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RESEARCH ARTICLE

# PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL EFFICACY OF OCIMUM GRATISSIMUM EXTRACTS ON MULTI-DRUG RESISTANT BACTERIA

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**Abstract:** The continuous indiscriminate use of antibiotics in treating animals and human diseases has led to an increase in the ability of bacteria to resist the effects of various antibiotics in use, and this has led to the study and screening of plants for their ability to treat various diseases. In this study, the bioactive components and the antibacterial efficacy of *Ocimum gratissimum* ethanolic and methanolic leaf extracts against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli S. marcescens* and *B. cereus* were determined using the Analytical Methods Committee's standard method and agar well diffusion method respectively. The phytochemical components, antibacterial activity, MIC, and MBC of *O. gratissimum* were determined against these isolates. The qualitative phytochemical analysis of the extracts revealed the presence of saponins, alkaloids, phenols, tannins, steroids, flavonoids, anthraquinones, terpenoids, glycoside and phlobatannins in both or either of the extracts. The result revealed ethanolic extract to be more potent than methanolic extract by producing inhibition ranges of 15-35mm, 8-27mm, 7-26mm, 9-27mm, 5-24mmm, 13-32mm (ethanol) and 12-33mm, 11-31mm, 8-23mm, 12-25mm, 8-26mm, 6-26mm (methanol) *against S. aureus, K. pneumoniae, P. aeruginosa, E. coli, S. marcescens* and *B. cereus* at 12.5-200mg/ml respectively. The MIC and MBC ranged between 12.5 - 50mg/ml and 50-100mg/ml for both extracts respectively. This study revealed that *O. gratissimum* possesses antibacterial efficacy on the selected isolates even at low concentrations; thereby its use is recommended for the treatment of various diseases caused by these organisms.

**KEYWORDS:** antibacterial efficacy, MIC, MBC, phytochemicals, resistance.

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## INTRODUCTION:

The daily increase in resistance of bacteria to conventional antibiotics has posed a serious challenge to researchers, pharmaceutical companies, and health practitioners worldwide. These resistant bacteria are usually transferred to humans from animals through the ingestion of contaminated food, leading to the exchange of resistant genes between these pathogenic strains and body microbiota in the host. Antibiotic resistance in bacteria often results from target site modification, overproduction of the target sites, decrease in cell membrane permeability, natural selection, and efflux pump of the antibiotics. This resistance ensures their survival in the presence of these bactericidal and bacteriostatic substances in low, mid, and high concentrations; antibacterial. Hence, the need and inspiration to search for new antimicrobial agents from other sources like plants to overcome the problem of resistance and improve the quality of life. For years, new antibiotics have not been developed despite the large number of bacteria that have become resistant to almost all antibiotics in use for treating various bacterial infections in animal husbandry and humans. In Africa, herbs have been in use for the treatment of ailments such as cough, fever, diarrhea, and sore throat for ages.[1] It was reported that *Vernonia amygdalina*, *Carica papaya* and *Carthamus tinctorius* possess antimicrobial efficacy against multi-drug resistant bacteria and fungi. [2-4] It is therefore worthy to study the antibacterial efficacy of *Ocimum gratissimum* on some multi-drug resistant bacteria that are becoming resistant to various antibiotics and posing threats to an individual's health. The increase in diseases caused by multi-drug resistant bacteria and the speed at which antibiotic-resistant genes are spreading in the environment, demand conservative efforts in searching for alternatives that are effective, available, affordable, and non-toxigenic to humans and animals.

*Ocimum gratissimum* is a perennial plant that is widely distributed in Africa and Asia. It belongs to the family Lamiaceae with 1-3m in height, dark brown stem, narrow, small, and oval-shaped leaves. In Nigeria, it is called "scent leaf" due to the fascinating aroma it produces that encourages its use as a spice in food[5] and when consumed, it serves as medicine for treating diseases in humans and animals.[6] This plant has been reported to possess antibacterial and antidiarrheal properties, antifungal, antidiabetic, treat blocked nostrils, regulate menstruation, ear infections and cough and remedy for constipation.[7-11] The essential oil contained in the leaves and flowers of *O. gratissimum* has been used in the preparation of tea, perfume, and infusion by Nigerians.[12] The whole plant is also used by Indians to treat headache, inflammation and pyretic.[13] It is also used by Nigerians to treat epilepsy, high fever, and diarrhea.[14] This study is conducted to evaluate the phytochemicals in *O. gratissimum* leaves and its antibacterial efficacy against some multi-drug resistant bacteria.

## MATERIALS AND METHODS:

### Collection of Plant Materials

Fresh leaves of the *O. gratissimum* were collected from the environment. Identification and authentication of the leaves were carried out at the herbarium section of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

### Preparation of Crude Extracts

The leaves were dried in an open space and ground into powder form. Fifty grams (50g) of the ground leaf material was soaked in 250ml of ethanol and methanol for 48 hours separately. The mixtures were sieved to remove debris. The filtrates were then filtered through Whatman No. 1 filter paper and opened to air for the solvent to evaporate to get the crude extract [15]. The crude methanol and ethanol extracts were stored at 4°C until needed.

### Preparation of Concentration of Plant Extract

Two grams (2g) each of ethanol and methanol extracts were added to the corresponding 10ml ethanol and

methanol separately to give an equivalent concentration of 200mg/ml while other lower concentrations (100, 50, 25 and 12.5mg/ml) were obtained by double fold dilution method of previous concentration.[15]

**Collection and Maintenance of the Test Organisms**

Pure cultures of two Gram-positive bacterial isolates (*Staphylococcus aureus and Bacillus cereus*) and four Gram-negative bacterial isolates (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Serratia marcescens)* were obtained from the Department of Microbiology, University of Ilorin and were re-identified by biochemical tests. The cultures were stocked on nutrient agar slants before they were sub-cultured onto freshly prepared nutrient agar plates for antimicrobial analysis.

**Qualitative Phytochemical Analysis**

The presence of bioactive agents such as saponins, alkaloids, phenols, tannins, steroids, flavonoids, anthraquinones, terpenoids, glycoside and phlobatannins in the extracts was determined according to the standard methods for the analysis of phytochemicals by Analytical Methods Committee of Royal Society of Chemistry.[16]

**Antibacterial Activity of *O. gratissimum* Extracts on Multi-drug Resistant Bacteria**

The antibacterial activity of ethanol and methanol extracts was determined by the agar well diffusion method. A flamed inoculating loop was used to pick a distinct colony from 24-hour old culture on nutrient agar into 10ml of sterile 0.85% normal saline in the test tube with gentle shaking to make the culture homogenized. The saline turbidity is then compared with 0.5 McFarland turbidity until it matches to obtain $10^7$CFU/ml. 0.1ml of the standardized aliquot was inoculated onto solidified Mueller Hinton agar, spread evenly by swap stick, and allowed to dry. A 4mm cork-borer was used to make two wells on the plate. 0.1ml of the extract was measured into these wells. The antibacterial activity of the extracts' concentrations against each isolate was determined by measuring the zone of inhibition around the well with meter rule in millimeters after 24 hours of incubation at $37^0$C. [17]

**Antibiotics Susceptibility Testing of the Test Organisms**

A swab stick moistened with standardized inoculum was used to spread the surface of solidified Mueller Hinton agar in a way that confluence growth can be obtained after incubation. A sterile forceps was used to place antibiotic discs on the surface of the dry inoculated plate. The diameter of inhibition around each disc was measured in millimeters after incubation at 37°C for 24 hours and interpreted as resistant, intermediate, or susceptible.[18]

**Determination of Minimum Inhibitory Concentration (MIC)**

Broth dilution method was used to determine the minimum inhibitory concentration (MIC) of ethanolic and methanolic extracts against each of the test isolates at varying concentrations of 200, 100, 50, 25 and 12.5mg/ml. 1ml of the extract was introduced into 10ml nutrient broth inoculated with 0.1ml of the standardized organism and the control tube was made the same way without the test organism. The tube with the lowest concentration of no growth or having the same turbidity as the control tube after incubation at 37°C for 24 hours was recorded as the MIC of that bacteria.[19]

**Determination of Minimum Bactericidal Concentration (MBC)**

MBC of the extracts was determined by sub-culturing from tubes having the same turbidity as the control tube from the MIC test. 0.1ml of the 24-hour culture was inoculated onto solidified nutrient agar plates spread by a sterile glass spreader and incubated at 37°C for 24 hours. The minimum concentration at which there was no bacterial growth on the plates was recorded as the MBC for the isolate.[19]

**Statistical analysis**

The results were expressed as mean ± SE of duplicate samples. Statistical analysis was performed by one-way ANOVA of SPSS version 20. The obtained results were considered statistically significant at $p \leq 0.05$.

## RESULTS:

**Effects of Extraction Solvent on Qualitative Phytochemical Constituents of *O. gratissimum* Leaves**

The qualitative analysis of *O. gratissimum* ethanolic leaf extract revealed the presence of saponins, alkaloids, phenols, tannins, steroids, flavonoids, terpenoids, glycoside and phlobatannins while the methanolic extract revealed the presence of saponins, alkaloids, phenols, tannins, steroids, flavonoids, anthraquinones and glycoside (**Table 1**).

**Table 1: Bioactive components of *O. gratissimum*.**

| Phytochemicals | Ethanol extract | Methanol extract |
|---|---|---|
| Alkaloids | + | + |
| Phenols | + | + |
| Saponins | + | + |
| Steroids | + | + |
| Flavonoids | + | + |
| Terpenoids | + | - |
| Glycoside | + | + |
| Phlobatannins | + | - |
| Tannins | + | + |
| Anthraquinones | - | + |

+ = present - = absent

**Antimicrobial Efficacy of *O. gratissimum* Ethanolic Extract against Test Bacterial Isolates**

The result of the *O. gratissimum* ethanolic leaf extract against *S. aureus, K. pneumoniae, P. aeruginosa, E. coli, S. marcescens* and *B. cereus* is shown in Table 2. All the isolates were susceptible to the extracts even at low concentrations without being resistant to any of the isolates. Ethanol extract produced an inhibition range of 15-35mm, 8-27mm, 7-26mm, 9-27mm, 5-24mm and 13-32mm at 12.5 to 200mg/ml *against S. aureus, K. pneumoniae, P. aeruginosa, E. coli, S. marcescens* and *B. cereus* respectively (**Table 2**).

**Table 2: Antibacterial Activity of *O. gratissimum* Ethanolic Leaf Extract against Test Bacterial Isolates**

| | Zones of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| Test isolates | 200mg/ml | 100mg/ml | 50mg/ml | 25mg/ml | 12.5mg/ml |
| *S. aureus* | 35±0.5 | 29±0.5 | 25±1.0 | 19±1.0 | 15±0.0 |
| *K. pneumoniae* | 27±1.1 | 21±1.0 | 18±0.0 | 13±0.0 | 8±0.4 |
| *P. aeruginosa* | 26±0.7 | 22±1.0 | 17±0.5 | 10±0.0 | 7±0.1 |
| *E. coli* | 27±0.0 | 22±0.0 | 18±0.5 | 15±1.1 | 9±0.0 |
| *S. marcescens* | 24±0.0 | 20±0.4 | 14±1.2 | 9±0.0 | 5±0.1 |
| *B. cereus* | 32±0.0 | 27±0.0 | 24±1.2 | 18±0.0 | 13 |

**Antimicrobial Efficacy of *O. gratissimum* Methanolic Extract against Test Bacterial Isolates**

Methanolic extract exerted inhibition in the range of 12-33mm, 11-31mm, 8-23mm, 12-25mm, 8-26mm and 6-26mm at 12.5 to 200mg/ml *against S. aureus, K. pneumoniae, P. aeruginosa, E. coli, S. marcescens and B. cereus* respectively (**Table 3**).

**Table 3: Antibacterial Activity of *O. gratissimum* Methanolic Leaf Extract against Test Bacterial Isolates**

| | Zone of inhibition | | | | |
|---|---|---|---|---|---|
| Test isolates | 200mg/ml | 100mg/ml | 50mg/ml | 25mg/ml | 12.5mg/ml |
| *S. aureus* | 33±1.1 | 30±1.0 | 25±0.0 | 20±0.0 | 12±0.5 |
| *K. pneumoniae* | 31±0.0 | 24±1.0 | 20±0.0 | 14±0.4 | 11±0.1 |
| *P. aeruginosa* | 23±1.2 | 19±1.0 | 16±0.0 | 12±0.5 | 8±0.0 |
| *E. coli* | 25±1.0 | 20±0.0 | 17±0.5 | 15±0.0 | 12±0.1 |
| *S. marcescens* | 26±1.0 | 22±1.0 | 17±0.0 | 13±1.0 | 8±0.0 |
| *B. cereus* | 26±0.0 | 23±0.5 | 14±1.0 | 10±0.0 | 6±0.0 |

**Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Ethanolic and Methanolic *O. gratissimum* Leaf Extracts**

| | MIC (mg/ml) | | MBC (mg/ml) | |
|---|---|---|---|---|
| Test isolates | Ethanol | Methanol | Ethanol | Methanol |
| *S. aureus* | 12.5 | 25 | 100 | 100 |
| *K. pneumoniae* | 25 | 50 | 100 | 100 |
| *P. aeruginosa* | 25 | 50 | 100 | 100 |
| *E. coli* | 25 | 12.5 | 50 | 50 |
| *S. marcescens* | 12.5 | 25 | 50 | 100 |
| *B. cereus* | 50 | 25 | 100 | 100 |

**Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *O. gratissimum* extracts**

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC) of *O. gratissimum* extracts on multi-antibiotic resistant *S. aureus, K. pneumoniae, P. aeruginosa, E. coli, S. marcescens* and *B. cereus* are presented in **Table 4**. The MIC of the ethanolic extracts were 12.5, 25, 25, 25, 12.5 and 50mg/ml on *S. aureus, K. pneumoniae, P. aeruginosa, E. coli, S. marcescens* and *B. cereus* respectively while methanol exerted MIC of 25, 50, 50, 12.5, 25 and 25mg/ml on *S. aureus, K. pneumoniae, P. aeruginosa, E. coli, S. marcescens* and *B. cereus* respectively. The MBC of the isolates on ethanolic extract was 100mg/ml for *S. aureus, K. pneumoniae, P. aeruginosa* and *B. cereus*, and 50mg/ml for *E. coli* and *S. marcescens* while the MBC on methanolic extract was 50mg/ml against *E. coli*, 100mg/ml against *S. aureus, K. pneumoniae, P. aeruginosa*, *B. cereus* and *S. marcescens*.

## DISCUSSION:

The rate at which pathogens are becoming resistant to more antimicrobial agents and the diseases caused by multi-drug resistant bacteria cannot be exaggerated. Thereby, the need to explore nature for alternatives, either for direct usage or synthesized from nature. Plants are well known as the major reservoir of chemical agents used by pharmaceutical industries to produce drugs used for the treatment of various diseases.[20] Also, several studies have been conducted on the therapeutic potential of various plant metabolites on disease-causing pathogens. The problem of antibiotic resistance is increasing and actions such as control measures in the use of antibiotics to treat diseases and improve livestock production should be promulgated as a high percentage of human disease-causing pathogens are gotten from animals.

In this study, we evaluated the phytochemical components of both methanol and ethanol extracts of scent leaf, the antibacterial activity of *O. gratissimum* extracts against multi-drug resistant bacteria and determined the least concentrations of both extracts required to kill and inhibit the bacterial growth. The phytochemical analysis revealed the absence of

anthraquinones in ethanolic extract, and terpenoids and phlobatannins in methanolic extract. These phytochemicals present in *O. gratissimum* leaves can be attributed to the plant's antibacterial activity.[21] Thereby, the reason for being used in the locality for treatment of many bacterial diseases. The finding on the presence of these bioactive agents in *O. gratissimum* ethanolic extract agrees with previous studies on the plant extract. [22, 23] These bioactive components have been reported by researchers to possess beneficial purposes to humans such as antitumor, anti-diuretic and treat gastrointestinal disorders. [24, 25]

The antibacterial analysis of the *O. gratissimum* extracts revealed that the extracts possess antibacterial efficacy against *S. aureus, K. pneumoniae, P. aeruginosa, E. coli, S. marcescens* and *B. cereus* by production of inhibition to their growth in in-vitro analysis. The extracts were found to have the highest inhibition on *S. aureus.* Despite the efficiency, the antibacterial activity of the extracts decreased with decrease in the concentration of the extracts which agrees with the previous studies on *O. gratissimum.* [22, 23] *O. gratissimum* extracts have also been reported to possess antibacterial ability against *E. coli, S. aureus* and *K. pneumonia*, *E. coli, Salmonella typhi* and *Yersinia enterolitica*. [26, 27] The extracts showed varied potential against the test isolates with ethanol being more efficient on Gram-positive than Gram-negative bacteria. The susceptibility of these isolates to these extracts shows the reason for their use for the treatment of gastrointestinal infections, cough, and sore throat. Various literature and the results of this research work have shown the therapeutic potential of this plant in treating diseases. It is interesting to reveal that even at low concentration of 12.5mg/ml, *O. gratissimum* extracts were effective against *B. cereus;* a known spore-forming bacterium and recalcitrant to harsh environmental conditions. Also, the extracts showed bacteriostatic and bactericidal ability against *S. aureus, K. pneumoniae, P. aeruginosa* and *E. coli* that have become multi-drug resistant. This study showed Gram-positive bacteria to be more susceptible to the extracts than Gram-negative bacteria. Past studies also arrived at the same conclusion about Gram-negative bacteria being more resistant to plant extracts and antibiotics than Gram-positive bacteria.[28] Plants exhibit antimicrobial activity on pathogens through mechanisms such as membrane disruption, inhibition of nucleic acids, proteins, and phospholipid synthesis.[29]

The MIC and MBC for both extracts ranged between 12.5-50mg/ml and 50-100mg/ml respectively. The possession of low MIC and MBC values by *O. gratissimum* extracts against these isolates was also revealed in a similar pattern by researchers. [21, 22, 26] These possession of high inhibition zones at low concentration, low MIC, and low MBC values by *O. gratissimum* extracts in this study against these pathogens show its medicinal ability in the treatment of diseases caused by the pathogens. This study therefore revealed the extracts of *O. gratissimum* to possess useful phytochemicals, low MIC, low MBC, and antibacterial ability on the selected infectious pathogens.

## CONCLUSION:

The results of this study revealed that *O. gratissimum* extracts possess bioactive substances that are responsible for its antibacterial activity, showed high antibacterial activity against the multi-drug resistant bacteria used in this study and could be used to treat various infections caused by these pathogens in animal husbandry and humans.

## RECOMMENDATION:

The extracts of *O. gratissimum* leaves are recommended for use in treating infections caused by *S. aureus, P. aeruginosa, E. coli, K. pneumoniae, B. cereus* and *S. marcescens*.

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