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Phytochemical screening and antibacterial activities of *Ocimum gratissimum* against some clinical isolates

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Abstract

Ocimum gratissimum is a common plant in the tropics and has been used in food and medicine. Its usage in food and medicine could be attributed to its phytochemical and antimicrobial properties. In this study the phytochemical and antibacterial attributes of aqueous and ethanolic extracts of *O. gratissimum* leaves was investigated against some selected clinical isolates. Susceptibility effects of these extracts were determined by agar well diffusion method against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Phytochemical compounds such as alkaloids, saponins, phenol, phytates, oxalate, glycosides, and tannins were detected in *O. gratissimum* leaf extract. The antibacterial results showed that the efficacy of the aqueous extracts was more pronounced against the test bacterial, especially *K. pneumoniae* at minimum inhibitory concentration (MIC) of 0.2 mg/mL. The ethanol extract was more pronounced against *S. aureus* with diameter of zones of inhibition ranging from 12.84±0.01 – 19.15±0.02 mm at concentrations of 0.2 – 0.8 mg/mL respectively. The significant antibacterial properties of the leaf extract could be attributed to the presence of these bioactive compounds. Thus, this investigation proves to an extent that the *O. gratissimum* extracts when used against microorganisms has sufficient antimicrobial property.

Keywords: Antibacterial; Clinical isolates; Crude extracts; *Ocimum gratissimum*; Phytochemical

1. Introduction

Medicinal plants play a vital role in the treatment and prevention of various diseases and their promotion and there is growing interest in the search for new drugs from natural resources (Ullah *et al.*, 2018). People living in many developing countries in Asia and Africa are mostly dependent on traditional medicines for healing purposes due to their limited access to modern medical facilities. They also have more cases of foodborne diseases because of their poor hygiene and exposure to contaminated drinking water and food materials (Odeyemi and Sani, 2016). Various studies have regularly reported the antimicrobial activities of traditional medicines from this part of the world. Medicinal plants offer a substantial opportunity as they contain various bioactive chemical constituents (phytochemicals) that can act as antimicrobial agents. Natural products are also reported to act as synergists along with many modern drugs to combat multidrug-resistant pathogens (Ayaz *et al.*, 2019).

Ocimum gratissimum belongs to the group of plants known as spices. The plant is an erect small plumb with many barnacles usually not more than 1 m high (Vierra and Simon, 2000). It is of the family Labiatea, genus *Ocimum* and species *gratissimum* (Iwu, 1993). In South East Asia, it is cultivated as a home garden crop but it is grown on a commercial scale in Vietnam. In Nigeria, it is *Efinrin* in Yoruba, *Diadoyal* in Hausa and *Nchuanwu* in Igbo (Owulade, 2004). It is used for a variety of reasons. In culinary, it is used in salads, soups, pastas, vinegars and jellies in many parts of the world. The Thai people are popularly known to use it in food flavoring. In traditional medicine, the leaves have

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been used as a general tonic and antidiarrheal agent and for the treatment of conjunctivitis by instilling directly into the eyes; the leaf oil when mixed with alcohol is applied as a lotion for skin infections, and taken internally for bronchitis. The dried leaves are snuffed to alleviate headaches and fever among other uses (Iwu, 1993).

Although, conventional antibiotics have been very useful in orthodox medicine, it has been argued by many that its concomitant use with herbal extracts is not desirable as one normally antagonizes the activity of the other. Considering the fact that *Ocimum gratissimum* is used in most local dishes/foods to achieve a variety of purposes, there is need to ascertain if its extract antagonizes or acts as a synergy when used together with conventional antibiotics. In addition, despite the fact that the various extracts of *O. gratissimum* have been tested *in vitro* and shown to be active against some bacterial and fungal isolates (Silva *et al*, 2005).

Nettle (*Urtica dioica*) which belongs to the family *Urticaceae* is recommended for complaints associated with rheumatoid arthritis, osteoarthritis and urinary tract infections, allergies, Alzheimer's, asthma, bladder problems, bronchitis, cough, bursitis, gingivitis, gout, hair growth and baldness, hives, kidney stones, prostate enlargement, sciatica, tendinitis (Banso and Adeyemo, 2006). New antimicrobial agents are needed to treat diseases in humans and animals caused by drug resistant microorganisms. Interest in plant-derived drugs has been increasing, mainly due to the current widespread belief that "green medicine" is safer and more dependable than costly synthetic drugs (Benkeblia, 2004). Nettle is stated to possess antihaemorrhagic and hypoglycemic properties. Traditionally, it has been used for uterine hemorrhage, cutaneous eruption, infantile and psychogenic eczema, epistaxis, and melena and specifically for nervous eczema (Banso and Adeyemo, 2006). The aim of this study is to comparatively study the antibacterial effect of aqueous and ethanolic extracts of *Ocimum gratissimum* and *Urtica dioica* on a spectrum of selected clinical isolates.

2. Material and methods

2.1. Collection of Plant Materials

Fresh leaves of *Ocimum gratissimum* and *Urtica dioica* were collected from farmland within the vicinity of the Federal Polytechnic, Ado-Ekiti, Nigeria, during December and were taken to the Department of Agricultural Technology for proper identification. The fresh leaves were washed with distilled water, air-dried at room temperature for 7 days, and then were crushed and subsequently extracted.

2.2. Extraction of bioactive components from the plant materials

The leaves of the two plant samples were separately extracted with distilled water and ethanol. One hundred grams (100 g) of the powdered plant materials (*O. gratissimum* and *U. dioica*) were separately weighed and poured into different beakers. 500 mL and 300 mL of distilled water and ethanol were poured into each beaker respectively. The contents are stirred using a sterile glass rod and allowed to stand for 24 hours at room temperature (25 ^{o}C ± 1). The contents were filtered through a filter paper (Whatman No. 1) and the filtrate concentrated and evaporated using water-bath at the temperature of +95^{o}C. Extracts are then kept at 20 ^{o}C prior use.

2.3. Phytochemical Analysis

2.3.1. Tannin determination

Two grams (2 g) of finely grounded plant samples were separately weighed into a 50 mL sample bottle. 10 mL of 70% aqueous acetone was added and properly covered. The bottle were put in an ice bath shaker and shaken for 2 hours at 30^{o}C. Each solution was then centrifuged and the supernatant store in ice. 0.2 mL of each solution was pipetted into the test tube and 0.8 mL of distilled water was added. Standard tannin acid solutions were prepared from a 0.5 mg/mL of the stock and the solution made up to 1 mL with distilled water. 0.5 mL of Folin ciocateau reagent was added to both sample and standard followed by 2.5 mL of 20% Na_2CO_3 the solution from a standard tannic acid curve was prepared.

2.3.2. Determination of terpenoids

Two grams (2 g) of finely grounded plant samples were separately weighed into a 50 mL conical flask, 20 mL of chloroform methanol 2:1 was added, the mixture was shaken thoroughly and allowed to stand for 15 minutes at room temperature. The suspension was centrifuged at 300rpm, the supernatant was discarded and the precipitate was dissolved in 40 mL of 10% SDS solution. 1 mL of 0.01M ferric chloride was added and allowed to stand for 30 minutes before taken the absorbance reading at 510 nm. The STD terpenoids (alphaterpineol) concentration ranging from 0 – 5 mg/mL from the stock solution.

2.3.3. Determination of cardiac glycoside

10 mL of the extract pipetted into a 250 mL conical flask. 50 mL chloroform was added and shaken on vortex mixer for 1 hour. The mixture was filtered into 100 mL conical flask. 10 mL of pyridine and 2 mL of 29% of sodium nitroprusside were added and shaken thoroughly for 10 minutes. 3 Ml of 20% NaOH was added to develop a brownish yellow colour. Glycosides standard (Digitoxin). A concentration which range from 0 – 50 mg/mL was prepared from stock solution, the absorbance was read at 510 nm.

2.3.4. Determination of alkaloid

Five grams (5 g) of the samples were separately weighed into 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and allowed to stand for 4 min, this was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is then alkaloid which was dried and weighed.

2.3.5. Determination of saponins

Two grams (2 g) of the finely grinded plant samples were separately weighed into a 250 mL beaker and 100 mL Isobutyl alcohol or (But-2-ol) was added. Shaker was used to shake the mixture for 5 hours to ensure uniform mixing. The mixture was then filtered with Whattman No. 1 filter paper into 100 mL beaker containing 20 mL of 40% saturated solution of magnesium carbonate ($MgCO_3$). The mixture obtained was filtered to obtain a clean colourless solution. 1 mL of the colourless solution was taken into 50 mL volumetric flask with pipette, 2 mL of 5% iron(III)chloride ($FeCl_3$) solution was added and made up to the mark with distilled water. It was allowed to stand for 30 minutes for colour to develop. The absorbance was read against the blank at 380 nm.

2.4. Collection of Test Organisms

Clinical isolates of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* were obtained from Ekiti State University Teaching Hospital, Ado-Ekiti.

2.5. Confirmation of Test Organisms

The identity of the clinical isolates was confirmed with phenotypic tests that had been previously described by Barrow and Feltham (2000).

2.6. Determination of the Degree of Antibacterial Potency

The agar well diffusion technique described by Perez and Bazevque (1990) was employed. Sterile Mueller Hinton agar plates were prepared, and the fresh standardized broth culture of each test organism was inoculated. Subsequently, a sterile cork borer of 5 mm diameter was used to punch 5 wells on each of the plates. One hundred micro-liters (100 µl) of each of the varying concentrations (0.20, 0.40, 0.60 and 0.80 mg/mL) of the test extracts was dropped into four of the wells, while the remaining one well was filled with sterile distilled water to serve as the control. The plates were then left for one hour to allow the contents in the well to diffuse into the agar, followed by incubation at 37 ^{o}C for 24 hours. The diameter zones of inhibition were then measured in millimeters (mm).

2.7. Statistical Analysis

The sensory scores were subjected to the analysis of variance (ANOVA) using Microsoft Excel Package 2010 and the treatment means separated using Fishers Less Significant difference (LSD) test.

3. Results

The quantitative analysis of *O. gratissimum* indicated that alkaloids (0.28 mg/g) and saponins (0.23 mg/g) had the highest concentrations compared to *U. dioica* which had 0.24 mg/g and 0.19 mg/g respectively, and in comparison to the other phytochemicals that were quantified (Table 1). Phytates, glycosides and oxalates were also present in moderate quantities with a concentration of 0.13 mg/g, 0.12 mg/g, and 0.11% respectively. The phytochemical with the lowest concentrations was tannin with a concentration of 0.012 mg/g and 0.010 mg/g respectively (Table 1). Comparatively, the quantitative phytochemical component of *O. gratissimum* was quite higher than that of *U. dioica*.

Table 1 Comparative quantitative phytochemical components in *O. gratissimum* and *U. dioica*

Phytochemicals (mg/g)	**Plant samples**	
	O. gratissimum	***U. dioica***
Alkaloid	0.28±0.001[a]	0.24±0.002[b]
Tannin	0.012±0.003[b]	0.010±0.001[a]
Glycoside	0.12±0.003[b]	0.11±0.002[a]
Saponin	0.23±0.002[b]	0.19±0.006[a]
Phenol	0.030±0.001[b]	0.028±0.004[a]
Phytate	0.13±0.002[a]	0.12±0.001[b]
Oxalate	0.11±0.001[a]	0.09±0.002[b]

Values are mean ± SD of triplicate determination. Samples with different superscripts within the same column were significantly (p≤0.05) different

Table 2 Comparative study of antibacterial activity of aqueous leaf extracts of plant samples on selected clinical isolates

Test organisms	**Diameter of zones of inhibition (mm)**							
	Concentrations (mg/mL)							
	0.2		0.4		0.6		0.8	
	O. gratissimum	*U. dioica*	*O. gratissimum*	*U. dioica*	*O. gratissimum*	*U. dioica*	*O. gratissimum*	*U. dioica*
E. coli	15.40±0.02[a]	12.20±0.01[a]	16.40±0.03[c]	13.70±0.02[a]	18.50±0.04[d]	15.10±0.03[c]	21.40±0.02[d]	17.30±0.03[c]
K. pneumoniae	20.10±0.02[a]	9.52±0.03[c]	22.60±0.02[a]	11.23±0.04[b]	24.50±0.01[a]	14.08±0.03[c]	25.10±0.02[a]	16.15±0.04[b]
P. aeruginosa	15.40±0.03[a]	9.15±0.04[b]	18.09±0.03[c]	11.42±0.03[c]	19.74±0.02[b]	13.63±0.03[c]	21.70±0.03[c]	15.15±0.03[c]
Staph. aureus	11.70±0.03[a]	8.98±0.02[a]	12.60±0.01[a]	11.09±0.02[b]	14.15±0.03[c]	12.54±0.02[b]	13.45±0.02[b]	14.51±0.02[d]

Values are mean ± SD of triplicate determination. Samples with different superscripts within the same column were significantly (p≤0.05) different

Table 2 represents the mean zones of inhibition derived from the antimicrobial susceptibility assays. Comparatively, the aqueous extract of *O. gratissimum* had the inhibition zones against the entire clinical isolates. It had the largest zone of inhibition compared to the other plant at a much lower concentration. There were also inhibition zones from the other extracts against the test organisms at that concentration or lower. *O. gratissimum* also had the best zone of inhibition against K. pneumoniae and also exhibited inhibition zones against all the test organisms at and to Staph aureus. *U. dioica* exhibited the highest zone of inhibition against E. coli as well as other clinical isolates.

The zones of inhibitions of the ethanolic extracts of *O. gratissimum* and *U. dioica* against E. coli, K. pneumoniae, P. aeruginosa, and S. aureus have been presented in table 3. The minimum inhibitory concentrations of *O. gratissimum* were 8.35 mg/ml, 9.43 mg/ml, and 12.84 for K. pneumoniae, P. aeruginosa, and S. aureus; while the minimum inhibitory concentrations of *U. dioica* extract for K. pneumoniae, P. aeruginosa, and S. aureus were reported to be 4.5 mg/ml, 7.50 mg/ml and 8.40 mg/ml respectively. However, there were no zones of inhibition of both plants for E. coli.

Table 3 Comparative study of antibacterial activity of ethanolic leaf extracts of plant samples on selected clinical isolates

Test organisms	**Diameter of zones of inhibition (mm)**							
	Concentrations (mg/mL)							
	0.2		0.4		0.6		0.8	
	O. gratissimum	*U. dioica*	*O. gratissimum*	*U. dioica*	*O. gratissimum*	*U. dioica*	*O. gratissimum*	*U. dioica*
E. coli	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}
K. pneumoniae	8.35±0.01^{b}	4.50±0.02^{c}	10.15±0.01^{c}	6.30±0.01^{c}	12.51±0.03^{b}	9.70±0.01^{d}	15.02±0.03^{b}	11.10±0.02^{d}
P. aeruginosa	9.43±0.04^{c}	7.50±0.02^{b}	10.93±0.02^{c}	8.40±0.01^{c}	12.74±0.04^{c}	9.80±0.02^{e}	13.70±0.01^{d}	11.07±0.02^{d}
Staph. aureus	12.84±0.01^{b}	8.40±0.02^{c}	14.08±0.01^{c}	10.80±0.03^{d}	16.52±0.01^{d}	11.57±0.01^{d}	19.15±0.02^{b}	13.71±0.01^{d}

Values are mean ± SD of triplicate determination. Samples with different superscripts within the same column were significantly ($p \leq 0.05$) different

4. Discussion

The antibacterial susceptibility assays performed in this study revealed that *O. gratissimum* and *U. dioica* could exhibit different forms of susceptibility to the bacterial organisms as corroborated by the antibacterial activities of these extracts. The aqueous extracts of these plants exhibited higher degrees of activity at higher concentrations than at lower concentrations indicating that the inhibition of bacterial growth might be dose dependent. The findings of the present research were consistent with previous studies regarding the spectrum of activity of these plant extracts. Nevertheless, these findings largely disagreed with the concentrations at which they were previously reported to have exhibited these activities (Jafari et al., 2012; Okwu et al., 2019). The antibacterial activities that were observed could be due to a combination of bioactive phytochemicals (alkaloids, cardiac glycosides, steroids, tannins, flavonoids and saponins) in these extracts (Ibrahim et al., 2009).

The resistance of microorganisms to most of the conventional antibiotics has led to more adverse clinical conditions, including morbidity and mortality induced by treatment failure. Moreover, it has resulted in the rise of health care costs. Even though a number of antibiotics are still efficient (Sharma and Kumar, 2009), the rising capability of microbes to develop resistance to several drugs has motivated researchers to conduct new studies dealing with new, safe and efficient bioactive therapeutic agents of herbal origin (Sharma and Kumar, 2009).

These findings suggest that susceptibility differences might not be induced by cell wall structural distinctions between the categories of bacteria that were examined. Since the inhibition zones might not be commensurate to the efficacy of the extracts due to the variable diffusivity of the extracts in the agar medium, the minimum inhibitory concentration was computed. The scientific evaluation and dissemination of the local ethnomedical preparations and prescriptions of plant origins is highly recommended. Hence, traditional herbs might prove to be new antimicrobial sources with stable, biologically active ingredients that could make a scientific basis for the use of plants in modern medicine.

5. Conclusion

Aqueous and ethanolic extracts of the leaves of *O. gratissimum* and *U. dioica* were active against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*, and the aqueous extract of the leaf was active against *P. aeruginosa* at the investigated concentrations. This brings to fore the role of solvent type in influencing the activity of *O. gratissimum* and *U. dioica* against microbes. This study suggests the exploration of *O. gratissimum* and *U. dioica* as sources of natural products for future use in the management of bacterial infections but not against resistant strains, except at high doses that must have been pharmaceutically determined. The findings could also be of commercial interest to both pharmaceutical companies and research institutes. Furthermore, further studies are required to be conducted concerning the botanical preparation of the traditional sources of medicinal plants in various fields, including pharmacology, phytochemistry, ethnobotany and other biological activities associated with drug recovery.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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