IJABR

International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-11, Issue-1, 2020, pp30-41
http://www.bipublication.com

Research Article

Evaluation of Aqueous Fruit Extract of *Vitex doniana* for Protective Effects in Rats Infected with Methicillin Resistant *Staphylococcus aureus*

Nwobodo, A. Humphrey

Department of Medical Laboratory Sciences
Enugu State University of Science & Technology
*Corresponding author: Email: humphreyafam@yahoo.com, Tel: +234-8036688783

[Received: 12/02/2020; Accepted: 23/02/2020; Published: 12/02/2020]

ABSTRACT:

Locally, *Vitexdoniana* is used in the management of a wide range of diseases. The aim of this research was to evaluate aqueous fruit extract of *Vitex doniana* for protective effect in rats infected with Methicillin Resistant *Staphylococcus aureus* (MRSA). Different concentrations (1%, 2%, 3% and 4%) of the aqueous fruit extract of *V. doniana* were prepared using Carbopol 940. Excision wound (6 mm) was induced on the dorsal skin region of 18 male Wistar rats put into six groups (A, B, C, D, E and F) and infected with 1 ml 10^6 cells /ml MRSA suspension. The negative (GP A) and positive (GP B) controls were topically treated with empty carbopol and 1% Vancomycin, while the experimental groups (C, D, E, and F) were treated with 1%, 2%, 3% and 4% carbopol gel of aqueous *V. doniana* fruit extract respectively for 12 days. Wound healing using wound contraction model was measured after every other day. Among the experimental groups, the rate of wound contraction was proportional to the percentage carbopol gel of aqueous *V. doniana* fruit extract used. Group C with wound diameter of 6.0±0.01 m on Day 0 contracted to 3.8±0.11 m on Day 12, while that of Group D contracted from 6.0±0.02 m to 2.4±0.01 m on Day 12 unlike the Group E contracting from 6.0±0.01 m to 0.8±0.13 and Group F from 6.0±0.01 to zero on Day 12. Hence complete wound healing evident in microbiological and histopathological analysis on the wound area occurred among group F treated with 4% aqueous fruit extract of *V. doniana*.Wound infection is detrimental to wound healing process. Findings from this study suggest that suppression of MRSA by the extract enhanced wound contraction and healing thereby conferring protective effect to the rats against MRSA. Further study should be conducted to identify the bioactive component of *V. doniana* fruit responsible for the observed antiMRSA activity.

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

[I] INTRODUCTION

Traditional and complementary medicine (TCM) has a long history and its practice is as old as man's existence (Nwobodo, 2014). 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).

Africa with its geographic position in the tropics has favoured the development of these plants (Wickens, 1973). *Vitex doniana* is an example of such plant. There are contradicting reports on the family of *Vitex* spp. *Vitex* has been classified differently. *Vitex* was classified as a genus of flowering plants in the family Lamiaceae (World Agroforestry Centre, 2019) and as a member of the family- *Verbenaceae* (Amegbor *et. al*., 2012). Linnaeus in 1975 described the genus *Vitex* (*Lamiaceae*) before *Verbenaceae* (Moke *et. al*., 2018).

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 and a clear bole up to 5 m (World Agroforestry Centre, 2019) [4]. The bark is rough, pale brown or greyish-white, rather smooth with narrow vertical fissures (World Agroforestry Centre, 2019). 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 (World Agroforestry Centre, 2019).

The flower petals are white except on largest lobe, which is purple and in 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 (Muhammad *et. al.,* 2013).

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 (Muhammad *et. al*., 2013; Amegbor *et. al.,* 2012). The *V. doniana* has antimicrobial, invigorating and anti-inflammatory actions [9]. 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 Staph 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. *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, diarrhoea and hearing problem among others (CDC, 2007).

In the recent years, research interest is focused on various plants due to downturn in the use of antibiotics derived from microorganism which include; limited life span of such agents, increasing incidence and severity of diseases caused by microorganisms as well as drug resistance due to fast development of resistant strains especially with the increasing development of resistance to available antibiotics as well as their adverse effect to humans and the environment (Collins and Jeffery, 1996). Various parts of *V. doniana* are used by traditional medicine practitioners in Nigeria and beyond in the management and treatment of several disorders such as rheumatism, hypertension, cancer and inflammatory diseases (Yakubu *et. al.,* 2016). Understanding the scientific basis for their use and action in traditional medicine is important.

Hence, ''Does aqueous fruit extract of *V. doniana* have protective effect against MRSA?''. The aim of this research is to evaluate the protective effect of aqueous fruit extract of *V. doniana* in mice with induced cutaneous MRSA infection.

[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*. Ripped fruits were collected through hand picking from the tree crown and transported to the laboratory in an airtight polyethylene bag.

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 following the method (Eloff, 1998) until processed further by solvent extraction.

2.2 Preparation of crude extract

Extraction of the fruit pulp was carried out using 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^{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.

2.3 Preparation of aqueous *V. doniana* fruit extract gel

Direct method was used to prepare gel of aqueous extract of *V. doniana*. One gram of Carbopol 940 (Amertek, UK) was thoroughly mixed with 100 ml of distilled water using vortexing machine (VWR) at 2500 rpm for 5 minutes to obtain lump-free dispersion. The mixture under continuous stirring was neutralized by drop-wise addition of NaOH 1 mol/l detected using pH meter. To make 1%, 2%, 3% and 4% equivalent to 10 mg/ml, 20 mg/ml, 30 mg/ml and 40 mg/ml aqueous fruit extract of *V. doniana* in gel, 1 g, 2 g, 3 g, and 4 g dried aqueous extract of *V. doniana* fruit was added and mixed continuous until a gel formed (Amegbor *et. al.*, 2012). Vancomycin (1%) was prepared as a positive control, while an empty gel of Carbopol 940 served as a negative control.

2.4Preparation of MRSA inoculum

Freeze-dried typed MRSA (Rosenbach ATCC® BAA-1680™) was obtained from Microbiology unit, department of Medical Laboratory Sciences, Enugu State University of Science and Technology, Enugu, Nigeria and identified by inoculating on CHROMagar™(Oxoid) for rose to mauve colonies indicating MRSA.

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. One millitreof 10^6 MRSA cells/ml was used to infect the induced wound on Winstar rat within 10 minutes of the excision (Baron and Finegold, 1990). Preparation of MRSA inoculum was under biosafety level 2 containment.

2.5 In vivo Assay of aqueous fruit extract of *Vitex doniana* for protective effect against MRSA

Eighteen (18) Wistar rats were put into six groups (A, B, C, D, E and F) of three rats per group. The rats were anesthetized by the open mask method using diethyl ether and their dorsal surface shaved using a shaving machine (Amegbor *et. al.*, 2012). Excision wounds were made with a 6-mm diameter biopsy punch on the shaved area on the dorsal skin region (Fan *et. al.*, 2018). The wounds were infected with 1 ml 10^6 cells /ml MRSA suspension prepared in section 2.6 (Preparation of MRSA inoculum) and observed daily. Group A and B served as negative and positive controls receiving carbopol empty gel and Carbopol vancomycin gel respectively. Groups- C, D, E and F mice served as the experimental groups topically receiving 1%, 2%, 3% and 4% aqueous V. doniana fruit extract carbopol gel respectively for 12 days. Wound healing activity was studied using wound contraction model (Fan *et. al.*, 2018).

2.6 Measurement of excision wound area infected with MRSA and treated with *V. doniana* aqueous fruit extract carbopol gel

The progressive changes in wound area were monitored by measuring the wound area every

other day. Wound healing was evaluated as Wound Contraction (WC) calculated as percentage of the reduction in wound area by using the mathematic expression:
$WC_d = (1\text{-}WA_d/WA_0)$ X 100% where WA_d is area of the wound on the day d and WA_0 the wound area on the zero day (Fan *et. al.,* 2018).

2.7 Qualitative microbiological analysis of excision wound infected with MRSA and treated with *V. doniana* aqueous fruit extract carbopol gel on day 12 of the experiment

The viability of MRSA after treatment with aqueous seed extract of *V. doniana* and vancomycin was examined using cultural method on day 12. Skin crumbs from the wound area were obtained by scraping and suspended in peptone water. The peptone water was subcultured on CHROMagar incubated at 37^oC for 24 hours for isolation of MRSA (Cheesbrough, 2010).

2.8 Histopathological Analysis of excision wound infected with MRSA and treated with *V. doniana* aqueous fruit extract carbopol gel on day 12 of the experiment

On day 12 of the study, all the animals were anesthetized using ketamine and specimens of wound tissue were collected and preserved in glass vials containing 10% formalin solution for histological examination following a standard method (Nagar *et. al.,* 2016). Sections of wound tissue specimens (about 5 µm thickness) were prepared by microtomy and stained with haematoxylin and eosin (H&E) dye for histological examination.

2.9 Animal sacrifice

The animals were sacrificed under chloroform anaesthesia and incinerated

2.10 Ethical issues and Ethical approval

The research was conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Academy of Science (National Academy of Sciences, 2011). Experimental ethical approval was obtained from Ethics Committee of Enugu State University Teaching Hospital.

2.11 Statistical Analysis

The data was presented as the mean and standard deviation (SD). The difference in the data of the different experimental groups was analyzed by the Kruskal-Wallis test. The *post-hoc* test for checking individual differences will be Dunn's test. Significance was indicated as $p< 0.05$.

[III] RESULTS

Results of the study were presented under the following subsections namely: Wound Contraction, Wound Healing, Qualitative microbiological and Histopathological study of induced excision wound model.

3.1. Wound Contraction in MRSA induced Excision wound treated with aqueous extract of V. doniana

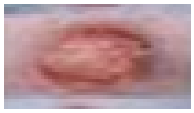
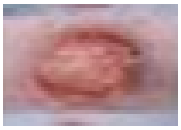
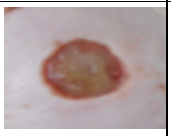
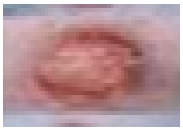
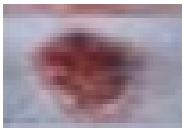
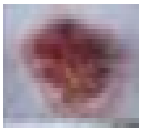
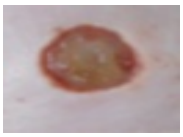
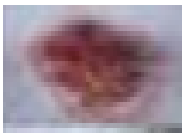
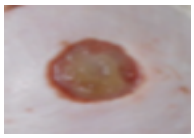
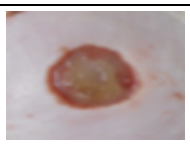
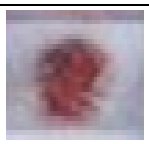
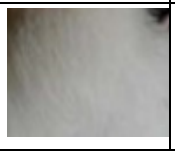
The result of percentage wound contraction estimated after every 24 hours presented in table 1 showed that there was 100% contraction among Group B and Group F on day 10 and 12 respectively. There was wound expansion which later became static until the end of the experiment among Group A. Groups C, D and E showed varying wound contractions as the experiment progressed which were never complete.

[Table-1: Percentage wound contraction in excision skin wound infected with MRSA and treated with aqueous fruit extract carbopol gel]*. (See at the bottom)**

3.2. **Wound Healing**

Table 2 is a pictorial representation of the wound healing and protective effect of aqueous fruit extract of *V. doniana* on MRSA induced excision wound infection. There was complete wound healing among the groups B and F treated with Vancomycin and 4% aqueous fruit extract of *V. doniana* in carbopol gel.

[Table-2: Picture of the excision wound infected with MRSA and treated with aqueous fruit extract carbopol gel***]***.

Day	GP A	GP B	GP C	GP D	GP E	GP F
0						
2						
4						
6						
8						
10						
12						

3.3 **Qualitative microbiological study of the induced excision wound infection**

To confirm the antiMRSA effect of aqueous *V. doniana* fruit extract on MRSA induced excision wound infection in Wistar rat, the of the swabbed wound area showed the presence of MRSA in Group A throughout the 12 day experiment unlike in Group B and Group F where there was no MRSA on day 2 and day 4 as could be seen in table 3.

[Table-3: Qualitative microbiological analysis of excision wound infected with MRSA and treated with aqueous fruit extract carbopol gel on day 12 of the experiment**].**

Day	Control		Experiment			
	Group A: Negative (Empty carbopol gel)	**Group B: Positive (Vancomycin gel)**	**Group C: Treated with 1% Aqueous fruit extract of *V. doniana* in carbopol gel**	**Group D: Treated with 2% Aqueous fruit extract of *V. doniana* in carbopol gel**	**Group E: Treated with 3% Aqueous fruit extract of *V. doniana* in carbopol gel**	**Group F: Treated with 4% Aqueous fruit extract of *V. doniana* in carbopol gel**
0	+	+	+	+	+	+
2	+	-	+	+	+	+
4	+	-	+	+	+	-
6	+	-	+	+	+	-
8	+	-	+	+	+	-
10	+	-	+	+	-	-
12	+	-	+	+	-	-

Key: +: MRSA Isolated, -: MRSA Not Isolated

3.4 **Histopathological Analysis**

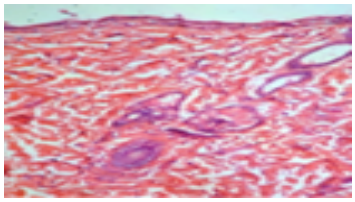
The result of histopathological studies of the tissue of the excision wound model on the day 12 is shown in figure 2. The section of Group A (Infected with MRSA without treatment) displayed necrotic cells and less collagen fibers and blood vessels (Figure 2a). The section of group B (Standard treatment with Vancomycin) showed complete tissue regeneration evident in increased fibroblast cell, collagen fibers, and blood vessels and reduced inflammatory cells (Figure 2b). Group C (1% aqueous fruit extract of *V. doniana* in carbopol gel) and Group D (2% aqueous fruit extract of *V. doniana* in carbopol gel) showed incomplete tissue regeneration, less cellular necrosis along with increased collagen fibers and blood vessels (Figure 2 c and d respectively), while Group E (3% aqueous fruit extract of *V. doniana* in carbopol gel) showed prominently increased fibroblast cells, blood vessels, and well organized collagen fibers as compared to Group II that received standard treatment (Figure 2 e) and Group F (4% aqueous fruit extract of *V. doniana* in carbopol gel) complete tissue regeneration with increased fibroblast cell, collagen fibers, blood vessels. Additionally, there was a reduced inflammatory cells in the skin section of Group F as could be seen in Figure 2 f.

Fig: 1. Micrograph of stained tissue of the excision wound infected with MRSA and treated with aqueous fruit extract carbopol gel day 12 of the experiment.

GP A

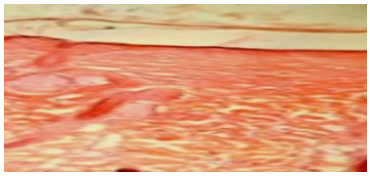
a. Necrotic cells and less collagen fibres and blood vessels

GP B

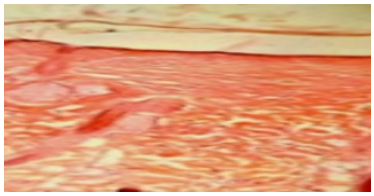


b. Fibroblast cell, collagen fibres, and blood vessels and reduced inflammatory cells

GP C

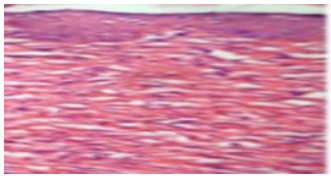


GP D



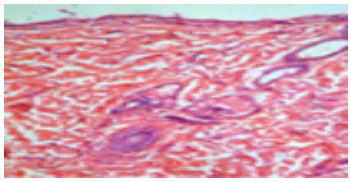
c and **d**. Incomplete tissue regeneration, less cellular necrosis along with increased collagen fibers and blood vessels

GP E



e. Increased fibroblast cells, blood vessels, and well organized collagen fibres

GP F



f. Complete tissue regeneration with increased fibroblast cell, collagen fibers, blood vessels and reduced inflammatory cells

[IV] DISCUSSION

In vivo assay of aqueous seed extract of *Vitex doniana* for protective effect against MRSA

Wound healing is an intricate process following damage to the skin and other soft tissues of the body and hence involves the dynamic process of multiple biochemical activities including; inflammation, proliferation, and remodelling aimed at the restoration of the damaged cellular structure to its regular and original state (Shamsuddin and Basri, 2018). There are two main phenomena involved in skin wound healing which include re-epithelialization and novel formation of granulation tissue (Sorg*et. al.*, 2007.). Re-epithelialization involves the replication and movement of epidermal cells to reconstitute tissue continuity, while novel formation of granulation tissue essentially composed of small vessels, fibroblasts, myofibroblasts and inflammatory cells. Hemostasis, inflammation, proliferation, and remodelling are the four phases that must occur in the proper sequence and time frame for wound to heal (Guo and Dipietro, 2010).

The process of wound healing could be affected by a number of factors including oxygenation, infection, age and sex hormones, stress, diabetes, obesity, medications, alcoholism, smoking, and nutrition (Guo and Dipietro, 2010). Wound infection is detrimental to wound healing process (Edwards and Harding, 2004). Topical application carbopol gel of aqueous fruit extract of *V. doniana* on rat with induced excision skin wound infected with MRSA reduced the MRSA load and improved wound healing via wound contraction. In a study to ascertain the Anti-methicillin resistant *Staphylococcus aureus* (MRSA) activity of an acetone extract from the leaves of *Canariumodontophyllum*, it was reported that the plant leaf exhibited antiMRSA activity (Shamsuddin and Basri, 2018).

Complete wound contraction which is a function of wound area with time occurred on day 12 of excision and treatment with 4% *V. doniana* aqueous fruit extract gel and on day 11 using Vancomycin gel. At other concentrations of the *V. doniana* aqueous fruit extract gel, wound contraction was gradual being both time and concentration dependent. At 1%, 2%, 3% and 4%, wound contraction was -89.75%, -77.73%, -74.56% and – 68.55% respectively on day 2 and -36.39%, -12.37%, 1.77% and 3.53% respectively on day 12 before the animals were sacrificed. The negative control which was infected without treatment did not experience wound contraction rather wound expansion from -96.5% to -314.1% on day 1 and 12 respectively. Finding from this study suggests that suppression of MRSA by the extract enhanced wound contraction and healing.

The anti MRSA activity of aqueous fruit extract of *V. doniana* was further verified through a skin swab culture qualitative assay. No bacteria was isolated from the infected skin sites on day 3 and beyond using vancomycin gel and as from day 5

and 11 using 4% and 30% *V. doniana* gel respectively.

Histological studies

The histopathological studies of the tissue of the excision wound model on the day 12 shown in figure 2 revealed that the section of Group A (Infected with MRSA without treatment) displayed necrotic cells and less collagen fibers and blood vessels (Figure 2a) suggesting damage by MRSA. According to Edwards and Harding (2004), the progression from wound colonization to infection depends not only on the bacterial count or the species present, but also on the host immune response, the number of different species present, the virulence of the organisms and synergistic interactions between the different species, arguing that there is increasing evidence that bacteria within chronic wounds live within biofilm communities, in which the bacteria are protected from host defences and develop resistance to antibiotic treatment.

The section of group B rats that received standard treatment with Vancomycin showed complete tissue regeneration which was evident by increased fibroblast cell, collagen fibers, and blood vessels and reduced inflammatory cells following inhibition of MRSA (Figure 2b).

Group C rats that were treated with 1% *V. doniana* gel and Group IV that received 2% *V. doniana* gel showed incomplete tissue regeneration, less cellular necrosis along with increased collagen fibers and blood vessels (Figure 2 c and d respectively). Group D rats that were treated with 3% *V. doniana* gel showed prominently increased fibroblast cells, blood vessels, and well organized collagen fibers compared to Group B that received standard treatment (Figure 2 e) and Group E treated with 4% *V. doniana* gel. There was complete tissue regeneration with increased fibroblast cell, collagen fibers, blood vessels and reduced inflammatory cells among group F rats (Figure 2 f). There was significant ($P<0.05$) increase in the rate of wound contraction following treatment with 4% *V. doniana* gel.

The finding from this study is consistent with that reported in a similar study in which *V. doniana* at 5% and 2.5% provided better wound contraction (91.14% and 86.38%) at day 12 post-excision when compared to control (51.15%) which is a panacea for wound healing [5]. Hence, it could be inferred that aqueous fruit extract of V. doniana has protective effect in Wistar rats with induced methicillin resistant *staphylococcus aureus* excision wound infection.

[V] CONCLUSION

Topical application of 4% carbopol gel of aqueous fruit extract of *V. doniana* on rat with induced excision skin wound infected with MRSA inhibited the bacterium and improved wound healing via wound contraction. The anti MRSA activity of 4% carbopol gel of aqueous fruit extract of *V. doniana* further verified through a qualitative skin swab culture yielded no bacteria Day 6 post-infection and treatment.

There was complete tissue regeneration with increased fibroblast cell, collagen fibers, blood vessels and reduced inflammatory cells after day 12 following topical application of 4% carbopol gel of aqueous fruit extract of *V. doniana*with significant ($P<0.05$) increase in the rate of wound contraction. Hence, it could be inferred that aqueous fruit extract of *V. doniana* has protective effect in Wistar rats with induced methicillin resistant *staphylococcus aureus* excision wound infection.

RECOMMENDATION

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

FINANCIAL DISCLOSURE

This research was funded by Enugu State University of Science & Technology via Institutional Based TETFund Research grant.

ACKNOWLEDGEMENT

I acknowledge with joy Enugu State University of Science & Technology/Tertiary Education Trust

Fund (TETFUND) for the research grant and all those who in one way or the other contributed to the success of this work.

REFERENCES

1. Abubakar, S. et al., (2015), Germination Trial, Proximate And Elemental Analysis Of *Vitex Doniana* (Linn) Fruits,*International Journal of Biological Sciences*. Vol-2, issue 2, pg 27 – 39.
2. Amegbor, K, et al., (2012), Preliminary evaluation of the wound healing effect of *Vitex doniana* sweet (Verbenaceae) in mice, Afr J Tradit Complement Altern Med. Vol-9, issue 4, pg584 590.
3. Baron, J. E. and Finegold, S. M, (1990), Methods for testing antimicrobial effectiveness. In Bailey Scotts Diagnostic Microbiology. Mosby C. V. (Ed). Missouri.pg 171-194.
4. CDC, (2007), Outpatient management of MRSA skin infections: www.cdc.gov/mrsa/pdf/Flowchart_pstr.pdf
5. Cheesbrough, M, (2010), District Laboratory Practice in Tropical Countries, Part 1, 2nd Edition. Cambridge University Press.
6. Chambers, H. F, (1997), Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications, Clin Microbiol. Rev. Vol-10, issue 4, pg 781 – 791.
7. Chambers, H. F, (2001),*Methicillin-resistant Staphylococcus aureus*. Mechanisms of resistance and implications for treatment. Postgraduate Medicine. 109 (2 Suppl): pg43-50. https://www.researchgate.net/publication/26729396_Methicillin-resistant_Staphylococcus_aureus_Mechanisms_of_resistance_and_implications_for_treatment
8. Collins, W. E. and Jeffery, G. M, (1996), Primaquine resistance in *Plasmodium vivax*, American Journal of Tropical Medicine and Hygiene. Vol-55, pg 243-249.
9. Edwards, R. and Harding, K. G, (2004), Bacterial Wound Healing,CurrOpin Infect Dis. Vol-17, issue 2, pg 91 – 96.
10. Egereonu, U. U, and Mokwe, N. R, (2005), Characterization of the Extract from *Bryophyllum pinnatum* Leaves, Journal of Chemical Society of Nigeria. Vol-30, issue 2, pg 192–196.
11. Eloff, J. N, (1998), A sensitive and quick microplate method to determine the minimum inhibitory concentration of plant extracts for bacteria, Planta Medica. Vol- 64, pg 711-713.
12. Fan, B, et al., (2018), Topical Application of Tat-Rax1 Promotes Cutaneous Wound Heraling in Normal and Diabetic Mice, Int J Biol Sci. Vol-14, issue 100, pg 1163 – 1174.
13. Guo, S. and Dipietro L. A, (2010), Factors Affecting Wound Healing, J. Dent Res.Vol-89, issue 3, pg219 – 229.
14. Kilani, A. M, (2006), Antibacterial assessment of whole stem bark of Vitex doniana against some Enterobacteriaceae, African Journal of Biotechnology. Vol- 5, issue 10,pg 958-959.
15. Moke, L. E, et al., (2018), Vitex madiensis Oliv. (Lamiaceae): Phytochemistry, Pharmacology and future directions, a mini-review, Pharmacogn Rev.Vol- 7, issue 14, pg188-198.
16. Muhammad, M. B, et al., (2013), Antimicrobial activity of the leaves and stem bark of Vitex doniana,*Intercontinental Journal of Biological Sciences.*Vol-3, issue 1, pg 1- 5.
17. Nagar, H. K, et al., (2016), Pharmacological Investigation of the Wound Healing Activity of *Cestrum nocturnum* Ointment in Wistar Albino Rats**,***Journal of Pharmaceutics*. https://www.hindawi.com/journals/jphar/2016/9249040/
18. National Academy of Sciences, (2011), Guide for the Care and Use of Laboratory Animals, 8th edition. National Academies Press, US.
19. Nwobodo, H. A, (2014), Antiviral and Antibacterial susceptibility of acetone leaf extract of *Combretum paniculatum*, World

Journal of Pharmacy and Pharmaceutical Sciences. Vol-3, issue 5,pg 1564-1575.

20. Nyiligira, E, (2008), Phytochemistry and *in vitro* pharmacological activities of South African *Vitex* (Verbenaceae) species, Journal of Ethnopharmacology. Vol-119, issue 3, pg 680 – 685.
21. Okerulu, I. O, and Ani, C. J, (2001), The phytochemical Analysis and Antibacterial Screening of Extracts of *Tetracarpidium conophorum*, Journal of Chemical Society of Nigeria. Vol- 26, issue 1, pg 53-55. 18.
22. Sadaf, F, et al., (2006), Healing potential of cream containing extract of *Sphaeranthusindicus* on dermal wounds in Guinea pigs, Journal of Ethnopharmacology. Vol-107, issue 2, pg 161 – 163.
23. Shamsuddin N. A. M. and Basri, D. F, (2018), Anti-methicillin resistant Staphylococcus aureus (MRSA) activity of an acetone extract from the leaves of *Canariumodontophyllum* (Miq.), The Journal of Phytopharmacology. Vol- 7, issue 3, pg 225-229.
24. Sorg. H, et al., (2007), Intravital insights in skin wound healing using the mouse dorsal skin fold chamber, J. Anat. Vol-211, issue 6, pg810 – 818.
25. Tramontina, V. A, et al., (2002), Effect of bismuth subgallate (local hemostatic agent) on wound healing in rats. Histological and histometric findings, Braz Dent J. Vol-13, issue 1, pg 11 – 16.
26. Wickens, G. E, (1973), *Combretum paniculatum*, Flora of Tropical East Africa.Vol- 1, pg 1 – 5.
27. World Health Organization, (2019), Traditional, complementary and integrative medicine. Traditional, Complementary, and Integrative Medicine. Geneva, Switzerland.
28. World Agroforestry Center, (2019), *Vitex doniana*. http://www.worldagroforestry.org/treedb2/speciesprofile.php?Spid=1687
29. Yakubu, O. E, et al., (2016), Effects of *Vitex doniana* leaf extract on aluminium-induced toxicity in male Wistar albino rats, Journal of Applied Biology & Biotechnology. Vol- 4, issue 05, pg 037-040.
30. Yang, S-C, et al., (2017), Pterostilbene, a Methoxylated Resveratrol Derivative, Efficiently Eradicates Planktonic, Biofilm, and Intracellular MRSA by Topical Application. Front. Microbiol., https://www.frontiersin.org/articles/10.3389/fmicb.2017.01103/full

Tables:*

[Table-1: Percentage wound contraction in excision skin wound infected with MRSA and treated with aqueous fruit extract carbopol gel].

Groups of Rats /Extract Concentration (mg/ml)/ Day	Negative Control (Empty carbopol gel)			Positive Control (Vancomycin)			Experimental Group											
	Group A			Group B			Group C (10mg/ml)			Group D (20 mg/ml)			Group E (30 mg/ml)			Group F (40 mg/ml)		
	WD (m)	WA (m^2)	WC (%)	WD (m)	WA (m^2)	WC (%)	WD (m)	WA (m^2)	WC (%)	WD (m)	WA (m^2)	WC	WD (m)	WA (m^2)	WC (%)	WD (m)	WA (m^2)	WC (%)
0.	6.0±0.02	28.3	-96.5	6.0±0.04	28.3	-96.5	6.0±0.01	28.3	-96.5	6.0±0.02	28.3	-96.5	6.0±0.01	28.3	-96.5	6.0±0.01	28.3	-96.5
2.	6.5±0.22	33.2	-113.8	5.0±0.01	19.6	-65.72	5.8±0.02	26.4	-89.75	5.5±0.10	23.8	-77.73	5.3±0.02	22.1	-74.56	5.1±0.01	20.4	-68.55
4.	7.6±0.03	45.4	-156.9	4.0±0.14	12.6	-40.99	5.7±0.10	25.5	-86.57	5.1±0.12	20.4	-68.55	4.0±0.02	12.6	-40.99	4.2±0.01	13.6	-44.52
6.	8.4±0.05	55.4	-192.2	3.0±0.01	7.1	-21.55	5.4±0.01	22.9	-77.39	4.8±0.12	18.1	-60.42	3.6±0.14	10.2	-32.51	3.2±0.13	8.0	-24.73
8.	9.2±0.03	66.5	-231.4	1.5±0.02	1.8	-2.83	4.8±0.02	18.1	-60.42	4.0±0.01	12.6	-40.99	2.2±0.02	3.80	-103.53	1.8±0.04	2.5	-5.300
10.	9.9±0.07	77.0	-268.6	0	0	3.53	4.0±0.01	12.6	-40.99	3.2±0.01	8.0	-24.73	1.8±0.04	2.5	-5.30	0.9±0.24	0.6	1.413
12.	10.7±0.10	89.9	-314.1	0	0	3.53	3.8±0.11	11.3	-1.06	2.4±0.01	4.5	-12.37	0.8±0.13	0.50	1.77	0	0	3.53