

*Full Length Research Paper*

# **Antibacterial activity of *Ocimum gratissimum* (scent leaf) on some pathogenic gastrointestinal bacteria**

**Agholor Kin<sup>1\*</sup>, Lucy M. Yaki<sup>2</sup>, Idris Abubakar<sup>1</sup>, Lucy F. Olusola<sup>1</sup> and Rakiya Zubairu<sup>1</sup>**

<sup>1</sup>Department of Biological Science, Niger state Polytechnic, Zungeru, Niger State, Nigeria.

<sup>2</sup>Department of Microbiology, Kaduna State University, Kaduna, Kaduna State, Nigeria.

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The emergence of antibiotic resistance as well as the recent undesirable side effect of some of the commercially available antibiotics has led to the screening of plant extract in order to discover new drug that could serve as alternative therapy for the treatment of various infections and diseases. Fresh leaf of *Ocimum gratissimum* (scent leaf) sample was collected, air-dried at room temperature and blended to powder using electric blender. The extraction was done using reflux extraction method and methanol as solvent. The phytochemical analysis and the antibacterial activity of *O. gratissimum* were determined to ascertain the different phytochemicals present in the plant extract. The extract was also tested against some selected Gram negative intestinal pathogenic bacteria; *Escherichia coli*, *Shigella* and *Salmonella* species, by reconstituting the extract in dimethyl sulphoxide (DMSO) to obtain different concentration (0.2, 0.1, 0.05 and 0.025 g/ml) and agar well diffusion techniques were used to evaluate the antibacterial susceptibility of the leaf extract. The qualitative phytochemical analysis of the extract revealed the presence of alkaloid, anthraquinone, flavonoid, glycoside, phenol, saponin, steroid and tannins. The result of antibacterial analysis showed that the extract of *O. gratissimum* has antibacterial activity against *E. coli*. This could be as a result of the presence of various phytochemicals or the interaction of one or more of the identified metabolites against the test organisms. However, there was no zone of inhibition (antibacterial effect) recorded on *Salmonella* and *Shigella* spp. as they were resistant to the extract. The results obtained from this research, suggest that *Escherichia coli* was susceptible to the leaf extract and the plant could be used as potential source of natural product for the treatment of infection.

**Key words:** Antibacterial activity, scent leaf, gastrointestinal bacteria, phytochemicals, plant extract.

## **INTRODUCTION**

Pathogenic gastrointestinal bacteria are bacteria that cause gastroenteritis (Okigbo and Igwe, 2007). They infect the gut leading to inflammation of the stomach and intestines (Ishiwu et al., 2014). This leads to vomiting, severe abdominal cramps, and diarrhea. They include

*Escherichia coli*, *Shigella* species, and *Salmonella* species. Bacterial gastroenteritis commonly occurs as a result of poor hygienic practices (Russell and Jarvis, 2011). However, infections can also occur after close contact with infected animals or consuming food or water

\*Corresponding author. E-mail: kinagholor@gmail.com. Tel: +2347062361650.

contaminated with bacteria or the toxic substances produced by bacteria (Opara et al., 2014).

*E. coli* are commonly found in fecaloid matters and can cause serious food poisoning in their hosts (Kotloff et al., 2013).

*Shigella* spp. causes shigellosis, commonly referred to as bacterial dysentery (Ram et al., 2008).

*Salmonella* spp. are facultative intracellular pathogens. They are two serotypes; the non-typhoidal and typhoidal serotypes. The non-typhoidal serotype invades only the gastrointestinal tract and cause *Salmonella* food poisoning while the typhoidal serotype spreads throughout the body, invades organs, and secretes endotoxins (Su and Chiu, 2007).

Antibacterial are forms of antimicrobial agent used especially against bacteria, for the treatment of bacterial infections (Prabhu et al., 2009). The discovery of antibiotics (a substance produced by microbes which inhibit or kill another microorganism at a very low concentration) has helped in the control of pathogenic bacteria until the recent development of resistance by most pathogens. Antibiotic resistance is becoming a worldwide problem posing danger to humanity as various diseases and infections that are formerly treated with this substance are now difficult to control (Suree and Pana, 2015).

In addition, some synthetic available antibacterial agents are becoming ineffective due to their side effects such as tendonitis, seizure, and Steven-Johnson syndrome (WHO, 2002). The emergence of antibiotic resistance as well as the recent undesirable side effect of some of the commercially available antibiotics has led to the screening of plant extracts in search for new drug that could serve as alternative therapy for the treatment of various infections and diseases (Effraim et al., 2013).

*Ocimum gratissimum* popularly referred to as scent leaf because of its aroma is commonly used as spices for food or soup preparation in Nigeria (Akinjogunla et al., 2009). It is a medicinal plant which has been used traditionally for the treatment of various infections (Abdullahi, 2012). The plant is cultivated in abundant in different part of Nigeria and it contains some bioactive substances such as tannis, saponins, alkaloids, glycosides, phenols and flavonoids, also referred to as phytochemicals.

These phytochemicals when consumed served as medicine for protection and treatment of human or animal disease (Abdullahi, 2012). The *in-vitro* antimicrobial screening of *O. gratissimum* against *Staphylococcus aureus*, *E. coli*, *Streptococcus fecalis*, *Pseudomonas aeruginosa* and *Lactobacilli* showed that the leaf extract is effective against human pathogens (Prabhu et al., 2009).

Hence, the 'Green' Movement in Western Society has established that naturally derived substances are safer and more desirable than synthetic chemicals products (Opara et al., 2014). Therefore, this study aimed at

determining the phytochemical constituent and antibacterial activity of *O. gratissimum* leaf extract on these pathogenic gastrointestinal bacteria; *Escherichia coli*, *Shigella* spp., and *Salmonella* spp.

## MATERIALS AND METHODS

### Collection and preparation of plant

Fresh samples of *O. gratissimum* leaves were collected from Niger State Polytechnic Staff Quarter, Wushishi Local Government, Niger State, on coordinate 9° 48'N 6° 9' E and elevation 149 m (489 ft) on 16 October, 2017. The plant was identified and authenticated in the Department of Biological Sciences, Niger State Polytechnic, Zungeru. The fresh leaves were dried completely for two weeks at room temperature (Figure 2) and were blended into powder form using an electric blender.

### Extraction process

The plant extraction was obtained using reflux extraction technique as described by Abdullahi (2012) and Sofowora (1993). 250 g of the blended plant leaf powder was weighed and wrapped in Whatman No. 1 filter paper and placed in the holding chamber of the reflux extractor. 600 ml of methanol was used for the extraction at 40°C for 48 h. Thereafter, the extract was concentrated by evaporating to dryness using water bath. The dark green coloured solid extract of the *O. gratissimum* was stored in an airtight container at 4°C in a refrigerator.

### Qualitative phytochemical analysis of the methanolic extract of *O. gratissimum*

The qualitative and quantitative phytochemical screening of the methanolic leaf extract were carried out following standard procedures according to the method described by Abdullahi (2012) and Okwu (2005) at the Centre for Genetic Engineering and Biotechnology Laboratory, Federal University of Technology Minna, Niger State.

### Determination of antibacterial activity of the plant extract

#### Reconstitution of the plant extract

The methanol plant extracts were reconstituted by weighing 0.2, 0.1, 0.050 and 0.025 g each into different sterile test tubes containing 1 ml of dimethyl sulphoxide (DMSO), respectively to obtain the following concentration of the extract: 0.2, 0.1, 0.05, and 0.025 g/ml.

#### Test for sterility of the plant extract

The plant extracts were tested for sterility by introducing 1 ml of the reconstituted plant extract into 5 ml of sterile nutrient broth and were incubated at 37°C for 24 h. The absence of turbidity of the broth (as compared to MacFarland standard) after incubation indicated that the extract was sterile (Preethi et al., 2010).

### Collection and confirmation of test organisms

The pure clinical isolates of some pathogenic gastrointestinal

**Table 1.** Qualitative and quantitative phytochemical constituent of the methanolic extract of *Ocimum gratissimum*.

Constituent	Qualitative analysis	Quantitative analysis
Saponin	+	52.48 ± 0.00
Flavonoid	+	5.33 ± 0.00
Steroid	+	N D
Terpenoids	-	N D
Glycoside	+	29.91 ± 0.03
Phenol	+	27.00 ± 0.01
Alkaloid	+	0.15 ± 0.03
Tannin	+	N D
Anthraquinones	+	N D

(+) = Positive, (-) = Negative; ND: Not determined.

**Table 2.** Antibacterial activity of methanolic extract of *Ocimum gratissimum* on the test organisms at different concentrations.

Test organism	Average zone of inhibition in diameters (mm)				Ciprofloxacin	DMSO
	0.20 g/ml	0.10 g/ml	0.05 g/ml	0.025 g/ml	10 mcg	
<i>Escherichia coli</i>	24	19	12	8	20	NI
<i>Salmonella</i> sp.	NI	NI	NI	NI	25	NI
<i>Shigella</i> sp.	NI	NI	NI	NI	23	NI

NI: No inhibition; Mcg: Micrograms.

bacteria belonging to the family Enterobacteriaceae: *Escherichia coli*, *Salmonella* spp. and *Shigella* spp. were obtained from the Department of Medical Microbiology, Kaduna State University, Kaduna State, Nigeria. All the isolates were checked for purity and confirmed by Gram staining and sub culturing them on selective media (*Salmonella shigella* Agar and MacConkey Agar); thus, observing the colony characteristics and morphology of the cells.

#### Antibacterial susceptibility assay

Agar well diffusion techniques were employed for the antimicrobial testing of the plant extracts. The 24 h old cultures were transferred into nutrient broth and incubated at 37°C for 5 h and standardized to Macfarland standard. Each of the test organisms from the broth cultures were streaked on 4 different Mueller Hinton agar plates under aseptic condition and were labeled accordingly.

Wells of approximately 5 mm in diameter were made on the surface of the inoculated agar medium using a sterile cork borer no. 1 and the wells labeled with a marker based on the concentration of the plant extract (0.2, 0.1, 0.05, and 0.025 g/ml) and the wells were filled with the different concentration of the extract. DMSO and ciprofloxacin were used as the negative and positive control, respectively as described by Lino and Deogracious (2006).

The plates were incubated at 37°C and the susceptibility of the test organisms to the plant extract were recorded after 24 h by measuring the average diameter of the clear zone of inhibition in millimeters (mm).

## RESULTS

The result of qualitative phytochemical analysis of methanolic extract of *O. gratissimum* leaves revealed the

presence of eight classes of secondary metabolites. However, the quantitative phytochemical analysis conducted on five classes of the identified metabolites showed that saponin has the highest phytochemical constituent (52.48 mg/g) of the extract, followed by tannin (29.91 mg/g), phenol (27.00 mg/g), flavonoid (5.33 mg/g) and alkaloid (0.15 mg/g) shown in Table 1. However, quantitative phytochemical analysis for anthraquinones, tannin, terpenoids and steroid were not determined.

The result of antibacterial activity of methanolic extract of *O. gratissimum* on *E. coli*, *Salmonella* spp. and *Shigella* spp. shown in Table 2, *E. coli* was sensitive to the extract while *Salmonella* spp. and *Shigella* sp. were resistant to the plant extract and there was no zone of inhibition of DMSO against the test organisms. However, the lower the concentration of the extract (from 0.20 to 0.025 g/ml), the lower the average zone of inhibition (from 24 to 8 mm) against *E. coli*.

The result of antibacterial activity of methanolic extract of *O. gratissimum* on *E. coli* shows decreased average zone of inhibition with decreased concentration of the extract (Figure 1), that is, the lower the concentration, the lower the antibacterial activity.

## DISCUSSION

The extraction of *O. gratissimum* leaf was obtained using methanol as solvent and the phytochemical analysis of the leaf extract revealed the presence of alkaloid,

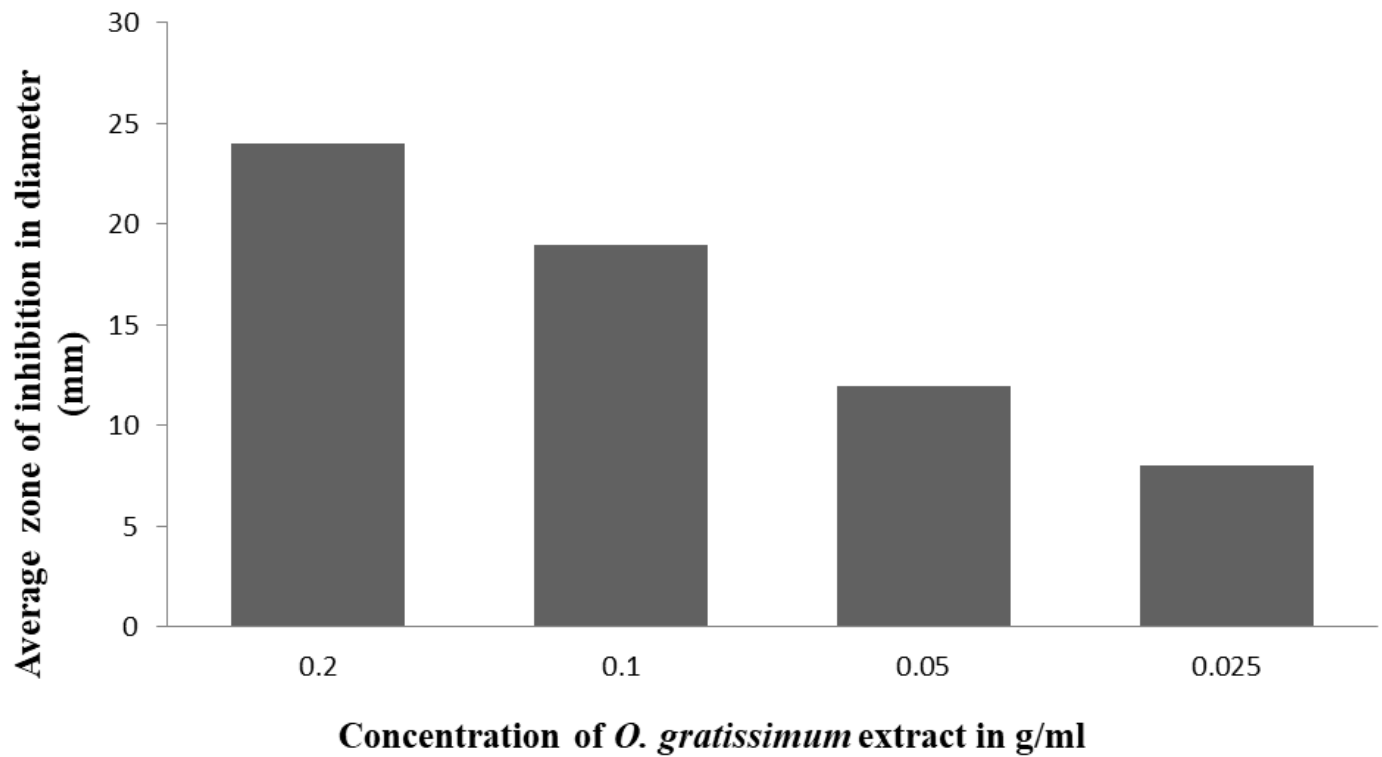


Figure 1. Antibacterial activity of methanolic extract of *O. gratissimum* leaf on *E. coli*.

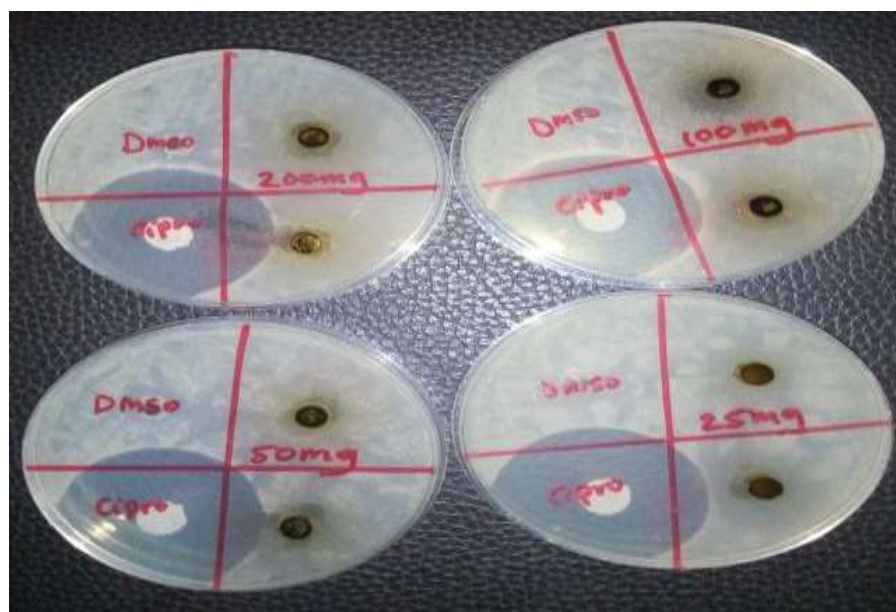


Figure 2. Collection and preparation of *O. gratissimum* leaf (scent leaf).





**Figure 3.** Antibacterial activity of *O. gratissimum* leaf extract on *E. coli*.



**Figure 4.** Antibacterial activity of *O. gratissimum* leaf extract on *Salmonella* sp.

anthraquinone, flavonoid, glycoside, phenol, saponin, steroid and tannins which agrees with the finding of Ladipo et al. (2010) that shows that *O. gratissimum* contains all the phytochemicals mentioned earlier.

The antibacterial analysis of the methanolic extract of *O. gratissimum* (Figure 3) revealed that the extract has antibacterial activity against *E. coli* shown in Table 2. This could be as a result of the presence of one of the phytochemicals or the interaction of two or more of the bioactive compounds against the test organism which agreed with the work of Abdullahi (2012) and also supported the traditional uses of the plant in the treatment of various bacterial enteric diseases such as

diarrhea, dysentery and other gastrointestinal infections (Nwinyi et al., 2009). Hence, from the result obtained, there was decreased in antibacterial activity with decreased in concentration of the extract as shown in Figure 1; as the concentration of the extract decreases from 0.20l to 0.025 g/ml, the average zone of inhibition also decreased from 24 to 8 mm. This also agreed with the finding of Ishiwu et al. (2014) who demonstrated that increase in the concentration of *O. gratissimum* extract reduces the number of viable *E. coli* from 36 to 5 cfu/ml.

However, there was no antibacterial effect of the extract on *Salmonella* (Figure 4) and *Shigella* spp. (Figure 5). This resistance may be due to the lipid content

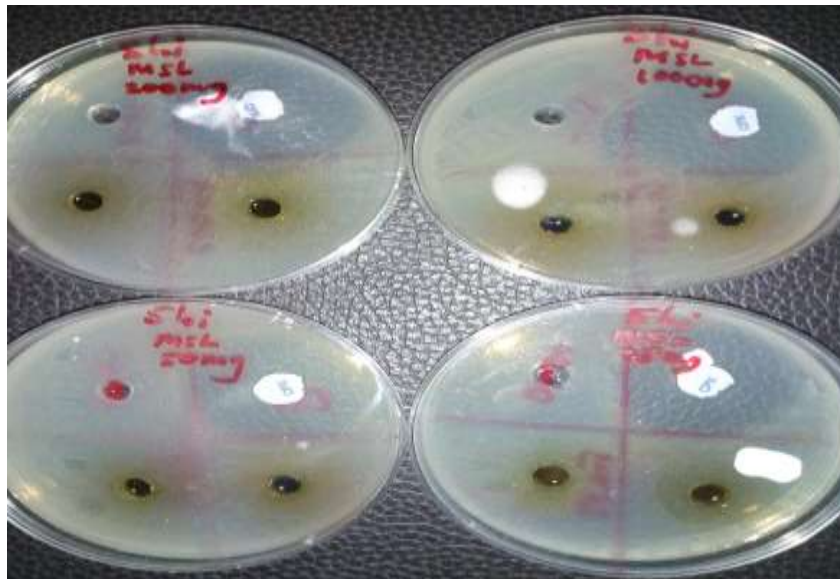


Figure 5. Antibacterial activity of *O. gratissimum* leaf extract on *Shigella* spp.

on the membranes of these bacteria that prevented the permeability of the active phytochemicals into the cell or the low quantity of alkaloid ( $0.15 \pm 0.03$ ) extracted with methanol (Abdullahi, 2012). However, this disagreed with the work of Ladipo et al. (2010), who reported that the methanol extract of *O. gratissimum* leaf has antibacterial activity against *Salmonella*, *Shigella* and *Klebsiella* spp. But Justina and Solomon (2017) suggested that difference in phytochemical constituent of the leaf of *O. gratissimum* could be as a result of the planting location, seasonal and environmental variations. This could also have effect on the antibacterial activity of the leaf extract on *Salmonella* and *Shigella* spp. Therefore, the leaf extract of *O. gratissimum* could serve as natural antibacterial agent and herbal drug against gastroenteritis.

## Conclusion

The results obtained in this study, suggest that *Escherichia coli* was susceptible to the plant leaf extract and the methanolic extract of *O. gratissimum* leaf contains phytochemicals that possess antibacterial property and could be used as potential source of natural product in industrial manufacturing of drugs, for the treatment of infections/diseases caused by *E. coli*. However, the toxicity and the side effects of the plant leaf extract should be determined even if the plant is consumed locally.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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