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Antibacterial Activity of Aqueous and Ethanolic Extracts of Leaf and Stem Bark of *Vitex doniana* on *Staphylococcus aureus*

Ubandoma Andefiki[1, *], Imarenezor Edobor Peter Kenneth[1], Brown Samuel Tamunoiyowuna Cockeye[1], Aregbe Elizabeth Adenike[2], Simon Shedrach[1]

[1]Department of Microbiology, Faculty of Pure and Applied Sciences, Federal University Wukari, Wukari, Taraba State, Nigeria

[2]Department of Microbiology, Faculty of Life Science, Ahmadu Bello University, Zaria, Nigeria

Email address

andefiki.ubandoma@fuwukari.edu.ng (U. Andefiki), andefiki13@gmail.com (U. Andefiki)

[*]Corresponding author

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Abstract

The aim of this study titled "antibacterial activity of aqueous and ethanolic extracts of leaf and stem bark of *Vitex doniana* on *Staphylococcus aureus*" was to verify the pharmacological property of the plant for possible use in the remedy of diseases especially against antibiotic-resistant agents. It is widely suggested that a vast array of plants serve medicinal purposes without scientific proofs. Materials used for the study include culture media, spectrophotometer and other reagents. The plant sample was collected from within the environment of Federal University Wukari before the extraction of its materials using the cold maceration method. Phytochemical screening was done with the plant being found to contain bioactive substances such as alkaloid, flavonoid, tannins, cardiac glycosides, resins terpenes and steroids. The pH of both leaf and stem bark extracts was between 5 and 7. The extracts of the stem bark had minimum inhibitory concentration (MIC) of 4000 µg/ml on *S. aureus* while the leaf extracts had MIC greater than 4000 µg/ml. From the spectrophotometric absorbance, it was seen that different concentrations of extracts had different degrees of antibacterial activity on the test organism. This implies that *V. doniana* has antibacterial activity on *S. aureus* and therefore suggests it can be used as an alternative remedy for treating bacterial diseases.

Keywords

Vitex doniana, *Staphylococcus aureus*, Antibacterial, Leaf and Stem Bark

1. Introduction

A lot of plants are believed to be of medicinal origin all over the world but the treatment of some disease conditions using these plants as claimed, have not been subjected to scientific methods for verification. This is why this study aims at assaying the antibacterial activity of *Vitex doniana* on *Staphylococcus aureus* isolate.

It is known that plants used as herbal remedy for curing various pathogenic diseases by man has been practiced for as long as man himself has existed [1]. Also, plant's derived bio-resources are widely utilized all over the world as food, medicine as well as being used for making shelter and clothing materials by man [2].

Vitex doniana commonly known as Black plum (the fruit), is in some indigenous Nigerian languages called Dinya (in Hausa), Ori-nla (Yoruba) and Uchakoro (Igbo) [2, 3]. *Vitex doniana* sweet is a perennial shrub widely distributed in tropical Africa and some East African countries including Kenya, Uganda, Tanzania, widely distributed in different parts of Nigeria [3]. The genus has 250 species [4].

Fruits from the plants are eaten raw and in some cases, cooked in some places when ripened. It has a sweet taste [5].

The plant has been reported to be used in the treatment of stomach and rheumatic pains, inflammatory disorder,

diarrhoea and dysentery. The leaves (foliage) are used for making soup, salad in parts of Nigeria (Adamawa, Taraba, Benue and Enugu). It is also used in curing nausea, colic and epilepsy i.e. the leaf and roots [6]. It is used by some locals in Nigeria to treat conditions such as gastroenteritis (diarrhea and dysentery), by decoction of the stem bark among the people in the western part of Nigeria [7]. A research conducted on the plants shows that the plant has antidiabetic activity which was tested on streptozotocin-induced diabetes rats [8]. The fruit is known to contain high nutritive value as it contains proteins, fats, carbohydrate, vitamin C and B complex's [9]. It is said to contain 16.6% moisture content, 11.50% Ash content, 8.24% crude protein content, 0.58% crude fiber content, 34.61% crude fat content and 28.40% carbohydrate content. The fruit also is determined to be rich in vitamins where it contain 0.27 mg/100 vitmain A, 18.33 mg/100 vitamin B_1, 4.8 mg/100 vitamin B_2, 20.45 mg/100 vitamin B_6 and 35.58 mg/100. Also, the fruit has been found to be rich in minerals with values, potassium content of 15.7 mg/Kg, Sodium 10.4 mg/Kg, Calcium 30.27mg/Kg, Phosphorus, 16.50 mg/Kg, Magnesium 20.10 mg/Kg, Iron 5.2 mg/Kg and Copper 2.70 mg/Kg [9].

In Nigeria, from information available from the indigenous traditional healers, a decoction of the chopped stem-bark part of *Vitex doniana* is prepared and taken orally [2]. It is administered for ailments including diarrhoea and dysentery. It is also taken to improved fertility and the juice may be squeezed into the eyes to treat eye troubles [7].

Staphylococcus aureus is a gram-positive cocci of the family *Micrococcaceae*, they are characteristically halotolerant bacteria which are usually highly sensitive to lysostaphin, these organisms are facultatively anaerobic and chemoorganotrophic [10]. They are non-motile and asporogenic. The cells occur in irregular bunches rather than ordered chains [10, 11].

According to [12], *Staphylococcus aureus* are associated with skin, skin glands and mucous membranes of almost all the warm blooded animals. These properties allow *Staphylococcus aureus* to be a normal inhabitant of the human skin, where it can sometimes give rise to dermatological conditions [13]. The bacterium is also widely present in a lot of environments. About one third human populations support the colonization of Staphylococcus aureus and are designated as carriers. The nasal carriage of *Staphylococcus aureus* occurs in 40-50% of human population. The major habitat of this bacteria on human is the anterior nare or on skin elsewhere. Patients in the hospitals and workers have the highest carriage of these organisms [12].

Staphylococcus aureus has unmatched versatility and exceptional adaptability to survive, propagate and produce disease in host because of a large number of antigens, toxins and enzymes liberated by his organisms. These surface antigens include capsule, polysaccharide A and protein A. Enzymes such as Coagulase, Staphylokinase, Hyaluronidase, Nuclease, Lipase, Phosphatase, Penicillinase are protease produced by some strains of *Staphylococcus* while toxins produced include Haemolytic toxins as Alpha-lysin, Beta-lysin, Gamma-lysin and Delta lysin, other toxins include leucocidins, enterotoxins, toxic shock syndrome toxin (TSST) and epidermolytic toxin. *Staphylococcus aureus* causes bronchitis, which is the inflammation of the bronchi; the condition may be acute or chronic and often follows) upper respiratory tract infection [10]. Osteomyelitis which is the Inflammation of bone is caused by some bacterium in which *Staphylococcus* is one via wounds or blood from another site of infection (e.g. skin, throat); it may occur as a complication of septicemia. The commonest causal agent is *Staphylococcus aureus*. Acute osteomyelitis occurs most commonly in children and affects mainly the long bones of the arms and legs [10].

This study is therefore important as it will justify to an extent, the medicinal quality of this plant when processed to provide a cheaper and effective means of handling disease conditions.

2. Materials and Methods

Materials used for the study include nutrient broth, peptone water tubes of *Staphylococcus aureus,* sterile test tubes, sterile pipettes, test tube holders, sterile distilled water, spectrophotometer, concentrated extract of stem bark, and leaf of *Vitex doniana,* Augmentin, Laclox, Tetracycline and Ciprofloxacin.

2.1. Collection of Plant Material

The leaf and stem bark of *Vitex doniana* were collected from Federal University Wukari environment in Wukari, Taraba state, Nigeria. The plant was identified in the Department of Forestry, Faculty of Agriculture and Life Science of the University. The leaves were selectively collected; leaves which were matured with noticeable colour changes, tender leaves and leaves which have patches on them were removed and discarded. The leaves were taken to the laboratory, rinsed with tap water and air dried on a clean surface to avoid contamination.

The stem bark was collected using a cutlass. The dead cells found at the outer layer of the stem bark were scraped using a clean cutlass washed with borehole water ensuring no rust or sign of corrosion was seen. The fleshy layer of the stem bark was collected and taken to the laboratory. The stem bark collected was put in an oven at temperature of 40°C to reduce water level for two days as well as maintaining the phytochemical components as described by [17]. The plants were pulverized using clean mortar and pestle and the powder stored in a clean bottle before its grinding with a grinding machine in the Food Science and Technology Laboratory of the Federal Unversity Wukari.

2.2. Extraction of Plant Material

Extraction of the plant sample was carried out using the cold maceration method. Two different solvents were used; aqueous (water) and organic (ethanol). For aqueous extraction the pulverized plant material i.e. both the leaves and stem bark were soaked into 1:10 volumes (W/V) of distilled water as

described by [2]. The plant extract was then kept for 4 days with vigorous shaking from time to time and then filtered using a cotton wool.

The organic (ethanol) extraction was carried out using 80% ethanol as described by, [17] where 1:10 weight per volume (W/V) of 80% ethanol was used, as described by [7] to extract both the leaf and stem bark. The resulting extract was then recovered from the mixture by filtration using a Whattman number 1 filter paper after a period of 48hrs. The extracts were dried by evaporation to dryness, where the ethanolic extracts were concentrated in open air, the aqueous extracts were evaporated to dryness at 60°C as described by [18], in which hot air oven was used instead of water bath.

2.3. Test Organism

Isolate of *Staphylococcus aureus* was collected from the National Veterinary Research Institute, (NVRI) Vom, Plateau State, which was taken to the laboratory in Federal University Wukari where it was confirmed using gram staining method and biochemical tests.

2.4. Phytochemical Screening

Phytochemical screening was performed on different extracts to ascertain the presence of bioactive components present in the leaves and stem bark of *Vitex doniana*.

2.4.1. Test for Alkaloids

Few drops of Wagner reagent were added to 2.0 ml of extract and observed for the formation of deep brown precipitate to indicate the presence of alkaloid as described by [19].

2.4.2. Test for Flavonoids

2 ml of extract was added to 10% lead acetate solution and observed for either cream or light yellow coloration confirming the presence of flavonoid.

2.4.3. Test for Tannins

2 ml of the aqueous extract filtrate and 3 ml distilled water were put into a test tube. A few drops of 0.1% ferric chloride was added to the mixture. The formation of a very dark precipitate indicated presence of tannin.

2.4.4. Test for Cardiac Glycosides

2 ml of concentrated H_2SO_4 was carefully added down the side of the tube containing 2.0ml of the extract and observing for the formation of a layer of interphase of reddish brown colour indicative of cardiac glycoside.

2.4.5. Test for Saponins

1 ml of extract was added to 4.0 ml of distilled water in a test tube and the tube was stopped and shaken vigorously for about 30 seconds and was then allowed to stand for half an hour. A honey comb-froth formation was an indication of the presence of saponins.

2.4.6. Test for Terpenes and Steroids

2 ml of the extract was mixed with 1.0 ml acetic anhydride followed by the addition of 1.0ml concentrated H_2SO_4 carefully down the side of the test tube while observing for the formation of interphase layer of reddish brown colour which showed the presence of terpenes and steroids.

2.4.7. Test for Resins

Small amount of the plant extract was dissolved in acetic anhydride and 1 drop of concentrated sulphuric acid was added. Development of a purple or violet colour indicated presence of resin.

2.5. Serial Dilution of Bacterial Isolate

Tenfold serial dilution was carried out as described by [20]. This dilution was carried out to reduce the number of viable cells.

2.6. Standardization of Extracts

8000 µg/ml of the plant extract was prepared by dissolving 0.08 g or 80 mg of the extract in 10 ml of distilled water to give the required concentration.

2.7. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by using the technique as described by [20]. Five rows of tubes were labeled and arranged in row as 1/2, 1/4, 1/8, 1/16 and 1/32. 5 ml of Nutrient broth was dispensed into each of the tubes. 5 ml of the prepared 8000 µg/ml extract was measured using a sterile syringe and serial dilution of the extract was done with the nutrient broth dispensed in the tubes to the tube with 1/32, and the last 5 ml pipette was discarded. Again 0.2 ml of the bacterial dilution (1×10^3 cfu/ml) was inoculated into the various test tubes and incubated for 24 hours at 37°C. The optical density was measured at 540 nm with a spectrophotometer. MIC was determined as the highest dilution (i.e. lowest concentration) of the extracts that showed no visible growth after 24 hours. For the the standard antibiotics, after inoculation it was left for 5 hours before the absorbance was taken in order for it to stabilize as described by [21]. The spectrophotometric absorbance was taken to determine and compare the level of bacterial growth in each of the tubes since the more the bacteria grow, the more turbid the becomes [20].

2.8 Determination of Minimum Bactericidal Concentration (MBC) of the Crude Extracts

MBC was determined by first selecting tubes that show no growth during MIC determination. A sterile wire loop was used to pick the extract from each of the tube with different concentration and was sub-cultured on the sterile Nutrient agar medium and incubated for 24 hours at 37°C. The MBC was determined as the highest dilution showing no visible growth in the nutrient broth.

3. Results

Table 1. *Phytochemical constituents of leaf and stem bark of Vitex doniana.*

PHYTOCHEMICAL COMPONENT	LEAF	STEM BARK
Alkaloid	+	+
Flavonoid	+	+
Tannins	+	+
Saponins	-	-
Terpenes and Steroids	+	+
Cardiac glycosides	+	+
Resins	+	+

Key: (+) Presence of phytochemical component; (-) Absence of phytochemical component

Table 2. *pH value of the extracts of leaf and stem bark of Vitex doniana.*

EXTRACT	pH VALUE
Aqueous extract of leaf	6.05
Aqueous extract of stem bark	6.03
Ethanolic extract of leaf	5.12
Ethanolic extract of stem bark	5.07

Table 3. *Minimum inhibitory concentration and minimum bactericidal concentration of commercial antibiotics on Staphylococcus aurues.*

ANTIBIOTICS	MIC	MBC
Tetracycline	>25 µg/ml	-
Ciprofloxacin	>25 µg/ml	>25 µg/ml
Augmentin	>25 µg/ml	-
Laclox	>25 µg/ml	>25 µg/ml

Table 4. *Minimum inhibitory concentration and minimum bactericidal concentration of extract of Vitex doniana on Staphylococcus aureus.*

EXTRACTS	MIC	MBC
Aqueous leaf	>4000 µg/ml	-
Ethanol leaf	>4000 µg/ml	-
Aqueous stem	4000 µg/ml	-
Ethanol stem	4000 µg/ml	>4000 µg/ml

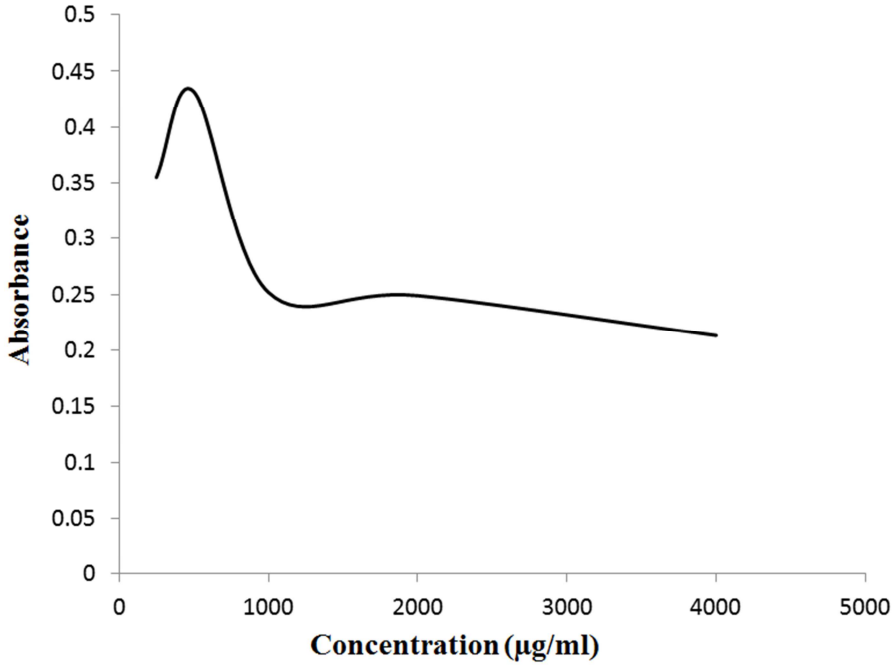

Figure 1. *Graph of concentration of aqueous leaf extract against growth absorbance of Staphylococcus aureus.*

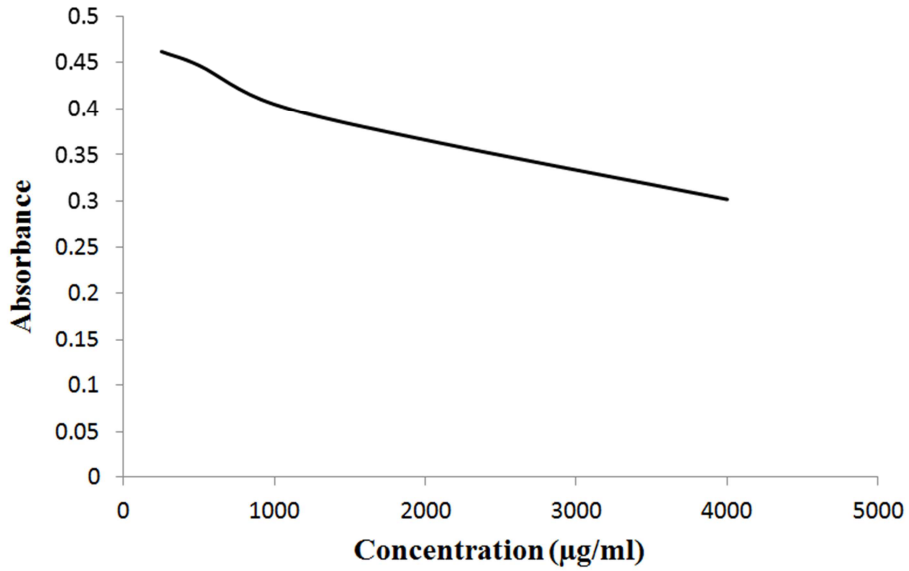

Figure 2. *Graph of concentration of ethanolic leaf extract against growth absorbance of Staphylococcus aureus.*

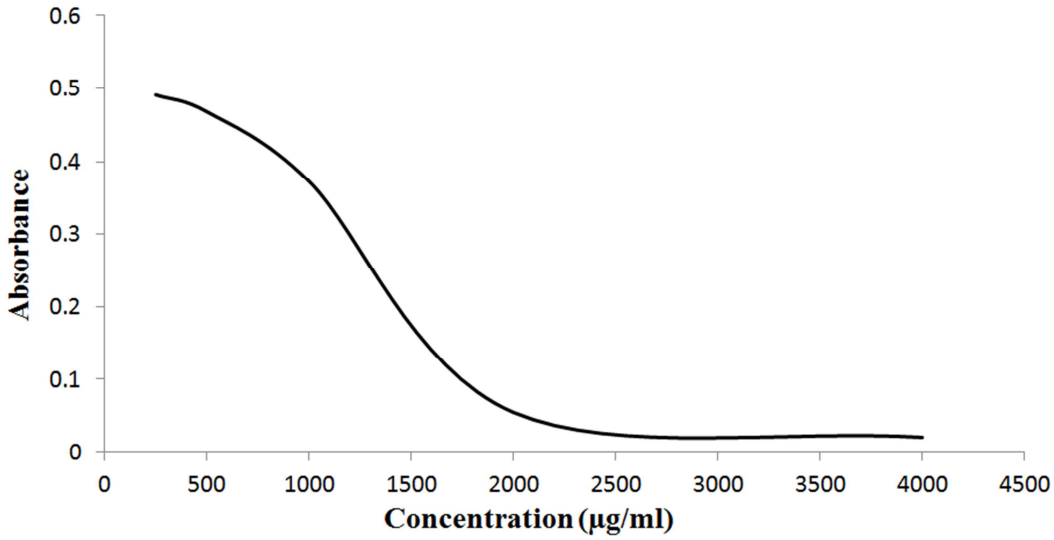

Figure 3. *Graph of concentration of aqueous stem bark extract against growth absorbance of Staphylococcus aureus.*

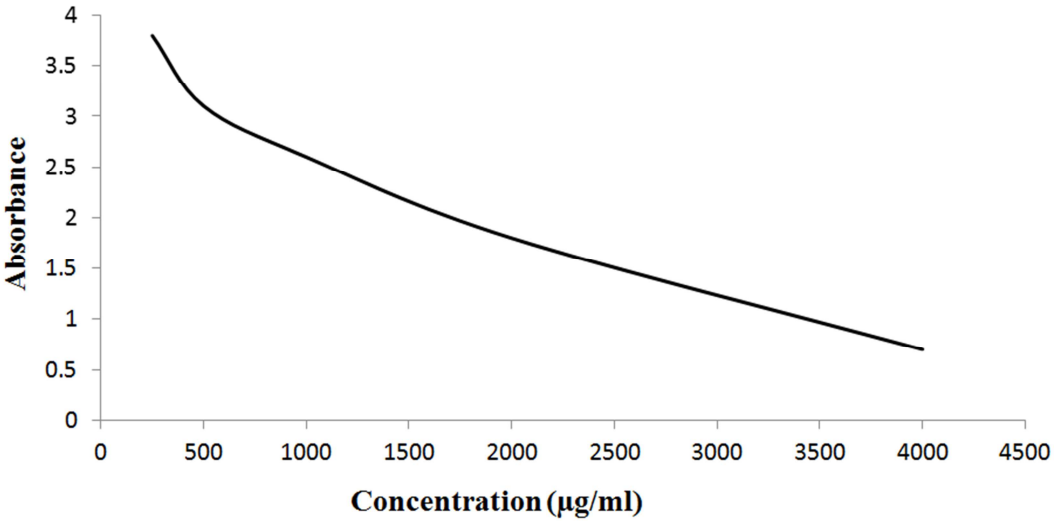

Figure 4. *Graph of concentration of ethanolic stem bark extract against growth absorbance of Staphylococcus aureus.*

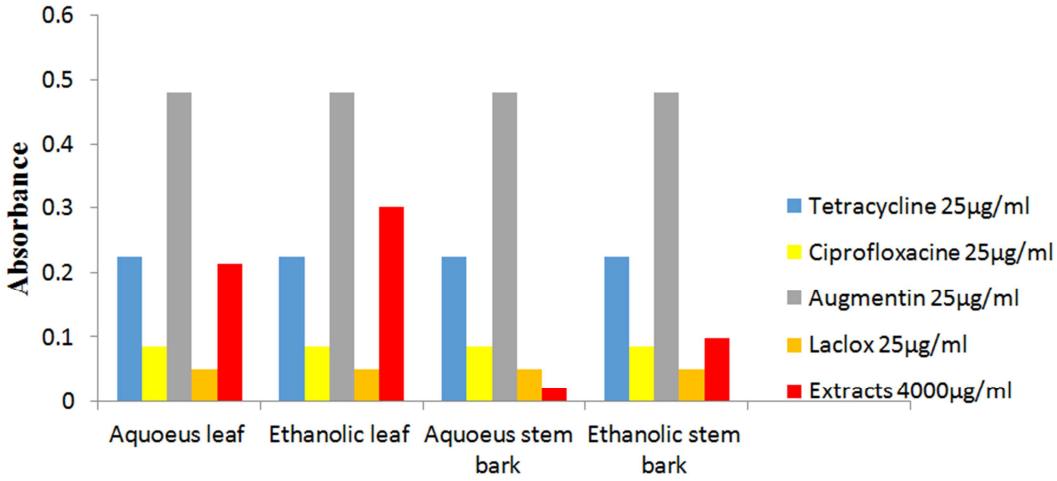

Figure 5. *Comparison of the growth absorbance of Staphylococcus aureus in commercial antibiotics and extracts of Vitex doniana.*

4. Discussion

The phytochemical results of the crude extracts of *Vitex doniana* showed the presence of Alkaloids, Flavonoids, Tannins, Terpenes and Steroids, Cardiac glycosides, and Resins, while Saponin was absent. The phytochemical components present in these crude extracts of the plant confer antibacterial activity to the plant [14]. The medicinal and therapeutic value of the plant lies on these plants secondary metabolites produced, hence resulting to varying degree of activity.

The pH value of the aqueous extracts is higher (>6) compared with the ethanolic extracts. Also, for both ethanolic and aqueous extracts, the pH value of the crude extract of the stem bark are lower than that of the leaf extracts. *Staphylococcus aureus* grows in pH range of 4-10, and an optimum pH value of 6-7 [15]. This therefore suggests that phytochemicals are the inhibiting component of the plant to microbial growth because the pH values of both leaf and stem bark extracts fall within the normal pH range of 4-10.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the standard antibiotics (standard) differs majorly due to the active group components of the antibiotics. Laclox (Amoxicillin + cloxacillin) are β-lactams together with Augmentin, which have more than 25 µg/ml MIC for *Staphylococcus aureus*. Tetracycline belongs to the group called the amynoglycoside and had MIC greater than 25 µg/ml while ciprofloxacine is a synthetic antibiotic belonging to the quinolone group and interferes with nucleic acid synthesis. Also, the β-lactams interferes with cell wall synthesis while tetracycline are protein inhibitors [13]. The nucleic acid inhibitor (ciprofloxacin) was more effective compared to the other standard antibiotics against *Staphylococcus aureus*.

The plant extracts showed varying degree of activity against the test organism. The activity of the stem bark was higher than that of the leaf on the organism. The MIC of both aqueous and ethanolic extracts of stem bark for the test organism is 4000 µg/ml and the crude extracts of the leaf have MIC greater than 4000 µg/ml. The phytochemical components could therefore be more concentrated in the stem than the leaf. This result is in agreement with that obtained by [21], in which the activity of the ethanolic extracts of the leaf on *Staphylococcus aureus* was 10.7 mg/ml (10700µg/ml).

Spectrophotometric absorption graph of the concentration of the plant extracts plotted against absorbance of growth of the organism showed a negative curve. The result obtained indicated that at higher concentration, lower bacterial growth was obtained and at lower concentration a higher bacterial growth was obtained hence higher absorbance. The extract had greater activity on the organism at higher concentration than at lower concentration.

In comparing of the activity of the extracts of *Vitex doniana* with some commercial antibiotics, it was seen that among the commercial antibiotics, *Staphylococcus aureus* had the highest resistance against Augmentin, with spectrophotometric growth absorbance of 0.482. Tetracycline had a growth absorbance of 0.255, Ciprofloxacin had growth absorbance of 0.085 and Laclox with absorbance of 0.051. This indicates that among the commercial antibiotics, the organism (*Staphylococcus aureus*) was most susceptibility to Laclox. Augmentin showed highest resistance against *S. aureus*. In comparing the plant extract at 4000 µg/ml to the commercial antibiotics, ethanolic leaf extract had absorbance of 0.304 which shows that it had more activity than Augmentin at 25 µg/ml and aqueous leaf had more activity than Augmentin and Tetracycline. Similarly, the ethanolic stem bark extracts had more activity than both Augmentin and Tetracycline. Again, the aqueous extract of the stem bark had more activity than stem bark ethanolic extracts and the aqueous and ethanolic extracts of the leaf. This suggests that aqueous extract of the stem bark is more effective in terms of antibacterial activity.

5. Conclusion

This study has shown that *Vitex donania* has an antibacterial activity on isolate of *Staphylococcus aureus* at least, in vitro. It has also shown that phytochemicals present in the plant are

responsible for the antibacterial activity of *Vitex doniana.* This result suggests that this plant can be used as an alternative future remedy for treating bacterial diseases with current resistance developed by bacteria against some antibiotics.

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