Research Article

ISSN 2320-480X
JPHYTO 2022; 11(2): 64-74
March- April
Received: 14-01-2022
Accepted: 24-02-2022
©2022, All rights reserved
doi: 10.31254/phyto.2022.11203

Bobai M
Department of Microbiology, Faculty of Science, Kaduna State University, Kaduna, Nigeria

Danjuma L
Department of Microbiology and Biotechnology, Federal University Dutse, Jigawa, Nigeria

Sani N.M
Department of Microbiology and Biotechnology, Federal University Dutse, Jigawa, Nigeria

Correspondence:
Bobai M
Department of Microbiology, Faculty of Science, Kaduna State University, Kaduna, Nigeria
Email: bobaimathkaya@yahoo.com

In vitro antibacterial activity of biologically synthesized silver nanoparticles using Terminalia avicenoides extracts against multidrug resistant Staphylococcus aureus strains

Bobai M, Danjuma L, Sani N.M

ABSTRACT

Antimicrobial resistance is currently one of the risen concerns to global healthcare in the 21st century. The search for new phytochemicals that could be developed as drugs for treatment of infectious diseases consequently increased with medicinal plants extracts derived nanoparticles receiving greater attention. This study was carried out to determine invitro antimicrobial activity of biologically synthesized silver nanoparticles using Terminalia avicenoides extracts against multidrug resistant Staphylococcus aureus strains isolated from wound infections. Isolation and characterization of Staphylococcus aureus was carried out using standard phenotypic and genotypic methods. Antimicrobial activity of selected antibiotics, Terminalia avicenoides extracts and biologically synthesized silver nanoparticles against multidrug resistant Staphylococcus aureus was carried out using standard procedures. The results of the susceptibility profile showed Staphylococcus aureus isolates resistant to 8.18% to 100% conventional antibiotics used, but 100% sensitive to imipenem. Phytochemical analysis of the extracts revealed the presence of tannins, alkaloids, flavonoids, cardiac glycoside, phenols, saponins and terpenoids. The antimicrobial activity of the biologically synthesized silver nanoparticles ranged from 28.25±1.90–30.65±2.21 mm and showed significant difference (p<0.05). Comparative analysis of Terminalia avicenoides extracts and their respective biologically synthesized silver nanoparticles activity showed significant difference (p<0.05) with antimicrobial activity of silver nanoparticles having larger zones of growth inhibition (29.60±2.83mm) compared to that of extracts (19.88±13.09mm). Remarkably, Terminalia avicenoides extracts derived silver nanoparticles exhibit higher inhibitory effects against the multidrug resistant Staphylococcus aureus strains, hence, can further be study and develop for wound infections therapy.

Keywords: Staphylococcus aureus, Multidrug Resistance, Nanoparticles, Wound, Antimicrobial, Terminalia avicenoides.

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]. Hence, the World Health Organization (WHO) recommended for a focus on discovery and development of new antibiotics specifically active against multidrug and extensively drug-resistant bacteria; and development of new types of antibiotics that lacks cross- and co-resistance to the existing classes of antibiotics [2].

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 [3,4]. Also, because of rising concern to antibacterial resistance, especially multidrug resistance, scientists are now exploring novel compounds including silver nanoparticles (AgNPs) to halt multidrug-resistant microorganisms, and silver nanoparticles have received much attention due to their unique high antibacterial activity against a broad range of bacteria without any toxicity to animal cell [5]. Current trends in enhancing the development of innovative wound care treatments includes the combining use of traditional healing agents and modern products/practices, such as nanofibers containing silver nanoparticles [6]. Hence, many medicinal plants including Terminalia cuneata have been used for synthesis of silver nanoparticles [7]. This study focused on evaluating the invitro antimicrobial activity of Terminalia avicenoides extracts as well as the biologically synthesized silver nanoparticles using the plant extracts against multidrug resistant Staphylococcus aureus isolated from wounds.
MATeRIALS AND METHODS

Ethical Consideration

Permission to collect patients’ wound swab samples for isolation of *Staphylococcus aureus* was obtained from the research ethics 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 swabs 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.

Collection and Transportation of Clinical 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.

**Isolation of Staphylococcus aureus from Wound Swabs**

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 Valli's et al. and Chesbrough [8,9]. 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 Mannitol 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 Bared 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 40°C for morphological and biochemical characterization.

**Morphological and Biochemical Characterization of presumptive Staphylococcus aureus Isolates**

Biochemical characterization of the pure isolates obtained was carried out as described by Aneja, Ochi and Kolhatkar, and Chesbrough [9,11]. 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.

**Molecular Identification of Staphylococcus aureus**

**Chromosomal DNA Extraction**

The DNA extraction was carried out using pioneer bacterial extraction kits (Genomic DNA extraction kits) protocols - “Bioneer Accuprep genomic DNA extraction kit (K-3032). Standard inoculum (a density of 1x10⁵ cells/ml) of *Staphylococcus aureus* were prepared from 24 hours broth culture.

Two milliliters (2ml) of the prepared standard inoculum were 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 milliliters (2ml) of the standard inoculum added and centrifuged at 10,000 rpm for 5 minutes., followed by carefully discarding the supernatant, and repeated once again to obtained more quantity of DNA.

The pellets obtained was resuspended in (200µl) of phosphate buffer saline (PBS) in the Eppendorf tube. Twenty microliters (20µl) of protease were added to the tube containing the pellet in PBS, followed by addition of 1µl of RNase, then mixed thoroughly by vortexing and incubated at room temperature.

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

Four hundred microliters (400µl) of absolute ethanol (Biological grade) were added and mixed well by pipetting, followed by careful transferred 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 minute. followed by discarding the solution from the collection tube and then reused the collection tube.

Five hundred microliters (500µl) of W2 buffer were 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 ensured 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 (100µl) of EA buffer (elution buffer) was added on to the binding column tube and then kept at room temperature (25°C) for 1 minute.

**Polymerase Chain Reaction (PCR) – Accupower Hotstart PCR premix  (Bioneer)**

Twenty microliters (20µl) reaction PCR set - up was prepared by adding; 6µl dH2O, 1µl forward primer- GGACTACAGGTATCTAAT 16S (RIBOSE-1), 1µl reverse primer - AGAGTTTGATCTCGG 16S (RIBOSE-2), and 2µl 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 (Bio-Rad). Amplified PCR products were sequence and the nucleotides sequences of the 16S rRNA genes were searched for sequences similarities using online BLASTn.

**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 [12]. 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 1x10⁵ cells/ml) before inoculation. Sterile cotton wool swabs were dipped in the suspensions adjusted to 1x10⁵ 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 (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 Sulphathiazole (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 guidelines [13].

Collection and Authentication of Terminalia avicenoides Plant Materials

Fresh Terminalia avicenoides 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 (900239) of the plant was obtained. Fresh Terminalia avicenoides 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 were 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.

Preparation of Terminalia avicenoides Plant Extracts

Water, acetone and ethanol were used as the extracting solvents. Twenty-five grams (25g), of the processed fine powder sample of plant was soaked in 250 ml 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 h. 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.

Qualitative 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 [14], Harborne [15], and Sofowara [16].

Biosynthesis of Silver Nanoparticles Using Terminalia avicenoides Extracts

The biogenic synthesis of silver nanoparticles was carried out according to Balasahamugam and Kalaichelvan [17], Henry et al. [18] and Suresh et al. [19]. Five milliliter (5mM) of silver nitrate solution was prepared by dissolving 0.0425g in forty-five milliliters (45mL) of sterile distilled water in 100ml conical flask. A magnetic stirrer was used to stir the mixture for 10 minutes. Five milliliters (5mL) of the extract was added to the silver nitrate solution drop-by-drop until an initial color changed was observed. For color shift control, mixture of silver nitrate solution and plant extract was held at 60°C for 60 minutes. The mixture was incubated for 24 hours at room temperature in clean dark cupboard. Plant extract solution was also incubated as a negative control. A final color changed to brown, different from the negative control indicating the formation of Terminalia avicenoides derived silver nanoparticles (AgNPs) was observed and recorded.

In vitro Determination of the Antibacterial Potency of the Terminalia avicenoides Extracts and (AgNPs) on Multi drug Resistant Staphylococcus aureus Isolates

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 Ochi and Kolhatkar [11], and Cheshire [9]. Zero-point eight grams of the extracts of Terminalia avicenoides 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 milliliters (20ml) of Sterile Muller-Hinton agar were 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 sterile distilled water, then adjusted by comparing with 0.5 McFarland turbidity standard (density of 1.0×10^8 cells/ml) against a light background. Sterile 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 5 minutes. Six millimeters (6mm) diameter corn borer was used to make wells on the inoculated culture plates and allowed to dry for 2 hours. An overnight broth cultures of each isolate was loaded 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).

Data Statistical Analysis

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

RESULTS

Morphological and Biochemical Characteristics of Presumptive Staphylococcus aureus

Presumptive Staphylococcus aureus colonies showed by table 1 appeared completely yellowish in color with raised, circular and smooth edges on Mannitol 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/bluesh in color (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.

Molecular Characteristics of Staphylococcus aureus Isolates

Plate 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 Gene Bank 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 | Gram reaction | Staphylococcus aureus |
| DR3, DR5, DR11, DR12, DR19, DR21, FSW1, FSW6, MSW2, MSW3, MSW4, | Complete yellow, raised, circular and smooth edges, and moderate colonies on MSA | Coccus appeared in cluster (gape-like) or bundle with fein in singles and pairs | - + + - + + + + |
|                            | Black shining colonies with lysis at their edge | Gram positive | Staphylococcus aureus |

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

Conflict of Interest

Plate 1: Gel electrophoresis of amplified PCR 16S rRNA genes bands of Staphylococcus aureus isolate at 800 bp of the 100 bp plus DNA marker. Key: M = 100 bp DNA marker, S = Staphylococcus aureus, bp = base pair, - Ve = Negative control, S1 = DR12, S2 = FSW1, S3 = DR11

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                     |

S1 = DR12, S2 = FSW1, S3 = DR11

Antimicrobial Activity of Selected Conventional Antibiotics Against Staphylococcus aureus Strains

Figure 1 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 sulphathiazole, and 11 (100.00%) resistant to cefazidine, ampicillin, oxacillin and cefoxitin. All 11 (100.00%) isolates were sensitive to imipenem. The resistant pattern of Staphylococcus aureus isolates showed by figure 2 indicated that four isolates (DR19, DR21, FSW1 and FSW6) were resistant each to 7 (58.33%) antibiotics used, five isolates (DR3, DR5, DR11, MSW2, and MSW3) were resistant each to 8 (66.64%) antibiotics used, and two isolates (DR12, and MSW4) 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.

Qualitative Phytochemical Characteristics of Root Barks, Stem Bark and Leaves Extract of Terminalia avicenoides

Table 3 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. Anthraquinone was not detected in all the extracts.
Figure 1: Susceptibility Profile of *Staphylococcus aureus* Strains against selected antibiotics

![Graph showing susceptibility profile of *Staphylococcus aureus* strains against selected antibiotics.](image)

Figure 2: Susceptibility Pattern of Selected Antibiotics Tested against *Staphylococcus aureus* Strain

![Graph showing susceptibility pattern of selected antibiotics tested against *Staphylococcus aureus* strain.](image)

Table 3: Phytochemical Characteristics of Root Barks, Stem Barks and Leave Extracts of *Terminalia avicenoides*.

| S/No | *Terminalia avicenoides* 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
Table 4: Visual Characteristics of Biologically Synthesised Terminalia avicenoides Extracts Derived Silver Nanoparticles.

| S/N | Plant extract code | Silver nanoparticle code | Extracts and Silver Nitrate Solution Held at 60°C for hour | Plant Extracts Derived Silver Nanoparticle after 24 hours Incubation |
|-----|--------------------|--------------------------|----------------------------------------------------------|---------------------------------------------------------------|
| 1   | AETL               | NP₁                      | Greenish brown                                           | Dark brown                                                   |
| 2   | EETL               | NP₂                      | Greenish brown                                           | Dark brown                                                   |
| 3   | AQTL               | NP₃                      | Greenish brown                                           | Light Brown                                                 |
| 4   | AETSB              | NP₄                      | Reddish brown                                           | Reddish brown                                              |
| 5   | EETSB              | NP₅                      | Light brown                                              | coffee brown                                                |
| 6   | AQTSB              | NP₆                      | Reddish brown                                           | Reddish brown                                              |
| 7   | AETRB              | NP₇                      | Light brown                                              | Dark brown                                                  |
| 8   | EETRB              | NP₈                      | Light brown                                              | coffee brown                                                |
| 9   | AQTRB              | NP₉                      | Light brown                                              | Dark brown                                                  |

Key: NP₁ to NP₉ = Synthesised Nanoparticles 1 to 9, EETL = ethanol Terminalia avicenoides Leave extract, AETL = acetone Terminalia avicenoides Leave extract, AQTL = Aquous Terminalia avicenoides Leave extract, EETSB = ethanol Terminalia avicenoides stem bark extract, AETSB = acetone Terminalia avicenoides stem bark extract, AQTSB = Aquous Terminalia avicenoides stem bark extract, EETRB = ethanol Terminalia avicenoides root bark extract, AETRB = acetone Terminalia avicenoides root bark extract, and AQTRB = aqueous Terminalia avicenoides root bark extract.

Table 5: Antibacterial Activity of Biologically Synthesised Terminalia avicenoides Extracts Derived Silver Nanoparticles Against Multi drug Resistant Staphylococcus aureus Strains.

| Organism          | Variable                                             | Mean ± SD Zone of growth inhibition (mm) | P-value at α = 0.05 | Interpretation                                                                 |
|-------------------|------------------------------------------------------|----------------------------------------|---------------------|--------------------------------------------------------------------------------|
| Staphylococcus aureus Strains | Leave, Stem and Root Bark Extracts NPs Activity | NP₁                                   | 29.20±2.66⁸         | 0.001                                                                             |
|                   | (DR₁, DR₂, DR₃, DR₄, DR₅, DR₆, DR₇, DR₈)             | NP₂                                   | 30.65 ± 2.21⁴       | (P < 0.05)                                                                      |
|                   |                                                       | NP₃                                   | 29.05 ± 2.10⁴       |                                                                                |
|                   | FSW₁, FSW₂, MSW₁, MSW₂, MSW₃                         | NP₄                                   | 30.10 ± 2.84⁴       |                                                                                |
|                   |                                                       | NP₅                                   | 28.50 ± 2.61⁴       |                                                                                |
|                   |                                                       | NP₆                                   | 30.25 ± 1.90⁴       |                                                                                |
|                   |                                                       | NP₇                                   | 30.60 ± 2.68⁸       |                                                                                |
|                   |                                                       | NP₈                                   | 30.45 ± 2.57⁷       |                                                                                |
|                   |                                                       | Standard (Ciprofloxacin)              | 28.25 ± 1.90⁴       |                                                                                |
|                   |                                                       |                                       | 40.55 ± 1.01¹       |                                                                                |
| Plant Parts Extracts NPs and Ciprofloxacin Activity | NP₁, NP₂, and NP₃ | NP₄                                   | 29.63 ± 2.37⁷       | 0.001                                                                           |
|                   |                                                       | NP₅                                   | 29.62 ± 2.53⁷       | (P < 0.05)                                                                     |
|                   |                                                       | NP₆                                   | 29.77 ± 3.31³       |                                                                                |
|                   |                                                       | Standard (Ciprofloxacin)              | 40.55 ± 1.01¹       |                                                                                |

There was a significant difference between silver nanoparticles and standard antibiotic (Ciprofloxacin) antibacterial activity, with ciprofloxacin having larger zone of growth inhibition compare to the AgNPs. However, the zone growth inhibitions between the AgNPs showed no significant difference.

There was a significant difference between silver nanoparticles and standard antibiotic (Ciprofloxacin) antibacterial activity, with ciprofloxacin having larger zone of growth inhibition compare to the AgNPs. However, the zone growth inhibitions between the AgNPs showed no significant difference.
The anti-bacterial activity between the extracts and their derived AgNPs showed significant difference. AgNPs showed larger zone of growth inhibition compared to extracts zone of growth inhibition.

The anti-bacterial activity between the standard antibiotic (ciprofloxacin) and extracts derived AgNPs showed significant difference. Ciprofloxacin showed larger zone of growth inhibition compared to AgNPs zone of growth inhibition.

| Organism          | Variable          | Mean ± SD Zone of growth inhibition (mm) | DF | t_{cal} | P-value at α = 0.05 | Interpretation                                                                                                                                 |
|-------------------|-------------------|------------------------------------------|----|---------|---------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| *Staphylococcus aureus* Strains (DR, DRc, DRt1, DRt2, DRt3, FSW1, FSW2, MSW1, MSW2, MSW3) | Extracts          | 19.88±13.09                              | 178| -6.89   | 0.0001 (P<0.005)    | The antibacterial activity between the extracts and their derived AgNPs showed significant difference. AgNPs showed larger zone of growth inhibition compared to extracts zone of growth inhibition. |
|                   | Nanoparticles     | 29.60±2.83                               |    |         |                     |                                                                                                                                             |
| Leave             | Extracts          | 17.40 ± 14.49                            | 58 | -4.51   | 0.0001              | The antibacterial activity between the extracts and their derived AgNPs showed significant difference. AgNPs showed larger zone of growth inhibition compared to extracts zone of growth inhibition. |
|                   | Nanoparticles     | 29.50 ±2.49                              |    |         |                     |                                                                                                                                             |
| Stem bark         | Extracts          | 19.63 ±12.45                             | 58 | -4.51   | 0.0001              | The antibacterial activity between the extracts and their derived AgNPs showed significant difference. AgNPs showed larger zone of growth inhibition compared to extracts zone of growth inhibition. |
|                   | Nanoparticles     | 29.60 ±2.50                              |    |         |                     |                                                                                                                                             |
| Root bark         | Extracts          | 22.60 ±12.09                             | 58 | -4.51   | 0.0001              | The antibacterial activity between the extracts and their derived AgNPs showed significant difference. AgNPs showed larger zone of growth inhibition compared to extracts zone of growth inhibition. |
|                   | Nanoparticles     | 29.70 ±3.49                              |    |         |                     |                                                                                                                                             |
|                   | Ciprofloxacin     | 40.50 ± 0.59                             | 13.41 | 0.0001 (p<0.005) | The antibacterial activity between the standard antibiotic (ciprofloxacin) and extracts derived AgNPs showed significant differences. Ciprofloxacin showed larger zone of growth inhibition compared to AgNPs zone of growth inhibition. |
|                   | Nanoparticles     | 28.399 ±2.9752                           | 34.76 | 0.0001 (p<0.005) |                                                                      |
DISCUSSION

This study isolated and identified Staphylococcus aureus strains from wound infected patients using both phenotypic and genotypic approaches. Cultural morphology of Phenotypic identification revealed the colonies 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 [20]. On Baird Parker medium, Staphylococcus aureus showed grey-black shining colonies with opaque halo surrounded by zone of clearing [20]. 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 [21]. 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 [22]. 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 [11], with characteristic formation of beta-hemolysis 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 used of the plant Terminalia avicennoides, it became imperative to characterized the Staphylococcus aureus using molecular identification methods. This is for reproducibility of studies according to VanVuren [23]. The molecular identification was employed to compare the genetic similarities of the Staphylococcus aureus [22]. 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 [11], with characteristic formation of beta-hemolysis on blood agar. The presence of the enzyme coagulase is phenotypically employed to differentiate between the strain of virulent and less virulent Staphylococcus aureus.

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 [24]. This supports 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 (figure 1 and 2), 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 the 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 of Staphylococcus aureus in a study by Rashedul et al. who reported imipenem as the most potent antibiotic with 90% sensitivity, and with 75% isolates also showing resistance to oxacillin, meticillin, ciprofloxacin and tetracycline [25]. Kitara et al. and Brown and Ngeno reported that Staphylococcus aureus is capable of producing many antibiotic resistant strains and that this organism has the ability to acquire resistance to many antibiotics [26,27]. 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 [25]. Also, this study reported multi drug resistance exhibited by Staphylococcus aureus to ceftazidime similar to report by Aisha et al. [28]. 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 [25], also, this current study also 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 Jultyan 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 [29,30].

Benjamin and Christopher recommended tetracycline, chloramphenicol and Gentamycin for treatment of wound infection cause by Staphylococcus aureus [31]. Similarly, Bowler et al. recommended Gentamycin, Vancomycin, Celoxitin and imipenem for effective treatment of wound infection [12]. 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 contradictory to the previous researches findings that recommended them. Moreso, Aisha et al. reported multidrug resistant Staphylococcus aureus against Gentamycin, Imipenem and Ciprofloxacin antibiotics, while in this study the Staphylococcus aureus strains were sensitive to these antibiotics [32]. The inconsistency might be due to some factors such as; bacterial acquisition of resistance genes, mutations, environmental changes, efflux pumps, 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 solvents ethanol, acetone and water. The qualitative phytochemical analysis of Terminalia avicennoides extracts showed the presence of tannins, flavonoids, cardiac glycosides, phenolic compounds, terpenoids and saponins. Anthraquinones was not detected from all the category of the plant extracts. Odebumin et al. and Alaje et al. reported similar findings [33,34]. Also, in previous studies of bioactive 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 [35]. Radhika et al. also stated that the most important of these bioactive compounds are the alkaloids, tannins, saponins, flavonoids and phenolic compounds [36]. 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 [37].

The important phytochemical constituents like steroids, tannins and
saponins have been detected in *Terminalia avicenoides* plant parts [38], and the presence of these compound is known to confer antibacterial activity against bacteria pathogens [39]. To confer antibacterial activity of plant, flavonoids has been reported to be singly responsible for antibacterial activity associated with some ethnomedicinal plant [40]. 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 [41]. The presence of the various phytochemical compounds is an indication that *Terminalia avicenoides* have potent antiseptic, bactericidal and other medicinal properties [42]. This may be due to the fact that each of the compounds identified has one or more therapeutic usage and may be acting singly or in consortiom 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 the in vitro antibacterial activity.

The in vitro antibacterial activity of the various *Terminalia avicenoides* extracts against multi drug resistant *Staphylococcus aureus* showed zone of growth inhibition. Antibacterial activity was characterized by a cleared zone between the wells (containing the samples) and certain distance. Formation of this inhibitory zones around the wells shows bacterial sensitivity to the extracts. The zone of growth inhibition ranged from 16.28±10.45–23.81±6.69 mm and showed significant difference (P<0.05). Udgure 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 [43].

Several studies have attributed the antibacterial and therapeutic activities of *Terminalia avicenoides* extracts to the presence of flavonoids and a mixture of phenolic compounds and tannins [44]. The phenolic compounds are said to act as protoplasmic poison which penetrate and disrupt bacterial cell wall in addition to precipitation of cell proteins. The present study revealed that the *Terminalia avicenoides* extracts showed potent antibacterial activity against the bacterial strains. This implies that the in vitro antimicrobial activity of the *Terminalia avicenoides* extracts recorded in this study was due to availability of the plant secondary metabolites required for antibacterial activity. The ability of the extracts of *Terminalia avicenoides* to inhibit the growth of the multi drug resistant *Staphylococcus aureus* has been linked by tetra peptides linked by amino acids and cross bacteria made up of peptidoglycan linked by amino acids and cross metabolites which act as reducing agents to produce nanoparticles from metal salts without producing any toxic by-product [46]. The presence of several polyphenolic components including flavonoids and terpenoids facilitated the reduction of Ag*+* ions, and also stabilized the surface of the resultant AgNPs.

This study showed antibacterial activity of biologically synthesized *Terminalia avicenoides* extracts derived silver nanoparticles (AgNPs) against multidrug resistant *Staphylococcus aureus* isolates. The zone of growth inhibition produced by the biologically synthesized silver nanoparticles tested against *Staphylococcus aureus* (isolates ranged from (28.25±1.90–30.65±2.21) mm and showed significant difference (P<0.05). Suresh et al. reported similar findings using biosynthesized silver nanoparticles derived from ethnolic extracts of *Coccinia indica* leaves against *Staphylococcus aureus* isolates [47]. Several studies including studies conducted by Skandalis et al. and Henry et al. also reported similar findings on the activity of silver nanoparticles derived from plant extracts against multidrug resistant *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and Escherichia coli [18,47], The mechanisms of action of silver nanoparticles against bacterial pathogens has been reported by different researchers. Skandalis et al. reported that silver nanoparticles are independent of cell wall structure; and that the silver nanoparticles do disrupt the integrity of the cell membrane and cell wall of Gram negative and Gram-positive bacteria [47]. Henry et al., also reported that the small size nature of silver nanoparticles makes it easier to penetrate the outer cell wall of bacteria, enter the respiratory chain and thus inhibit cell respiration and bacterial death [18]. In the same manner, plant extracts derived biologically synthesised silver nanoparticles has been reported by Skandalis et al. to exert damage to bacterial cell membrane through membrane disruption affecting the bacterial cell shape, hence, leading to shrinkage of bacterial membrane and induction of holes observed with membrane damage [47].

The findings from the comparative antibacterial activity of *Terminalia avicenoides* extracts and their biologically synthesised silver nanoparticles (AgNPs) tested against multidrug resistant *Staphylococcus aureus* strains generally revealed zones of growth inhibition. Generally, the zone of growth inhibition between the extracts and their derived AgNPs showed significant difference (P<0.05), with the antimicrobial activity of the extracts having lower zone of growth inhibition (19.88±13.09 mm) compared to the lager zone of growth inhibition (29.60±2.83 mm) produced by the AgNPs [18,19]. This study revealed that silver nanoparticles possess higher antibacterial activity against multidrug resistant *Staphylococcus aureus* strains with larger zones of growth inhibition compared to *Terminalia avicenoides* extracts antimicrobial activity. High antibacterial activity of biologically synthesised plant derived silver nanoparticles has been reported in series of studies [18], and this high antimicrobial activity may be attributed to the silver nanoparticle’s ability to penetrate through flexible cell walls of bacteria made up of peptidoglycan linked by amino acids and cross-linked by tetra peptides [18]. Interestingly, the inhibition zones produced by the antibacterial activity of the biologically synthesised silver nanoparticles in this study can be categorized into strong inhibitory activity according to Davis and Stout [18]. This established the broad-spectrum antibacterial activity of the plant extracts derived silver nanoparticles against the studied bacterial isolates, hence, the *Terminalia avicenoides* extracts can be recommended as an effective biomaterial for biogenic synthesis of silver nanoparticles for...
therapeutic applications. By and large, this study provided scientific evidence on the potent antimicrobial activity of Terminalia avicenoides extracts derived silver nanoparticles against multidrug resistant Staphylococcus aureus isolates from wound infection that may further be explored for therapeutic purposes.

CONCLUSION

Staphylococcus aureus strains were isolated from wounds of out - and in - patients attending Baran Dikko Teaching Hospital Kaduna, Nigeria. All the Staphylococcus aureus were multidrug resistant strains. Out of all the antibacterial agents used, imipenem, ciprofloxacin, kanamycin and gentamicin were the most effective antibiotics against these wound pathogens. The Terminalia avicenoides extracts contain significant phytochemical compounds requires to exert bacteriostatic and bactericidal effects, and hence, exhibit noteworthy antibacterial activity against the multidrug resistant Staphylococcus aureus strains. However, the Terminalia avicenoides extracts derived silver nanoparticles exhibited higher antibacterial activity against the Multidrug Resistant Staphylococcus aureus strains in comparison to the plant extracts activity. The efficacy of the Terminalia avicenoides extracts and their derived silver nanoparticles against the multidrug resistant Staphylococcus aureus strains indicated that this plant extracts can be used to produce nanomaterial for effective therapy of drug 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 needed.

Conflict of Interest

None declared.

Financial Support

None declared.

REFERENCES

1. Ileka AEK, Mukes M, Engelbrecht F, Moyo SR. Antimicrobial Susceptibility Patterns of Staphylococcus aureus strains Isolated at the Namibia Institute of Pathology from 2012 to 2014. Journal of Medical Microbiology. 2016; 6: 116-124. http://dx.doi.org/10.4236/jmm.2016.63016
2. World Health Organization, (WHO). Antimicrobial Resistance: Global Report on Surveillance 2014; Available from: http://www.who.int/drugresistance/documents/surveillancecereport/en/
3. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiol Review. 1999; 12:564–82.
4. Adelbayo JO, Krettli AU. Potential antimalarial from Nigerian plants: A review. Journal of Ethnopharmacology, 2011; 133:289–302
5. Pirtarighat S, Ghannadnia M, Baghsahi S. Green synthesis of silver nanoparticles using the plant extract of Salvia spinoza grown in vitro and their antibacterial activity assessment. International Journal of Nanostructures in Chemistry. 2019; 9(1): 1-9.
6. Reuben A, Terrie EM, Ayszhalom C, Daniel WB, OnahdeeH, Felix S, Sean H, Sandhya R, Richie P, Andrea D. Lest we forget: Comparing retrospective and prospective assessments of adverse childhood experiences in the prediction of adult health. J Child Psychol Psychiatry. 2016; 57(10): 1103–1112.
7. Velze E, Campillo G, Morales G, Hiricate C, Osoria J, Armache O. Silver nanoparticles obtained by aqueous or ethanolic Aloe vera extracts: An assessment of the antibacterial activity and Mercury removal capability. Journal of Nanomaterials. 2018; http://doi.org/10.1155/2018/7215210
8. Vallès SJ, Nacente BJ. Hand book of Microbiological culture media, 9 edition. 2006; export@scarlau.com Pp 68.
9. Cheesbrough M. District Laboratory practice in tropical countries, part 2, low price edition, Cambridge university press. 2010; Pp 63-70, 91-105, 137-142, 178-186, 194-197.
10. Anepa KR. Experiment in Microbiology plant pathology biotechnology, 4th edition, new age international (p) Ltd, new Delhi new York. 2007; www.new age publisher.com Pp390.
11. Ochaj OJ, Kolhatkar A. Medical Laboratory Science and practice, Tata McGraw Hill publishing limited new Delhi, New York. 2008; Pp535, 539, 632-635.
12. Arora DR, Arora B. A text book of Microbiology; 3rd edition, CBS Publisher, New Delhi. 2011; Pp 75-80, 213, 418.
13. CLSI. Performance standard for antimicrobial susceptibility testing; thirty edition 2020.
14. Trease GE, Evans WC. Pharmacognosy. 15th edition, London: Saunders publishers. 2000; Pp 42-44, 221-229, 246-249, 304-306, 331-332, 391-394.
15. Harborne JB. Phytochemical methods. London Chapman and Hall Ltd. 1996; Pp 52-105.
16. Sofofara EA. Research on medicinal plants and traditional medicine in Africa. Journal of alternative and complementary Medi medi. 1996; 2(3): 365-372.
17. Balashannugam P, Kalaiachelvan PT. Biogenic synthesis of silver nanoparticles from Dodneae viscou and its effective Antibacterial activity. Journal of scientific transaction, environment and technology. 2014; 13(2):67-71.
18. Henry FA, Henry K, Andy D. Synthesis of silver nanoparticles Using Aqueous extracts of medicinal plants (Impatien balsamina and Lantana camera) fresh leaves and Analysis of Antimicrobial Activity. International Journal of Microbiology. 2019; doi.org/10.1055/s-2019-662303
19. Suresh VC, Subash CBG, Periasamy A, Neeraj F, Shivkanya F, Proveena M, et al. Characterization and Antibacterial Response of Filer Nanoparticles Biosynthesized using an ethanolic extract of Cocaine indica leaves, Crystals, 2021; 9:7; doi.org/10.3390/crystal0020097
20. Fitzgerald N, Ogunjobi AA, Ogunjobi TE. Comparative of antibacterial Activities of ethanol extracts of the Band seeds Garania kola and Carica papaya. African Journal of Biomedicine. 2004; 14:14-152.
21. Silva WP, Destra MT, Landgraf M, Franco DGM. Biochemical Characteristics of typical and atypical Staphylococcus aureus in mas titular and environmental samples. Brazilian Journal of Microbiology. 2000; 31:103-106.
22. Tong D, Njume C, Afolayan AJ, Clarke AM, Ndip RN. Crude ethanol extracts of Garcinia Kola Seeds Heckel prolong the lag phase of Helicobacter pylori Inhibitory and bactericidal potential. Journal of medical food. 2015; 14 (7-6); 822-827.
23. VanVuuren SF. Antimicrobial activity of South African medicinal plants. Journal of Ethnopharmacology. 2008; 119:462–72.
24. Prescott LM, Harley JP, Klein AD. Microbiology; 7th edition, McGraw-Hill, New York, 2008; pp852-853, 53-54, 446-853, 832-838.
25. Rashded H, Mrtiyunjay O, Rashd N. Prevalence of vancomycin resistant Staphylococcus aureus VRSA) in methicillin resistant S. aureus (MRSA) strains isolated from burn wound infections. Tzu Chi Medical Journal. 2016; 28(2) 49-53. https://doi.org/10.1016/j.tcmj.2016.03.002
26. Kitara LD, Anyvar Ad, Acullu D, Odongo-Aginya E, Aloyo J, Fendu M. Antibiotic Susceptibility of Staphylococcus aureus in Suppurative Lesions in Lacor Hospital, Uganda. African Health Sciences 2011; 11; S34-S39.http://dx.doi.org/10.4314/ahs.v11i3.70068
27. Brown PD, Ngemo C. Antibacterial Resistance in Clinical Isolates of Staphylococcus aureus from hospital and community sources in Southern Jamaica. International Journal of Infectious Diseases. 2007; 11(3), 220-225.
28. Aisha N, Abdul R, Sadia I, Aisha N, Arfan A, Afshan K. Characterization of antibiotic resistant gene in Staphylococcus aureus isolated from surgical wounds. Advances Life Sciences. 2016; 3(3): pp. 83-88.
29. Khan Z, Faisal S, Hasnain S. The continuing threat of Methicillin Resistant Staphylococcus aureus—past, present, future. Journal of Scientific Research. 2010, 40, (2); pp. 31–34.
30. Juayang AC, delosReyes GB, delaRama AJG, Gallega CT. Antibiotic Resistance Profiling of Staphylococcus aureus Isolated from Clinical Specimens in a Tertiary Hospital from 2010 to 2012. Interdisciplinary Perspectives on Infectious Diseases. 2014; ID: 898457.http://dx.doi.org/10.1155/2014/898457
The Journal of Phytopharmacology

31. Benjamin AL, Christopher H. Topical Antimicrobial therapy for treating chronic wounds. Clinical infectious diseases. 2009; 49:1541-9. Doi:10.1086/644732.
32. Bowler PG, Duerden BI, Armstrong DG. Wound Microbiology and Associated approach to wound management. Clinical Microbiology Review. 2001.
33. Odebunni EO, Oluwaniyi OO, Awolola GV, Adediji OO. Proximate and nutritional composition of kolanut (colantrida), Bitter Cola (Garcinia kola) and Alligator pepper (Aframomum eclequeta). Polish African Journal of Biotechnology. 2009; 8(2):308-310.
34. Alaje AF, Yoon I, Hovde CJ. A brief review of Escherichia coli 0157:H7 and its plasmad 0157. Journal of microbiology and biotechnology. 2014; 20(1): 5-14.
35. Ishad S, Butt M, and Younis H. In vitro antibacterial activity of two medicinal plants: neem (Azadirachta indica) and peppermint. International Research Journal of Pharmaceuticals. 2011; 01(01):9-14.
36. Radhika B, Murthy N, Nirmala D. Preliminary phytochemical analysis and antibacterial activity against clinical pathogens of medically important Orchid Cymbidium aloifolium (L) SW. International Journal of pharmaceutical Sciences and Research. 2013; 4(10):3925-3931.
37. Cragg GM, Newman DJ. Biodiversity: A continuing source of novel drug leads. Pure and Applied Chemistry. 2005; 77 (1): 7 – 24.
38. Mann A, Kuta YA. Antibacterial activity of methanolic extracts of Terminalia avicennioides against fish pathogenic bacteria. Am J Res Commun. 2014;2:133-46.
39. Pavithra PS, Janani VS, Charumathi KH, Indumathy R, Potala S, Verma RS. Antibacterial activity of plants used in Indian herbal medicine. International journal of green pharmacy. 2010; 4:22-8.
40. Keshebo DL, Choudhug MK. Phytochemical investigation of Securidoca longipeduncilara (polygalacaceae) and Structure elucidation of benzyl 2-hydroxy-5-11/21/4024benzoate. International Journal of Current microbial and applied Science. 2015; 4(1): 490-65.
41. Onaja SO, Ezeja MIM, Omeh YN, Onwukwen BC. Antioxidant, anti-inflammatory and antinociceptive activities of methanolic extract of Justicia secenda Vahl leaf. Alexander Journal of Medicine. 2016; 14(6):56-63.
42. Ali SS, Ayuba A, Ali SN, Begum S, Siddiqui BS, Mahmou M, Khan KL. Antibacterial activity of methanol extracts from some selected mechanical plants. FULAST Journal of Biological Sciences. 2017; 7(1):123-125.
43. Udigire MS, Pathade GR. Evaluation of antimicrobial activities and phytochemical constituents of extracts of Valeriana wallichii. Asian Journal of Plant Science and Research. 2013; 3(5):55-59.
44. Anevehb B, Sofowora AO. Qualitative phytochemical screening and invitro antimicrobial effect methanol steam bark of Ficus thonningii. Journal Complementary and Alternative machine. 2006, 3: 269-295.
45. Qwidwai AJ, Kumar R, Dikshit A. Green Synthesis of Silver Nanoparticles by seed of phoenix syvestris L, and their role in the management of cometics embarrassment, Green Chemistry letters and Review. 2018; 11(2): 176-188. doi: 10.1080/17518253, 2018, 144530
46. Khwaja SS, Azamal H, Rifaqat AKP. A review on Biosynthesis of silver nanoparticles and the biocide properties. Journal of Nano biotechnology. 2018; 16:14.
47. Skandalis M, Dimopoulou A, Georgorgopoulou A, Gallious N, Papadopoulas D, Tsipas D, et al. The effect of filler nanoparticles and the size, produced using plant extract from Arbutus unedo on their antibacterial efficacy, Nanomaterials. 2017; 7 (7): 178.
48. Marcinkiewiez J, Ibedron R, Bialacka A, Kasprowicz A, Mak M, Targosz M. Susceptibility of propionic bacterium acnes and Staphylococcus epidermichs to Killing by MPO - Haloide system Product. Implication for Taurineboramminse as a new Candidate for topical therapy in treating Acne vulgaris. Arch Immunol. Ther exp. 2006; 54:61-68.
49. Wang L, Li H, Tian J, Sun X. Monodisperse, micrometer-scale highly crystalline. Nanoparticles Ag dendrites, rapid, large-scale wet-chemical synthesis and their application as SERS substrates SACS Applied mattes Interfaces. 2010; 21 2987. 2991.
50. Davis MW, and Stout TR. Disc plate method of microbiological antibiotic assay. Journal of homeobiology.1971; 22 (4) 666-670.

HOW TO CITE THIS ARTICLE
Bohai M, Danjuma L, Sani NM. In vitro antibacterial activity of biologically synthesised silver nanoparticles using Terminalia avicennioides extracts against multidrug resistant Staphylococcus aureus strains. J Phytopharmacol 2022; 11(2):64-74. doi: 10.31254/phyto.2022.11203

Creative Commons (CC) License-
This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license. This license permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. (http://creativecommons.org/licenses/by/4.0/).