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**Research Article**

# Invitro Susceptibility Assay of Aqueous Fruit Extract of *Vitex doniana* for Antimethicillin Resistant *Staphylococcus aureus* Activity

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## ABSTRACT

The use of *Vitexdoniana* in herbal medicine has a long standing history. This research assessed the *invitro* susceptibility of Methicillin Resistant *Staphylococcus aureus* (MRSA) to aqueous fruit extract of *V. doniana.* Proximate and phytochemical compositions of *Vitex doniana* fruit were determined and various concentrations of the aqueous extract tested for i*nvitro* antiMRSA activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). *V. doniana* fruit has high lipid content of 31.40±1.24%/100g Dry Weight (DW) with fibre content of 0.90±0.10%/100g DW. Potassium, sodium, phosphorus, copper, magnesium and iron were present in the fruit with phosphorus accounting for 36% per 100g DW and copper accounting for 2.10±0.01%/100g DW. Zones of inhibition of aqueous *V. doniana* fruit extract against MRSA at 10mg/ml, 20 mg/ml, 30 mg/ml and 40 mg/ml were 17.5±0.20mm, 22.0±0.02mm, 25.0±0.10mm and 28.0±0.45mm respectively. The zone of inhibition of methicillin (30µg) was 6.0±0.23mm and that of vancomycin (30µg) was 37.6±0.10 mm. Against Methicillin Sensitive *Staphylococcus aureus* (MSSA) at the same concentrations of 10 mg/ml, 20 mg/ml, 30 mg/ml and 40 mg/ml, zones of inhibition of 17.0±0.13 mm, 23.0±0.40 mm, 24.5±0.11 mm and 29.0±0.20 mm were recorded, whereas 30µg methicillin (negative control) and 30µg vancomycin (positive control) zones of inhibition were 36.0±0.21 mm and 38.0±0.11 mm respectively. The MIC of the extract against MRSA and MSSA was 250 µg/ml while the MBC was 500 µg/ml, values higher than 1.36 µg/ml MIC and 1.85 µg/ml MBC of methicillin and 0.49 µg/ml MIC and MBC of vancomycin against MRSA. The MIC and MBC of methicillin and vancomycin against MSSA were equal (0.24 µg/ml). Aqueous *V. doniana* fruit extract exhibited significant antimicrobial activity against MRSA ($p>0.05$), which increased proportionally with increase in concentration of the extract, although the MIC and MBC were high. Further research should be conducted to evaluate the *invivo* activity of the extract while isolating the bioactive substance responsible for the observed *invitro* antiMRSA activity of the extract.

**Keywords**: *Susceptibility,Aqueous, Fruit, Extract, Vitex doniana, Methicillin Resistant Staphylococcus aureus (MRSA)*

## [I] INTRODUCTION

For decades, natural products have been a wellspring of drugs and drug leads. Sixty-one percent (61%) of the 877 new molecular entities introduced as drugs worldwide during 1981 – 2002 and even beyond can be traced to or inspired by natural products (Balunas and

Kinghorn, 2005). Accordingly, these include natural products (6%), natural product derivatives (27%), synthetic compounds with natural product derived pharmacophores (5%), and synthetic compounds designed on the basis of knowledge gained from a natural product (23%).

Traditional and complementary medicine (TCM) has a long history and its practice is as old as man's existence (WHO, 2019). It is the sum total of knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO, 2019). Thus, plants with medicinal value play a major role in the primary healthcare of about 80% of the world population (WHO, 2019). Despite the ravaging effects caused by herbivores and microorganisms, some of these medicinal plants although without protective structures, are untouched in their habitats. This is perhaps due to distasteful or toxic substance they contain which offers them protection against natural enemies and in addition are toxic to cells(Koduru *et. al.*, 2007).

In the recent years, research interest is focused on various plants due to downturn in the use of antibiotics derived from microorganism including the limited lifespan of such agents (Balunas and Kinghorn, 2005). Again, the incidence and severity of diseases caused by microorganisms is on the increase, while drug resistance as a result of fast development of resistance strain is a big threat to the management of infectious diseases (Nwobodo, 2014).

Consequently, considerable attention has been focussed on screening of plant extracts for possible antimicrobial activity. Such endeavours have been undertaken with the aim of isolating bioactive compounds as an alternative to chemotherapeutic agents. This of course has given interesting results in that most plant-derived antimicrobial compounds kill or inhibit the growth of microorganisms by interfering with one or more of the steps in their growth and developmental cycle (Vlietinck*et. al.*, 1995).

Extracts of *V. doniana* has been found effective in the treatment of infectious diseases caused by diverse groups of microorganisms- bacteria, fungi, viruses, and parasites (Kilani, 2006).

Since most microbial infections possess a great threat to global health and food security, the need for an increasing research for newer, safer and affordable drugs to supplement the existing ones can never be overemphasized (Nwobodo, 2014; Mack, 1982).

Traditional medicine has been an important source of products for developing countries in treating common infections including disorders of gastrointestinal tract, skin, bronchopulmonary, and urinary systems (Tahraoui*et. al.*, 2007).

Africa with its geographic position in the tropics has favoured the development of these plants [9]. *Vitex doniana* is an example of such plant used in traditional medicine, a genus of flowering plants in the family Lamiaceae (World Agroforestry Center, 2019).

*V. doniana* has various local names; plem in Amharic, Mufutu in Bemba, black plum, vitex, African oak in English, Prunier noir in French, galbihi in Fula, Dinya in Hausa, uchakoro in Igbo, munyamazi in Luganda, kashilumbulu in Lunda, mfutu, msimsya, mfifya, mfimfya in Nyanja and mfudu, mfulu, mfuru in Swahili (World Agroforestry Center, 2019). It is a medium-sized deciduous tree, 8-18 m high, with a heavy rounded crown (Wickens, 1973). The bark is rough, pale brown or greyish-white, rather smooth with narrow vertical fissures (Wickens, 1973). The bases of old trees have oblong scales. The leaves are opposite and glabrous measuring 14-34 cm long, usually with 5 leaflets on stalks that are 6-14 cm long. Leaflets are distinctly stalked, ovate, obovate-elliptic or oblong, entire and measure 8-22 cm long and 2-9 cm wide. Leaf tips are rounded or emarginated, while the bases are cuneate. The leaves are dark green above, pale greyish-green below, thickly leathery, with

or without a few scattered stellate hairs on the upper surface (Wickens, 1973).

The flower petals are white except on largest lobe, which are purple, dense opposite and axillary cymes (Abubakar*et. al.,* 2015). Flowers are small, blue or violet measuring 3-12 cm in diameter, only a few being open at a time. The fruit of *V. doniana* which measures 3 cm long is oblong, green when young, turning purplish-black on ripening and with a starchy black pulp. Each fruit contains 1 hard, conical seed of size 1.5-2 cm long and 1-1.2 cm wide (World Agroforestry Center, 2019).

*Vitex* species (*V. piramidata, V. pubescens, V. agnus-castus, V. doniana, V. gaumeri, V. trifolia, V. cienkowskii, V. rehmannii*) have been reported to be used in traditional medicine to treat a wide range of ailments, such as depression, venereal diseases, malaria, asthma, allergy, wounds, skin diseases, snake bite, inflammation and body pains as well as gastroenteritis, diarrhoea, dysentery, infertility, eye diseases, anaemia, jaundice and leprosy (Amegbor*et. al.*, 2012; Muhammad*et. al.,* 2013). Additionally, *V. doniana* have antimicrobial, invigorating and anti-inflammatory actions (Moke*et. al.,* 2018). An ethanolic extract of *V. doniana* leaf was found to reduce spontaneous motor activity and produced significant inhibition of granulation tissue formation while a cold aqueous infusion reduced total serum cholesterol (Moke*et. al.,* 2018). In acute inflammation, a cold aqueous infusion as well as a mixture of flavonoids of *Vitex leucoxylon* exhibited an anti-inflammatory activity without any effect on chronic inflammation (Moke*et. al.,* 2018). Decoction of young leaves is used to manage cough, cold, diarrhoea and dysentery. The roots cooked in water are used for the treatment of diabetes, anaemia, conjunctivitis, dysentery, diarrhoea, fatigue, headaches, mental disorders, respiratory problems, evil back among women, leprosy, fever and jaundice (Moke*et. al.,* 2018). The plant extract showed a good antimicrobial activity especially against bacteria and parasite (Nyiligira *et. al.,* 2008). Anecdotal evidence showed that the edible portion of the fruit (endocarp) is used in local treatment of wound infections.

*Staphylococcus aureus* is a versatile and dangerous bacterial pathogen (Yang*et. al.,* 2017). It is a major cause of skin, soft tissue, respiratory, bone, joint and endovascular disorders (CDC, 2007). The most common types of *Staphylococcus aureus* are the *Methicillin-SensitiveStaphylococcus aureus* (*MSSA*) - a common type of Staphylococcus that is vulnerable to the methicillin class of antibiotics and therefore easier to treat (CDC, 2007). Others include; *Vancomycin-Resistant Staphylococcus aureus* (VRSA), a rare type of *Staphylococcus aureus* that has become immune to a common "last resort" antibiotic called vancomycin as well as *Vancomycin-Intermediate Staphylococcus aureus* (*VISA*) which are only partially resistant to the vancomycin (CDC, 2007), and the *Oxacillin-ResistantStaphylococcus aureus* (*ORSA*) which are resistant to Oxacillin, an antibiotic of the same class as methicillin (CDC, 2007).

Treatment of Staphylococcal infections has become more difficult due to the emergence of multidrug resistance strains such as *Methicillin Resistant Staphylococcus aureus* (*MRSA*) (Edwards and Harding, 2004). The *S. aureus* resistance to methicillin confers resistance to all penicillinase resistant penicillins and cephalosporins (CDC, 2007). The high level of resistance requires the presence of the mec gene that encodes penicillin-binding protein 2a (Chambers, 1997). The expression of this gene is often heterogenous and the percentage of bacterial population that expresses the resistance phenotype varies according to the environmental conditions and antimicrobial testing modified to enhance the detection of the resistance phenotype (Chambers, 2001).

Multidrug resistance notwithstanding, a number of antibiotics such as vancomycin, linezolid, daptomycin and clindamycin have been effective

in treating MRSA infections, although with resistance by some strains like VRSA to vancomycin and occasional severe side effects that can include ringing in ears, diarrhea and hearing problem among others (Yakubu*et. al.*, 2016).

Various parts of *V. doniana* are used by traditional medicine practitioners in Nigeria and beyond to manage several disorders such as cough, rheumatism, hypertension, cancer and inflammatory diseases (Yakubu*et. al*., 2016). With the purported medicinal applications and efficacy of *V. doniana* locally, there is need to prove or establish a scientific basis for this practice. In the study therefore, aqueous fruit extract of *V. doniana* was assayed for invitro antiMRSA activity.

## [II] MATERIALS AND METHODS

### 2.1 Plant fruitsource, identification and preparation

*Vitex doniana* fruits were collected from ObuoffiaAwkunanaw in Nkanu-West Local Government Area of Enugu State, Nigeria in the month of August, 2019 and taken to the department of Botany, University of Nigeria where it was authenticated by plant taxonomists as *Vitex doniana*. Riped fruits (see figure 1) were collected through hand picking from the tree crown and transported to the laboratory in an airtight polyethylene bag.


Figure 1: a. Ripe and unripe *V. doniana* fruit on the plant. b. Harvested ripe *V. doniana* fruit

The fruits were washed with distilled water and air dried at room temperature until they became friable (Egereonu and Mokwe, 2005). The endocarp, referred to as the fruit in this work, was separated from the seed by mechanically crushing the fruit in between the palms of the hands. The dried fruit was ground with porcelain mortar and pestle to fine particles and stored in an air tight plastic container at room temperature using a standard method (Egereonu and Mokwe, 2005) until processed further by solvent extraction.

### 2.2Proximate analysis

Proximate analysis of moisture, protein, fat, crude fiber and carbohydrate contents of *V. doniana* fruit were carried out according to the procedure described by the Association of Official Analytical Chemist (Association of Official Analytical Chemists, 1990).

### 2.3Phytochemical analysis

The aqueous fruit extract was evaluated for the presence of alkaloids, tannins, glycosides, saponins, steroids and flavonoids using a standard method (Agomuo*et. al.*, 2004; Sofowora, 2005).

### 2.4 Mineral analysis

Mineral content of the extract was analyzed (Association of Official Analytical Chemists, 1990; Umar*et. al.*, 2007). One hundred (100) gram of the ground sample was digested with 24 $cm^3$ mixture of the conc. $HNO_3$, Conc. $H_2SO_4$ and 60% $HClO_3$ (9:2:1 v/v) and mineral constituents (Sodium, Potassium, copper, iron and magnesium) analyzed using LI-180 Spectrometer.

### 2.5 Preparation of crude extract

The active ingredient in the fruit was extracted using a standard method (Okerulu and Ani, 2001). Forty grams (40g) of the ground seed was added into 250ml of distilled water in a conical flask and the mixture stirred six hourly for 24 hours. The mixture was filtered through Whatman No. 1 filter paper and the filtrate concentrated by evaporation in a water bath at 40$^oC$ until all the solvent was

removed. Concentrated filtrate was weighed and stored in a dark air-tight vial at room temperature until used. The extract was reconstituted by dissolving 5g in 10 ml of sterile phosphate buffered saline (PBS) to obtain a stock solution of 500 mg/ml. The stock solution was sterilized using a millipore membrane filter (0.45 µm pore diameter) and diluted to 10 mg/ml, 20 mg/ml, 30 mg/ml and 40 mg/ml for invitro susceptibility testing using agar well diffusion method.

**2.6 Preparation of crude extract**

Extraction of the fruit pulp was carried out using standard method (Chambers, 2001). Forty grams (40g) of the ground seed was added into 250ml of distilled water in a conical flask and the mixture stirred six hourly for 24 hours. The mixture was filtered through Whatman No. 1 filter paper and the filtrate concentrated by evaporation in a water bath at 40$^{o}$C until all the solvent was removed. Concentrated filtrate was weighed and stored in a dark air-tight vial at room temperature until used.

**2.7 AntiMRSA susceptibility assay**

Freeze-dried typed MRSA (Rosenbach ATCC® BAA-1680™) and MSSA (ATCC 29213) were obtained from Microbiology unit, department of Medical Laboratory Sciences, Enugu State University of Science and Technology, Enugu, Nigeria. The stock MRSA and MSSA were identified by inoculating on CHROMagar™(Oxoid) for rose to mauve colonies indicating MRSA or no growth indicating MSSA.

**2.7.1Preparation of MRSA and MSSA inoculum**

A loopful of MRSA broth culture was transferred into a fresh Mueller-Hinton broth medium and incubated for 24 hours. The Muller Hinton broth culture was diluted 1:200 by mixing 0.1 ml of the culture and 19.9 ml of freshly prepared Muller Hinton broth. McFarland turbidity standard of 0.5 was prepared by mixing 99.5 ml of 1% v/v sulphuric acid and 0.5 ml of 1.175% w/v barium chloride ($BaCl_2.H_2O$) and dispensed in 4 ml amounts in test tubes. The turbidity of the inoculum was compared with that of the standard to get $10^6$ cells /ml (Baron and Finegold, 1990). Preparation of MRSA inoculum was under biosafety level 2 containment.

**2.7.2Preparation of MRSA inoculum**

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**2.8*In vitro* Antibacterial sensitivity assay**

Antibacterial sensitivity assay was performed by the agar-well diffusion (Okwori*et. al.,* 2007; Adegoke*et. al.,* 2010) [29, 30]. Muller Hinton agar was freshly prepared and tempered to 45$^{o}$C. Nine millilitres (9 ml) of the molten agar was inoculated with 100µl of 1.0 X $10^6$ cells/ml of the test organisms (MRSA and MSSA respectively) in a 9 cm diameter petri dish in 3 replicates. Three wells (6 mm) were punched out of the solid agar using pipette tips. One hundred microliter (100µl)

of the extract concentration, positive (vancomycin) and negative (methicillin) control antibiotics was placed in each well. The Petri dishes were incubated at 37°C for 24 hours. Controls (positive- 30µg vancomycin (**Oxoid™)),** negative control (30 µg methicillin (**Oxoid™**))were set up alongside the test. The average diameter of the inhibition zone surrounding the wells was measured and recorded.

**2.9Macrodilution assay for determining Minimum Inhibitory Concentration (MIC)**

Thirteen (13) sterile test tubes were set up in a test tube rack. Mueller-Hinton broth was dispensed in 1 ml amount into tubes 2-12, while 2 ml was dispensed into tube 13 (broth control). The Forty milligram (40mg/ml) stock aqueous seed extract of *V. doniana* was pipetted into tubes 1 and 12 (tube 12 serving as aqueous seed extract of *V. doniana* control) in 1 ml amount. From tube 1, two fold serial dilutions were made up to tube 10. The inoculum was then pipetted into tubes 1 to 11 (tube 11 being inoculums control) in 1 ml amount. The tubes were incubated at 37°C for 24 hours. MIC was determined by comparing the test with the controls and recording the lowest concentration showing no growth for the test organism (Baron and Finegold, 1990).

*Sensitive Staphylococcus aureus* was used as bacterial control, while Vancomycin and Methicillin were used as antibiotics control.

**2.10 Minimum Bactericidal Concentration (MBC)**

The minimum bactericidal concentration was determined by first selecting tubes that showed no growth during MIC determination. A loopful from each tube was subcultured onto extract free agar plates, incubated for further 24 hours at 37°C. The least concentration at which no growth was observed was recorded as the MBC (Baron and Finegold, 1990). *Sensitive Staphylococcus aureus* was used as bacterial control, while Vancomycin and Methicillin were used as antibiotics control.

**[III] RESULTS**

Results of the study were presented in sections.

**3.1. Proximate analysis**

Result of the proximate composition of *V. doniana* fruitexpressed as percentage dry weight (% DW) is presented in table 1. *V. doniana* fruit has high lipid content of 31.40±1.24%/100g DW with fibre (0.90%/100g DW ±0.10) being the proximate with the least content in the fruit.

Table 1: Proximate Composition of fruit Extract of *V. doniana*

| **Parameter** | **Composition (% /100g DW)** |
|---|---|
| Moisture | 18.09±0.91 |
| Protein | 9.61±0.63 |
| Lipids | 31.40±1.24 |
| Ash | 13.30±1.81 |
| Fibre | 0.90±0.10 |
| Carbohydrate | 26.70±0.02 |

Proximate analysis was done in triplicates and presented as mean values ± Standard deviation (SD) expressed as %/100g Dry Weight.

**4.2 Mineral analysis**

Analysis of the elemental constituents of the extract revealed the presence of potassium, sodium, phosphorus, copper, magnesium and iron in varying amounts as shown in table 2. The fruit contains 36% phosphorus per 100g DW which is the highest, and a low concentration of copper (2.10±0.01%/100g DW).

**Table 2:** Mineral composition of fruit Extract of *V. doniana*

| **Parameter** | **Composition (mg/100g DW)** |
|---|---|
| K | 17.1±0.10 |
| Na | 11.9±0.01 |
| P | 36.30±0.20 |
| Cu | 2.10±0.01 |
| Mg | 18.04±0.12 |
| Fe | 4.95±0.70 |
| Ca | 28.4±0.41 |

The analysis was also done in triplicates and presented as mean values ± Standard deviation (SD) expressed as mg/100 g DW

### 4.3 Phytochemical analysis

Phytochemical analysis of the aqueous seed extract of *V. doniana* revealed the presence of Flavonoids, Glycosides and saponins, while alkaloids, phenols and steroids were not detected (See table 3).

Table 3: Qualitative test for Phytochemical in Aqueous seed extract of *V. doniana*

| Parameter | Composition (%/100g DW) |
|---|---|
| Flavonoids | + |
| Glycosides | + |
| Saponins | + |
| Alkaloids | - |
| Phenols | - |
| Steroids | - |

### Antibacterial sensitivity assay

Evaluation of the antimicrobial effect of aqueous seed extract of *V. doniana* using an *in vitro* culture model and an *in vivo* mouse model of cutaneous infection showed that aqueous fruit extract of *V. doniana* exhibited antiMRSA activity.

Table 4 shows the result of antibacterial activity of the extract using disc diffusion method. At 10mg/ml, there were 17.5±0.20mm and 17.0±0.13mm zones of inhibition of the extract against MRSA and MSSA respectively, which increased proportionally with increase in concentration of the extract.

### Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of aqueous *V. doniana* fruit extract compared with vancomycin and methicillin on MRSA and MSSA

The MIC and MBC of aqueous *V. doniana* fruit extract against MRSA and MSSA were 250 µg/ml and 500 µg/ml respectively (see table 5). The values were higher than 1.36 µg/ml and 0.24 µg/ml MIC of Methicillin as well as 0.49 µg/ml and 0.49 µg/ml MIC of Vancomycin against MRSA and MSSA respectively. The MIC and MBC of methicillin and vancomycin against MSSA were 0.24 µg/ml.

**Table 4:** In vitro Sensitivity of aqueous *V. doniana* fruit extract against MRSA and MSSA

| Extract Concentration (Mg/ml) | Zone of Inhibition (mm) MRSA | MSSA |
|---|---|---|
| 10 | 17.5±0.20 | 17.0±0.13 |
| 20 | 22.0±0.02 | 23.0±0.40 |
| 30 | 25.0±0.10 | 24.5±0.11 |
| 40 | 28.0±0.45 | 29.0±0.20 |
| **Controls (Oxoid)** | | |
| Blank | 0 | 0 |
| Methicillin (30µg) | 6.0±0.23 | 36.0±0.21 |
| Vancomycin (30µg) | 37.6±0.10 | 38.0±0.11 |

Each value represents the ±SD (n=3)

**Table 5:**MIC and MBCofaqueous *V. doniana* fruit extract, Methicillin and Vancomycin against MRSA and MSSA

| *Staph Strain* | *V. doniana* | | Methicillin | | Vancomycin | |
|---|---|---|---|---|---|---|
| | MIC (µg/ml) | MBC (µg/ml) | MIC (µg/ml) | MBC(µg/ml) | MIC (µg/ml) | MBC (µg/ml) |
| MRSA | 250.0 | 500.0 | 1.36 | 1.85 | 0.49 | 0.49 |
| MSSA | 250.0 | 500.0 | 0.24 | 0.24 | 0.24 | 0.24 |

## 4.0 DISCUSSION

Findings of this study were discussed under proximate composition, phytochemical analysis, Mineral analysis, and antiMRSA susceptibility assay.

### 4.1 RESULTS PROXIMATE COMPOSITION

*V. doniana* fruit has a high lipid, carbohydrate, moisture and ash contents constituting 31.40±1.24%, 26.70±0.02%, 18.09±0.91% and 13.30±1.81% /100g dry weight of the fruit respectively relative to protein and fibre which accounted for 9.61±0.63% and 0.90%. The result is in agreement; although with slight differences in the values obtained in a study to estimate the proximate, vitamins and mineral composition of *Vitex doniana* (black plum) fruit pulp [31]. In the study, the crude fat, carbohydrate, moisture and

ash content of *V. doniana* fruit were 34.62 ± 0.56%, 28.40 ± 1.06%, 16.66 ± 1.06% and 11.50 ± 1.10% /100g dry weight respectively, with crude protein and crude fibre accounting for 8.24 ± 0.24% and 0.58 ± 0.08% respectively (Vunchi*et. al.,* 2011). Varying proximate of 55% carbohydrate, 16% ash, 12% crude fat, 8% moisture, 7% crude protein and 2% crude fibre per 100g dry weight were reported in a germination trial, proximate and elemental analysis of *Vitex Doniana* (Linn) Fruits (Abubakar *et. al.,* 2015). The values were higher and in contrast with that obtained in this study. Accounting for the variance in plant proximate were the method of processing, place and time of collection of plant specimen (Nwobodo, 2014).

The proximate composition (%) of *Sidaacuta*, a similar plant to *V. doniana* was 9.03+0.06, 19.13+0.15 0.67+0.06, 6.33+0.06, 9.50+0.01 and 55.30+0.10 per 100g DW moisture, protein, fat, ash, fibre and carbohydrate respectively (Raimi et. al., 2014). Although *Sidaacuta* has lower moisture of 9.03+0.06% against 18.09±0.9% in the fruit of *V. doniana*, its carbohydrate content (55.30+0.10%) was higher than 26.70±0.02% obtained in this study. The observed difference may depend on the unit of measurement which was not expressed in the study.

**4.2 Phytochemical analysis**

Out of the six phytochemicalsnamely; flavonoids, glycosides, saponins, alkaloids, phenols and steroids screened in this study, only three (flavonoids, glycosides and saponins) were detected. This finding was in line with that already documented in which flavonoids, glycosides and saponins were detected while alkaloids, phenols and steroids were absent (Amegbor*et. al.,* 2012). Alkaloids, saponins, tannins, anthraquinones, terpenoids, and flavonoids were detected in aqueous leaf extract of *V. doniana*, with no glycosides expressing the likelihood of differences in phytochemical composition of different parts of a plant(Amegbor*et. al.,* 2012; Agbaforand Nwachukwu, 2011).

**4.3 Mineral analysis**

Elements found in aqueous fruit extract of *V. doniana* include; phosphorus (36.30±0.20 mg/ per 100g DW), calcium (28.4±0.41 mg/ per 100g DW), magnesium (18.04±0.12 mg/ per 100g DW), potassium (17.1±0.10 mg/ per 100g DW), sodium (11.9±0.01 mg/ per 100g DW), iron (4.95±0.70 mg/ per 100g DW), and copper (2.10±0.01 mg/ per 100g DW) in the order of their magnitude. Similar range of values (K-15.70 ± 0.26, Na-10.40 ± 0.26, Ca- 30.27 ± 0.30, P- 16.50 ± 1.00, Mg- 20.10 ± 0.10, Fe- 5.20 ± 0.36, and Cu- 2.70 ± 0.45) in mg/100g DW was obtained in a study to determine the proximate, vitamins and mineral composition of *V. doniana* (black plum) fruit pulp (Vunchi*et. al.,* 2011).

Calcium which is necessary for blood clotting and muscle contraction is contained in *V. doniana* fruit pulp at 28.4±0.41 mg/100g DW. This value was lower than 30.27 ± 0.30 mg/100g DW and 139 mg/100g DW obtained in other studies (Vunchi*et. al.,* 2011).

The quantity of phosphorous (36.30±0.20mg/100g DW) obtained is appreciably higher than 16.50 mg/100g DW (Vunchi*et. al.,* 2011) and lower than 38.5mg/100g DW from *V. doniana* leaves (Nnamani*et. al.,* 2009). The differences may be attributable to environmental factors, the part of plant and the method of processing used. Magnesium plays an important role in blood circulation and angiogenesis (Vunchi*et. al.,* 2011). The value of magnesium (18.04±0.12 mg/100g DW) reported in this study was lower than 20.10mg/100g DW reported in another study (Vunchi*et. al.,* 2011) and 45.0mg/100g DW for *V. doniana* leaves(Nnamani*et. al.*,2009). The potassium content was 17.1±0.10 mg/100g DW, a value higher than 15.70 mg/100g DW and lower than 36.0 mg/100g DW for the leaves in two independent studies (Vunchi*et. al.,* 2011; Nnamani*et. al.*,2009).

Aqueous fruit extract of *V. doniana* contains 4.95±0.70 mg /100g DW of iron, an essential micronutrient for haemoglobin formation, normal functioning of central nervous system (CNS) and

oxidation of carbohydrate, protein and fat. The values are in agreement with 5.20mg/100g DW obtained in another study ((Vunchi*et. al.,* 2011). although lower. The 11.9±0.01 mg/100g DW sodium content of the fruit according to this study was higher than 10.40 mg/100g DW obtained in a similar study (Vunchi*et. al.,* 2011), while the concentration of copper in the fruit (2.10±0.01 mg/100g) was slightly lower than 2.70 mg/100g DW, but higher than 65.0 mg/100g DW reported in *V. doniana* leaves (Vunchi*et. al.,* 2011; Nnamani*et. al.,*2009).

**4.4 AntiMRSA susceptibility assay**

Evaluation of the antimicrobial effect of aqueous fruit extract of *V. doniana* using an *in vitro* culture model showed that MRSA and MSSA were susceptible to aqueous extract of *V. doniana* fruit at 10 mg/ml, 20 mg/ml and 30 mg/ml with zones of inhibition of 27.5±0.20/27.0±0.13 mm, 32.0±0.02/33.0±0.40 mm and 35.0±0.10/34.5±0.11 mm respectively in concentration dependent manner. There was an increase in resistance with increase in concentration of the extract implying that the fruit extract exhibited concentration dependent antibacterial activity. Hence, at 40 mg/ml, a higher zone of inhibition of 28.0±0.45/ 29.3±0.20 mm was obtained as against 17.5±0.20/17.0±0.13 mm at 10 mg/ml for both strains of *Staphylococcus aureus*. The 6.0±0.23 mm zone of inhibition of Methicillin at 30 µg against MRSA was less than 22.0±0.02/23.0±0.40 mm, 25.0±0.10/24.5±0.11 mm and 29.0±0.45/ 29.3±0.20 mm obtained using 20 mg/ml, 30 mg/ml and 40 mg/ml of the extract, but higher against MSSA with 36.0±0.21 mm zone of inhibition. Both strains of *Staphylococcus aureus* (MRSA and MSSA) exhibited high zones of inhibition of 37.6±0.10 mm and 38.0±0.11 mm against vancomycin.Using methicillin sensitivity and resistance standard set as zone of inhibition ≥ 4 mm and ≤ 8 mm respectively, it could be inferred that MRSA is susceptible to crude aqueous extract of *V. doniana* at the various concentrations used. This observation collaborated the fact that *V. doniana* has antibacterial activity in a study to assess the antimicrobial activity of the leaves and stem bark of *V. doniana* (Muhammad*et. al.,* 2013).

The leaf and stem bark extracts inhibited the growth patterns of the tested microorganisms with 21.5 ± 0.71 mm, 17.5 ± 0.71mm, and 14.5 ± 0.71mm zone of inhibition for *E. coli, S. pyogenes* and *S. typhi* respectively (Muhammad*et. al.,* 2013).

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the aqueous fruit extract of *V. doniana* were determined. The minimum inhibitory concentration of 250 µg/ml and minimum bactericidal concentration of 500 µg/ml obtained with the crude extract of *V. doniana* were high compared with the MIC of 1.36 µg/ml and 1.0 µg/ml of methicillin and 0.49 µg/ml and 0.24 µg/ml of vancomycin against MRSA and MSSA respectively. The MBC of 1.85 µg/ml and 0.24 µg/ml of methicillin and 0.49 µg/ml and 0.24 µg/ml of vancomycin against MRSA and MSSA respectively were lower than that obtained in this study. The MIC and MBC confirmed the bacteriostatic and bactericidal effects of *V. doniana* against both MRSA and MSSA strains. The difference in MIC and MBC of the crude extract of *V. doniana* compared with methicillin and vancomycin could be attributed to the presence of other components in the crude extract increasing the quantity required to make a given concentration or dosage. In a similar study, the MIC and MBC of acetone extract of *C. odontophyllum* leaves against Mu50 were 312.5 µg/ml and 625 µg/ml respectively, whereas the MIC and MBC values for ATCC 33591 were 625 µg/ml and 1,250 µg/ml respectively (Shamsuddin and Basri, 2018).

These findings suggest that an aqueous fruit extract of *V. doniana* inhibits growth of MRSA and could be utilised as an alternative anti-MRSA agent in immune uncompromised hosts. Purifying the crude extract through fractionation could help isolate the active ingredient responsible for the

observed antiMRSA activity and further reduce the concentration required to achieve same action. The MIC of 12.5 mg/ml, 25 mg/ml, 50 mg/ml, and 100mg/ml for *S. typhi, E. coli* and *S. pyogenes* respectively was obtained using aqueous leaf extract, and 25 mg/ml (*S. aureus* and *Salmonella typhi*) and 50 mg/ml (*E. coli* and *S. pyogenes*) using methanolic leaf extract. With aqueous bark extract, MIC was 100 mg/ml and 200 mg/ml for *E. coli* and *S. pyogenes* respectively while methanolic bark extract had MIC of 50mg/ml for *S. aureus* and *S. typhi*, 100mg/ml for *E. coli* and 200mg/ml for *B. subtilis* and *S. pyogenes*. MBC of the aqueous and methanolic stem and bark extract of *V. doniana* obtained in a similar study were lower than that obtained in this study (Muhammad*et. al.*, 2013). The MBC of the stem bark aqueous extract was 100mg/ml for *E. coli* and *S. typhi* and 200 mg/ml for, *S. pyogenes* while that of stem bark methanolic extract was 100 mg/ml (*E. coli*, *S. aureus*, *S. typhi*) and 200 mg/ml (*S. pyogenes*)

## [V] CONCLUSION

Aqueous fruit extract of *V. doniana* exhibited significant anti MRSA activity which was concentration dependent ($P<0.05$).

Further research should be conducted to assess the invivo antiMRSA activity of the extract and the bioactive substance responsible for the observed invitro antiMRSA activity.

## RECOMMENDATION

Further study should be carried out to identify the bioactive component of *V. doniana* fruit responsible for the observed antiMRSA activity.

## FINANCIAL DISCLOSURE

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