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Phytochemical Constituents and Antimicrobial and Grain Protectant Activities of Clove Basil (*Ocimum gratissimum* L.) Grown in Nigeria

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Abstract *Ocimum gratissimum* Linn (Lamiaceae) is an herbaceous plant reputed for many medicinal and agronomic practices amongst Nigerian peasant farmers. *O. gratissimum* was investigated for antimicrobial activity against ten micro-organisms and for grain protectant activity against *Callosobrochus maculatus*. The phytocontituents of the aerial part of *O. gratissimum* were extracted with 95% ethanol using the percolation method. The crude ethanol extracted was further fractionated into hexane, chloroform and methanol fractions. The fractions obtained were screened for phytoconstiteunts, antimicrobial and grain protectant properties. Result showed that hexane fraction exhibited the highest antimicrobial activity against *Vibrio cholera* and *Klebsiella pneumonia*. Similarly hexane fraction also showed the highest grain protectant activity. The other extracts of the *O. gratissimum* did not significantly inhibit both bacterial growth and grain infestation. However, the methanol fraction contains phytocompounds such as phenolic compounds associated with antioxidant properties. The study shows that *O. gratissimum* extractants are potential sources of antimicrobial and preservative agents.

Keywords Antimicrobial Activity, Grain Protectant Activity, *Ocimum Gratissimum*, Weevil Perforation Index, *Callosobruchus Maculatus*, Traditional Medicine, Phytoconstituents

1. Introduction

Traditional medicine continues to provide health coverage for over 80% of the world population, especially in the developing world[1]. Plants are the major constituents of traditional medicine[2]. Many of the plant materials used in herbal medicine are readily available in rural areas and this has made it relatively cheaper than orthodox medicine[3]. The upsurge in the prevalence of side effects of many synthetic antimicrobial agents and incidence of multi-drug resistant bacteria and pests has spurred scientists onto the research for plant based antimicrobial of therapeutic and pesticidal potentials[4-7].

Ocimum gratissimum Linn (Lamiaceae) is an herbaceous shrub notably found in tropical countries including Nigeria, where it is commonly called Clove basil, Sweet basil, teabush, Scent leaf or fever plant; but it is also popularly known with different local names in Nigeria (Nupe: Tanmotsungi-wawagi; Ebira: Ireru; Hausa: Dai doya ta gida; Yoruba: Efinrin ajase; Ibo: Nchanwu)[8-10].

Many species of the genus *Ocimum* namely: *Ocimum americanum*, *Ocimum basilicum*, *Ocimum canum*, *Ocimum gratissimum, Ocimum sanctum* and *Ocimum suave* have been reputed for various medicinal uses[10-13].

Several ethnobotanical surveys show that *Ocimum gratissimum* was among the plants reported in Nigeria communities to be used traditionally to treat bacterial infections such as enteric diseases viz: diarrhoea, dysentery and other gastrointestinal infections; upper respiratory tract infections associated with coughing pneumonia, asthma and bronchitis; urogenital infections including sexually transmitted diseases, skin infections (dermatitis, eczema, scabies), wounds and ulcers; headache, ophthalmic, insect bites, nasal bleeding, stroke, measles, paludism; and bacterial fevers such as typhoid fever and diabetes and veterinary problems[13-36]. It is also used in the treatment of epilepsy, shigellosis, trypanosomiasis, convulsion, pile and anaemia in Nigeria[37]. It is also implicated in the oral hygiene and veterinary in Nigeria[38, 39].

Comprehensive biological activities of *O. gratissimum* has been reviewed[14] and it is associated with antibacterial, antifungal, hypoglycaemic, antipyretic, anti-nociceptive, antioxidant, anti-inflammatory, anthelmintic, chemo- preventive, anti-carcinogenic, free radical scavenging, radio protective, antidermatophytic activities, and numerous others pharmacological use[40-61]. Earlier reports have also shown the smooth muscle contracting and antimutagenic activity[62] as well as its anti-diarrhoeal effects in experimental animals[63], high antiviral indices against HIV-1 and

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HIV-2[64]; shigellocidal properties[65,66], anti- trypanosomal effects[67], immunobiologicalactivity[68], gastroprotective properties[69], controlling agent for food spoilage and mycotoxin producing fungi[70], disintegrant properties of its seed mucilage[71], and as a relaxant on isolated ileum from guinea pig[72]. Its essential oil has mosquito repellant, insecticidal properties[73,74]. The essential oil of *O. gratissimum* and its main component eugenol were reported to be efficient in inhibiting Haemonchus contortus[75,76]. Currently, basil is mainly used as a culinary herb as well as perfumes and cosmetics[77]. It is therefore important that phytochemical composition be correlated to the antimicrobial activity in order to verify the therapeutic value proclaimed by the traditional healers.

One major factor responsible for promoting grain production in developed countries has been attributed to the usage of insecticides in grain protection and storage. Many plant components are now known to possess herbicidal, insecticidal or fungicidal properties[78-81]. Preparations made from Nigerian plants are identified with pesticidal activities[82-88]. The discovery and development of these products were based on the significance of the Nigerian plants in folklore medicine and agronomic practices amongst our peasant farmers. Peasant farmers in northern Nigeria indigenously use various plants to protect cereals and legumes against pest damage during storage with O. gratissimum being one of such plants[7]. It is based on this view that, bioassay screening method[87] was adopted for screening Ocimum gratissimum for grain protectant activities. Despite these scientific and medicinal values, comparative analyses of its phytochemical evaluation vis-à-vis the antimicrobial and pesticidal potentials have not been investigated. Therefore, the present study reports phytochemical, antimicrobial and grain protectant activities of O. gratissimum used for the treatment of human infections and agronomic pests.

2. Materials and Methods

2.1. Collection and Identification of Materials Used

The aerial parts of *O. gratissimum* were collected from Kuchi-gbako, a village along Bida-Doko road of Lavun Local Government, Niger State as described by the traditional healers and farmers. The plant was botanically identified by Mal Muazzim Ibrahim of the Herbarium Unit of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, Garki – Abuja, Nigeria where voucher specimen (No. NIPRDH 1285) was deposited. *Callosobruchus maculatus* was obtain from national cereal research institute, Baddegi, Niger State and maintained on seeds of the cowpea[*Vigna unguiculata (L.) Walp*] cultivar life brown[87].

2.2. Extraction Procedure

The plant material was air-dried under the laboratory room conditions for one week and then milled into coarse powder by using clean mortar and pestle. The powdered material (200g) was percolated with 95% ethanol (2.5L) for two weeks. The extract was filtered and evaporated to dryness using a rotavapor to give a dark greenish residue (43.5g)[89].

2.3. Test procedure for Antimicrobial Activity

The crude ethanol extract of the aerial parts of *O. gratissimum* was screened *in vitro* for antimicrobial activity against ten pathogenic microorganisms (Table 2) using Agar-dilution streak technique[90] as follows: the test organisms were prepared by incubating them in freshly prepared nutrient broth at 37^{o}C for 8 h the cultures were serially diluted with sterile normal saline. 48 mg of the test extract was dissolved in 1ml of absolute ethanol and made up to 3ml with sterile distilled water to give a concentration of 16 mg/ml of extract. 1ml of the prepared extract was then introduced into 15 ml of molten Agar placed in water at 54 ^{o}C these were mixed well and poured into sterile petri-dish plates to give a concentration of 1000 µg/ml of Agar. Other concentrations were similarly prepared. The plates were then hardened in a refrigerator for 15 min. Thereafter the standardized test organisms (1000 ml each) were inoculated onto the nutrient Agar plate and incubated at 37℃ for 24-48 h the results of the tests done in triplicate are shown in Table 2.

2.4. Fractionation of Extracts

The crude extract (100 g) was extracted with hexane and 70% aqueous methanol (150 ml, 1:1), hexane soluble fraction was separated and concentrated in *vacuo* to give a hexane residue (15.56 g), and the methanol layer was then extracted with chloroform (150 ml). The resultant chloroform soluble and methanol soluble portions were separated and concentrated *in vacuo* to give chloroform residue (5.4 g) and methanol residue (3.2 g) respectively.

2.5. Phytochemical Screening of Extracts

The plant extracts were phytochemically screened using standard techniques for the detection of Sterols, saponins, phenolics, tannins, flavonoids, terpenoids andalkaloids[91-93].

2.6. Cowpea Weevil Bioassay with the Plant Extracts

The residues obtained from the fractionation of *O. gratissimum* were screened for grain protectant activity against *C. maculatus* using cowpea weevil bioassay techniques[87] as follows: Unperforated cowpea seeds (50 g) from newly harvested dry pods were weighed out and from 10 g were transferred to each of four Erlenmeyer flasks. The cowpea seeds in each of these three flasks were separately treated with the various extracted residues (1 g) each. The untreated seeds in the fourth flask served as a control.

Freshly emerged adults of *C. maculatus* (age, 0-8 h) were used to infest the cowpea seeds in each flask. The flasks were covered with mesh net and left on the shelf at room temperature. The control and the three treated samples are trip-

licated. After 4 months the cowpea seeds in each flask were examined for perforations. The number of cowpea seeds perforated in treated and control were counted for determination of weevil perforation index (WPI). The weevil perforation index, defined as percentage of treated cowpea seeds perforated X 100/percentage of control cowpea seeds perforated + percentage of treated cowpea seeds perforated, was calculated for each extract for comparison of grain protectant properties of *O. gratissimum*.

2.7. Test Organisms

Stock culture of *Neisseria gonorrheae, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae, Vibrio cholerae, Streptococcus faecalis* and *Bacillus anthracis* were obtained from Microbiology Laboratory, Federal University of Technology, Minna. These cultures were checked for viability and purity and maintained on nutrient agar slopes.

2.8. Statistical Analysis

The data was analyzed with ANOVA and the means were separated using Duncan Multiple Range Test at a probability level of 5%.

3. Results and Discussion

The crude 95% ethanol extract of *O. gratissimum* has demonstrated antimicrobial activity against *N. gonorrhoeae S. typhi, P. aeruginosa, K. pneumoniae*, and *V. cholera* at a concentration of 1000 µg/ml (Table 1). Most of the fractions obtained had exhibited broad-spectrum antimicrobial activity for many test organisms at 1000 µg/ml which is a good inhibitory concentration. Since crude extracts with activity at concentrations of 1000 µg/ml and below are considered as promising bioactive agents for further work[94].

Table 1. *In vitro* agar streak dilution antimicrobial activity test of *Ocimum gratissimum* extracts

Microbes	Diameter of zone of inhibition (mm)/ Extract concentration (1000µg/disc)				
	CE	HS	CS	MS	SS
N. gonorrheae	11.5	14.3	13.0	14.2	25.0
S. typhi	10.2	12.5	0	0	25.0
P. aeruginosa	10.2	15.5	0	0	25.0
E. coli	12.2	0	0	17.0	25.0
Staph. aureus	12.0	0	0	17.0	25.0
P. vulgaris	0	0	0	0	20.0
K. pneumoniae	12.5	18.0	0	0	25.0
V. cholerae	12.5	18.5	0	0	20.0
Strept. faecalis	0	0	0	0	25.0
B. anthracis	0	0	0	0	25.0

Key: Activity = [a]Results are means ($P < 0.5$) of three replicate values, CE = Crude ethanolic extract, HS = Hexane soluble, CS = Chloroform soluble, MS = Methanol soluble, SS = Streptomycin sulphate

The antimicrobial potency of plants is associated with the secondary metabolites found in its extracts. Fractionation of the crude extracts allows the distribution of these metabolites in to petroleum ether, chloroform, ethyl acetate and methanol fractions according to their polarity[95]. By this, phytocompounds can easily be separated and the associated activity might be correlated to the presence of alkaloids, flavonoids, saponins, sterols and tannins in the individual fraction[96-99]. The result of the preliminary phytochemical screening revealed the presence of tannins, phenolic compounds, terpenoids, sterols, saponins and alkaloids in *O. gratissimum*. In particular the flavonoids, terpenoids and alkaloids were detected in most of the extracts of the basil grown in Nigeria. However, ketones, cardiac glycosides, and flavonols were not detected at all (Table 2).

Carbohydrates, anthraquinones, phenolics, tannins and saponins were present in alcoholic fractions only. Sterols were found in crude ethanolic extract as well as hexane chloroform soluble fractions. The antimicrobial activity of flavonoids is may be due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall; thereby disrupting their membrane integrity[100]. Antioxidants are molecules that can delay or prevent an oxidative reaction catalysed by free radicals. It is noteworthy that phytochemicals are the most important antioxidants in dietary. Such vital metabolites include polyphenols, quinones, flavonoids, catechins, coumarins, terpenoids and in addition to the smaller molecules like ascorbic acid (Vitamin C) and alpha-tocopherol (Vitamin E)[101]. Therefore, the presence of these phytochemicals could support the herbal medicine uses of *O. gratissimum* as antioxidant and its edible leaves being used to prepare soup and tea. The antioxidant effect is mainly due to the presence of phenolic components such as flavonoids and phenolic acids[101]. On further fractionation, flavonoids and phenolic compounds were found to be predominantly present in the methanol fraction of *O. gratissimum*. Saponins which are glycosides with soapy characteristic are often reported to possess bioactive agents[102]. Tannins have been reported to hinder the development of micro-organisms by their ability to precipitate and inactivate microbial adhesions enzymes and cell envelope proteins[4]. The significant activity observed in this study could thus be attributed to the interaction of one or more of the identified metabolites against the test organisms. On further fractionation, the hexane soluble fraction obtained demonstrated the highest sensitivity towards *V. cholera* (18.5 mm) and *K. pneumoniae* (18.0 mm), followed by the methanol soluble fraction with *Escherichia coli* (17.0 mm) and *Staphylococcus aureus* (17.0 mm). The present results justify the traditional medical uses of *O. gratissimum* for treating diarrhoea, respiratory tract infection and fever. In addition, it could also be used for the range of organisms inhibited, for which the plants is not traditional used for. A full investigation of the toxicity of this plant is required, even though many ethnic groups of Nigeria use the edible leaves *O. gratissimum* to prepare soup and tea for decades.

Table 2. Detection of phytocontituents by different colour indications

Phytocontituents	Reagent used	Positive colour indication	CE	HS	CS	MS
Carbohydrates	Fehling's reagent	Deep blue to red or brick red	+	-	-	+
Ketones	Seliwanoff's reagent	Red	-	-	-	-
Alkaloids	Dragedorff'sreagent	Orange	+	+	+	+
Terpenoids	Anisaldehyde	Purple to blue	+	+	+	+
Cardiac glycosides	10% Ammonium hydroxide	Purple	-	-	-	-
Phenolics	5% Aqueous Iron (III) Chloride	Green to blue	+	-	-	+
Tannins	Ferricchloride	Blue-black to green	+	-	-	+
Flavonoids	Ammonium hydroxide vapour	Deep yellow	+	+	+	+
Anthraquinones	10% Ammonium hydroxide	Violet	+	-	-	+
Flavonols/ Flavones / Chalcones	Aluminium chloride	Yellow	-	-	-	-
Sterols	50% Acetic anhydride in H_2SO_4	Green to blue	+	+	+	-
Saponins	Shaking in water	Froth formation	+	-	-	+

Key: + = Present, - = Absent, CE = Crude ethanolic extract, HS = Hexane soluble, CS = Chloroform soluble, MS = Methanol soluble

The 'Green' movement in Western society has changed attitudes of the general public who now viewed naturally derived substances and extracts as being inherently safer and more desirable than synthetic chemicals products thereby leading to the net increase in sales of herbal preparations[95]. Therefore, 80% of people in the developing world rely on natural products for primary healthcare for man[1-3].

Of the various screening procedures, the cowpea weevil bioassay[87] is the most convenient for general use in a small laboratory. Hence it was adopted for the activity screening of *O. gratissimum*. Weevil perforation index values are recorded for tests in which the damage levels of control seeds are not less than 50%. In this bioassay, a WPI value of 45 or less after 4 months of storage with plant extracts at a dosage of 10% (wt/wt) is considered to be a strong activity, a WPI value of 50 shows that the equal amounts of treated and untreated cowpea seeds were performed. Of all the extracted fractions screened, hexane fraction with 3WPI value showed the best grain protectant activity (Table 3).

Table 3. Residues from fractionation and grain protectant activity of *Ocimum gratissimum* extracts

Solvent	Weight(g)	WPI* (after 4 month)
Crude ethanolic extract	24.5	19
Hexane soluble	15.6	3
Chloroform soluble	5.4	45
Methanol soluble	3.4	50

*Weevil perforation index (WPI) value greater than 50 indicates negative grain protectant effect or enhancement of infestation by weevil

From the results of the phytochemical screening, some phytocompounds identified have previously been associated with repellency activity[73,74,103]. The principal constituent of the essential oil of *O. gratissimum* is eugenol which is responsible for the biological activities observed. The volatile oil of *O. gratissimum* also contain phenols, particularly thymol which was earlier reported to be responsible for the antimicrobial action[11,104]. The studies of the chemical composition of a related species *O. suave* showed that the major components of the oil are p-cymene, α-thujene, myrcene and thymol, in addition to phenols[105-106]. Therefore, these compounds may be responsible for the grains protectant and antimicrobial activities of the hexane fraction of *O. gratissimum* and it could serve as a potential source of pharmaceutical applications and in the control of agronomic pests.

4. Conclusions

The preliminary biological and phytochemical screenings of *O. gratissimum* results are quite promising and have strongly indicated the grain protectant property as well as the antimicrobial activity spectra of aerial parts of the plant. The present result also showed the possible phytocompounds to which the biological activity may be attributable. Further work is ongoing to isolate and elucidate the structure of the bioactive compounds and to screen its pure active constituents against agronomic pests.

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