



## Phytochemical Screening and Antibacterial Investigation of The Extract of *Ocimum Gratissimum* (Scent Leaf) On Selected Enterobacteriaceae.

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### Abstract

Antibacterial properties of *Ocimum gratissimum* was determined and tested against pure cultures of clinical isolates of *Escherichia coli*, *Klebsiella*, *Shigella* and *Salmonella* species. Water and Ethanol were used for the extraction of the active constituent of the plants. Water extract of *Ocimum gratissimum* was not as effective as the ethanolic extracts against the tested organisms. The phytochemical analysis carried out on the plant revealed the presence of alkaloids, tannins, saponin, steroids, cardioglycoside, flavonoid, terpenoids and phenol. The results of this study suggest the possibility of using the ethanolic extract in treating the diseases caused by the test organisms.

**Keywords:** Antibacterial activity, *Ocimum gratissimum*, Phytochemical, Organism

### Introduction

Medicinal plants are of great importance to the health of individual and the communities. The medicinal values of some plants lies in some chemical substances that produce definite physiological actions in the human body. The most important of these bioactive constituents are alkaloids, tannins, flavonoids and phenolic compounds. Many of these indigenous medicinal plants are used as spices and food plants (Okwu, 1999, 2001). An Ethno botanical and ubiquitous plant serves as rich resources of natural drugs for research and development (Kong *et al*, 2008). Medicinal plants based drugs owe the advantage of being simple, effective and exhibit broad spectrum activity. The revival of interest in the use and importance of African medical plants by WHO and many developing countries has led to intensified efforts on the documentation of ethnomedical data of medicinal efforts. This is because most traditional healers keep no records and their information is passed on mainly verbally from generation to generation. Researchers are increasingly turning their attention to natural products looking for new leads to develop better drugs against cancer, as well as viral and microbial infections. The phytochemical evaluation of *Ocimum gratissimum* shows that it is rich in alkaloid, tannins, phytates, flavonoids and Oligosaccharides (Ijeh, *et al* 2004). In the coastal area of Nigeria, the plant *Ocimum gratissimum* is used in the treatment of epilepsy, high fever and diarrhea (Sofowora 1993). *Ocimum gratissimum*

(Scent leaf) is a perennial plant which is widely distributed in the tropics of Africa and Asia. It belongs to the family Labiatae and it as the most abundant of the genus *Ocimum*. In the southern part of Nigeria, it is called “Efirin nla” by the Yoruba speaking tribe. “Nichonwu” in Igbo while in the northern part of Nigeria, it is called “Daidoga” (Effraim, et al; 2003). Leaf extract of *Ocimum gratissimum* and *Xylopi aethiopia* were analyzed against five pathogenic organisms. *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus fecalis*, *Pseudomonas aeruginosa* and *Lactobacilli* (Ijeh et al, 2004). The findings justifies the application of *Ocimum gratissimum* in dermatological cream and indicate the effective doses could be achieved at very low concentration and also shows that the aqueous fractions of both plants have more potential as antimicrobial agents than their ethanolic fractions (Ijeh et al., 2004). The findings of Silva, et al (2005) show that the extracts of *Ocimum gratissimum* are active in vitro against human pathogenic dermatophytes. The aim of this present work therefore is to study the antibacterial effects of the medicinal plant leaf extract of *Ocimum gratissimum* on some selected Enterobacteriaceae and to carry out phytochemical screening of the sample .

## Materials and Methods

### Collection and identification of plant materials

*Ocimum gratissimum* (scent leaf) was collected from a farm at Ikorodu, Ikorodu Lagos Nigeria, the fresh plants were allowed to dry completely for two weeks at a room temperature before using them for this study. The plant was identified in the Department of Biological Sciences, Yaba College of Technology.

### Source of Test Organisms

The test organisms for this study were members of the family Enterobacteriaceae namely *Escherichia coli*, *Klebsiella spp*, *Salmonella spp* and *Shigella spp*. The pure clinical isolates were obtained from the Department of Medical Microbiology, and Parasitology, University of Lagos, Teaching Hospital (LUTH) Idi-araba Lagos. Nigeria. All the clinical isolates were checked for purity and are maintained on Nutrient broth at 4<sup>0</sup>C in the refrigerator until required for use.

### Sample Preparations and Extraction

The plant sample was grinded using an electric blender into a powder form. An aliquot of 100g of the plant was added into a 100ml of distilled water and 70% w/v ethanol, in order to obtain the water and ethanol extract (100mg/ml) respectively. This crude extraction was done at a room temperature. The ground sample (100g) was soaked in

1000ml of distilled water and ethanol in order to obtain water and Ethanol extracts at room temperature for 24 hours. Muslin cloth was then used to filter the plant residues and the filtrate thus obtained was further purified by filtration through Whatman No 1 filter paper under aseptic conditions. The filtrate collected was then concentrated by using rotary evaporator. The extract was then collected in fresh sterile universal bottles and stored in the refrigerator at 4°C until when required for use (Atata *et al* 2003). The extract was tested for sterility by introducing 2ml of the supposed sterile extract into 10ml of sterile nutrient broth. Incubation was done at 37°C for 24 hours. A sterile extract was indicated by absence of turbidity or clearness of the broth after the incubation period (Ronald, 1995).

### **Standardization of Bacteria Cell Suspension**

The nutrient broth cultures of the organisms for this study were taken and inoculated on a fresh agar plate of nutrient agar for 24 hours. Sterile distilled water ( 2ml ) was poured on it and then mixed with the inoculums, 1ml of each was taken and were transferred into 9ml of sterile distilled water and was diluted to 10<sup>4</sup>fold. One hundred microlitre of this was taken and poured on the surface of the agar and then spread evenly with the use of a spreader on the plate to be used for study. The different extracts of the sample were reconstituted with sterile distilled water and ethanol. The initial concentration of each plant extracts (5g) was diluted using 50ml of sterile water and Ethanol to obtain the stock culture. From this stock culture, different concentration were gotten such as 30mg/ml, 20mg/ml, 10m/1ml and 5mg/ml for each extracts (water and ethanol).

### **Antibacterial Susceptibility Assay**

Two methods were employed for the antimicrobial testing which are the, Agar diffusion method and Disc diffusion method.

### **Agar Diffusion Method**

The antimicrobial screening of the ethanolic extract was done as described by Lino and Deogracious (2006). Nutrient agar was poured in sterile Petri dishes and was allowed to solidify. 1ml of the test culture was dropped on the solidified agar and the organism was spread all over the surface of the agar using a spreader. Wells of approximately 5mm in diameter were made on the surface of the agar medium using a sterile cork borer. The plates were turned upside down and the wells labeled with a marker. Each well was filled with 0.2ml of the extract. The plates were incubated aerobically at 37<sup>0</sup>c for 24 hours. Sensitivity of the organisms to the extract was recorded.

### **Disc Diffusion Method**

The locally prepared sterile discs were soaked in the water extract for some hours and nutrient agar medium was poured in sterile Petri Dishes and it was allowed to solidified. 1ml of the test organisms was placed on the solidified agar and it was spread all over the surface of the agar. The soaked disc was picked using sterile forceps and it was dropped on the surface of the agar. The plates were incubated at 37<sup>0</sup>C for 24 hours. Sensitivity of the organisms was recorded.

### **Phytochemical Analysis**

Phytochemical screening was carried out on the obtained plant extract, according to Okwu, (2005).

### **Qualitative Analysis of The Constituents.**

#### **Test for Tannins**

About 0.5g of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue black colouration.

#### **Test for Phlobatannin**

An aqueous extract of the plant sample was boiled with 1% aqueous hydrochloric acid and deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

#### **Test for Saponins**

About 2g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil, shaken vigorously and then observed for the formation of emulsion.

#### **Test for Flavonoids**

5ml of 10% dilute ammonia solution was added to a portion of the aqueous filtrate of the plant extract, followed by addition of concentrated H<sub>2</sub>SO<sub>4</sub>. A yellow coloration observed in the extract indicated the presence of flavonoid.

#### **Test for Cardiac Glycosides**

5ml of the extract was treated with 2ml of glacial acetic acid containing 1 drop of ferric chloride solution (0.1%). This was underlayered with 1ml of concentrated H<sub>2</sub>SO<sub>4</sub>. A brown ring of the interface indicated a deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring, while in the acetic layer, a greenish ring may form just gradually throughout thin layer.

### **Determination of Total Phenols By Spectrophotometer Methods**

The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15minutes. 5ml of the extract was pipetted into a 50ml flask and then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amylochohol were made up to mark and left to react for 30min. Colour development was measured at 505nm.

### **Alkaloid Determination Using Harborne (1973) Method**

5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

### **Tannin Determination By Van-Burden And Robinson Method (1981)**

500mg of the sample was weighed into a 50ml and shaken for 1 hour in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtered was pipetted out into a test tube and mixed with 2ml of 0.1M  $FeCl_3$  in 0.1M HCl and 0.008m potassium Ferrocyanide. The absorbance was measured at 120nm within 10min.

### **Saponin Determination**

The method used was that of Obadoni and Ochuko (2001). Twenty grammes of ground sample was put into a conical flask and 100ml of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 hours with continuous stirring at about 550C. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 900C. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Sixty milliliter (60ml ) of n-butanol was added to the extracts and washed twice with 10ml of 55 aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the extracts were dried in the oven to a constant weight, and percentage saponin content determined.

### Flavonoid Determination by The Method of Boham And Kocipaiabyazan (1974)

Ten grammes(10g) of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over water bath and weighed to a constant weight.

### Results

The result revealed that the aqueous extract had no inhibitory activity on the test organisms, Table 1. The ethanol extracts from *Ocimum gratissimum* have antimicrobial activity against the isolates tested . At 5mg/ml concentration , the ethanolic extract showed greater antimicrobial activity than the aqueous extract as indicated by zones of inhibition in (Table 1) and (Table 2).

**Table 1:** Diameter (mm) of mean zone of inhibition of water extract of scent leaf, *Ocimum gratissimum* in tested organisms.

Test organisms	Concentrations in mg/ml				
	50	30	20	10	5
<i>E.coli</i> (mm)	NIL	NIL	NIL	NIL	NIL
<i>Klebseilla spp</i> (mm)	NIL	9	9	NIL	NIL
<i>Salmonella spp</i> (mm)	NIL	10	10	NIL	NIL
<i>Shigella spp</i> (mm)	NIL	NIL	NIL	NIL	18

Mean Zones of inhibition in millimeters, NIL =No inhibition

**Table 2:** Diameter of mean zone of inhibition of Ethanolic extract of scent leaf , *Ocimum gratissimum* on the test organisms.

Test organisms	Concentrations in mg/ml				
	50	30	20	10	5
<i>E.coli</i> (mm)	19	17	20	18	18
<i>Klebseilla spp</i> (mm)	16	33	13	20	NIL
<i>Salmonella spp</i> (mm)	9	NIL	NIL	NIL	8
<i>Shigella spp</i> (mm)	13	12	8	8	9

Mean Zones of inhibition in millimeters, NIL =No inhibition

At 5mg/ml concentration, the largest zones of inhibition occurred with *Escherichia coli* for the ethanolic extract and *Shigella* for the aqueous extract. *Echerichia coli* & *Klebsiella spp* showed highest zones of inhibition at high concentration of 50mg/mg with the ethanolic extract . At 5mg/ml concentration, the ethanolic extract is effective on *Escherichia coli*, *Salmonella spp* and *Shigella spp* and nil inhibition for *Klebsiella spp* but at 50mg/ml, the ethanolic extract is effective on all the isolates. At 50mg/ml , the aqueous extract was not effective on all the isolates. This indicates that the

antimicrobial activity of this plant is concentration dependent. Ethanolic extract showed high inhibitory zones than aqueous extracts. The phytochemical analysis revealed the presence of Tannin, saponin, flavonoid, steroid, terpenoids, cardiac glycoside, alkaloid and phenol as shown in Table 3. The percentage composition of the phytochemical constituents of *Ocimum gratissimum* is shown in Table 4.

Table 3: Phytochemical constituents of the seeds of , *Ocimum gratissimum*

Constituents	Results
Tannin	+
Saponin	+
Flavonoid	+
Steroid	+
Terpenoids	+
Cardiac glycoside	+
Alkaloid	+
Phlobatannis	-

+ = present, - = Not present

Table 4: Percentage composition of the Phytochemical constituents of , *Ocimum gratissimum* .

Constituents	Results
Phenol	0.17
Alkanoids	0.82
Tannins	1.95
Flavonoid	0.68
Saponin	1.92

## Discussion

The result of this work showed that the ethanolic extract showed high inhibitory zones than aqueous extracts. (Table 1 & 2). This observed difference between these plants extracts may be due to insolubility of active compounds in water or the presence of inhibitors to the antimicrobial components Okigbo and Ogbonnanya (2006), Amadioha and Obi (1999), Okigbo and Ajale (2005). They have attributed this observation to the high volatility of ethanol which tends to extract more active compound from the sample than water, hence, this study follow similar trends. Okigbo et al(2005) reported that inactivity of plant extracts may be due to age of plant, extracting solvent, method of extraction and time of harvesting of plant materials. Conversely, the ethanol extracts of *O. gratissimum* showed a concentration dependent gradient decrease in the level of

inhibition against isolates. *E.coli* , *Klebsiella*, *Salmonella*, and *Shigella* showed inhibition zones ranging from 8.0 to 33.0 mm. Other report have shown results similar to ours (Nwinyi et al 2009). Lima et al. (1993) tested *in vitro* antifungal activity of some plants against dermatophytes. Of the tested plants, *O.gratissimum* was found to be the most active, inhibiting 80% of the dermatophyte strains tested and producing zones greater than 10 mm in diameter.

Ethanol extracts of *O. gratissimum* have been reported to show more antibacterial activity against *S. aureus* than *E. coli*( Agatemor 2009). Nweze et al. (2004) reported phytochemical screening of *O. gratissimum* in the presence of alkaloids, tannins, glycoside, saponin, resins, cardiac glycoside, steroidal terpens and flavonoids, this is similar to the results of this study.

The findings of this study work agrees with that of several workers that demonstrated that *Ocimum* plant has activity against several species of bacteria including *Staphylococcus aureus*, *Listeria monocytogenes* *Escherichia coil*, *Shigella*, *Salmonella* and *Proteus* species and fungi (Nwosu and Okafor, 1995. Akinyemi *et al.*, 2004; Janine de Aquino Lemos *et al.*, 2005; Lopez *et al.*, 2005). The future use of *O. gratissimum* antibiotic combinations as a therapeutic measure against shigellosis can be envisaged (Iwalokun *et al.*, 2003).

## Conclusion

From this study, it was observed that ethanol extracts exhibited high inhibitory activity on the test organisms. This can be deduced to the ability of ethanol to extract more of the essential oils and secondary plant metabolites which are believed to exert antibacterial activity on the test organisms. This study however can justify the use of the leaf in traditional medicine practice as a therapeutic agent and can explain the long history use of these plants.

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