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PHYTOCHEMICAL CONSTITUENTS AND ANTIBACTERIAL ACTIVITIES OF SCENT LEAF EXTRACTS

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ABSTRACT

Scent leaf (*Ocimum gratissimum*) is a plant that is widely distributed in the tropics of Africa and Asia. It is rich in a good number of ethnomedicinal applications including treatment of diarrhea, pneumonia and upper respiratory tract infection. In this study, we carried out phytochemical screening to detect the chemical components present in methanol and n-hexane extracts of *Ocimum gratissimum* and tested the anti-microbial activities of these extracts against four different species of bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. The phytochemical screening revealed the presence of alkaloids, saponins, tannins, flavonoids, steroids and triterpenes in the methanol extract, but only steroids and triterpenes in the n-hexane portion. The methanol extract showed zone of inhibition that ranged from 12-29 mm, and minimal inhibitory concentration (MIC) of 6.25, 12.5 and 25 mg/ml were recorded against *B. subtilis*, *P. aeruginosa* and *E.coli* respectively. However, the n-hexane extract had zone of inhibition of 14-28 mm, and MIC of 6.25 mg/ml against *B. subtilis* and *E. coli*, and 12.5mg/ml against *P. aeruginosa*. In all cases, twice the MICs were recorded as the respective minimal bactericidal concentration (MBC). These results indicate that consumption of scent leaves is a good source of nutrients and can sufficiently confer physiological protection against different disease-causing organisms.

KEYWORDS: *Phytochemical screening, Ocimum gratissimum, ethnomedicinal activities, antibacterial activity, minimal inhibitory concentration*

INTRODUCTION

Traditional medicine is the oldest form of medicine [1, 2]. This is defined by the World Health Organization as "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" [3]. The knowledge of medicinal plants, applications and contraindications or toxic potentials has been passed down through generations [4]. A lot of reasons can be given to explain why in Africa a large segment of the population still prefers to patronize traditional practitioners and their arts than to seek orthodox medical attention [5, 6]. These include accessibility, cost and convenience.

In the last two centuries or thereabout when the world witnessed advancement of chemistry that allowed for extraction of active ingredients from herbs/plants, standardization of the extracts from these sources has increased tremendously. The old French word for herb, drogue, has since been Anglicized to drug, the chemical preparation that is prescribed as treatment for many disease conditions. The active principles in many drugs are either isolated from plant sources or are synthetic analogs of the natural constituents of plants. Example, aspirin is an analgesic closely related to the chemical in the bark of willow tree, digoxin from foxglove (*Digitalis purpurea*), quinine from the bark of cinchona tree and morphine from *Papaver somniferum* [7]. The bioactive micronutrients in plants include alkaloids, tannins,

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flavonoids and phenolic compounds which are generally called phytochemicals [8-10]. Phytochemicals may contain either antioxidants or hormone-like actions for treating health conditions such as cancer, heart diseases, diabetes, high blood pressure and preventing the formation of carcinogens on their target tissues [11].

The leaf of *Ocimum gratissimum* is widely used as food seasoning because of its aromatic appeal [12]. In West Africa, the leaves are also used as anti-cancer, anti-diabetic, mosquito repellant and for the treatment of malaria, convulsion, catarrh, gastrointestinal disorders, stomach pains, cold, dysentery, high fever, diarrhea, etc. In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy [13], high fever [14] and diarrhea [15], while in the savannah areas decoction of the leaves is used to treat mental illness [16]. In North-Central Nigeria, The Ebira people of Kogi State use leaves of *Ocimum gratissimum* to prepare a local dish that is given to a woman after childbirth.

Orthodox antibiotics are drugs that have contributed immensely in the fight against infectious diseases. Unfortunately, however, infections are now on the increase largely due partly to the increased resistance of disease causing agents to these antibiotics [17]. However, according to Parekh *et al.* [18], antimicrobials of plant origin are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often linked with synthetic antimicrobials.

The knowledge of the chemical constituents that are present in plants is indispensable in the quest to harness their ample potentials in solving many of our health challenges. In this regard, it is important to ascertain the possible medicinal values of plants through investigation of their chemical compositions. This study was, therefore, conducted in order to investigate the potentials of the plant *Ocimum gratissimum* for antibacterial activity in relation to its chemical composition.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals used were of analytical grade and were purchased from reliable suppliers. Reagents were prepared in accordance with standard protocols.

Microbial species

The test microorganisms used in this study were clinical isolates of bacteria obtained from the Department of Microbiology, Ahmadu Bello University, Zaria. The isolates were *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.*

Collection and Preparation of Plant Samples

The scent leaves were obtained from a farm in Basawa village, Kaduna State, Nigeria in the month of June, 2015. It was identified and authenticated as *Ocimum gratissimum* (Figure 1) at the herbarium of Biological Sciences Department, Ahmadu Bello University, Zaria, Nigeria. The specimen was deposited with voucher number 1285. The leaves were washed and air dried at room temperature for two weeks after which they were ground into powder with a wooden mortar and pestle and stored in an air-tight container until needed.

Figure 1: Full grown plant of *Ocimum gratissimum*

Extraction of Leaf Samples

Exactly 150 g of ground leaves of *Ocimum gratissimum* was extracted sequentially using solvent of increasing polarity, n-hexane and methanol, by cold maceration for 48 hours. The sample was first extracted with 600 ml of n-hexane. This was to remove components such as fats and oils present in the plant. The residue was then extracted with 600 ml methanol. The extract was initially light green, and then deep green, and became almost colorless on completion of extraction. Muslin cloth was used to filter the extract. The filtrate thus obtained was further purified by filtration using Whatman filter paper. Thereafter, both extracts were concentrated under reduced pressure on a rotary evaporator to yield crude extracts of *Ocimum gratissimum.*

Phytochemical screening

Phytochemical screening to detect the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, glycosides, steroids and triterpenes was carried out according to protocols reported by Sofowora [15] and Trease and Evans [19].

Culture Media for Antibacterial Screening of the Methanol and n-Hexane Extracts

The culture media used for the study include Muller Hinton Agar (MHA), Muller Hinton Broth (MHB) and nutrient agar (NA). The mentioned media were used for sensitivity test, determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). All media were prepared according to manufacturer's instruction and sterilized by autoclaving at 121 °C for 15 minutes.

Determination of Inhibitory Activity (Sensitivity Test) of the Extract Using Agar Well Diffusion Method

Sensitivity of the microbial isolates to the extracts of *O. gratissimum* was tested in accordance with Ericsson and Sherris [20]. The standardized inocula of the bacterial isolates were streaked on sterilized Muller Hinton agar plates with the aid of sterile swap sticks. Four wells were dug on each inoculated agar plate with a sterile cork borer. The wells were properly labeled according to different concentrations of the extracts prepared: 50, 25, 12.5 and 6.25 mg/ml respectively. Each well was filled up with approximately 0.2 ml of extract. The inoculated plates containing the extract were allowed to stay on the bench for about one hour; this was to enable the extract to diffuse on the agar. The plates were then incubated at 37 ^{o}C for 24 hours.

At the end of the incubation period, the plates were observed for any evidence of inhibition which appeared as a clear zone that was completely devoid of bacterial growth around the wells (zone of inhibition) (Figures 2 and 3). The diameters of the zones were measured using a transparent ruler calibrated in millimeter and the result was recorded.

Determination of Minimal Inhibitory Concentration

The minimal inhibitory concentration (MIC) of the extracts was determined using tube dilution method with Muller Hinton broth as diluent [21]. The lowest concentration of the extract showing inhibition for each organism when the extract tested positive during sensitivity test was serially diluted in the test tubes containing Muller Hinton broth. The organisms were inoculated into each tube containing the broth in the extract. The inoculated tubes were then incubated at 37 ^{o}C for 24 hours. At the end of the incubation period, the tubes were examined for the presence or absence of growth using turbidity as a criterion; the lowest concentration in the series without visible sign of growth (turbidity) was considered to be the MIC.

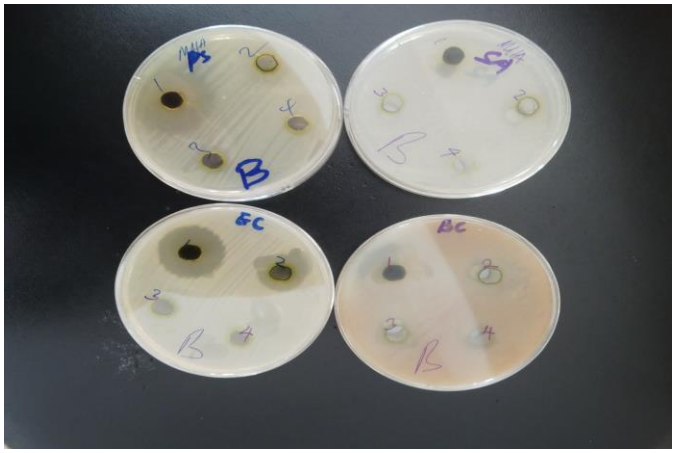

Figure 2: Petri dishes containing culture of B (methanol extract) for sensitivity test.

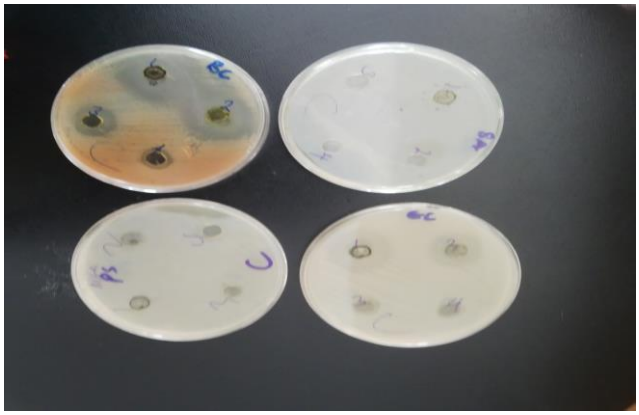

Figure 3: Petri dishes containing culture of C (n-hexane extract) for sensitivity test.

Determination of Minimal Bactericidal Concentration

The result from the minimal inhibitory concentration (MIC) test was used as the basis for the determination of the minimal bactericidal concentration (MBC) of the extract. A sterilized wire loop was dropped into the test tube(s) that did not show turbidity (clear) in the MIC test and a loopful was taken and streaked on sterile nutrient agar plates (Figure 4). The plates were incubated at 37 °C for 18-24 hours. At the end of the incubation period, the plates were examined for the presence or absence of growth. This was to determine whether the antimicrobial effects of the extracts were bacteriostatic or bactericidal.

Figure 4: Petri dishes with culture to determine the minimal bactericidal concentration

RESULTS AND DISCUSSION

From the original 150 g of dried plant sample, the methanol extract of the plant was weighed and found to be 9.12 g while the n-hexane extract was 5.38 g which translated to 6.08 % and 3.59 % yield respectively (Table 1).

Table 1: Methanol and n-hexane extraction yields

Extract	Weight of extract (g)	Yield (%)
n-Hexane	5.38	3.59
Methanol	9.12	6.08

The phytochemical screening of the methanol extract showed the presence of alkaloids, flavonoids, tannins, saponins, steriods and triterpenes with the absence of glycosides and anthraquinones. The n-hexane extract contained only steriods and triterpenes (Table 2).

Table 2: Results of phytochemical screening of n-hexane and methanol extracts of *O. gratissimum*

Metabolites	n-Hexane extract	Methanol extract
Glycosides	-	-
Anthraquinones	-	-
Saponins	-	+
Tannins	-	+
Alkaloids	-	+
Flavonoids	-	+
Steroids	+	+
Triterpenes	+	+

+ = Present - = Absent

The difference in the types of phytochemicals present in the methanol and n-hexane extracts of *Ocimum gratissimum* may be due to the variation in polarity of the solvent indicating that not all phytochemicals can be extracted by one solvent. This finding is supported by report of Mbatchou *et al*. [22]. These metabolites have been known to show physiological and medicinal activities [15]. Flavonoids are free radical scavengers, antioxidants which prevent oxidative cell damage and have strong anti-cancer activity [23]. This justifies the use of *Ocimum gratissimum* as an anti-cancer agent in traditional medicine [15]. Alkaloids have significant therapeutic action and are condiments for many important medicines [17]. Tannins have beneficial effects on vascular health. Studies have shown that they suppress the generation of atherosclerosis and they possess anti-microbial effects; this justifies the use of *O. gratissimum* by the Igbos in the management of a baby's cord [24]. Therefore, the use of *Ocimum gratissimum* for soup and tea could give a lot of medicinal advantages to consumers [12].

The antimicrobial screening of both methanol and n-hexane extracts showed marked antimicrobial activity on *Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* (Table 3). Methanol extract showed zone of inhibition ranging from 12-29 mm when varying concentrations of 6.25-50mg/ml were used, whereas n-hexane extract showed zone of inhibition of 13-28 mm accordingly (Table 4). The results indicate that *B. subtilis* and *E. coli* were more sensitive to the n-hexane extrac than the methanol extract as determined by higher zones of inhibition of 28 mm and 23 mm respectively, while *P.aeruginosa* was more sensitive to the methanol extract as indicated by the higher zone of inhibition of 29 mm.

Table 3: Result of sensitivity test for methanol and n-hexane extracts

Test organisms	Methanol	n-Hexane
Staphylococcus aureus	R	R
Bacillus subtilis	S	S
Escherichia coli	S	S
Pseudomonas aeruginosa	S	S

S= Sensitive R= Resistant

Table 4: Zone of inhibition (mm) at varying concentrations (mg/ml) of the extracts

Species	Methanol extract				n-Hexane extract				CP*
	50	25	12.5	6.25	50	25	12.5	6.25	10 μg
S. aureus	0	0	0	0	0	0	0	0	42
B. subtilis	23	20	17	12	28	25	20	14	32
E. coli	21	18	0	0	23	20	13	10	38
P. aeruginosa	29	23	12	0	26	20	15	0	32

*Ciprofloxacin: positive control

Table 5: Results of MIC and MBC in mg/ml of the methanol and n-hexane extracts

Test organism	MIC		MBC	
	Methanol	n-Hexane	Methanol	n-hexane
B. subtilis	6.25	6.25	12.5	12.5
E. coli	25	6.25	50	12.5
P. aeruginosa	12.5	12.5	25	25

MIC= Minimal Inhibitory Concentration, MBC= Minimal Bactericidal Concentration

The minimal inhibitory concentration (MIC) of both methanol and hexane extracts was found to be 6.25 mg/ml for *B. subtilis* and 12.5 mg/ml for *P. aeruginosa.* For *E. coli*, however, 12.5 mg/ml and 6.25 mg/ml were recorded for methanol and n-hexane extracts respectively (Table 5). In all cases, twice these concentrations were found as the respective minimal bactericidal concentration (MBC).

These results show that the methanol and n-hexane extracts from *O. gratissimum* were active against different bacterial isolates. This might be the rationale for using the plant in many infection-related conditions such as pneumonia and urinary tract infections [25]. Our findings are in agreement with reports of Ekhaise and Okoruma [26], Aluyi *et al*. [27] and Nwze *et al.* [28] who found that plant extracts inhibited the growth of microbial isolates. The difference in the results obtained for the two types of extract could be linked to their different phytochemical constituents. This further shows that bioactive pigments and phytochemicals in plants can be good sources of broad spectrum and specific conventional antimicrobials [29].

CONCLUSIONS

Results from this work showed the presence of different phytochemicals that have been previously linked with medicinal potentials, especially antibacterial activities. Methanol and n-hexane extracts of O. *gratissimum* possessed different phytochemical constituents, an indication that no single solvent may be effective in extracting all plant components. This difference in constituents has led to the difference in anti-microbial potentials as indicated by difference in sensitivity and MIC/MBC values.

Consumption of scent leaves is a good source of nutrients and can sufficiently confer

physiological protection against different disease-causing organisms. Isolation and purification of these phytochemicals to establish the specific component(s) responsible for defined physiological/medicinal application (s) is a highly important goal of future research works.

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