Socio-Demographic Distribution of Multidrug Resistant Staphylococcus aureus from Clinical Sources and their Comparative Control with White and Red Roselle (Hibiscus sabdariffa) Calyces

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Article info
Received 31 January 2022
Revised 30 March 2022
Accepted 30 March 2022
Published online 4 April 2022

Regular article

Keywords:
Staphylococcus aureus, phytochemicals, antioxidant, DDPH, clinical samples, antimicrobial sensitivity pattern

Abstract
Background: Increasing resistance of bacterial infections to current treatment threatens to derail progress made to reduce the global burden of diseases. However, there is insufficient research on effective ways to target information or provision of alternative method of mitigating multidrug resistance that could increase public knowledge toward improvement of antibiotics stewardship. Identification, antimicrobial sensitivity pattern and antimicrobial activity was determined using standard microbiological method.

Results: Among the 50 Staphylococcus aureus isolated from different clinical samples, 38% and 62% were recovered from male and female patients respectively. Largest proportions were from age-group 21-30 years (47.5%). Urine had the highest number of occurrence of S. aureus 16 (32%). The isolates showed high resistance to Cefoxitin (FOX), Ceftazidime (100%), Ciprofloxacin (100%), Cefoxolin (100%), Cephalixin (12%) and erythromycin (36.8%). White and red calyces contain the same type of phytochemicals except Tannin which was absent in the red calyces. The Red calyces (73.45) contain higher phenol than white calyces (38.60). White calyces (163.05) contain higher amount of Flavonoids than in Red calyces (98.30). DPPH inhibition observed in red calyces (76.50) was higher than white calyces (45.33). NO% Inhibition has higher value in the white calyces (162.13) than red calyces (148.35). Both white and red calyces contain equal Tbars (0.136). % Iron chelation observed in white calyces (125.49) was higher than in the red calyces (110.43). Red calyces contain higher amount of Vitamin-C than in the white calyces. Red Hibiscus sabdariffa extract shows greater inhibitory property against isolates than the white Hibiscus sabdariffa extracts. Although, they showed less inhibitory potential on the studied isolates in comparison with commercial antibiotics.

Conclusions: To prevent further emergence and spread of MDR Staphylococcus aureus, rational use of antibiotics and regular monitoring of antimicrobial resistance patterns are made easily through social demographic investigation of the affected population.

1. Introduction

The inappropriate use of antimicrobials such as antibiotics accelerates the emergence of antibiotic-resistant pathogenic process and diminishes drug efficacy. Particularly, widespread misconceptions about the potency of antibiotics for various ailments have led to unnecessary use and self-medication practices in communities. Knowledge about antibiotics and resistant infections has been found to influence antibiotic use (Fair and Tor, 2014; Gualano et al., 2015). Understanding the different factors that lead to inappropriate antibiotic use may help mitigate this global health problem through effective antibiotic stewardship programs to promote rational use of medicines in communities. Age, gender, education level, and socio-economic status have been hypothesized to predict typology of antibiotic knowledge and use (Demoré et al., 2017). For instance, studies have shown that younger individuals; those with lower educational attainment, rural residents, and men exhibited lower knowledge and were more likely to misuse antibiotics, although it is unclear the extent to which the conclusions are generalizable (Pavydėt al., 2015). Identifying predictors of inappropriate antibiotic use could provide important information about the specific knowledge and behaviors to target during the development and implementation of public health interventions and will subsequently promote prudent use of antibiotics in communities.

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https://doi.org/10.36547/ae.2022.4.124-40
For several decades, the emergence of multidrug resistant *Staphylococcus aureus* has been an obstacle to the control of the disease. Early detection of drug resistance is crucial to prevent transmission of drug-resistant *staphylococcus aureus* and avoid mortality (*Erku et al.,* 2017). In low- and middle-income countries, the implementation of robust resistance diagnostic programs using molecular tools remains a challenge. *Staphylococcus aureus* are Gram-positive cocci ranging from 0.5 to 1.5 mm in diameter, which may or may not contain a polysaccharide capsule. They are non-motile, non-spore forming facultative anaerobes that produce catalase and coagulase enzymes (*Fisher and Paterson, 2020*). *Staphylococcus aureus* is a commensal of humans, it is also a frequent cause of human infections which may become serious if caused by antimicrobial resistant strains (*Lee, 2003*). Antibiotic resistant *S. aureus*, especially MRSA, are equally adopted to hospitals and outer environments evolving as major pathogens of public health concern (*Shah et al.,* 2013).

The fact that *S. aureus* is resistant to multiple classes of antimicrobial agents in the hospital environment is a challenge currently facing clinicians when treating *S. aureus* infections (*Saba et al.,* 2017). This resistance stems from a history of over 50 years of recurrent adaptation of *S. aureus* to different antibiotics introduced into clinical practice over the years. Abuse of as well as indiscriminate use of antimicrobials are contributing factors to the spread of resistance (*wang et al.,* 2017). Antibiotic-resistance genes are carried on plasmids and transposons, and can be transferred from one staphylococcal species to another and among other Gram-positive bacteria. Antimicrobials act by targeting important bacterial functions such as cell wall synthesis (beta-lactams and glycopeptides), protein synthesis (aminoglycosides, tetracyclines, macrolides, lincosamides, chloramphenicol, mupirocin and fusidic acid), nucleic acid synthesis (quinolones), RNA synthesis (rifampin), and metabolic pathways such as folic acid metabolism (sulphonamides and trimethoprim) (*Schmitt et al.,* 2021). The overuse of antimicrobials elicits resistance either by the emergence of point mutations or by the acquisition of foreign resistance genes, which leads to alteration of the antimicrobial target and the degradation of the antimicrobial or reduction of the cell’s internal antimicrobial concentration (*Fisher and Paterson, 2020*).

*Staphylococcus aureus*. *S. aureus* is a potentially harmful human pathogen associated with both nosocomial and community-acquired infections, and it is increasingly becoming resistant to most antibiotics. Previous studies of *S. aureus* in marine environments have linked swimmers to the dissemination of *S. aureus* in marine water, via the shedding of the bacterium from their nose, skin, and respiratory tract. On recreational beaches, *S. aureus* has occasionally been found in high abundance in both water and sand, which can be directly associated with bather density and human activities around the beach (*Akambo et al.,* 2017).

Plants are rich sources of several classes of bioactive compounds that have been responsible in the prevention and treatment of chronic health pathologies such as hypertension, cardiovascular diseases, inflammation and cancer (*Bresciani et al 2015*). *Hibiscus sabdariffa* (Figure 1), a member of Malvaceae family, is a known medicinal plant with a worldwide fame (*Abbas et al, 2011*) and the plant can be found in almost all warm countries such as India, Saudi Arabia, Malaysia, Indonesia, Thailand, Philippines, Vietnam, Sudan, Egypt and Mexico.

![Hibiscus sabdariffa](image1.png)

**Figure 1 Hibiscus sabdariffa Linn**

There are health and nutritional claims that *Hibiscus sabdariffa* possess health benefits such as soothing colds, opening blocked nose, clearing up mucus, promoting proper kidney function, helping digestion and helping reduce fever. *Roselle* is known for its antibacterial, antifungal and anti-parasitic actions. Oil extract, Aqueous and ethanol extracts from seeds of *Roselle* has been shown to have an *in vitro* inhibitory effect on microorganisms. However, there are limited studies to substantiate these health/nutritional claims. There is need for scientific information to substantiate the claims and validate its applicability in the treatment of infectious diseases.

### 2. Material and methods

#### Sample Collection

**Clinical samples:**

Clinical isolates were collected from State Specialist Hospital. The isolates were isolated from fifty (50) patients with vary sources which include ear, hair, toe, high vaginal swab (HVS) and urine. The isolates were subcultured into slants and were transported to Laboratory of the Department of Microbiology, Federal university of technology Akure.

**Plant samples**

*Hibiscus sabdariffa* used for this project were gotten from the market in Ado-Ekiti (Oja-Bisi), Ekiti State, Nigeria.

**Reagent used**

- Mercuric chloride, 100ml distilled water, Biamuth nitrate, iodine, 2% Hydrochloric acid, 5% Fecl3, 10% Lead acetate, 1N Sodium hydroxide, Concentrated Tetraoxosulphate(IV) acid (H2SO4), Ammonia solution, Methanol, Chloroform, Sulphuric acid, 10% Acetic acid, 10% Thio urea, 2,4-Dinitrophenyl Hydrazine, 85% Sulphuric acid, 5-Bromine water, 10% potassium iodide, 1M potassium iodate (KI03), Sodium thiosulphate and 3% Starch solution.

**Materials**

- Test tube, Filter paper, Glass slide, Air oven, Desiccator, Porcelain crucible, Round bottom flask, pipette, weighing balance, Atomic Absorption Spectrophotometer, Apple-UV-Visible spectrophotometer.

**Preparation of plant Materials for Extraction**

The dried samples were grinded using electric blender. The powdered samples were then kept in an air-tight plastic container, further Soxhlet extraction process using water as solvent of extraction was carried out using standard method as described by *Ibisannii and Arbisala, 2022*. 
Preparation of Culture Media
All glass wares and culture media were sterilized by autoclaving at 121°C for 15 minutes at 15psi. Mannitol salt agar and Nutrient Agar used in this study were all prepared according to manufacturer's instructions.

Morphological identification of isolates
The morphology of the clinical isolate was based on the physical appearance of the colonies on the Mannitol Salt Agar (MSA), the size, the color, the shape, the edge, the elevation, the texture and the transparency.

2.1 Biochemical identification of isolates
Gram staining
This was carried out to establish the Gram reflection of the isolates. A smear of each isolate was made on glass slides, crystal violet stain was added to the smear for 30-60 seconds after which the stain was washed off with sterile distilled water, then iodine was added to the smear for another 30-60 seconds to fix the stain and washed off with sterile distilled water. Alcohol (70%) was added to the smear for 60 seconds to decolorize the stain, safranin red was finally added to the smear as a counter stain. The glass slide was mounted on a microscope and observed under oil immersion objectives lens (x100). A purple color indicated the presence of Gram-positive microorganism while red/pink color indicates presence of Gram-negative microorganism.

Biochemical tests
The various biochemical tests were carried out on the organisms to aid in their identification. They include the following:

Citrate test
This test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. The subsequent increase in the pH of the medium is demonstrated by the color change of pH indicator. The isolates were inoculated into citrate slants and incubated at room temperature (28±2°C) for 24 hours. Organisms that are able to utilize citrate as their source will be indicated by a color change of the agar from green to deep blue while microorganisms that cannot utilize citrate will be indicated by no color change.

Indole test
The indole test screens for the ability of the organism to degrade the amino acid tryptophan and produce indole. It is used as part of the IMViC (indole, MR-VP citrate) procedures. A positive test indicates the formation of a pink to red color (“cherry-red ring”) in the reagent layer on top of the medium within seconds of adding the reagent.

Oxidation test
This is a test that assays for the presence of cytochrome oxidase, an enzyme sometimes called indophenols oxidase. In the presence of an organism that contains the cytochrome oxidase enzyme, the reduced colorless reagent becomes an oxidized colored product. It is Oxidase positive when the color changes to dark purple within 5 to 10 seconds and Oxidase negative if the color does not change or it takes longer than 2 minutes.

Methyl red
This test demonstrates the ability of an organism to oxidize glucose with the production and stabilization of high concentrations of acid end products. The methyl red indicator will turn red throughout the tube, which is indicating of a positive test at pH6, still indicating the presence of acid but with a lower hydrogen ion concentration, the indicators turn yellow, which is indicating the negative test.

Voges-Proskauer
This test determines the ability of the organism to produce acetone (acetyl methyl carbinol) during fermentation of glucose. The reagent used in this test, Barrett’s reagent, consists of a mixture of alcoholic alpha-naphthol and 40% potassium hydroxide solution. Development of a deep rose colour in culture within a minute following the addition of Barrett’s reagent is indicative of presence of the acetyl methyl carbinol and represents a positive result. The absence of rose colouration is a negative result.

Sugar fermentation test
This test is used to determine the ability of the organisms to degrade and ferment carbohydrates with the production of acid and gas. In fermentation, substrate and alcohols undergo anaerobic dissimilation and produce an organic acid. The pH indicator red is used to detect the production of acid, which is red at a neutral pH 7 and changes to yellow at a slightly acidic pH of 6.8. This indicates a positive reaction. The following sugars were used for the experiment: Galactose, Lactose, Sucrose and Maltose.

Triple sugar iron test
The triple sugar-iron test is designed to differentiate among the different groups of organisms capable of fermenting glucose with the production of acid. Carbohydrate fermentation is indicated by the presence of gas and visible color change of the pH indicator, phenol red. The production of hydrogen sulphide in the medium is indicated by the formation of a black precipitate that will blacken the medium in the butt of the tube.

Coagulase test
A drop of sterile distilled water was placed on clean slides, a sterile loop was used to pick colonies and a hick suspension was made. A loopful of plasma was added to the suspension. Formation of clumps within 10 seconds indicates positive reaction and absence of clumps indicates negative reaction.

Mannitol motility test
This test is used to detect if an organism is motile and also mannitol is fermenting or not. A motile organism typically diffused, hazy growth that spreads throughout the medium rendering it slightly opaque. This test also helps to identify whether the organisms ferment mannitol or not. It produces acidic end products which in turn change the red colour of phenolred indicator to yellow.

2.2 Phytochemical analysis (Fresh samples)

Detection of alkaloids
Mayer’s test
A fraction of the extract is treated with Mayer’s reagent (1.36g of mercuric chloride and 5g of potassium iodide in 100ml distilled water and noted for a cream-colored precipitate).

Dragendorff’s test
A fraction of the extract is treated with Dragendorff’s reagent and observed for the formation of reddish orange precipitate. (Bismuth nitrate 1.7g, glacial acetic acid 20ml, water 80ml, and 100ml of 50% solution of KI in water, mix together and keep as stock solution, 10ml of stock, 20ml of glacial acetic acid make up for 100ml in water for working solution)
Wagner's test
A fraction of the extract is treated with Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of distilled water) and observed for the formation of reddish-brown precipitate.

Alternative test for Alkaloids
For the purpose of phytochemical analysis of the selected plants, 0.2g of the selected plant samples were added in each test tube and 3ml of hexane were mixed in it, shaken well and filtered. Then took 5ml of 2% HCL and poured in a test tube having the mixture of plant and hexane. Heated the test tube having the mixture, filtered it and pour few drops of picric acid in a mixture. Formation of yellow colour precipitate indicates the presence of alkaloids.

Detection of phenolic compounds
Ferric chloride test
A fraction of the extract was treated with 5% FeCl₃ solution and observed for the formation of deep blue colour.

Lead acetate test
A fraction of the extract was treated with 10% lead acetate solution and observed for the formation of white precipitate.

Determination of Flavonoids
Aqueous NaOH test
To a fraction of the extract, 1N aqueous NaOH was added and observed for the formation of yellow-orange colour

Concentrated H₂SO₄ test
To a small fraction of the extract, concentrated H₂SO₄ was added and observed for the formation of orange colour.

Schinodo's test
To a small fraction of the extract, a piece of magnesium turnings was added, followed by concentrated HCl and then heated slightly and the formation of dark pink colour was observed.

Alternative test for flavonoids
For the confirmation of flavonoid in the selected plants, 0.5g of each selected plant extract were added in a test tube and 10ml of distilled water, 5ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of 1ml concentrated H₂SO₄. Indication of yellow colour shows the presence of flavonoid in each extract.

Detection of saponins
Foam test
A fraction of the extract was vigorously shaken with water and observed for persistent foam.

Haemolytic test
A fraction of the extract will be added to a drop of blood placed on a glass slide and observed for the haemolytic zone.

Test for terpenoids
An amount of 0.8g of selected plant sample will be taken into a test tube, then 10ml of methanol will be poured into it, shaken well and filtered to take 5ml extract of plant sample. Then 2ml of chloroform is mixed with the extract of selected plant sample followed by 3ml of sulphuric acid in selected sample extract. Formation of reddish brown colour indicates the presence of terpenoids in the selected plants.

Test for tannins
0.5g of the dried powdered samples is boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride is added and observed for brownish green or a blue-black colouration.

2.3 Some in-vitro antioxidant analyses of the plants samples

Determination of DPPH free radical scavenging ability
The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging ability of the extract was determined using the modified method of (Gyamfi et al., 1999). Briefly, 1.0 mL of different concentrations (20, 40 and 80 mg/mL) of the extracts was placed in respective test tubes. 1.0 mL of 0.1M methanolic DPPH solution was added to the samples. These samples were vortexed, and incubated in dark at room temperature for 30 minutes before absorbance measured at 516nm. Decreased absorbance of the sample indicates DDPH free radical scavenging capability. Further calculation was carried out according to standard method (Ibisanmi and Aribisala, 2022).

Determination of Nitric oxide (NO) scavenging ability
The modified methods of (Jagetia and Baliga, 2004) was used to determine the Nitric oxide radical scavenging ability. Sodium Nitroprusside in acqueous solution at physiological PH 7.0 spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent (1.0mL sulfuric acid reagent (0.33% prepared in 20% glacial acetic acid at room temperature for 5 minutes with 1mL of naphthylethylenediamine dichloride (0.1% w/v). Further calculation was carried out according to standard method (Ibisanmi and Aribisala, 2022).

Determination of ferric reducing antioxidant power
The reducing property of the extract was determined by the modified method of (Pulido et al., 2002). This method is based on the reduction of (Fe3+) ferricyanide in stoichiometric excess relative to the antioxidants. Different concentrations of the methanolic extract of the sample and its various fractions (10-50 g/Ml) was added to 1.0mL of 200Mm of sodium phosphate buffer PH 6.6 and 1.0mL of 1% potassium ferricyanide [K₃Fe (CN)₆]. The mixture was incubated at 50⁰C for 20 minutes, thereafter 1.0mL of freshly prepared 10% TCA was quickly added and centrifuged at 2000rpm for 10 minutes. 1.0mL of the supernatant was mixed with 1.0mL of distilled water and 0.25ml 0f 0.1% of FeCl₃ solution was added. Distilled water was used for blank without the test sample while control solution contained all other reagents except the 0.1% potassium ferricyanide. Absorbances of these mixtures were measured at 700nm using a spectrophotometer. Decreased absorbance indicates ferric reducing power capability of sample. Further calculation was carried out according to standard method (Ibisanmi and Aribisala, 2022).

Determination of Fe²⁺ Chelation
The ability of the extract to chelate Fe²⁺ was determined using a modified method of Minotti and Aust (1987) by (Puntel et al., 2005). Freshly prepared 500 M FeSO₄ (150 L) was added to a reaction mixture containing 168 L of 0.1M Tris - HCl (PH 7.4), 218 L saline and the different concentrations of extracts (0-25 L). The reaction mixture was incubated for 5 minutes before the addition of 13 L of 0.25% 1, 10- phenanthroline (w/v). The absorbance was subsequently measured at 510nm in a
spectrophotometer. Further calculation was carried out according to standard method (Ibisanmi and Aribisala, 2022).

Quantification of Total Phenolic Content and flavonoids

Estimation of Total Phenolic Content

The extractable phenol content was determined on the extracts using the method reported by Singleton et al., (1999). 0.2ml of the extract was mix with 1.5ml of 10% Polinciocealtea's reagent and 2ml of 7.5% Sodium carbonate. The reaction mixture will be subsequently incubated at 45 °C for 40 minutes, and the absorbance was measure at 700nm in the spectrophotometer, garlic acid would be used as standard phenol. Further calculation was carried out according to standard method (Ibisanmi and Aribisala, 2022).

Determination of total flavonoid

The total flavonoid content of the extract was determined using a colorimeter assay developed by (Bao, 2005). 0.2 ml of the extract was added to 0.3mL of 5 % NaNO3 at zero time. After 5 minutes, 0.6ml of 10% AlCl3 was added and after 6 minutes, 2mL of distilled water. Absorbance was read at 510nm against the reagent blank and flavonoid content was expressed as gallic acid equivalent. Further calculation was carried out according to standard method (Ibisanmi and Aribisala, 2022).

Determination of Vitamin C (ascorbic acid) Contents in various fruit and vegetable by UV- spectrophotometry and titration methods

Determination of Vitamin C (ascorbic acid) Contents in various fruit and vegetable by UV-spectrophotometry and titration methods carried out according to standard method as described by ibisanmi and Aribisala, 2022.

Antibiotic Sensitivity Test Using Agar Diffusion Method (discs)

The disk diffusion method was used and after 16-18 hours of incubation at 37°C zone of inhibition was measured and interpreted as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2018). Using a sterile wire loop, 3-5 pure colonies were picked from Nutrient agar for Gram positive then emulsified in nutrient broth. Standard inoculums adjusted to 0.5 McFarland standard using McFarland Densitometer was swabbed onto Muller-Hinton agar (dispensed on 100mm plate). Accordingly, detailed CLSI guidelines for each category of Gram-positive bacteria, isolates were tested against. The zone of inhibition was measured to the nearest millimeter and all bacterial isolates were classified as sensitive, intermediate and resistant according to the standardized table supplied by CLSI (2018).

Antimicrobial Activity of White and Red Roselle Sun-Dried Calyces (Hibiscus Sabdariffa) Extracts

Agar well diffusion method was used to test the antimicrobial activity of the extracts. By making used of fresh medium, bacterial suspensions were streaked on petri dishes containing freshly prepared Muller Hinton Agar using a sterile swab stick. Five 6mm wells were bored on the already inoculated MHA plates using a sterile cork borer, the susceptibility test was carried out in triplicate. Each of the wells were filled up with 200mg/ml and 100mg/ml of the reconstituted extracts respectively. The plates were incubated at 25 °C for 18 to 40 hours and zones of inhibition were measured with milliliter ruler at 24 hours interval.

3. Results

3.1 Identification of Staphylococcus aureus

Fifty (50) target organisms (Staphylococcus aureus) were isolated from different clinical sources were identified by standard microbiological methods. Results obtained for biochemical test is shown in Table 1.

3.2 Socio-demographic characteristics

A total of fifty (n=50) eligible out-patients attending Image Diagnostic Centre were investigated during this study period. Of these patients who developed infections from different clinical sources 38% (n=19/50) of them were male and 62% (n=31/50) were female shown in Figure 1. The majority of these patient 30% (n=15/50) were between the ages of 21-30 years as shown in Figure 2. Among all study participants, the highest rate of occurrence of target organism 32% (n=16/50) were urine, 24% (n=12/50) HVS, 22% (n=11/50) ear, 12% (n=6/50) hair, 10% (n=5/50) were toe as shown in Figure 3.

3.3 Antibiotic Sensitivity testing for Staphylococcus aureus from clinical sources

The frequency of occurrence of antibiotics susceptibility and multirud-resistant Staphylococcus aureus from different clinical sources are indicated in figure 4 to figure 7. Among the urine samples Ofloxacin (OFL) (100%) was found to have a very good efficacy on almost all the isolates whereas the isolates were able to completely resist the effect of Cefoxitin (FOX), Amoxillin (AUG), Cefazidime (CAZ), Ciprofloxacin (ORX), Ceftriaxone (CTR) and Cloxacillin (CXC). Among the Toe samples Ofloxacin (OFL) (100%) and Gentamicin (GEN) (100%) was found to have a very good efficacy on almost all the isolates whereas the effect of others antibiotics varied as shown in figure 4. Among the hair samples Ofloxacin (OFL) (100%) was found to have a very good efficacy on almost all the isolates whereas the effect of others antibiotics varied. Result obtained from HVS sample shows that the isolates were able to resist the effect of Cefoxitin (FOX), Amoxillin (AUG), Cefazidime (CAZ), Ciprofloxacin (ORX), Ceftriaxone (CTR) and Cloxacillin (CXC) whereas Gentamicin (GEN) (100%) were found to be most effective as shown in figure 5. Among the ear samples one of the isolates 17% resistance was observed for Ofloxacin (OFL), the result shows that effect of antibiotics used varied among the isolates as shown in figure 6. Among all the clinical samples Ofloxacin (OFL) (94%) was found to have a very good efficacy on almost all the isolates, followed by Gentamicin (GEN) (73%) and Erythromycin (ERY) (42%) (figure 7). The resistance patterns of these isolates were little bit low to OFL, GEN and ERY. The antibiotic susceptibility profile showed that the isolates were most resistant to FOX, CAZ, ORX, CTR, and CXC. Multi drug resistant pattern of Staphylococcus aureus from clinical sources is shown in Table 2.
<table>
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<tr>
<th>S/N</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Citrate</th>
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</tr>
</tbody>
</table>

**Figure 1** Frequency of *Staphylococcus aureus* among genders

U= urine sample, HV= HVS sample, T= toe sample, E= ear sample, H = hair sample
Figure 2 Frequency of *Staphylococcus aureus* among age in year of patients

Figure 3 Frequency of *Staphylococcus aureus* among the different types of specimen collected
Table 2 Multidrug resistant pattern of *Staphylococcus aureus* from clinical sources

<table>
<thead>
<tr>
<th>S/N</th>
<th>Source</th>
<th>Antibiotics</th>
<th>No of MDR</th>
<th>Percentage of MDR S. aureus (n=50)</th>
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<td>N=6</td>
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</tr>
</tbody>
</table>

Ceftazidime=CAZ (30 mg/ml), Amoxicillin=AUG (30 mg/ml), Gentamicin=GEN (10 mg/ml), Cloxacillin=CXC (3.8-6.4 mg/ml), Ofloxacin=OFL (200-800 mg/ml), Ciprofloxacin=ORX (5 mg/ml), Erythromycin=ERY (5 mg/ml), Cefoxitin = Fox samples (30 mg/ml), Cefolaxime=CTR (81-102 mg/ml).
Figure 4 frequency of occurrence of antibiotics susceptibility and multidrug-resistant *Staphylococcus aureus* from urine and toe samples.

Figure 5 frequency of occurrence of antibiotics susceptibility and multidrug-resistant *Staphylococcus aureus* from hair and hvs samples.
Figure 6 frequency of occurrence of antibiotics susceptibility and multidrug-resistant *Staphylococcus aureus* from ear samples

Figure 7 frequency of occurrence of antibiotics susceptibility and multidrug-resistant *Staphylococcus aureus* from all the clinical samples

3.4 Extract Yield

After the extraction process, the white *Hibiscus sabdariffa* showed yields of 25.23% while the yields from red *Hibiscus sabdariffa* is 24.85%.

3.5 Phytochemicals composition of *Hibiscus sabdariffa*

Table 3 shows the Qualitative analysis of *Hibiscus sabdariffa* (Sun dried calyces of white and red roselle). All the phytochemicals were present in the white and red calyces except Tannin which was absent in the red calyces.

3.6 Quantitative Phenolics and Flavonoids composition

Table 4 shows the quantitative composition of Total Phenolics and Flavonoid in the sun dried white and red calyces of *Hibiscus sabdariffa*. Higher Total Phenolics was observed in the Red calyces (73.45) while lower value was recorded in the White calyces (38.60). However, Flavonoids has higher value in the White calyces (163.05) and lower value in the Red calyces (98.30).

Table 3 Qualitative phytochemicals composition of *Hibiscus sabdariffa*

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Red calyx</th>
<th>White calyx</th>
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<tr>
<td>Saponin</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Flavonoid</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Phenolic</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
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</tbody>
</table>

+++ = Present in an appreciable amount  
++ = Present in a moderate amount  
+ = Present in a minute amount  
- = Completely absent

Table 4 Quantitative Phenolics and Flavonoids composition of calyces of *Hibiscus sabdariffa* at 100mg/ml

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total Phenolics</th>
<th>Flavonoids</th>
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</thead>
<tbody>
<tr>
<td>Red calyx</td>
<td>73.45a</td>
<td>98.30b</td>
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<tr>
<td>White calyx</td>
<td>38.60b</td>
<td>163.05a</td>
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</tbody>
</table>

Means with the same letter within a column are not significantly different at P≤0.05
3.7 Quantitative composition of DDPH% Inhibition, NO% Inhibition, TBars and % Iron chelation analysis

Table 5 shows the quantitative composition of DDPH% Inhibition, NO% Inhibition, TBars and % Iron chelation analysis in the sun dried white and red calyces of *Hibiscus sabdariffa*. DDPH% Inhibition has higher value in the red calyces (76.50) while lower value was recorded in the white calyces (45.33). NO% Inhibition has higher value in the white calyces (162.13) while lower value was recorded in the red calyces (148.35). For TBars, the same result was recorded both in the white and red calyces (0.136). % Iron chelation has higher value in the white calyces (125.49) while lower value was recorded in the red calyces (110.43).

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Red calyx</th>
<th>White calyx</th>
</tr>
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<tbody>
<tr>
<td>DPPH% Inhibition</td>
<td>76.50a</td>
<td>45.33b</td>
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<tr>
<td>NO% Inhibition</td>
<td>148.35b</td>
<td>162.13a</td>
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<tr>
<td>TBars</td>
<td>0.136a</td>
<td>0.136a</td>
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<tr>
<td>% Iron chelation</td>
<td>110.43b</td>
<td>125.49a</td>
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</tbody>
</table>

Means with the same letter within rows are not significantly different at P≤0.05; DPPH - Diphenyl Picryl Hydrazyl Radical; NO - Nitric oxide TBars - Thiobarbituric acid reactive species

3.8 Concentration of vitamin C in *Hibiscus sabdariffa*

Table 6 presents the concentration of vitamin C in *Hibiscus sabdariffa*. In all the concentration, vitamin C was higher in the red calyces than in the white calyces (Figure 8).

<table>
<thead>
<tr>
<th>Conc.</th>
<th>Vit. C std</th>
<th>Red calyx</th>
<th>White calyx</th>
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</thead>
<tbody>
<tr>
<td>20mg/ml</td>
<td>0.424</td>
<td>0.411</td>
<td>0.314</td>
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<tr>
<td>40mg/ml</td>
<td>0.572</td>
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<tr>
<td>80mg/ml</td>
<td>0.644</td>
<td>0.472</td>
<td>0.457</td>
</tr>
<tr>
<td>100mg/ml</td>
<td>0.730</td>
<td>0.486</td>
<td>0.475</td>
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</table>

3.9 Antimicrobial activity of white and red *Hibiscus sabdariffa* on multidrug resistance *Staphylococcus aureus* from clinical sources

According to the result obtained from this study, *Hibiscus sabdariffa* extract shows a great antimicrobial activity against 50 *Staphylococcus aureus* isolated from different clinical sources, only one of the isolates was able to resist the effect of red *Hibiscus sabdariffa* at 200mg/ml whereas 2 different isolates were able to resist the effect of both red and white *Hibiscus sabdariffa* at 100mg/ml. The trend of the results shows that red *Hibiscus sabdariffa* extract shows a greater inhibitory property against isolates used than the white *Hibiscus sabdariffa* extracts. (Figure 9 & 10)
Figure 9  Zone of inhibition (mm) of ethanol extracts of white and red roselle (Hibiscus sabdariffa) calyces (200mg/ml) on Staphylococcus aureus from clinical sources.
Figure 10 Zone of inhibition (mm) of ethanol extracts of white and red roselle (*Hibiscus sabdariffa*) calyces (100mg/ml) on *Staphylococcus aureus* from clinical sources
4. Discussion

The overall prevalence of bacterial isolates from out-patients attending the attending Image Diagnostic Laboratory at Rumuola from different clinical sources was 100% (n=50/50). The incidence of bacterial infection was higher in female 62% (n=31/50) than in male 38% (n=19/50). Our study found that Staphylococcus aureus infection was common among female patients. This might be explained by the fact that traditionally, in Nigeria, females are predominantly involved in contracting bacterial infections due to their exposure to the outside environment than males. It is also speculated that male sex hormones may modify the immune response and impact contracting infection (Soe et al., 2021).

According to our study association between Staphylococcus aureus infection and age was observed, the frequency of occurrence was higher in age of 21 to 30 years of age and a systematic literature review from India also showed similar evidence. Older age groups are more prone to get infected due to decreased host resistance and increased exposure to healthcare settings (Ghia et al., 2020). Among the different clinical isolates, the highest proportion of Staphylococcus aureus in urine specimens was 32% (n=16/50) whereas toe had the lowest occurrence of the target organism 10% (n=5/50). The result was in harmony to the one obtained by Soe et al. in 2021. This may be due to urinary catherization practice and the colonization by MRSA of indwelling urinary catheters (Soe et al., 2021).

The overall multidrug resistance level of all Staphylococcus aureus isolates from different clinical samples was 100% (n=50/50). Among the urine samples Ofoxacin (OFL) (100%) was found to have a very good efficacy on almost all the isolates whereas 100% resistant was recorded for Cefoxitin (FOX), Amoxillin (AUG), Ceftazidine (CAZ), Ciprofloxacin (ORX), Ceftriaxone (CTR) and Cloxacillin (CXC). Resistance to these antibiotics. Among isolates isolate from this source has been reported in two previous studies, one of which Danianyn et al. (2011) reported a complete resistance to Ceftriaxone (CTR) and Ciprofloxacin (CXC). Among the Toe samples Ofoxacin (OFL) (100%) and Gentamicin (GEN) (100%) was found to have a very good efficacy on almost all the isolates whereas the effect of other antibiotics varied. Among the hair samples Ofoxacin (OFL) (100%) was found to have a very good efficacy on almost all the isolates whereas the effect of other antibiotics varied. Result obtained from HVS sample shows that the isolates were able to resist the effect of Cefoxitin (FOX), Amoxillin (AUG), Ceftazidine (CAZ), Ciprofloxacin (ORX), Ceftriaxone (CTR) and Cloxacillin (CXC) whereas Gentamicin (GEN) (100%) were found to be most effective. Among the ear samples one of the isolates 17% resistance was observed for Ofoxacin (OFL), the result also shows that effect of antibiotics used varied among isolates from this source. This high rate of antibiotic resistance might reflect the inappropriate use of antibiotic, lack of laboratory diagnostic tests for appropriate antibiotic selection, unavailability of guideline for the selection of antibiotics, unskilled practitioners, expired antibiotics, self-medication, counterfeit drugs, or inadequate hospital control measures.

All Staphylococcus aureus isolates tested in this study were completely resistant to Cefoxitin (FOX), Ceftazidine (CAZ), Ciprofloxacin (ORX), Ceftriaxone (CTR), and Cloxacillin (CXC). A similar result was also reported in a study where complete resistance of S. aureus to ceftazidime was observed. According to the result obtained from these studies the isolates were moderately to erythromycin (ERY) and gentamicin (GEN) this result is in harmony to the one obtained by Agbawga and Jirigwa (2015) who reported 95.65% susceptibility to ofloxacin. Similar result was also obtained by Abdelghafar et al. (2020). Their work was in agreement with the study carried out by Hanif et al. (2019), where results obtained suggested ceftriaxone, ciprofloxacin, augmentin, ofloxacin and gentamicin as drugs of choice for Staphylococcus aureus.

The type of sample used had significant effect on the percentage recovery in the studied Hibiscus sabdariffa extracts. The yield of 25.23% was obtained for the white while the yields from red Hibiscus sabdariffa is 24.85%. Phytochemicals present in red and white Hibiscus sabdariffa are Saponin, Alkaloid, Flavonoid, Tanin Flavonoid and Terpenoids whereas Tanin was only found in the white Hibiscus sabdariffa, similar result was obtained by Adegunloye et al., 1996. Plant bioactive compounds/ Phytochemicals are known to for their ability protect the plant against bacterial and fungi causing harm to the plant, they are known to play important roles in bioactivity of medicinal plants. (Ibisanni and Aribisala, 2022). Flavonoids were, however, more in the red calyces than in the white calyces. Flavonoids which are part of the phytochemical constituents of Hibiscus sabdariffa exhibit a wide range of biological activities, one of which is their ability to scavenge for hydroxyl radicals, and superoxide anion radicals, and thus health-promoting action. Flavonoids also exhibit anti-inflammatory, antiangiogenic, antiallergic, analgesic and antioxidant properties (Hodek et al., 2002). In summary, these phytochemicals were present as the concentration increases.

According to the result obtained DPPH% Inhibition potential of DPPH% inhibition (2,2-diphenyl-1-picrylhydrazyl) (DPPH) is 25.23% was obtained for the white while the yields from red Hibiscus sabdariffa is 24.85%. Phytochemicals present in red and white Hibiscus sabdariffa exhibit a wide range of biological activities, one of which is their ability to scavenge for hydroxyl radicals, and superoxide anion radicals, and thus health-promoting action. Flavonoids also exhibit anti-inflammatory, antiangiogenic, antiallergic, analgesic and antioxidant properties (Hodek et al., 2002). In summary, these phytochemicals were present as the concentration increases.

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% Iron chelation is a therapy that prevents the accumulation of iron reaching harmful levels by matching iron intake from blood transfusion with iron excreted by iron chelation. The higher, the better. Therefore, white calyces are highly recommended for iron chelation. All the roselle calyces samples used were good sources of Vitamin C, which makes it a good source of disease combating compounds for the body. The effectiveness of antimicrobial agent varies with organism and type of extract used. Since microorganisms differ markedly in their susceptibility. The presence of the active principles in plants is influenced by several factors such as age of the plants, method of extraction and extracting solvent (Ibisanmi and Aribisala, 2022). It was observed that phytochemical constituents of extract of both white and Hibiscus sabdariffa are almost the same varied. Table 1 shows the Qualitative analysis of Hibiscus sabdariffa (Sun dried calyces of white and red roselle). All the phytochemicals were present in the white and red calyces except Tannin which was absent in the red calyces. These bioactive compounds that are known for their ability to protect the plant against bacterial and fungi in combating bacteria were confirmed in the extracts of sample after phytochemical screening (Amit and Ranjeeta, 2018). According to the result obtained from this studyHibiscus sabdariffa extract shows a great antimicrobial activity against50 Staphylococcus aureus isolated from different clinical sources, only one of the isolates was able to resist the effect of red Hibiscus sabdariffa at 200mg/ml whereas 2 different isolates were able to resist the effect of both red and white hibiscus sabdariffa at 100mg/ml, similar results was reported by Lim et al, 2020 red Hibiscus sabdariffa extract shows a greater inhibitory property against isolates used than the white Hibiscus sabdariffa extracts. This investigation had shown that the white and red Hibiscus sabdariffa extracts exhibited a potent inhibitory ability against studied isolates, although they showed less inhibitory potential on the studied in comparison with commercial antibiotics. This investigation had shown that the white and red Hibiscus sabdariffa extracts exhibited a potent inhibitory ability against studied isolates, although they showed less inhibitory potential on the studied in comparison with commercial antibiotics.

5. Conclusion
The overall prevalence of Staphylococcus aureus from different clinical sources was high. In this study, it was observed that S. aureus was a major pathogenic agent prevalent in clinical samples. Staphylococcus aureus isolates tested in this study were completely resistant to Cefoxitin (FOX), Cefazidime (CAZ), Ciprofloxacin (ORX), Cefotaxime (CTR), and Cloxacillin (CXC), while a fewer of them showed resistance to erythromycin (ERY) and gentamicin (GEN). The high level of resistance could be associated with exposure of these drugs to isolates which may have enhanced development of resistance. There is a high level of antibiotic abuse in developing countries such as Nigeria arising from self-medication associated with inadequate dosage and failure to comply with treatment and availability of antibiotics to consumers across the counters with or without prescription. Hence, appropriate action is needed to enhance the infection control programs in healthcare settings and to focus more on the appropriate use of antibiotics. Although complete eradication of Staphylococcus aureus infections is not possible, proper precautions should be taken to minimize the occurrence by strictly adhering to the choice of drugs for the treatment of Staphylococcus aureus from Staphylococcus aureus infections was quite narrow especially for strains which were resistant to most classes of antibiotics which have been used previously. To prevent further emergence and spread of multi drug resistant Staphylococcus aureus, rational use of antibiotics and regular monitoring of antimicrobial resistance patterns are essential and mandatory.

The incidence of bacterial infection was higher in female than in male. This might be explained by the fact that traditionally, in Nigeria, females are predominantly involved in contracting bacterial infections due to their exposure to the outside environment than males. It is also speculated that male sex hormones may modify the immune response and impact contracting infection. According to our study association between Staphylococcus aureus infection and age was observed, the frequency of occurrence was higher in age of 21 to 30 years of age although the new born and older age groups are more prone to get infected due to under developed immunity, decreased host resistance and increased exposure to healthcare settings. Among the different clinical isolates, the highest proportion of Staphylococcus aureus in urine specimens with frequency whereas toe had the lowest occurrence of the target organism. This may be due to urinary catheterization practice and the colonization by MRSA of indwelling urinary catheters.

The study shows that both red and white Hibiscus sabdariffa contain almost the same bioactive compound except tannin present in only white Hibiscus sabdariffa. According to the result obtained from this study, red Hibiscus sabdariffa extract shows a greater inhibitory property against isolates used than the white Hibiscus sabdariffa extracts. This investigation had shown that the white and red Hibiscus sabdariffa extracts exhibited a potent inhibitory ability against studied isolates, although they showed less inhibitory potential on the studied in comparison with commercial antibiotics.

Authors’ contributions
TAL: conceived and designed the experiments contributed to sample preparation. TAI & EAO & OBO & PKO & UAP: carried out the experiment, processed the experimental data, performed the analysis, drafted the manuscript, designed the figures and contributed to the interpretation of the results. TAI: involved in planning and supervised the work, contributed to the interpretation of the results, other contribution. All authors discussed the results and commented on the manuscript. All authors read and approved the final manuscript.

Conflict of Interest
The authors declare no Competing interest.

Acknowledgement
The Department of Microbiology, School of Life Sciences, Federal University of Technology Akure, Akure Nigeria, is acknowledged for providing the basic infrastructure for carrying out the research work.

List of abbreviation

References


