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Analysis of Bactericidal and Bacteriostatic Effect of the Crude Extract of Scent Leaf (Ocimum gratissimum) on Escherichia coli and Staphylococcus aureus

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Abstract
The antibacterial activity of the extract of *Ocimum gratissimum* was determined against *Escherichia coli* and *Staphylococcus aureus*. The phytochemical analysis of the leaf extract revealed the presence of bioactive bases responsible for antibacterial property, such as Saponin, Alkanoid, Flavonoid, Tanin and Steroid. The methanolic extraction of the active ingredient of the leaves was carried out using the method as described by Fatope, *et al.,* (1993). The minimum inhibitory concentration (MIC) was 12.5mg/ml for *E. coli* and 25mg/ml for *S. aureus* and also had minimum bactericidal concentration of 6.25mg/ml for *E. coli* and 12.5mg/ml for *S. aureus*. The concentration 1.0mg/disc inhibited the isolates with highest diameter zone of inhibition as 15mm for *E. coli* and 13mm for *S. aureus* while the lowest diameter zone of inhibition as 8mm for *E. coli* and 7mm for *S. aureus* were recorded at concentration of 0.1mg/disc. Statistical analysis of the extract showed no significant difference (P> 0.05) the test isolates were sensitive to leaf extract of *Ocimum gratissimum* which implies that the leaf extract of *Ocimum gratissimum* can serve as a source of therapeutic agent and possess antibacterial properties.
Keywords- *Ocimum gratissimum, Escherichia coli*, *Staphylococcus aureus* Bactericidal, Bacteriostatic, Crude extract

1. INTRODUCTION

Medicinal plants are a source of many important specific drugs of this modern world and about 80% of the present day medicines are directly or indirectly from plants (Sofowora, 2007). Ijeoma and Umar (2009) noted that herbal treatment is the earliest form of medicine. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. In developed countries about 80% of plants are used in traditional medicine. Therefore, such plants have been investigated for the betterment of understanding of their medicinal properties. The antimicrobial properties of many plants have been investigated by a number of researcher's worldwide (Adamu *et al*., 2005). Plants have been a source of medicine in pharmacopoeia (British, United State's or any other). Herbal medicine can be served as an alternative treatment for some commercial drugs (Anyamene and Ezeadila, 2010). Medicinal plants provide inestimable projections for new drug discoveries because of the matchless availability of chemical range. The practice of herbal medicines in Asia signifies a long antiquity of human interactions with the environment (Sasidharan *et al*., 2011).

Scent leaf belongs to kingdom plantae, order lamiales, family lamiaceae, genus *Ocimum,* species *Ocimum gratissimum* and binomial name *Ocimum gratissimum.* It is a tropical shrub that grows to between 50cm to 80cm tall in height. The leaves are oval in shape, serrated at margins and opposite in arrangement. The flowers are often white in colour (Obvute, 2006).

Phytochemical substances such as alkaloids, saponins, tannins, flavonoids, alkaloids, flavonoids, and terpenoids may be present, which are bioactive bases responsible for antibacterial property (Obadoni *et al.,* 2001). These medicinal properties exert bacteriostatic and bactericidal effects on some bacteria (Effraim *et al*., 2000; 2003; Okigbo and Ogbonnanya, 2006; Funmilayo *et al*., 2010).

The test organisms for this study are causal agents of various human diseases. Infections caused by these and other microorganisms have contributed a lot to health problems especially in developing countries including Nigeria. The research for new antibacterial agents is a continuous exercise and the target microorganisms often evolve new genetic variants causing the emergence of antibiotic resistance among microorganisms (Okeke *et al*., 2008).

MATERIAL AND METHODS

Collection of Plant Materials

The fresh form of scent leaf (*Ocimum gratissimum*) used in this project research was obtained directly from the farm, at Malam Inna of Akko Local Government Area of Gombe State. This was identified in the department of biological sciences, faculty of science, Gombe State University. The confirmed fresh leaves of the plant were rinsed with tap water to remove dirt and separated from the stalk, and were dried at room temperature for 2

weeks and made into powdered form using a clean pestle and a mortar.

2.2 Extraction procedure

The extraction was carried out according to the method of Fatope *et al.,* (1993). In this, 40g of the powdered plant samples were percolated at room temperature with 400ml of methanol (thus achieving 1:10). The beaker was covered with aluminium foil, shaken and left to stand for 2 weeks with regular shaking. After two weeks the suspension was filtered and the filtrate was concentrated using Rotary-evaporating machine at 40^{o}C. The extract was stored in the refrigerator for further analysis at 4 ^{o}C.

The Test Organisms

The test organisms obtained from Federal Teaching Hospital (FTH) Gombe, Gombe State were subjected to standard identification procedures by Cheesbrough (2000). They were identified as *Escherichia coli,* and *Staphyloccocus aureus.*

Inocula Preparation (Standardization)

The inoculum was prepared using the procedure described by Cheesbrough (2000). Using a sterile wire-loop, 3-5 well isolated colonies of the test organisms were touched and emulsified into about 3ml of physiological saline. Turbidity of the suspension of test organism was compared with 0.5 McFarland turbidity standards.

Preparation of Sensitivity Discs

Discs of 6mm in diameter were punched out using Whatman's No. 1 filter paper with the aid of a paper punch and placed in Bijou bottles. The discs were then sterilized by autoclaving using an autoclave at 121^{o}c for 15mins after which they were allowed to cool (Garba *et al*., 2013).

Stock solution of the plant methanolic crude extract (that was recovered) was prepared by dissolving 0.1g (i.e. 10mg) of the plant extract in 1ml of Dimethyl sulphoxide (DMSO). Therefore, the stock solution had a concentration of 10,000µg/ml. from these stock four (4) different concentrations of the plant extract was prepared as 10000µg /ml, 30,000 µg /ml, 50000 µg /ml, and 100000 µg /ml which finally yielded disc potencies of 100 µg /disc, 300 µg /disc, 500 µg /disc and 1000 µg /disc respectively, as a result of introducing 100 sterile discs into each concentration. The discs were allowed to absorb the solution and kept for further analysis. Each paper disc was capable of absorbing 0.01ml of the prepared concentration (Kirby-Bauer, 1966).

2.6 Antibacterial Susceptibility Test (Bioassay Procedure)

The bioassay was carried out to determine the antibacterial activity of the methanolic extract of *Ocimum gratissimum* against the test organisms. Disc-agar diffusion method described by Cheesbrough (2000) was used. Sterile swab sticks were used to swab the standardized inocula of the test organisms and was inoculated onto sterile prepared Mueller-Hinton agar plates. The inoculated plates were then allowed to stay for about 4-5 minutes for the surface of the agar to air-dry. Prepared discs of the four different concentrations (i.e. 100µg, 300µg, 500µg and 1000µg) of the methanolic extract were then placed on the inoculated Mueller Hinton agar plates. Within 30 minutes of discs application, the plates were inverted and incubated aerobically at 37^{0}C for 24hrs. Ciprofloxacin and Gentamycin were used as control. After overnight incubation, the plates were observed or examined for zones of inhibitions. The zones of inhibition were measured in mm using a plastic meter rule.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration was carried out by diluting the initial concentration of the extract using double fold serial dilution (a two-fold serial dilution reduces the concentration of a solution by a factor of two that is, reduces the original concentration by one half) by transferring 50mg of sterile plant extract into 1ml of nutrient broth to obtain 50mg/ml concentration. The above process was repeated several times to obtain other dilutions; 25mg/ml, 12.5mg/ml, and finally 6.25mg/ml (Ibekwe *et al*., 2001).

Having obtained the different concentrations of the extract, each concentration was inoculated with 0.1ml of the standardized bacterial suspension and incubation was carried out at 37^{0}C for 24hrs. The growth of the inocula in the broth was indicated by turbidity or cloudiness of the broth. The lowest concentration of the extract which inhibited the growth of the test organism was taken as the minimum inhibitory concentration (MIC).

The Minimum Bactericidal Concentration (MBC) was determined by selecting tubes that showed no growth during MIC determination. A loopful from each tube was sub-cultured onto extract free agar plates and was incubated for twenty four hours (24hrs) at 37^{0}C. The least concentration at which no growth was observed was noted as the MBC. Readings were taken in mg/ml (Garba *et al.,* 2013).

Phytochemical Screening

Phytochemical screening for the presence of alkaloids, steroids, flavonoids, saponins, resins etc was carried out according to the method described by Trease and Evans, (2007).

RESULTS

Table 1 showed the results of susceptibility test of the extracts on the test isolates. The extract was generally observed to have less activity on *Staphylococcus aureus* than on *Escherichia coli* used in the analysis. The results obtained as shown in table 1 indicated that the higher the concentration of the extract, the higher the zone of inhibition and this varies according to organism while the lowest concentration, which was 100µg /disc, showed the lowest zone of inhibition. Gentamycin and Ciproflox were used as positive controls with zones of 20mm and 30mm, on *Staphylococcus aureus* and *Escherichia coli* respectively.

The minimum inhibitory concentration (MIC) results as shown in table 2 indicated that the leaf extract had a minimum inhibitory concentration of 25mg/ml to *inhibit Staphylococcus aureus* and a minimum concentration of 12.5mg/ml to inhibit *Escherichia coli*. The minimum bactericidal concentration (MBC) showed 6.25mg/ml on *Escherichia coli* and 12.5mg/ml on *Staphylococcus aureus* respectively

The phytochemical screening showed the important classes of compounds like saponins, steroids, Alkaloids, Tanins, and Flavonoids to be present in the leaf of *Ocimum gratissimum* as shown in table 3.

TABLE 1: Anti-bacterial Activity of Methanolic Extract of Scent leaf (*Ocimum gratissimum*) on Some Clinical Isolates Using Agar-Disc Diffusion Method

	Concentration (mg/disc)					
Test organism	0.1	0.3	0.5	1.0	Mean Total	Control
	Zone of inhibition (mm)					
Escherichia coli	8	10	12	15	11.75	30- CN
Staphylococcus aureus	7	9	11	13	10	20-CPX

Key:
CN- Gentamycin
CPX- Ciproflox.

TABLE: 2 **Antibacterial Activity of the Leaves Extract of *Ocimum gratissimum* using Macro Broth Dilution Technique**

Bacteria		Concentrations (mg/ml)							
		MIC				MBC			
		50	25	12.5	6.25	50	25	12.5	6.25
1.	*E. coli*	-	-	-	+	-	-	-	-
2.	*S. aureus*	-	-	+	++	-	-	-	*

KEY: MIC=Minimum Inhibitory Concentration.
MBC=Minimum Bactericidal Concentration
* =Growth observed
\- =Not turbid
\+ =Turbid

TABLE 3: Some Phytochemical Contents of methanolic Leaf Extracts of *Ocimum gratissimum*

Active principle	Inference
Flavonoids	+
Saponins	+
Steroids	+
Tanins	+
Alkaloids	+

Key Present = + Absent = -

4. DISCUSSION

This research was conducted on the antibacterial activity of the crude extract of *Ocimum gratissimum* tested against *Escherichia coli* and *Staphylococcus aureus*; pathogenic bacteria that cause urinary tract infection, diarrhea, minor skin infections such as pimples, impetigo, boils, scalded skin syndrome to life threatening disease such as pneumonia.

The general aim of this research work was to determine the minimum inhibitory concentration that is, the concentrations at which all the bacteria strains were inhibited, the minimum bactericidal concentration, that is, concentration at which all the bacterial strains were killed.

Table1 showed the results of the antibacterial activity of the plant at various concentrations. The table also showed the mean inhibition zone sizes on the test organisms. The highest diameter zone of inhibition occurred with a zone diameter of 15mm at a concentration of 1000µg/disc on *Escherichia coli*, while the lowest

zone of growth inhibition occurred with a zone diameter of 7mm on *Staphylococcus aureus* at a concentration of 100µg /disc. The above findings pointed out that, the higher the concentrations of the extracts, the higher the zone of inhibition.

Sensitivities to the extracts as shown by the increased size of the bacterial growth inhibition zones, was not in agreement with Agatemor (2009) who reported that gram negative bacteria are more resistant than gram positive bacteria to the extracts of *Ocimum gratissimum* but was in conformity with Eyob *et al*., (2008), Okigbo and Ogbonnanya (2006), Jagtap *et al*., (2009) and Nwiyi *et al*., (2009) who reported that the extracts of *O. gratissimum* showed more antibacterial activity against *E. coli*. This may not be unconnected with the factors like differences in geographical settings of the places where the different researches were conducted the species of the *O gratissimum* used as well the strains of the bacteria tested.

Table 2 showed the minimum inhibitory concentration (MIC) of the extract. The MIC was determined to be 12.5mg/ml of extract on *Escherichia coli* and it was found out to be 25mg/ml on *Staphylococcus aureus*. In essence, the MIC on *E. coli* was found to be low in comparison with that on *S. aureus*. The results obtained from this study showed that the leaf extract of the plant inhibited the growth of the test isolates at varying concentrations. This is similar to the findings of Nwinyi *et al*., (2009) who reported that the extracts of *O.* gratissimum showed more antibacterial activity against *E. coli*. And the minimum bactericidal concentration (MBC) of the extract were determined to be 6.25mg/ml on *Escherichia coli* and 12.5mg/ml on *Staphylococcus aureus*, these were the concentrations at which all the bacterial strains were killed, which is similar to the work of Ibekwe *et al.*, (2001) where similar values were obtained for *E. coli* and *S. aureus.*

Table 3 shows the antibacterial activities of these extracts appeared to be of broad spectrum since both the Gram-positive and Gram negative bacteria were sensitive to their inhibitory effects. The presence of these phytochemicals in *O. gratissumum* accounts for usefulness as a medicinal plant (Obadoni *et al.,* 2001).

5.1 Conclusion

The results of this present study signify the potentiality of *O. gratissimum* leaf as a source of therapeutic agents which may pave way in the ongoing search for antimicrobial botanicals. The antimicrobial activity of *O. gratissimum* extract showed a remarkable activity against Gram negative bacteria (*E. coli,*) and Gram positive bacteria (*S. aureus*). This indicates that the plant can be used as herbal medicine in the management of ailments caused by these microbes as well as in pharmaceutical industries for the production of antibiotics.
It was clearly evident from the study that the *O. gratissimum* possess antibacterial properties.

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REFERENCES

Adamu HM, Abayeh OJ, Agho MO, Abdullahi AL, Uba A, Dukku HU, Wufem BM. (2005). An ethnobotanical survey of Bauchi State herbal plants and their antimicrobial activity. *J Ethno Pharma*, 13, 99(1), 1-4.

Agatemor C (2009). Antimcrobial activity of aqueous and ethanol extracts of nine Nigerian spices against four food borne bacteria. *Elec. J. Environ. Agric. food chem.* 8(3): 195-200.

Anyamene CO and Ezeadila JO. (2010). Antibacterial activity of water, ethanol and methanol extracts of *Ocimum gratissimum, Vernonia amygdalina* and *Afromomum melegueta*. *Journal of Applied Sciences*, 13 (1), 8940-8948.

Cheesborough M. (2000). *District Laboratory practice in tropical countries*. Published by Press syndicate of the University of Cambridge, the Edinburgh Building, Cambridge United Kingdom, New York. Pp. 194 – 201.

Effraim ID, Salami HA and Osewa TS, (2000). the effect of aqueous leaf extract of *Ocimum gratissium* on haematological and biochemical parameters in rabbits. *Afr J Biomed Res*, 175-179.

Effraim KD, Jacks TW, Sodipo OA. (2003) Histopathological studies on the toxicity of *Ocimum gratissimum* leave extract on some organs of rabbit. *Afr J Biomed Res* 6: 21-5.

Eyob S, Appelgren M, Rohloff J, Tsegaye A, Messele G. (2008). "Traditional medicinal uses and essential oil composition of leaves and rhizomes of korarima (*Aframomum corrorima* (Braun P.C.M. Jansen) from southern Ethiopia". *South African Journal of Botany*. 74: 181-185.

Fatope, A. O., Ibrahim, H. and Takeda, Y. (1993): "Screening of higher plants reputed as pesticides using brine shrimp lethality bioassay". *International Journal of Pharmacognosy 31: 250-256*.

Funmilayo VD, Olaniran OO, Kanife UC, (2010). Antimicrobial Activities of *Aframomum melegueta* (Alligator Pepper) *International Journal of Biology*, 2(2), 126-131.

Garba L., Isa S. and Hafsat G.L (2013). Bacteriostatic effect of Terminalia catappa leaves extracts on clinical

isolates of Gram-negative bacteria, *Asian Journal of Applied Sciences* 1:(4), 113-118.

Ibekwe, V.I., Nnanyere, N.F. and Akujobi, C.O. (2001). "Studies on Antibacterial activity and phytochemical quality of extracts of orange peel", *Int. J. Environ.,Health and human Development 3:* 077-081.

Ijeoma, J.U. and Umar, A.H. (2009) "Medical plant in use by the Fulani Traditional Herbalists in Yola-North Local Government area of Adamawa State". *Journal of applied science and management* 1(1) 59-63.

Jagtap SD, Deokule SS, Pawar PK, Harsulkar AM. (2009). Traditional Ethnomedicinal Knowledge Confined to the Pawra Tribe of Satpura Hills, Maharashtra, India. *Ethnobotanical Leaflets,* 13: 98-115.

Kirby – Bauer (1966): "Antimicrobial sensitivity testing by agar diffusion method'. *African Journal of Clinical Pathology.* Published by Fibiger United Kingdom 44:493.

Nwinyi OC, Chinedu NS, Ajani OO, Ikpo CO, Ogunniran KO. (2009). Antibacterial effects of extracts of *Ocimum gratissimum* and *Piper guineense* on *Escherichia coli* and *Staphylococcus aureus*. *African Journal of Food Science*, 3:(3), 077-081.

Obadoni, B.O., Ochuko, P.O. (2001). Phytochemcial studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure Applied Science*, 7(3): 455-459.

Obvute, G.C. (2006). *Ethan medical plant resources of south-Eastern Nigeria*. In: http://www.ethnoleaflets.com/leaflets/obute.htm. Retrieved 24/6/2014

Okigbo RN and Ogbonnanya, OU. (2006). "Antifungal effects of two tropical plants extract *Ocimum gratissimum* and *Afromaomum melegueta* on post harvest yam *Discorea spp.* rot". *African Journal of Biotechnology*. 5 (9): 727-731.

Sasidharan S, Chen Y, Saravanan D, Sundram K. M., Latha L.Y. (2011). Extraction, Isolation and Characterization of Bioactive Compounds from Plants' extracts. *Afr J Tradit Comp Altern Med*, 8(1), 1-101.

Sofowora, E. A. (2007). An investigation in the efficacy of *Ocimum gratissimum* as used in Nigerian Native medicine. *Platea medica* 97: 195-200.

Trease, G. E. and Evans, W.O. (2007).. *Pharmacology* 11th Ed. Bailliere Tindall Ltd, London. (1989) pp 60-75.