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Research Article

Phytochemical Constituents and Antimicrobial Activity of Various Concentrations of Ethanolic Extracts of Different Plant Parts of *Ocimum gratissimum*

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ABSTRACT

Recent trend of high percentage of microbial resistance to conventional antibiotics necessitates intensified efforts towards the search for more sources of antimicrobial agents. This study investigated phytochemicals and antimicrobial activity of aqueous extracts of *Ocimum gratissimum* against *Staphylococus aureus* and *Candida albicans* using standard techniques. Phytochemical screening of *Ocimum gratissimum* extracts indicates presence of alkaloids, flavonoids, tannins, saponnins, cardiac glycosides, and phlobatannins. This showed that *Ocimum gratissimum* produces secondary metabolites that make the plant useful in medicine. Alkaloids, tannins and phlobatannins were absent in the seeds while flavonoids was absent in the stem. Antimicrobial activity of *Ocimum gratissimum* against *Staphylococcus aureus* and *Candida albicans* using agar well diffusion method showed zones of inhibition of Leaf extract against *Staphylococcus aureus* at 100mg/ml, 50mg/ml and 25mg/ml concentrations were 22.00±0.26mm, 20.00±0.26mm and 16.00±0.26mm respectively. Zones of inhibition of Seed extract against *Staphylococcus aureus* at 100mg/ml and 50mg/ml were 20.00±0.26mm and 18.00±0.26mm respectively. Zones of inhibition of Leaf extract against *Candida albicans* at 100mg/ml, 50mg/ml and 25mg/ml concentration were 18.00±1.73mm, 16.00±1.73mm and 14.00±1.73mm respectively. Seed extract exhibited an antimicrobial activity at 100mg/ml and 50mg/ml at 17.00±1.73mm and 20.00±1.73mm respectively. While zones of inhibition of Stem extract against *Candida albicans* at 100mg/ml, 50mg/ml and 25mg/ml concentrations were 19.67±1.73mm, 18.33±1.73mm and 16.67±1.73mm respectively. Antibiotic sensitivity of the bacteria was tested with commercially available antibiotic Ciprofloxin® which produced a zone of inhibition of 38.00±0.26mm against *Staphylococus aureus*. Antibiotic sensitivity of the fungi was tested with commercially available antibiotic Terbinafine® which produced zone of inhibition of 20.00±1.73mm against *Candida albicans.* Results of the minimum inhibitory concentration (MIC) of aqueous extracts of *Ocimum gratissimum* indicate that only 100mg/ml and 50mg/ml had MIC while for the MBC, it was bacteriostatic at all concentrations. Only concentrations at 100mg/ml and 50mg/ml had MIC for fungi and 100mg/ml was fungicidal.

Key words: *Ocimum gratissimum*, plant parts, aqueous extracts, antimicrobial activity, bacteriostatic, fungicidal.

Introduction

The increased prevalence of microorganisms which are resistant to the available antibiotics is one of the major challenges for the healthcare systems worldwide. Antibiotic-resistant infections are associated with one to two-fold increases in mortality compared to antibiotic-susceptible infections (Cosgrove and Carmeli, 2003). With the recent trend of high percentage resistance of microorganisms to the present day antibiotics, efforts have been intensified by researchers towards the search for more sources of antimicrobial agents (Dionisi *et al*. 2012). The genus *Staphylococcus* is made up of gram positive cocci with diameter of 0.5 – 1.5 µm. *Staphylocoocus aureus* is the most pathogenic spp of the genus *Staphylococcus.* It is implicated in both communities acquired and nocosomial infections. It often symptomatically colonizes the skin and mucus membrane of healthy individuals in particular the anteronaires.

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Phytochemical evaluation of *Ocimum gratissimum* has shown that it is rich in alkaloid, tannis, phytates, flavonoids and oligosaccharides (Akinmoladun *et al.,* 2007). It has tolerable cyanogenic content (Ijeh *et al.,* 2004). The volatile aromatic oil from the leaves consists mainly of thymol (32-65%) and eugenol; it also contains xanthones, terpenes and lactones (Ezekwesili *et al.,* 2004). Characterization of its ethanolic extracts revealed the presence of non – cyclic sesquiterpenes, phenols (Esvanzhuga, 1986).

Antimicrobial activities of the aqueous and ethanolic extracts of plant potentials have been evaluated both *in vitro* and *in vivo* against *Aspergillus niger* and *Escherichia coli* (Albayrak *et al*., 2010). Due to importance of *Staphylococcus aureus* on the increasing prevalence of antibiotic resistance stains, these bacteria have become the most studied *Staphylococcus* species. However, not many reports are available on the exploitation of antifungal or antibacterial property of *Ocimum gratissimum* plant for developing commercial formulations for application in human health. *Staphylococcus aureus,* and *Candida albicans*.

Materials and Methods

Sample Collection of *Ocimum gratissimum* Plant and Microorganisms

The seed, leaves, stem and roots of *Ocimum gratissimum* were collected from Wusasa and Samaru in Zaria, Kaduna State and were taken to the herbarium of the Department of Biological Science, Ahmadu Bello University Zaria, Kaduna State, for identification after which a voucher number (ABO01285) for the plant was given. The laboratory isolates of *Staphylococcus aureus* and *Candida albicans* were collected from the Department of Biological Sciences, Ahmadu Bello University after identification.

Preparation and Phytochemical Screening of Extracts of *Ocimum gratissimum*

The samples of seeds, leaves, stem and roots of *Ocimum gratissimum* were dried separately at room temperature to preserve the active phytochemical constituents. After drying, it was pounded using mortar and pestle and stored in plastic containers until when required for analysis.

The powdered samples of the seeds, leaves, stems and roots of *Ocimum gratissimum* were separately soaked in seventy-five percent (75%) ethanol at room temperature for 72h for the extraction of phyto-chemical constituents from the plant parts. The contents were filtered with filter paper and the filtrate was evaporated with an evaporating dish in a hot water bath (Harborne, 1973). The extracts were screened for alkaloids, flavonoids, tannins, saponins, cardiac glycosides, and phlobatannins using methods of Sofowara (1993), and Trease and Evans (1989).

Microbiological Media Used for the Study

Nutrient agar, Nutrient broth, Sabouraud dextrose agar and Sabouraud liquid media were used for culturing and preparing of the bacteria and fungi. The media were both used for Zone of Inhibition, Minimum Inhibitory Concentration, Minimum Bacteriosidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC).

Test for Minimum Inhibitory Concentration (MIC)

The organisms were cultured and standardized as earlier described. The growth requirements of the organisms were considered, for the bacteria culture (*staphylococcus aureus*), nutrient broth was used while for the fungi culture *(Candida albicans*) Sabouraud liquid medium was used. Double strength nutrient broth (Oxoid) and Sabouraud liquid media and single strength solutions were separately prepared according to the manufacturer's instructions. 2.5mls of each of double and single strengths were dispensed into each set of test tubes capped and sterilized by autoclaving at 121^0 for 15minutes. The sterilized tubes containing the nutrient broth were then removed to the aseptic screen and allowed to cool. Ten (10) tubes of nutrient broth and Sabouraud liquid medium were arranged in rows. The first tube however contained double strength nutrient broth. To the first tubes 2.5mls of the extract were added using the sterile pipette and mixed properly with the whirl mixer. From this first tube 2.5mls were withdrawn and added to the second tube and mixed properly too, this dilution was continued serially to the last tube from which 2.5mls were withdrawn and discarded into a disinfectant container. Similar operation was carried out for the fungal culture but using the Sabouraud liquid medium in place of nutrient broth. The dilutions of the extracts were as follows, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512 and 1:1024.

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To the test tubes of nutrient broth and Sabouraud liquid medium containing the leaf extract, two drops of the standardized cultures of the respective test organisms were added to each of the 10 test tubes and were incubated at 37^0 for 24hours for bacteria and at 30^0 for 48hours for fungi.

For each experiment, three controls were set up;

(1) to show the sterility of the media,
(2) to show the sterility of the extract and
(3) to ascertain the growth promoting property of the media.

For 1 and 2, a sterile tube of media and extract were incubated at 37ºC for 24hours while for 3, a sterile tube of nutrient broth and sabouraud liquid medium were inoculated with test cultures respectively and incubated appropriately and the results recorded.

Test for Minimum Bacteriocidal Concentration and Minimum Fungicidal Concentration

From the Test for minimum inhibitory concentration (MIC) above, a loopful from the tube containing the least concentration of the extract without growth were streaked on a sterile nutrient agar plate for bacterial tests and Sabouraud dextrose agar for fungal test and incubated at the appropriate temperature and time.

This is to test for mode of action of the extract, whether bacteriostatic or bacteriocidal and whether fungistatic or fungicidal.

Data collection and statistical analysis

Data obtained were subjected to Analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) using statistical package for social science SPSS to know the significance in the zone of inhibition, effectiveness of each extract and the susceptibility of the test organism. Least significant difference (LSD) of p=0.05 was used to compare means.

Results

The result of the phytochemical constituents present in the seeds, leaves, stem and roots of *Ocimum gratissimum* is shown in Table 1 below. The studies of phytochemical screening of roots, seeds and leaves showed that all the plant parts contain alkaloids, flavonoids, saponnins, and cardiac glycosides, but flavonoids was absent in the stem and phlobatannins, tannins and alkaloids were absent in the seeds.

Table 1: Phytochemical constituents present in seeds, leaves, stem and roots of *Ocimum gratissimum*

Phytochemical Constituent	Seeds	Leaves	Stem	Roots
Alkaloids	-	+	+	+
Flavonoids	+	+	-	+
Tannins	-	+	+	+
Saponins	+	+	+	+
Cardiac glycosides	+	+	+	+
Phlobatannins	-	+	+	+

Key: - = Absent, **+** = Present

The result of the zone of inhibition of *Staphylococcus aureus* using various concentrations of extracts of the different plant parts of *Ocimum gratissimum* is shown in Table 2. Significant difference was noted in the zone of inhibition at varying concentrations in different parts of *Ocimum gratissimum*. The control concentration had the highest zone of inhibition of 38.00mm in all the plant parts. The control concentration value is followed by 100 mg/ml in leaf (22.00mm) and seed (20.00mm). No inhibition was observed in other concentrations in stem and root of *Ocimum gratissimum*.

The result of the zone of inhibition of *Candida albicans* using various concentrations of extracts of the different plant parts of *Ocimum gratissimum* is shown in Table 3. Significant difference was noted in the zone of inhibition at varying concentrations in different parts of *Ocimum gratissimum* using *Candida albicans*. The control concentration had the highest zone of inhibition of 20.00 for leaf, root and seed while 100mg/ml (19.67) had the highest in stem. Meanwhile, 6.25 mg/ml had the least mean inhibition throughout the test using *Candida albicans*.

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Table 2: Zone of Inhibition of *Staph. aureus* using various concentrations of extracts of different plant parts of *Ocimum gratissimum*

Concentration of Extract	Plant Part Extract of *Ocimum gratissimum*							
	Leaf		Root		Seed		Stem	
	No. Examined	Mean inhibition±S.E	No. Examined	Mean inhibition±S.E	No. Examined	Mean inhibition±S.E	No. Examined	Mean inhibition±S.E
0	3	38.00±0.26	3	38.00±0.26	3	38.00±0.26	3	38.00±0.26
6.25	3	0.00±0.26	3	0.00±0.26	3	0.00±0.26	3	0.00±0.26
12.5	3	0.00±0.26	3	0.00±0.26	3	0.00±0.26	3	0.00±0.26
25	3	16.00±0.26	3	0.00±0.26	3	0.00±0.26	3	0.00±0.26
50	3	20.00±0.26	3	0.00±0.26	3	18.00±0.26	3	0.00±0.26
100	3	22.00±0.26	3	0.00±0.26	3	20.00±0.26	3	0.00±0.26
	18	**16.00**	**18**	**6.33**	**18**	**12.67**	**18**	**6.33**

Note: Using ciprofloxacin as control. P value = 0.000**

Table 3: Zone of Inhibition of *Candida albicans* using various concentrations of extracts of different plant parts of *Ocimum gratissimum*

Concentration of Extract	Plant Part Extract of *Ocimum gratissimum*							
	Leaf		Root		Seed		Stem	
	No. Examined	Mean inhibition±S.E	No. Examined	Mean inhibition±S.E	No. Examined	Mean inhibition±S.E	No. Examined	Mean inhibition±S.E
0	3	20.00±1.73	3	20.00±1.73	3	20.00±1.73	3	20.00±1.73
6.25	3	0.00±1.73	3	0.00±1.73	3	0.00±1.73	3	0.00±1.73
12.5	3	0.00±1.73	3	0.00±1.73	3	0.00±1.73	3	0.00±1.73
25	3	14.00±1.73	3	0.00±1.73	3	0.00±1.73	3	16.67±1.73
50	3	16.00±1.73	3	0.00±1.73	3	20.00±1.73	3	18.33±1.73
100	3	18.00±1.73	3	0.00±1.73	3	17.00±1.73	3	19.67±1.73
	18	**11.33**	**18**	**3.33**	**18**	**9.50**	**18**	**12.45**

Note: Using Terbinafine as control. P value = 0.000**

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The result of the minimum bactericidal concentrations (MBC) of *Staphylococcus aureus* using various concentrations of *Ocimum gratissimum* extracts is shown in Table 4. One hundred percent (100%) growth was observed at varying concentrations of *Ocimum gratissimum* lead and stem. No bactericidal effect was observed at all. While the control had 60.00% growth (18 positive) with 40.00% no growth (12 negative).

The result of the minimum fungicidal concentrations (MFC) of *Candida albicans* using various concentrations of *Ocimum gratissimum* extracts is shown in Table 5. The highest growth was observed in *Candida albicans* when leaf was used. Although, the seed and stem results in 90.00% growth noticed respectively which is similar to what was obtained in the control.

Table 4: Minimum bactericidal concentrations of *Staphylococcus aureus* using various concentrations of *Ocimum gratissimum* extracts

Concentration of Extract	Dilution	SA1 - *Staph aureus* Leaf Extract		SA3 - *Staph aureus* Seed Extract		Control		Total	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
0.1953125	1:1024	3	0	3	0	3	0	9	0
0.390625	1:512	3	0	3	0	3	0	9	0
0.78125	1:256	3	0	3	0	3	0	9	0
1.5625	1:128	3	0	3	0	3	0	9	0
3.125	1:64	3	0	3	0	3	0	9	0
6.25	1:32	3	0	3	0	3	0	9	0
12.5	1:16	3	0	3	0	0	3	6	3
25	1:8	3	0	3	0	0	3	6	3
50	1:4	3	0	3	0	0	3	6	3
100	1:2	3	0	3	0	0	3	6	3
Total		**30 (100.00%)**	**0 (0.00%)**	**30 (100.00%)**	**0 (0.00%)**	**18 (60.00%)**	**12 (40.00%)**	**78 (86.67%)**	**12 (13.33%)**

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Table 5: Minimum fungicidal concentration of *Candida albicans* using various concentrations of *Ocimum gratissimum* extracts

MFC Fungi		CA1 - *Candida albicans*		CA3 - *Candida albicans*		CA4 - *Candida albicans*					
Concentration	Dilution	Leaf		Seed		Stem		Control		Total	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
0.1953125	1:1024	3	0	3	0	3	0	4	0	13	0
0.390625	1:512	3	0	3	0	3	0	4	0	13	0
0.78125	1:256	3	0	3	0	3	0	4	0	13	0
1.5625	1:128	3	0	3	0	3	0	4	0	13	0
3.125	1:64	3	0	3	0	3	0	4	0	13	0
6.25	1:32	3	0	3	0	3	0	4	0	13	0
12.5	1:16	3	0	3	0	3	0	4	0	13	0
25	1:8	3	0	3	0	3	0	4	0	13	0
50	1:4	3	0	3	0	3	0	4	0	13	0
100	1:2	2	1	0	3	0	3	0	4	2	11
Total		**29 (96.67%)**	**1 (3.33%)**	**27 (90.00%)**	**3 (10.00%)**	**27 (90.00%)**	**3 (10.00%)**	**36 (90.00%)**	**4 (10.00%)**	**119 (99.17%)**	**11 (9.17%)**

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Discussion

The studies of phytochemical screening of roots, seeds and leaves showed that all the plant parts contain alkaloids, flavonoids, saponnins, and cardiac glycosides, but flavonoids was absent in the stem and phlobatannins, tannins and alkaloids were absent in the seeds. The studies therefore showed that secondary metabolites that makes the plant useful in medicine especially traditional medicine (Musa *et al*., 2018). Fungi are important pathogens of plants than animals with significant yield losses while others spoil crops by Preliminary phytochemical screening revealed the presence ofalkaloids, tannins, glycosides, saponnins, resins, cardiac glycosides, steroids and terpenes and flavonoids. The presence of these phytochemical bases in *Ocimum gratissium* accounts for its usefulness as a medicinal plant. Alkaloids, Tannins and Phlobatannins are absent in seeds of *O. gratissimum* while flavonoids, saponins and cardiac glycosides are present. Flavonoids are absent in the stem of *O. gratissimum* and Alkaloids, phlobatanins, Tannins, Saponins and Cardiac glycosides were present. All other phytochemical constituents were present in both the leaves and roots of *O. gratissimum*. Phytochemical screening of the leaves, stem and roots extracts of *Ocimum gratissimum* showed similarity in their phytoconstituents. The seeds however showed a little disparity. These are believed to be responsible for the antibacterial effects observed. Like in the treatment of diarrhoea and dysentery, some workers have also attributed to their observed antimicrobial effect of plant extracts in the presence of these secondary plant metabolites (Nweze et al., 2004). This is also in agreement with the presence of tannins, alkaloids, flavonoids, terpens, saponins, carbohydrates and cyanogenetic glycosides that was reported in the findings of Doughari and Manzara (2008). The presence of these phytochemical bases in *Ocimum gratissimum* accounts for its usefulness as medicinal plants. The secondary metabolites found in the seeds, roots, and stems have earlier been reported in the leaves as extract of *Ocimum gratissimum* (Afolabi *et al*., 2007). The presence or absence of some secondary metabolites in the plants extracts is usually dependent on the soil type or environmental conditions where the plant is found growing. This may be as a result of the growing season, harvesting methods, processing and storage conditions (Sulistiarini, 1999). The seeds of *Ocimum gratissimum* contained no alkaloids, tannins and phylobatannins while flavonoids, saponins and cardiac glycosides were present (Table 1). This could be related to the environmental conditions or the nature of the soil. Absence of flavonoids can be related to the soil nature and environmental conditions. It can also be related to the fact that flavonoids are organic pigments that gives the plants a red, violet or blue coloration producing potent toxins, causing mycotoxicosis in immunocompromised animal and human when infected foods are ingested. In addition, some individuals display strong and dangerous allergic reactions to molds (Boundless, 2016).

The phytochemicals revealed in *O. gratissimum* L. plant extracts in this present study had been documented in previous works (Ezeokeke *et al*., 2015; Sulistiarini, 1999; Dubey *et al*., 2000; Holets *et al*., 2003). These were cardiac glycosides, flavonoids phlobatan, steroids, saponins, alkaloids, tannins and terpenoids. The present work reveals that the extract from the leaves of *O. gratissimum* possesses good antioxidant potential than the other parts of the plants. This is because of its phytochemical constituents (Thabrew *et al*., 1998; Halliwell and Gutteridge, 1992). The higher activity of Itraconazole was expected since the extracts have various impurities as compared to the drug that is already a synthetically processed molecule and has undergone refining processes that have established it as a standard antifungal. The zones of inhibition of the standards and extracts, though of same volume varied slightly in the replicates, this might be due to uneven distribution of test organisms on agar surfaces or slight difference in temperature and the flatness of the plates at the time it was used. The MIC values found to be lower than the MBC values suggesting that extracts were fungistatic at lower concentrations and fungicidal at higher concentrations.

In conclusion, the presence of these phytochemical constituents could be responsible for the therapeutic claims elicited by *Ocimum gratissimum* which make this plant useful as therapeutic agents in the treatment of diseases and malfunctions to humans. The quantitative analysis of the plants parts of *Ocimum gratissimum* can be done in the knowledge of health using these plants that are commonly found in the wild and in our environment. Inhibition was observed using different parts of *Ocimum gratissimum*. Although the highest inhibitory effect was observed in the leaf at 100 mg/ml concentration, it inhibited the growth of the bacteria *Staphylococus aureus* and kills the fungus *Candida albicans* at 100 mg/ml concentration of the extract.

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