ANTIBACTERIALACTIVITY OF VITAMIN C AND SWEET ORANGE JUICE (*Citrus sinensis*) EXTRACT AGAINST *Staphylococcus aureus* ISOLATED FROM WOUND INFECTION AMONG PATIENT ATTENDING BODINGA GENERAL HOSPITAL, SOKOTO, NIGERIA

ABSTRACT

Staphylococcus aureus is a major human pathogen responsible for a wide range of clinical infections, including bacteremia, infective endocarditis, osteoarticular infections, skin and soft tissue infections, pleuropulmonary infections, and device-related infections. This study aimed to evaluate the antibacterial activity of Vitamin C and Sweet Orange Juice (Citrus sinensis) extract against Staphylococcus aureus isolated from wound infections in patients at Bodinga General Hospital, Sokoto. Ten samples were collected and analyzed using Gram staining and standard biochemical tests. The results indicated that both Vitamin C and Sweet Orange Juice exhibited the highest zones of inhibition at a 100% concentration, followed by 70%, 50%, and the lowest at 25% concentration. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for both Vitamin C and Sweet Orange Juice were determined to be 50% and 70%, respectively. Maintaining good personal hygiene and environmental sanitation can help reduce the infection rates in the study area, as well as in Nigeria and globally.

Key word:ANTIBACTERIAL ACTIVITY, JUICE extract, human pathogen, Gram Staining, antimicrobial properties, antibiotic resistance,

1. INTRODUCTION

Medicinal plants offer untapped potential for new drug discovery with their diverse chemical makeup [1]. Utilized since ancient times in Africa and Asia, these plants, from roots to seeds, have shown antimicrobial properties due to their chemical constituents [1]. Recent attention focuses on extracting active compounds from common plant species, which produce defense mechanisms like phenolic compounds and terpenoids [2]. These plants have been used for centuries to treat ailments, with ongoing interest in their effectiveness. In India, traditional medicinal plants have long been used for specific purposes [2].Clinical microbiology studies plant extracts for their antimicrobial properties, aiming to find new drugs and address concerns about traditional medicine misuse [3].

Oranges, globally beloved, are prized for their health benefits and antioxidants [3]. Packed with nutrients like vitamin C, they're studied for potential antibiotic development and cancer risk reduction [3]. Originally from China, sweet oranges are now widely cultivated, with research exploring their antibacterial properties against microbial resistance [4]. With rising resistance, fruit-derived alternatives gain importance, particularly oranges with their therapeutic potential. Further research is needed to understand their effectiveness against various strains [4].

Vitamin C, a powerful antioxidant, has antibacterial properties and can enhance the effects of antibiotics [5]. It's considered a cheap and readily available alternative for treating infections, including urinary tract infections [5]. High concentrations of vitamin C have immunomodulatory functions, reducing infection risks [6]. L-ascorbic acid, a form of vitamin C, has shown antibacterial effects against various pathogens. Its pharmacological use is crucial for a well-functioning immune system [6].

Wounds, caused by skin damage, are a major challenge in preventing internal infections by harmful microorganisms. Trauma, whether accidental or deliberate, is the main cause [8]. Deliberate wounds include those from medical procedures like surgery or from medical devices [8]. Hospital-acquired wounds, such as pressure sores, can also become infected, often hosting multiple bacterial species [9]. Clinical guidelines advise monitoring microbial load as a key indicator of infection when obvious signs are absent [9]. The aim of this research is to determine the antibacterial activity of Vitamin C and Sweet Orange (*Citrus sinensis*) extract against *Staphylococcus aureus* isolated from wound infection among patients attending Bodinga General Hospital, Sokoto, Nigeria.

2.0 MATERIALS AND METHOD

2.1 Study Area

All samples for this study were collected from patients at General Hospital Bodinga, located behind Bodinga Police Divisional Headquarters. Established in 1975, it began operating in 1976 under the Ministry of Health, initially as a primary healthcare facility during the administration of former military governor Usman Faruk. The primary healthcare unit preceded Bodinga status as a local government area, initially staffed with 1 ward, 3 nurses, 3 health workers, and 1 doctor. After two years, in 1977, Bodinga became a local government area. In 2007, the primary healthcare facility was upgraded to a general hospital, equipped with a one-site laboratory, pharmacy, ambulance, and four wards, including labor and emergency rooms. Bodinga General Hospital operates 24/7, open every day of the week [7].

Sokoto State, one of Nigeria's seven northwestern states, spans an area of about 25,973 square kilometers and has a population of over four million people according to the 2006 census. It shares borders with Zamfara, Kebbi states, and Niger Republic, consisting of 23 local government areas with Sokoto as its headquarters. The majority of its people are Hausa-Fulani, mainly engaged in farming and animal husbandry. The state's vegetation is Sudan Savannah, characterized by short grasses and scattered trees, with annual rainfall averaging about 1000mm, typically occurring from May to September [7].

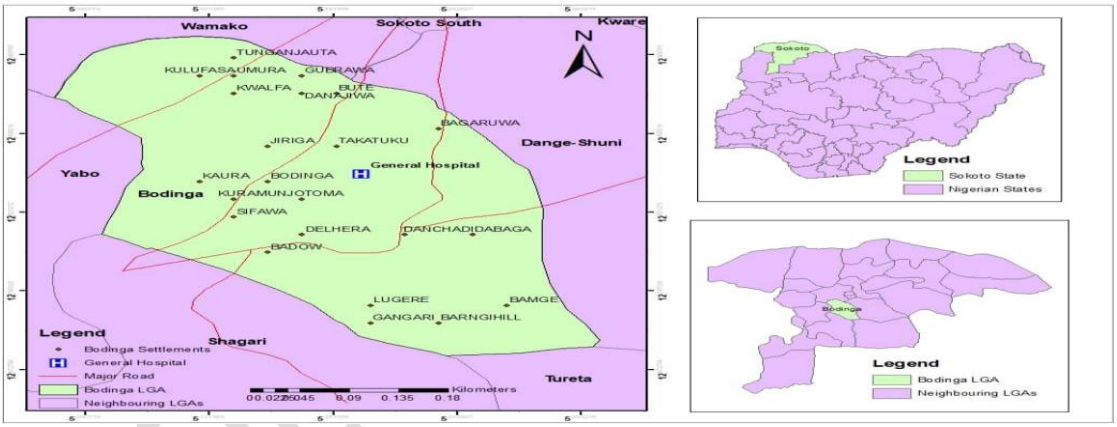

Figure 1: Map of Bodinga and its environs Showing the Study Area

Source: GIS Laboratory, Sokoto State University Sokoto

2.2 Sample Collection

Samples were collected using sterile swab sticks, which were gently swiped on the surface of the patients' wounds. A drop of normal saline was then added to the swab sticks, and they were promptly transported to the Microbiology Laboratory of Sokoto State University for analysis [10].

2.3 Media Preparation

Nutrient agar was prepared by dissolving 28.0g of powdered nutrient agar into 1 liter of distilled water (28.0g/l), following the manufacturer's instructions. The solution was sterilized by autoclaving at 121°C for 15 minutes. After sterilization, it was cooled to 45-50°C, poured into sterile petri dishes, and left to solidify at room temperature [11].

2.4 Extraction of Orange Juice

Fresh, ripe oranges were purchased from the market and taken to the laboratory for juice extraction. The oranges were thoroughly washed with distilled water, then cut in half and squeezed into a sterile conical flask [12].

2.5 Preparation of Vitamin C

Twenty milligrams (20mg) Of Vitamin C were dissolved in a sterile conical flask containing 50ml of distilled water [12].

2.6 Inoculation of the Swab Stick into Nutrient Media

Each swab stick utilized for collecting samples from infected wounds was inoculated into prepared nutrient media via the spreading method, followed by an incubation period at 37°C for 24 hours. Subsequently, the colonies were sub-cultured onto a fresh plate of nutrient agar using a sterile wire loop to obtain pure isolates. Gram staining and biochemical tests were then employed to characterize the pure isolate of Staphylococcus aureus [13].

2.7 Gram Staining Procedure

The Gram staining process begins with preparing a smear on a clean glass slide, which is then air-dried and heat-fixed. The slide is sequentially stained with crystal violet and iodine, washed, decolorized with acetone, and washed again. Neutral red is then applied, followed by a final wash. After wiping the slide clean and air-drying it on a draining rack, microscopic examination is performed first at x40 magnification to observe material distribution, and then at x100 oil immersion to identify bacteria. [14].

2.8 Biochemical Test

The biochemical tests were carried out at Microbiology laboratory of Sokoto state university using the following parameter [15].

2.8.1 Catalase Test:

This was be done by using a clean sterile wire loop to pick a small amount of the growth on the plate and placed on drops of h2o2 on a clean grease free microscope slide. Production of bubbles indicate positive [15].

2.8.2 Coagulase Test:

A drop of saline is deposited on a clean, grease-free slide, and a small amount of the test organism is collected with a sterile wire loop to create a smooth suspension. The loop is then dipped into undiluted plasma, prepared by centrifuging human blood with added sodium oxalate to a concentration of 0.3%. Coagulation becomes visible to the naked eye within 10 seconds, indicating a positive result, while slower reactions are considered potentially negative [16].

2.8.3 Motility Test:

Three drops of peptone water are placed on a glass slide, and a small amount of the test organism is introduced using a sterile wire loop. A cover slip is then placed over the slide and left for observation. The presence of motile organisms indicates a positive result. The peptone water is inoculated and incubated for 48 hours at 37°C. After incubation, Kovac's reagent is added to each tube; a red color in the alcohol layer signifies a positive result. [17].

2.8.4 Urease test:

The sterile Christenson’s urea media was inoculated with the test organism and incubated at 37^{o}C for 48hours. Tubes were examined and re-examine after over-night incubation [18].

2.9 Antibacterial Activity of Sweet Orange

The antibacterial activity was evaluated using the disc diffusion technique. The isolate was inoculated onto prepared Maule Hinton agar plates. Discs soaked in orange juice solution were aseptically placed on the agar plates using sterile forceps. The inoculated plates were then incubated at 37°C for 24 hours. The diameter of the inhibition zone was measured and recorded in millimeters (mm) [18].

2.10 Antibacterial Activity of Vitamin C

The same method used above was used using 0.5ml, 1m land 1.5ml of vitamin C preparation [18].

3.0 RESULTS

3.1 Zone of Inhibition in Triplicate Using 6mm Cock Barrier Size

The results present the biochemical tests conducted to identify the type of *Staphylococcus aureus* isolated from wound infections and the zone of inhibition measured in triplicate using a 6mm corn borer. The findings reveal that the highest inhibition is recorded at 100%, followed by 75%, 50%, and the lowest inhibition at 25%, as shown in Table 1.

3.2 Concentration of Sweet Orange and Vitamin C Extract

The results indicate that both sweet orange extract and vitamin C have similar effects on *Staphylococcus aureus*. However, vitamin C demonstrates a larger zone of inhibition at higher concentrations. This suggests that vitamin C, beyond its nutritional value, can be beneficial in treating diseases caused by *Staphylococcus aureus* and may serve as an alternative to certain antibiotics, as illustrated in Table 2.

Table 1: Zone Of Inhibition in Triplicate Using 6mm Cock Barrier Size

	Zone Of Inhibition in Triplicate Using 6mm Cock Barrier Size.			
Antibiotic	**100%**	**70%**	**50%**	**25%**
Orange	12mm	9mm	7mm	6mm
	11mm	7mm	6mm	6mm
	14mm	8mm	6mm	6mm
Mean Count	12:33	8	6.33	6
Vitamin C	15mm	12mm	8mm	6mm
	13mm	12mm	7mm	7mm
	16mm	10mm	8mm	6mm
Mean Count	14.66	12	7.66	6.33

Table 2: Concentration of Sweet Orange and Vitamin C Extract

Antibiotic	**Minimum Inhibitory Concentration (MIC)**	**Minimum Bactericidal Concentration (MBC)**
Orange	50%	70%
Vitamin C	50%	70%

4.0 DISCUSSION

The study revealed active antibacterial properties in both Vitamin C and Sweet Orange extracts against Staphylococcus aureus found in wound infections at Bodinga General Hospital, Nigeria. Orange and Vitamin C showed significant inhibition, with the highest at 100% concentration: Orange had a mean inhibition zone of 12.33mm and Vitamin C had 14.66mm. This aligns with previous studies by [8] and [10] showing similar inhibition patterns. However, [11] found different results, with higher inhibition at 25% concentration. Despite differences, both Vitamin C and Sweet Orange showed effectiveness against Staphylococcus aureus, indicating potential as alternatives to antibiotics.

The study suggests that Vitamin C and Sweet Orange extracts have similar effects on Staphylococcus aureus, with Vitamin C showing the highest inhibition at higher concentrations. This indicates Vitamin

C's potential beyond nutrition, possibly replacing some antibiotics for Staphylococcus aureus infections. This aligns with [9] and [12] findings on antibacterial properties. However, [11] reported different effects in their study on various bacteria.

4.1 Conclusion

The result of this study reveals *Staphylococcus aureus* as agent that causes staphylococcal wound infections. The zones of inhibition of both orange extract and Vitamin C on the organism were minimal because the effect of Vitamin C is on bacterial organism is found to be active involved (inside the body) by increasing the production of B and T cells and other white blood cells including those that destroy microorganism. The sensitivity pattern showed that the higher the concentration of the antibacterial agents the more the zone of inhibition. Since Vitamin C is found to boost the immune system and promotes healing of wounds, fractures and bruises, its intake as food supplement will go a long way in preventing wound infections.

4.2 Recommendations

1. The antibiotic profile of the bacteria isolated from wound should be determined prior to this study.
2. Isolation and purification of major orange juice components should be performed.
3. The mechanisms of extract isolated and purified should be investigated against the bacteria used.
4. Further safety studies using orange juice extract in greater concentration should be investigated.
5. Wounds should be treated as early as possible.
6. Good personal hygiene and good sanitary environment should be adopted.
7. The juice should be processed hygienically so as to avoid contamination by spore forming or capsule forming bacteria.
8. Natural fruits such as orange should be taken because it boosts the immune system in fighting against bacteria and viruses.
9. The juice should also be preserved in the fridge so as to avoid fermentation which will lead to serious increase in acidity of the juice and this will have negative impact on human if consumed.

REFERENCES

1. Abalaka, M. E., Adeyemo, S. O., Daniyan, S. Y. (2011). Evaluation of The Antimicrobial Potentials of Leaf Extracts of *Khaya senegalensis*. *Journal of Pharmaceutical Research and Opinion*. 1(2): 48-51
2. Ahmad, O., Razak, F. A., Agboda, A., Adeiza, S. S., Ungukore, H. Y. and Nuhu, T. (2017). Incidence and Antibiotic Susceptibility Profile of *Staphylococcus aureus* Isolated from Wound of Patients at Specialist Hospitals, Sokoto, Nigeria. *Journal of Medical Biotechnology*. 6(4): 44-50.
3. Alo, M. N., Anyim, C., Igwe, J. C., Elom, M., Uchenna, D. S. (2012). Antibacterial activity of water, ethanol and methanol extracts of *Ocimumgratissimum*, *Vernoniaamygdalina* and *Aframomummelegueta*. *Advances in Applied Science Research*. 3(2): 844-848.
4. Anitha M, Hemapriya J, Mathivathani P, Ramya K, Monisha D. M. (2016). A study on effectiveness of sweet orange against bacterial wound Isolates. *International Journal Plant, Animal andEnvironmental Science*. 6(1): 1-7.
5. Baba, J., Mohammed, S.B., Ya'aba, Y. (2018). Antibacterial activity of Sweet Orange (*Citrus sinensis*) on some clinical bacteria species isolated from wound. *Journal of Family Medicine and Community Health*. 5(4):1154.
6. Ehler, S. A. (2011). Citrus and its benefits, *Journal of Botany*, 5: 201-207.
7. Cambak, D. (2008) *Cambak Income Global Distribution Database Wikipedia*, the Free Encyclopedia. 08-20

8. Hassan, I. A., Ashiru, W. A., Ajetunmobi, O. A. (2013). Sensitivity of *Vernoniaamygdalina* and *Ocimumgratissimum* on Some Selected Bacteria. *International Journal of Scientific Research and Reviews*. 2(4):188-196.
9. Hassan, I. A., Ekum, M. I. and Ogunsanya, A. S. (2021). Antibacterial Activity of Sweet Orange (*Citrus sinensis*) Juice Extract on Selected Bacteria. *Journal of Microbiology Research*. 15(4): 178-182.
10. Israa, A. and Ibrahim, J. (2015). The Activity of Medical Plant Extracts with Al2O3 Nanoparticles on the Vitality of Bacteria and their Genomes. *Journal of Chemical and Pharmaceutical Research*,7(9):645-652
11. Iwuji1, J. C., Chukwuemeka, S. C., Ogbodo, E. C., Ogbonye, E. E., Amah, A. K., Uduchi, I. O., Okezie, A. O. and Okebalama, A. A. (2022). Antimicrobial activities of sweet orange (*Citrus sinensis*) juice on isolated *Staphylococcus aureus, Escherichia coli, Pseudomonas spp*. and *Klebsiella spp*. *International Journal of Clinical Biochemistry and Research*. 9(3) 246-249.
12. Kumar, K. A., Narayani, M., Subanthini, A. (2011). Antimicrobial Activity and Phytochemical Analysis of Citrus Fruit Peels-Utilization of Fruit Waste. *International Journal of Engineering Science and Technology*. 3(6):5414-5421
13. Madhuri, S., Ashwini, U. H., Srilakhmi, N. S., Prashith, K. T. R. Antimicrobial activity of *Citrus aurantum* peel extract. *Journal of Pharmaceutical and Scientific Innovation*. 2014; 3: 366-368.
14. Neenu, A., Santhosh-Anto, P. V., Neethu-Baby, N. (2015). Evaluation on antimicrobial activity of fruit peels of selected citrus species against human pathogenic microorganisms. *Journal Pharmacology and Phytochemicals*. 4: 278-281.
15. Nwankwo, I.U., Onwuakor, C.E., Aninweze, O.N. (2014). Antibacterial Activity of Ethanolic Extract of *Citrus Sinensis* Peel on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* Isolated from Wound Infections. *International Journal of Advances in Pharmacy, Biology and Chemistry*. 3(4):941-947
16. Sonali, P. P., Vishruthi, M., Lalitha, A., Rahul, R., Jayadeepika, S., Rajash, T. and Mehenthirani, R. (2021). Antibacterial Activity of Sweet Orange (*Citrus sinensis*) *Staphylococcus aureus* and *Escherichia coli* Isolate form Wound Infection. *Journal of Emergent Technology and Innovative Research*. 8(7): 640-644.
17. Sulaiman, G.M., Mohammed, W.H., Marzoog, T.R., Al-Amiery, A.A.A., Kadhum, A.A.H., Mohamad, A.B. (2013). Green Synthesis, Antimicrobial and Cytotoxic Effects of Silver Nanoparticles Using Eucalyptus Chapmaniana Leaves Extract. *Asian Pacific Journal of Tropical Biomedicine*, 3(1): 58-63
18. Tariq H., Muhammad A., Sarzamin K., Hamid S. and Muhammad S. Q. (2011). Invitro Screening of Methanol Plant Extracts for Their Antibacterial Activity. *Pakistani Journal Botany*, 43(1): 531-538.