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The antimicrobial and phytonutrient profile of *Ocimum gratissimum*

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Abstract

Many plant-derived compounds have been used as drugs, either in their original or semi-synthetic forms. Plant derived metabolites can also serve as lead compounds, which may be used as templates for the development of new drugs. Therefore, the aim of this study was to evaluate the phytochemical and antimicrobial activities of extract of *O. gratissimum* leaves on selected clinical pathogenic organisms. The plant materials were obtained through successive extractions using solvents of different polarities such as petroleum ether, ethanol and distilled water. The phytoconstituents were analyzed using standard procedures while antibacterial activities of the extracts were then evaluated by the hole-agar-well diffusion method. The minimum inhibitory concentration of the extract was determined against the isolated microorganism by agar dilution method. The presence of tannins, flavonoids, saponins, glycosides, steroids, triterpenoids and alkaloids in the different leaf extracts was established at varying concentration from the results. The ethanol extract was found to be the most potent, followed by the petroleum ether extract, while aqueous extract was the least potent. *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Candida albican* were highly susceptible to the ethanol extracts, since it showed the most susceptible higher zones of inhibition. The ability of the extracts to inhibit the growth of several bacterial and fungal species is an indication of the broad spectrum antimicrobial potential of leaf extract of *O. gratissimum*, which suggests the plant as a tool for bioprospecting for antibiotic and antifungal drugs.

Keywords: Antibacteria; Antidiarrhoeal; Antifungal; Antimalarial; Anti-Inflammatory; Analgesic Activity

1. Introduction

There is growing interest in exploiting plants for medicinal purposes especially in Africa. This stems from the fact that microorganisms are developing resistance to many drugs and as such created situation where some of the common and less expensive antimicrobial agents are losing effectiveness. Herbal medicine which uses medicinal plants primarily presents as an alternative to such situation [1]. These medicinal plants have immensely contributed to the development of human health and welfare. Concomitantly, there is an increase in data and huge patronage to herbal products round the world [2]. Medicinal plants such as *Ocimum gratissimum* have been asserted to provide various culinary and medicinal properties. These medicinal properties exert bacteriostatic and bactericidal effects on some bacteria. These effects have been attributed to the peptides, alkaloids, essential oils, phenols and flavonols which are major components in these plants [3]. *O. gratissimum* belongs to the family *Leguminosae*, commonly known as "alfavaca". Some of the vernacular names in Nigeria include: (Ncho-anwu,) Igbo, (Efinrin,) Yoruba, (Aramogbo) Edo and (Daidoya) Hausa [4]. *O. gratissimum* has been described to have other species in the flora of tropical West Africa. *O. gratissimum* is found in the tropical and warm temperature regions such as India and Nigeria [5]. These include: *Ocimum viride* Linn, *Ocimum suave* Linn, *Ocimum basilicum* Linn and *Ocimum canum* Sims. Dasofunjo *et al.* [6] reported their numerous medicinal uses

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which include: the management of upper respiratory tract infections, diarrhea, headache, conjunctivitis, skin disease, pneumonia, tooth and gum disorder, fever and as mosquito repellants.



Figure 1 Leaves and flowers of *O. gratissimum*

The *Ocimum* oil has been described to be active against several species of bacteria and fungi. These include *Listeria monocytogenes*, *Shigella*, *Salmonella* and *Proteus*, for fungi *Trichophyton rubrum*, *Trichophyton menta-grophytes*, *Cryptococcus neoformans*, *Penicillium islandicum*, and *Candida albicans* [7,8]. From recent findings, *O. gratissimum* has also proved to be useful in the medication for people living with Human Immuno deficiency Virus (HIV), and Acquired Immuno Deficiency Syndrome virus (AIDS), gonorrhoeal infection, vaginal douches for *metritis* and *vaginitis* and used in treatment of mental illness [8]. The plant exhibited various biological activities including antidiabetic, muscle relaxant, anthelmintic, antinociceptive, antihypotensive, antileishmanial, antioxidant activity and anticonvulsant, antidiarrhoeal, anti-inflammatory; analgesic activity [5]. Thus, this present study seeks to investigate the scientific basis for the traditional use of this medicinal plant locally in Nigeria, for treating ailments associated with microbial infections.

2. Material and methods

2.1. Plant Materials

Fresh leaves of *O. gratissimum* were collected from the Federal Secretariat Farms, Calabar, Cross River State, Nigeria. The leaves were taken to the University of Calabar, Department of Botany for identification and authentication. The voucher number of 201 has been deposited for future reference at the department's herbarium.

2.2. Method

2.3. Preparation of methanol extract of *O. gratissimum* leaves

The leaves of *O. gratissimum* were collected and air dried at room temperature for a period of 21 days until constant weight was obtained. The dried leaves were then pulverized to powdered form by a machine blender and sieved. Thereafter, 400g of the pulverized plant material (*O. gratissimum*) was dissolved in 1200ml of 70% methanol for 72 hours. This was followed with vacuum filtration and extracts were concentrated using an evaporator water bath at 40°C to obtain a solvent free extract, and stored in a refrigerator at 4°C.

2.4. Procedures for antimicrobial screening

2.4.1. Antimicrobial assay

The following bacteria were used in this study; *Candida albicans*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli*. These organisms were collected from the microbiology laboratory of University of Nigeria Nsukka. Cultures of these bacteria were grown in nutrient agar at 37°C and maintained on slopes of nutrient agar. Each of the organisms was transferred into a separate test-tube containing nutrient broth to reactivate them by culturing overnight at 37°C. The different prepared extracts of the leaves of *O. gratissimum* were tested for antimicrobial activity against the test organism using the agar diffusion method of [9]. The organisms were used to seed different nutrient agar plates;

one organism per plate, wells were made on the plates with a sterile cork borer of 6mm diameter to contain the different extracts and the plates were incubated at 37°C for 24 h. The zones of inhibition were measured at the end of the incubation period

2.4.2. Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the compound to inhibit the growth of microorganisms.

To the prepared nutrient agar plates containing specific bacteria on which different wells have been made, different dilutions of the extracts of *O. gratissimum* were introduced. The plates were incubated at 37°C for 24 h. Zones of inhibition were measured at the end of the incubation period. The MIC was taken to be the lowest dilution inhibiting the growth of the organism.

2.4.3. Minimum bactericidal concentration (MBC)

MBCs were determined by first selecting tubes that showed no growth during MIC determination, a loopful from each tube was subcultured onto extract free agar plates, incubated for further 24 h at 37°C. The least concentration, at which no growth was observed, was noted as the MBC [10].

2.5. Statistical analysis

Data obtained were analyzed using One Way Analysis of Variance (ANOVA) followed by post hoc test at $P < 0.05$. The Statistical Package for Social Science (SPSS) Software version 20.0 was used for the analysis

3. Results

The results of phytochemical screening of *O. gratissimum* indicates the presence of flavonoids, tannins, phlobatannins, triterpenoids, saponins, steroids glycosides and alkaloids at varying degree of concentrations. Ethanol extract appeared to have higher concentrations of phytochemicals than aqueous and petroleum ether extracts (Table 1).

The result from table 2 below depicts the antimicrobial potential of aqueous, petroleum ether and the ethanol extract of *O. gratissimum* leaves. Though, the effect of the extract was not significantly ($P < 0.05$) different especially for aqueous and petroleum ether extracts when compared individually.

Zones of inhibitions were wider at 100mg/ml with ethanol extract than aqueous and petroleum ether extracts. This shows that ethanol extract inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Candida albicans* with zones of inhibition 8.89 ± 01 , 7.28 ± 01 , 10.10 ± 04 , 10.10 ± 04 respectively. Comparatively, all the extracts were less effective when compare to the standards but ethanol extracts of *O. gratissimum* were more effective than the aqueous and petroleum ether extracts due to wider zone of inhibition (Table 2).

Table 1 The qualitative phytochemical composition of the aqueous, petroleum ether and ethanol extracts of *O. gratissimum* leaf

Phytochemicals	Aqueous Extract	Petroleum ether Extracts	Ethanol Extract
Flavonoids	++	+	+++
Tannins	+	++	++
Steroids	++	++	+++
Phlobatannins	++	++	+++
Saponins	++	+	+++
Triterpenoids	++	++++	+++
Glycosides	++	+++	+++
Alkaloids	+	++	+++

Where: + = sparingly present; ++ = moderately present; +++ = highly present; and ++++ = very highly present.

Table 2 Zones of Microbial inhibitions by aqueous, petroleum ether and ethanol extracts of *O. gratissimum* leaf at 100mg/ml.

Materials	A.e	P.E.e	E.e.	Std
Microorganisms	Inhibition Zone (mm)			
<i>S. aureus</i>	2.21±012	2.91±021	8.89±011	13.78± 021 *
<i>E. coli</i>	1.14±032	1.59±021	7.28±013	12.22±041 ***
<i>K. pneumonia</i>	0.1±021	1.32±030	10.10±041	11.22±021 **
<i>C. albicans</i>	0.00	2.23±012	6.50±011	18.0±013

A.e: Aqueous extract, P.E.e: Petroleum ether extract, E.e: Ethanol extract, Std: Standard, S: *Staphylococcus*, E: *Escherichia*, K: *Klebsiella*, C: *Candida*, *: Nystatin, **: Gentamicin, ***: Ampicillin.

4. Discussion

O. gratissimum is a plant of great medicinal importance and it is also valued high in the field of ethnomedicine. The therapeutic values of medicinal plants lie in the various chemical/phyto constituents present in them. For instance, plants rich in tannins have antibacterial potentials due to their basic character that allow them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane [11]. Flavonoids are a major group of phenolic compounds reported for their antiviral, antimicrobial and spasmolytic properties [12,13,14]. Alkaloids isolated from plants are commonly found to have antimicrobial properties [15].

The qualitative difference of phytochemical analysis observed in plant extracts may be attributed to different solvents used for extraction. This observation is in line with the findings of [16] that reported different solvents have different spectrum of solubility for the phytoconstituents. In all the three extracts, tannins were present resulting in the inhibition of cell protein synthesis as it forms irreversible complexes with proline rich proteins [17]. Tannin containing herbs have been reported in treating intestinal disorders such as diarrhoea and dysentery, [18], treatment of inflamed or ulcerated tissues. Parekh, [19] reported that the extract of the seeds of *O. gratissimum* possess antimicrobial activity which was associated with its alkaloids, saponins, tannins, flavonoids, and glycosides contents [20]. Various researchers have already shown that gram-positive bacteria are more susceptible towards plants extracts as compared to gram negative. These differences may be attributed to the fact that the cell wall in gram-positive bacteria is of a single layer, whereas the gram negative cell wall has a multi layered structure.

The antibacterial activity of the leaf extracts of *O. gratissimum* as recorded in this present study may therefore be attributed to the presence of the above mentioned phytochemicals *i.e.* flavonoids, terpenoids, steroids, tannins alkaloids, phlobatannins among others.

It can therefore be inferred that the plant extracts possess both antifungal and antibacterial activity against tested organisms. The zone of inhibition varied with solvent used in extraction suggesting the varying degree of efficacy and different phytoconstituents of herb on the target organisms. The antifungal and antibacterial activity of the plants may be due to the presence of various active principles in their leaves.

5. Conclusion

The inhibitory effect of the extract of *O. gratissimum* against pathogenic bacteria and fungi strains can introduce the plant as a potential tool for drug development for the treatment of ailments caused by human pathogens. The ability of the extracts to inhibit the growth of several bacterial and fungal species is an indication of the broad spectrum antimicrobial potential of leaf extract of *O. gratissimum*, which makes the plant a tool for bioprospecting as antibiotic and antifungal drugs. These findings tend to lend credence to the traditional use of *O. gratissimum* for the treatment of microbial infections.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have not declared any conflict of interests.

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