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Larvicidal Effects of Methanolic Extract of *Ocimum gratissimum* Leaves on *Anopheles* spp. Larva in Gombe, Gombe State Nigeria

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Abstract
Mosquitoes are among the most notorious insect vectors responsible for transmitting numerous life-threatening diseases, posing serious public health challenges and hindering development in many of the world's poorest nations. Effective vector control remains a crucial component in managing these diseases. However, the widespread emergence of insecticide resistance, along with the negative impacts of chemical insecticides on non-target organisms and the environment, underscores the urgent need for safer, eco-friendly, and sustainable mosquito control strategies. This study evaluated the larvicidal potential of *Ocimum gratissimum* (scent leaf) methanolic extract against *Anopheles* species larvae. The extract was prepared using the cold extraction method, while phytochemical screening was carried out through standard qualitative procedures. Larvicidal assays were performed following the World Health Organization (WHO) guidelines. The methanolic extract yielded 8.14% and revealed the presence of several bioactive compounds, including alkaloids, flavonoids, tannins, steroids, saponins, anthraquinones, phenolics, and glycosides. Bioassay results indicated strong larvicidal activity, with LC_{50} and LC_{90} values of 4.38 mg/ml and 25.40 mg/ml after 72 hours, respectively. These findings suggest that *O. gratissimum* possesses significant potential as a natural larvicide against *Anopheles* species, offering a promising alternative to synthetic insecticides. The aromatic plant extract could serve as a safe, economical, and environmentally friendly mosquito control agent.

Keywords: Vector control, Insecticide resistance, Phytochemicals, Natural insecticides, larvicides.

Introduction

Mosquitoes are recognized worldwide as major disease vectors, responsible for transmitting illnesses that lead to millions of deaths annually (Bagavan *et al.,* 2021). Their widespread distribution and role in disease transmission make them a significant public health concern, especially in low-income countries where they impede socioeconomic development (Hassan *et al.,* 2022). Effective mosquito control strategies focus primarily on breaking the transmission cycle, either by eliminating mosquitoes or preventing their bites. Among various control measures, larval control plays a pivotal role (Bagavan *et al.,* 2021). This can be achieved by environmental modification—such as draining stagnant water, sealing soakaways, and removing containers like old tins or tires—or through the application of larvicides when physical control is not feasible. Chemical insecticides typically act by weakening the larval cuticle, allowing easier penetration of pathogens and ultimately reducing mosquito populations (Dua *et al.,* 2024; Pandey *et al.,* 2021). However, the continuous and indiscriminate use of synthetic insecticides has led to several challenges, including the development of resistance among mosquito species, accumulation of toxic residues through food chains, and harmful effects on humans and other non-target organisms (Senthilkumar *et al.,* 2021; Bagavan *et al.,* 2020; Mishra *et al.,* 2021). Consequently, there is a growing global demand for environmentally sustainable, cost-effective, and biodegradable alternatives that maintain high efficacy in vector control (Senthilkumar *et al.,* 2021).

Plant-based insecticides, or botanicals, have emerged as promising alternatives due to their broad insecticidal properties, biodegradability, and minimal environmental impact (Senthilkumar *et al.,* 2021). These botanical insecticides often contain multiple bioactive compounds that act synergistically on both physiological and behavioral processes of pests, thereby reducing the likelihood of resistance development (Goselle *et al.,* 2021). Given their adaptability to different ecological conditions, botanical insecticides are increasingly regarded as valuable tools in integrated mosquito management programs (Geetha & Geetha, 2024; Ghamba *et al.,* 2024; Ghosh *et al.,* 2022). One such promising plant is *Ocimum gratissimum* (commonly known as scent leaf), belonging to the family Lamiaceae. Widely cultivated in Nigeria, this aromatic herb is traditionally used as a mosquito repellent and for treating respiratory ailments such as bronchitis, catarrh, and sinusitis. The leaves are also used for managing tooth and gum infections and serve as a culinary herb among the Igbo people due to their distinctive aroma. Medicinally, *O. gratissimum* has been reported to exhibit antifungal, antibacterial, antimalarial, and anticonvulsant activities (Singh *et al.,* 2023; Ghosh *et al.,* 2022; Anuagasi *et al.,* 2020). Given its wide medicinal applications and potential as a natural insecticide, this study investigates the larvicidal efficacy of *Ocimum gratissimum* methanolic extract against Anopheles species larvae in Gombe, Gombe State, Nigeria.

Collection of Plant Material

Fresh leaves of *Ocimum gratissimum* (commonly known as scent leaf) were collected from a garden located in Malam Inna Quarters, behind Gombe State

University, Gombe, Nigeria. The plant specimen was authenticated by a botanist at the University Herbarium, where it was identified as *Ocimum gratissimum* and assigned the voucher number GSU H36 (Senthilkumar *et al.,* 2021).

Sample Preparation and Extraction

The collected leaves were thoroughly rinsed with clean water to remove dust and debris, then air-dried on a clean surface at room temperature (28 ± 2°C) for five days. The dried leaves were ground into fine powder using an electric blender. A 100 g portion of the powdered sample was measured with an electronic balance and transferred into a clean container. Methanol (1000 ml) was added at a 1:10 (w/v) ratio, and the mixture was stirred thoroughly. The mixture was then transferred into a conical flask, sealed with aluminum foil, and secured with masking tape to prevent contamination. The flask was placed on an electric shaker for 24 hours to facilitate extraction. The resulting mixture was filtered first through muslin cloth and then through Whatman filter paper to obtain a clear filtrate. The filtrate was left to evaporate at room temperature for seven days to yield the crude methanolic extract, which was stored in sterilized bottles at 4°C until further use (Bagavan *et al.,* 2020).

The percentage yield of the extracts was determined as:

$$\% \text{ Yield} = \frac{\text{Weight of sample extracted}}{\text{Weight of dry extract}} \times 100.$$

A stock solution of 5.00 mg/ml was prepared by dissolving 5000 mg of the crude extract in 1000 ml of distilled water. From this stock, serial dilutions of 4.00, 3.00, 2.00, 1.00, and 0.50 mg/ml were prepared in accordance with WHO (2005) standard procedures.

Phytochemical Screening

Preliminary phytochemical analysis of the methanolic and aqueous extracts of *O. gratissimum* leaves was conducted following the methods of Evans (2020) to identify major bioactive components.

Flavonoids Test: To 4 ml of extract, a small piece of magnesium ribbon and a few drops of concentrated HCl were added. The appearance of a crimson to magenta coloration confirmed the presence of flavonoids (Maryam *et al.,* 2022).

Glycosides Test (Keller–Killiani): To 2 ml of extract, 1 ml of glacial acetic acid containing $FeCl_3$ was added, followed by concentrated H_2SO_4. Formation of a blue coloration indicated glycosides (Maryam *et al.,* 2022).

Saponins Test: One milliliter of extract was shaken vigorously with 5 ml of distilled water. Persistent frothing lasting 15 minutes and emulsification upon addition of olive oil indicated saponins (Maryam *et al.,* 2022).

Tannins Test: Two milliliters of extract were mixed with distilled water and 2–3 drops of 5% $FeCl_3$. The development of greenish-black or blue-black coloration confirmed tannins (Maryam *et al.,* 2022).

Terpenoids Test: Two milliliters of chloroform and 3 ml of concentrated H_2SO_4 were added to 5 ml of extract to form a layer. A reddish-brown coloration at the interface signified terpenoids (Ejikeme et al., 2024).
Steroids Test: Two milliliters of extract were treated with 1 ml of concentrated H_2SO_4. The presence of a red coloration in the chloroform layer indicated steroids (Maryam *et al.,* 2022).
Phlobatannins Test: Ten milliliters of extract were boiled with 5 ml of 1% HCl. Red precipitates confirmed phlobatannins (Maryam *et al.,* 2022).
Anthraquinones Test: Five grams of sample were boiled with 10 ml of aqueous H_2SO_4 and filtered while hot. The filtrate was shaken with 5 ml of benzene, and the benzene layer was treated with 10% ammonia solution. A pink to violet coloration in the ammonia phase indicated anthraquinones (Maryam *et al.,* 2022).

Collection of *Anopheles* spp. Larvae
First and second instar larvae of *Anopheles* mosquitoes were collected from stagnant water bodies in Malam Inna, Abacha Quarters, and Gladimary areas of Gombe, Nigeria. The larvae were transported to the Insectary Laboratory at Gombe State University, where they were identified by an entomologist. Environmental conditions in the insectary were maintained at 27 ± 2°C and 70–80% relative humidity. The larvae were then used for larvicidal bioassays following Maryam et al. (2022).

Preparation of Stock Solution
A 5 g portion of the plant extract was dissolved in 1000 ml of distilled water and shaken thoroughly to ensure complete dissolution. The solution was left to stand for 24 hours to ensure homogeneity before use in bioassays.

Larvicidal Bioassay
Larvicidal activity was assessed against 1st and 2nd instar Anopheles larvae using the WHO (2005) protocol. Test concentrations of 50.0, 40.0, 30.0, 20.0, 10.0, and 5.0 mg/ml were prepared from the stock solution, with four replicates for each concentration, along with a control (distilled water only). Larvae were fed uniformly with carbine powder biscuit every 24 hours. Mortality was recorded after 24, 48, and 72 hours of exposure. Dead larvae were identified by lack of movement when probed with a needle, while moribund larvae were those unable to reach the water surface. Mortality percentages were calculated, and the data were subjected to probit analysis following Finney (1971) using SPSS version 23 to determine LC_{50} and LC_{90} values.

RESULTS
Phytochemical screening of *O. gratissimum* extract revealed the presence of alkaloids, flavonoids, tannins, steroids, saponins, anthraquinones, phenolics, and glycosides. Among these, alkaloids, steroids, saponins, and glycosides were found in relatively higher

concentrations, while flavonoids, tannins, anthraquinones, and phenolics were present in moderate amounts.

The methanolic extract of *Ocimum gratissimum* demonstrated significant larvicidal activity against Anopheles mosquito larvae across all tested concentrations and exposure periods (24, 48, and 72 hours). At the lowest concentration (T1), mortality was consistent at six larvae after 24, 48, and 72 hours. In T2, mortality increased progressively from 13 to 20 larvae across the same intervals. Treatments T3 and T4 showed substantial larval mortality, reaching 20 dead larvae after 72 hours. The highest concentrations (T5 and T6) resulted in near-complete mortality (96.6% and 98.3%, respectively) by the end of the 72-hour period. The control group, which contained only distilled water, showed no mortality, confirming the absence of extraneous toxicity.

The LC_{50} and LC_{90} values obtained after 72 hours were 4.38 mg/ml and 25.40 mg/ml, respectively, indicating strong larvicidal potency.

Table 1: QUALITATIVE PHYTOCHEMICAL RESULT

PHYTOCHEMICALS	INFERENCE
ALKALOIDS	+
FLAVANOIDS	++
TANNINS	+
STEROIDS	+
SAPONINS	++
ANTRAQUINONES	+
PHENOLICS	++
GLYCOSIDES	+

KEY: += PRESENT, ++ =MODERATE, +++= EXCESS and - = ABSENCE

Table 2: QUANTITATIVE PHYTOCHEMICAL RESULTS

Parameters	Concentration
Saponins (mg/100g Gravimetric)	208.0
Tannins (mg/100g Tannic acid eqv)	81.30
Steroids (mg/100g Cholesterol eqv)	125.58
T. Phenols (mg/100 Gallic acid eqv)	123.0
Alkaloids (mg/100g Gravimetric)	169.0
Flavonoids (mg/100g Quarcetineqv)	98.45

Table 3: Mortality rate of the *Anopheles* larva at different concentrations of the *Occimum gratissimum* plant extracts

Plant extract	Concentration (mg/ml)	Number of mosquito larva used	Mortality rate at 24hrs	Mortality rate at 48hrs	Mortality rate at 72hrs
T1	5.0	20	6	7	6
T2	10.0	20	18	20	12
T3	20.0	20	20	17	19
T4	30.0	20	20	19	18
T5	40.0	20	19	19	20
T6	50.0	20	20	19	20
Control	0	20	0	0	0

Table 4: Percentage Mortality and Probit of Mortality of *Occimum grattissimum* plant extracts against *Anopheles* larva

Concentration (mg/ml)	Log Conc.	Total Larva	Mean of Mortality(±SD)	Percentage Mortality (%)	Probit of Mortality	LC_{50}	LC_{90}
5	0.698	20	6±0.1	30	4.48		
10	1	20	17±2.9	85	6.04	4.38	25.40
20	1.301	20	18.7±1.2	93	6.48		
30	1.477	20	19±0.8	95	6.64		
40	1.602	20	19.3±0.5	96.6	6.82		
50	1.698	20	19.7±0.5	98.3	7.12		
Control	0	20	0	0	0		

DISCUSSION

This research investigated the larvicidal potential of *Ocimum gratissimum* (scent leaf) leaf extract against the larvae of Anopheles mosquitoes. The results (Tables 4.1 and 4.2) clearly demonstrated that the extract exhibited significant larvicidal effects, with the degree of mortality varying according to concentration and exposure time. These variations in larval susceptibility are consistent with earlier findings suggesting that different mosquito species display diverse levels of sensitivity to various botanical extracts (Anuagasi *et al.,* 2020).

Among the tested concentrations, Treatment 6 (T6; 50 mg/ml) caused the highest larval mortality, completely eliminating all larvae within 24 hours. This was followed by T5 (40 mg/ml) with 19 deaths, T4 (30 mg/ml) eliminating 18 larvae, T3 (20 mg/ml) eliminating 17 larvae, and T2 and T1 (10 mg/ml and 5 mg/ml) producing mortalities of 13 and 6 larvae, respectively. The results indicate a clear dose-dependent response, where higher extract concentrations led to greater larval mortality. This observation supports the report of El-Akhal et al. (2019), who found that the toxicity of *O. gratissimum* extracts increased proportionally with concentration.

The percentage mortality of Anopheles larvae also increased progressively over time, reaching its peak at 72 hours of exposure. The calculated LC_{50} and LC_{90} values (4.28 mg/ml and 24.40 mg/ml, respectively) confirmed the strong larvicidal efficacy of O. gratissimum extract. Comparable studies by El-Akhal et al. (2019) reported LC_{50} and LC_{90} values of 17 mg/ml and 48.29 mg/ml, respectively—values higher than those obtained in this study. This difference highlights the superior potency of the extract used in the present investigation and supports its potential as an effective natural larvicide (Bagavan *et al.,* 2020).

The phytochemical screening (Table 1) revealed the presence of several bioactive compounds, including alkaloids, flavonoids, tannins, steroids, saponins, anthraquinones, phenolics, and glycosides. Among these, alkaloids, saponins, steroids, and glycosides were more abundant than other compounds. Previous research by Anuagasi et al. (2020) reported that alkaloids, saponins, phenolics, and glycosides possess strong insecticidal and larvicidal properties, which may account for the high mortality observed in this study. Flavonoids are known to play a vital role in plant defense mechanisms against environmental stress and pest attacks. Singh et al. (2023) also documented that flavonoid-rich plant extracts show larvicidal activity against

Anopheles species and could serve as an eco-friendly alternative to chemical insecticides. Similarly, Abu Raihan (2024) observed that flavonoids contribute to plant resistance by acting as natural toxins against insect pests. Furthermore, tannins, as noted by Johnson (2021) and Ashraf (2019), are toxic plant metabolites that can alter aquatic habitats and cause mortality in aquatic organisms.

Therefore, the observed larvicidal effect of O. gratissimum may not be due to a single compound but rather to the combined and synergistic action of multiple phytochemicals—such as alkaloids, tannins, flavonoids, steroids, and glycosides—that together enhance the plant's insecticidal potential.

CONCLUSION

The findings of this study clearly demonstrate that the methanolic extract of Ocimum gratissimum leaves exhibits strong larvicidal activity against Anopheles mosquito larvae. The extract showed considerable efficacy with LC_{50} and LC_{90} values of 4.28 mg/ml and 24.40 mg/ml after 72 hours of exposure, respectively. These results highlight the potential of O. gratissimum as a promising source of environmentally safe and cost-effective mosquito control agents. Given that Anopheles mosquitoes are major vectors of malaria—a disease of significant public health concern in Nigeria and other parts of the world—the application of plant-based larvicides such as O. gratissimum could serve as a sustainable alternative to synthetic insecticides, which often pose health and environmental risks.

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