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Antibiogram of *Staphylococcus Aureus* and its Sensitivity to *Ocimum Gratissimum* Extract

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ABSTRACT

In this study, clinical isolate of *Staphylococcus aureus* was subjected to susceptibility tests against commercially available antibiotics and *Ocimum gratissimum* (scent leaf) leaf extracts. disc diffusion nmethod was adopted for the antibiotics test while agar well diffusion technique was employed for the antimicrobial screening of the leaf extracts. The result shows that the organism was sensitive to Gentamycin, Zinnacef, Ciprofloxacin and Streptomycin representing 40 % of the antibiotics while showing outright resistance to Ampiclox, Amoxicillin and Erythromycin (30 %) and intermediate results for the rest. Also, the cold water extract of *O. gratissimum* did not show any activity against the bacterium. However, both hot water extract and ethanol extract of the plant had varying degree of activity against the organism with ethanol extract recording a zone of inhibition of 25.33 mm compared with 32.00 mm recorded for Gentamycin. Furthermore, the minimum inhibitory concentration recorded ofr the extract against the organism were 10 mg/ml and 2.5 mg/ml respectively for hot water and ethanol extracts. These results indicate that ethanol extract of *O. gratissimum* leaf is very effective against *Staphylococcus aureus* and could be used as potential source of natural product for the treatment of infections caused by the organism especially the antibiotic resistant strains.

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INTRODUCTION

Staphyloccoccus aureus a Gram-positive bacterium is part of the normal flora of the human skin. However, it has been implicated in several opportunistic infections such as skin infection and inflammation, soft tissue infection, and septicaemia. It is also one of the major causes of nosocomial infections in surgical wards. Some strains of this organism are capable of producing toxins which may have severe implications like toxic shock syndrome and food poisoning (Kotloff *et al*., 2013).

At the introduction of antibiotics, they were a game changer in the continuous struggle between man and microbes leading to overwhelming improvement in health and disease management in humans. However, the microbes have developed strategies to evade these antibiotics, particularly those with the penicillin G ring (Pruden *et al*., 2013). This resistance was reported to be mediated by the production of a special enzyme called betalactamase which helps the organisms to inactivate antibiotics like penicillin, ampicillin and amoxicillin. Antibiotic resistance to this set of antibiotics and other classes is on the rise and health care delivery systems are at a loss to how to combat this phenomenon (Morgan *et al*., 2011).

There is a strain of *S. aureus* presently that possess genes that helps it to resist the action of many antibiotics and this strain is termed mutidrug resistant *S. aureus* (MRSA). The treatment of infection caused by this MRSA is more complicated and costly since it requires a second line antimicrobial agents which are mostly more expensive and more toxic (Lino and Deogracious, 2006).

The ongoing search for new and more effective sources of antimicrobials has led to a humongous research efforts among the professionals and academic community. The plant kingdom seems to be the last resort in the search for alternative treatment resources for infectious agents, some of which are resistant to the available antibiotics. Although, the use of plant and its product for management of infectious diseases has been in practice since time immemoria, recent developments has necessitated going back to the use of medicinal plants in this century owing to

their accessibility, effectiveness and cheaper nature (Preethi *et al*., 2010).

Ocimum gratissimum L. (Scent leaf) is a common plant that is extensively distributed in Nigeria. It is usually planted in the backyard as vegetable and handy remedy especially for stomach discomfort (Abdullahi, 2012). The plant has also been reported to contain phytochemicals like alkaloids, tannins, phytates, flavonoids which are known to possess antimicrobial properties against bacteria and fungi (Ishiwu *et al.,* 2014). The present study was therefore designed to investigate the antibacterial effects of *Ocimum gratissimum* leaf extract against clinical isolate of antibiotic resistant *Staphylococcus aureus*.

Materials and Methods

Sample collection

O. gratisimum leaves were collected from surrounding gardens in Rufus Giwa Polytechnic, Owo. The leaves were washed with distilled water and then air dried under shade for about three weeks. Therafter, the dried leaves were ground to powder using an electronic blender.

Collection of test organism

Clinical isolates of *Staphylococcus aureus* were collected from the Microbiology Laboratory of Federal Medical Centre, Owo. They were transported in ice packs to the Microbiology Laboratory of the Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo for further analysis.

Sterilization protocol

All glasswares were washed with detergent and water, and rinsed thoroughly with distilled water and dried. Petri dishes, MacCartney bottles, test tube and conical flasks with media dispensed into them were sterilized by autoclaving at 121^{o}C, 15Ibs (1Kg/cm^{3}) pressure or 15 minutes. The inoculating wireloop was sterilized by flaming under a spirit till red-heat and then allowed to cool before use. The top of the workbench was swabbed with cotton soaked in 95% alcohol before and after use.

Preparation of different extracts of *O. gratissimum*

1. About 200 g of the fresh leaves were placed in a pot and distilled water (500 ml) was added, this was brought to boil for 30 minutes to get the hot water extract.
2. About 200 g of the fresh leaves was macerated in a sterile mortal and pestle with addition of 500 ml of water. The cold water extract was obtained by sieving with a sterile muslin cloth.
3. To make the ethanol extract, the powdered dried leaf sample was soaked in ethanol for 48 hrs and sieve with muslin cloth to collect the extract.

Standardization of Innoculum

About 5 loopfuls of the test organism was aseptically inoculated in 5ml of sterile saline (0.85% NaCl). The inoculum suspension was shaken for 15 seconds and the inoculum density was adjusted to the turbidity of a 0.5 McFarland standard (equivalent to 1.5×10^{6} cfu/ml) with sterile saline before use according to the method of Akinnibosun and Oyetayo (2018).

Antibiotic Susceptibility Test

The prepared molten Mueller-Hinton agar was dispensed in Petri plates containing 0.1 ml of the inoculum and allowed to solidify in Petri plates while sterile forceps was used to aseptically place the commercially available antibiotics sensitivity disc on it. The plates were incubated for 24 hrs at 37 ^{o}C. The zones of inhibition indicating the susceptibility of the organisms to the antibiotics were observed and recorded (Suree and Pana, 2015).

Antibacterial Activity Test

The antibacterial activity of the plant extracts was determined using the agar-well diffusion method. The inoculum suspensions were tested against the effect of the extracts at a concentration of 20 mg/ml. Two hundred microliter of the standardized cell suspensions were spread on a Muller-Hilton agar. Wells were ten bored into the agar using a sterile 5mm diameter cork borer. Approximately 100 µl of the crude extracts were dispensed into the wells and properly labeled. The preparations were left to diffuse before incubation (Owoseni and Ogunnusi, 2006). The inoculated plates were incubated at 37 ^{o}C for 24 hrs. The plates were then observed for clearing around wells, that is zones of inhibition.

Determination of the Minimum Inhibitory Concentration (MIC)

The MIC of different concentrations of the extracts was determined using two-fold dilutions method. The lowest concentration (dilution) of antimicrobial agent that completely prevented the visible growth of the microorganisms was taken as the minimum inhibitory concentration (MIC) of the extract (Okigbo and Igwe, 2007).

Results and Discussion

The results presented in Table 1 revealed that the clinical isolate of the test *S. aureus* was sensitive to four (4) of the tested antibiotics namely Gentamycin, Zinnacef, Ciprofloxacin and Streptomycin while showing resistance to Ampiclox, Amoxicillin and Erythromycin; and having intermediate sensitivity against the others.

Table 1: Antibiotics susceptibility of *Staphylococcus aureus* against commercially available antibiotics

S/N	ANTIBIOTICS	RESULT
1	Refloxacin	I
2	Gentamycin	S
3	Ampiclox	R
4	Zinnacef	S
5	Amoxicillin	R
6	Rocephin	I
7	Ciprofloxacin	S
8	Streptomycin	S
9	Septrin	I
10	Erythromycin	R

Key: S = sensitive, I = intermediate, R = resistant

The resistance of the isolate to Ampiclox, Amoxicillin and Erythromycin is instructive since they are among the most widely distributed and available antibiotics in Nigerian market. Also, the organism has been widely reported to have mechanisms for antibiotics resistance with a report of many strains having multidrug resistance gene. Another explanation may be due to the fact that the organism may have been exposed to these antibiotics previously in the hospital and may have developed a way to circumvent the actions of these antibiotics.

The antibacterial activity of different leave extracts of *O. gratissimum* against *S. aureus* is shown in table 2 while its minimum inhibitory concentration (MIC) is presented in table 3. These tables revealed that both hot water extract and ethanol extract possess activity against the organism recording 12.67 mm and 25.33 mm zones of inhibition respectively. Albeit, cold water extract showed no activity at the concentration used. The MICs reccorded against the bacterium were 10 mg/ml and 2.5 mg/ml respectively for hot water and ethanol extract.

Table 2: Comparison of the antibacterial activity of the different *O. gratissimum* leave extracts against *Staphylococcus aureus* at 20 mg/ml

Sample	Zone of inhibition (mm)
Cold extract	0.00
Hot extract	12.67
Ethanol extract	25.33
Gentamycin	32.00

Table 3: The minimum inhibitory concentration of different *O. gratissimum* leave extracts against *Staphylococcus aureus*

Sample	concentration (mg/ml)
Cold extract	ND
Hot extract	10
Ethanol extract	2.5

Ethanol extract was observed to show higher effectiveness with a wider zone of inhibition when compared to hot water extracts whereas, no effects were observed in the extracts prepared with cold water. In this study, ethanol extracts have showed the most effective result among the extracts tested, this observation is similar to results obtained by earlier researchers. Earlier, Opara *et al.* (2014) reported antibacterial activity of the *O. gratissimum* against *P. aeruginosa. Although, this bacterium is a gramnegative organism, it is one of the most notorious antibiotic resistant organisms. Therefore, the activity of the plant against S. ureus in this study suggests that the plant may cotain wide spectrum of activity against both gram positive and gram negtive bacteria.*

The observed antibacterial activity of the plant extract may be due to the reported presence of arrays of phytochemicals inherent in the plant. Ladipo *et al.* (2010) had reported the presence of alkaloid, anthraquinone, flavonoid, glycoside, phenol, saponin, steroid and tannins in the leaf extract of *O. gratissimum*. Some of these phytochemicals like alkaloids, phenols, tannins and flavonoids are known to possess antimicrobial properties. These result supports the folkloric claim of the plant being used in the management f various infectious diseases such as diarrhoea (Nwinyi *et al.,* 2009).

The MIC is defined as the least concentration of a substance that is needed to cause visible inhibition of growth of a microbial agent. The lower the value of the MIC, the more effective the plant extract. Therefore the very low MIC value obtained for the crude extract of the *O. gratissimum* leaf suggest that it might possess potent antibacterial principles against *S. aureus*. The plant extract may be particularly useful in the management of drug resistant strains of

CONCLUSION

The demonstration of antibacterial activity of *O. gratissimum* leaf ethanol extract against *S. aureus* is an indication that the plant is a potential source of drugs with a high level of activity against bacteria. Further pharmacological evaluation, toxicology studies and possible mechanism of action of the plant extract are the future challenges that require further research.

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