Phytochemical and antibacterial activity of *Ocimum gratissismum* L. against three selected gastrointestinal tract pathogens

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Abstract

The rise in the incidence of side effects of many synthetic antimicrobial agents and prevalence of multidrug resistant bacteria has spurred scientists on the research for plant based antimicrobial of therapeutic potentials. *Ocimum gratissimum* L. presents such potential of high medicinal value. This plant is used in Nigeria traditionally as condiments and for treatment of various ailments. This study is aimed to determine the phytochemical and antimicrobial property of ethanol and aqueous extracts of *O. gratissimum* L. leaves. Phytochemical screening of the leave extracts confirmed the presence of Steroid, Tannins, Flavonoid, Terpenoid, Alkaloid, Saponin and Phlobatannis. Ethanol and aqueous extracts were obtained from the powdered leaves of *O. gratissimum* using ethanol and distilled water respectively. These were later filtered and the filtrates were concentrated in water bath at 50°C. Mueller-Hinton agar was used for the antibacterial susceptibility using the agar well diffusion method. For ethanol extract, all the isolates were susceptible at 0.2g/mL, 0.1g/mL and 0.05g/mL concentrations. However, for aqueous extract, all the isolates were resistant at 0.05g/ml and 0.025g/ml but at 0.2g/ml concentration all the isolates were susceptible. *Staphylococcus aureus* was resistant at 0.1g/ml, except *Pseudomonas aeruginosa* and *Bacillus cereus* which were susceptible with a diameter 15mm, and 14mm respectively. Ciprofloxacin used as positive control also inhibited the growth of the bacterial isolates with the diameter of zone of 25mm. Results obtained from this study showed that the extracts of *Ocimum gratissimum* L. has antibacterial activities against the selected gastrointestinal tract pathogenic bacteria and also possess some bioactive compounds.

***Keywords*:** Phytochemicals, antibacterial, *Ocimum gratissimum, gastrointestinal pathogens*

1. Introduction

Pathogenic gastrointestinal bacteria are bacteria that cause gastroenteritis (Okigbo and Igwe, 2007). They infect the gut leading to gastroenteritis (inflammation of the stomach and intestines) (Ishiwu *et al*., 2014). This leads to vomiting, severe abdominal cramps, and diarrhea. They include *Bacillus cereus, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* Bacterial gastroenteritis commonly occurs due to poor hygienic practices (Russell and Jarvis, 2011). However, infections can also occur through contact with infected animals; a process known as zoonosis or through consumption of food or water contaminated with bacteria or the metabolic waste that is been released by them (Opara *et al.,* 2014). Antibacterial agents are substances employed for the treatment of bacterial infections (Prabhu *et al.,* 2009). The discovery of antibiotics (a substance that slow down or kill bacteria at a very low

concentration) has helped in the control of pathogenic bacteria until the recent increase in resistance exhibited by most pathogens. Antibiotic resistance is becoming a worldwide problem posing risk to life of humans as this substances which are previously used to treat various diseases and infections have now gain resistance by the causal agents (Suree and Pana, 2015). The development of antibiotic resistance and the recent unacceptable side effect of some antibiotics have led to the search of plant that could be used for the formulation of drug that can serve as alternative therapy to treat various infections and diseases (Effraim *et al.*, 2013).

Medicinal plants are plants that possess therapeutic properties or exert beneficial pharmacological effect on the human or animal body. They are the richest bio-resource of drugs used in folk medicine and modern medicines. It can also be used as food supplements, pharmaceutical intermediates, nutraceuticals and chemical entities for synthetic drugs (Ncube *et al.,* 2008). The medicinal plants value lies on some chemical compounds that bring about a definite physiological action on the human body and these chemical compounds are called phytochemicals (Omojoyegbe *et al.,* 2020). These are non-nutritive chemicals that have disease preventive or protective property (Subhashini *et al.,* 2010). The most significant of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Edeoga *et al.,* 2005).

Ocimum gratissimum L. often called Scent leaf is known to be one of the leading plants that could be used to treat infections and diseases. Because of its aroma, it is commonly used as spices for preparation of food or soup in Nigeria (Akinjogunla *et al*., 2009). It is a perennial plant that is widely distributed in the tropics of Africa and Asia. It is naturally found in almost all parts of Nigeria. The common names of the plant include; Fever plant, Basil and Tea bush and vernacular names include 'Daidoyatagida' (Hausa), 'Nichonwu' (Igbo), 'Tanmotswangiwawagi' (Nupe), 'Efinrin' in Yoruba (Abdullahi *et al.,* 2003; Idris *et al.,* 2011) and 'Ebalabhonkhokho' (Esan). The plant is woody at the base and it average height range from one to three meters (1-3 meters). The leaves are narrowly ovate and broad, usually from 3 to 9cm wide and 5 to 13cm long. It is a scented plant with lime-green leaves. The leaves are commonly consumed by Nigerians as leafy vegetables because of the importance nutrition the plant contains and its aromatic flavor make it useful as a seasoning. Edeoga and Eriata (2001) in their experiment showed that the leaf juice of this plant is used for the treatment of stomach pain, convulsion and catarrh. Edeoga and Eriata (2001) also discovered that oil from the leaves possess antibacterial, antifungal and antiseptics activities. *Ocimum gratissimum* L. is active against human pathogenic dermatophytes (in vitro) (Silva, *et al.,* 2005). *Ocimum gratissimum* L. has been used traditionally for the treatment of numerous infections (Abdullahi, 2012). The plant contains various bioactive substances such as tannins, saponins, alkaloids, glycosides, flavonoids and phenols, also

known as phytochemicals. These phytochemicals when taken served as medicine for treatment and protection of human or animal disease (Abdullahi, 2012). The antimicrobial screening (in-vitro) of *Ocimum gratissimum L.* against *Pseudomonas aeruginosa, Escherichia coli, Streptococcus fecalis, Staphylococcus aureaus* and *Lactobacilli* proved that the extract of the leaf is active against human pathogens (Prabhu *et al.,* 2009). Therefore, the 'Green' Movement in Western Society has shown that substances that are derived naturally are desirable and safer than synthetic chemical products (Opara *et al.,* 2014). The recent undesirable side effect of some of the commercially available antibiotics and the emergence of antibiotic resistance has led to the search for a plant that could be used to formulate new drug which could serve as an alternative therapy for the treatment of various infections and diseases. Therefore, this present study is designed to investigate the phytochemical constituent and antibacterial activity of *O. gratissimum* L. leaf extract on three selected pathogenic gastrointestinal bacteria.

2. Methods

2.1 Collection of bacterial isolates

Bacterial isolates of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus cereus* were obtained from Microbiology Laboratory, at Irrua Specialist Hospital Irrua, Edo State, Nigeria. The isolates were resuscitated in buffered peptone broth, after which they were sub-cultured into nutrient agar medium and incubated at 37^{o}C for 24 hrs. The isolates were sub-cultured in a nutrient broth at 37^{o}C for 18 hours prior to antibacterial testing.

2.2 Collection of Plant Sample and Identification

Fresh sample of *Ocimum gratissimum L.* leaves used in the research were obtained from the natural habitats in Samuel Adegboyega University Ogwa, Edo State, Nigeria located on Latitude: 60'0 and Longitude: 60'5. All the collections were done in the month of April, 2019. The plant materials were validated by Prof. Mrs. F. M. Ogbe; Head of Department of Biological Sciences, College of Basic and Applied Sciences, Samuel Adegboyega University Ogwa.

2.3 Processing of Plant Samples (*Ocimum gratissimum L.)* Leaves

The method of Atata *et a*l., (2003) was used to process the leaves. Fresh leaves of *O. gratissimum L.* were collected by hand plucking from plant and cleaned of debris. The leaves were thoroughly washed using tap water and rinsed with distilled water. The leaves were then air-dried at room temperature for

14 days and milled to a fine powder with the aid of a manual grinder. The powder sample was stored in airtight containers protected from sunlight until required for analysis.

2.4 Phytochemical Analysis (Qualitative Analysis of the Constituents)

Phytochemical was carried out using standard procedures to identify the constituents as describe by Okwu, (2005) and Ladipo, *et al.* (2010). The leaves were screened for tannins, steroids, anthraquinones, xanthoproteins phlobatanin, alkaloids, triterpenes, flavonoids, saponins, terpenoids and cardiac glycosides.

2.5 Preparation of plant samples (*Ocimum gratissimum* L.*)* extracts

The plant extraction was obtained using the aqueous and ethanol extraction technique as described by Doughari and Manzara, (2008)

Aqueous Extraction: The aqueous extract was prepared by weighing out twenty grams (20g) of the powdered leaves of *Ocimum gratissimum* L. into 200ml of distilled water in a sterile conical flask and stirring vigorously with a glass rod and left for 72 hours (3 days). It was filtered off with sterile filter paper (Whatman No 1 filter paper) into a clean conical flask and the filtrate was allowed to heat in a water bath at 50^{o}C to concentrate it better. After the complete evaporation of water, the weight of the extract was obtained and stored under antimicrobial conditions until bioassayed.

Ethanol Extraction: 20g portion of the leaf powder was weighed into two hundred milliliters (200 mL) of ethanol in sterile conical flasks and stirring vigorously with a glass rod and was allowed to soak at room temperature for 72 hours (3 days). The extracts were then filtered using filter paper (Whatman No 1) into a conical flask and the filtrate was allowed to heat in water bath at 50^{0}C to concentrate it better. After complete evaporation of ethanol, the weight of the extract was obtained and stored under antimicrobial conditions until bioassayed.

2.6 Standardization of inocula

The method of Dalitha (2008) was used to standardize the cultures. Pure isolates were transferred into the sterile prepared nutrient broth and the cultures were then adjusted to 0.5 McFarland standards by checking the turbidity level in comparison with the standard.

2.7 Antimicrobial activity (agar well diffusion test)

Agar well diffusion technique as described by Dalitha (2008).was employed for the antibacterial activity of the plant extracts. The 24 hours old cultures were transferred into nutrient broth incubated at 37^{o}C for 18hrs and standardized to McFarland standard. Each test organism was streaked on Mueller

Hinton agar plates aseptically and was labeled accordingly. Wells of approximately 6mm in diameter were made on the surface of the inoculated agar medium using a sterile cork borer and the wells were labeled with a marker based on the concentration of the plant extract (0.2, 0.1, 0.05, and 0.025g/ml) and the wells were filled with the different concentrations of the extracts. Ciprofloxacin was used as the control, as described by Lino and Deogracious (2006). The plates were incubated at 37^{o}C and the susceptibility of the test organisms to the plant extract were recorded after 24hrs by measuring the diameter of the clear zone of inhibition in millimeters (mm).

3. Result

The phytochemical screening of the leaf sample showed the presence of saponin, tannins, phlobatannins, steroids, cardiac glycosides, terpenoid and flavonoids (Table 1). Table 2 shows the antibacterial activity of ethanol extract of *Ocimum gratissimum* L. leaf at a concentration of 0.2 g/ml, 0.1 g/ml, 0.05g/ml and 0.025g/ml. *Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus cereus* were sensitive to the ethanol extract at a concentration of 0.2g/ml (the higher concentration), *Pseudomonas aeruginosa* was sensitive to 0.1g/ml concentration, intermediate to 0.05g/ml but resistant to 0.025g/ml concentration. *Staphylococcus aureus* was intermediate to 0.1g/ml concentration but resistant to 0.05g/ml and 0.025g/ml concentrations. *Bacillus cereus* was intermediate to 0.1, 0.05 and 0.025g/ml concentrations. Ciprofloxacin used as positive control. Inhibited all the isolates (Table 2). Table 3 shows the result obtained from the antibacterial activity of aqueous extract of *Ocimum gratissimum* L. leaf. *Pseudomonas aeruginosa* and *Bacillus cereus* were sensitive to 0.2g/ml concentration, intermediate to 0.1g/ml concentration but resistant to 0.05g/ml and 0.025g/ml concentration. *Staphylococcus aureus* was intermediate to 0.2g/ml concentration, but resistant to 0.1g/ml, 0.05g/ml and 0.025g/ml concentrations

Table 1: Qualitative phytochemical constituents of *Ocimum gratissimum*

Plant part	Phytochemical properties	Result
Leaf	Tannins	+ve
	Phlobatannis	+ve
	Triterpenes	-ve
	Saponin	+ve
	Terpenoid	+ve
	Flavonoid	+ve
	Anthraquinones	-ve
	Steroid	+ve
	Xanthoproteins	-ve
	Cardiac Glycosides	+ve

Table 2: Susceptibility patterns exhibited by the test organisms against ethanol extract of *Ocimum gratissimum* at different concentrations and ciprofloxacin

Test Organisms	Zone of Inhibition in Diameters (mm)				Ciprofloxacin
	0.20gml	0.10g/ml	0.050g/ml	0.025g/ml	10 mcg
Pseudomonas aeruginosa	S(25)	S(24)	I(17)	R	S(25)
Staphylococcus aureus	S(25)	I(17)	R	R	S(25)
Bacillus cereus	S(25)	I(17)	I(15)	I(14)	S(25)

S = Sensitivity; I = Intermediate; R = Resistance; Mcg = Micrograms

Table 3: Susceptibility patterns exhibited by the test organisms against aqueous extract of *Ocimum gratissimum* at different concentrations and ciprofloxacin

Test Organisms	Zone of Inhibition in Diameters (mm)				Ciprofloxacin
	0.20gml	0.10g/ml	0.050g/ml	0.025g/ml	10 mcg
Pseudomonas aeruginosa	S(25)	I(15)	R	R	S(25)
Staphylococcus aureus	I(15)	R	R	R	S(25)
Bacillus cereus	S(22)	I(14)	R	R	S(25)

S = Sensitivity; I = Intermediate; R = Resistance; Mcg = Micrograms

Discussion

The results obtained from this present study confirmed the presence of flavonoids, steroids, tannins, terpenoids, saponins, phlobatannins and cardiac glycosides as reported by Ladipo *et al*. (2010), Kin *et al*. (2018 and Hamma *et al.,* 2020)). This is also in agreement with the findings of Shukla *et al*. (2015) and Bello *et al*. (2022) which show that *Ocimum gratissimum* contains all the phytochemicals mentioned earlier. It is suggested that the differences in the phytochemical constituent of the leaf of *O. gratissimum* could be a result of the planting location, seasonal and environmental variations (Talabi and Makanjuola, 2017). The phytochemicals in medicinal plants have been reported to be the active principles responsible for the pharmacological potentials of medicinal plants (Edeoga *et al*., 2005). The presence of these chemicals in the leaves of *O. gratissimum* justifies the local use of this plant for the treatment of various ailments (Hamma *et al*., 2020).

Flavonoids are reported to exhibit antioxidant activity and are effective scavengers of superoxide anions. Hence this can significantly affect the cell wall of *Staphylococcus aureus* which invariably may lead to the destruction of the cell wall and overall, affect the entire mechanism of the bacterium. *In vivo* studies have proven that flavonoids have antimicrobial, anti-inflammatory and anti-allergic, anti-diarrheal and anti-cancer activities (Cushnie and Lamb, 2005: Schuier *et al.,* 2005). Saponins are natural glycosides that act as hypoglycemic, antifungal and serum cholesterol-lowering agents in

animals (Osuntokun and Ogunleye, 2017. Saponins are also essential elements in ensuring hormonal balance and the synthesis of sex hormones (Ladipo, *et al.,* 2010). Tannins are bitter polyphenolic compounds that hasten the healing of wounds. They also possess antidiuretic and anti-diarrhea properties (Okwu, 2004).

From the investigation, the aqueous extracts of *Ocimum gartissimum* showed minimal antibacterial activity against the three bacterial isolates; *Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus cereus* compared to ethanol concentration. Ethanol extracts of *O. gratissimum* had more antibacterial activity against the three bacterial isolates which agrees with the finding of Agatemor (2009). The observed difference between the two extracts may be due to the presence of inhibitors to the antimicrobial components or the insolubility of active compounds in water. This is in agreement with the investigation of Okigbo and Ogbonnanya (2006). Okigbo *et al.,* (2005b) reported that the inactivity of plant extracts may be caused by the time of harvesting of plant materials, method of extraction, age of plant and extracting solvent.

In addition, the ethanol extracts of *O. gratissimum* showed a concentration-dependent gradient decrease in the level of inhibition against isolates as the concentration decreased which is in line with the findings of Hamma *et al.* (2020). 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*scherichia. coli* and *S. aureus.* The antibacterial analysis of the aqueous and ethanol extracts of *Ocimum gratissimum* revealed that the leaf extract has antibacterial activity against *Pseudomonas aeruginosa* and *Bacillus cereus* with maximum zone of inhibition of 25 mm at 0.2g/ml concentrations. This could be a result of the interaction of two or more of the bioactive compounds against the test organisms or the presence of one of the phytochemicals or which is in line with the work of Abdullahi (2012). It also supported the traditional usage of the plant for the treatment of various bacterial enteric diseases including dysentery, diarrhea and other gastrointestinal infections (Nwinyi *et al.,* 2009). On the other hand, for the Gram-positive bacteria isolates, *Staphylococcus aureus* showed sensitivity to aqueous extract at a concentration of 0.2g/mL (higher concentration). This agreed with the previous studies of Hamma *et al*. (2020) and Oladele and Ologundudu (2022) that the aqueous extract *O. gratissimum* has an inhibitory effect on *Staphylococcus aureus*. However, there was no antibacterial effect of the aqueous extract on *Staphylococcus aureus* at lower concentrations. Thus, from the result obtained from the present study, there was variation in the degrees of antibacterial activities of the extracts on the isolates. Also, there was a decrease in antibacterial activity as the concentration of the extract decreased. This also agreed with the findings of Nwinyi *et al* (2009) and Ishiwu *et al.,* (2014).

The results revealed that the leaves of *Ocimum gratissimum* contain phytochemicals such as saponins, tannins, flavonoids and terpenoids. The leaf extract of *Ocimum gratissimum* also showed varying degrees of antibacterial activity on the bacteria isolates tested. The result indicated that the leaf of the plant contains some major bioactive compounds that can be used to treat infections caused by gastrointestinal pathogens.

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