Antibacterial Activity of Epicarp of Sweet Orange (*Citrus sinensis)* against Bacterial Isolates from Wound Infections

[1]kashari O., [1]Danjumma, B.J., [1]Osesua, B.A and **[2]Famubo .A**
[1]Department of Science Technology, Waziri Umaru Federal Polytechnic, Birnin Kebbi, Nigeria.
[2]Basaura Institute of Comprehensive Education, Birnin Kebbi, Kebbi State, Nigeria.
Correspondence e-mail: Kasharioyibo@yahoo.com

Abstract

The antibacterial activity of epicarp of sweet orange (Citrus sinensis) against clinical bacteria from wound infection was carried out using disc diffusion method. The test bacteria were isolates from the wound of patients. Varying concentrations (50,100,150 and 200 mg/ml) of the Citrus sinensis extracts showed activity against the test bacteria. The methanol crude extracts of the epicarp of sweet orange (Citrus sinensis) showed activity against all the test bacteria with highest activity of 22.0mm against E. coli at the highest concentration of 200 mg/ml used but the methanol extract failed to exhibit activity against B. macerane at lower concentration of 50 and 100 mg/ml used. The aqueous extract showed activity against the organism at all the concentrations used with highest activity of 20.0 at 200mg/ml of the concentration used but did not produce any activity on B. macerane. Chloramphenicol was used as positive control which also showed varying degree of activities against the bacteria. MIC and MBC study of both the methanol and aqueous extract showed that the epicarp of sweet orange (Citrus sinensis) can be bacteriostatic and bacteriocidal against the test bacteria except against B. macerane. The phytochemical screening carried out revealed the presence of some important bioactive compound such as Alkaloids, Flavonoids, etc. but tannins and Terpenoids were absent. These results suggest that the epicarp of Citrus sinensis has great antibacterial potentials and can be used to formulate remedies that could treat disorders caused by these test bacteria.

Keywords: *Epicarp, Citrus sinensis, highest, activity, fail, except, bioactive.*

Introduction

Sweet orange is a strum belonging to the plant family Rutaceae with a botanical name *Citrus sinensis*.

Sweet oranges originate from Southern China thousands of years ago. Now they are most popular and widely spread.

Citrus sinensis (Sweet orange) can be grown in most part of the tropics where more than five months and where there is fairly even distribution of rainfall throughout the year. The trees can be grown from seeds but it's more preferable to buy budded. *Citrus sinensis* is a spreading, evergreen, sometimes spiny trees of up to 12m tall with ovale elliptic leaves which are commonly 7-10cm long dark green and routed at the base. The leaves are strongly scented, the white sweet smelling flowers are smaller. Deep yellow to orange or in humid climate remain green when ripe. Sweet oranges *(Citrus sinensis)* are tropical crops and are also annual crops (Bailey, 2002).

In a typical sweet orange, the exocarp and mesocarp are leathery and protect the juicy inner tissue received from the endocarp from desiccation. The epidermis of the fruit has a thick cuticle and varying number of stomata, the exocarp or flavedo is a layer of irregular photosynthetically active parenchyma cell which is green in young fruit and becoming orange intercellular space. The mesocarp is known as the albedo. It is rich in vitamin C and sugar, cellulose and in pectin. The mesocarp and exocarp together form the flesh of the fruit. The center of the fruit is occupied by the development of carpels of the ovary which are disposed around the pithy axis in form of several closely packed segments. Each segment develops from a single carpel and is surrounded by thin, transparent endocarp or "ray" from which multi-cellular hairs grow to fill each segment. Each huge cell pulp vesicle of these hairs fills with the juice and they form the edible part of the fruit for which the crops are grown. The seed lies on axle placenta close to the central axis and in the mature fruit is about 40-45% juice, 30% rind, pulp and seeds which if taken together consist of about 90% water, 5-10% sugar, 1-2% petunia, various acids, essential oil proteins and minerals (Aschoff *et al.*, 2015).

Generally, the fruit contains 80-90% of sugar and acids with relative proportion varying between other species of citrus. Citrus acid is the abundant acid in the sap. Pectin in the juice gives it a cloudy colloidal appearance. *Citrus sinensis* contain mineral salts, glycosides, small amount of protein and vitamin. It is a good source of citrus.

The medical potency of sweet orange *(Citrus sinensis)* is due to its high content in vitamin C which is believed to stimulate the production of white blood cells, primarily neutrophile, which attack foreign antigens such as bacteria and virus. It also boosts the body's production of antibodies and interfere on the protein that helps protect us from viral invaders and cancer cells. This importance of vitamin C from citrus fruits in prevention of scurvy was scientifically proven in 1756 by John Lind (Perescacho and Rouseff, 2008).

The skin is normally an effective barrier to pathogens, but skin may be broken; example, by wounding, surgery or the "bites" of insects, etc. Wounds may admit any of the variety of potential pathogens capable of causing systemic disease (disease affecting the whole body) or localized disease. Bacterial pathogens can enter via "bites". There are many organisms associated with wound infections which are propionibacterium, Klebsiella, Staphylococcus, Escherichia Coli etc. Superficial infections are skin postutes, boils, carbunicles, impetize,

penphigus, neonatorum, syeosis barbae, paronycha styles, blephritis and conjunctivitis, 'infection of accidental and surgical wounds" (Perescacho and Rouseff, 2008).

Wound infections are known to be caused by micro-organisms such as Staphylococcus aureus, Escherichia coli and many others. Infection is a significant problem for most people with wound as it can delay healing, result in unpleasant symptoms such as exudates and pain, increased length of treatment, can result in hospital admissions with prolonged stays, raising the costs of care. It can also be responsible for turning acute wound into chronic ones and if unchecked, it can lead to serious consequences such as Osteomyelitis, amputation, sepsis, multiple organ failure and death.

Treatments of these infections are often expensive that many people cannot afford it due to high level of poverty. According to Spreen and Thomas (2010), sweet orange has been one of the major sources of vitamin C which plays important role in the protection against bacterial and viral infections. The uptake of orange juice can stimulate the production of white blood cells primarily neutrophilis and speeds up healing of wound.

Spreen also said that consumption of sweet orange is safer and more effective than medical drugs since some bacteria are often resistant to many of these drugs and many people often experience side effect of these drugs. Therefore, different plants can be used in the formulation of existing ethnomedicines, one of which is the epicarp of sweet oranges *(citrus sinensis)* because they are available, cheap and safer alternative sources for antimicrobial drugs.

Justification

This research work is carried out to determine the various micro-organisms that cause wound infection and how to kill or inhibit the bacteria present in wounds with available and affordable plant product in our environment *(Citrus sinensis),* that contains a high level of victims C which makes it suitable to be used as an antibacterial agent against clinical isolates from wound infections and this brought about the quest of finding an alternative source for the treatment of these infections caused by these pathogenic microbes that are found in wounds. Therefore, the aim of this research work is to determine the antibacterial activity of epicarp of sweet orange *(Citrus sinensis)* on bacterial isolates from wound infections.

Materials and Methods

The Epicarp of sweet orange (*Citrus sinensis)* used in this research work were obtained from Birnin Kebbi Central Market and transported to Microbiology Laboratory of Waziri Umaru Federal Polytechnic, Birnin Kebbi, Kebbi State.

Preparation of Plant Material

The freshly collected epicarps of sweet oranges (*Citrus sinensis)* were washed thoroughly with running tap water, pealed and then dried under shade at room temperature for a period of seven days until they were completely dry. The small pieces were pounded into powder using wooden mortar and pestle (Matu *et al.*, 2012).

Extraction of Epicarp of *Citrus sinensis*

The extraction of the epicarp of sweet orange (*Citrus sinensis)* sample was done in accordance to the method proposed by Oyeleke and Manga (2008). 200g of the powdered extract of epicarp of sweet orange (*Citrus sinensis)* was weighed, soaked in 500ml of methanol for 72hours and the mixture was shaken after some interval of time, the mixture were filtered

using Whattman No.1 filter paper and the filtrate was concentrated using water bath at 40^0C to obtain the crude methanol extract.

The epicarp of the sweet orange (*Citrus sinensis)* was extracted using warmed water; 200g of powdered sample was soaked in 500ml of distilled water and was allowed to stay for 24hours and was sieved and evaporated to obtain the crude aqueous extract. In this way the methanol extract and the aqueous extract obtained were used for antibacterial testing.

Formulation of Concentrations
Extracted 5g of the prepared methanol crude extract of *Citrus sinensis* was measured using weighting balance and poured into 10ml of distilled water. The same procedure was repeated for 10g, 15g, and 20g to obtain various concentrations of 50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml respectively. The same procedure was repeated for the aqueous extract.

Preparation of the Media
The media used in this research work are prepared in accordance to the manufacturer's instructions. The media used are nutrient broth, nutrient agar, Mueller Hington agar and mannitol salt agar.

Test Bacteria
The micro-organisms used for the study are *Staphycocus aureus, Bacillus Macerane, Pseudomonas aeruginosa* and *Escherichia coli.* Swab sticks were used to swab the wound area of patients. The swab stick was then put into test tubes containing nutrient broth and was incubated at 37^o C for 8 hours. This was then sub-cultured into nutrient agar to obtain pure colonies.

Gram Staining Techniques
From the colonies that developed on nutrient Agar, a smear was made on a clean glass slide using sterile wire loop. It was dried and heat fixed. The smear was flooded with crystal violet solution for 60 seconds and washed, tipped off and covered with Lugol's iodine for 2 minutes. The stain was then decolorized with acetone and washed off immediately with distilled water. It was slide was wiped clean; the smear was placed on a draining rack and allowed to air dry. The smear was then viewed under the microscope using oil immersion objectives x 100. Biochemical test was carried out as described in Oyeleke and Manga (2008).

Antibacterial Activity
The antibacterial testing was carried out according to the method proposed by Mohan *et al.* (2011) as well as Oyeleke and Menga (2008). Mueller-Hington agar was prepared and the plates were allowed to solidify, the sterilized filter paper discs were soaked into the various concentration of the *Citrus Sinensis* extracts (methanol and aqueous). The soaked discs were dried and then placed on the plates containing the test bacteria (*Staphycocus aureus, Bacillus Macerane, Pseudomonas aeruginosa* and *Escherichia coli).* This was done in duplicate. Chloramphenicol antibiotic impregnated disc was used as a positive control. The plates were then incubated at 37^oc for 24 hours and the zone of inhibition was measured, recorded and expressed in millimeters (Mohan et al., 2011).

Determination of (MIC) and (MBC) of the crude extracts
The minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) of the crude extracts of *Citrus senensis* on the test bacteria (*Staphycocus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Escherichia coli)* were determined

according to the method proposed by Samie *et al.* (2005) and Omori *et al.* (2012). Twelve sterile test tubes were used and 1ml of sterile nutrient broth was dispensed from test tube 2 to test tube 12, a stock solution of the seed extracted (methanol and aqueous) of *Citrus sinensis* was prepared, i.e. 400mg of each crude extract was dissolved in 2ml distilled water, 1ml of the stock solution was dispensed aseptically into tube 1 and 1ml transferred to tube 10, leaving tubes 11 and 12, 1ml was taken out from tube 10 and discarded. Broth cultures of each the organisms (*Staphycocus aureus, Bacillus Macerane, Pseudomonas aeruginosa* and *Escherichia coli)* was prepared separately and 1m of the prepared broth culture was dispensed into each test tube with the exception test tube 11, and were then incubated at 37oC for 24hours. After 24hours, the tubes were examined for turbidity in order to determine the MIC and MBC. The MIC was the concentration in the tube that failed to show evidence of growth (turbidity), just immediately after the last one that showed growth. MBC were the tubes that failed to show any growth including the MIC and were cultures on nutrient agar. The absence of growth after incubation indicated a positive result for MBC.

Phytochemical screening

The phytochemical screening of the plant extracts were conducted using standard procedure of Harborne (1984). The extracts were tested for the presence of alkaloids, saponin, tannins, flavonoids, anthraquinones, glycosides and phenolic compounds.

Results and Discussion

The results of this research work are presented in tables, which are as follows:

Table 1: shows the morphology and biochemical characteristics of the bacteria isolates from wound infection

Grams Reactions	Species
Positive cocci	*Staphylococcus aureus*
Negative Rod	*Pseudomonas aeruginosa*
Negative Rod	*Escherichia coli*
Positive Rod	*Bacillus macerane*

The biochemical test carried out are cat = catalase, coa = coagulate, lac = lactose, Glu = glucose, Suc = sucrose, Cit = citrate, Mot = motility, Ind = indole, Ure = urease, MR = methyl red, VP = Voge's Proskauer

Table 2: shows the means zone of inhibition of epicarp of *Citrus Sinensis* extracts against bacteria isolates from wound infection.

Concentration of extracts (mg/ml)		Means zone of inhibition in (mm) test bacteria *S. aureus*	*Ps. aeruginosa*	*E. coli*	*B. macerane*
Methanol Extracts	50	12.0	10.0	14.0	6.0
	100	14.0	10.0	20.0	6.0
	150	18.0	16.0	20.0	10.0
	200	18.0	16.0	22.0	10.0
Aqueous extract	50	14.0	12.0	16.0	6.0
	100	14.0	10.0	16.0	6.0
	150	18.0	10.0	12.0	6.0
	200	20.0	14.0	16.0	6.0
Chloramphenicol control	18.0		18.0	16.0	18.0

Key: - mm = Millimeter mg/ml = milligram per ml

Table 3: Shows the MIC and results and MBC of epicarp of *Citurs sinensis* extract against the test bacteria isolates from wound infections

Extracts/ test bacteria	**MIC values mg/ml**	**MBC values mg/ml**
Methanol extract		
S. aureus	12.5	25.0
Ps. aeruginosa	25.0	50.0
E. coli	6.25	12.5
B. macerane	100	200
Aqueousextract		
S. aureus	6.25	12.5
Ps. aeruginosa	25.0	50.0
E. coli	12.5	25.0
B. macerane		

Key: = + = Presence of growth, - = Absence of growth, mm = millimeter, mg/ml = milligram per ml.

Table 4: Shows the Phytochemical Compounds of Epicarp of *Citrus Sinensis* Extracts

	Level of presence	
Phytochemical compounds	Methanol extract	Aqueous extract
Alkaloids	+	+
Tannins	-	-
Flavoniods	+	+
Saponins	+	-
Steroids	++	+
Terpeniods	-	-
Glycosides	+	-
Anthraquinones	++	++

Key:

++ = highly present

+ = slightly present

- = Absent

Discussion

The result of the antibacterial activities of both the methanol crude extract and aqueous extract of the epicarp of sweet orange (*Citrus sinensis)* using disc diffusion method at different concentrations (50, 100, 150 and 200 mg/ml) used against the bacteria isolates *(Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Bacillus macerane)* from wound infection of patients. The results revealed that the epicarp of *Citrus sinensis* has good activity against the bacteria isolates.

From table 2, the methanol and crude extract indicated high activity against *S. aureus* with the highest activity of 18.0mm at concentration of 150 and 200mg/ml, 16.0mm on *Ps. aeruginosa* at same concentration recorded on *S. aureus* 22.0mm on *E. coli* at the highest concentration of 200mg/ml used. The methanol crude extract shows appreciable inhibitory effect of 10.0mm only at the highest concentration of 150 and 200mg/ml used but fails to show activity at the lower concentrations of 50 and 100mg/ml used and this may be due to the extraction procedure used or the concentrations may be too low to exert inhibitory effect. Also from table 2, the aqueous extract shows appreciable inhibitory effect on the test bacteria with highest zone of inhibition of 20.0mm, 14.0mm, and 16.0mm on *S. aureus, Ps. aeruginosa* and *E. coli* respectively at the highest concentration of 200 mg/ml while the aqueous extract did not show any activity on *B. mecarane* used. This finding corresponds with the findings of motamedi *et al.* (2009) who reported *Citrus sinensis* pearls to be effective against a wide range of microorganisms. The result of their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) study reveals that both the methanol crude extract and the aqueous extract of the epicarp of sweet orange *(Citrus sinensis)* are highly potent against the bacteria isolates and it was also observed that the extract has both bacteriostatic and bacteriocidal effect against the test bacteria except the aqueous extract that fails to produce any bacteriostatic or bacteriocidal effect against *Bacillus macerane.* This result is in the line with findings of Amarda *et al.* (2007) who reported that higher concentrations of antimicrobial substance of the same extract could show appreciable inhibitions.

The result of the phytochemical screening carried out in table 4, of both the methanol and crude extract and the aqueous extract of the epicarp of sweet orange (*Citrus sinensis)* revealed the presence of some important bioactive compounds such as Alkaloids, Falvonionds, Steriods, glycoside and Anthraquiones while Tannis, saponin and terpeniods were absent. The inhibitions of growth of the bacteria isolates could be as a result of the presence of the above named biologically active compounds. Some of these phytochemicals have been reported to possess antimicrobial properties as shown by many researchers (Anderson, 2004; liu, 2004).

Conclusion

The test bacteria used in this research were obtained from the wound of patients attending Federal Medical Center, Birnin Kebbi. The antibacterial activity observed indicated high activity of both the extract of *Citrus sinensis* against the test bacteria except the aqueous extract that fails to produce any inhibitory effect against *Bacullis macerane*. The MIC and MBC study revealed that citrus sinensis extract has both bacteriostatic and bactericidal effect in the extract of citrus sinensis. These results suggest that the epicarp of sweet orange (*Citrus sinensis)* has great antibacterial potentials and can be used to formulate remedies that could treat wound infections or any disorders caused by these test bacteria.

Reference

Anderson, G.D (2004): phytochemical dynamics, conservation of medical important leaf vetable in Nigeria. http://siv.Edu/elb.ayodele.html.

Angrew, H.f: (2007). Antimicrobial and toxicological activities of five medicinal plants species from Cameroon traditional medicine. Biomedical central complementary and alternative medicine, 11 (70): 1-11

Aschoof, C., Mensah, A.Y. and Asante, O.S (2015). Antimicrobial activity and phytochemical studies of some medicinal plant from Ghana, bulletin Latinomericano plantas medicinal Aromaticas, 5 (6): 113-117

Bailey, J.A (2002): Ethnobotanical observations on cuna Indians, Econ. Bot. 29:278.

Liu R.H (2004): Potential synergy of phytochemicals in cancer prevention mechanism of action. Journal of nutrition. 13(4)-34795-34855

Matu, E, N., Nicolosi J.S., Sultan, M., and Srivastava SK (2008): two new glyeoflavones from the roots of phyllanthus niruri, planta Med., 32, 2.

Mohan, N., Jassal, P.S., Kumar, V. and Singh, R.P. (2011): Recent research in Science Technology; vol.3(9): pp 78-83.

Motamedi, H., Safary, A., Maleki, S and Seyyednejad, S.M (2009): Potential source for discovery of new antimicrobial agents, Asian Journal of plant science, 8(2) 187-190

Ogunjobi, D., and Elizerbeth, B. (2011): Medicinal plants of the Admiraly Islands, Papu New Guinea, Part 1, International Journal of crude and Drug Research**20**(4): 169-181.

Omori, R., Cowling, B.J. and Nishiura H. (2012): How vaccine effectiveness scaled by the transmission dynamics of interacting pathogen strains with cross-protective immunity? Journal pone, 7(11), 507-51

Ostergen, G.F and Lebosse, P (2011): medicinal plants of Jamaica. II, *West Indian Medical Journal* 4: 69-82

Oyeleke, B.S. and Manga, S.B. (2008): Essential Laboratory Practical in Microbiology, 1st Ed, Tobest Publisher Minna, 23-62.

Perecacho, W., and Rouseff. C. (2008): herbs of the genus phylanthus in treatment of chronic hepatitis B observations with three preparations from different geographic sites, J, Lab.

Samie, A. Obi, C., Bessong, P.O and Namrita. L. (2005): activity profiles of fourteen selected medical plants from Rural Venda Communities in South A.