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Phytochemical, antimicrobial and antioxidant preliminary screening of a traditional Nigerian medicinal plant, *Ocimun gratissimum*

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Abstract The aim of this work is to investigate and compare the phytochemical screening, antioxidant and antimicrobial activities of crude extracts obtained from dry leaves of *Ocimun gratissimum*. Different solvents including methanol, dichloromethane were used to prepare the crude extracts from the dry leaves. Antioxidant and antimicrobial activities of different crude extracts from dry leaves of *Ocimun gratissimum* were determined by DPPH method and agar disc diffusion method with minor modifications. *In vitro* phytochemical screening for all crude extracts from both dry leaves was tested and shown positive result for alkaloid, flavonoid, saponin and tannin compounds. The antioxidant activity results of the crude extracts showed direct proportionality relationship between the sample concentration and absorbance. However, the methanol and dichloromethane crude extracts showed small and moderate antibacterial potential with one gram positive (*Staphylococcus aureus*) and three gram negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). In conclusion, all crude extracts from the dry leaves of *Ocimun gratissimum* could be used as potential sources of new antioxidant and antimicrobial agents.

Keywords Phytochemical, antimicrobial, antioxidant, *Ocimun gratissimum*

Introduction

Medicinal plants continue to be an important resource to fight serious diseases, especially in developing countries [1]. Malignant and infectious diseases are still a serious problem to public health, despite the great development in modern medicine. The treatment of infectious diseases caused by resistant bacterial strains, represent one of the main challenges of medicine today, especially due to the inefficacy of long-term drug therapy [2]. The relative unavailability of medicines in developing countries and the appearance of widespread multiresistant bacterial strains let the effect of these diseases particularly large and considerable [3]. In the search for new alternatives to treat these infections, many researchers have been looking for novel compounds derived from natural products to replace, or be used in combination with conventional antibiotics [2,4]. Consequently, medicinal plants have served as an important source of effective antimicrobial and antioxidant agents.

Ocimun gratissimum L. (Labiate) is widely distributed in tropical and warm temperature regions. The plant is commonly used in folk medicine to treat different diseases e.g. upper respiratory tract infections, diarrhea, headache, skin diseases, pneumonia and also as treatment for cough, fever and conjunctivitis [5-6]. Previous studies shows that the essential oils (*Ocimun gratissimum L.* have been reported to have antimicrobial [6-7] and antidiarrhoea activity, the extract also inhibited castor oil-induced diarrhea in rats as judged by decrease in the number of wet faeces in the extract-treated rats. In addition, the extract inhibited the propulsive movement of intestinal contents.

Generally, considerable part of researches performed today focuses on the development of new drugs to treat microbial infections as well as cancers and other diseases [2]. Therefore, this work aimed to evaluate the antimicrobial, anticancer and antioxidant activities of selected medicinal plants such as *Ocimun gratissimum*

Materials and Methods

Plant Preparation

The fresh leaves of *Ocimum gratisimum Lamiaceae* were washed with tap water and then rinsed with distilled water, after which it was air for the period of 14 days. The dried leaves were then transferred into an oven at 60 ^{o}Cand were then crushed into powder.

Extraction

Total weight of the powdered plant material was 240g. The powdered material was divided into two halves 120g each. One portion (120g) was macerated in dichloromethane (900ml) and the other portion (120g) was also macerated in methanol (900ml). After 72 hours each extract was first filtered using Whatman filter paper (number 4) and solvent was removed *in-vacuo*.

Phytochemical/Qualitative Screening Test

The test for the presence of phytochemicals where carried out using the methods outlined in Treas and Evans [8].

Test for Saponin: Aqueous extract (1 ml) was added to few volumes of distilled water in a test tube. Presence of frothing upon agitation which persist for 10-15 minutes shows the presence of saponin.

Test for Tannins: Few drops of water was added to the dry extract; after which alcoholic ferric chloride ($FeCl_3$) reagent (3 drops) was added. A dark green color indicates the presence of tannins.

Test For Flavonoids: About 2ml of the aqueous extract was added into the test tube and few drops of concentrated ammonia solution was added. The Formation of yellow coloration indicated presence of flavonoids.

Test for Alkaloids: The aqueous extract (2ml) was transferred into the test tube and few drops of Mayer's reagent was added. The Presence of creamish precipitate indicates the presence of alkaloids.

Test For Steroids: Chloroform(2ml)was added to the extract and then few drops of Aceticanhydride were added followed by concentrated sulphuric acid (H_2SO_4). Blue green coloration indicate the presence of steroid.

Test For Phenolics: The plant extract about 1 g was added to test tube containing 2ml of ferric chloride. Presence of blue, violent, purple, green or reddish brown colour showed the presence of phenols.

Test For Triterpenoids: The crude extract (1 g) was dissolved in chloroform (2ml); then acetic anhydride (1ml) was added and Reddish-violent colour indicates the presence of triterpenoid.

Quantitative Analysis

Estimation of alkaloid content

The total alkaloid content was estimated using the method described by Unuofin *et al* [9]. 5 g of the pulverized *Ocimum gratisimum Lamiacea e*was immersed in 200 mL of 10% acetic acid in ethanol. The mixture was allowed to stand for 4hr at room temperature. It was subsequently filtered and the filtrate was concentrated using a water bath at 55 ^{o}C to a quarter of its original volume. Concentrated ammonium hydroxide was added in single drops until completion of the precipitation process. The solution was then washed with dilute ammonium hydroxide and filtered again. The residue obtained was first dried and then weighed. The alkaloid content was calculated using the equation:

% Alkaloid = Weight of precipitate / Weight of original sample ×100

Saponin Determination

The method used was that of Onyesife *et al* [10]. The sample *Ocimum gratisimum Lamiaceae* was ground and 20 g was put into a conical flask and 20% aqueous ethanol (100 ml) was added. The sample was heated over a hot water bath for 4 h with continuous stirring at about 55 ^{o}C. The mixture was filtered and the residue re-extracted with another 20% ethanol (200 ml). The concentrate was transferred into a 250 ml separatory funnel and diethyl ether (20 ml) was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and n-butanol (60 ml) was added. The combined n-butanol extracts were washed twice with 5% aqueous sodium chloride (2 x 10 ml). The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight; the saponin content was calculated thus:

% Saponins contents = Weight of residue /Weight of sample ×100

Estimation of Tannin content

The total tannin content was estimated using the Folin - Ciocalteu method of Vijay and Bhambar, 2014. Distilled water (7.5 ml) was added to a tube containing 0.1ml of *Ocimum gratisimum Lamiaceae*. 0.5 ml of Folin-Ciocalteu phenol reagent and 1 ml of 35 % Na_2CO_3 solution was then added. The whole solution was made up to 10 ml with distilled water. The mixture was vortexed and kept at room temperature for 30 min. The absorbance was read at 725 nm using a spectrophotometer. The total tannin content was expressed as mg/g GAE equivalent.

DPPH radical scavenging assay

DPPH radical scavenging activity of *Ocimum gratisimum Lamiaceae* extracts were determined according to the method described by Wintola and Afolayan, [11] with some modifications. A preparation of 1 ml of 0.135 mM DPPH in methanol was mixed with 1ml of various concentrations (1 – 7 mg/ml) of the plant extracts and vitamin C. The mixture was left in the dark at room temperature for 30 min after being vortexed. The absorbance of the mixture was then measured spectrophotometrically at 517 nm. Vitamin C was used as standard. The DPPH radical scavenging activity was calculated from the equation:

DPPH radical scavenging activity = Abs control - Abs sample/ Abs control ×100

Where Abs control was the absorbance of DPPH radical + methanol.

Abs sample was the absorbance of DPPH radical + sample extract or standard (Vitamin C).

Test microorganisms

For antimicrobial studies: The test organisms (*Staphylococcus aureus, Klebsiella Pnuemoniae, Pseudomonas Aeruginosa and Escherichia coli*) were procured from the Medical Microbiology and Parasitology Department, Niger Delta University, Bayelsa State and stored at -20 °C for further studies.

Antibacterial activity assay

The antibacterial potential test was carried out using the agar disc diffusion method [12]. Negative controls were prepared by using the same solvents employed to dissolve the samples. Inhibition zones were measured and compared with the standard reference antibiotic ciprofloxacin. Each extract was subjected to serial dilution by using dimethyl sulphoxide (DMSO) as a solvent to give 50 mg/ml, 10 mg/ml, 2 mg/ml, and 1 mg/ml solutions of as *Ocimun gratissimum.* The concentration of ciprofloxacin standard used for this study was at 1 mg/ml. Each prepared concentration of the different extracts was tested for its antimicrobial activity against the test organisms (*Staphylococcus aureus, Klebsiella Pnuemoniae, Pseudomonas Aeruginosa and Escherichia coli*) on nutrient agar plates using disc diffusion method. Whatman (No. 1), sterile filter paper discs (6 mm diameter) were impregnated with methanol and dichloromethane extracts of *Ocimun gratissimum* and placed on the inoculated agar. All the plates were incubated at 37 ^{o}C for 24 h. Evaluation of antibacterial activity was measured showing the diameter of the zones of inhibition against the tested bacteria. Each method in this experiment was replicated three times.

Results

Table 1: Results of phytochemical screening of *Ocimun gratissimum*

Phytochemicals	Methanol	DCM
Tannins	+	+
Saponins	++	_
Alkaloid	+	_
Terpenoids	_	+
Flavonoids	+	_
Steroids	+	+

Key: - = not present + = present in little quantity ++ = present in high quantity +++ = present in large quantity DCM = Dichloromethane

Table 2: Results of quantitative phytochemical of *Ocimun gratissimum*

Phytochemicals	Quantitative value	
	Dichloromethane	Methanol
Alkaloids	10.04 %	3.89 %
Tannins	1.3 mg/GAE	2.3 mg/GAE
Saponins	2.9 %	7.8 %

Table 3: Zone of inhibition (mm) of methanolic extract

Conc. (mg/ml)	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. aureus*
50	21	-	15	-
20	-	-	-	-
10	-	-	-	-
2	-	-	-	-

Table 4: % scavenging activity of the *Ocimun gratissimum* extracts

Conc. (mg/ml)	% Inhibition		
	Ascorbic acid	Methanol	Dichloromethane
1	66.96	55.67	44.33
3	71.55	59.93	69.17
5	82.39	74.16	73.48
7	91.13	86.44	79.23

Discussion

The percentage yield of the extracts obtained were (10%) formethanolic extract and 3% for dichlormethane extract. The Phytochemical Screening carried out on *Ocimum gratisimum* showed the presence of Saponin, Tannins, Flavonoids, Alkaloids, Steroids, Phenolics in the methanolic extract while triterpenoids was absent. For the Dichloromethane extract tannins, steroids and triterpenoids were present while saponin, flavonoids, alkaloids and phenolics were absent. These compounds as earlier stated confer activity on the plant including antibacterial activity. Antibacterial activity of *Ocimum gratisimum* has been evaluated *in vitro* against four clinical bacterial isolates which are frequently encountered in human infections. The results obtained showed that the methanol extract of *Ocimum gratisimum* had more antibacterial activity on *E. coli, K. pneumoniae, P. aeruginosa*. The organisms showed varying degree of sensitivity to the extract at the concentration used. These findings have been found to be consistent with those obtained in previous studies by Ajibesin *et al* [13].

Also the quantitative determination of alkaloid showed 10.04 % for dichloromethane and 3.89 % for methanolic extracts of *Ocimum gratisimum*. The saponin quantitative content also showed 2.9 % and 7.8 % for dichloromethane and methanolic extracts of *Ocimum gratisimum* respectively. In case of antioxidant activity, methanolic extract of the samples showed effective scavengers of DPPH at 7 mg/ml 86.44 % of the radical DPPH was scavenged and at same concentration dichloromethane extract and Ascorbic acid scavenged 79.23 % and 91.13 % respectively.

Extraction of antimicrobial compound of *Ocimum gratisimum* with DCM was poor as the microorganisms weren't susceptible at all except for *Pseudomonas* with relative small zones of inhibition. Previous studies reports that ethyl acetate, butanol, aqueous extracts of the plant demonstrated antibacterial activity on *Pseudomonas aeruginosa* NCIB 950 (8mm, 5mm & 3mm) *E. coli NCIB 86 (7mm, 4mm & 3mm) and S. aureus NCIB 85 (8mm, 5mm and 3mm) respectively [13]*. *Pseudomonas aeruginosa* was susceptible to the methanol extract of the plant with a zone of inhibition of 15mm at the concentration of 8mg/0.4ml. Comparing the result with that of standard antibiotics, gentamicin had an inhibition of 25mm.

Klebsiella pneumoniae had an intermediate susceptibility with zone of inhibition 21mm. Comparing with standard antibiotics used, *K. pneumoniae* was susceptible to all the antibiotics used with highest zones of inhibition obtained with ciprofloxacin (30mm), gentamicin (26mm) and Cotrimoxazole (21mm).The zone of inhibition for *E. coli* was 18mm and the standard antibiotic was 25mm for Levofloxacin, 34mm for Gentamicin, 24mm for Cotrimoxazole. The activity on *Staph. aureus* at this concentration was evident with a zone of inhibition of 18mm.In conclusion, activity of methanol and dichloromethane extract against Klebsiella, Pseudomonas, E. coli which are among the organisms indicated in upper respiratory tract infections was evaluated. If the concentration of these extract are further increased, there may be more activity nearly equal to the activity of the standard. These extracts can be further studied on other cough causing microorganisms as a future alternative for combating upper respiratory tract infections.

The aforementioned plant *Ocimum gratisimum* has promising antibacterial properties and may be a potential drug for the treatment of infections. Further studies on this plant should be carried out on to determine its activity. Minimum inhibitory concentrations and maximum biocidal concentration needs to be determined for this plant. The toxicity study of the plant extract needs to be performed in order to determine the risk and benefits of potential applications of this plant as drug in human medicine.

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