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Antimicrobial efficacy and phytochemical screening of aqueous and ethanolic extracts of *Ocimum gratissimum* (scent leaf) leaf against some clinical isolates

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Abstract

Medicinal and aromatic plants form a numerically large group of economically important plants which provide basic raw materials for medicines, perfumes, flavours and cosmetics in Nigeria. This study investigated the antimicrobial activity of scent leaves (*Ocimum gratissimum*) extracts on four pathogenic microorganisms using the agar well diffusion method. These bacteria include; *Pseudomonas aeruginosa, Salmonella typhi, Candida albicans,* and *Escherichia coli*. Two different extracts were obtained from the plant samples (water-soluble and ethanol-soluble extracts). Both extracts showed the presence of some important phytochemicals, however screening of the ethanolic extracts showed that it contained high levels of saponin, tannins, phenolics, steroids and glycosides when compared with the aqueous extract. There were zones of inhibitions observed around the wells of both water and ethanol extract of *Ocimum gratissimum*. The results showed that the isolates behaved differently in their sensitivity to different extracts added to their growth medium ranging between $3 \pm 0.01 - 35 \pm 0.02$ mm for ethanolic extract and $6 \pm 0.02 - 45 \pm 0.12$ mm for the aqueous extract with significance ($p < 0.05$). Ethanol extract was strongly effective against the four clinical pathogens. The MIC, MBC and MFC test showed that the ethanolic and aqueous extract of the plants are bactericidal and fungicidal but their fungicidal activities are lower when compared with their bactericidal activities. This investigation indicates that *Ocimum gratissimum* had antimicrobial effect thus confirming its use in traditional medicine.

Key words: Minimum bactericidal concentration, minimum fungicidal concentration, *Ocimum. gratissimum*, leaf extract, agar well diffusion

1. Introduction

World Health Organization (WHO) estimates that approximately 80% of the world's inhabitants depend on traditional or herbal medicines for their primary health care and medicinal plants have long formed the basis of sophisticated traditional medicine systems and purportedly provide excellent leads for new drug developments [1-5]. Medicinal plants have been used for centuries before the advent of modern medicine. Leaves, flowers, stems, roots, seeds, fruit, and bark can all be constituents of herbal medicines. The medicinal values of these plants lie in their component phytochemicals, which produce definite physiological actions on the human body [6].

Ocimum gratissimum (Scent leaf) also known as "alfavaca" is a medicinal plant belonging to the family Lamiaceae. It is a very important herbal medicine found in the tropical and warm regions such as India and sub-Sahara Africa especially in Kenya, Ghana and Nigeria [7-9]. In Nigeria, *O. gratissimum* is called "Efinrin" in Yoruba; "Nchoanwu" or "Ahuji" in Igbo; "Aramogbo" in Edo and "Daidoya" in Hausa [5, 10].

O. gratissimum is used as a good choice for upset stomach. It is used as an emetic and for hemorrhoids. This important plant is also used for the treatment of rheumatism, paralysis, epilepsy, high fever, diarrhea, sunstroke, influenza, gonorrhea and mental illness [1, 6]. In addition, the plant is used as a spice and condiment in the southern part of Nigeria. It is also used in toothpastes, mouth washes and some topical ointments, also used as an excellent gargle for sore throats and tonsillitis, expectorant and a cough suppressant [11].

Facts on its wide use by the traditionalist in treating some infections and diseases in Nigeria have encouraged us to further study this plant for in order to ascertain its antimicrobial efficacy in treating infections and diseases caused by some pathogenic microorganisms.

2. Materials and methods

2.1 Test organisms

The microorganisms used in this study, *Pseudomonas aeruginosa, Salmonella typhi, Candida albicans,* and *Escherichia coli* were obtained from the Department of Microbiology, University of Ilorin Teaching Hospital (UITH), Ilorin, Kwara state. Purity of the cultures was

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checked at regular intervals as described by Acheampong [12].

2.2 Plant collection and identification

Leaf samples of *Ocimum gratissium* were collected from the plants within the campus of Ladoke Akintola University of Technology, Ogbomoso. The plant samples were identified macroscopically as described by Dalziel [13] and confirmed at the herbarium unit of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. The fresh samples were sundried for a week, after which an electric blender was used in grinding them into fine powder and kept in plastic containers until further use at room temperature (28 ± 1 °C).

2.3 Cold aqueous extract

One hundred grams (100 g) of leaves were soaked into 100 mL of water in different airtight sterile jars. The solution was heated to boil using hot plate with intermediate shaking for about 25-30 min. The content was then filtered with Whatman No.1 filter paper and the filtrate was sterilized using autoclave and concentrated in a water bath at 60 °C for about 2 hr. A pinch of activated charcoal was added to remove the pigments. The concentrated decolorized extract was then stored at 4 °C for future use [14].

2.4 Preparation of ethanolic extract

One hundred grams (100 g) of each of the plant leaves were soaked into 100 mL of the solvent (95% ethanol) in different airtight sterile jars respectively at room temperature and kept on a shaker (90 rpm) with uniform shaking for 24 hr. The solvents containing the extracts were decanted, filtered with a muslin cloth initially and then with Whatman no. 1 filter paper respectively. The solutions were heated at 55 °C on water bath for 5 min, sealed with the glass stopper and kept on the rotary shaker for 24 hr. After 24 hr, the solutions were concentrated under reduced pressure at 45 °C using the rotary evaporator to $1/10^{th}$ of the initial volume, and finally dried at 55 °C in the oven. Dried extracts were weighted and stored at 4 °C in refrigerator for future use [14].

2.5 Sterility of extracts

Each of the extracts was tested for growth of contaminants. This was done by making serial dilution of 1 g of each extract up to 10^{-1}. Twenty microliters (20 µL) of the diluents were aseptically inoculated on nutrient agar plates and incubated at 37 ^{0}C for 24 hr. The plates were observed for growth. Absence of microbial growth in the extract indicated their sterility. Sterile extracts were used to test for antimicrobial efficacy.

2.6 Standardization of inoculums

Standardized inoculums of each tested organism were obtained by making their respective suspension up to 0.5 McFarland Standard as observed in the spectrophotometer and as described by Barry and Thornsberry [15].

2.7 Antimicrobial assay of the plant extracts

Prepared sterile nutrient agar plate was inoculated with standardized organisms of 0.1 mL of a day old culture. Glass spreader was used in spreading the inoculum evenly on the surface of the agar and excess are drained off. A sterile cork borer of 5 mm diameter was used to make five (5) ditches on the plates. The bacteria were inoculated into nutrient agar with varying concentrations of the extracts 200 mg/mL, 100 mg/mL, 50 mg/mL and 25 mg/mL were prepared from the stock concentration of the extracts. 0.5 mL of each concentration of the extracts was dispensed into each of the ditches on the plates that are appropriately labeled. The fifth ditch in the plates was picked as control by adding 0.5 mL of appropriate solvent used for the different extraction. The plates were done in duplicates and left on the bench for few minutes for the extract to diffuse into the agar and later incubated at 37 ^{0}C for 24 hr for bacteria and at 30 ± 1 °C for 72 hr for fungi. After incubation, the zone of clearance around each ditch was measured using a metric ruler by taking measurement of the zone of clearance around the ditch. The diameter of the cork borer was deducted from the diameter of the zone of clearance and this made or represented the antibacterial and antifungal activities measured or diameter of the zone of inhibition [16-17].

2.8 Determination of minimum inhibitory concentration (MIC) of the extracts

Broth dilution method was used to determine the MIC. Varying concentrations of the extracts was used that ranged from 10.0 mg/mL to 100 mg/mL. 0.5 mL of each concentration of the extracts were dispensed to 9 mL of nutrient broth containing 0.5 mL of standardized test organisms (bacteria and fungi) cells. The tubes were incubated aerobically at 37 ^{0}C for 24 hr for bacteria and 30 ± 1 ^{0}C for 48 hr for fungi. Controls were equally set up by using solvents and test organisms without the extract. The tube with least concentration of extract that does not show growth after incubation was picked as the minimum inhibitory concentration (MIC) [18-19].

2.9 Determination of minimum bactericidal concentration (MBC)

The culture tubes used in MIC (Minimum Inhibitory Concentration) determination that did not show turbidity or any visible growth after the period of incubation were drawn with syringe (0.5 mL) and dispensed onto the surface of nutrient agar. The inoculum were seeded on the surface of the media. The plates were incubated at 37 ^{0}C. The lowest concentration of the extract that inhibits the growth of the organisms on the plates after incubation was regarded as Minimum Bactericidal Concentration (MBC) [18-19].

2.10 Determination of minimum fungicidal concentration (MFC)

The culture tubes used in MIC (Minimum Inhibitory Concentration) determination that did not show turbidity or any visible growth after the period of incubation were drawn with syringe (0.5 mL) and dispensed onto the surface of potato dextrose agar. The inoculum were seeded on the surface of the media. The plates were incubated at 30 ^{0}C. The lowest concentration of the extract that inhibit the growth of the organisms on the plates after incubation was regarded as Minimum Fungicidal Concentration (MFC) [19].

2.11 Phytochemical screening of the extracts of the leaves

Chemical tests were carried out using aqueous extract to identify various constitutes using standard methods [20-22].

Test for alkaloid: 3 mL aqueous extract was stirred with 3 mL of 1% HCl on steam bath. Mayer and Wagner's reagent were added to mixture separately. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid.

Test for tannins: About 2 mL of the aqueous extract was stirred with 2 mL of distilled water and few drops of $FeCl_3$ solution were added. Formation of green precipitate was indication of presence of tannins.

Test for saponins: 5 ml of aqueous extract was shaken vigorously with 5 mL of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

Test for phlobatannins: About 2 mL of aqueous extract was added to 2 mL of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

Test for flavonoids: To 1 mL of aqueous extract, 1 mL of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoids.

Test for terpenoids: 2 mL of the organic extract was dissolved in 2 mL of chloroform and evaporated to dryness. 2 mL of concentrated sulphuric acid was then added and heated for about 2 min. Development of a greyish color indicates the presence of terpenoids.

Tests for glycosides: Liebermann's test: 2 mL of the organic extract was dissolved in 2 mL of chloroform and then 2 mL of acetic acid was added in it. The solution was cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus (that is, aglycone portion of glycoside).

Tests for steroids: I. A red colour produced in the lower chloroform layer when 2 mL of organic extract was dissolved in 2 mL of chloroform and 2 mL concentrated sulphuric acid was added in it, indicates the presence of steroids. II. Development of a greenish colour when 2 mL of the organic extract was dissolved in 2 mL of chloroform and treated with sulphuric and acetic acid indicates the presence of steroids.

2.12 Statistical Analysis

The data obtained in this study were expressed as mean ± SEM and subjected to analysis by one way ANOVA. *p* values of 0.05 or less were considered significant using SPSS software version 17.0.

3. Results

3.1 Antimicrobial effects of aqueous and ethanolic extract of Ocimum gratissium leaves

The screening for antimicrobial activity of the plant samples used in this study revealed that the plant extracts had varying effects on the growth of the clinical isolates. All the plant extracts have inhibiting strength on the test organisms. The ethanolic extract of *O. gratissimum* had the highest zone of inhibition at the concentration of 200 mg/mL for the clinical isolates tested, having 45 mm, 39 mm, 30 mm, and 36 mm for *P. aeruginosa*, *E.coli*, *S. typhi* and *C. albicans* respectively. The aqueous extract of *O. gratissimum* had the highest zone of inhibition at (35 mm) on *P aeruginosa* at the concentration of 200 mg/mL and also showed the best resistance on all the clinical isolates tested (Table 1).

Table 1 Antimicrobial effects of aqueous and ethanolic extract of *Ocimum gratissium* (Scent leaf) on selected microorganisms

Name of organisms	ZONE OF INHIBITION (mm)									
	Aqueous extract					Ethanolic extract				
	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	Control	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	Control
Pseudomonas aeruginosa	35 ± 0.02	31 ± 0.24	22 ± 0.04	3 ± 0.01	-	45 ± 0.12	38 ± 0.01	31 ± 0.05	15 ± 0.04	-
Escherichia coli	31 ± 0.04	27 ± 0.07	19 ± 0.15	5 ± 0.02	-	39 ± 0.08	32 ± 0.06	26 ± 0.09	13 ± 0.03	-
Salmonella typhi	24 ± 0.08	15 ± 0.5	10 ± 0.31	-	-	30 ± 0.01	23 ± 0.07	16 ± 0.01	6 ± 0.02	-
Candida albicans	30 ± 0.03	21 ± 0.04	13 ± 0.14	4 ± 0.01	-	36 ± 0.15	28 ± 0.02	14 ± 0.12	7 ± 0.01	-
***p* level (0.05)**	***	***	***	***		***	***	***	***	

KEY: - = No Zone of inhibition; Values of three replicates. Values expressed as mean ± SEM; *** = Means square significant at P<0.001

3.2 Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal (MBC/MFC) concentration of

aqueous and ethanolic extracts of Ocimum gratissimum leaves on tested organism

The MIC, MBC and MFC values obtained for the plant extracts on *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi* varied from one plant to another and from one extract to another. The ethanolic extract had the best MIC, MBC and MFC on all the clinical isolates tested. The aqueous extract of all the plant sample extracts also had tangible MIC, MBC and MFC values for all the clinical pathogens tested. The values ranged from 24 - 70 mg/mL for the aqueous extract, while ethanolic extract has ranged from 22 - 44 mg/mL. These concentrations gave bactericidal and fungicidal effects after 24 hr of incubation. They are therefore regarded as the lowest concentrations of the extract sufficient to kill defined proportion of viable organisms at a specified period (Table 2).

Table 2. Minimum Inhibitory concentration and minimum bactericidal/ fungicidal concentration of aqueous and ethanolic extracts of *Ocimum gratissimum* leaves

Name of Organisms	*Ocimum gratissimum*					
	Aqueous			Ethanolic		
	MIC (mg/mL)	MBC (mg/mL)	MFC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MFC (mg/mL)
Pseudomonas aeruginosa	24 ± 0.04	59 ± 0.015	-	22 ± 0.25	28 ± 0.11	-
Escherichia coli	32 ± 0.02	70 ± 0.24	-	24 ± 0.12	39 ± 0.14	-
Salmonella typhi	35 ± 0.07	53 ± 0.21	-	30 ± 0.09	44 ± 0.21	-
Candida albicans	37 ± 0.01	-	68 ± 0.31	23 ± 0.03	-	52 ± 0.21
***p* Level (0.05)**	***	***	***	***	***	***

Values of three replicates. Values expressed as mean ± SEM
*** = Means square significant at P<0.001
KEY: - = Not determined, MIC = Minimum Inhibitory concentration, MBC = Minimum bactericidal concentration, MFC = Minimum fungicidal concentration.

3.3 Phytochemical analysis of Ocimum gratissimum leaves

The phytochemical analysis of the plant leaves showed that the tested plant parts contain some active components. The aqueous and ethanolic extracts of *Ocimum gratissimum* showed that it contains glycosides, flavonoid, terpenoids saponin, phenolics, and tannins in tangible amounts but higher presence in the ethanolic extract was observed when compared to the aqueous extract, while alkaloids and phenolics were absent in the aqueous extract but present in the ethanolic extract as shown in Table 3.

Table 3. Phytochemical analysis of aqueous and ethanolic extract of *Ocimum gratissimum*

S. No.	*Ocimum gratissimum*		
	Chemical constituent	**Aqueous**	**Ethanolic**
1	Alkaloids	-	+
2	Tannins	+	++
3	Saponins	+	+
4	Phlobatanins	+	+
5	Flavonoids	+	+
6	Terpenoids	+	+
7	Glycosides	+	++
8	Steriods	+	-
9	Phenolics	-	+

KEY + ++ = More Strongly Present, + + = Strongly Present, + = Present, - = Absent

4. Discussion

The antimicrobial effect of medicinal plants on microorganisms may depend on the type of medium used to culture to microorganisms [23]. The antimicrobial agent may be incapable of diffusing through the cell wall or membrane of the microorganism as a result of the complexity in the organism.

In this study, the four microorganisms studied were sensitive to the plant extract. The plant extract showed varying degrees of antimicrobial activity on the microorganisms. This is in agreement with the work of Rojas et al. [24] on ten medicinal plants. The inhibitory activities of the plant extract agree with the report of Leven et al. [25]. The antimicrobial activities of plants can be linked to the presence of tannins, alkaloids, flavonoids and saponins. It has been reported that various plants that are rich in alkaloids, tannins and glycosides possess antimicrobial activity against a number of microorganisms [26]. This suggests that the plant extract used in this study has a general antimicrobial activity. The antifungal potential of *Ocimum gratissimum* extract is more pronounced than its antibacterial properties and the best for all the plant samples tested. The various sizes of inhibition zones obtained in this study is an indication of the potency of the bioactive principles contained in the plant extract.

The ability of ethanolic extract of *Ocimum gratissimum* to inhibit *E. coli, C. albicans* and *P. aeruginosa* may be due to the active component it contains such as saponin, flavonoid, phenolics and glycosides. Flavonoids have ability to complex with bacterial cell wall which often lead to inactivation of the protein and loss of function [27]. Phenolics inhibit enzymes by reaction with sulfhydryl groups or through non-specific interaction with protein thus toxic to microorganisms [28]. The mechanisms described above likely to be responsible for inhibition of *E. coli* by ethanolic leaf extract of *Ocimum gratissimum* which have been resistant to other plant extract used in the research work.

The varied MICs of the plant extract on the test organisms correlates with the report indicating that microorganisms exhibits varied level of susceptibility to plant extracts. The MIC result in this study agrees with Banso, and Adeyemo [29] showing that antimicrobial agent with low activity against an organism has a high MIC, while a highly active antimicrobial agent gives a low MIC. The antimicrobial substances in the extracts were bactericidal at

higher concentrations of the extracts. The result of the MBC and MFC of this research work is in agreement with the observation reported by Olorundare *et al*. [30].

The present study therefore shows that the plant extracts have useful antimicrobial properties in the ethanolics than the aqueous extracts. The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are of then associated with synthetic antimicrobials [31].

Study of the phytochemical screening of aqueous and ethanol extracts of the plants used in this study, showed that the extracts contained secondary metabolites. The presence of these biologically active substances may have been responsible for the antibacterial effect reported in this particular work.

Furthermore, the phytochemicals reported in this study for *Ocimum gratissimum* concur with an earlier reports [11, 32] where alkaloid, steroids and phenolics are either not detected or absent. The results of the study have shown that aromatic plants possess pharmacologically active component capable of inhibiting or stopping the growth of pathogenic microorganisms used. The result showed that the extracts of *Ocimum gratissimum* can be better purified to manufacture drugs for use in the treatment of stomach upset, typhoid, candidiasis and other diseases causes by the tested isolates.

5. Conclusion

It is concluded that extract of *O. gratissimum* may be useful in treating the diseases of which the test organisms are aetiological agents. This finding is significant because most bacteria and fungi have been reported to be resistant to the action of most antimicrobial agent available. Therefore, further work is needed to isolate the active principles from the plant in order to test the specific antimicrobial activity of the respective phytochemical components. Also, the active components identified in the extracts should be purified. Secondary screening should be carryout on the purified active components.

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