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Comparative Evaluation of the Larvicidal Properties of Methanol Extracts and Fractions of *Ocimum gratissimum* L. and *Ocimum basilicum* L. Leaves (Lamiaceae) on the Fourth Instar Larvae of *Culex quinquefasciatus* L. and Control of Filariasis

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RAU and IIJ designed the study, performed the experimental procedures, statistical analysis. Author RAU wrote the first draft of the manuscript. Authors RAU and IIJ supervised lab experiments. Authors AEU, AAE, OTU and LEE organized data, managed the literature searches, assisted in plant material preparation. All authors read and approved the final manuscript.

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ABSTRACT

Background: Botanical insecticides may serve as suitable alternatives to synthetic insecticides because they contain bioactive chemicals. They are relatively safe, biodegradable and readily available in many areas of the world.

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Objective: The aim of this study is to comparatively evaluate the larvicidal properties of methanol extracts and fractions of *Ocimum gratissimum* and *Ocimum basilicum* leaves on fourth instar larvae of *Culex quinquefasciatus*, the vector of filariasis.
Methods: The plants were identified, collected, air-dried and pulverized. The powdered leaves were macerated in 100% redistilled methanol for 72 hours, filtered and concentrated to thick extracts. After the preliminary work was done as activity-guided process, the crude extracts were partitioned into different solvents and again concentrated to thick fractions. The percentage yields were calculated and recorded. The larvae were collected from a location at Ewet Housing Estate, Uyo, Akwa Ibom State. Toxicity was evaluated by exposing fourth instar larvae of *Culex quinquefasciatus* to a concentration range of 0.0625 to 1.000mg/mL of the methanol crude extracts and fractions. The larval mortalities were recorded after 24 hours of exposure and LC_{50} values were determined using the non-linear regression analysis of a statistical package graph pad prism®.
Results: The results of percentage mortalities of methanol crude extracts ranged from 2.5±0.50 to 67.50±1.05% for *O. gratissimum* and 2.5±0.50 to 100±0.00% for *O. basilicum* with their LC_{50} of 0.79mg/mL and 0.18mg/mL respectively. The solvent partitioned fractions also showed that the percentage mortalities of n-hexane and chloroform of *O. gratissimum* ranged from 5±1.00% to 100±0.00% and 17.5±1.5% to 97.5±0.5% with their LC_{50} of 0.29mg/mL and 0.32 mg/mL respectively, while those of *O. basilicum* ranged from 0.25±0.5% to 100±0.00% for n-hexane and 7.5±0.5% to 92.5±1.5% for chloroform fractions with their LC_{50} of 0.42mg/mL and 0.39 mg/mL compared to that of Nicotine (positive control) with LC_{50} of 0.01mg/mL with a percentage mortality range of 10±1.00 to 100±0.00%.
Conclusion: *O. basilicum* leaf methanol crude extract was more than four times active than that of *O. gratissimum,* but n-hexane and chloroform solvent partitioned fractions of *O. gratissimum* were more active than those of *O. basilicum* considering their percentage mortalities and LC_{50}. Hence, both methanol crude extracts of *O. basilicum* and n-hexane fraction of *O. gratissimum* have greater potentials as larvicides which can also be used in the control of *C. quinquefasciatus* larvae.

Keywords: Culex quinquefasciatus; Larvicide; mortality; Ocimum basilicum; Ocimum gratissimum.

1. INTRODUCTION

Mankind has been totally dependent on plants, as a source of food, shelter and drug. Plants are valuable sources of a wide range of secondary metabolites which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives. In the United States, where chemical synthesis dominates, the pharmaceuticals are based on plant-derived chemicals [1,2].

The use of plants with larvicidal properties in the control of different vectors have been reported recently by Umoh *et al.* [3] where the methanol extracts and fractions of *Myristica fragrans* Houtt Seed and *Thymus vulgaris* L. leaf were comparatively evaluated to show their larvicidal properties on the fourth instar larvae of *Culex quinquefasciatus*. Umoh *et al* [4] also reported on the larvicidal toxicity of extracts and fractions of *Trichilia monadelpha* (Thonn.) J. J. de Wilde and *Trichilia emetica* Vahl on larvae of *Anopheles gambiae.* The findings of Ileke and Adesina [5] using *O. basilicum* and *O. gratissimum* have also demonstrated to be one of the alternative approaches to manage mosquito vectors than the use of synthetic chemical insecticides that causes adverse effect on humans, environment and on non-target aquatic organisms.

Ocimum gratissimum is an aromatic, perennial herb,1-3m tall, stem erect, round-quadrangular, much branched, glabrous or pubescent, woody at the base; leaves are opposite, petiole 2-4.5cm long, slender, pubescent, blade elliptic to ovate, 1.5-16 cm × 1-8.5 cm, membranous sometimes glandular punctuate, base cuneate, entire margin. The fruit consists of 4 dry, one-seeded nutlets enclosed in the persistent calyx (the lower lip closing the mouth of the fruiting calyx). *Ocimum gratissimum* is a variable polymorphic complex species, often subdivided into subspecies and varieties, mainly based on the differences in chemical content, the morphology of the fruiting calyx and on different degrees of hairiness, but the variation forms a continuum [6]. It is an herbaceous plant which belongs to the family Lamiaceae. The plant is indigenous to tropical areas especially India and West Africa. In

Nigeria, it is found in the Savannah and Coastal areas. It is cultivated in Ceylon, South Sea Islands, Nepal, Bengal, Chittagong and Deccan [7].

Ethnomedicinally, it is used as medicinal condiment and culinary purpose. It is used for preparation of teas, infusion and in the treatment of epilepsy, high fever and diarrhoea. It is also used in the treatment of mental illness, management of baby's umbilical cord to keep the wound surfaces sterile. *O. gratissimum* is used in the treatment of fungal infections, cold, catarrh, influenza, pneumonia and conjunctivitis. It possesses diaphoretic, antiseptic, antitussive, antiplasmodial, antipyretic and anti-inflammatory properties. It is also used in the treatment of gonorrhoea, warm infection, elephantiasis, malaria and rheumatism [8,9,10,11,12,13,14, 15,16,17]. It leaves are used to prepare pepper soup and porridge for women after delivery among the Ibibio people in Nigeria.

Pharmacological studies revealed its uses as antimicrobial, antifungal, ovicidal, Leishmanicidal, antidiarrhoeal, antihypertensive and antidiabetic. The chemical constituents include flavonoids, alkaloids, tannins, saponin, steroids, phenolic compounds. Also contains vitamins and minerals [18,19]. It contains essential oil which gives the unique fragrance of the plant.

Ocimum basilicum is an aromatic annual herb, 0.3 to 0.5 m tall, but some cultivars can reach up to 1 meter. Some cultivars such as the "Dark Opal" have leaves and stems deep purple in colour. The leaves are ovate, often puckered flowers, white or pink and fruits have small nutlets which are mucilaginous when wet [20]. It is an important economic crop depending on the species and cultivar, the leaves may taste somewhat like anise with strong pungent, often sweet smell. There are many varieties of *O. basilicum* as well as several related species or species hybrids also called basil. The type used in Italians' food is typically called sweet basil as opposed to Thai basil (*O. basilicum* var. *thyrsiflora*), lemon basil (*Ocimum citriodorum*) and holy basil (*Ocimum tenuiflorum*), which are used in Asia. While most common varieties of basil are treated as annuals, some are perennial in warm, tropical climates including holy basil and a cultivar known as "African Blue". It is used ethnomedicinally as flavour in soups and sauces. it is used to treat flatulence, stomach cramps, diarrhoea, dysentery, kidney complains and infections, whooping cough and various types of fever. The leaves are also used externally to make an insecticide that can protect stored crops from beetle damage [21]. Its chemical constituents include alkaloids, flavonoids, tannins, saponin and cardiac glycosides. It also contains essential oil.

Table 1. Phylogeny of *Ocimum gratissimum* and *Ocimum basilicum* (scientific classification) according to Angiosperm Phylogeny Group (APG) system [22]

Ocimum gratissimum		***Ocimum basilicum***	
Kingdom –	Plantae	Kingdom-	Plantae
Clade-	Tracheophytes	*Clade*-	Tracheophytes
Clade-	Eudicots	*Clade*-	Eudicots
Clade-	Asterids	*Clade*-	Asterids
Order-	Lamiales	Order-	Lamiales
Family –	Lamiaceae	Family-	Lamiaceae
Genus –	*Ocimum*	Genus-	*Ocimum*
Species -	*O. gratissimum* L	Species-	*O. basilicum* L
English Name –	African basil	Common Name-	Sweet basil
Local names-	Ibibo/Efik – Ntong	Local Names-	Ibibio/Efik-Iko
Yoruba –	Efinrin	Yoruba-	Efinrin wewe
Igbo –	Nchu-anwu	Igbo-	Nchu-anwu
Hausa –	Daidoya tagida	Hausa-	Daidoya tagida

Fig. 1. *Ocimum gratissimum* in its natural habitat

Fig. 2. *Ocimum basilicum* in its natural habitat

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

The fresh leaves of *O. gratissimum* and *O. basilicum* were collected from Afaha Oku, in Uyo Local Government Area of Akwa Ibom State, Nigeria (Latitude: 5.0463174 and longitude: 7.9352819). They were identified by Prof (Mrs.) M. E. Bassey in the Department of Botany and Ecological Studies, University of Uyo.

2.2 Larval Collection

Larvae were collected from a breeding site in Ewet Housing Estate in Uyo Local Government Area, Akwa Ibom State, Nigeria (Latitude: 5.0192202 and Longitude: 7.9528028) and were reared in plastic buckets.

2.2.1 Preparation of methanol crude extracts and larval toxicity tests

The dried leaves powders of *O. gratissimum* of 935.81g and 1150.24g of *O. basilicum* were separately extracted with 100% redistilled methanol by cold maceration at room temperature for 72 hours. They were filtered and the filtrates were concentrated to thick extracts. These extracts were used in larval toxicity tests.

2.2.2 Bioassay-guided fractionation of methanol crude extracts

After confirmation of the significant larvicidal activity of the methanol crude extracts, the extracts of 30g were separately dissolved in methanol - water in the ratio 3:1 and partitioned successively with n-hexane, chloroform, ethyl acetate and the residues were considered as aqueous fractions. All the fractions were concentrated to solid residues and percentage yields were obtained and recorded [23].

2.2.3 Preparation of stock solutions

Stock solutions of crude methanol extracts and fractions of n-hexane, chloroform, ethyl acetate and aqueous were prepared as follows: The methanol extract and the fractions of 0.2g were dissolved in 1mL of ethanol and subsequently into 99 ml of dechlorinated water to make up to 100 ml and a stock solution of 2 mg/mL [23].

2.2.4 Larval toxicity test

The prepared stock solution of each extract and fraction of 50 mL, 25 mL, 12.5 mL, 6.25 mL. and 3.125 mL were dispensed into sterile cups and were serially diluted with declorinated water to 100 mL in each disposable cup to the test concentration of 0.0625 to 1.000 mg/mL as activity-guided screening. 20 fourth instar larvae were released into each cup of 100mL solution and toxicity of the extracts and solvent partitioned fractions were obtained by percentage (%) mortality. After 24 hours contact, the number of dead larvae in cups were counted and recorded. Positive and negative experiments were also done with Nicotine and 1% of ethanol respectively. All the experiments were done in duplicates [4].

2.3 Statistical Analysis

Results were expressed as means ± SEM of two independent experiments. Larval toxicities were reported as LC_{50} obtained from Graph Pad Prism® Statistical Software.

3. RESULTS

The results for the percentage mortalities, percentage yields for the crude methanol extracts, standard (Nicotine) and fractions are as summarized in Tables 2 – 7.

Table 2. Showing the percentage mortalities of methanol crude extract, n-hexane, chloroform, ethylacetate and aqueous fractions of *O. gratissimum*

Conc.(mg/mL)	Methanol crude extract	N-hexane	Chloroform	Ethylacetate	Aqueous
1.0000	67.5±1.50	100±0.00	97.5±0.50	15.0±1.00	12.5±1.50
0.5000	25.0±1.00	87.5±2.50	95.0±0.00	10.0±1.00	12.5±0.50
0.2500	10.0±1.00	42.5±0.50	30.0±2.00	5.0±0.00	5.00±0.00
0.1250	5.0±0.00	10.0±0.00	27.5±0.5	5.0±0.00	5.00±0.00
0.0625	2.5±0.50	5.0±1.00	17.5±1.5	5.0±0.00	2.5±0.50

Table 3. Showing the percentage mortalities of methanol crude extract, n-hexane, chloroform, ethylacetate and aqueous fractions of *O. basilicum*

Conc.(mg/mL)	Methanol crude extract	N-hexane	Chloroform	Ethylacetate	Aqueous
1.0000	100±0.00	100±0.00	92.5±1.50	12.5±0.50	17.5±1.50
0.5000	90±0.00	70±1.00	60.0±2.00	10.0±0.00	12.5±0.50
0.2500	85±1.00	7.5±0.50	37.5±0.50	7.5±0.50	12.5±0.50
0.1250	17.5±0.50	2.50±0.50	7.5±1.50	5.0±0.00	10.0±0.00
0.0625	2.5±0.50	2.50±0.50	7.5±0.50	5.0±1.00	7.0±0.50

Table 4. Showing quantity and percentage yields of methanol crude extracts of *O. gratissimum* and *O. basilicum*

Plant	Morphological part used	Quantity extracted(g)	Quantity yield (g)	Percentage yield (%)
O. gratissimum	Leaves	935.81	81.2	8.7
O. basilicum	Leaves	1150.24g	60.41	5.3

Table 5. Solvents partitioned fractions yields

Plants	Quantity used(g)	Solvents used in Partitioning	Yield(g)	Percentage yield (%)
O. gratissimum	30	N-hexane	8.22	27.40
		Chloroform	8.11	27.03
		Ethylacetate	6.85	22.83
		Methanol/water	5.12	17.07
O. Basilicum	30	N-hexane	14.4	48.00
		Chloroform	5.68	18.93
		Ethylacetate	5.61	18.70
		Methanol/water	4.14	13.80

Table 6. Showing the percentage mortality of nicotine (Umoh *et al.* [3])

Conc. (mg/mL)	0.0020	0.0039	0.0078	0.0156	0.0313	0.0625	0.1250	0.2500	0.5000	1.000
%Mortality ±SEM	10 ±1.00	15 ±1.00	45 ±1.00	60 ±1.00	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00

1% ethanol 5±0.00

Table 7. Showing LC_{50} of methanol crude extracts, n-hexane, chloroform, ethyl acetate and aqueous solvent partitioned fractions of *O. gratissimum* and *O. basilicum*

Conc.(mg/mL)	Methanol crude extracts	N-hexane	Chloroform	Ethylacetate	Aqueous
O. *gratissimum*	0.97	0.29	0.32	N.F	N.F
O. *basilicum*	0.18	0.42	0.39	N.F	N.F

N.F – Not Feasible

4. DISCUSSION

Plants even though are served as source of food, drug or shelter, but also serve as active larvicides/ insecticides [3]. Vector control is facing a threat due to emergence of resistance of vector (mosquitoes) to conventional synthetic insecticides, warranting either counter measures or development of newer insecticides [24]. With this development, botanical insecticides may serve as suitable alternatives to synthetic insecticides because of their bioactive chemicals.

The observed larvicidal properties of the leaves extracts and solvent partitioned fractions of these plants therefore could have resulted from the synergistic effects of these chemical compounds present in them which were toxic to larvae of *Culex quiquefasciatus* mosquitoes, the vector of filariasis. Filarial infection involves asymptomatic, acute and chronic conditions. This asymptomatic infection causes damage to the lymphatic system and the kidney as well as alters the body immune system. Acute episodes of local inflammation involving skin, lymph nodes and lymphatic vessels often accompany the chronic lymphatic vessels followed by the chronic lymphoedema or elephantiasis. Some of these episodes are caused by the body immune response to the parasites or mostly as the result of bacterial skin infection where normal defenses have been partially lost due to underlying lymphatic damage.

Elephantiasis may affect male and female genital organs. In male, there may be enlargement of the scrotum and the penis may be retracted under skin which becomes thickened, non-elastic, hot and painful and spermatic cord may become thickened [25]. The external parts of the female genital organs (vulva) may also be affected. A long tumorous mass covered by thickened and ulcerated skin may develop between the thighs. There may also be enlargement of the lymph nodes of the legs. Such body deformities lead to social stigma, financial hardship from loss of income and increased medical expenses. The social economic burdens of isolation and poverty are immense [26].

From the results obtained, methanol crude extracts at a concentration range of 0.0625 to 1.0000mg/ml, the percentage mortalities of *O. gratissimum* recorded 2.5±0.50% to 67.5±1.50% with LC_{50} of 0.79mg/ml, while that of *O. basilicum* was 2.5±0.50 to 100±0.00% with LC_{50} of 0.18mg/ml. Moreover, It was observed that every concentration of *O. basilicum* methanol extract produced higher percentage mortality than that of *O. gratissimum* as shown in tables 2 and 3. This indicated that *O. basilicum* has a higher larvicidal activity than *O. gratissimum* when comparing their percentage mortalities, median lethal concentrations (LC_{50}) as shown in table 7. Just like other reports on larvicidal potential of plants [3,4,5] these findings have demonstrated to be one of the alternative approach to manage mosquito vectors than the use of synthetic chemical insecticides that causes adverse effect on humans, environment and on non-target aquatic organisms.

However, the larvicidal activities of the different solvent partitioned fractions of the two plants were also carried out along with the same concentration range of 0.0625 to 1.0000mg/ml. The results showed that even though the n-hexane fractions of the two plants had 100% mortality at 1.0000mg/mL concentration, n-hexane fraction of *O. gratissimum* recorded a higher activity with LC_{50} of 0.29 mg/mL than n-hexane of *O. basilicum* with LC_{50} of 0.42mg/mL and the chloroform fraction of *O. gratissimum* with LC_{50} of 0.32mg/mL was a little more active than than that of *O. basilicum* with LC_{50} of 0.39mg/mL (Table 7), while the LC_{50} of ethylacetate and aqueous fractions were not feasible, but in a whole the methanol crude extract of *O. basilicum* was more active than the solvent partitioned fractions of *O. gratissimum* considering their percentage mortalities and median lethal concentrations of Nicotine used as positive control and 1% ethanol as negative control.

Hence, it may be necessary to exploit larvicidal activity of crude natural products for rapid vector eradication programmes since the crude extracts of plants are known to have dual advantages of minimal adverse impact on non target beneficial organisms and unlikely event of emergence of pest resistance.

5. CONCLUSION

There are many serious drawbacks with the use of synthetic insecticides for vector control and so alternative means of control are needed. Besides the adverse environmental effect present as micro pollutants from conventional insecticides, most major vectors have become physiologically resistant to many of these compounds. These factors have created the need for environmentally safe, degradable and target specific insecticides against mosquitoes. Most times, specific treatment, the urgent need in preventing and controlling this mosquito-borne disease may be successful if efforts are targeted at the interruption of the transmission cycle of the vector involved. Elimination of mosquitoes is a must in developing countries as they act as a primary vector for the pathogens that cause severe infestations and infections. Hence, the methanol crude extract of *O. basilicum*, n-hexane and chloroform fractions of *O. gratissimum* examined in this study offer great potentials as new control agents against *Culex quinquefasciatus* larvae, the vector of filariasis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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