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Phytochemical Screening and Antibacterial Activity of the Crude Extract of Scent Leaf (*Ocimum gratissimum*) on *Escherichia coli* and *Staphylococcus aureus*

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Authors' contributions

This work was carried out in collaboration among all authors. Author IIH designed the study, make collection of samples and their analyses. Author IYT performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AA and JT managed the literature searches and its development. While, author MA help in the analyses of the samples. All authors read and approved the final manuscript.

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ABSTRACT

Scent (*Ocimum gratissimum)* leaves are known for their medicinal values for a long. The study was carried out on phytochemical screening and antibacterial activity of scent leaf extracts on *E. coli* and *S. aureus.* Antibacterial assay of the plant extracts was carried out on the test isolates, by inoculation on the surface of freshly gelled sterile nutrient agar plates by streaking using sterilized swab stick and the potent extracts was determined according to the macro broth dilution technique. Phytochemical screening of *O. gratissimum* leaves revealed the presence of steroids, saponins, flavonoids, alkaloids, cardiac glycosides and tannins in all the extracts. There was decreased in

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antibacterial activity with decreased in concentration of the extract as the concentration of the extract decreases from 200 – 25 mg/ml, the zones of inhibition also decreased from 20 to 11 mm (ethanol extract) and 17 – 10 mm (aqueous extract) for *S. aureus;* 19 – 10 mm (ethanol extract) and 15 – 8 mm (aqueous extract) for *E. coli.* The Minimum Inhibitory Concentration (MIC) of *O. gratissimum* extracts against the selected clinical isolates revealed no growth (clear) in all the test organisms at the concentration of 200 mg/ml. Also, the Minimum Bactericidal Concentration (MBC) showed no growth of bacterial colonies at the concentration of 200 mg/ml. It was observed from the study that ethanol and aqueous extracts exhibited high inhibitory activities on *Escherichia coli* a representative of enteric coliforms and Gram negative bacteria and *Staphylococcus aureus* a representative of Gram positive bacteria. Ethanolic extract had higher inhibition compared to the aqueous extract. 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.

Keywords: Antibacterial activity; phytochemical; Ocimum gratissimum; extracts; clinical isolate; MIC; MBC.

1. INTRODUCTION

There is growing interest in exploiting plants for medicinal purposes especially in Africa. This is due to the fact that microorganisms are developing resistance and continue to multiply in the presence of therapeutic levels of the antibiotics [1]. The use of plants in the treatment of human disease is as old as the disease themselves [2]. Ijeoma and Umar [3] noted that herbalism is the earliest form of medicine. Herbal medicine was the major form of medicine in Nigeria during the pre-colonial days [4]. Medicinal plants are sources of many important specific drugs of this modern world and about 80% of the present day medicines are directly or indirectly from plants [5]. The various plants parts are concocted in a dosage which may be taken in the form of a liquid, solid or semi – solid [6]. Based on these perspectives, investigations of antimicrobial activities, mode of action and potential uses of plants have regained momentum. There appears to be a revival in the use of traditional approaches to protecting human beings, livestock and food from diseases, pest and spoilage in industrial environments. This is particularly important with regard to man whose health and longevity provides the impetus for antimicrobial evaluation of plants vis – a vis their medical values [7].

Scent leaf is naturally used in the treatment of different diseases which include: upper respiratory tract infections, diarrhoea, headache, conjunctivitis, skin disease, and pneumonia, tooth and gum disorder, fever and as mosquito repellents [8]. It is also used in the treatment of fungal infections, cold and catarrh. The plant is consumed as a leafy vegetables and the nutritional importance of this plant centered on its usefulness as a seasoning because of its aromatic flavour. It is also used in the management of the baby's cord. It is believed to keep the baby's cord and wound surface sterile. In recent years, scientific research has focused more in discovering and identifying local plant species with bioactive compounds as an essential oil, their characterization, extraction and purification processes and application in drug industries [9]. The Ocimum oil has been described to be active against several species of bacteria and fungi. These include *Escherichia coli*, *Staphylococcus aureus*, *Shigella, Salmonella and proteus,* for fungi: *Trichophyton rubrum, Trichophyton mentagrophytes, Cryptococcus neoformans, Penicilium islandicum and Candida albicans* [10,11,12]. Oil from the leaves have been found to possess antiseptics, antibacterial and antifungal activities [13]. Most phytochemicals when consumed served as medicine for protection and treatment of human or animal disease [14]. The study was carried out on phytochemical screening and to investigate the antibacterial activity of scent leaf extracts on *E. coli* and *S. aureus.*

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

Leaves of *Ocimum gratissimum* (scent leaf) was collected from a farm at Bajoga, in Funakaye Local Government Area of Gombe State, Nigeria. The plant was identified and authenticated in the herbarium of the Department of Science Laboratory Technology, Gombe State

Polytechnic, Bajoga and was given a voucher number of GSPB/SLT/19/0019. The fresh plant was air dried for two weeks at room temperature. The plant was pulverized using mortar and pestle to obtain the powder form.

2.2 Aqueous Extraction and Ethanol Extraction

Fifty grams (50 g) of the powdered leaves was suspended in 500ml of distilled water in one litre conical flask. It was shaken vigorously for 30 minutes and allowed to stand for 48 hours at room temperature. Muslin cloth of 0.4 mm size was used to filter the plant and the filtrate was further purified by filtration through filter paper (Whatman No 1) under aseptic conditions. The filtrate collected was evaporated to dryness using a water bath. The extract was collected in fresh sterile universal bottles and stored in the refrigerator at 4° C until when required for use. The same procedure was conducted for the ethanolic extract using 95% ethanol in one litre conical flask described by Atata et al. [15].

2.3 Phytochemical Screening

Phytochemical screening was carried out to test for the presence of secondary metabolites which include tannins, phlobatannins, alkaloids, flavonoids, saponins, cardiac glycosides, reducing sugar and steroids on both extracts (aqueous and ethanol) using standard procedures at room temperature as adopted by [16,17].

2.4 Qualitative Analysis of the Constituents

2.4.1 Test for tannins

Two grams (2 g) of each extract was dissolved in 10 ml of distilled water in separate test tubes and 3 drops of 10% Ferric Chloride ($FeCl_3$) was added to 2 ml of the solution. The occurrence of blackish – blue, green or blackish green colouration indicates the presence of tannins.

2.4.2 Test for phlobatannins

Zero point two grams (0.2 g) of each extract was boiled with an equal volume of 1% HCl, the deposition of a red precipitate indicates the presence of phlobatannins.

2.4.3 Test for saponins

Zero point one grams (0.1 g) of each extract was dissolved in 5 ml of distilled water and shaken vigorously. The formation of frothing bubbles which lasted for 10 minutes indicate the presence of saponins.

2.4.4 Test for alkaloids

Zero point five grams (0.5 g) of each extract was dissolved in 3 drops of Dragendoffs reagent. An orange precipitate indicates the presence of alkaloid.

2.4.5 Test for flavonoids

Zero point two grams (0.2 g) of each extract was dissolved in 2 ml of Sodium Hydroxide solution. The occurrence of a yellow solution which disappears on addition of HCl acid indicates the presence of flavonoids.

2.4.6 Test for cardiac glycosides

Zero point five grams (0.5 g) of each extract was dissolved in 3 ml of Fehling solution. A brick red precipitate indicates the presence of glycosides.

2.4.7 Test for steroids

Five (5) drops of concentrated H_2SO_4 was added to 0.1 g of each extract in test tube, a reddish brown colouration indicates the presence of steroids.

2.4.8 Test for reducing sugar

Zero point one grams (0.1 g) of each extract was dissolved in 2 ml of distilled water in separate test tubes. This was followed by addition of Fehling solution A and B and then the mixture was warmed. A brick red precipitate at the bottom of the test tube indicates the presence of reducing sugar.

2.5 Sterilization of Materials

Glass wares used were properly washed and sterilized in an autoclave at 121°C at 15 psi for 15 minutes before use. The work was carried out under aseptic condition. The work bench was disinfected with 70% ethanol.

2.6 Media Preparation

Nutrient agar medium and nutrient broth were used during the course of this work, it was prepared according to manufacturer's instructions which was sterilized by autoclave at 121°C at 15 psi for 15 minutes, after which it was

allowed to cool and then poured into sterile plate and allowed to solidify.

2.7 Test Microorganisms

The bacterial strains used in this study were pure clinical isolates (*Staphylococcus aureus* and *Escherichia coli*) obtained from the Microbiology Laboratory, from Gombe State Federal Teaching Hospital. The isolates were tested for viability by sub – culture into nutrient broth at 37°C in an incubator for 24 hours prior to antibacterial testing.

2.8 Biochemical Identification of the Test Organism

2.8.1 *Escherichia coli*

The *E. coli* was placed on Eosine Methylene Blue agar for 18 hours. Colonies with green metallic sheen were observed which indicate a positive result for *E. coli* [18].

2.8.2 *Staphylococcus aureus*

The *S. aureus* was placed on Manitol Salt Agar (MSA) for 18 hours. Colonies with green metallic sheen were observed which indicate a positive result for *S. aureus* [18].

2.9 Extract Dilution

The method adopted by Akujobi et al. [19] was used. The crude extract was diluted with the Dimethyl Sulfoxide ($DMSO^4$) to obtain concentration of 200, 150, 100 and 50 mg/ml respectively.

2.10 Antibacterial Assay

The antibacterial assay of the plant extracts was carried out on the test isolates and were inoculated on the surface of freshly gelled sterile nutrient agar plates by streaking using sterilized swab stick. Four wells were aseptically bored on each agar plate using a sterile cork borer (6 mm) and wells were properly labelled. Fixed volumes (0.1 ml) of different concentrations of the extracts (aqueous and ethanol) were then introduced into the wells in the plates respectively. A control well was in the centre with 0.01 ml of the extracting solvent. The plates were allowed on the bench for 40 minutes for pre – diffusion of the extract to occur and then incubated at 37°C for 24 hours. The resulting zone diameter of inhibition was measured using a transparent ruler calibrated in millimetres (mm). The readings were taken to be the zone diameter of inhibition of the bacterial isolate in question at that particular concentration [20].

2.11 Minimum Inhibitory Concentration (MIC)

The MIC of the potent extracts was determined according to the macro broth dilution technique. Standardized suspensions of the test organism were inoculated into a series of sterile tubes of nutrient broth containing two – fold dilutions of leaf extracts and incubated at 37°C for 24 hours. The MICs were read as the least concentration that inhibited the growth of the test organisms [20]. The lowest or least concentration of the extract that shows no growth in the test tubes is the MIC of the extract tested.

2.12 Minimum Bactericidal Concentration (MBC)

The MBCs were determined by first selecting tubes that showed no growth during MIC determination; a loopful from each tube were sub – cultured onto already gelled nutrient agar plates using spread plate technique and incubated for 24 hours at 37°C. The least concentration, at which no growth was observed, was noted as the MBC [20].

3. RESULTS AND DISCUSSION

The result of the phytochemical screening of *O. gratissimum* leaves revealed the presence of steroids, saponins, alkaloids in the aqueous extract, flavonoids, cardiac glycosides and tannins were found in the ethanolic while alkaloids in both the aqueous and ethanolic extracts as shown in Table 1.

Table 1. Phytochemical analysis of dried leaf extract of *Ocimum gratissimum*

Phytochemicals	Aqueous leaf extract	Ethanol leaf extract
Steroids	+	-
Saponins	+	-
Alkaloids	+	+
Flavonoids	-	+
Cardiac glycosides	-	+
Tannins	-	+
Phlobatannin	-	-
Reducing sugar	-	-

Key: + = Present, - = Absent

The results obtained as shown in Table 2 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 25 mg/ml showed low zone of inhibition.

The phytochemical analysis result of the leaf of this plant is similar to that of [21]. The phytochemicals in medicinal plants have been reported to be the active principles responsible for the pharmacological potentials of medicinal plants [13]. The presence of these chemicals in the leaves of *O. gratissimum* justifies the local use of this plant for the treatment of various ailments.

Flavonoids are compounds that are biologically active against liver toxins, microorganisms, inflammation, tumor and free radicals [22]. Saponins are natural glycosides that act as hypo-glycemic, antifungal and serum cholesterol lowering agents in animals [9]. Saponins are also essential elements in ensuring hormonal balance and synthesis of sex hormones [17]. Tannins are bitter polyphenolic compounds that hasten the healing of wounds. They also possess anti-diuretic and anti-diarrhea properties [22]. Conversely, condensed tannins can inhibit herbivore digestion by binding to consumed proteins, thereby making it indigestible for animals. Its concentration in the leaves might be the reason why animals do not graze on this plant [7].

The result obtained, there was decreased in antibacterial activity with decreased in concentration of the extract as shown in Table 2; as the concentration of the extract decreases from 200 – 25 mg/ml, the zones of inhibition also decreased from 20 to 11 mm (ethanol extract) and 17 – 10 mm (aqueous extract), 19 – 10 mm (ethanol extract) and 15 – 8 mm (aqueous extract) for *S. aureus* and *E. coli* respectively. This also agreed with the finding of Ishiwu et al. [23] who demonstrated that increase in the concentration of *O. gratissimum* extract reduces the number of viable *E. coli* and *S. aureus.* Other reports such as Nwinyi et al. [24] and Nwinyi et al. [25] have shown results similar to this work.

The quantitative measure of the *in vitro* activity of antibiotics and non–antibiotic antibacterial agents including those of plant origin with antibacterial potentials are the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. Table 3 shows the MIC of *O. gratissimum* extracts against the selected clinical isolates and the results revealed that all the test organisms at the concentration of 200 mg/ml showed no growth (clear). This is similar to the findings of Olanrewaju and Oju [21], which shows no much significant difference with the results obtained in this work.

Table 2. Zones of inhibition at various concentrations of leaf extract on test organisms

Microorganism	Concentration of ETE (mg/ml)				Concentration of AQE (mg/ml)				Control (mg/ml)
	200	100	50	25	200	100	50	25	20
	Zone of Inhibition (mm)								
Staphylococcus aureus	20	17	16	11	17	15	13	10	22
Escherichia coli	19	18	15	10	15	13	12	8	24

KEY: ETE = Ethanol Extract, AQE = Aqueous Extract

Table 3. Minimum Inhibitory Concentration (MIC) of ethanolic and aqueous extracts of *Ocimum gratissimum* on *S. aureus* and *E. coli*

Extract	Test organism	Concentration in mg/ml					
		200	100	50	25	12.5	6.25
	S. aureus						
Ethanolic		-	-	+	+	+	+
Aqueous		-	-	+	+	+	+
	E. coli						
Ethanolic		-	-	+	+	+	+
Aqueous		-	+	+	+	+	+

KEY: - = No growth (clear), + = Bacteria growth (Very turbid)

Table 4. Minimum Bactericidal Concentration (MBC) of ethanolic and aqueous extracts of *Ocimum gratissimum* against *S. aureus* and *E. coli*

Extract	Test organism	Concentration in mg/ml					
		200	100	50	25	12.5	6.25
	S. aureus						
Ethanolic		-	+	+	+	+	+
Aqueous		-	+	+	+	+	+
	E. coli						
Ethanolic		-	+	+	+	+	+
Aqueous		-	+	+	+	+	+

KEY: + = Bacteria growth of colonies, - = No growth of bacteria colonies

The MBC was defined as the concentrations at which all the bacteria strains were killed. Table 4 shows the MBC of *O. gratissimum* extracts against the test organisms and the results showed no growth of bacteria colonies at the concentration of 200 mg/ml. This also agreed with the findings of Olanrewaju and Oju [21].

4. CONCLUSION

This study concludes that ethanol and aqueous extracts had high inhibitory activities on *E. coli*; a representative of enteric coliforms and Gram negative bacteria and *Staphylococcus aureus*; a representative of Gram positive bacteria. Ethanolic extract had higher inhibition effects compared to the aqueous extract. This can be concluded that 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.

5. RECOMMENDATIONS

1. *O. gratissimum* leaf contains phytochemicals that possess antibacterial property and could be used as potential source of natural product in industrial manufacturing of drugs.
2. Scent leaf, (*O. gratissimum*) leaves could help fulfil the growing demands of plant based foods for human nutrition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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