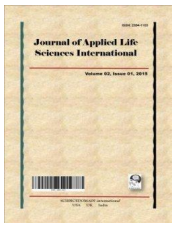

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Larvicidal Activities of N-Hexane Fraction of *Ocimum gratissimum* Leaf against Mosquito Larvae and Its GC-Ms Analysis of Phytoconstituents

F. S. Adefolalu[1*], E. O. Ogbadoyi[1], I. S. Ndams[2] and A. Mann[3]

[1]Department of Biochemistry, Federal University of Technology, Minna, Nigeria.
[2]Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.
[3]Department of Chemistry, Federal University of Technology, Minna, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors FSA and EOO designed the study. Author FSA wrote the protocol and wrote the first draft of the manuscript. Author FSA managed the literature searches. Authors FSA and AM analysed the data, performed the spectroscopy analysis and authors FSA and ISN managed the experimental process and author AM identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the larvicidal efficacy and identify the active phytochemical of *Ocimum gratissimum* n-hexane leaf extract on mosquito larvae.
Study Design: Reflux extraction, standard qualitative phytochemical tests, Column and Thin layer chromatographic methods and GC- MS analysis.
Place and Duration of Study: Department of Biochemistry, STEP-B (Genetic Engineering) Laboratory, Federal University of Technology, Minna between August, 2013 and July 2014.
Methodology: The plant leaves was extracted with 70% methanol, crude extract was partitioned into fractions and healthy mosquito larvae subjected to Standard WHO insecticide susceptibility protocol for the extract efficacy. Column and Thin layer Chromatographic methods were used for phyto-constituent separation of plant extract. The most effective larvicidal column chromatography fraction was subjected to GC-MS analysis for further quantitative and identification of the most

**Corresponding author: Email: funadefolalu@futminna.edu.ng; adeffun@gmail.com;*

effective phytochemical isolates. The phytochemical constituents were determined qualitatively.
Results: Maximum larvae mortality (100%) was observed in 2, 4 and 8% w/v concentrations of leaf extract of *O. gratissimum* 24 h post exposure. Column chromatographic fraction F_{42} gave 100% larvicidal activity at 0.5% concentration. The predominant phytochemical constituents detected in the leaf extract of this plant were terpenes, saponins and tannins. Steroids and phenols were detected in all extracts but absent in the ethanolic leaf extracts. Flavonoids were not detected in any extracts of the plant. The GC-MS analysis of fraction F_{42} from *O. gratissimum* reflected thymol, caryophyllene and terpenoids were present.
Conclusion: The study clearly indicates that *O. gratissimum* possesses chemical compounds that are active against mosquito larvae. Therefore these chemicals can be formulated into mosquito larvicides to prevent mosquito borne diseases.

Keywords: Mosquito; larvicide; plant extract; phytochemicals; chromatography.

1. INTRODUCTION

Mosquitoes transmitted pathogens, (protozoa, viruses, and nematode worms) indirectly cause higher morbidity and mortality among human than any other group of organisms. Malaria, yellow fever, filariasis, West Nile virus, Chikungunya, Elephantiasis, Japanese encephalitis, Rift valley fever, Hemorrhagic fever, Dengue fever, are diseases transmitted by mosquitoes which are responsible for millions of deaths in different parts of the world [1,2].

Mosquitoes borne diseases especially malaria present an obstacle to national development because of its high cost to human and the economy. These diseases impose substantial costs on both individuals and governments [3]. It is responsible for low productivity at work; reduced physical growth and economic turnover [4].

Mosquitoes control measures using insecticides selectively as residual insecticides for indoor spraying and insecticides treated bednets, and repellent for personal protection are *accepted* globally as a recommended strategy by WHO [5,6]. Synthetic insecticides however may have a direct or indirect effect on non target organisms including human. Plant sourced insecticides and repellents are believed to have promising potential because they contain compounds that can eliminate the quick resistance development, are biodegradable and of low toxicity compared to synthetic insecticides.

Plants of Lamiaceae family such as *Ocimum* species (basil) and *Hyptis* species (bushmint) are used traditionally as repellent, fumigants, by rubbing on the skin, hanging on walls, burning of leaves to provide the only economically viable form of personal protection against mosquito in the local communities [7].

The aim of this study is to determine the bio-activity and chemical constituents of extract of *Ocimum gratissimum* leaf used traditionally in Nigeria as repellents and mosquitocides.

2. MATERIALS AND METHODS

2.1 Plant Material and Extraction

Through ethnobotanical study *Ocimum gratisimum* proclaimed to be used as a mosquitocide by inhabitants of Bosso community, Minna, Niger state were collected from a location behind the Federal University of Technology, Minna in the month of October, 2010. The plant was identified and deposited at the herbarium of the National Institute of Pharmaceutical Research Idu (NIPRID), FCT, Nigeria, with the Voucher number NPRID/H/6557.

The leaf part of *Ocimum gratisimum* was washed under running tap water drained, sorted out and dried at room temperature for five days. It was powdered with electric blender (Moulinex) and stored in Polyethylene bottles at room temperature [8].

Extraction of the plant samples were carried out by cold maceration for 72 h but decanted every 24 h; the plant powder to solvent was mixed in a ratio 1:5 (w/v) using 70% aqueous methanol (v/v). Each of the resulting extracts was concentrated using rotary evaporator. The remaining solvent in the extract was allowed to evaporate at room temperature. A 10% stock solution of the 70% methanolic extract *Ocimum gratisimum* leaf were each prepared by dissolving 1g of crude extract in 9 ml acetone [8]. The percentage yield of crude extract was calculated as:

$$\% \text{ Yield} = \frac{\text{Crude extract}}{\text{Crude extract} + \text{dried residue (marc)}} \times 100$$

2.2 Larvicidal Bioassay

Standard WHO larvicidal protocol was employed for the assay. Healthy and wriggling 3rd instars mosquito larvae (25) were introduced into each plastic bowls containing 100 ml of distilled water. A range of different concentrations (0.2, 0.4, 0.8, 1, 2, 4 and 8%) of the stock plant extract solution were introduced and exposure terminated after 2 h, control was in distilled water only. At the end of 2 h exposure time larvae in extract solutions were transferred into plastic bowls containing only 100 ml of distilled water. The number of dead and living larvae in each bowl was recorded at 24 and 48 h post exposure. The larvae were considered dead if they become immobile, do not show the characteristic diving reaction to disturbance of the water or it was in an unnatural position and not capable of rising to the surface after probing with a syringe needle [9]. The larvae were fed on a meal compounded with fish feed and yeast in a 3:1 ratio [9]. Mortality was then calculated.

% Mortality was calculated as:

$$\frac{\text{Number of dead larva}}{\text{Number of larva alive} + \text{Number of dead larva}} \times 100$$

2.3 Partition Fractionation of Crude Extracts

The crude extract of 70% methanolic *Ocimum gratisimum* leaf extract each was separately dissolved in 100 ml of water in a separating funnel, 100 ml of n-hexane was introduce and slowly mixed and allowed to stand for phase separation. The n-hexane fraction was carefully decanted after partitioning while more n-hexane solvent was added and same process repeated until no further colour change was observed with n-hexane. The same procedure was repeated for chloroform and ethyl acetate. Each fraction obtained was concentrated using a rotary evaporator, the remaining solvent in the extract was allowed to evaporate at room temperature to a constant weight. The fractions derived were used to carry out the larvicidal bioassay as described above.

2.4 Column Chromatography

A slurry of silica gel was prepared by dissolving 150 g the gel in 200 ml of n-hexane and packed into a column (2.0 cm x 30 cm). The n-hexane crude extract was dissolved in 20 ml of methanol and 10 g of silica gel were mixed, it was allowed to dry on filter paper and the column was loaded with the extract and silica gel mixture. Sequential elution was carried out starting with n-hexane the least polar solvent and polarity was gradually increased by combining n-hexane and ethylacetate in the ratios 95:5; 90:10; 80:20 and 75:25. A volume of 25 ml of eluted fractions were continuously collected, concentrated and thin layer chromatography (TLC) was carried out. The weights of the recovered extracts were recorded.

2.5 Thin layer chromatography

Thin layer chromatography (TLC) of the n-hexane *Ocimum gratissimum* partitioned fraction was performed using UV active Whatman's thin layer chromatography plates Lk 6D silica gel 60^A, with a thickness of 250 µm and size of 7 x 3.0 cm as the stationary phase. Normal hexane and increased polarity of n-hexane/ ethylacetate (9:1, 4:1, 3:1 and 1.1) were used as the solvent systems for the mobile phase to separate and move the components. Each developing solvent (2 ml) was poured into the developing chamber one at a time, and allowed to saturate the tank. The plates were spotted with the concentrated fractions in three to five places on the pre-coated aluminium plate (7 cm x 3 cm) using capillary tubes. The spotted plate was allowed to dry before placing inside the thin layer chromatographic tank and allowed to run up to the solvent front marked line. The plates were removed and the solvent was allowed to evaporate before viewing under UV light. Visible spots were marked and using a CAMAG UV lamp TL- 600(s0-60H_z, 14VA) at 254 nm (short wave length) and 366 nm (long wave length) other spots were visualized and marked with a soft pencil [10]. The UV is able to reveal functional carbons which absorb at the UV wavelength. The plates were equally placed in iodine tanks to further identify separated spots. The retardation or retention factor (R_f value) was calculated by dividing the distance moved by the developing solvent system front from the origin by the distance moved by the sample zone from the origin. The weights of recovered fractions were recorded. Testable (sizeable) fractions were subjected to larvicidal activity. GC-MS analysis was carried out on the most effective fraction.

3. RESULTS

Tables 1 presents the larvicidal efficacy result of the leaf extract of *Ocimum gratissimum* plant 24

h and 48 h post exposure respectively. The highest larvicidal mortality (100%) for *O. gratissimum* was obtained for the 70% aqueous methanolic leaf extract (2%), while the lowest mortality (96%) was obtained by the acetone leaf extract (8%) this was significantly different at ($p<0.05$). After the 48 h exposure the mortality significantly increased ($p<0.05$) in the acetone and ethanolic leaf extracts of *O. gratissimum*, but not with the n-hexane and 70% methanolic leaf extracts.

Table 2 presents the lethal concentrations (LC_{50} and LC_{90}) values of the *O. gratissimum* plant extracts using the regression equation. The *O. gratissimum* plant 24 h post exposure gave the lowest LC_{50} and LC_{90} of 0.11% (n-hexane solvent extract) and 1.62% for 70% aqueous methanolic extract while the highest were 2.89 and 6.71% for LC_{50} and LC_{90} respectively with acetone solvent extract.

The highest coefficient of determination (R^2) and correlation coefficient values were 0.931 and 0.97 respectively for *O. gratissimum* acetone leaf extract 48h post exposure while the lowest values were 0.724 and 0.85 for ethanolic extract.

Tables 3 shows the larvicidal mortality of mosquito larvae exposed to the partitioned fractions of the 70% aqueous methanolic leaf extract of *O. gratissimum* at 24 h and 48 h post exposure time. Chloroform (2% concentration) gave the highest larvicidal activity 24 h and with 2% concentration 48h post exposure. The lowest mortality was obtained with the aqueous extract. At 24 h and 48 h post exposure time the mortality values for the different solvents were significantly different ($p<0.05$) at lower concentrations but not significantly different for the higher concentrations of extracts and the reference insecticide.

3.1 Phytochemistry of *Ocimum gratissimum* Extract

The qualitative phytochemical constituent of the different solvent leaf extracts of *O. gratissimum* are as shown in Table 4. It shows that only terpenes were detected in all the solvent used to extract the plant but flavonoid was not detected. Saponins and tannins were detected in the acetone, ethanolic and 70% aqueous methanolic leaf extracts but absent in the n-hexane leaf extract of the plant. Steroids and phenols were detected in the n-hexane, acetone and 70% methanolic leaf extracts but absent in the ethanolic leaf extract.

Tables 1. Mortality (%) of *Culex quiquefasciatus* larvae 24 h and 48 h post exposure to leaf extract of *Ocimum gratissimum*

Concentration (%)	n-Hexane	Acetone	Ethanol	70% Methanol
24 h				
0.0	00.00±0.00^a	0.00±0.00^a	0.00±0.00^a	00.00±0.00^a
0.5	54.27±3.05^b	9.33±0.67^a	18.31±0.50^b	33.19±1.57^b
1.0	67.42±2.78^c	22.67±1.33^b	40.58±3.13^b	80.00±2.31^c
2.0	95.33±2.69^d	64.00±2.30^c	57.32±0.17^c	100.00±0.0^d
4.0	100.00±0.00^d	69.33±4.81^c	92.92±1.39^d	100.00±0.0^d
8.0	100.00±0.00^d	96.00±2.31^d	100.00±0.00^e	100.00±0.00^d
Cypermethrin (10%)	100.00±0.00^d	100.00±0.00^d	100.00±0.00^d	100.00±0.00^d
48 h				
0	00.00±00.00^a	00.00±00.00^a	00.00±00.00^a	00.00±0.00^a
0.5	61.33±7.06^b	54.66±5.81^b	41.33±3.52^b	46.66±3.52^b
1.0	81.33±2.67^c	62.66±4.81^b	54.66±7.42^c	85.33±8.11^c
2	97.33±2.67^d	85.33±7.42^c	78.66±1.33^d	100.00±0.00^d
4	100.00±0.00^d	100.00±0.00^d	97.33±1.33^e	100.00±0.00^c
8	100.00±0.00^d	100.00±0.00^d	100.00±0.00^e	100.00±0.00^d
Cypermethrin (10%)	100.00±0.00^d	100.00±0.00^d	100.00±0.00^e	100.00±0.00^d

Values are mean of three determinations and ± standard error of mean. Values in the same column with different superscript are significantly different ($p<0.05$)

Table 2. Larvicidal LC_{50} and LC_{90} (%) Values of leaf extract of *Ocimum gratissimum* on *Culex quiquefasciatus* 24 h and 48 h post exposure

	LC_{50}	LC_{90}	R^2	R	Regression Equation
24 h					
n-Hexane	0.11	2.85	0.797	0.89	y=14.66x+48.26
Acetone	2.89	6.71	0.809	0.90	y=10.49x+19.66
Ethanol	1.94	5.89	0.799	0.89	y=10.16x+30.29
70% Methanol	0.66	1.62	0.834	0.91	y=41.14x+23
48 h					
n-Hexane	0.01	2.77	0.747	0.86	y=14.47x+49.89
Acetone	0.63	3.13	0.931	0.97	y=16x+40
Ethanol	0.49	5.41	0.724	0.85	y=8.129x+46
70% Methanol	0.35	1.56	0.817	0.90	y=33x+38.5

Table 3. Larvicidal mortality of *Culex quiquefasciatus* larvae 24 h and 48 h post Exposure to partitioned fractions of the methanolic leaf extract of *Ocimum gratissimum*

	Mortality (%)			
Concentration g/100ml	**n-Hexane**	**Chloroform**	**Ethyl acetate**	**Aqueous**
24 h				
0	2.00±0.50^{a}	4.00±0.50^{a}	4.00±0.50^{a}	0.00±0.00^{a}
0.5	35.00±2.31^{b}	76.00±3.53^{b}	58.00±2.53^{b}	15.00±1.50^{b}
1.0	68.00±1.33^{c}	94.00±2.31^{c}	65.00±2.67^{b}	20.00±2.31^{bc}
2.0	75.00±1.33^{c}	100.00±0.00^{d}	78.00±2.31^{c}	24.00±1.33^{c}
4.0	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}	26.00±2.67^{c}
8.0	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}	28.00±1.20^{c}
Cypermethrin (10%)	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}
48 h				
0	4.00±1.00^{a}	2.00±0.00^{a}	4.00±0.50^{a}	5.00±0.50^{a}
0.5	40.00±0.30^{b}	86.00±2.00^{b}	66.00±2.3^{b}	20.00±2.31^{b}
1.0	75.00±1.33^{c}	100.00±0.00^{c}	85.00±4.62^{c}	54.00±0.50^{c}
2.0	90.00±1.50^{d}	100.00±0.00^{c}	96.00±2.31^{d}	60.00±1.33^{c}
4.0	100.00±0.00^{e}	100.00±0.00^{c}	100.00±0.00^{e}	66.00±2.50^{d}
8.0	100.00±0.00^{e}	100.00±0.00^{c}	100.00±0.00^{e}	68.00±1.33^{d}
Cypermethrin (10%)	100.00±0.00^{e}	100.00±0.00^{c}	100.00±0.00^{e}	100.00±0.00^{e}

Values are mean of three determinations and ± standard error of mean. Values in the same column with different superscript are significantly different (p<0.05)

Table 4. Qualitative phytochemical constituents of *Ocimum gratissimum* leaf extracts

	n-Hexane	Acetone	Ethanol	Methanol
Alkaloids	+	-	-	-
Flavonoids	-	-	-	-
Steroids	-	-	+	+
Saponin	-	+	+	+
Tannin	-	+	-	+
Terpenes	+	+	+	+
Phenols	-	+	-	+

- Not detected; + Detected

3.2 The Derived Fractions and their Physical Properties

Table 5 presents the pooled fractions of the Column chromatographic separations of the partitioned n-hexane of *O. gratissimum* leaf extract based on the thin layer Chromatogram obtained. Fractions F_9, F_{10}, F_{162} and F_{230} gave two spots while the rest fractions gave multiple spots on the thin layer chromatogram. The physical appearances of the fractions were oily and brownish (F_9), oily and straw coloured (F_{10}),

others ranged from yellowish (F_{162}) to different shades of green ($F_{42,}$ F_{71}, F_{169} - F_{300}).

Table 6 shows the mortality (%) of *Culex quiquefasciatus* larvae exposed to F_{42} column chromatography fractions of n-hexane leaf extract of *O. gratissimum* 24 and 48h post exposure. The 100% activity obtained at 0.5% fraction concentration was comparable with the standard activity. The activity was not significantly different ($p< 0.05$) for the higher fraction concentrations but was significantly different at lower concentration than activity of standard larvicide (cypermethrin) at $p<0.05$.

Table 7 present the GC-MS analysis of n-hexane fractions of *O. gratissimum* leaf extract showing the presence of oleic acid (16.33%), 2-isopropyl-5-methylphenol (10.62),hexadecanoic acid (9.82%), phytol (9.46%), docosenoic acid (8.42%), methyl hexadecanoate (6.26%), carophyellene oxide (3.44%), lancerol (2.57%) in high occurrence while terpin-1-en -4-ol (1.13%) and tetrahydronaphthalene (0.57%) are in low occurrence.

3.3 GC-MS Analysis of Derived Fractions of *O. gratissimum*

Fig. 1 shows the GC-MS chromatogram for fraction F_{42} of n-hexane leaf extract of *O. gratissimum*. Twenty three peaks corresponding to 23 compounds were identified with their retention time (RTS), area % and the fragmentation patterns. The suggested compounds includes: thymol, caryophyllenes, phytol terpineol and tetrahydronaphthalene, Benzenemethanoic acid, 2-Isopropyl-5-methylphenol, 5,6-dihydro-4-(2,3-dimethyl-2-buten-1-yl)-2 H-pyran-2-one and others.

Table 5. Pooled column chromatographic fractions of n-hexane *Ocimum gratissimum* leaf extract after TLC analysis

Serial no.	Fraction	Eluting solvent system	No. of spots	Weight (mg)
1	F_{1-2}	100% n-hexane	2	54
2	F_{3-9}	100% n-hexane	2	1553.1
3	F_{10-16}	n-hexane/ethylacetate (19:1)	2	70.9
4	F_{17-23}	n-hexane/ethylacetate (19:1)		1052
5	F_{24}	n-hexane/ethylacetate (19:1)	More than 2	92.1
6	F_{25-42}	n-hexane/ethylacetate (19:1)	More than 2	2331.4
7	F_{43-71}	n-hexane/ethylacetate (19:1)	More than 2	2845
8	F_{72-78}	n-hexane/ethylacetate (19:1)	More than 2	2057.3
9	F_{79-80}	n-hexane/ethylacetate (19:1)	More than 2	21.6
10	F_{81-105}	n-hexane/ethylacetate (19:1)	More than 2	55.5
11	$F_{106-117}$	n-hexane/ethylacetate (9:1)	More than 2	132.4
12	$F_{118-156}$	n-hexane/ethylacetate (9:1)	More than 2	5.2
13	$F_{157-161}$	n-hexane/ethylacetate (8:2)	More than 2	58.2
14	$F_{162-168}$	n-hexane/ethylacetate (8:2)	2	69.6
15	$F_{169-171}$	n-hexane/ethylacetate (8:2)	More than 2	39.3
16	$F_{172-196}$	n-hexane/ethylacetate (7:3)	More than 2	223.1
17	$F_{197-209}$	n-hexane/ethylacetate (7:3)	More than 2	149.3
18	$F_{210-213}$	n-hexane/ethylacetate (7:3)	More than 2	51.2
19	$F_{214-220}$	n-hexane/ethylacetate (7:3)	More than 2	90.7
20	$F_{221-224}$	n-hexane/ethylacetate (7:3)	More than 2	62.5
21	$F_{225-232}$	n-hexane/ethylacetate (7:3)	More than 2	104.4
22	$F_{233-240}$	n-hexane/ethylacetate (7:3)	More than 2	116.8
23	$F_{241-253}$	n-hexane/ethylacetate (7:3)	More than 2	224
24	$F_{254-259}$	n-hexane/ethylacetate (7:3)	More than 2	37.5
25	$F_{260-261}$	n-hexane/ethylacetate (7:3)	More than 2	154.2
26	$F_{262-276}$	n-hexane/ethylacetate (7:3)	More than 2	200.5
27	$F_{278-279}$	n-hexane/ethylacetate (7:3)	More than 2	2.5
28	$F_{280-286}$	n-hexane/ethylacetate (7:3)	More than 2	2.8
29	$F_{287-294}$	n-hexane/ethylacetate (7:3)	More than 2	5.6
30	$F_{295-296}$	n-hexane/ethylacetate (7:3)	More than 2	1.32
31	$F_{297-300}$	n-hexane/ethylacetate (7:3)	More than 2	2.10

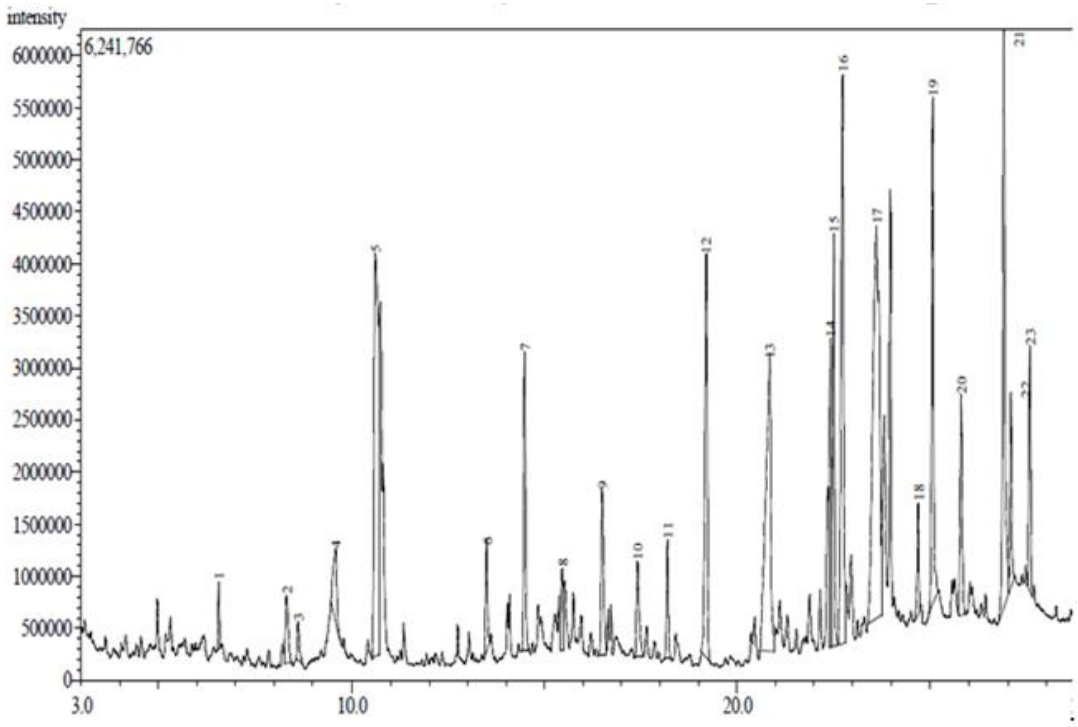


Fig. 1. GC-MS chromatogram for fraction F_{42} of n-hexane leaf extract of *Ocimum gratissimum*

4. DISCUSSION

4.1 Phytochemistry

The presence of saponins, tannins, alkaloids, terpenes and phenols in the extracts of *O. gratissimum* could account for their wild acclaimed medicinal, repellent and mosquitocidal activities traditionally. These phytochemical are known to exhibit medicinal as well as physiological activity [11]. Terpenes, alkaloids, tannins, flavonoids and phenolics compounds are the most effective and important bioactive compounds of plant as insecticide [10] and can be used to control mosquitoes [2,12-15].

The presence of lipophilic groups in phytochemicals give an overall increase in potency. Terpenes are indicated to be the most potent compounds of comparably good larvicidal activity. The presence of terpenoids in plants acts as an anti-feedant, growth disruptor and possesses considerable toxicity toward insects [16]. Terpenoids confer hydrophobicity; hydrophobic compounds are associated with protein deactivation leading to enzyme inhibition [17]. Hence terpenoids are capable of inhibiting the acetyl cholinesterase enzyme and disrupting the function of the neurotransmitters in insects like mosquito and inducing knockdown effects1 [18]. Edeoga, [8] described eugenol, a terpene as the major active component of *H. suaveolens* oil with minor components of thymol, methyl eugenol and β-caryophyllene. Eugenol is also known to disrupt cell membranes of bacteria by the inhibition of ATPase, increasing its permeability and enhancing the uptake of other toxic materials into the cells [19]. Eugenol had been reported to cause blockage of octopamine receptor binding site causing neurotoxicity to the target insects [20]. α-Pinene component acts synergistically with the other active components of *O. gratissimum* and is reported to inhibit respiratory activity [21]. The synergistic action of various active components have been suggested especially that of caryophyllene which is reported to shorten the action potentials of muscle nerves by blocking the calcium and potassium channels of the neurons [22]. Secondary metabolites induce repellent activity via the olfactory system [23]. Essential oil known as 1, 8- cineole has been reported to cause loss of membrane integrity and function, facilitating the uptake of other active ingredients like caryophyllene to effect mortality [24]. *O. gratissimum* being abundant and a locally available plants offers promise as a potential bio-control agent against mosquitoes particularly as larvicides and repellent.

Table 6. Mortality (%) of *Culex quiquefasciatus* larvae 24 h and 48 h post exposure to F_{42} column chromatography fractions of n-hexane leaf extract of *Ocimum gratissimum*

Concentration g/100 ml	24 h	48 h
0	6.67±1.33[a]	17.33±1.33[a]
0.125	64.00±4.00[b]	74.67±4.67[b]
0.25	92.00±6.11[c]	96.67±1.67[c]
0.50	100.00±0.00[d]	100.00±0.00[d]
1.0	100.00±0.00[d]	100.00±0.00[d]
2.0	100.00±0.00[d]	100.00±0.00[d]
Cypermethrin (10%)	100.00±0.00[d]	100.00±0.00[d]

Values are mean of three determinations and ± standard error of mean. Values in the same column with different superscript are significantly different (p<0.05)

Phytochemicals detected in this studied plant may be responsible for the positive larvicidal and repellent effects recorded these chemicals have previously been reported to have insecticidal properties by other workers; Okoli and his team [25] reported that methanolic extracts of *O. gratissimum* to contain: alkaloids, carbohydrates, flavonoids, glycosides, resins, saponin and tannins while petroleum ether leaves extract have steroids and terpenoid present. Ofoegbu and co [26] reported the presence of tannin, alkaloids, flavonoids, cyanogenic glycosides, cardiac glycoside and saponin in ethanolic and methanolic extracts of *O. gratissimum*. The entire absence of flavonoids reported in this study agrees with the findings of [27].

The difference in the phytochemicals present in each extract with respect to solvent used for the extraction may be due to the variation in polarity of the solvents indicating not all phytochemicals can be extracted by all solvents. This finding is supported by reports of other workers, [28] reported the presence of alkaloids and cardiac glycosides in *O. gratissimum* n-hexane leaf extract. Tannin and glycosides are found in chloroform and saponin in ethanol crude extract [29].

4.2 Larvicidal Activity of the Plant Extract

The highest larvicidal activity induced by the 70% methanolic and n-hexane leaf extract of *O. gratissimum* suggest the active principle may be highest in these extracts. This is accounted for by the presence of phytochemical such as alkaloids, terpenes, saponin, tannin and phenol present in the tested extracts.

The demonstrated increase in the larvicidal activity with increased post exposure time as observed in this study is expected as toxicity increases with post exposure time, which may be due to biotransformation or accumulation effect of the active principles, this is in agreement the observation of [30] that active compounds may also have been transformed into more toxic substance resulting in a higher effect on the mosquito larvae.

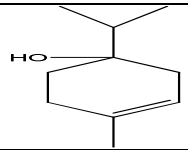
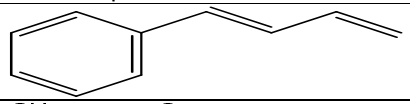
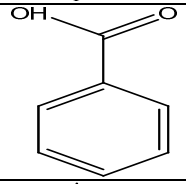
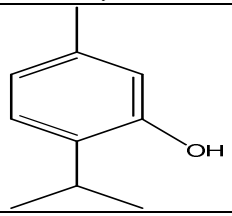
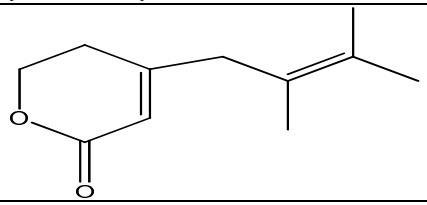
Alkaloids have been known to have larvicidal effect [31], this might account for the high larva mortality of *O. gratisimum* extract. Saponins are known to destroy cell membranes causing haemolysis of red blood cell [32], phenolics compounds aggregate proteins, [33], Tannins cause coagulation of proteins and DNAs. The prevalence of these metabolites in the extracts tested in this work may account for the high efficacy recorded, which is in agreement with the works of [8].

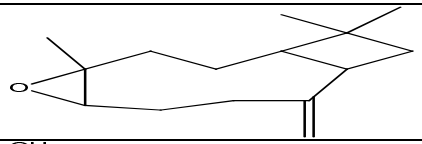
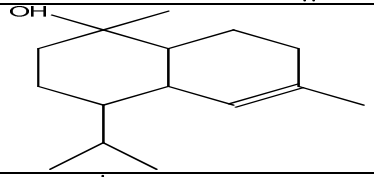
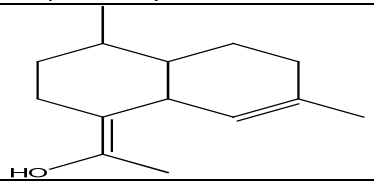
The variations of these phytochemicals (Table 4) in *O. gratissimum* leaf extract being higher may account for the observed high larvicidal activity. This is in agreement with the findings of other authors, who reported that methanolic extracts of different plant parts act as mosquito larvicide [2]. Bansal and his team [34] found that the methanolic extract of different parts of *Solanum xanthocarpum* was of higher larvicidal efficacy than it aqueous extract.

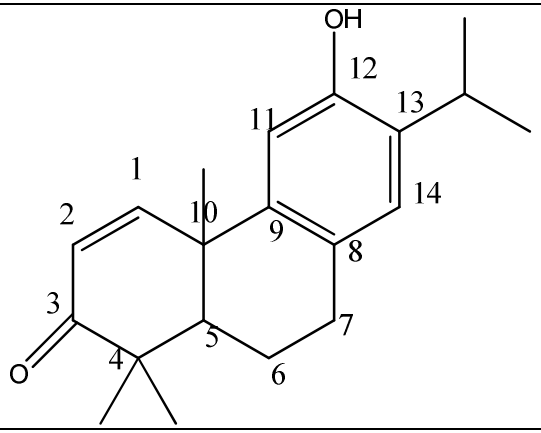
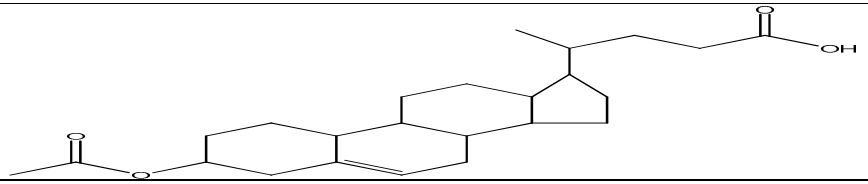
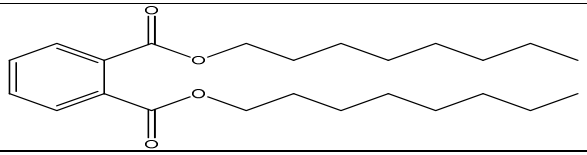
The higher the lethal concentration the less effective is the extract. The lower larvicidal LC_{90} value of tested extracts depicts higher mortality at lower concentration. The LC_{90} in this study is lower compared to the findings of [26] who evaluated the larvicidal LC_{90} for ethanolic leaf extract of *O. gratissimum* to be 464.4 mg/ml while LC_{90} of 1021 mg/ml was reported for the methanolic leaf extract when tested on *An. Gambiae* [35].

Partitioned fractions that had high larvicidal effect were that of the chloroform and ethylacetate and were comparable with the activity obtained for the crude extract. Purification appeared not to have enhanced larvicidal effect. The larvicidal activity of chloroform and ethylacetate were the same because the polarity of these solvents is similar which means the two solvents may have extracted the same phytochemicals. Crude extract may be more effective compared to the individual active compounds separated into fractions due to natural synergism that discourages the development of resistance in the vector [36].

Table 7. Parameters deduced from fraction F_{42} GC-MS spectrum of *Ocimum gratissimum* n-hexane leaf extract

Peak no.	IUPAC Name and RT of compound	Molecular formula	Peak area (%)
1	Decane 6.656	$CH_3(CH_2)_8CH_3$	0.66
2	Terpin-1-en-4-ol 8.324		1.13
3	1,4,5,8-tetrahydronapthalene 8.619		0.57
4	Benzenemethanoic acid 9.589		1.69
5	2-Isopropyl-5-methylphenol 10.603		10.62
6	5,6-dihydro-4-(2,3-dimethyl-2-buten-1-yl)-2H-pyran-2-one 13.490		1.50

Peak no.	IUPAC Name and RT of compound	Molecular formula	Peak area (%)
7	Caryophylene oxide 14.484		3.44
8	α –Cardinol 15.465		1.14
9	Lanceol 16.502		2.57
10	1-octadecyne 17.417	$CH_3(CH_2)_{15}C{\equiv}CH$	1.52
11	9-octadecanone 18.192	$CH_3(CH_2)_8CO(CH_2)_7CH_3$	1.49
12	Methylhexadecanoate 19.204	$CH_3OCO(CH_2)_{14}CH_3$	6.26
13	Hexadecanoic acid 20.857	$CH_3(CH_2)_{14}COOH$	9.82
14	Methyl-11-octadecenoate 22.429	$CH_3OCO(CH_2)_9CH{=}CH(CH_2)_5CH_3$	3.87
15	9,12,15-octadecatrien-1-ol 22.517	$OH(CH_2)_8CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH_3$	4.56
16	Phytol 22.738	$OH(CH_2)CHCH_3(CH_2)_3CHCH_3(CH_2)_3CHCH_3(CH_2)_3CHCH_3CH_3$	9.46
17	Oleic acid 23.629	$COOH(CH_2)_7CH{=}CH(CH_2)_7CH_3$	16.33

Peak no.	IUPAC Name and RT of compound	Molecular formula	Peak area (%)
18	Podocarpa-1,8,11,13-tetraen-3-one 24.704		1.31
19	Glycerin-1,3-distearate 25.066	$CH_3(CH_2)_{16}COOCH_2CHOHCH_2OCO(CH_2)_{17}CH_3$	6.16
20	B –Aceto-5-bisnorcholenic acid 25.800		3.14
21	13-Docosenoic acid 26.905	$CH_3(CH_2)_{20}COOH$	8.42
22	Glycerol-1,2-dipalmitate 27.081	$CH_3(CH_2)_{14}COOCH_2CHOHCOO(CH_2)_{14}CH_3$	2.05
23	1,2-benzenedicarboxylic acid dioctylester 27.572		2.32

The high activity obtained for the purified column fraction of *O. gratisimum* may be due to the presence of thymol, terpineol, caryophyllene, phytol and tetrahydronapthalene. Thymol is known for membrane distruption [37]. Caryophyllenes is a sesquiterpene that acts as a cytotoxic compound in anti-inflamatory and anticancer drugs [38]. This therefore accounts for the larvicidal activity recorded in *O. gratisimum*.

5. CONCLUSION

The n-hexane leaf extract of *O. gratissimum* showed high larvicidal effect which agrees with WHO acceptable standards. This extract had lower larvicidal LC_{50} and LC_{90} and was considered the most effective. The larvicidal activity of *O. gratissimum* leaf extract is due to the demonstrated presence of thymol and caryophyellene, which are known to possess larvicidal properties. The *O. gratissimum* plant was found to contain many bioactive compounds and can therefore be used to formulate mosquito larvicides that are of lower cost and with lesser risk environmental effect which will further benefit the local economy.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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