative Phytochemical Analysis of Root Bark, Stem Bark, and Leave Extracts of *Terminalia avicennoides*.

	T. Phenols (mg/100g)	Flavonoids (mg/100g)	Tannins (mg/100g)	Terpenoids (mg/100g)	Saponins (µg/g)	Alkaloids (mg/100g)
EET L	176.00 ± 10.50	84.00 ± 3.30	120.00 ± 2.60	887.00 ± 4.20	24.31 ± 0.76	129.52 ± 1.96
EET RB	273.00 ± 10.70	84.40 ± 1.30	102.00 ± 1.50	68.00 ± 1.20	47.27 ± 1.72	298.33 ± 1.12
EET SB	123.00 ± 20.80	88.00 ± 4.20	89.00 ± 11.00	Not Detected	45.93 ± 2.20	122.48 ± 4.96
AQET L	362.00 ± 20.10	77.00 ± 8.10	104.00 ± 1.60	35.00 ± 1.70	19.90 ± 1.02	312.43 ± 0.96
AQET RB	34.00 ± 10.12	100.00 ± 13.00	83.00 ± 3.70	Not Detected	37.35 ± 3.14	236.40 ± 0.48
AQET SB	540.00 ± 20.10	111.00 ± 10.00	112.00 ± 10.00	Not Detected	22.72 ± 1.31	275.28 ± 1.48
AET L	2331.00 ± 23.00	106.00 ± 4.30	114.00 ± 3.50	388.00 ± 3.00	37.76 ± 3.20	131.73 ± 1.21
AET RB	96.00 ± 10.10	104.00 ± 13.00	91.00 ± 3.60	Not Detected	37.79 ± 2.30	127.60 ± 0.72
AET SB	1660.00 ± 12.00	104.00 ± 17.00	108.00 ± 3.50	56.00 ± 3.00	45.22 ± 4.21	323.82 ± 3.12

Key:

EET L: Ethanol Extract *Terminalia* Leaves; EET RB: Ethanol Extract *Terminalia* Root Back;

EET SB: Ethanol Extract *Terminalia* Stem Back; QET L: Aquoues Extract *Terminalia* Leaves;

AQET RB: Aquoues Extract *Terminalia* Root Back; AQET SB: Aquoues Extract *Terminalia* Stem Back;

AET L: Acetone Extract *Terminalia* Leaves; AET RB: Acetone Extract *Terminalia* Root Back;

AET SB: Acetone Extract *Terminalia* Stem Back.

3.5. Qualitative and Quantitative Phytochemical Characteristics of Root Barks, Stem Bark and Leaves Extract of *Terminalia avicennoides*

Table 6 showed the presence of flavonoids, tannins, saponins and phenol in all the root bark, stem bark and leaves extracts obtained using both ethanol, acetone and water solvents. Alkaloids was detected only in ethanolic extracts of root bark, stem bark and also acetone aqueous stem bark

extracts. Cardiac glycoside was detected only in all stem bark, ethanolic and aqueous root bark extracts and also ethanolic leaves extracts. Terpenoids was present in all leave extracts acetone stem bark and ethanol root bark extracts. Anithroquinone was not detected in all the extracts. The quantitative analysis (Table 7) showed that the extracts generally had higher phenol content (2331 – $34\text{mg}/100\text{g}$), followed by terpenoids (887 – $35\text{mg}/100\text{g}$), and then Saponins (47.27 – $22.72 \mu\text{g}/\text{g}$) as the lowest.



Zone of growth inhibition produce by *Terminalia avicennoides* Extracts

Figure 2. Showing zone of growth inhibition of *Terminalia avicennoides* extract.

Table 8. Antimicrobial Activity of *Terminalia avicennioides* Extracts Against Multidrug Resistant *Staphylococcus aureus* Strains.

Organism	Variably	Mean \pm SD zone of growth inhibition (mm)	P-value at $\alpha = 0.05$	interpretation
<i>Staphylococcus aureus</i> Strains (DR ₃ , DR ₅ , DR ₁₁ , DR ₁₂ , DR ₁₉ , DR ₂₁ , FSW ₁ , FSW ₆ , MSW ₂ , MSW ₄)	Leaves, Stem and Root Bark Activity			
	AETL	16.45 \pm 11.38 ^c	0.0003 (P < 0.05)	Generally, zone of growth inhibition showed significant difference.
	EETL	18.56 \pm 11.63 ^{bc}		
	AQTL	17.02 \pm 10.92 ^c		
	AETSB	23.38 \pm 5.98 ^{ab}	-	
	EETSB	16.28 \pm 10.45 ^c		
	AQTSB	20.86 \pm 6.38 ^{abc}		
	AETRB	23.81 \pm 6.69 ^a	-	
	EETRB	23.25 \pm 6.51 ^a		
	AQTRB	21.00 \pm 6.99 ^{abc}		
	Plant Parts Extracts Activity			
	Leaves	17.34 \pm 11.24 ^b	0.0003 (P < 0.05)	There was a significant difference between leave, stem and root bark activity. Stem and root bark extracts showed larger zone of growth inhibition compared to leave extracts
	Stem bark	20.17 \pm 8.33 ^a		
	Root bark	22.69 \pm 6.77 ^a		
	Concentration (mg/ml)			
	200	25.21 \pm 3.45 ^a	0.0364 (P < 0.05)	There was significant difference between the zone of growth inhibition for four concentrations tested against the organisms. 200mg/ml, 100mg/ml showed larger zone of growth inhibition compared to 50mg/ml and 25mg/ml activity
	100	24.71 \pm 5.34 ^a		
	50	23.15 \pm 7.00 ^{ab}		
	25	21.15 \pm 4.37 ^a		

Key:: DR = dressing room wound isolate, FSW = female surgical ward wound isolate, and MSW = male surgical ward isolate, EETL = ethanol *Terminalia avicennioides* Leave extract, AETL = acetone *Terminalia avicennioides* Leave extract, AQTL = Aqueous *Terminalia avicennioides* Leave extract, EETSB = ethanol *Terminalia avicennioides* stem bark extract, AETSB = acetone *Terminalia avicennioides* stem bark extract, AQTSB = aqueous *Terminalia avicennioides* stem bark extract, EETRB = ethanol *Terminalia avicennioides* root bark extract, AETRB = acetone *Terminalia avicennioides* root bark extract, and AQTRB = aqueous *Terminalia avicennioides* root bark extract.

Table 9. Antimicrobial Activity of *Terminalia avicennioides* Extracts Against Multidrug Resistant *Staphylococcus aureus* Strains.

Organism	Variable	Mean \pm SD zone of growth inhibition (mm)	P-value at $\alpha = 0.05$	interpretation
DR ₃ , DR ₅ , DR ₁₁ , DR ₁₂ , DR ₁₉ , DR ₂₁ , FSW ₁ , FSW ₆ , MSW ₂ , MSW ₄ , MSW ₄	Leave Extracts Activity			
	AETL	16.45 \pm 11.39 ^a	0.7431 (P > 0.05)	There was no significant different between the activity of acetone, ethanol and aqueous extracts
	EETL	18.56 \pm 11.64 ^a		
	AQTL	17.02 \pm 10.92 ^a		
	Concentration (mg/ml)			
	200	21.81 \pm 12.93 ^a	0.0298 (P < 0.05)	There was significant different between activity at 200mg/ml and 100ml, and 50mg/ml and 25mg/ml. Higher activity was recorded at 200mg/ml and 100mg/ml compare to 50mg/ml and 25mg/ml
	100	19.00 \pm 11.63 ^a		
	50	15.81 \pm 9.89 ^{ab}		
	25	12.75 \pm 8.68 ^a		
	Stem Bark Extract Activity			
	AETSB	23.38 \pm 5.98 ^a	0.0019 (P < 0.05)	There was significant different between acetone, aqueous extract, and ethanol extract activity. Acetone and aqueous extracts showed larger zones compared to ethanol extract activity.
	EETSB	16.28 \pm 10.45 ^b		
	AQTSB	20.85 \pm 6.38 ^a		
	Concentration (mg/ml)			
	200	24.63 \pm 8.39 ^a	0.0003 (p < 0.05)	Three was a significant difference between the extracts activity for all the concentrations, with larger zone of growth inhibition at 200mg/ml, followed by 100mg/ml, 50mg/ml and then 25mg/ml
	100	22.00 \pm 7.67 ^{ab}		
	50	18.88 \pm 7.23 ^{bc}		
	25	15.19 \pm 7.27 ^c		
	Root Bark Extracts Activity			
	AETRB	23.81 \pm 6.69 ^a	0.2146 (p < 0.05)	There was no significant difference between both acetone, ethanol, and aqueous extracts
	EETRB	23.25 \pm 6.51 ^a		
	AQTRB	21.00 \pm 6.99 ^a		
	Concentration (mg/ml)			
	200	28.29 \pm 4.14 ^a	0.0001 (p < 0.05)	Three was a significant difference between the extracts activity for all the concentrations, with larger zone of growth inhibition at 200mg/ml, followed by 100mg/ml, 50mg/ml and then 25mg/ml
	100	25.10 \pm 4.49 ^b		
	50	20.96 \pm 5.53 ^c		
	25	16.39 \pm 6.17 ^d		

Key:: DR = dressing room wound isolate, FSW = female surgical ward wound isolate, and MSW = male surgical ward isolate, EETL = ethanol *Terminalia avicennioides* Leave extract, AETL = acetone *Terminalia avicennioides* Leave extract, AQTL = Aqueous *Terminalia avicennioides* Leave extract, EETSB = ethanol *Terminalia avicennioides* stem bark extract, AETSB = acetone *Terminalia avicennioides* stem bark extract, AQTSB = aqueous *Terminalia avicennioides* stem bark extract, EETRB = ethanol *Terminalia avicennioides* root bark extract, AETRB = acetone *Terminalia avicennioides* root bark extract, and AQTRB = aqueous *Terminalia avicennioides* root bark extract.

3.6. Antimicrobial Activity of *Terminalia avicennoides* Extracts Against Multi drug Resistant *Staphylococcus aureus* Strains

Figure 2 showed the zone of growth inhibition produced by the activity of *Terminalia avicennoides* extracts. Antimicrobial activity of *Terminalia avicennoides* extracts against multidrug resistant *Staphylococcus aureus* isolate result in tables 8 and 9 showed in vitro activity of the

acetone, ethanol and aqueous extracts of stem bark, root bark and leave extracts as zone of growth inhibition in millimeter for four varying concentrations: 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml. The zone of growth inhibition ranged from 16.28 ± 10.45 – 23.81 ± 6.69 mm and showed significant difference ($P < 0.05$), with acetone root and stem bark, ethanol root bark and aqueous leave extracts showing larger zone of growth inhibition.

Table 10. Minimum inhibitory Concentration (MIC) of *Terminalia avicennoides* Extracts against Multidrug Resistant *Staphylococcus aureus* strains.

Organism	Variable	Mean \pm SD MIC (mg/ml)	P-value at $\alpha = 0.05$	Interpretation
<i>Staphylococcus aureus</i> strains (DR ₃ , DR ₅ , DR ₁₁ , DR ₁₂ , DR ₁₉ , DR ₂₁ , FSW ₁ , FSW ₆ , MSW ₂ , MSW ₃ , MSW ₄)	Leave, Stem and Root Bark Extracts Activity			
	AETL	31.25 ± 22.16^a	0.7804 ($P > 0.05$)	Generally, there was no significant difference between the MIC for all the extracts irrespective of the parts of the plant and type of the extracting solvents extracts tested against all the multidrug resistant <i>Staphylococcus aureus</i> strains.
	EETL	37.50 ± 32.73^a		
	AQTL	43.75 ± 32.04^a		
	AETSB	56.25 ± 29.12^a		
	EETSB	43.75 ± 39.52^a		
	AQTSB	53.12 ± 31.16^a		
	AETRB	43.75 ± 25.87^a		
	EETRB	43.75 ± 11.57^a		
	AQTRB	53.12 ± 31.16^a		
	Plant Parts Extracts Activity			
	Leaves	37.50 ± 28.55^a	0.2480 ($P > 0.05$)	No Significant difference between the MIC for the leave, stem and root bark extracts activity against all the bacterial strains
	Stem bark	51.04 ± 32.54^a		
	Root bark	46.87 ± 23.67^a		
	Leave Extracts Activity			
	AETL	31.25 ± 22.16^a	0.7005 ($P > 0.05$)	No Significant difference between the MIC for the acetone, ethanol and aqueous leave extracts activity against all the bacterial strains
	EETL	37.50 ± 32.73^a		
	AETL	43.75 ± 32.04^a		
	Stem Bark Extracts Activity			
	AETSB	56.25 ± 29.12^a	0.7437 ($P > 0.05$)	No Significant difference between the MIC for the acetone, ethanol and aqueous stem bark extracts activity against all the bacterial strains
	EETSB	43.75 ± 39.53^a		
	AQTSB	53.13 ± 31.16^a		
	Root Bark Extracts Activity			
	AETRB	43.75 ± 25.87^a	0.6778 ($P > 0.05$)	Significant difference between the MIC for the acetone, ethanol and aqueous stem bark extracts activity against all the bacterial strains
	EETRB	43.75 ± 11.57^a		
	AQTRB	53.12 ± 31.16^a		

Key:: DR = dressing room wound isolate, FSW = female surgical ward wound isolate, and MSW = male surgical ward isolate, EETL = ethanol *Terminalia avicennoides* Leave extract, AETL = acetone *Terminalia avicennoides* Leave extract, AQTL = Aqueous *Terminalia avicennoides* Leave extract, EETSB = ethanol *Terminalia avicennoides* stem bark extract, AETSB = acetone *Terminalia avicennoides* stem bark extract, AQTSB = aqueous *Terminalia avicennoides* stem bark extract, EETRB = ethanol *Terminalia avicennoides* root bark extract, AETRB = acetone *Terminalia avicennoides* root bark extract, and AQTRB = aqueous *Terminalia avicennoides* root bark extract.

3.7. Minimum Inhibitory Concentration (MIC) of *Terminalia avicennoides* Extracts Against Multi Drug Resistant *Staphylococcus aureus* Strains

As presented in table 10 the MIC of leave, stem and root bark extracts for all types of solvent extracts tested against multi drug resistant *Staphylococcus aureus* isolate strains ranged from 56.25 ± 29.12 – 31.25 ± 22.16 mg/ml and showed no significant difference ($P > 0.05$). However, acetone extracts showed higher MIC value of 31.25 ± 22.16 mg/ml, and acetone stem bark extracts showed the lower MIC values of 56.25 ± 29.12 mg/ml.

3.8. Minimum Bactericidal Concentration (MBC) of *Terminalia avicennoides* Extracts Against Multidrug Resistant *Staphylococcus aureus* Strains

As presented in table 11, the MBC of leave, stem and root bark extracts for all types of solvent extracts tested against multi drug resistant *Staphylococcus aureus* isolate strains ranged from 175.00 ± 46.29 – 68.75 ± 45.81 mg/ml and showed no significant difference ($P > 0.05$). However, acetone leave extracts showed higher MBC (68.75 ± 45.81 mg/ml), and aqueous stem bark extracts showed the lower MBC values of 175.00 ± 46.29 mg/ml.

Table 11. Minimum Bactericidal Concentration (MBC) of *Terminalia avicennioides* Extracts against Multidrug Resistant *Staphylococcus aureus*.

Organism	Variable	Mean \pm SD MBC (mg/ml)	P-value at $\alpha = 0.05$	Interpretation
<i>Staphylococcus aureus</i> Strains (DR ₃ , DR ₅ , DR ₁₁ , DR ₁₂ , DR ₁₉ , DDR ₂₁ , FSW ₁ , FSW ₆ , MSW ₂ , MSW ₃ , MSW ₄)	Leave, Stem and Root Bark Extracts Activity			
	AETL	68.75 \pm 45.81 ^b	0.0388 (P < 0.05)	The MBC showed significant difference. However, acetone stem bark and ethanol root bark showed lower MBC compared to other extracts.
	EETL	75.00 \pm 46.29 ^b		
	AQTL	125.00 \pm 88.64 ^{ab}		
	AETSB	112.500 \pm 64.09 ^{ab}		
	EETSB	106.25 \pm 86.34 ^b		
	AQTSB	175.00 \pm 46.29 ^a		
	AETRB	115.00 \pm 45.28 ^{ab}		
	EETRB	125.00 \pm 46.29 ^{ab}		
	AQTRB	106.25 \pm 57.37 ^b		
	Plant Parts Extracts Activity			
	Leaves	89.58 \pm 65.90 ^b	0.0872 (p > 0.05)	MBC showed no significant difference. However, leave extracts showed higher MBC compared to stem and root bark extracts.
	Stem bark	131.25 \pm 71.95 ^a		
	Root bark	112.50 \pm 53.67 ^a		
	Leave Extracts Activity			
	AETL	68.75 \pm 45.81 ^b	0.1765 (p > 0.05)	MBC showed no significant different. However, acetone extracts showed higher MBC compared to stem and root bark extracts.
	EETL	75.00 \pm 46.29 ^a		
	AQTL	125.00 \pm 88.64 ^a		
	Stem Bark Extracts Activity			
	AETSB	112.50 \pm 64.09 ^a	0.1036 (p > 0.05)	MBC showed no significant different.
	EETSB	106.25 \pm 86.34 ^a		
	AQTSB	175.00 \pm 46.29 ^a		
	Root Bark Extracts Activity			
	AETRB	115.00 \pm 45.28 ^a	0.4320 (p > 0.05)	MBC showed no significant different.
	EETRB	125.00 \pm 46.91 ^a		
	AQTRB	106.25 \pm 57.37 ^a		

Key: DR = dressing room wound isolate, FSW = female surgical ward wound isolate, and MSW = male surgical ward isolate, EETL = ethanol *Terminalia avicennioides* Leave extract, AETL = acetone *Terminalia avicennioides* Leave extract, AQTL = aqueous *Terminalia avicennioides* Leave extract, EETSB = ethanol *Terminalia avicennioides* stem bark extract, AETSB = acetone *Terminalia avicennioides* stem bark extract, AQTSB = aqueous *Terminalia avicennioides* stem bark extract, EETRB = ethanol *Terminalia avicennioides* root bark extract, AETRB = acetone *Terminalia avicennioides* root bark extract, and AQTRB = aqueous *Terminalia avicennioides* root bark extract.

4. Discussion

This study isolated and identified *Staphylococcus aureus* strains from wound infected patients using both phenotypic and genotypic approaches. Cultural morphology of *Staphylococcus aureus* on mannitol salt Agar (MSA) as yellow, with flat and moderate shape. The production of yellow colonies on MSA has been reported by Fitzgerald to be as a result of fermentation of mannitol salt with consequent production of acid [26]. On Baird Parker medium, *Staphylococcus aureus* showed grey-black shining colonies with opaque halo surrounded by zone of clearing. Silva *et al.* reported similar characteristics of *Staphylococcus aureus* on Baird parker medium, where it was reported that the formation of grey black shining colonies is due to reduction of potassium tellurite and the proteolytic activity through breaking down of egg yolk by Lecithinase causing clear zone around respective colonies, while the opaque halo surrounding zone of clearing is as a result of Lipase activity [27]. The gram stain cell revealed a characteristic of Gram positive cocci which appeared in grape-like (cluster) under microscopic examination using x100 objective lens. Tong *et al.* reported similar cellular appearance of *Staphylococcus aureus* [28] The biochemical characteristic showed that this organism is catalase and coagulase positive with

characteristic production of beta-hemolysis on blood agar – a unique characteristic for phenotypic identification of pathogenic *Staphylococcus aureus* strains. Studies have reported that *Staphylococcus aureus* isolated from human have bound and free form of coagulase [16], with characteristic formation of beta-haemolysis on blood agar. The presence of the enzyme coagulase is phenotypically employed to differentiate between the strain of virulent and less virulent *Staphylococcus aureus*.

The phenotypic identification approach in this study generally revealed cultural and biochemical characteristics related to *Staphylococcus aureus* isolates. However, due to the need for ethnobotanical studies to be conducted on pathogen-specific wound infection in this study with the selection of the organisms related directly to the reported traditional use of the plant *Terminalia avicennioides*, it became imperative to characterise the *Staphylococcus aureus* using molecular identification methods. This is for reproducibility of studies according to Vanvuuren [5]. The molecular identification was employed to compare the genetic similarities of the *Staphylococcus aureus* isolated from wound infections with Genbank database according to Prescott *et al.* [29]. The results of the molecular analysis in this study showed the gel electrophoresis of amplified PCR 16S rRNA genes bands of *Staphylococcus aureus* isolates at 800bp of the 100 bp plus DNA marker. The sequences BLAST results of the presumptive *Staphylococcus aureus*

isolates; S1, S2 and S3 16SrRNA genes revealed the percentage identity and similarity of these isolates to those from the GeneBank database as 76.87%, 91.64% and 86.94% respectively, confirming the identity of these isolates as *Staphylococcus aureus* strains.

Generally, the percentage identity and similarity revealed by the sequences BLAST results for all the *Staphylococcus aureus* strains ranged from 76.87%, -997.67% and Prescott *et al.* reported that since 1970s, it has been widely accepted that Prokaryotes whose genomes are at least 70% homologous belongs to the same species [5]. This support the confirmation of identity of these isolates as *Staphylococcus aureus* in this study.

The findings in this study revealed all the *Staphylococcus aureus* as multi drug resistant isolates. According to the results (3 and 4), imipenem was revealed to be the most potent antibiotic against the *Staphylococcus aureus* isolates followed by Gentamycin, because all the isolates were sensitive to these antibiotics. This means that imipenem must be carefully prescribed by clinicians to avoid development of resistance by the organism. Also, sensitivity result should always be used as the basis for the prescription of these drugs to patients. There is also need to educate clinicians on this finding and the public health importance. Findings from this study are similar to that of susceptibility profile studied of *Staphylococcus aureus* by Rashedul *et al.* who reported imipenem as the most potent antibiotic with 90% sensitivity, and with 75% isolates also showing resistance to oxacillin, methicillin, ciprofloxacin and tetracycline [30]. Kitara *et al.*, and Brown and Ngeno reported that *Staphylococcus aureus* is capable of producing many antibiotic resistant strains and that this organism have the ability to acquire resistance to many antibiotics [31, 32]. Brown and Ngeno also stated that *Staphylococcus aureus* resistance to antibiotics is a worldwide problem.

This study reported *Staphylococcus aureus* isolates to be resistant to chloramphenicol similar to findings by Rashedul *et al.* who reported *Staphylococcus aureus* isolates resistant to chloramphenicol [30]. Also, this study reported multi drug resistance exhibited by *Staphylococcus aureus* to ceftazidime similar to report by Aisha *et al.* [33]. Moreso, as Rashedul *et al.* studied reported that only 4 (36.63%) *Staphylococcus aureus* showed sensitivity to vancomycin and that it is considered as a serious threat to clinical setting [30], also, this current study reported vancomycin resistance to *Staphylococcus aureus*. The vancomycin resistance by *Staphylococcus aureus* isolates in this study indicated that the strains of this bacteria pathogens may presently be a serious problem to successful treatment of wound infections and may be an additional problem to the health system especially at the community level. According to Khan *et al.* and Juayan *et al.*, vancomycin resistant *Staphylococcus aureus* (VRSA) is currently one of the great threats mankind faces because the antibiotic, vancomycin is the last resort for treating Staphylococcal infections [34, 35].

Benjamin and Christopher recommended tetracycline, chloramphenicol and Gentamycin for treatment of wound infection cause by *Staphylococcus aureus* [36]. Similarly,

Bowler *et al.*, recommended Gentamycin, Vancomycin, Cefoxitin and imipenem for effective treatment of wound infection [37]. Findings from this study showed Imipenem, Gentamycin and Ciprofloxacin to be the most potent antibiotics indicating that they are still effective as recommended. Other recommended antibiotics were not potent to the tested bacterial isolates contrarily to the previous researches findings that recommended them. Moreso, Aisha *et al.* reported multidrug resistant *Staphylococcus aureus* against gentamycin, imipenem and ciprofloxacin antibiotics [38], while in this study the *Staphylococcus aureus* strains were sensitive to these antibiotics. The inconsistency might be due to some factors such as; bacterial acquisition of resistance genes, mutations, environmental changes factors, efflux pump mechanism, biofilm formation, possession of beta-lactamase, among others. This indicated the need for an alternative drug for effective therapy of bacterial wound infections, due to failure of the existing antibiotics.

In this study, extracts from *Terminalia avicennoides* were obtained from dried processed powdered of stem bark, root bark and leaves using three extracting solvent; ethanol, acetone and water. The percentage extracts yields ranged from 5.19 ± 1.61 – $15.98 \pm 3.95\%$ and showed significant difference ($P < 0.05$). Based on extracting solvents, the percentage extracts yield ranged from 8.40 ± 3.66 – $14.09 \pm 6.42\%$ and showed no significant difference ($P > 0.05$). The variations in percentage yield could be due to the used of different solvents for the extraction. Mule *et al.* reported that types of solvents used for extraction have some effect on the nature of the compound extracted and it implies that extracting solvent polarity (non-polar, polar and less polar) plays crucial role on the types of bioactive compounds of plants that can be extracted from plant parts [39]. This study reported low percentage extracts yield with aqueous solvent compared to acetone and ethanol solvent. Afolayan *et al.* reported that most active antimicrobials components are generally insoluble in universal polar solvent (water) [40] and hence, it is expected that organic polar solvents such as ethanol and acetone would yield more active antimicrobial extracts. This may be attributed to the higher percentage extract yield from ethanol and acetone compared to water extracts in this study.

The qualitative and quantitative phytochemical analysis of *Terminalia avicennoides* extracts in this study showed the presence of tannins, alkaloids, flavonoids, cardiac glycosides, phenolic compounds, terpenoids and saponins. Anthroquinones was not detected from all the category of the plant extracts. The quantitative analysis showed that the extracts generally had higher phenol content (2331-34mg/100g), followed by terpenoids (887-35mg/100g), and the Saponins (47.27-22.72 $\mu\text{g/g}$) as the lowest. Odebumin *et al.* and Alaje *et al.* reported similar findings [41, 42]. Also, in previous studies of biochemical compounds of medicinal plant, Irshad *et al.* reported that most chemical constituents of plant contain many bioactive compounds including alkaloids, tannins, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones [43]. Radhika *et al.* also stated that the most important of these bioactive compounds

are the alkaloids, tannins, saponins, flavonoids and phenolic compounds [44]. According to Cragg and Newman, the presence of important phytochemical constituents is the bioactive bases for plant medicinal properties as these secondary metabolites are the chemical substances used by the plants for defense system and serve as bioactive principles for various drugs and modern therapy [45].

The important phytochemical constituents like steroids, tannins and saponins have been detected in *Terminilia avicennooides* plant parts [46], and the presence of these compound is known to confer antibacterial activity against bacteria pathogens [47]. To confer antibacterial activity of plant, flavonoids has been reported to be singly responsible for antibacterial activity associated with some ethnomedicinal plant [48]. It has also been reported that plants that are rich in tannins or phenolics compounds are inhibitory to wide range of bacteria, thus capable of conferring protection against some microbial infections [49]. The presence of the various phytochemical compounds is an indication that *Terminilia avicennooides* have potent antiseptic, bactericidal and other medicinal properties. This is due to the fact that each of the compounds identified has one or more therapeutic usage and may be acting singly or in consortium to bring about cidal or static effect on the organism. Thus, the presence of the phytochemical compound recorded in this study could be responsible for *in vitro* antibacterial activity.

The *in vitro* antimicrobial activity of the various *Terminilia avicennooides* extracts against multi drug resistant *Staphylococcus aureus* showed zone of growth inhibition on the various concentrations, extracting solvents and parts of the plant. Antibacterial activity was shown by an inhibitory activity characterized by a cleared zone between the wells (containing the samples) and certain distance. Formation of inhibitory zones around the wells shows bacterial sensitivity to the extracts. The antimicrobial activity of *Terminalia avicennioides* extracts against multidrug resistant *Staphylococcus aureus* isolates showed *in vitro* antimicrobial activity of the acetone, ethanol and aqueous extracts of stem bark, root bark and leave extracts as zone of growth inhibition in millimeter for four varying concentrations: 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml. The zone of growth inhibition ranged from 16.28 ± 10.45 – 23.81 ± 6.69 mm and showed significant difference ($P < 0.05$), with acetone root and stem bark, ethanol root bark and aqueous leave extracts showing larger zone of growth inhibition in comparison to others. Udgire and Pathade suggested that plant extracts exhibiting inhibitory zones diameter greater than or equal to 10 mm and above against selected microbial pathogens should be considered to possess antimicrobial activity, whereas, those showing inhibitory zones greater than 20 mm against selected microbial pathogens should be considered noteworthy [50]. The level of the extracts *in vitro* antibacterial activity against the multi drug resistant *Staphylococcus aureus* isolates revealed the presence of the important bioactive ingredients, the strength concentrations of these ingredients and their capacity to diffuse into the agar medium. In this study, the

zone of inhibition of the extracts increases as the extract concentration increases, thus, the linear relationship between the concentrations of the extract zone of inhibition could be that the extracts used were able to diffuse into the inoculated nutrient agar. This however, may explain why even though there were cleared zone of growth inhibition for some extracts against some bacteria strains, there were also no detectable zone for different solvents extracts and different extracts concentration against different bacteria isolates.

Several studies have attributed the antibacterial and therapeutic activities of *Terminilia avicennooides* extracts to the presence of flavonoids and a mixture of phenolic compounds and tannins [51]. The phenolic compounds are said to act as protoplasmic poison which penetrate and disrupt bacterial cell wall in addition to precipitation of cell proteins. More so, it has been confirmed that secondary metabolites such as alkaloids and tannins inhibit enzymes and protein synthesis, while glycosides are antidiarrhea [52]. The *Terminilia avicennooides* extracts were found to be active against the *Staphylococcus aureus* strains with greater inhibitory activity at concentration of 200 mg/ml and 100 mg/ml and this is similar to findings by Shedidi [53]. The present study revealed that the *Terminalia avicennioides* extracts showed potent antibacterial activity against the bacterial strains. This implies that the *in vitro* antimicrobial activity of the *Terminilia avicennooides* extracts recorded in this study was due to availability of the plant secondary metabolites required for antibacterial activity. The ability of the extracts of *Terminilia avicennooides* to inhibit the growth of the multi drug resistant *Staphylococcus aureus* explains why it is been effectively used in folk medicine for treatment of wound infection. The *Terminalia avicennioides* is the most widely used plants for traditional medicinal purposes worldwide including wound healing. It is known for local used in form of; leaf and root bark medicine, pain killer root bark medicine, and skin and mucosae root bark medicine [53, 54]. Because of its potential antimicrobial activity, it is harvested locally and used for treatment of burn and wound infection. The pulverized leaves are used in Northern Nigeria on burns and bruises. In north Eastern Nigeria, the Jukun in Taraba state use the roots in treatment of syphilis. The root bark is made into a decoction along with other medicinal plants by the Baule of Ivory coast for severe jaundice and non-healing old sores. In Casamance of Senegal, the root bark is considered cleasing and healing on refractory sores. The powdered root bark is applied topically to sores and ulcers and is rubbed on the gums of toothache in Ivory Coast. The root bark is being used for treatment of skin infection and separate examination of antimicrobial activity against *Sarcina lutea*, *Staphylococcus aureus*, *Mycobacterium phlei*, and some Gram positive organisms [53, 54]. It can therefore, be deduced from the result obtained in this study that *Terminilia avicennooides* is a source of bioactive compounds with potential therapeutic benefit, because it portrays a good inhibitory effect against the multi drug resistant *Staphylococcus aureus*.

The *Terminilia avicennooides* extracts showed MIC values

at different concentration depending on extracting solvent and parts of the plants. The MIC of the plant extracts tested against multi drug resistant *Staphylococcus aureus* isolate strains in this study ranged from $56.25 \pm 29.12 - 31.25 \pm 22.16$ mg/ml, and showed no significant difference ($P > 0.05$) with acetone extracts having higher MIC value of 31.25 ± 22.16 mg/ml, and acetone stem bark extracts showed the lower MIC values of 56.25 ± 29.12 mg/ml. Similarly, the MBC of the extracts tested against multi drug resistant *Staphylococcus aureus* isolate strains ranged from $175.00 \pm 46.29 - 68.75 \pm 45.81$ mg/ml and showed no significant difference ($P > 0.05$) with acetone leave extracts having higher MBC (68.75 ± 45.81 mg/ml), and aqueous stem bark extracts having the lower MBC values of 175.00 ± 46.29 mg/ml. These values represent the in vitro bacteriostatic and bactericidal concentrations of these crude extracts against the multi drug resistant *Staphylococcus aureus* strains. The high concentrations of the secondary metabolites such as tannins, alkaloids, flavonoids, saponins, terpenoids, cardiac glycosides, among others in this plant extracts could be attributed to the high antimicrobial activity recorded in this study. The findings are indicative of the various efficacy levels of *Terminalia avicennoides* extracts that can be enhanced by further separation, purification and concentration of the bioactive compounds of the plants.

5. Conclusion

Staphylococcus aureus strains were isolated from wounds of in - and out - patients attending Barau Dikko Teaching Hospital Kaduna, Nigeria. All the *Staphylococcus aureus* were multidrug resistant strains. Out of all the antimicrobial agents used, only imipenem, ciprofloxacin, kanamycin and gentamycin were effective antibiotics against these wound pathogens. The *Terminalia avicennoides* extracts contain significant phytochemical compounds requires to exert bacteriostatic and bactericidal effects, and hence, exhibit noteworthy antimicrobial activity against the multidrug resistant *Staphylococcus aureus* strains. The efficacy of the *Terminalia avicennoides* extracts against the multidrug resistant *Staphylococcus aureus* strains indicated that this plant extracts can be used to produce therapeutic agent for effective treatment of multidrug resistant bacterial wound infections. However, exhaustive studies involving isolation and concentration of the specific bioactive or inhibitory compounds active against the multidrug resistant *Staphylococcus aureus* strains is required.

Declarations

Competing Interests

All authors have no conflict of interest to disclose.

Study Limitations

Insufficient funds and standard equipment to explore characterisation of nanoparticles.

Ethical Approval

Permission to collect patients' wound swab samples for isolation of *Pseudomonas aeruginosa* was obtained from the research ethic committee (Reference number: HREC - 20-0004) of the Barau Dikko Teaching Hospital, Kaduna State University, Kaduna, Nigeria. Informed consent forms were administered to patients with wound infections for their consent before obtaining relevant data and wound swab samples.

Informed Consent

Prior to collection of wound swab from patients, the hospital ethical committee approval and appropriate intended research information were disseminated to the nurses of the selected hospital wards and units. A brief explanation of the aim and objectives of the research was done to enlighten the patients. Patients were also informed of their freedom to consent or decline participation. Guardian or parents of children with wound infection were requested to give assent for the children.

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