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Phytochemical screening and larvicidal effects of methanolic leave extract of *Ocimum gratissimum* on *Anopheles* species larva in Gombe, Gombe State Nigeria

Maryam, Mairo Musa [1,*], Isa Shu'aibu [2], Alhassan Alkali [3], Hamma I. I [1] and Inuwa Alhaji Umar [1]

[1] *Department of Intergrated Science, State Polytechnic Bajoga, Gombe State, Nigeria.*
[2] *Department of Biochemistry, Gombe State University, Gombe, Gombe State, Nigeria.*
[3] *Department of Biological Science, Yobe State University, Damaturu, Yobe State, Nigeria.*

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Abstract

Mosquito vectors are among the well-known group of insect that transmit a number of deleterious human diseases, which has pose a major public health challenges affecting development of poorest countries in the world. Vector control is therefore an essential requirement in the control of these diseases. The emergence of insecticide resistance and their effects on non-target population and the environment has necessitated an urgent search for development of new and improved vector control methods that are ecofriendly, economical and effective as well as safe for non-target organisms and the environment. The present study aims to access the larvicidal efficacy of *Ocimum gratissimum* extracts against *Anopheles* species larvae. The *Ocimum* (scent) leaf methanolic extract was obtained using a cold method of extraction, the phytochemical investigation was studied using a simple qualitative analysis and a biological test to determine it larvicidal properties as per the WHO standard protocol. The percent yield of the methanolic extract from leaves of *Occimum gratissimum* was 8.14%. Presence of alkaloids, flavonoids, tannins, steroids, saponins, antraquinones, phenolics and glycosides have been observed. The biological test revealed that the methanolic extract from *Occimum gratissimum* has remarkable larvicidal properties with an LC_{50} and LC_{90} values of 4.28 and 24.40 mg/ml after a period of 72 hours respectively. This may offer great prospective as new control agents against Anopheles spp which is considered a threat to human health in the World especially Nigeria. The *Ocimum gratissimum* plant extracts tested in our study showed potent larvicidal properties that have potential to be developed as a natural insecticides.

Keywords: Vector; Resistance; Phytochemicals; Insecticides

1. Introduction

Mosquito vector transmit serious human diseases that caused millions of deaths every year (Kamaraj *et al.*, 2011). Mosquitoes are among the well-known group of insect vectors that transmit various human diseases, which poses a major public health challenge affecting development in the poorest countries of the world (Redwani *et al.*, 2012). Thus, one of the approaches for control of these mosquito-borne diseases is the interruption of the disease transmission pathway, by destroying the vectors or preventing vectors from biting humans. One of such key to mosquito control is by larval control (Kamaraj *et al.*, 2011) which can be achieved through modification of habitat and breeding sites of the vectors with insecticides. Another way of controlling this infections is by minimizing the larval habitat especially in urban environment which include sealing of drains and soak away, removing receptacles containing water such as old tins, tires etc. In cases where physical control measures are not possible, larvicides may be employed. These insecticides work by weakening the cuticle defense system of the insect larvae causing penetration of pathogenic organisms, thus reclining the mosquito population (Batabya *et al.*, 2014; Dua *et al.,* 2011). However, persistent application of these synthetic insecticides results in development of resistance in vector species and magnification of biological toxic

* Corresponding author: Maryam, Mairo Musa
Department of Intergrated Science, State Polytechnic Bajoga, Gombe State, Nigeria.

substances through the food chain as well adverse effects on environment and non-target organisms including human health (Kannathasan *et al*., 2011; Rahuman *et al.*, 2018; Kishore *et al.*, 2011). Identification of these bio-insecticides that are efficient, as well as adaptive to ecological conditions, is important for continued vector control management. Through the years, plants have been studied to have widespread insecticidal properties and will obviously work as a new tool in the arsenal of synthetic insecticides and in future may serve as suitable alternative product to fight against vector borne diseases (Chowdhury *et al.*, 2017).

In almost all tropical and subtropical countries of the world, to avert proliferation of mosquito borne diseases and to improve the quality of environment and public health, mosquito control is essential. The major tool used in mosquito control operation is the application of chemical insecticides such as organochlorides and organophosphate compounds (Mgbemena, 2010; Mohammed *et al.*, 2010). But this has been unsuccessful due to human, technical, operational, ecological, and economic factors. In recent years, the use of many of the former synthetic insecticides in mosquito control programme has been limited. This may result due to lack of novel insecticides, high cost of synthetic insecticides, their non-biodegradable nature, concern for environmental sustainability, harmful effect on human health, and other non-target populations, increasing insecticide resistance on a global scale and higher rate of biological magnification through ecosystem (Musa *et al.*, 2015). These factors have resulted in a need to search for eco-friendly, cost-effective, biodegradable and target specific insecticides against mosquito vectors. Considering these, the application of eco-friendly alternatives such as biological control of vectors has become the focus of the control programme (Nzelibe and Chintem, 2015).

The most effective alternative methods under the biological control program is aimed at exploring the floral biodiversity and enter the field of using safer insecticides of plants origin as a simple and sustainable method of mosquito vector control. Furthermore, unlike the conventional insecticides which are built on a single active ingredient, plant derived insecticides comprises botanical blends of chemical compounds which will act concertedly on both physiological and behavioural processes (Goselle *et al.*, 2017). Thus, pests may have a little chance of developing resistance to such substances. Identifying these bio-insecticides that are efficient, as well as suitable and adaptive to ecological conditions, is important for continued effective vector management (Geetha T and Geetha N, 2014; Ghamba *et al.*, 2014; Ghosh *et al.,* 2012).

The scent leaf (*Ocimum gratissimum*), is a member of the Family "Labiatae". It is commonly grown as a mosquito repellant. The leaf of *Ocimum* serves as a decongestant for head colds, bronchitis and sinusitis. It is usually chewed in traditional medicine for all tooth and gum disorders and has various other medicinal usages. *Ocimum gratissimum* is used throughout West Africa as anti-malarial and anti-convulsant. The leaves when crushed into juice is used in the treatment of convulsion, stomach pain and catarrh. Oil from the leaves have been found to hold antiseptics, antibacterial and antifungal properties (Ghosh *et al.,* 2012). The findings of Okigbo *et al.* (2010) showed that the extracts of *Ocimum gratissimum* are active in-vitro against human pathogenic dermatophytes.

This study therefore access the larvicidal efficacy of *Ocimum gratissimum* extracts against *Anopheles species* larvae in Gombe, Gombe State, Nigeria.

2. Material and methods

2.1. Collection of Plant Materials

Fresh leaves of *Ocimumm gratissimum* (scent leaf) were collected from a garden in Malam Inna Quarters behind Gombe State University, Gombe, Nigeria. The plant was taken to the school Herbarium and was identified by a botanist as *Ocimum gratissimum* and was given a voucher number; VN: GSU H36. (Kannathasan, 2011).

2.2. Preparation and Extraction of Sample

Leaves of *O. gratissimum* were rinsed with water to remove dirt and spread on a clean surface to dry at room temperature (35 ± 2 °C) for period of five days. The dried leaves were then blended using an electric blender. One hundred gram (100 g) of the powdered sample was measure with an electronic weighing balance and was recorded. The weighed sample was subjected into a clean container and 1000 ml of methanol was poured into the sample and stirred vigorously. It was at the ratio 1:10. The mixture was then transfer into a clean conical flask with constant stirring, the conical flask was sealed with an aluminum foil and a masking tape was used to hold firm the mouth of the flask.

The mixture was placed on an electric shaker for 24 hours and was sieved with muslin cloth, the filtrate was filtered again using filter paper. The final filtrate was then subjected for evaporation for 7 days to obtain the residue (the crude extract). The crude extracts obtained was stored in sterilized container and maintained at 4 °C in a refrigerator.

The percentage yield of the extracts was determined as:

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

$$= \frac{100\text{g (leaf of Ocimum)}}{184.6\text{ (dry extract)}} \times 100\text{ g}$$

$$= 54.34\ \%$$

5000 mg of the methanolic extracts was measured and dissolved in 1000 ml of distilled water to obtain a stock solution of 5.00mg/ml. From the stock, various graded concentrations of 4.00 mg/ml, 3.00 mg/ml, 2.00 mg/ml, 1.00 mg/ml and 0.50 mg/ml was obtained. As described by WHO (2005).

2.3. Phytochemical Screening of Ocimum leaf Extract

Crude methanolic and aqueous extracts of the leaves of *Ocimum grattisimum* was screened for their phytochemical comstituents using the methods described by Evans, (2012).

- Test for Flavonoids: - 4 ml of the plant extract was measured, a piece of magnesium ribbon was added followed by few drops of concentrated hydrochloric acid (HCl). The presence of colour ranging from crimson to magenta indicated that flavonoids are present (Chowdhury *et al.*, 2017).
- Test for Glycosides: - Keller Killianoi test was used to test for the presence of glycosides. To 2 ml of plant extract, add 1 ml of glacial acetic acid with Iron (III) chloride and conc. H_2SO_4. The appearance of blue colour indicates the presence of glycosides (Chowdhury *et al.*, 2017).
- Test for Saponins: - To 1ml of plant extract in a test tube and 5ml of distilled water was added and vigorously shaken. A persistent froth that lasted for at least 15 minutes and on addition to few drops of olive oil formed an emulsion indicated that saponins are present (Chowdhury *et al.*, 2017).
- Test for Tannins: - Measure 2 ml of the extract diluted with distilled water in separate test tubes and 2-3 drops of 5% Iron (III) chloride ($FeCl_3$) solution added. A green - black or blue - black coloration indicated the presence of tannins (Chowdhury *et al.*, 2017).
- Test for Terpenoids: - A mixture of (2 ml) chloroform and 3 ml concentrated H_2SO_4 acid was added to 5 ml of each extract to form a layer. The presence of a reddish-brown colouration at the interface shows positive results for the presence of terpenoids (Ejikeme *et al*., 2004).
- Test for Steroids: - To 2.0 ml of the plant extract, 1ml of concentrated H_2SO_4 was added carefully along the sides of the test tube. A red colour produced in the chloroform layer infers the presence of steroids (Chowdhury *et al.*, 2017).
- Test for Phlobatannins: - 10 ml of plant extract of each sample was boiled with 5 ml of 1 % aqueous hydrochloric acid (HCl). Deposits of red precipitates showed positive result (Chowdhury *et al.*, 2017).
- Test for Anthraquinones: - 5 g of each of the sample will be boiled with 10 ml aqueous sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of benzene. The benzene layer was separated, and 10% ammonia solution was added to half of its volume. A pink, red or violet colouration in the ammonia phase (lower layer) indicates the presence of anthraquinone (Chowdhury *et al.*, 2017).

2.4. Collection of *Anopheles* mosquito larvae

The 1st and 2nd instar Larvae of *Anopheles* spp were used in this investigation and the larva were collected from stagant waters in Malaminna, Abacha quarters and Gladimary within Gombe, Gombe State State. The collected larvas were taken to the insectary laboratory, Gombe State University where it was then transferred to enamel larval trays for identification by an entomologist. Mean room temperature of (35± 2 °C) and a relative humidity of 75-80 percent were maintained in the insectary. A 1st and 2nd instar larva was then picked for larvicidal bioassay (Chowdhury *et al.*, 2017).

2.5. Preparation of Stock Solutions

Stock solutions was prepared by dissolving 5 g of plant extract in 1000 mls of distilled water. It was then shake vigoursily for proper dissolving of extract and allowed to stay for 24 hour.

2.6. Larvicidal Bioassay

Larvicidal bioassay of individual plant extracts was tested against 1st and 2nd instar larvae of *Anopheles*. spp. The larvae in all the bowls were fed every twenty-four hours with the same quantity of carbine powder biscuit which was spread evenly across the water surface. Four replicates of the test concentration and control (without the plant extracts) were tested for anti-larval effects with extract concentrations of 50.0, 40.0, 30.0, 20.0, 10.0, and 5.0 mg/ml. The mortalities of the larva was recorded at intervals of 24, 48, and 72 hours of exposure to extract and the percentage mortality was obtained. Larvicidal activity of each extract was determined by counting the number of dead larvae on daily basis (24 hrs interval).

The dead larvae in the three replicates was averaged and expressed as percentage mortality for each concentration. Dead larvae were recorded when they failed to move after pricking with a needle. (Chowdhury *et al.*, 2017).

2.7. Determination of Lethal Concentration (LC_{50} and LC_{95})

The 24, 48 and 72 hrs lethal concentration value (LC_{50} and LC_{95}) was determined by probit analysis as described by Finney (1971). SPSS version 23 was employed in the analysis.

3. Results

The effects of the plants *Ocimum gratissimum* against the larvae of *Anopheles* mosquito after 24, 48, and 72, hours of exposure are shown in Table 1 below. From the results obtained, Treatment 1 (T1) showed mortality of 6, 6 and 6 after a period of 24, 48 and 72 hrs respectively. Treatment 2 (T2) showed mortality of 13, 18 and 20 after 24, 48 and 72 hrs respectively. Treatment 3 (T3) showed mortality of 17, 19 and 20 in 24, 48 and 72 hrs respectively. Treatment 4 (T4) showed mortality of 18, 19 and 20 after 24, 48 and 72hrs respectively. Treatment 5 (T5) showed mortality of 19, 19 and 20 after periods of 24, 48 and 72 hrs respectively while Treatment 6 (T6) showed mortality of 20, 20 and 19 after periods of 24, 48 and 72 hrs respectively.

Table 2 shows the percentage mortality and probit of mortality of *Occimum gratissimum* plant extracts against *Anopheles spp* larva. From the table, 30% mortality of the *Anopheles* mosquito larvae was observed in T1, 85 % mortality was observed in T2, 93 % was observed in T3, 95 % in T4, 96.6 % in T5 and 98.3 % in T6 for periods of 24, 48 and 72 hrs respectively. The control treatment which contains only the mosquito larvae in distilled water and no extract showed no toxicity.

Table 1 Qualitative phytochemical result

Phytochemicals	Inference
Alkaloids	++
Flavanoids	+
Tannins	+
Steroids	++
Saponins	++
Antraquinones	+
Phenolics	+
Glycosides	++

Key: += present, ++ =moderate, +++= excess and - = absence

Table 3 shows the result of the phytochemical sreening carried out to determine the presence of active ingredients and their concentration in the examined sample which include Alkaloids, Flavonoids, Tannins, Saponins, Phenol, Steroids, and Glycosides in the *Ocimum gratissimum*extracts. The outcome revealed a presence alkaloid, flavonoids, tannins,

steroids, saponins, antraquinones, phenolics, and glycosides. However, alkaloids, steroids, saponins and glycosides were found in more concentration than flavonoids, tannins, antraquinones, and phenolics as shown in table 4.

Table 2 Quantitative phytochemical results

Parameters	**Concentration**
Saponins (mg/100 g Gravimetric)	258.0
Tannins (mg/100 g Tannic acid eqv)	91.50
Steroids (mg/100 g Cholesterol eqv)	135.78
T. Phenols (mg/100 g Gallic acid eqv)	131.0
Alkaloids (mg/100 g Gravimetric)	179.0
Flavonoids (mg/100 g Quarcetineqv)	94.54

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	6	6
T2	10.0	20	13	18	20
T3	20.0	20	17	19	20
T4	30.0	20	18	19	20
T5 T6	40.0 50.0	20 20	19 20	20 20	20 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	30	4.48		
10	1	20	17±2.9	85	6.04	4.28	24.40
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		

Step1: Photography of Collected *Ocimum gratissimum* leaves

Step 2: Sample Preparation and extraction
Step 3: Crude Extract of *Ocimum gratissimum*

Step 4: Photography during phytochemical screening 1
Step 5: Photography during phytochemical screening 2

Step 6: *Anopheles* larva for larvicidal bioassay
Step 7: Photography during larvicidal bioassay

Figure 1 Phytochemical Screening and Larvicidal Bioassay of *Ocimum gratissimum* (Survey, 2021)

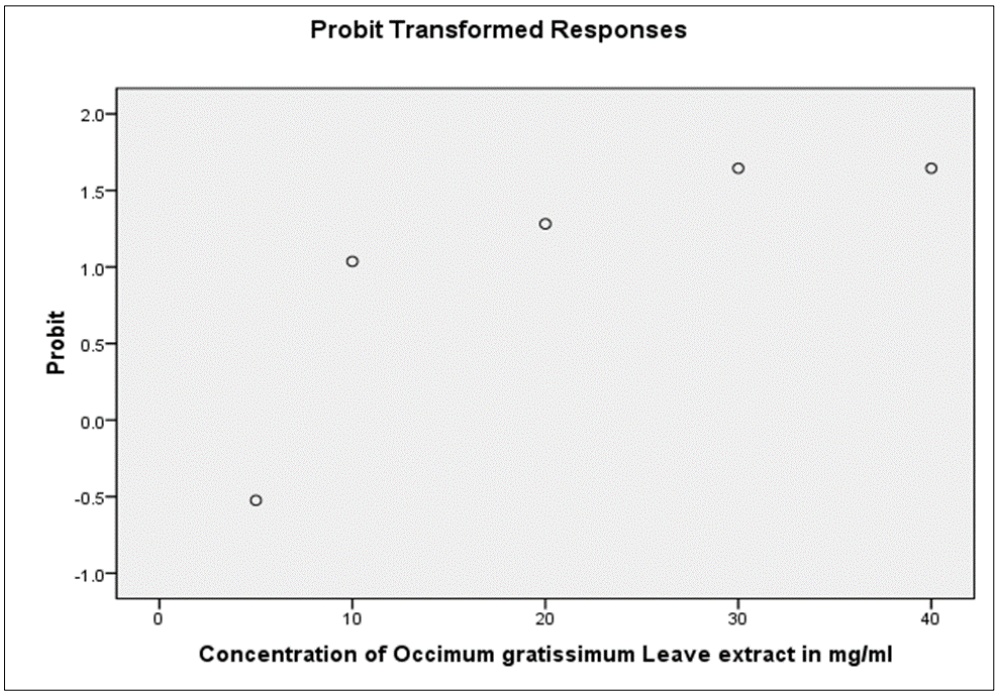

Figure 2 Scatterplot indicating the relationship between log concentration and their corresponding probit mortality figures (Survey, 2021)

4. Discussion

This study was carried out to examine the larvicidal efficacy of *Ocimum gratissimum* leave extract against the larvae of mosquito (*Anopheles*spp). The results as presented in tables 4.1 and 4.2 showed that the leave extracts exhibited good larvicidal activities on the mosquito larvae with varying susceptibility. The varying susceptibility observed was in line with reports from previous findings that various mosquito species showed differential susceptibility to different plant extracts (Das *et al.*, 2012). The 6th treatment (T6) exhibited the highest mortality after 24 hrs of exposure at the concentration of 50mg/ml by eliminating all the larvae at once. T5 (T5) had mortality rate of 19 concentration of 40.0 mg/ml. Treatment 4 (T4) was able to eliminate nineteen (18) mosquito larvae at concentration of 30.0 after 24 hours. Treatment 3 (T3) eliminated 17 larva at concentration of 20.0 mg/ml. Where Treatment 2 (T2) and Treatment 1 (T1) eliminated 13 and 6 mosquito larva at concentrations of 10.0 and 5.0 mg/ml respectively. Average mortality indicate that the *Occimum gratissimum* extracts exhibited significant mortality rate on the targeted mosquito larvae. This is in support of the findings of El-Akhal*et al.,* (2016) which showed that the toxic effect of the *Occimum gratissimum* extracts were proportional to the concentrations with the highest concentration being the most effective.

Also from results observed in Table 4.2 showed the percentage mortality for the *Anopheles* larva increases with increasing concentration of the *Occimum gratissimum* leave extract within 72 hours of exposure. The lethal effect for the instar larva of the malaria vectors showed LC_{50} and LC_{90} values of 4.28 and 24.40 mg/ml respectively. Similarly Studies by El-Akhal*et al.,* (2016) showed LC_{50} and LC_{90} values of 17 and 48.29mg/ml respectively. Thus, these larvicidal activities in literatures are much when compared with the current investigations and this indicates the applicability of the leave extarct as a potential larvicide.

Table 4.4 showed the phytochemical composition of the extract *O. gratissimum* where alkaloids, flavanoids, tannins, steroids, saponins, antraquinones, phenolics and glycosides where found to be present. However, *O.gratissimum* has more of alkaloids, steroids, saponins and glycosides than flavonoid, tannins, antraquinones and phenolics. It was previously reported by Adeniyi *et al*., (2010) that the alkaloids, saponins, phenolics and glycosides exhibit larvicidal properties as such may be responsible for the mortality recorded. Also, flavonoids have been reported to have a key role in stress response mechanisms in plants. Recently, Keerti *et al.,* (2013) in his study reported that flavonoid extracts from different plants exhibit larvicidal activity against *Anopheles* mosquitoes and could be utilized for developing flavonoid-based, eco-friendly insecticide as an alternative to synthetic insecticides. Abu Raihan (2014) in his paper, reported that many of the plant toxins are flavonoids and that they are generally responsible for resistance of plants to insect attack. In contrast,

Johnson (2011) and Ashraf (2017) have confirmed that, tannins, a toxic component present dominantly in plants and vegetables, and there by-products are well known in degrading aquatic habitat and inflict mortalities in aquatic organisms. It is ultimately the mixture of compounds such as flavonoids, tannins, steroids and alkaloids and their synergy which is responsible for the activity of the plant.

5. Conclusion

The extract of *Occimum gratissimum* (scent leaf) examined in this study showed a remarkable activity on larvae of mosquito (*Anopheles* spp) in stage 3 (3^{rd} instar). It was found effective, with an LC_{50} and LC_{95} values of 4.28 and 24.40 mg/ml respectively. It offers great potential as new control agents against *Anopheles* spp which is considered as a serious threat to human health in World and especially Nigeria. The aromatic plant extracts tested in our study demonstrated potent larvicidal properties that have potential to be developed further as natural insecticides.

Recommendation

In the current study, it may be suggestive that the rapid synthesis of leave extract of *Occimum gratissimum* would be proper for developing a biological method for mosquito control. Nevertheless, more studies are required to be done before the *Occimum gratissimum* extract could be exploited at commercial scale.

Compliance with ethical standards

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Disclosure of conflict of interest

There was no conflict of interest in the work as all authors contributed towards publishing the article.

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