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Preliminary Phytochemical, Physicochemical, and Comparative Antibacterial Evaluation of Methanolic Extracts of *Ocimum gratissimum* Stem against Methicillin-Resistant *Staphylococcus aureus* and *Proteus vulgaris*

Lawrence Uchenna Nwankwo[a*], Efemena Curtbeth Obokare[a]

a. Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmacy, Delta State University, Abraka, Delta State, Nigeria.

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* **Corresponding Author:**
Lawrence Uchenna Nwankwo, Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmacy, Delta State University, Abraka, Delta State, Nigeria.
Email: lunwankwo@delsu.edu.ng

Abstract:

Herbal medicine, as defined by WHO, is finished labeled medicinal products that contain active ingredients of plants, whether in the crude state or as plant preparation. *Ocimum gratissimum* has been traditionally used to alleviate symptoms of illness such as stomach disorders, fever, and cough. This study aimed to evaluate the phytochemical, physiochemical, and comparative antibacterial activity of the methanol and n-hexane extracts of *Ocimum gratissimum* stems against Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Proteus vulgaris*. Phytochemical screening was conducted using standard methods. Physicochemical analysis was carried out, including moisture content, extractive values, and ash values. Biochemical tests such as catalase, coagulase, indole, and fermentation were carried out to identify the organisms. The minimum inhibitory concentration (MIC) and the susceptibility of the organisms to the various concentrations of the two extracts were determined using the agar well diffusion technique. Phytochemical screening results revealed the presence of phytoconstituents such as alkaloids, saponin, tannins, cardiac glycosides, and terpenoids. The physicochemical evaluation results confirm the quality of the crude drugs as the results obtained fall within standard ranges for the different parameters used for crude drug standardization. Susceptibility test results revealed the zones of inhibition of methanol extracts of *Ocimum gratissimum* stem against MRSA according to the various concentrations as (19.20, 17.33, 14.66, 14.33, 13.6, and 12.33) with MIC at 25 mg/ml while against *P. vulgaris* as (16.6, 16.6, 14.30, 13.6, 13 and 12.6) with MIC at 12.5 mg/ml. This study has clearly shown that the methanol extract of the stem of *Ocimum gratissimum* has a moderate degree of inhibition on both MRSA and *P. vulgaris.* However, this crude drug is seen to be slightly more effective against MRSA in comparison to its antibacterial activity against *P. vulgaris.* Further investigations should be conducted to develop newer and more effective drugs for *in vivo* and *in vitro* use.

Keywords: Bacteria, Inhibition, Physicochemical, Standardization, Susceptibility, Phytoconstituents.

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1. Introduction

Plants are mainly multicellular organisms, predominantly photosynthetic eukaryotes of the kingdom Plantae. Plants have many cultural and other uses, such as ornaments, building materials, and writing materials, and in great variety, have been the source of medicines and psychoactive drugs [1, 2]. The scientific study of

Medicinal plants, also called medicinal herbs, has been discovered and used in traditional medicine since prehistoric times. Plants synthesize hundreds of chemical compounds for defense against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain.

Further, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety [3]. *Ocimum gratissimum* L. belongs to the Labiateae family, also known as clove basil, African basil [4], and in Hawaii, wild basil [3] is a species of Ocimum. *Proteus vulgaris* is a gram-negative bacteria reported as a major causative agent of wound infections, burn infections, urinary tract infections, bloodstream infections, and respiratory tract infections [5]. On the other hand, Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a gram-positive *staphylococcus aureus* usually resistant to most antibiotics used in treating ordinary staph infections. Different extracts from *Ocimum gratissimum* leaves show antibacterial activity when tested against *Staphylococcus aureus*, *Salmonella typhi*, and *Salmonella typhimurium*, pathogenic bacteria that cause diarrhea. Extract included cold water extract, hot water extract, and steam distillation extract. Only steam distillation extract and the minimum inhibitory concentration inhibit the selected bacteria ranged from 0.1% for S. aureus to 0.01% for *E. coli* and *S. typhimurium* and 0.001% for *S. typhi* [6]. *Ocimum gratissimum*, ethanolic extract was tested for antimicrobial activity against *Actinobacillus actinomycetemcomitans* in human dental plaque and compared with 0.2% chlorhexidine as the positive control and dimethyl sulfoxide (DMSO) as the negative control. Maximum antimicrobial potential was at 0.6% concentration level [7]. Antimicrobial activity was carried out against *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, and *Porphyromonas gingivalis* and found that 0.5 and 1.0 % extract showed maximum zone of inhibition. Doxycycline was taken as the positive control and DMSO as the negative control [8]. The objectives of this study encompass the determination of the phytochemical constituents of the stem extracts of *Ocimum gratissimum*, analyzing the physiochemical properties of the stem extracts of *Ocimum gratissimum*, determining the antibacterial activity of the stem extracts of *Ocimum gratissimum* against MRSA and *Proteus vulgaris* as well as comparing the efficacy of the methanol extracts of the stem of *Ocimum gratissimum* and deducing the microorganism more susceptible to *Ocimum gratissimum* stem extract.

2. Materials and Methods

2.1. Chemicals and Reagents

Ferric chloride solution (M/S.Bhumi Chem, India), Glacial acetic acid (Jiangsu Sopo, China) Fehling's solution A and B, Concentrated and diluted Hydrochloric acid (HCl) (Ideal Chemicals, India), Sodium hydroxide (NaOH) (CJ Chemicals, U.S.A), Tetraoxosulphate (VI) acid (H2SO4) (Ideal Chemicals, India), Hydrogen peroxide(H2O2) (Univar Solutions, U.S.A), Ammonia solution (Yara, Texas), Meyer's reagent, Dragendoff's reagent, Wagner's reagent, Methanol (Sigma Aldrich), Chloroform (Sigma Aldrich), N-hexane (Sigma Aldrich), Distilled water. MRSA, Proteus vulgaris.

2.2. Plant Collection

Leaves of *Ocimum gratissimum* were collected from the botanical garden of the Faculty of Pharmacy, Delta State University, Abraka, Delta State, Nigeria. The stems were carefully removed from the leaves and subjected to drying at room temperature. After drying, the stems were pulverized and stored in a cool, dry place until it is needed for extraction.

2.3. Extraction of plant material

The pulverized plant material was subjected to extraction via maceration. A total of 200 g of the plant material was extracted by cold maceration with n-hexane for 72 hours. Another 200 g of the plant material was extracted by cold maceration using 80% methanol. The total methanol and n-hexane extracts obtained after filtration were concentrated to dryness under vacuum at 40-60 degrees centigrade using a rotary evaporator. The concentrated extracts were

weighed, kept in a glass container, and stored in a refrigerator.

2.4. Qualitative Phytochemical Screening

Phytochemical screening was carried out to use standard methods to determine the presence of phytoconstituents such as alkaloid, saponin, tannin, cardiac glycosides, terpenoid, and anthraquinonoid glycosides [9].

2.5. Physicochemical Evaluation

The following physicochemical parameters were used to evaluate the qualities of the crude drug (*Ocimum gratissimum* stem extract). They include moisture content, total ash value, Acid insoluble ash value, water-soluble ash value, alcohol-soluble extractive value, and water-soluble extractive value [10].

2.6. Determination of Moisture Content

About 2 g of the powdered sample was weighed into a dried and weighed porcelain dish. The porcelain dish containing the sample was placed inside the hot air oven at 105°C for 15 minutes. The sample was removed from the hot air oven and placed in a desiccator to cool for 15 minutes. After cooling, the weight of the dried extract was obtained. The process was repeated until a constant weight was obtained, and then the moisture content was calculated in percentage.

2.7. Determination of Extractive Value

2.7.1. Water Soluble Extractive Value

About four grams of the crude plant sample was weighed and placed into a conical flask. Then, 100 ml of water was added to the conical flask, allowed to sit for 24 hours, and filtered. Then, 25 ml of the extract was added to a porcelain dish and concentrated to dryness in a hot air oven. After completely drying, the porcelain dish was placed in a desiccator, allowed to cool, and then weighed, and the extractive value was calculated in percentage.

2.7.2. Alcohol Soluble Extractive Value

About 4 g of the crude plant sample was weighed and placed into a conical flask. Then, 100 ml of Ethanol was added to the conical flask, allowed to sit for 24 hours, and filtered. Then, 25 ml of the extract was added to a porcelain dish and concentrated to dryness in a hot air oven. After completely drying, the porcelain dish was placed in a desiccator, allowed to cool, and then weighed, and the extractive value was calculated in percentage.

2.7.3. Hexane Soluble Extractive Value

About 4g of the crude plant sample was weighed and placed into a conical flask. Then, 100 ml of n-Hexane was added to the conical flask, allowed to sit for 24 hours, and filtered. Then, 25ml of the extract was added to a porcelain dish and concentrated to dryness in a hot air oven. After completely drying, the porcelain dish was placed in a desiccator, allowed to cool, and then weighed, and the extractive value was calculated in percentage.

2.7.4. Determination of Total Ash Value

An empty crucible was placed in a hot air oven at 105°C for 20 minutes to remove any trace of moisture and then placed in a desiccator to cool. The crucible was weighed, and about 4 g of the plant sample was placed into the crucible and then covered with a lid. The crucible was placed in the muffle furnace at 700°C for 2 hours. After 2 hours, the crucible was removed from the muffle furnace using stainless steel locking thongs and placed in a desiccator to cool. The crucible was weighed again, and the ash value was calculated in percentage.

2.7.5. Determination of Acid Insoluble Ash

HCl (40%) was prepared by adding 60 ml of water to 40 ml of HCl. The 40% HCl was then stored in a glass bottle until needed. The crucible was placed on a weighing balance, and 2 g of the powdered sample was measured correctly into the crucible. The crucible was covered with the lid and placed in a muffle furnace. The powdered sample was burned at 550 degrees centigrade for 30 minutes. After 30 minutes, the crucible was ejected from the furnace, the lid was removed, and the ash was observed; a few drops of distilled water were added to the ash content in the crucible to check for any possible development of black color which usually indicates that the ash still has much carbon. Upon adding the distilled water, a black color was observed, prompting the need to burn again. Before placing the crucible in a furnace to burn again, the crucible was placed on a hot plate to dry the moist ash completely. The crucible containing the dried ash was placed back into the furnace for another 40 minutes, after which it was observed that it was carbon-

free after the addition of distilled water failed to produce any black color.

After confirming the carbon-free ash, 25 ml of the 40% HCl solution was measured and emptied into the ash crucible. The ash content in the HCl solution was boiled for 5 minutes by placing the crucible on the hot plate. After boiling, an ashless filter system was prepared; the ash solution was filtered while still warm. After filtration, the residues trapped on the filter paper are counted as the acid insoluble ash. The residue on the filter is washed off with hot water to ensure no trace of the acid is left within the filter paper.

A crucible was placed in a hot air oven for 30 minutes to remove any trace of moisture. After drying, it was cooled in a desiccator for 10 minutes. The weight of the crucible was noted, and then the filter paper containing the acid-insoluble ash was placed inside the crucible, covered with the lid, and pushed into the furnace set at a temperature of 550 degrees centigrade for 90 minutes. The crucible was taken out of the furnace after 90 minutes and allowed to cool. The crucible containing the acid-insoluble ash was weighed, and the percentage of acid-insoluble ash was calculated.

2.7.6. Determination of Water-Soluble Ash

The crucible was dried in a hot air oven. After cooling, the empty crucible and lid were weighed, and the weight was noted correctly. Two grams of the powdered sample was measured into the crucible, and then the weight of the lid and the crude drug was also noted. At this point, the crucible containing the crude drug was placed in the muffle furnace until it became red hot, then switched off, and the crucible containing the ash was removed and kept in the desiccator to cool for 20 minutes. The weight of the crucible, lid, and ash was noted, and 25 ml of distilled water was added to dissolve the ash. It was later poured into the beaker and placed in a heating mantle for 15 minutes. It was subjected to filtration using ashless filter paper; after filtration, the filter paper retained the water-insoluble ash while the filtrate contained the water-soluble ash. The insoluble ash is retrieved, placed in a crucible, and kept in an incinerator until red hot, and it is carefully ejected from the incinerator and placed in a desiccator to allow cooling. The water-soluble ash is the difference between the water-insoluble ash and the total ash. Finally, the percentage of water-soluble ash was calculated accordingly.

2.8. Biochemical Tests

The following biochemical tests confirmed the identity of Methicillin-resistant *Staphylococcus aureus* and *Proteus vulgaris*. The tests include catalase, coagulase, indole, and fermentation test.

2.9. Evaluation of the Anti-Bacterial Activity of Extracts of Ocimum Gratissimum Stem

Nutrient broth cultures of MRSA and *Proteus vulgaris* were prepared using standard procedures. The microorganisms were obtained from the Pharmaceutical Microbiology Department, Faculty of Pharmacy, Delta State University, Abraka. Overnight broth and Mueller-Hinton agar were also prepared.

2.10. Susceptibility Test

The organisms used were methylene-resistant *Staphylococcus aureus* (MRSA) and *Proteus vulgaris* (MRPV). The surface of the solidified agar was swapped with the various organisms., Holes were bored on the surface of the agar according to the different concentrations of each plant extract using a 6 mm cork borer. A few drops of each plant extract were dropped into the holes according to the serial dilution and then allowed to sit for 15 minutes before incubating at 37°C for 24 hours using a pasture pipette [11, 12].

2.11. Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC), the least concentration of the fraction of the extract that inhibits the growth of a microorganism, was determined. The different concentrations of the plant extract were prepared by serial dilution. The different concentrations of the plant extract (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml) were poured into Petri dishes containing the sterilized nutrient agar and allowed to solidify, the organisms (MRSA and *P. vulgaris*) were inoculated into the nutrient agar and plant extract mixture Using a sterilized wire loop. Each organism was labeled accordingly underneath the plates.

Then, it was allowed to stand for some time, inverted, and incubated for 24 hours at 37°C [13].

3. Results and Discussion

Table 1 shows two extracts of *Ocimum gratissimum* containing alkaloid, saponin, reducing sugar, cardiac glycosides, and tannins. Both methanol and hexane extract did not contain anthraquinones.

Table 1: Phytochemicals detected in the extract of *O. gratissimum* stem.

S/N	Phytoconstituents	Methanol extract	Hexane extract
1	Alkaloids	+	+
2	Saponin	+	+
3	Tannin	+	+
4	Cardiac glycosides	+	+
5	Terpenoids	+	+
6	Anthraquinone glycosides	-	-

Table 2 shows the diameter of the zones of inhibition of the methanolic extract of *Ocimum gratissimum* against the test organisms (MRSA and *Proteus vulgaris*). The plant extract with the highest concentration (200 mg/ml) showed the highest zone of inhibition against both organisms, 19.20 mm and 16.6 mm for MRSA and Proteus vulgaris, respectively.

Table 2: Zone of inhibition of methanol extract of *O. gratissimum* stem.

Concentration of plant extract (mg/ml)	Zones of inhibition in mm (Mean ± SEM)	
	MRSA	*P. vulgaris*
200	19.20± 0.16	16.6± 0.16
100	17.33±0.33	16.6 ± 0.32
50	14.66 ± 0.32	14.30± 0.32
25	14.33 ± 0.87	13.6 ± 0.32
12.5	13.6 ± 0.33	12.6 ± 0.32
6.25	12.33 ± 0.32	13 ± 0.56

Our results showed that the concentration of *Ocimum gratissimum* extract did not inhibit MRSA and *Proteus vulgaris* growth.

Table 3 shows the minimum inhibitory concentration of *Ocimum gratissimum* methanol extract against the test organism in mg/ml. The MIC of the methanol extract against MRSA is 25 mg/ml, while the MIC of the methanol extract against *P. vulgaris* is 12.5 mg/ml.

Table 3: Minimum inhibitory concentration of methanol extract of *O. gratissimum*stem.

Concentration of plant extracts (mg/ml)	MRSA	*P. vulgaris*
200	-	-
100	-	-
50	-	-
25	-	-
12.5	+	-
6.25	+	+
3.125	+	+

The physicochemical analysis of the stem of *Ocimum gratissimum* was carried out to determine the moisture content, total ash content, acid insoluble ash, water soluble ash, and extractive values of different solvents (water, acid, alcohol, and n-hexane) (Table 4). The average moisture content obtained from the *Ocimum gratissimum* stem was 9.33%, which falls within the limits for water content (8 to 14%) for vegetable drugs. The result indicated a moderate shelf life of the fresh plant. Results for ash analysis on the dry matter of the stem indicated low inorganic contents, though the values were primarily subject to the soil type/mineral composition of the soil used to cultivate the plant.

Table 4: Physicochemical analysis of *Ocimum gratissimum* stem.

Parameters	Mean±SEM (%)
Moisture content	9.33± 0.32
Total ash	3.08± 0.08
Acid insoluble	0.0129± 0.009
Water soluble	5.84± 0.006
Extractive value	
Water soluble	26.67± 1.76
Alcohol soluble	19.67± 0.32
Hexane	10.67± 0.66

Several research works have been done on the antimicrobial activity of *Ocimum gratissimum* in previous years. However, the degree of inhibitory activity of the extract depends on the type of bioactive ingredients present in the extract. These active ingredients include alkaloids, saponins, tannins, and cardiac glycosides. The various zones of inhibition exhibited by *Ocimum gratissimum* stem extracts are due to the ability of the bioactive compounds to interfere with

the microbial enzymes. The different bioactive components have different levels of solubility in the two solvents. The plant extracts exhibiting diameters of zones of inhibition > 10 mm are considered active. It can be observed from the methanol extract activity against MRSA that decreasing concentration results in a decrease in activity (lowering zones of inhibition). The methanol extract action on *Proteus vulgaris* shares a similar pattern with that of MRSA; decreasing concentration results in decreased activity (lowering inhibition zones). It has been reported from previous studies that the volatile oil of this plant contains mostly phenols, particularly thymol, and these are responsible for its reported antimicrobial action [14]. Phenolic compounds possess antimicrobial activity and seem to be important for anti-MRSA activity. However, secondary metabolites such as alkaloids, steroids, tannins, and terpenoids have curative activity against pathogens such as *Staphylococcus aureus* and *Escherichia coli*. Therefore, they could suggest using this plant to treat diseases caused by these pathogens [15, 16]. Specifically, tannins have drawn significant interest due to the recently reported broad-spectrum antibacterial activities. Tannins are multidentate ligands with a high tendency to bind to proteins via hydrophobic interactions and hydrogen bonds [17]. Generally, the antibacterial activity of tannins is mainly via interference with bacteria metabolic processes. The results showed slightly better inhibition zones of the crude drug against MRSA than that of *Proteus vulgaris*. It can be attributed to gram-negative bacteria being more challenging to treat because of their cell structure, which is mainly linked to their tough cell wall. In addition to those above, it is essential to note that all concentrations of the hexane extract failed to inhibit both test organisms due to the volatile nature of the solvent of extraction and the poor infusibility of the solvent into the culture media.

The minimum inhibitory concentration was the lowest concentration of an antimicrobial agent that inhibits growth. The methanol extract against MRSA (anti-MRSA activity) was at 25 mg/ml concentration and 12.5 mg/ml concentration for *Proteus vulgaris*. It is important to note that the variation in the antimicrobial properties of a plant is attributed to the age of the plant used, the freshness of the plant material, physical factors (temperature, light, water), contamination by field microbes, adulteration and substitution of plants, incorrect preparation and dosage, Consideration should also be given to time and temperature of extraction as these, as well as the solvent, determines the extraction yield.

The physicochemical evaluations have confirmed the quality of the crude drug by revealing the moisture content, ash values, and extractive values. The moisture content of stem bark is 9.3%. This value falls within the normal range; hence, it can be affirmed that this crude drug exhibits a minimal tendency of microbial spoilage; hence, long-term storage can be encouraged. The ash value revealed a low amount of inorganic contents with a value of 3.08, 0.0129, and 5.84 for total ash, acid-insoluble ash, and water-soluble ash, respectively. Therefore, these deficient impurities indicate a low contaminant level on the crude drug. The very low acid insoluble ash and water soluble ash indicates less siliceous impurities and inorganic contents respectively, hence *Ocimum gratissimum* stem can be considered safe for die and punches of tableting machine because it will exhibit little or no abrasive effect on it during formulation of this crude drug into tablet dosage form. The extractive values indicate that the polar solvents will give the best yield for this crude drug, since water and methanol produced a better yield when compared to hexane.

4. Conclusion

In this present study, evaluation of the phytochemical and physicochemical properties provides useful information that aids pharmacognostic standardization and establishes a scientific base for buttressing the good quality of both crude drugs. This study has clearly shown that the methanol extract of the stem of *Ocimum gratissimum* has a moderate degree of inhibition on both MRSA and *Proteus vulgaris*. It is, therefore, important to note that the methanol extract of *Ocimum gratissimum* has shown slightly more potent antibacterial activity against *MRSA* than against *Proteus vulgaris*. Often, the stem of the *Ocimum gratissimum* plant is usually handled with little or no clinical importance, and this study has been able to prove the potential of the stem in the management of infections caused by MRSA and Proteus vulgaris such as pneumonia, skin infections, urinary tract infections, bloodstream infections, respiratory tract infections. With these activities, further investigation into its toxicity, chemical constituents, purification, and activity against various microorganisms such as viruses and fungi should be carried out to develop newer and more effective drugs for *in vivo* and *in vitro* use.

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Conflict of interest

The authors of this article announce that we have no conflict of interest.

Authors' ORCIDS

Lawrence Uchenna Nwankwo:
https://orcid.org/0000-0003-1612-0114
Efemena Curtbeth Obokare:
https://orcid.org/0000-0002-1682-0906

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