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Experimental Investigation of the Antimicrobial Effects of Terminalia avicennioides Extracts on Staphylococcus aureus Strains Resistant to Multiple Drugs

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Abstract: As the prevalence of antibiotic resistance rises, fewer effective treatments are available for infections caused by MRSA and other multidrug-resistant bacteria. As a result, there was a surge in interest in medicinal plant extracts as a potential source of novel phytochemicals for the treatment of infectious disorders. The purpose of this research was to identify the in vitro antibacterial activity of Terminalia avicennioides extracts against MRSA strains that have developed resistance to many drugs used in wound infections. Patients from Nigeria's Barau Dikko Teaching Hospital in Kaduna provided the wound swab samples. We used conventional phenotypic and genotypic identification techniques to isolate and characterize Staphylococcus aureus. Following established protocols, we determined the antimicrobial susceptibility profile of the Staphylococcus aureus isolates. Standard protocols were also followed to synthesize Terminalia avicennioides extracts and test them for antibacterial activity against MRSA in vitro. Staphylococcus aureus isolates exhibited resistance to a wide range of conventional antibiotics, from 8.18% to 100%, according to the susceptibility profile. Nevertheless, imipenem was effective against all of the isolates. Phytochemical studies conducted on the extracts, both qualitative and quantitative, have shown that they include tannin, alkaloids, flavonoids, cardiac glycoside, phenols, saponins, and terpenoids, but no anthraquinones. Terminalia avicennioides extracts shown a strong antimicrobial effect against MRSA isolates, with growth inhibition zones ranging from 16.28±10.45 - 23.81±6.69 mm and a p-value less than 0.05. The range of the extracts' minimum inhibitory concentrations (MIC) was 56.2500 ± 29.1241 - 31.2500 + 22.16013 gm/ml, and there was no significant difference ($p > 0.05$). There was no significant difference ($p > 0.05$) in the minimum bactericidal concentration (MBC) of the extracts, which varied from 175,000 ± 64.2910 to 68.7500 ± 45.8063 mg/ml. Surprisingly, the antimicrobial properties of Terminalia avicennioides extracts show stronger inhibitory effects against MRSA strains, suggesting they might be developed and studied further for the treatment of wound infections.

Keywords: Staphylococcus aureus, Multidrug Resistance, Wound, Antibacterial Terminalia avicennioides

1. Introduction

Staphylococcus aureus persists in evading antibiotic control efforts and has a history of developing resistance to new medications. There has been a worldwide pandemic of infections caused by Staphylococcus aureus strains that are resistant to antibiotics, and the rates of antimicrobial resistance are on the rise. are reducing the number of available treatments [1]. The global economic and health burden of multidrug-resistant (MDR) diseases is immense and terrible. It has only lately come to light that antimicrobial-resistant illnesses cause the deaths of around 700,000 people every year [2-4]. Unrecognized costs of multidrug-resistant infections (MDR) are disproportionately high in underdeveloped countries like Nigeria. therapy of antibiotic-resistant diseases and related fatalities. Antimicrobial resistance infections are a growing problem in modern medicine, and many important variables, including shifting demography, increased international trade, and extreme weather events, are exacerbating the problem [3, 4]. It is well acknowledged that the majority of antibacterial agents on the market, particularly synthetic ones, have been improperly utilized and no longer work [5-7]. So, the World Health Organization (WHO) said that we should be looking for new antibiotics that work against bacteria that are resistant to multiple drugs, as well as ones that don't cross- or co-resist with other antibiotic classes [3]. The world over, people have been using traditional herbal remedies to cure a variety of infectious ailments for thousands of years [8, 9], and it's interesting to note that medicinal plants are also seen as possible sources of novel antimicrobial compounds. Because chemically produced drugs often cause side effects and microbial resistance, ethnopharmaconosy has become the preferred method of drug discovery involving natural products, such as plant extracts (either as pure compounds or as standardized extracts) [10, 11]. The simplicity, low cost, and high clinical performance of traditional healing agents make them ideal for use in wound care. These treatments provide a more economical option for treating various wounds that are difficult to heal, such as burns, ulcers, and infected wounds. They have a variety of therapeutic actions that speed up the healing process and enhance the quality of the new skin [12]. The purpose of this research was to determine whether or not Terminalia avicennioides extracts had any antibacterial effect on MRSA, a kind of bacteria often seen in wounds, when cultured in a laboratory setting.

2. Materials and Methods

2.1. Ethical Consideration

2.2. At the Barau Dikko Teaching Hospital, Kaduna State University, Kaduna, Nigeria, we got approval from the research ethics committee (Reference number: HREC: 20-0004) to take swabs from patients' wounds in order to isolate Staphylococcus aureus. Kaduna State University, Barau Dikko Teaching Hospital, Nigeria. Patients diagnosed with wound infections were asked to fill out an informed consent form before any pertinent data or wound swab samples were collected. The nurses of the

chosen hospital wards and units were informed of the study's purpose and the ethical committee's clearance before wound swabs were taken from patients. In order to educate the patients, a concise description of the research's goals and purposes was given. Patients were also made aware that they might choose to participate or not. Parents or guardians of children who have a wound infection are kindly asked to provide their consent on behalf of the children.Collection of Wound Swabs and Isolation of Staphylococcus aureus from the Wound Swabs A total of sixty wound swabs samples were collected from

2.3. 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 Baired 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.

2.4. Morphological and Biochemical Characterisation ofPresumptive 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.

2.5. Molecular Identification of Staphylococcus aureus

2.5.1. 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 inoculumn (a density of $1x10^8$ cells/ml) of *Staphylococcus aureus* were prepared from 24 hours broth culture.

Two millilitre (2 ml) of the prepared standard inoculumn was transfered 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 inoculumn added and centrifuged at 10,000 rpm for 5 min., followed by carefully discarding the supernatant, and repeated once again to obtained 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 10µl 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 transfered 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 thenresused the collection tube.

Five hundred micro litres (500 µl) of W_2 buffer was added without wetting the rim, followed by closing the tube andthen 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 checkingto ensured that there were no droplets clinging to the bottom of the binding column tube. The binding column tube was transfered 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.

2.5.2. Polymerase Chain Reaction (PCR) - AccupowerHotstart PCR Premix (Bioneer)

Twenty microlitres (20µl) reaction PCR set - up was prepared by adding; 16ul dH_2O, 1µl forward primer - GGACTACAGGGTATCTAAT 16S (RIBOSE-1), 1µl reverse primer - AGAGTTTGATCCTGG 16S (RIBOSE-2), and 2ul
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, primerannealing 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 16SrRNA genes were searched for sequences similarities using online BLASTn.

2.6. Antimicrobial Susceptibility Tests Using SelectedConventional Antimicrobial Agents Used for Treatments of Wound Infections

Antimicrobial susceptibility test against *Staphylococcus aureus isolates* was carried out using Kirby-Buaer 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

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diluted in steps of 1:10 to give turbidity equivalent to the 0.5 McFarland standards (a density of $1x10^8$ cells/ml) before inoculation. Sterile cotton wool swabs were dipped in the suspensions adjusted to $1x10^8$ 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 MullerHinton agar plates. The inoculated plates were allowed to dry for about 5 minutes. Using disc dispenser, single disc Gram positive antibiotics (Oxoid); Gentamycin (10µg), Amoxicillin- Clavulanic acid (30µg), Nalidixic acid (30µg), Kanamycin (30µg), Ciprofloxacin (5µg), Vancomycin (30µg), Ampicillin (10µg), Oxacillin (1µg), Chloramphenicol (30µg), Imipenem (10µg), Cefoxitin (30µg), and Sulphamethaxole (25µg) 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].

2.7. Collection and Authentication of Terminaliaavicennioides 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 smallpieces and dried under shade at 30°C in a clean laboratory cabinet. The dried plant materials wasl first pounded in a mortar, followed by dry-milling with an electric blender and then sieved to obtained fine powder using 20µm mesh size sieve.

2.8. 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.

2.9. 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]**.**

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

2.10.1. 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 steriled distilled water, then adjusted by comparing with 0.5 Mcfarland turbidity standard (density of 1.0×10^8 cells/ml) against a light background. Steriled 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 0.2mleach 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 zonesof growth inhibition (mm).

2.10.2. Determination of Minimum Inhibitory Conentration(MIC)

The concentrations that showed antimicrobial activity fromthe 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-poit 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 steriled 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 10^8cfu/ml isolate suspension was transfered 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.

2.10.3. Determination of the Minimum BactericidalConentration (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].

2.11. 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 ofPresumptive Staphylococcus aureus

Presumptive *Staphylococcu aureus* colonies showed by table 1 appeared completely yellowish in colour with raised, circular and smooth edges on Manitol Salt agar (MSA). On Baired 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-hymolysis. Gram stains cell appeared purple/bluewish in colour (Gram-positive characteristics) and cocci in shape, arranged inclusters (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-heamolytic, lactose utilization negative, mannitol utilization positive and sucrose utilization negative.

3.2. Molecular Characteristics of Staphylococcus aureusIsolates

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

Staphylococcus aureus strains.

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

Isolate Idenfication Code	Morphological Characteristics	Biochemical Characteristics	Probable Organism

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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	*Staphylococcus aureus*	16SrRNA	134	76.87	8e-29	44	LT6805131
2.	S2	*Staphylococcus aureus*	16SrRNA	878	91.64	0.0	99	LC429749.1
3.	S3	*Staphylococcus aureus*	16SrRNA	360	86.94	9e-94	43	LC57519.1

Key: S1 = DR_{12}, S2 = FSW_1, S3 = $DR_{11,}$ DR = dressing room wound isolate, FSW = female surgical ward wound isolate, and MSW = male surgical ward isolate.

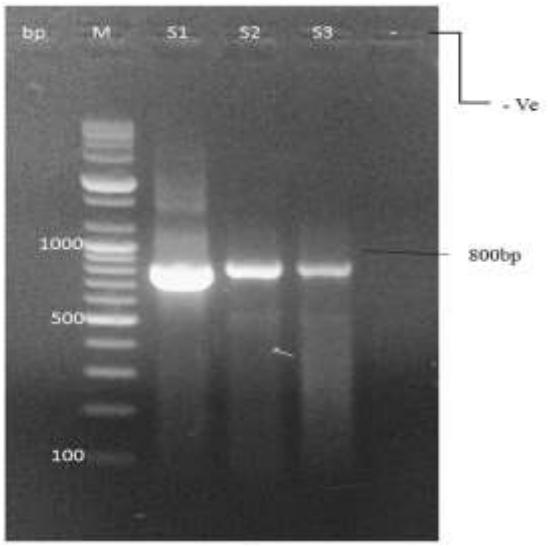

Figure 1. *Gel electrophoresis of amplified PCR 16SrRNA genes bands of Staphylococcus aureus isolates at 800bp of the 100 bp plus DNA marker.*

Key: M = 100bp DNA marker, S = *Staphylococcus aureus*, bp = base pair, - Ve = Negative Control$_{12}$,
S2 = FSW_1, S3 = DR_{11}

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 *Staphylococcu 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 *Staphylococcu aureus* isolates showed by table 4 indicated that four isolates (DR_{19}, $DR_{21,}$ FSW_1 and FSW_6) were resistant each to 7 (58.33%) antibiotics used, five isolates ($DR_{3,}$ DR_5, $DR_{11,}$ MSW_3 and MSW_4) were resistant each to 8 (66.64%) antibiotics used, and two isolates (DR_{12}, and MSW_2) 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	*Staphylococcus aureus* (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)

Ceftazidine	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 α = 0.05**	**Comment**
1	Leave, Stem and Root Bark Extracts			The percentage extracts yield based on plant extracts showed significant difference. Stem and root bark extract showed higher percentage yield compared to leave extracts
	Leaves	5.19 ±1.61[b]	0.0052	
	Stem bark	15.98 ± 3.95[a]	(P< 0.05)	
	Root bark	13.28 ± 3.75[a]		
2	Acetone, Ethanol and Aqueous Extracts			Percentage extracts yield based on extracting solvents showed no significant difference.
	Acetone	11.95±6.90[a]	0.5209	
	Ethanol	14.09 ± 6.42[a]	(P> 0.05)	
	Aqueous	8.40 ±3.66[a]		

Table 6. *Phytochemical Characteristics of Root Barks, Stem Barks and Leave Exracts of Terminelia avecenoides.*

S/No	***Terminelia avecenoides* 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 extractsyields ranged from 5.19±1.61– 15.98 ±3.95%., with stem bark extracts having high percentage yied (15.98 ±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 ± 6.42%, with ethanol stem bark having high percentage yield (14.09±6.42%).

Table 7. *Quantitative Phytochemical Analysis of Root Bark, Stem Bark, and Leave Extracts of Terminelia avecenoides.*

	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;

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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 LeavesExtract 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 barkextracts. 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 (2331-34mg/100g), followed by terpenoids (887-35mg/100g), and then Saponins (47.27-22.72 µg/g) as the lowest.

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

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

Organism	Variably	Mean ± SD zone of growth inhibition (mm)	P-value at α = 0.05	interpretation
	Leaves, Stem and Root Bark Activity			
	AETL	16.45 ± 11.38^{c}	0.0003	
	EETL	18.56 ± 11.63^{bc}	(P < 0.05)	
	AQTL	17.02± 10.92^{c}		
	AETSB	23.38 ± 5.98^{ab}		Generally, zone of growth inhibition showed significant difference.
	EETSB	16.28 ± 10.45^{c}	-	
Staphylococcus aureus Strains (DR_3, DR_5, DR_{11}, DR_{12}, DR_{19}, DR_{21}, FSW_1, FSW_6, MSW_2, MSW_4)	AQTSB	20.86 ± 6.38^{abc}		
	AETRB	23.81 ± 6.69^{a}		
	EETRB	23.25 ± 6.51^{a}	-	
	AQTRB	21.00 ± 6.99^{abc}		
	Plant Parts Extracts Activity			There was a significant difference between leave, stem and root bark activity. Stem and root bark extracts showed larger zone of growth inhibition compered to leave extracts
	Leaves	17.34 ± 11.24^{b}	0.0003	
	Stem bark	20.17 ± 8.33^{a}	(P < 0.05)	
	Root bark	22.69 ± 6.77^{a}		
	Concentration (mg/ml)			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
	200	25.21 ± 3.45^{a}	0.0364	
	100	24.71 ± 5.34^{a}	(P < 0.05)	
	50	23.15 ± 7.00^{ab}		
	25	21.15 ± 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 =Aquoeus*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 ± SD zone of growth inhibition (mm)	P-value at α = 0.05	interpretation
	Leave Extracts Activity			
	AETL	16.45 ± 11.39^{a}	0.7431	There was no significant different between the activity of acetone, ethanol and aqueous extracts
	EETL	18.56 ± 11.64^{a}	(P > 0.05)	
	AQTL	17.02 ± 10.92^{a}		
	Concentration (mg/ml)			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
	200	21.81 ± 12.93^{a}	0.0298	
	100	19.00 ± 11.63^{a}	(P < 0.05)	
	50	15.81 ± 9.89^{ab}		
	25	12.75 ± 8.68^{a}		
	Stem Bark Extract Activity			There was significant different between acetone, aqueous extract, and ethanol extract activity. Acetone and aqueous extracts showed larger zones compared to ethanol extract activity.
	AETSB	23.38 ± 5.98^{a}	0.0019	
DR_3, DR_5, DR_{11}, DR_{12}, DR_{19}, DR_{21}, FSW_1, FSW_6, MSW_2, MSW_4,	EETSB	16.28 ± 10.45^{b}	(P < 0.05)	
	AQTSB	20.85 ± 6.38^{a}		
	Concentration (mg/ml)			Three was a significant differennce between the extracts activity for all the concentrations, with larger zone of growth inhibition at 200mg/ml,
	200	24.63 ± 8.39^{a}	0.0003	
	100	22.00 ± 7.67^{ab}		

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MSW_4	50	18.88 ± 7.23^{bc}	(p < 0.05)	followed by 100mg/ml, 50mg/ml and then 25mg/ml
	25	15.19 ± 7.27^{c}		
	Root Bark Extracts Activity			
	AETRB	23.81 ± 6.69^{a}	0.2146	There was no significant difference between both acetone, ethanol, and aqueous extracts
	EETRB	23.25 ± 6.51^{a}	(p < 0.05)	
	AQTRB	21.00 ± 6.99^{a}		
	Concentration (mg/ml)			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
	200	28.29 ± 4.14^{a}	0.0001	
	100	25.10 ± 4.49^{b}	(p < 0.05)	
	50	20.96 ± 5.53^{c}		
	25	16.39 ± 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 =Aquoeus*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 Staphylococcusaureus Strains

Figure 2 showed the zone of growth inhibition producedby the activity of *Terminalia avicennioides* extracts. Antimicrobial activity of *Terminalia avicennioides extacts* against multidrug resistant *Staphylococcus aureus* isolate result in tables 8 and 9 showed in vitro activity of theacetone, 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 avicennioides Extracts against Multidrug Resistant Staphylococcus aureus strains.*

Organism	Variable	Mean ± SD MIC (mg/ml)	P-value at α = 0.05	Interpretion
Staphylococcus aureus 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 *Staphylococcus aureus 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 avicennioides* Leave extract, AETL =acetone*Terminalia avicennioides* Leave extract, AQTL =Aquoeus*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.

3.7. Minimum Inhibitory Concentration (MIC) of Terminalia avicenode Extracts Against Multi DrugResistant 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 valuesof 56.25±29.12 mg/ml.

3.8. Minimum Bactericidal Concentration (MBC) of Terminalia avicennioides Extracts Against MultidrugResistant 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 showedno significant difference (P> 0.05). However, acetone leav 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 ± SD MBC (mg/ml)	P-value at α = 0.05	Interpretation
Staphylococcus aureus 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 ±45.81^{p}		
	EETL	75.00 ±46.29^{p}		
	AQTL	125.00 ±88.64^{ap}		The MBC showed significant difference. However, acetone stem bark and ethanol root bark showed lower MBC compared to other extracts.
	AETSB	112.500 ±64.09^{ap}	0.0388	
	EETSB	106.25 ±86.34^{p}	(P < 0.05)	
	AQTSB	175.00 ±46.29^{a}		
	AETRB	115.00 ±45.28^{ap}		
	EETRB	125.00 ±46.29^{ap}		
	AQTRB	106.25 ±57.37^{u}		
	Plant Parts Extracts Activity			
	Leaves	89.58 ±65.90^{b}	0.0872	MBC showed no significant difference. However, leave extracts showed higher MBC compared to stem and root bark extracts.
	Stem bark	131.25 ±71.95^{a}	(p > 0.05)	
	Root bark	112.50 ±53.67^{a}		
	Leave Extracts Activity			
	AETL	68.75 ±45.81^{b}	0.1765	MBC showed no significant different. However, acetone extracts showed higher MBC compared to stem and root bark extracts.
	EETL	75.00±46.29^{a}	(p > 0.05)	
	AQTL	125.00 ±88.64^{a}		
	Stem Bark Extracts Activity			
	AETSB	112.50 ±64.09^{a}	0.1036	MBC showed no significant different.
	EETSB	106.25 ±86.34^{a}	(p > 0.05)	
	AQTSB	175.00 ±46.29^{a}		
	Root Bark Extracts Activity			
	AETRB	115.00 ±45.28^{a}	0.4320	MBC showed no significant different.
	EETRB	125.00 ±46.91^{a}	(p > 0.05)	
	AQTRB	106.25 ±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 avicenioides* Leave extract, AQTL =Aquoeus*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

Using phenotypic and genotypic methods, this research isolated and identified strains of Staphylococcus aureus from individuals with wound infections. Staphylococcus aureus colonies on mannitol salt agar (MSA) were found to be yellow, flat, and moderately shaped, according to cultural morphology of phenotypic identification. Fitzgerald said that the fermentation of mannitol salt, leading to the creation of acid, is responsible for the formation of yellow colonies on MSA [26]. Colonies of Staphylococcus aureus appeared dark grey-black and shiny on Baired Parker medium. They had an opaque halo and a clearing zone around them. In a study conducted by Silva et al., similar phenomena were observed in Staphylococcus aureus on Baired Parker medium. The authors found that the colonies' greyish-black shine is caused by a decrease in potassium tellurite, while the clear zone around each colony is the outcome of proteolytic activity caused by Lecithinase breaking down egg yolk. The opaque halo surrounding this clearing is thought to be caused by Lipase activity [27]. Under the microscope with an x100 objective lens, the gram stain cell showed a characteristic of Gram positive cocci, which is that they appeared in clusters like grapes. Staphylococcus aureus has a comparable cellular morphology, according to Tong et al. [28]. This organism was shown to be catalase and coagulase positive according to the biochemical characteristics.

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Beta-hemolysis, a distinctive phenotypic marker for pathogenic Staphylococcus aureus strain identification, is characteristically produced on blood agar. Research has shown that human Staphylococcus aureus isolates contain both bound and free coagulase forms [16], and they produce the telltale beta-haemolysis when cultured on blood agar. One way to tell harmful Staphylococcus aureus strains apart from less dangerous ones is by looking for the presence of the enzyme coagulase.

This study's phenotypic identification method uncovered biochemical and cultural traits associated with Staphylococcus aureus isolates. But because this ethnobotanical study had to focus on pathogen-specific wound infections, and because the organisms chosen had to be directly related to the traditional uses of the plant Terminalia avicennioides, it was necessary to use molecular identification methods to characterize Staphylococcus aureus. According to Vanvuuren [5], this is done to ensure that research can be reproduced. As per Prescott et al. [29], the Staphylococcus aureus bacteria that were found in wound infections were compared using molecular identification to the Genbank database to see how similar they were genetically. The gel electrophoresis of amplified PCR 16SrRNA gene bands of Staphylococcus aureus isolates at 800 bp of the 100 bp plus DNA marker was shown by the findings of the molecular analysis in this research. Presumptive Staphylococcus aureus sequences from BLAST 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*s trains.

According to Prescott et al., it has been generally acknowledged since the 1970s that prokaryotes with genomes that are at least 70% homologous belong to the same species [5]. The percentages of identity and similarity shown by the sequence BLAST results for all of the Staphylococcus aureus strains ranged from 76.87% to -997.67%. This lends credence to the study's conclusion that the isolates in question are, in fact, Staphylococcus aureus. All of the Staphylococcus aureus strains identified in this investigation were shown to be resistant to several drugs. All of the Staphylococcus aureus isolates tested were susceptible to imipenem and Gentamycin, respectively, suggesting that these antibiotics were the most effective against the bacteria (3 and 4). This implies that doctors need to be very cautious when prescribing imipenem to patients in order to prevent the organism from developing a resistance. The prescription of these medications to patients should always be based on sensitivity results. The discovery and its significance to public health should also be communicated to practitioners. Similar results were observed in a study of Staphylococcus aureus susceptibility profiles by Rashedul et al. [30], who found that 90% of the isolates were sensitive to imipenem and that 75% of the isolates were resistant to oxacillin, methicillin, ciprofloxacin, and tetracycline. According to research by Kitara et al. and Brown and Ngeno, among other sources, Staphylococcus aureus may develop resistance to a wide variety of medicines and can generate several strains that are resistant to these drugs [31, 32]. The authors Brown and Ngeno agreed that antibiotic-resistant Staphylococcus aureus represents an international health crisis.

Consistent with previous research, this investigation found that some strains of Staphylococcus aureus were resistant to the antibiotic chloramphenicol [30]. In line with the findings of Aisha et al. [33], our investigation also documented that Staphylococcus aureus displayed multidrug resistance to ceftazidime. Also, this research found that Staphylococcus aureus is resistant to vancomycin, which is concerning since Rashedul et al. found that only 4 out of 66.63 percent of Staphylococcus aureus strains were sensitive to the antibiotic [30]. This study's findings of vancomycin resistance in Staphylococcus aureus isolates suggest that some strains of this bacterium pose a significant threat to the efficacy of wound infection treatments and pose an extra burden on healthcare systems, particularly in communities. Since vancomycin is still only effective against some strains of Staphylococcus aureus, researchers Khan et al. and Juayan et al. have identified VRSA as a major global health concern [34, 35].

When it came to treating wound infections caused by Staphylococcus aureus, Benjamin and Christopher suggested tetracycline, chloramphenicol, and Gentamycin [36]. On a similar note,

For the efficient treatment of wound infections, Bowler et al. suggested the following medications: imipenem, cefoxitin, gentamycin, and vancomycin [37]. The study's results showed that the most effective antibiotics, as advised, are Imipenem, Gentamycin, and Ciprofloxacin. Despite the earlier study recommending them, the other antibiotics tested were ineffective against the tested bacterial strains. Even more concerning is the fact that the Staphylococcus aureus strains tested here were susceptible to gentamycin, imipenem, and ciprofloxacin, in contrast to the multidrug-resistant bacteria described by Aisha et al. [38]. Bacterial resistance gene acquisition, mutations, environmental conditions, biofilm development, presence of beta-lactamase, efflux pump mechanism, and other factors could all contribute to the inconsistency. Because current antibiotics are ineffective against bacterial wound infections, this demonstrated the need for a new medication. Extraction solvents used in this research were ethanol, acetone, and water, while the plant material used was dried, processed powdered Terminalia avicennoides stem bark, root bark, and leaves. The percentage yields of the extracts demonstrated a significant variation ($P<0.05$), ranging from 5.19±1.61 to 15.98 ±3.95%. The percentage yield of the extracts varied from 8.40 ± 3.66 to 14.09 ± 6.42% depending on the solvents used for extraction, and there was no significant difference ($P>0.05$). Possible explanations for the observed % yield discrepancies include the use of various extraction solvents. According to research by Mule et al., the polarity of the extracting solvent (non-polar, polar, or less polar) has a significant impact on the kinds of bioactive compounds that can be extracted from plant components [39]. The results showed that acetone and ethanol solvents produced higher percentage yields of extracts than water, according to this research. Since most active antimicrobial components are insoluble in water, a

universal polar solvent, according to Afolayan et al. [40], it stands to reason that organic polar solvents like acetone and ethanol would produce more potent antimicrobial extracts. This could be because ethanol and acetone had a greater percentage yield from their extracts than water did in this investigation.

The research found that the extracts of Terminalia aveicennioides included tannins, alkaloids, flavonoids, cardiac glycosides, phenolic compounds, terpenoids, and sapponins, according to the quantitative and qualitative phytochemical examination. None of the plant extract categories showed any signs of anthroquinones. Following terpenoids (887-35mg/100g) and saponins (47.27-22.72 µg/g), the quantitative analysis revealed that extracts often exhibited a greater phenol content (2331-34mg/100g). Alaje et al. and Odebumin et al. both found the same thing [41, 42]. The majority of the plant's chemical components include many bioactive substances, such as alkaloids, tannins, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids, and ketones, according to earlier research on the biochemical components of medicinal plants by Irshad et al. [43]. According to Radhika et al., the bioactive chemicals in question are very significant. are the alkaloids, tannins, sapponins, flavonoids and phenoliccompounds [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 theplants for defense system and serve as bioactive principles forvarious drugs and modern therapy [45].

The important phytochemical constituents like steroids, tannins and saponins have been detected in *Terminilia avecennoides* 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 confering protection against some microbial infections [49]. The presence of the various phytochemical compounds is an indication that *Terminilia avecennoides* 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 avecennoides*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 avicennoides extacts* 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 inhibitionin 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 andstem 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, thezone 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 avecennoides* 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 avecennoides* 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 avecennoides*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 avecennoides 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 rootbark is considered cleasing and healing

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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 avecennoides* 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 avecennoides* 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±29.12 – 31.25±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±46/29 – 68.75±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 *Terminilia avecennoides* extracts that can be enhanced by further separation, purification and concentration of the bioactive compounds of the plants.

5. Conclusion

Patients both in and out of Barau Dikko Teaching Hospital Kaduan, Nigeria, had Staphylococcus aureus strains extracted from their wounds. The Staphylococcus aureus strains were all resistant to more than one antibiotic. Only four antibiotics—gentamycin, imipenem, ciprofloxacin, and kanamycin—were effective against the wound infections tested. Noteworthy antimicrobial activity against multidrug resistant Staphylococcus aureus strains is shown by the Terminalia avicennioides extracts, which include important phytochemical components necessary for bacteriostatic and bactericidal activities. The plant extracts of Terminalia avicennioides showed promise as a potential therapeutic agent for the treatment of bacterial wound infections caused by strains of Staphylococcus aureus that are resistant to several drugs. Nevertheless, rigorous research is necessary to identify the particular bioactive or inhibitory chemicals that are effective against the strains of Staphylococcus aureus that are resistant to multiding.

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