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Antibacterial Activity of *Ocimum gratissimum* L. against Some Selected Human Bacterial Pathogens

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Authors' contributions

This work was carried out in collaboration between both authors. Author ADJ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OOM managed the analyses of the study under the supervision of author ADJ. Authors ADJ and OOM managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was designed to investigate the bioactive spice to determine its activity against some selected human bacterial pathogens. The spice studied was *Ocimum gratissimum* (clove basil).
Place and Duration of study: Microbiology laboratory of Bells University of Technology between October, 2016 to July, 2017.
Methodology: The plant extracts was tested for their activity against *Staphylococcus* species, *Escherichia coli, Enterococcus faecalis, Proteus mirabilis* and *Klebsiella pneumoniae* collected from microorganism bank of Bells University of Technology. Antibacterial activity was carried out using agar diffusion method while agar dilution method was employed for determining the minimum inhibitory concentration (MIC) on Mueller-Hinton agar.
Results: The findings of this study showed that the spice contained phytochemicals which made it active against the tested microorganisms. Hot water extract was more active against all tested isolates with MIC of 5.0 mg/ml against *Klebsiella pneumoniae* to 10 mg/ml against *Escherichia coli, Proteus mirabilis, Staphylococcus aureus* and *Enterococcus faecalis.* Zone of inhibition was

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between 18 mm for *Staphylococcus aureus* and *Enterococcus faecalis* and 31 mm for *Proteus mirabilis.*

Conclusion: *Ocimum gratissimum* has a broad spectrum antibacterial activity against all tested isolates and thus has a potential as a source of natural drugs. However, *in vivo* studies are recommended.

Keywords: Spice; Ocimum gratissimum; human pathogen; agar diffusion.

1. INTRODUCTION

Herbs and plants are abundant in nature which form the main source of traditional medicines used for relief in illnesses and are still widely used all over the world. Medicinal plants have a long history of use and their use is widespread in both developing and developed countries Kavitha [1]. According to the report of the World Health Organization (WHO), 80 per cent of the world's populations rely mainly on traditional therapies which involve the use of plant extracts or their active substances [2]. The antimicrobial properties of medicinal plants are now being reported from all over the world [3,4,5].

Ocimum gratissimum also known as clove basil, African basil, and in Hawaii as wild basil, is is known for its various uses in traditional medicine. Indeed, it has been reported for its action against gastrointestinal infections (diarrhoea, dysentery), infections of the skin (dermatitis, eczema, scabies), infections of the upper respiratory tract, associated with cough, asthma and bronchitis, wounds and sores, insect bites, nosebleeds, stroke, anaemia [6]. The essential oil of *Ocimum gratissimum* contains eugenol and shows some evidence of antibacterial activity [7]. The essential oil has potential for use as a food preservative [8] and is toxic to *Leishmania* [9]. Extracts of the leaves are documented to possess antidiabetic properties [10], anti-hyperlipidemic effect [11] and recently, it was shown to improve heamatological variables in experimental diabetes mellitus via its well reported antioxidant property [12].

Nowadays, multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. Moreover, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation has thus engaged scientists in the search for new antimicrobial substances. This study was therefore channelled towards investigating the bioactive spice; *Ocimum gratissimum* on selected human pathogens.

2. MATERIALS AND METHODS

Mueller-Hinton Agar (Oxoid, UK CM0337) was used to cultivate the bacteria. Gram-negative and Gram-positive (Oxoid, UK) multi discs were procured. Sterilin Petri dishes were used. Dimethyl Sulphoxide (DMSO) (Qualikems)

2.1 Sample Collection and Study/ Sampling Site

Fresh leaves of *Ocimum gratissimum* was collected locally from Benja Village, Ota, Ogun State. The plant was identified by Miss Babalola Sadat; a botanist in the Department of Biological Sciences, College of Natural And Applied Sciences, Bells University of Technology, Ota, Ogun State.

2.2 Preparation and Processing of Plant

Fresh leaves of *Ocimum gratissimum* was harvested and properly washed and rinsed in sterile distilled water and left to air dry in a room for two (2) weeks and micronized using an electric blender. The powdered sample was extracted using hot water, methanol and ethanol. The extract procedure in this study was as described by [13]. The mixture was mechanically shaken every 30 minutes for 6 hrs and then allowed to stand for about 24 hrs for extraction. The solution was then filtered using Whatman No.1 filter paper.

2.3 Preliminary Phytochemical Screening

The different extracts like rthanol extract, methanol extract, and the aqueous extract were subjected to qualitative tests for the identification of various phytochemical constituents like alkaloids, steroids, terpenoids, flavonoids, tannins and saponins using standard procedure [14].

2.4 Antibiotic Sensitivity Testing

Antimicrobial susceptibility tests were performed using the disk diffusion method as recommended by Kirby-Bauer method according to the CLSI guidelines [15]. This method is suitable for organism that grows rapidly over night at 35 – 37℃. The antibiotic (specific concentration) impregnated disc absorbs moisture from the agar and antibiotic diffuses in to the agar medium. The rate of extraction of the antibiotic from the disc is greater than the rate of diffusion. As the distance from the disc increases there is a logarithmic reduction in the antibiotic concentration. Zone of inhibition of bacterial growth around each disc is measured and the susceptibility is determined. The medium used was Muller Hinton Agar.

The antibiotics tested are: Gram-negative disc (Oxoid); Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg), Augmentin (50 µg), Nitrofurantoin (300 µg), Ampicillin (10 µg); Gram-positive disc (Oxoid); Ceftazidime (30 µg), Cloxicillin (5 µg), Erytromycin (30 µg), Caftriaxone (30 µg), Gentamycin (10 µg), Cefuroxime (30 µg), Ofloxacin (5 µg), Amoxycillin (30 µg).

A sterile swab stick was used to take inoculums from a solution of the test organism (stock solution) and the excess was drained off by pressing the swab stick against the inside of the test tube above the level of the suspension. The entire surface of the Mueller Hinton agar (MHA) plates were uniformly swabbed with the inoculums of test organisms and allowed to dry for 3-5 minutes. Carefully a sterile forceps was used to pick the appropriate antibiotic discs and placed at the center of the inoculated Petri-dishes labelled accordingly. The plates were incubated over night at 37℃ in an inverted position and the zones of inhibition were measured and interpreted using the standard recommendation of the Clinical Laboratory Standard Institute [16] guidelines.

2.5 Antimicrobial Preliminary Screening of Crude Extracts

The plant extract was reconstituted in sterile distilled water for the hot water extraction and concentrated DMSO for both methanol and ethanol extracts to give required dilutions of extracts which ranged from 12.5 to 100 mg/ml. The crude extracts were screened for antibacterial activity using the agar well diffusion method. This is according to [17].

2.6 Preparation of Bacterial Seeded Plates

A 0.1 ml of the overnight broth culture was transferred into 9.9 ml of sterile distilled water to produce a 1 in 100 (10^{-2}) dilution. A 0.2 ml of this dilution was then transferred aseptically into 20 ml sterile Mueller-Hinton agar. This was mixed gently and poured into sterile Petri dishes. With the aid of a sterile cork borer of 8 mm diameter, equidistant wells were bored into the seeded agar and the wells labeled accordingly after the plates had been allowed to dry.

Using a micro pipette approximately 100 µL of the reconstituted extracts at the various dilutions (100 to 12.5 mg/ml) were dropped into each well which filled them respectively to fullness.

Sterile distilled water and concentrated DMSO were used as controls in each case. The plates were allowed to stand for one hour on the bench to allow for proper diffusion of extracts into the medium and then incubated uprightly at 37℃ for 24 hours. Zones of inhibition were measured to nearest millimeter (mm). All experiments were applied in triplicates.

2.7 Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MIC) were determined by standard agar dilution method [18] in a series of doubling extract concentrations.

2.8 Determination of Minimum Inhibitory Concentration (MIC) of Crude Extract against Selected Pathogens

Different dilutions were prepared to give concentrations of 100, 50, 25 and 12.5 and 6.25 mg/ml. About 1 ml of each dilution of the extract was mixed with 19 mL of molten Mueller-Hinton agar (1 in 20 dilutions) to give final concentrations of 10, 5, 2.5 and 1.25 mg/ml, poured into Petri dishes and allowed to set. The surfaces of the set agar plates were allowed to dry before streaking with an overnight broth culture of the selected isolates and then incubated appropriately. The plates were then examined for the presence/absence of growth. The lowest concentration of the extract inhibiting the visible growth of each organism on the agar plate was regarded as the minimum inhibitory concentration. All experiments were done in triplicates.

2.9 Statistical Analysis

Data generated in this study were subjected to statistical analysis using Analysis of variance (ANOVA). Duncan's Multiple Range test method (1955) was then used to determine the significant differences between the means of the values using SPSS 16.0 for Windows Inc, Chicago.

3. RESULTS AND DISCUSSION

In this study, the plant was screened for the presence of secondary metabolites. Results from this present study showed which phytochemicals ranging from tannins, alkaloids, flavonoids, saponins, terpenoids to sterols were present in the study plant sample as represented in Table 1.

Analyzing the sensitivity and resistance pattern of isolates to different antibiotics, it was found that all tested Gram-negative bacteria isolates were totally resistant to all tested antibiotics. On the other hand, for the Gram-positive bacteria isolates *Staphylococcus aureus* showed resistance to caftriaxone while *Enterococcus faecalis* was resistant to erythromycin and cefuroxime; Tables 2a and b.

Table 3 shows the ethno-pharmacological claims of the plant. Zones of inhibition were determined. All tested isolates showed varying pattern to the various dilutions (concentration) of the plant extracts.

Other tested parameter such as the minimum inhibitory concentration of extracts against selected isolates expressed in milligram per milliliter (mg/ml) is indicated in Table 4.

Table 1. Preliminary phytochemical analysis of various extracts of *Ocimum gratissimum* L. leaves

Chemical test	Methanol extract	Ethanol extract	Aqueous extract
Alkaloids	+	+	+
Saponin	+	+	-
Tannins	+	+	+
Terpenoid: Liebermann-Burchard	+	+	+
Flavonoids	+	+	+
Steroids	+	+	+

+ present; - absent

Table 2a. Antimicrobial susceptibility testing of the gram negative isolates

Organism	Antibiotics (± SD)							
	Caz	Crx	Gen	Cpr	Ofl	Aug	Nit	Amp
Pm	-	-	-	-	-	-	-	-
Ec	-	-	-	-	-	-	-	-
Kp	-	-	-	-	-	-	-	-

NOTE: all readings are average of triplicate experiments; zones of inhibition are represented in millimeter (mm)
Key : Caz = Ceftazidime 30 µg, Crx = Cefuroxime 30 µg, Pm = Proteus mirabilis, Amp = Ampicillin 10 µg, Ec = Escherichia coli, Nit= Nitrofurantoin 300 µg, Kp = Klebsiella pneumonia, Gen = Gentamycin 10 µg, Cpr = Ciprofloxacin 5 µg, - = resistant, Ofl = Ofloxacin 5 µg, Aug = Amoxycillin 30 µg

Table 2b. Antimicrobial susceptibility testing of the gram positive isolates

Organism	Antibiotics (± SD)							
	Ctr	Ery	Cxc	Ofl	Aug	Caz	Crx	Gen
Sa	-	21±0.97	24±0.43	20±0.60	20±0.22	23±0.47	21±0.34	23±0.40
Ef	20±0.48	-	26±1.52	21±0.21	21±0.74	20±0.45	-	20±0.38

NOTE: all readings are average of triplicate experiments; zones of inhibition are represented in millimeter (mm)
Key: Caz = Ceftazidime 30 µg, Cxc = Cloxicillin 5 µg, Ery = Erytromycin 30 µg, Sa = Staphylococcus aureus, Ctr = Caftriaxone 30 µg, Ef = Enterococcus faecalis, Gen = Gentamycin 10 µg, Crx = Cefuroxime 30 µg, - = resistant, Ofl = Ofloxacin 5 µg, Aug = Amoxycillin 30 µg

Table 3. Antimicrobial susceptibility of test isolates to crude extracts

Organism					
Ethanol					
Conc of extract	***Ec***	***Pm***	***Sa***	***Kp***	***Ef***
25	18±0.55	20±0.22	14±0.44	21±0.56	-
50	23±1.06	22±0.69	16±0.72	-	-
100	25±0.89	26±0.36	19±0.51	-	-
200	28±0.52	29±0.55	22±0.37	-	-
D	-	-	-	-	-
Hot Water					
	Ec	***Pm***	***Sa***	***Kp***	***Ef***
25	20±0.38	24±0.56	18±0.82	21±0.48	19±0.58
50	21±1.59	26±0.45	20±1.43	22±0.58	22±0.24
100	23±1.20	29±0.69	23±1.53	25±0.59	24±0.26
200	25±1.59	31±0.27	25±0.26	27±0.99	26±0.79
D.S	-	-	-	-	-
Methanol					
	Ec	***Pm***	***Sa***	***Kp***	***Ef***
25	23±0.23	25±1.21	13±0.50	16±1.48	20±0.44
50	25±0.91	26±0.59	16±0.26	19±1.59	21±0.38
100	26±1.50	29±0.75	20±0.5	21±0.34	24±0.66
200	29±0.82	32±0.33	21±0.75	23±0.52	26±0.86
D	-	-	-	-	-

NOTE: all readings are average of triplicate experiments and are represented in millimeter (mm)
Key: - = resistant; Ec = Escherichia coli; Pm = Proteus mirabilis; Sa = Staphylococcus aureus; Kp = Klebsiella pneumonia; Ef = Enterococcus faecalis; D = DMSO; D.S = Distilled water

Table 4. Minimum inhibitory concentration of methanolic, ethanolic and hot water extracts of *O. gratissimum* against selected isolates

Organism	Concentration (mg/ml)				MIC
	10.0	**5.0**	**2.5**	**1.25**	
			Ethanol		
Ec	-	-	+	+	5.0
Pa	-	+	+	+	10.0
Sa	+	+	+	+	< 1.25
Kp	-	-	+	+	5.0
Ef	-	+	+	+	10.0
			Methanol		
Ec	-	-	+	+	5.0
Pa	-	-	+	+	5.0
Sa	-	+	+	+	10.0
Kp	-	-	+	+	5.0
Ef	-	+	+	+	10.0
			Hot water		
Ec	-	+	+	+	10.0
Pa	-	+	+	+	10.0
Sa	-	+	+	+	10.0
Kp	-	-	+	+	5.0
Ef	-	+	+	+	10.0

NOTE: all readings are average of triplicate experiments
Ec = Escherichia coli; Pm = Proteus mirabilis; Sa = Staphylococcus aureus; Kp = Klebsiella pneumoniae; Ef = Enterococcus faecalis

The phytochemical composition and antimicrobial properties of the leaves of *Ocimum gratissimum* have been studied. The positive results for carbohydrates, reducing sugars, glycosides, saponins, tannins, flavonoids, proteins, steroids and terpenoids confirms the presence of these secondary metabolites in the leaf extract of *O. gratissimum*. Antimicrobial effects of plant extracts have been attributed to the presence of these secondary metabolites [19]. The presence of these metabolites in the investigated plant part account for its usefulness as a medicinal plant. *Ocimum gratissimum* is known for its various uses in traditional medicine. Indeed, the drug is reported for its action against gastrointestinal infections (diarrhoea, dysentery), infections of the skin (dermatitis, eczema, scabies), infections of the upper respiratory tract, associated with cough, asthma and bronchitis, wounds and sores, insect bites, nosebleeds, stroke, anaemia [6]. This could be attributed to the presence of essential oil. *Ocimum gratissimum* contains eugenol and shows some evidence of antibacterial activity [7]. Spices are aromatic flavourings made from parts of plants. The "Spice" is a culinary term not a botanical category, it does not refer to a specific kind of plant or plant part [20]. Each spice has a unique aroma and flavour which derive from compounds known as phytochemicals or secondary compounds. These chemicals evolved in plants to protect them against herbivorous insects, vertebrates, fungi, pathogens, and parasites [21]. Spices are used as substances that increase the taste and variation of food [22]. It frequently also includes herbs, which are the fragrant leaves of herbaceous plants, many of which are native to temperate regions. For thousands of years, aromatic plant materials have been used in food preparation and preservation, as well as for embalming, in areas where the plants are native, such as Hindustan and the Spice Islands [23]. Furthermore, some spices are reported to have bactericidal or bacteriostatic activities. The inhibitory effects of spices are mostly due to the volatile oils present in their composition [24].

The activity of the extract against the selected pathogens shows that the plant extract has a broad spectrum of activity since it inhibited the growth of Gram-positive (*S. aureus, E. faecalis*) and gram negative bacteria *(E. coli, Klebsiella pneumoniae and P. mirabilis*). The results yielded a zone of inhibition that are within the intermediate (15- 19 mm) through the susceptible (≥20 mm) range. These established a good support to the use of this plant in herbal medicine and a base for the development of new drugs and phytomedicine [25]. This is based on the standards of the Clinical and Laboratory Standards Institute [16]. The pattern of growth of the gram negative isolates observed against the standard antibiotics could be to resistance (acquired or innate) of the isolates against the antibiotics. Many microorganisms have developed resistance against many antibiotics due to the indiscriminate use of antimicrobial drugs [26]. This creates problems in the treatment of infectious diseases [26,27]. From 1980 to 1990, [28] documented a high incidence of resistant microorganisms in clinical microbiology. This fact has also been verified in other clinics around the world. The extensive use of antimicrobial drugs has resulted in drug resistance that threatens to reverse the medical advances of the last seventy years (personal communication). Clinically important microbes that are rapidly developing resistance to available antimicrobials include bacteria that cause pneumonia, ear infections, and meningitis (e.g., *Streptococcus pneumoniae*), skin, bone, lung, and bloodstream infections (e.g., *Staphylococcus aureus*), urinary tract infections (e.g. *Escherichia coli*), foodborne infections (e.g. *Salmonella* or *E. coli* acquired from meat, eggs, nuts, fresh produce etc.), and infections transmitted in healthcare settings (e.g., Enterococci, *Acinetobacter baumanii, Pseudomonas aeruginosa*, and *Klebsiella* spp.) (Adeniyi and Ajose, *In Press*). Minimum inhibitory concentration was as low as 5.0 mg/ml for mostly Gram-negative organisms. This could be attributed to the polarity of these solvents. However, this is in contrast to the study of [29] who reported that Gram-negative organisms contain a high level of lipid material. These materials are thought to make a substantial contribution to the mechanism whereby injurious chemicals are prevented from reaching their sites of action within the cell.

4. CONCLUSION

In line with the Clinical and Laboratory Standards Institute (CLSI) antimicrobial susceptibility test interpretive category and the results obtained from this study, the tested sample; *Ocimum gratissimum* has a broad spectrum antibacterial activity against all tested isolates and thus has a potential as a source of natural drugs that may be employed in combating ailments and inconveniences resulting from microbial attacks. Assessing the therapeutic potentials of plants from the traditional African system of medicine

could insight us as to how best these plants can be used in the treatment of diseases. As a result of the emergence of bacteria which are resistant to multiple antimicrobial drugs indigenous plants; useful herbs which are used as spices in flavouring food can be used as an alternative therapy. However, *in vivo* studies are suggested in further work.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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