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PHYTOCHEMICAL, ANTIMICROBIAL AND TOXICITY STUDIES OF *Ocimum gratissimum* LEAF (SCENT LEAF)

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ABSTRACT

The present study investigated the phytochemical, antimicrobial and toxicity studies of *Ocimum gratissimum* leaf. The leaves were extracted using n-hexane, ethyl acetate and methanol. Phytochemical screening was carried out on the three extracts to check for the presence of plant metabolites. Antimicrobial activity of the methanolic leaf extract was carried out using disk diffusion method and the toxicity of the leaves extracts was carried out using *Drosoplila* assay. The results showed the plant leaves contains secondary metabolites such as tannins, saponins, flavonoids, alkaloids, glycosides and steroids/terpenes among others. The *in vitro* antimicrobial screening of the plant extracts showed that they are potential antimicrobial agents against the tested microorganisms with *S. pneumonia* showing highest zone of inhibition of 16 mm at concentration of 240 mg/cm^3. Toxicity using *Drosophila melanogaster* flies showed the plant extracts are safe for oral consumption up to concentration of 320 mg/10g diet. The presence of these secondary metabolites suggests great potentials of the plants as a source of useful phytomedicine.

Keywords: Antimicrobial activity, *Drosophila melanogaster, Ocimum gratissimum*, Phytomedicine

INTRODUCTION

Natural products in particular medicinal plant have impacted positively on humans in many aspects of their health such as the digestive system, nervous system, respiratory system, immune system, circulation, muscles, and joints (Ogwuche and Edema, 2020). Natural products have played central role in prevention and treatment of human diseases during thousands of years and remedies based on natural substances that come from different sources such as terrestrial plants and microorganisms, sea macro and microorganisms, as well as terrestrial invertebrates and vertebrates (Dar *et al*., 2017).

Plant based antibacterial agents have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic counterparts. Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens (Kumaraswamy *et al*., 2008).

The plant species belonging to *Ocimum* genus are widely used for medicinal purposes (Chinedu and Ofili, 2021). *O. gratissimum* has been used extensively by traditional

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healers in many parts of the world. In Nigeria, the plant is used in the treatment of diabetes, cancer, inflammation, anemia, pain, inflammation, diarrhea, fungal and bacterial infections (Ugbogu *et al*., 2021). Figure 1 showed *O. gratissimum* in its natural habitat.

Figure 1: *Ocimum gratissimum* (Maddi *et al*., 2019).

Ocimum species such as *O. gratissimum, O. americanum, O. sanctum, O. Kilimandscharicum* and *O. basilicum* have been reported traditionally to possess medicinal activities and in literature have been reported to exhibit pharmacological activity (Maddi *et al*., 2019).

The farmers in Ekiti state of Nigeria preserve *O. gratissimum* which grows on their farmland in large quantities and is also believed to repel termites (Kayode and Akande, 1998). The plant grows slowly with an average height of 20 to 25m and diameter of 1m, and had average life span of sixty to two hundred years (Sheneni *et al*., 2018).

MATERIALS AND METHODS

Extraction of Plant Material

Extraction was carried out using serial extraction method. Powdered plant material (300 g) was weighed and soaked in 1 liter of n-hexane for 24 hours followed by ethyl acetate and then methanol. The extracts were filtered using Whatman 1 filter paper and the solvent was evaporated using a rotary evaporator. The filtrates were dried and then weighed to determine percentage yield.

Phytochemical Screening

Phytochemicals screening of all the evaporated solvent extracts of *O. gratissimum* were tested for the presence of alkaloids, anthraquinones, cardiac glycoside, steroids/terpenes, flavonoids, saponins and tannins were carried out in all the fraction in accordance with the standard procedure as follows:

Test for Alkaloids

Dragendorff's Reagent: Three drops of Dragendorff's reagent were added to the 2 cm^3 crude extract in the test tube. The formation of a reddish-brown precipitate indicates the presence of alkaloids (Trease and Evans, 2008).

Wagner's Reagent: Three drops of Wagner's reagent were added to crude extract (2 cm^3) in a test tube; formation of dark brown precipitate reveals the presence of alkaloids (Trease and Evans, 2008).

Mayer's Reagent: Few drops of Mayer's reagent were added to crude extract (2 cm^3) in a test tube, an orange precipitate indicates the presence of alkaloids (Trease and Evans, 2008).

Test for Anthraquinones

Borntrager's Test: Two (2) cm^3 of methanol and 2 cm^3 of 10 % ammonia solution were

added to 2 cm^3 of crude extract, formation of rose pink coloration confirmed the presence of Anthraquinones (Trease and Evans, 2008).

Test for Cardiac glycosides

Keller Kiliani's Test: Fifty milligrams (50 mg) of crude extract was dissolved in 1 cm^3 of glacial acetic acid containing some ferric chloride solution. The solution was transferred to a cleaned test tube followed by addition of 2 cm^3 of sulfuric acid. Formation of brown ring at the interface indicates the presence of deoxysugars (Trease and Evans, 2008).

Test for Steroids/Terpenes

Liebermann-Burchards' Test: Two (2) cm^3 of the crude extract was for melted in chloroform (2 cm^3). Then 5 cm^3 acetic anhydride and concentrated sulfuric acid were added to the solution, formation of green coloration showed the presence of steroids (Silva *et al*., 1998).

Salkowski's Test: The crude extract (2 mg) was dissolved in chloroform (5 cm^3) followed by addition of concentrated sulfuric acid (2 cm^3) to the mixture, formation of a red precipitate confirms steroids (Sofowara, 2008).

Test for Flavonoids

Ferric Chloride Test: Two (2) cm^3 of 5 % ferric chloride solution was mixed with crude extract (2 mg), formation of green precipitate shows the presence of flavonoids (Trease and Evans, 2008).

Test for Saponins

Two (2) cm^3 of crude extract was diluted with 5 cm^3 of distilled water and shaken vigorously and allowed to stand for 15 minutes, persistent foaming indicates the presence of saponins (Silva *et al*., 1998).

Test for Tannins

Ferric Chloride Test: five (5) cm^3 distilled water was added to 2 mg of the crude extract. The mixture was heated to boil for five (5) minutes, then two drops of 5% $FeCl_3$ were added. Formation of greenish precipitate showed the presence of tannins (Trease and Evans, 2008).

Antimicrobial Susceptibility Test

The antibacterial activity of the 3 extracts of *Ocimum gratissimum* was determined by a susceptibility test using the agar disk diffusion method as describe by Kirby-Bauer Agar diffusion method (1966). Antimicrobial testing was performed using four (4) bacterial pathogens isolated from clinical practice, namely *Escherichia coli*, *Staphylococcus aureus, Streptococcus pneumonia and Salmonella. typhi* and Ciprofloxacin as positive control while sterile distilled water as negative control. Microorganisms were maintained at 37 ^{O}C on nutrient agar slants, which were sub cultured and reconfirmed by gram staining technique.

Preparation of Media

The Nutrient Agar medium used for the study was prepared according to the manufacturer's instructions (Titan Biotech Limited, India). Twenty-eight gram (28 g) of powdered nutrient agar (CM0003B) was suspended in 1 dm^3 of distilled water which was allowed to mix and dissolved completely and sterilized by autoclaving at 120 ^{O}C for 15 minutes. The slurry was poured into petri dish and allowed to solidify before inoculation (Fawole and Osa, 2001).

Preparation of Paper Disc for Sensitivity Test

Four (4) Whatmann No. 1 filter papers (6 mm diameter) were placed in different concentrations of 30, 60, 120 and 240 mg/ cm^3 for 24 hours. The adsorbed paper disks

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were dried and each was placed on the surface of the media contained in the Petri dishes (Akinpelu *et al*., 2011).

Antibacterial Sensitivity Testing

The four organisms were inoculated into the medium in a separate plate. The paper disks prepared earlier were sufficiently poured unto the plates containing the bacterial cultured using sterilized forceps dipped in alcohol, flamed and cooled at room temperature (27 ^{O}C). The disks were placed on each spaced 15 mm from each other to prevent the resulting zones of inhibition from overlapping. The plates were incubated at a temperature of 37 ^{O}C for 24 hours before being examined for zone of inhibition (ZI) of growth, presence of zone of inhibition (ZI) indicates activity (Hudzicki, 2009). The zones of inhibition were measured using Vernier scale (Panda and Singh, 2014).

Toxicity Testing

Seven-day old adult *Drosophila melanogaster* flies (Harwick strain) were obtained and identified from the Department of Pharmacology, Faculty of Pharmacy University of Maiduguri, Borno state. They were reared under controlled temperature of 25-35 ^{O}C by use of air conditioner (Syed *et al*., 2017). The flies were kept in a glass bottle of height 10 cm^3 and weight 5 cm^3 with the lid closed using cotton wool. The bottles were kept away from sunlight, heat source, chemicals, predator and parasitic organisms.

Preparation of *D. melanogaster* Feed

Tap water (850 cm^3) was used for preparation of the media. One-liter portion of the water was boiled in a container; 350 cm^3 of the water was used to prepare 5 g of agar and corn flour slurries were added to the boiling water and stirred for 10 min. Yeast (10 g) and sugar were also added. After 20 to 30 min of cooking time, heat was turned off and allowed to cool to 55 ^{O}C. Then 0.059 M propionic acid (2.5 cm^3) was added. While still stirring, the feed was quickly transferred into sterilized bottles and further allowed to cool before introducing the flies (Bloomington *Drosophila* Center, 2018).

Solvent Extracts for Toxicity Assay

The *D melanogaster* flies were anesthetized by introducing 1 cm^3 chloroform vapour soaked with cotton wool for 2 min. Twenty adult flies (10 males and 10 females) each were introduced with the aid of a magnifying lens and the lid of the bottles covered with cotton wool. After 10 min, the flies woke up from the sleep and started feeding on the extracts mixed with the food. Percentage death was calculated on day one and recorded until it reaches day seven, the concentrations of the solvent extracts were varied in 8 bottles (Syed *et al*., 2017).

RESULTS AND DISCUSSION

The most useful phytochemicals of plant materials typically result from the metabolites present in the plant. A significant number of studies have been carried out for screening of phytochemicals for traditional as well as orthodox medicinal plant study.

The percentage recovery of the extracts used for the analysis showed that methanol had the highest percentage recovery of 14.68%, followed by ethyl acetate (10.13%) and then n-hexane (7.51%) (Table 1).

Table 1: Percentage Yield of *O. gratissimum*

Extract	Mass (g)	Yield (%)
n-Hexane	37.56 ± 10	7.51
Ethyl acetate	46.00 ± 06	10.13
Methanol	59.90 ± 03	14.68

Each value is presented as Mean ± SD (n=3)

These variations in the percentage yield could be due to differences in polarity of which methanol is greater than ethyl acetate and the least polar solvent being n-hexane. This result

is in line with findings obtained from (Halilu *et al*., 2017).

The preliminary phytochemical screening conducted on the n-hexane, ethyl acetate and methanol leaf extracts of *O. gratissimum* reveals the presence of alkaloids, saponins and tannins in all the three extracts. Anthraquinones, cardiac glycosides, steroid/terpene and flavonoids were found to be present in only the methanol extracts (Table 2).

Table 2: Phytochemical Constituents of the Three Extracts of *O. gratissimum*

Phytochemicals	Test	n-Hexane	Ethyl acetate	Methanol
Alkaloids	i. Mayer's	+	+	+
	ii. Wagner's	+	+	+
	iii. Dragendorff's	+	+	+
Anthraquinones	Borntrager's	-	-	+
Cardiac glycosides	Keller-Kilianis	-	-	+
Steroids/terpenes	i. Salkowski's	-	-	+
	ii. Lieberman-Burchards'	-	-	+
Flavonoids	i. NaOH	-	-	+
	ii. Shinoda's	-	-	+
	iii. Ammonia	-	-	+
Saponins	Frothing	+	+	+
Tannins	i. Bromine water	+	+	+
	ii. Lead acetate			

Key: + = present, - = absent

This result is in line with findings of Uba *et al*. (2021) where the methanolic extracts of *O. gratissimum* was found to contain tannins, flavonoids, steroids, cardiac glycoside, alkaloids terpenoids, and anthraquinones. Alkaloids are one of the main reported medicinal compounds present in different parts of medicinal plants. They are reported to have analgesic, antispasmodic and bactericidal properties (Arpita, 2017). Flavonoid was present in all the three extracts of the plants materials. The abundance of flavonoids which are polyphenolic phytochemicals substances might be responsible for reduced risk of diseases such as cardiovascular, neurodegenerative, cancer and hypertension among others (Mondal and Rahaman, 2020).

Table 3 showed the antibacterial activity of n-hexane, ethyl acetate and methanol extracts of the crude *O. gratissimum* plant maintain at concentration of 30 to 240 mg/cm^3. *O. gratissimum* showed remarkable sensitivity against bacterial isolates used in this study. Higher zones of inhibition of 16 mm were recorded from methanol extract against *S. pneumonia* and no inhibition was recorded against *S. typhi*. The present of phytochemicals may be responsible for the biological activity of the leaf (Devendran and Balasubramanian, 2011).

Table 3: Antibacterial Activity of *O. gratissimum* Methanol Extract

Extract Conc.	Zone of Inhibition (mm)				
(mg/cm^3)	*E. coli*	*S. aureus*	*S. pneumonia*	*S. typhi*	Ampicillin
30	8	7	14	-	28
60	10	9	14	5	25
120	12	12	15	5	24
240	15	13	16	6	21

Fruit flies (*D. melanogaster*) has been a very useful tool in study of the toxicity of plant material, which is a dynamic yet an everlasting subject in medical and

paramedical research that heavily relies on the application of model organisms, pre-clinical testing of new drugs can be carried out extensively before testing on humans (Nainu *et al*., 2019). Table 4 showed the toxicity of all the three extracts against *D. melanogaster*.

Table 4: Toxicity of *O. gratissimum* Extracts against *Drosophila* flies for 5 days

Extract	10 mg/10g diet	20 mg/10g diet	40 mg/10g diet	80 mg/10g diet	160 mg/10g diet	320 mg/10g diet
n-Hexane	0	0	0	0	0	0
Ethyl acetate	0	0	0	0	0	0
Methanol	0	0	0	0	0	0

From the results, it could be observed that the plant extracts are safe for oral consumption up to the concentration of 320 mg/cm^3 for 5 days. The findings this research is in line with findings of Oguanobi *et al*. (2019) where the toxicity of crude leaf extract of *O. gratissimum* was tested against normoglycaemic and diabetic rats and found that at extract dose of 300 mg/kg body weight there was no significant organ toxicity observed.

CONCLUSION

These findings demonstrated the potentials of *Ocimum gratissimum* leaf as a source of phytochemicals with antimicrobial activities and non-toxic against Drosophila melanogaster flies. Further research is warranted to explore the underlying mechanisms and further enhance the therapeutic potential of the plant.

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