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Antibacterial Activity of *Citrus sinensis* (Orange) Peel on Bacterial Isolates from Wound

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Abstract

The antibacterial activity of aqueous, ethanolic and ethyl acetate extracts of *Citrus sinensis* against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, and Pseudomonas aeruginosa* was determined. This work aimed to discover the effects of orange peels extracts in the treatment of wounds contaminated by bacteria. Four different concentrations (50mg/ml, 100mg/ml, 150mg/ml, and 200mg/ml) of each extract were used against the test organisms. The test isolates demonstrated susceptibility to the varying concentrations of the crude extracts; this was evident in the different diameters of zones of inhibition displayed by the extracts. *Staphylococcus aureus* was the least susceptible to the various extracts at different concentrations. The aqueous extract showed a zone of inhibition of 7±0.0mm at 50mg/ml and 16±2.0mm at 200mg/ml against *Escherichia coli*; *Pseudomonas aeroginosa* at 7±0.0mm, 50mg/ml and 15±1.0mm at 200mg/ml; *Klebsiella pneumonia*e 6±0.0mm at 50mg/ml and 15±1.0mm at 200mg/ml. Ethyl acetate produce the zone of inhibition of 9±0.0mm and 19±1.0mm with *Klebsiella pneumoniae* at 50mg/ml and 200mg/ml; *Escherichia coli* at 8±0.0mm and 14±1.0mm at 50mg/ml and 200mg/ml respectively; *Pseudomonas aeroginosa* was susceptible to ethanol extract giving zones of inhibition of 6±0.0mm and 14±0.0mm at the concentrations of 50mg/ml and 200mg/ml respectively. The result showed that the potency and efficacy of the orange peel extracts on the organisms that cause wound infection had different hierarchy of susceptibility among the test organisms. The crude extracts from orange peels may be used to disinfect wounds and probably lead to cure if it can be properly refined.

Keywords: Antibacterial activity; *Citrus sinensis;* orange peel; bacterial isolates; wounds.

INTRODUCTION

Medicinal plants are essential curative agents for different types of ailments. The experiment carried out by scientists has shown the antimicrobial ability and capacity of plant components which was discovered first in the 19th century (Odebiyi and Sofowora, 1978). In India, from the antediluvian era, plants which have medicinal importance have been used for therapeutic purpose of specific ailments. There are two reasons why clinical Microbiology has interest in the antimicrobial activity of plants extract. First, it is possible that the phytochemicals can be found as the components of antimicrobial drugs prescribed by the doctors or pharmacists. Second, the awareness of the public about the problem of the over prescription and misuse of traditional medicine. To know the degree of effectiveness and enhance the use of herbal medicine, therefore, is necessary to ensure intensive study of plants that are of medicinal importance.

Traditional medicine is important and has shown to be of great promise as an easy source and effective treatment of different diseases to people especially in the tropical developing countries including Nigeria. Different people in different locality use several plants to derive different preparation as curative agent for different diseases (Satyayati *et al.,* 1990).

The World Health Organization (WHO) has defined the word traditional medicine as: the sum total of the skills and practices based on the theories, beliefs, and experiences indigenous to different order, whether explainable or not, used in the maintenance of health as well as in the prevention and diagnosis of physical and mental illness (WHO, 2008).

Traditional medicine which consists of herbal medicine and spiritual therapies has been used for millennium by various people to treat chronic and acute diseases. In many developing countries they remain the most accessible and most commonly used form of medical care (WHO, 2008) while pharmaceutical medicines are commonly used in developed countries to treat a wide range of infectious diseases and chronic conditions. Patient in developing countries depend exclusively on traditional medicine for numerous reasons (WHO, 2008).

Herbal medicine is a long in the tooth form of health-care known to mankind. It has been used by all culture throughout history. It was an inherent part of the development of modern order. Undisputedly, modern medicine and the history of herbology have been inextricably intertwined. Many drugs listed as orthodox medication were originally derived from plants Leslie *et al.* (2005) Today, research confirms that the herbs boost the immune system by stimulating the production of disease-fighting white blood cells (Chong, 2003).

The world health organization estimates that four billion people, 80% of the world population, presently use herbal medicine for some part of primary health-care. Herbal medicine plays a major role in the indigenous people's traditional medicine and a common element in Ayuvedic, homeopathic, naturopathic, traditional oriental and Native American Indian medicine Leslie *et al.* (2005).

Plant derived components are a major area of interest to source for safer and more effective antibacterial agents Mann *et al.* (2008). Phytochemicals are in the most discipline sense of the world, chemicals produced by plants. Commonly, the word phytochemical refer to only those chemicals which may have an impact on health or on flavour, texture, or color of the plants but are not required by humans as essential nutrients (Semiz and Sen 2007).

A wound is as a result of physical disruption of the skin, one of the major hurdles to the establishment of infections by pathogenic microorganism in internal tissues. Once bacteria disrupt this barrier, it may result to infection [Bisno Stevens 1996; Janda *et al.,* 1997]. The most common underlying event for all wounds is trauma. Trauma may be intentionally or accidentally induced Janda *et al.,* (1997). The former category includes hospital-acquired wounds, which can be grouped according to how they are acquired, such as surgically and by use of intravenous medical devices. Notwithstanding not intentionally induced, hospital-acquired wounds can be the pressure sores caused by local ischemia, too. They are also referred as decubitus ulcers, and when such wounds become infected, they are often colonized by multiple bacterial species [Janda *et al.,* 1997]. According to NCCLS (1997), NNISS, (2002), in the absence of clinical signs of infection, the amount of organisms, or microbial load, is believed to be the best indicator of wound infection.

Sweet orange (*Citrus sinensis*) is a small evergreen tree 7.5 m high and sometimes up to 15 m. Its origin is China and it has been cultivated over the years, but is grown commercially worldwide in tropics, semi-tropical and some warm temperate regions and has become the most widely planted tree fruit in the world today according to Nicolosi *et al.* (2000), Ehler, (2011).

In this research work, *Cirus sinensis* peels extract was studied for their antibacterial activity against organisms that cause wound infections.

MATERIALS AND METHODS

Collection of Orange Peel and Drying

The plant used in this study Citrus sinensis was obtained from fruit sellers at Hanyan Gwari, Bosso, Minna, Niger state. After collection, the orange was peeled and the peel was shade dried at room temperature

for seven days after which it was pulverized into powder using mortar and pestle and then packed into clean bottles for further analysis.

Wound swab

The wound samples swabs used were collected by the medical laboratory scientists in the respective diagnostic laboratories, using swab sticks, at General Hospital, Minna and IBB specialist Hospital, Minna, Nigeria. A total of 20 wound samples were collected (10 each) from the two hospitals.

Preparation of Extract

Extraction was done using Soxhlet extraction method with three different solvents; Ethanol, Ethyl acetate and water respectively. Orange peel was ground into fine powder and 150g was weighed into a beaker containing 1000ml (1 litre) of each solvent. The mixture of each was left standing for 73 hours with shaking at regular intervals of 5 hourly. At the end of this period, it was filtered using Whatman filter paper No. 11. 2g of the extract was dissolved in 5ml of Dimethyl sulfoxide (DMSO) to obtain 2000mg/5ml which was used as stock solution. The use of antibiotics such as Gentamycin, Septrin, Ampicilin e.t.c. was used as negative control against the wound isolates in comparison to the orange peel extracts.

Culture Media

After the collection of the wound swap, it was inoculated into nutrient agar. The different colonies were sub cultured into MacConkey agar and blood agar using the streak method and was incubated at 37°C for 24hours. The pure isolates were identified using some standard biochemical methods such peroxidase, citrate utilization, coagulase, indole as well as sugars fermentation tests. After identification, the isolates were further sub cultured into bijou bottle containing nutrient agar and stored in the refrigerator for further analysis.

EVALUATION OF ANTIBATERIAL ACTIVITY

Preparation of Stock Solution of Extract

About 2g of the resultant residue of the extract was dissolved into 5ml of Dimethyl sulfoxide (DMSO) to give a concentration of 2000mg/5ml to have 200mg/ml as stock. The resultant stock was further serially diluted to give four different dilutions at the concentrations of 200mg/ml, 150mg/ml, 100mg/ml and 50mg/ml respectively. The tubes containing the various concentrations were labeled and stored in the refrigerator until they were needed for further analysis.

Susceptibility Test

Antibacterial screening of crude extract using the following steps:

a) Preparation of bacterial suspension. Four organisms isolated from the wounds swabs namely; *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia and Pseudomonas aeruginosa* were used for the tests. After the collection of the wound swap, it was inoculated into nutrient agar. The different colonies were sub cultured into nutrient agar to obtain pure isolates. After 24hours incubation, the organisms where sub cultured in to a sterile nutrient broth of 10ml each respectively and incubated for 24hours and was re-inoculated into Muller Hinton agar for 3hours to standardize the organisms at microbial suspension of 10^6CFU/ml.

b) Antibacterial screening of crude extract using agar cup plate technique as described by [Silva *et al.,* (1997), Abalaka *et al.,* (2011)] was used. Using a cork borer of 7mm/diameter, four holes were made on the surface of the agar medium and each cup was sealed at the bottom with molten nutrient agar to avoid seepage of the extract. The holes were then filled with various orange peel extract at varying test concentrations.

An 18 hour culture of each test organism in nutrient broth was used to inoculate the agar medium.

The following concentrations of extract; 200mg/ml, 150mg/ml, 100mg/ml and 50mg/ml were used to challenge the organisms and their reactions to the extracts after incubation for 24hours were observed and recorded. Antibiotics such as chloramphenicol, septrin, ampicilin etc were also used as a negative control on various organisms in comparison to the extract.

Minimum Inhibitory Concentration

The minimum inhibitory concentration was determined using the tube dilution method in which 9ml of sterile nutrient broth was dispensed into test tubes and 1ml of the extract of varying concentrations was added into the different test tubes and 0.1ml of standardized organism was inoculated and incubated for 24hours at 37^{o}C. The test tube with least concentration of extract that showed no turbidity was taken as the minimum inhibitory concentration.

Minimum Bactericidal Concentration

The test tubes that contained 0.1ml of the test organism, 9ml of sterile nutrient broth and 1ml of the plant extract at different concentration that showed no turbidity was inoculated into nutrient agar plate and incubated for 24hours and the plate that contained the lowest concentration of extract that showed no growth was taken as the minimum bactericidal concentration.

Phytochemical Analysis of Plant Extracts

Phytochemical screening of the extracts was carried out according to the methods described by [Odebiyi and Sofowora (1978), Trease and Evans (1989)] for the detection of active components like saponins, tannins, alkaloids, glycosides.

RESULTS

Tables 1,,2 & 3 show diameter zones of inhibition produced against the test organisms by the ethanol ethyl acetate and aqueous extracts at various test concentrations which indicate potency of the extracts against the organisms. Table 4 shows the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the extracts against the test isolates. Tables 5 & 6 contain information on the activities of the control (standard antibiotics) against the organisms and the types of plant secondary metabolites found in the extracts.

Table 1. Mean diameter of zone of inhibition (mm)* of Ethanol Extract of orange peel

Concentration (mg/ml)	***K. pneumoniae***	***E. coli***	***P. aeruginosa***	***S. aureus***
200	19±4.00	15±1.00	14±1.00	NA
150	16±2.00	11±1.00	12±2.00	NA
100	15±2.00	10±0.00	9±0.00	NA
50	9±0.00	4±0.00	6±0.00	NA

KEY: NA= No Activity

Table 2. Mean diameter of zone of inhibition (mm)* of Ethyl Acetate Extract of orange peel

Concentration (mg/ml)	***K. pneumonia***	***E. coli***	***P. aeruginosa***	***S. aureus***
200	19±1.00	14±1.00	19±2.00	25±4.00
150	17±2.00	14±1.00	15±1.00	22±4.00
100	16±1.00	13±3	11±1.00	17±4.00
50	9±0.00	8±0.00	9±0.00	NA

Key: NA= No Activity

Table 3. Mean diameter of zone of inhibition (mm)* of Aqueous Extract of orange peel

Concentration (mg/ml)	***K. pneumonia***	***E. coli***	***P. aeruginosa***	***S. aureus***
200	15±1.00	16±2.00	15±1.00	14±2.00
150	11±1.00	13±2.00	11±2.00	10±0.00
100	9±0.00	11±1.00	11±1.00	10±0.00
50	6±0.00	7±0.00	7±0.00	NA

KEY: NA= No Activity

Table 4. Comparing the Susceptibility of the test isolates to the extracts minimum inhibitory concentration and the minimum bactericidal concentration

Bacteria	**Extracts**			
	Conc mg/ml	**Aqueous**	**Ethanol**	**Ethyl acetate**
S. aureus	50	-	-	-
	200	+*	-	+*
E.coli	50	+	+	+
	200	+*	+*	+*
P.aeroginosa	50	+	+	+
	200	+*	+*	+*
K. pneumonia	50	+	+	+
	200	+*	+*	+*

KEY: - = NO ACTIVITY, +*= MBC, + = MIC

Table 5. Positive control in mean diameter per zone of inhibition (mm)*

Antibiotics	***S. aureus***	***K. pneumonia***	***E. coli***	***P. aeruginosa***
GENTAMYCIN (CN)	S	S	I	S
SEPTRIN (SXT)	S	__	__	__
CHLORAMPHENICOL	S	S	S	S
NALIDIXIC ACID (NA)	__	S	__	S
REFLACINE (PEF)	S	__	__	__
CIPROFLOX(CPX)	S	S	S	S
STREPTOMYCIN (S)	S	__	S	__
AMPICILIN (PN)	S	__	S	__

KEYS: Sensitive(S) ≥ 20 (++ to +++), Intermediate (I) 15-19 (+), Resistant(R) ≤ 14

Table 6. Phytochemical analysis of crude extract of *Citrus sinensis* peel

Phytochemical	**Ethanol**	**Ethyl acetate**	**aqueous**
Alkaloids	+	+	+
Flavonoid	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Terpenes	+	+	-
Anthraquinones	-	-	-
Glycosides	-	+	+
Reducing sugar	-	-	+

KEY; + = PRESENT, - = NOT PRESENT

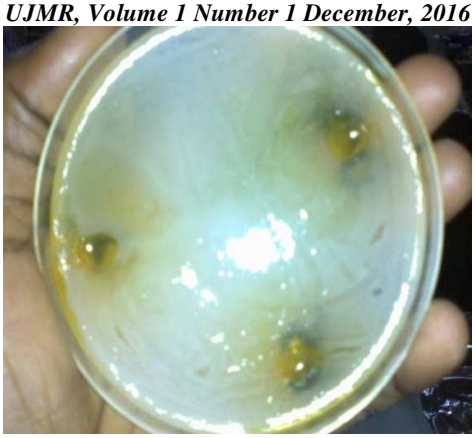

Figure 1 Zones of inhibition shown by the extracts against isolates

DISCUSSION

The result obtained from the antimicrobial assay showed characteristic zones of inhibition around the test organisms isolated from wounds. These organisms include *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, and Pseudomonas aeruginosa.* Amongst all the isolates used in this experiment, *Staphylococcus aureus is* the least susceptible to extracts. The aqueous extract showed no activity against *Staphylococcus aureus* at a concentration of 50mg/ml but was active with a zone of inhibition of 14±2 at a concentration of 200mg/ml, *Klebsiella pneumoniae* was susceptible with a zone of inhibition of 6±1.0 at 50mg/ml and 15±1.0 at 200mg/ml, *Escherichia coli* showed a zone of clearing of 7±0.0 at 50mg/ml and 16±2.0 at 200mg/ml, *Pseudomonas aeruginosa* showed a zone of inhibition of 7±0.0 at 50mg/ml and 15±1.0 at 200mg/ml. The ethanol and ethyl acetate extracts exhibit a significant activity against *Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* but no activity on *Staphylococcus aureus* with the ethanol extract. The Ethanol extract was active against *Escherichia coli* with zones of inhibition of 4±0.0 at a concentration of 50mg/ml and 15±1.0 at a concentration of 200mg/ml, *Pseudomonas aeruginosa* 6±0.0 at 50mg/ml and 14±1.0 at 200mg/ml, *Klebsiella pneumoniae* 9±0.0 at 50mg/ml and 19±4.0 at 200mg/ml. The ethyl acetate has the highest activity against *Staphylococcus aureus* displaying a zone of inhibition of 25±4.0 at 200mg/ml, *Pseudomonas aeruginosa*at 9±0.0 at 50mg/ml and 19±2.0 at 200mg/ml, *Klebsiella pneumoniae* 9±0.0 at 50mg/ml and 19±1.0 at 200mg/ml, *Escherichia coli* 8±0.0 at 50mg/ml and 14±1.0 at 200mg/ml.

The results showed that the potency of the orange peel extracts on the organisms that cause wound infection had different hierarchy of susceptibility among the organisms. Generally, against the isolated bacteria, higher concentration of the extract shows a greater zone of inhibition; this results is in agreement with the report of Bisno and Stevens (1996) which states that the higher the concentration of antibacterial substance, the higher it shows an appreciable zone of inhibition. Israa and Ibrahim (2015) in their studies on the antibacterial activities of plant extracts on *S. aureus* and *E. coli* reported that the extracts from alumina had profound activities on the test organisms. Bag *et al.* (2013) studied the therapeutic usefulness of an Indian medicinal plant (*Terminalia chebula* Retz. and some of its isolated compounds, along with their safety evaluation) and clearly demonstrated its activities against the test organisms just as in the present study. Studies of the activities of Eucalyptus chapmaniana leaves extracts on *Escherichia coli* were carried out by Sulaiman *et al.* (2013) and concluded that the plant extracts is useful against the diseases caused by the organism.

The growth of the entire organisms was inhibited with the extracts at Minimum Inhibitory Concentration of 50mg/ml and 100mg/ml.

The Minimum Bactericidal Concentration of the extracts was 200mg/ml. From the results it is clear that *Staphylococcus aureus* was least susceptible to the different fractions of extracts used while *Klebsiella pneumoniae and Pseudomonas aeruginosa* are the highest susceptible to the crude extracts. Tariq *et al.* (2011) tested the extracts of *Carum copticum, Mallotus philippensis, Citrullus colocynthis, Calotropis procera, Embelli ribes* and *Ricinus communis* against the organisms *Pasteurella multocida, Escherichia coli, Bacillus cereus, Corynebacterium bovis* and *Staphylococcus aureus* and reported similar experience as in the present research with regards to their minimum inhibitory concentrations.

The phytochemical screening of the orange peel revealed that it contains active compounds such as alkaloids, terpenens, flavonoids, reducing sugar, saponins, tannins and glycocides. The presence of these components may be responsible for the antibacterial activity of the orange peel. For example, studies have shown that the saponin present in the orange peel is known to cause interference with the multiplication of DNA and glycogen present is hydrolyzed to produce products such as phenol compounds and acids with antiseptic action. Semiz and Sen.(2007);. Kumar *et al,* (2011), Amandeep and Ahmed (2009) and Nwankwo *et al,* (2014) all have also reported similar results for the various activities of citrus fruits extracts. Thus the present work is in agreement with theirs.

CONCLUSION

This present work has shown that extracts from *Citrus sinesis* have activity against the clinical isolates from wounds used in this experiment. The rate at which pathogenic bacteria are developing resistance to common conventional antibiotics is alarming therefore it is heartwarming to note that we could find succor in abundantly available local remedy like orange peels for the treatment of wounds. It is hoped that therapeutics can be developed from orange peels to which these organisms are yet to develop resistance. Therefore, the orange peel extract that has an antimicrobial property against these organisms isolated from infected wounds may be harnessed as one of the highly needed drugs for wounds treatment in the developing world.

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