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IDENTIFICATION OF MOSQUITOCIDAL COMPOUNDS FROM THE LEAF EXTRACTS OF *OCIMUM GRATISSIMUM* (LAMINACEAE) AGAINST DENGUE AND CHIKUNGUNYA VECTOR *AEDES AGYPTI* (L.)

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A B S T R A C T

Dengue and Chikungunya are mosquito-borne disease, transmitted mainly by the *Aedes aegypti* mosquito. The control of mosquito larvae worldwide depends primarily on continued applications of synthetic insecticides. Repeated use of synthetic insecticides in agriculture and public health programs has caused multifarious problems, including toxic hazards to human and non-target organisms. Alternatively, extracts or essential oils from plants may be served as an alternative source of mosquito control agents. Hence, the present study chose various organic solvent extracts (Hexane, chloroform, acetone, ethyl acetate, methanol and water) of *Ocimum gratissimum* leaves for control of mosquito vectors. Results showed the chloroform extract has remarkable pupicidal and adulticidal activity and the values are LC_{50} 19.28mg/l and LC_{50} 16.08mg/l, respectively. Results of Thin Layer Chromatography (TLC) fractions of chloroform extract indicate the presence of phenolic group of compounds. A total of 35 peaks was identified by Gas Chromatography and Mass spectroscopy (GC/MS) analysis, five as considered as major compounds, *i.e,* Hentriaconate, Hepta 2-1 trimethyl, tetracosahexane, Hexamethyl, Benzopyran and Dihydro tetramethyl trimethyl acetate. The present study shows that *O. gratissimum* can act as an efficient toxic agent against mosquitoes. These results suggest that the leaf extracts of *O. gratissimum* have a potential to be used as an ideal eco-friendly approach for the control of mosquitoes.

Keywords: *Ocimum gratissimum; Aedes agypti*; mosquito control, GCMS.

INTRODUCTION

Mosquitoes are major vectors for the transmission of several life-threatening diseases. Dengue and Chikungunya are mosquito-borne viral diseases and transmitted mainly by the *Aedes aegypti* mosquito. They cause an acute illness, including fever, headache, skin rash, and incapacitating arthralgia. The control of mosquito larvae worldwide depends primarily on continued applications of synthetic insecticides (organophosphates such as temphos, fenthion, and insect growth regulators such as diflubenzuron and methoprene). Repeated use of synthetic insecticides in agriculture and public health programs has caused multifarious problems, including insecticide resistance, environmental pollution, destabilization of the ecosystem, and toxic hazards to human and non-target organisms. Alternatively, extracts or essential oils from plants may be served as alternative sources of mosquito control agents, since they constitute a rich source of bioactive compounds that are biodegradable into less toxic products and are potentially suitable for use in the control of mosquito. *Aedes aegypti* (L.) is generally known as a vector for arbovirus responsible for dengue and Chikungunya fever, which are endemic to Southeast Asia, the Pacific island area, Africa, and the Americas (Chretien *et al*., 2007). To prevent the proliferation of mosquito borne diseases, the environment pollution and deficiency of public health, mosquito control is essential. Nowadays, the control of mosquitoes in particular stages (larval, pupa and adults) by using the synthetic chemicals has been an efficient way in the integrated

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vector management. However, a major drawback with the use of chemical insecticides, they are non-selective and could be harmful to other organisms in the environment, cause effects on human health, non-biodegradable nature and also expensive than bioactive substances. These problems, together with the growing incidence of insect resistance, have called attention to the need for novel insecticides, and for more detailed studies of naturally occurring insecticides. Plants may be a source of alternative agents for control of mosquitoes, because they contain rich bioactive chemicals. Mosquito repellent properties of plants were well known from ancient periods. Plant derived products have received increased attention from scientists and more than 2000 plant species are already known to have insecticidal properties and 344 plant species exhibited mosquitocidal activity (Shaallan *et al*., 2005 and Balandrin,1985). Plant extracts/products are considered to be a potential alternative approach against various stages and species of mosquitoes, due to presence of ingredients/phytocompounds which can act as ovicidal, larvicidal, insect growth regulators, repellent, oviposition deterrent and fecundity and fertility reducer (Rajkumar and Jebanesan 2005). *Ocimum gratissimum* L. (Labiatae) is widely distributed in tropical and warm temperature regions. The plant is commonly used in folk medicine to treat different diseases, e.g. upper respiratory tract infections, diarrhea, headache, ophthalmic, skin diseases, pneumonia, and also as a treatment for cough, fever, and conjunctivitis (Corrêa 1932, Onajobi 1986). Previous study stated that the essential oils of *O. gratissimum* have potential antimicrobial activity (Janssen *et al*., 1989; Nwosu and Okafor, 1995; Nakaruma *et al*., 1999). The volatile oil of this plant contains mostly phenols, particularly thymol (Olivier 1960, Sainsbury and Sofowora 1971). Hence, the present study chose various organic solvents (Hexane, chloroform, acetone, ethyl acetate, methanol) and water extracts of *Ocimum gratissimum* for control of mosquito vector *A. aegypti*.

MATERIAL AND METHODS

Plant source and Extraction: The fresh leaves of *O. gratissimum* were collected (during the month of November and December 2013) from Kalvarayan hills, Salem District, Tamilnadu, India. The plant was botanically identified by Dr. D. Natarajan, Assistant Professor, Dept. of Biotechnology, Periyar University, Salem. The voucher specimen has been deposited in the Laboratory for further reference. The collected plant leaves were washed with tap-water to remove unnecessary solid dust particles and they were shade-dried at room temperature. The dried plant material was powdered separately using commercial electrical blender. The processed plant materials (500g) were sequentially extracted by hot extraction methods in a soxhlet apparatus using various organic solvents (Hexane, chloroform, acetone, ethyl acetate and methanol) as well as water for 48 to 74 hours until the mixture solvent become colorless. The plant extracts were filtered through Whatman filter paper No. 1. Extracts were concentrated under reduced pressure at 40°C using rotary vacuum evaporator. The dried crude extracts were weighed for calculating their extractive value and stored in an air tight container at 4°C for further bioassays.

Mosquito source and maintenance: *Aedes aegypti* larvae, pupae and adults were collected from National Centre for Disease Control (NCDC), Connoor, Tamil Nadu, India. It was maintained in Natural Drug Research Laboratory, Department of Biotechnology, Periyar University, Salem. The larvae were kept in plastic trays containing tap-water, and maintained at 27 ± 2°C with 75-85% relative humidity under 14:10 hours light and dark. Larvae were fed with yeast. While adult mosquitoes, dog biscuits and sugar solution were used as feed.

Larvicidal bioassay: The larvicidal activity of plant crude extracts was tested against the larvae of *Aedes aegypti* as per method of WHOPES, (1981). Briefly, in a container, 25 fourth instar larvae was kept in 249 ml of distilled water with 1ml of different concentrations (100, 200, 300, 400 and 500 mg/L) of plant extracts. The chamber containing the control larvae received 1ml of Dimethoxy sulfoxide DMSO served as negative control. After 24 hours exposures the dead larvae were counted and corrected by Abbott's formula (Abbott's, 1925) and the percentage mortality was recorded from the average of three replicates. The average mortality percentage of three replicates was used to carry out lethal concentration (LC_{50}, LC_{90} and LC_{99}) by Probit analysis (Finney, 1971).

Pupicidal bioassay: The pupicidal activity of crude extracts against pupa of *A. aegypti* was evaluated as per the modified method of Kovendan et al., **(**2012**).** For the bioassay, 25 pupae were kept in a container with 249 ml

of distilled water and 1 ml of extracts at different concentrations (100, 200, 300, 400 and 500 mg/L) along with DMSO, which is served as negative control. All containers were maintained at room temperature (28±2ºC) with naturally prevailing photoperiod (12:12h/L: D) in the laboratory. Any pupa was considered to be dead if did not move when probed repeatedly with a soft brush. After exposure period, the dead larvae were counted and mortality was corrected by Abbott's (1925) formula. The percentage mortality was recorded from the average of three replicates (Finney, 1971).

Adulticidal bioassay: About 3-7 days old 15 adult female mosquitoes of *A. aegypti*, were treated with different concentrations (100, 200, 300, 400 and 500 mg/L) of plant crude extracts impregnated filter papers (WHO 1981). The mosquitoes were allowed to acclimatize in the holding tube for 1h and then exposed to test paper for 1h. Mortality was recorded every 10 minutes throughout the exposure period. Mortality of mosquitoes was determined at the end of 24 h recovery period. Percentage of mortality was corrected by using Abbott's formula. LC_{50}, LC_{90} and LC_{99} with 95 % confidence limits were determined using Probit analysis (Finney, 1971) and Chi-squared test was used to compare experimental and control groups, with a significance level established at $P<0.05$ calculated using the SPSS14.0 (Statistical Package of Social Sciences).

TLC profile: The promising plant extract (chloroform extract) showing larvicidal/publicidal/adulticidal activity was selected for the purification of bioactive principles using thin layer chromatography (TLC) and Gas Chromatography and Mass Spectroscopy Gas Chromatography-Mass Spectroscopy analysis. TLC was performed as per the modified method of Wojciech and Teresa, (2002). The glass plates were cleaned and dried in hot air oven. Silica powder was added to distilled water and mixed continuously using a magnetic stirrer. The slurry was poured into a clean dried slide and scattered all over the slide to make a thin film. The silica plates were activated by heating them in hot air oven at 120^0C for 3 hrs. After 3h, the silica plates were allowed to cool at room temperature and marked about 1cm from the bottom. The extracts were loaded in the bottom center of the slide. A beaker was mixed with suitable solvent system (methanol: chloroform (7:3). The plate was kept in the beaker without touching the baseline of solvent and left for development. The final solvent front was marked and the plate was dried. The developed TLC plates were dried and visually observed for various bands. The retention factor (Rf) value was calculated as follows:

$$Rf = \frac{The\ distance\ travelled\ by\ compund}{The\ distance\ travelled\ by\ the\ solvent}$$

Few pieces of iodine crystals were kept in the iodine vapor container. The plates were kept in iodine vapor and left for a few hours. Brown colored bands were visualized. The bands were photographed under UV trans-illuminator (low beam and high beam UV light).

GC MS of Bioactive extracts: The chemical nature of selected extracts was analyzed by gas liquid chromatography (Polaris Q Ion Trap GC/FID) and mass spectrometry (Perkin Elmer Q-700 equipment) as per the protocol of Cheng et al. (2009). Column temperature was programmed at 35°C for 2 min, increased to 180°C at 4 °C/min, then increased to 280°C at 20 °C/min. Helium was used as the carrier gas at a 0.9 ml/min. The mass spectrum was obtained at a 70 eV ionization voltage. The identification of individual compounds was done using Wiley/ NBS Registry of mass spectral database (the NIST (version 3.0) database). Furthermore, the Retention Time (RT) and Kovats Index (KI) values of reference compounds were compared with isolated compounds for identification.

RESULTS AND DISCUSSION

The different solvent extracts of *O. gratissimum* tested against 4^{th} instar larva of *A. aegypti* exhibits broad spectrum of larvicidal activity. The ethyl acetate leaves extract of *O. gratissimum* showed highest activity with LC_{50} value 24.57 mg/l. The moderate activity was observed in acetone extract with the LC_{50} value of 28.70 mg/l. The least larvicidal property was obtained in water extract with LC_{50} values of 52.56 mg/l. The entire process was noticed after 24hours of exposure (Table 1) of plant extracts to mosquito larvae. Results of pupicidal activity of *O. gratissimum* extracts reflect substantial activity against *A. aegypti* pupa. The chloroform extract showed the highest activity and LC_{50} values of 19.28mg/l (Table 2). A moderate activity was seen in ethyl acetate extract with the LC_{50} values of 21.07mg/l. Water extracts of *O. gratissimum* expressed minimum pupicidal activity (LC_{50} values of 38.11mg/l). Different solvent extracts of *O. gratissimum* expressed potential adulticidal activity against the female adult mosquitoes of *A.*

aegypti. Among the different solvents tested the chloroform leaf extract of *O. gratissimum* having a maximum adulticidal property with the LC_{50} values of 16.08mg/l. The moderate activity was observed in acetone extract with the LC_{50} values of 19.16mg/l. Least adulticidal activity was shown in hexane extract with LC_{50} values of 32.01mg/l. The overall LC50 and LC90 values are presented in (Table 3).

Table 1. Larvicidal activity of *O. gratissimum* against *A. aegypti*.

Extracts	Concentration (mg/ml)	% of mortality	LC_{50} (UCL-LCL) mg/l	LC_{90} (UCL-LCL) mg/l	LC_{99} (UCL-LCL) mg/l	Slope	Chi- Square
Hexane	100	35.52±3.84	39.86 (32.75-74.10)	82.19 (61.87-121.69)	215.39 (134.57-401.50)	-4.60	2.85
	200	48.84±6.66					
	300	68.82±3.84					
	400	93.24±6.66					
	500	100.0±0.00					
Chloroform	100	33.3±3.84	37.21 (24.87-70.21)	74.90 (50.83-94.16)	263.70 (18.65-463.76)	8.67	3.31
	200	53.28±6.66					
	300	79.92±6.66					
	400	91.24±3.84					
	500	100.0±0.00					
Ethyl acetate	100	68.82±6.66	24.57 (19.23-32.49)	56.01 (44.27-79.68)	160.83 (98.20-236.62)	6.02	4.47
	200	79.92±6.66					
	300	93.24±3.84					
	400	100.0±0.00					
	500	100.0±0.00					
Acetone	100	48.84±6.66	28.70 (21.67-33.75)	64.97 (46.59-88.17)	197.28 (135.72-376.91)	-10.11	5.69
	200	68.82±3.84					
	300	91.02±7.69					
	400	100.0±0.00					
	500	100.0±0.00					
Methanol	100	22.2±6.66	30.38 (26.63-47.35)	96.77 (72.814139.56)	367.49 (216.26-549.17)	-5.84	6.27
	200	53.28±3.89					
	300	75.48±3.84					
	400	93.24±7.69					
	500	100.0±0.00					
Water	100	13.32±6.66	52.56 (46.23-65.30)	192.69 (97.85-378.10)	368.09 (210.57-673.48)	-6.83	2.88
	200	26.64±3.84					
	300	46.62±7.81					
	400	75.48±6.66					
	500	91.02±3.84					

LC50 lethal concentration that kills 50% of the exposed larvae, LC90 lethal concentration that kills 90% of the exposed larvae, UCL upper confidence limit (95 % fiducial limit), LCL lower confidence limit (95 % fiducial limit).

Table 2. Pupicidal activity of *O. gratissimum* against *A. aegypti*.

Extracts	Concentration (mg/ml)	% of mortality	LC_{50} (UCL-LCL) mg/l	LC_{90} (UCL-LCL) mg/l	LC_{99} (UCL-LCL) mg/l	Slope	Chi- Square
Hexane	100	57.12±3.84	24.86 (17.20-29.38)	34.71 (123.74-429.58)	2962.11 (586.32-4892.11)	-3.82	4.38
	200	82.24±6.66					
	300	95.46±3.84					
	400	100.0±0.00					
	500	100.0±0.00					
Chloroform	100	48.84±6.66	19.28 (15.40-26.47)	67.92 (48.94-94.50)	173.67 (89.13-238.0)	-8.61	2.49
	200	53.28±3.84					
	300	68.82±3.84					
	400	93.24±3.84					
	500	100.0±0.00					
Ethyl acetate	100	68.82±3.84	21.07 (17.36-24.82)	487.26 (165.86-874.93)	2346.51 436.28-3664.37)	-11.81	4.42
	200	75.48±6.66					
	300	95.46±3.84					
	400	100.0±0.00					
	500	100.0±0.00					
Acetone	100	57.72±3.84	22.63 (18.94-26.12)	432.94 (116.24-804.79)	3014.37 (671.32-4218.53)	-5.96	7.06
	200	68.82±6.66					
	300	93.24±6.66					
	400	100.0±0.00					
	500	100.0±0.00					
Methanol	100	22.2±3.84	34.83 (28.61-40.88)	543.20 (17.48-757.89)	3678.14 (813.01-8065.83)	-6.43	3.90
	200	48.28±6.66					
	300	77.7±6.66					
	400	95.46±6.66					
	500	100.0±0.00					
Water	100	17.76±13.22	38.11 (29.77-49.28)	481.98 (173.52-840.507	3207.48 620.20-4647.77)	-8.27	5.42
	200	42.18±6.66					
	300	71.04±3.84					
	400	93.24±3.84					
	500	100.0±0.00					

LC50 lethal concentration that kills 50% of the exposed larvae, LC90 lethal concentration that kills 90% of the exposed larvae, UCL upper confidence limit (95 % fiducial limit), LCL lower confidence limit (95 % fiducial limit).

Table 3. Adulticidal activity of *O. gratissimum* against *A. aegypti*.

Extracts	Concentration (mg/ml)	% of mortality	LC_{50} (UCL-LCL) mg/l	LC_{90} (UCL-LCL) mg/l	LC_{99} (UCL-LCL) mg/l	Slope	Chi- Square
Hexane	100	22.0±1.02	32.01 (11.72-42.18)	72.01 (69.38-81.12)	125.01 (115.03-135.11)	-11.61	3.82
	200	36.0±0.00					
	300	56.0±1.02					
	400	78.1±0.22					
	500	100.0±0.00					
Chloroform	100	24.1±0.22	16.08 (11.72-20.18)	32.28 (29.78-40.31)	98.12 (83.21-108.21)	-8.613	1.85
	200	32.1±0.81					
	300	38.1±1.21					
	400	83.0±0.32					
	500	100.0±0.00					
Ethyl Acetate	100	22.1±1.02	29.21 (23.21-38.31)	65.81 (55.83-62.01)	88.12 (79.03-93.48)	-7.21	7.03
	200	31.3±1.02					
	300	42.0±0.00					
	400	70.0±1.02					
	500	100.0±0.00					
Acetone	100	22.0±0.00	19.16 (10.08-38.11)	42.01 (37.08-51.16)	79.11 (62.01-92.11)	-5.01	5.83
	200	28.0±0.00					
	300	39.0±0.00					
	400	68.5±1.22					
	500	100.0±0.00					
Methanol	100	33.0±0.00	31.18 (26.78-46.21)	78.41 (69.83-82.41)	99.31 (89.24-125.01)	-3.82	1.49
	200	38.0±0.00					
	300	54.0±0.00					
	400	67.5±0.82					
	500	100.0±0.00					
Water	100	33.0±0.00	30.41 (28.31-58.21)	72.41 (69.24-98.11)	112.41 (102.41-132.41)	-4.01	0.221
	200	35.0±0.00					
	300	43.0±0.00					
	400	65.0±1.20					
	500	100.0±0.00					

LC50 lethal concentration that kills 50% of the exposed larvae, LC90 lethal concentration that kills 90% of the exposed larvae, UCL upper confidence limit (95 % fiducial limit), LCL lower confidence limit (95 % fiducial limit).

The TLC has been performed in the chloroform extract of *O. gratissimum* to analyze the band separation in the solvent system of Chloroform: Methanol (by the ratio of 9:1, 8:2 and 7:3). The 7: 3 ratios produced the clear band separation under UV and in the Iodine vapor saturated tank. The Rf values of the separated fractions were calculated. Fraction 1. Rf = 3.4/5.7 = 0.59649cm, 2. Rf = 4.8/5.5 = 0.87273cm and 3. Rf = 3.8/5.6 = 0.67857cm. Based on the Rf values the fractions were found to be as a phenolic group of compounds (Figure 1 A & B).

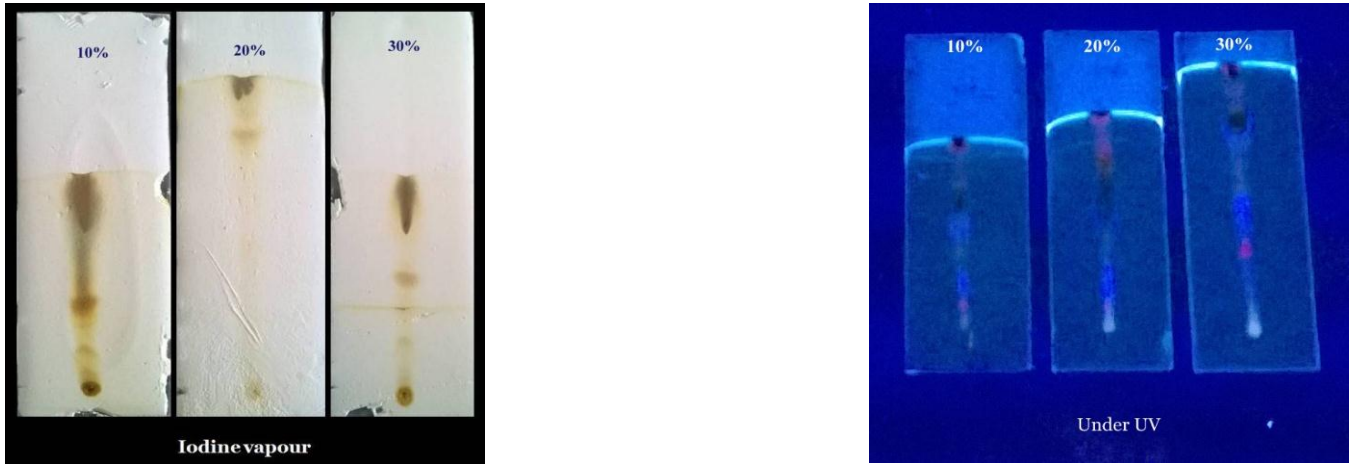


Figure 1. Thin layer chromatography of chloroform extracts of *O. gratissimum*.

A. Spot identification of phenolic compounds in iodine vapour, **B.** Spots visualized under Low beam UV light 10% (methanol 10ml: chloroform 90ml), 20% (methanol 20ml: chloroform 80ml) and 30 % (methanol 30ml: chloroform 70ml).

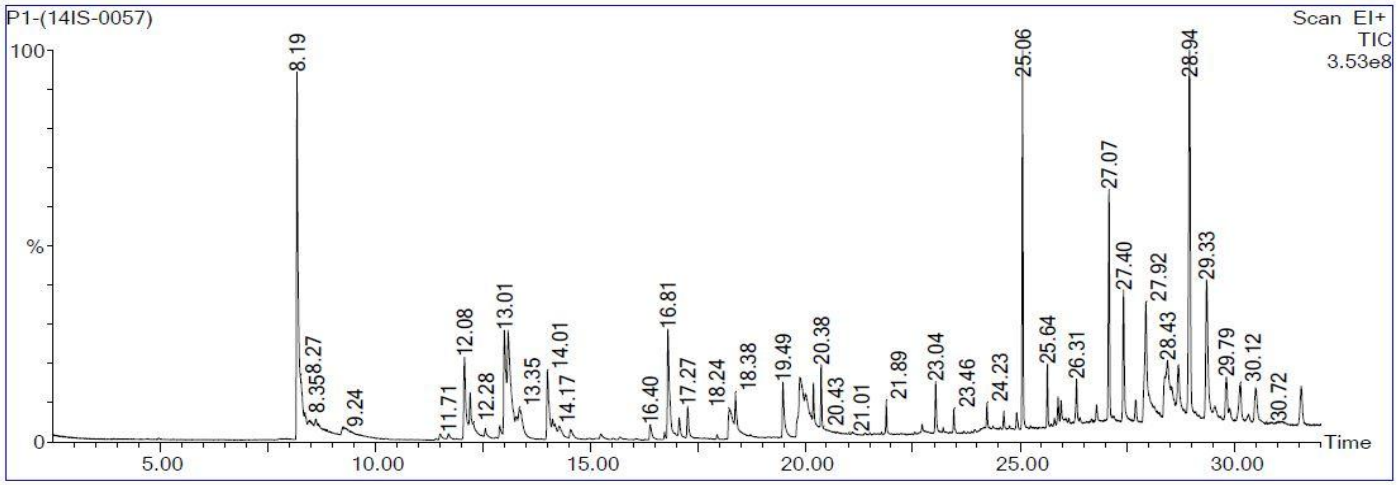
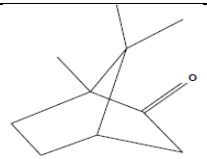
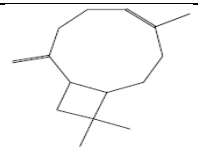
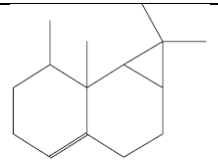
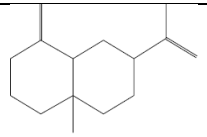
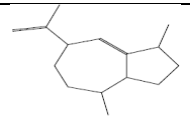
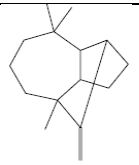
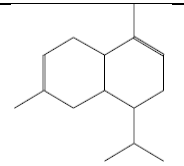
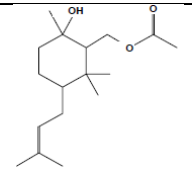
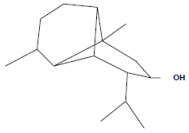
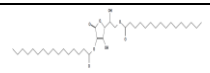


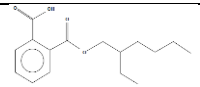
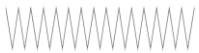
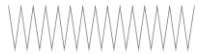
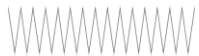
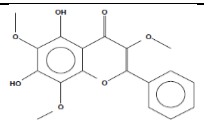
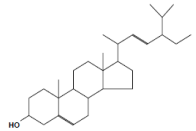
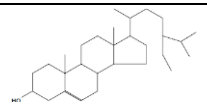
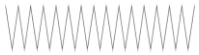
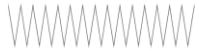
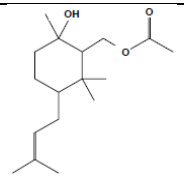
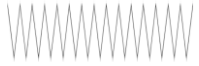
Figure 2. Chromatogram of the components identified in the chloroform extracts of *O. gratissimum* by GC-MS.

The results of thin layer chromatography profile show different band formation (figure 1 A & B) based on the solvent systems (methanol and chloroform) percentage of 10%, 20% and 30%, which are denoted that the polarity of the solvent system and show reliable separation of fractions from the crude extracts. The Rf values are 0.596, 0.678 and 0.872cm respectively. The GC-MS chromatogram of chloroform extract of *O. gratissimum* (Figure 2) show 35 peaks indicate the presence of thirty three phytochemical constituents. The retention time of compounds identified in GC – MS was from 8.186 to 31.535. In comparison, of the mass spectra of the constituents with the NIST library, the abundant phytoconstituents with major peak were characterized and identified. The five major compounds were identified from GC-MS analysis (Table 4). i.e Hentriaconate, Hepta 2-1 trimethyl, tetracosahexane, Hexamethyl, Benzopyran and Dihydro tetramethyl trimethyl acetate. The remaining compounds were identified as minor compounds.

Table 4. GC-MS analysis of chloroform extracts of O. *gratissimum*.

S. No	Retention Time	Peak Area	Name of the Compound	Molecular Weight	Molecular Formula	Structure
1	8.186	10.980	Bicyclo[2.2.1]Heptan-2-One, 1,7,7-Trimethyl-, (1s)-	152	$C_{10}H_{16}O$	
2	12.082	2.790	Caryophyllene	204	$C_{15}H_{24}$	
3	12.212	2.041	1h-Cyclopropa[A]Naphthalene, 1a,2,3,5,6,7,7a,7b-Octahydro-1,1,7,7a-Tetramethyl-, [1ar-(1a.Alpha.,7.	204	$C_{15}H_{24}$	
4	13.008	3.514	Naphthalene, Decahydro-4a-Methyl-1-Methylene-7-(1-Methylethenyl)-, [4ar-(4a.Alpha.,7.Alpha.,8	204	$C_{15}H_{24}$	
5	13.093	5.988	Azulene, 1,2,3,3a,4,5,6,7-Octahydro-1,4-Dimethyl-7-(1-Methylethenyl)-, [1r-(1.Alpha.,3a.Beta.,4.Alp	204	$C_{15}H_{24}$	
6	13.283	0.659	1,4-Methanoazulene, Decahydro-4,8,8-Trimethyl-9-Methylene-, [1s-(1.Alpha.,3a.Beta.,4.Alpha.,8a.Bet	204	$C_{15}H_{24}$	

7	13.353	1.687	Naphthalene, 1,2,4a,5,8,8a-Hexahydro-4,7-Dimethyl-1-(1-Methylethyl)-, [1s-(1.Alpha.,4a.Beta.,8a.Alp	204	C15H24	
8	14.008	2.309	2r-Acetoxymethyl-1,3,3-Trimethyl-4t-(3-Methyl-2-Buten-1-Yl)-1t-Cyclohexanol	282	$C_{17}H_{30}O_3$	
9	14.128	0.677	Dihydro-Cis-.Alpha.-Copaene-8-Ol	222	$C_{15}H_{26}O$	
10	16.809	3.342	Z,Z-6,28-Heptatriactontadien-2-One	530	$C_{37}H_{70}O$	
11	17.269	0.782	Z,Z-6,28-Heptatriactontadien-2-One	530	$C_{37}H_{70}O$	
12	18.240	1.954	L-(+)-Ascorbic Acid 2,6-Dihexadecanoate	652	$C_{38}H_{68}O_8$	
13	18.380	1.761	1-Heptacosanol	396	$C_{27}H_{56}O$	
14	19.485	1.568	Phytol	296	$C_{20}H_{40}O$	
15	19.875	4.215	Methyl 8,11,14-Heptadecatrienoate	278	$C_{18}H_{30}O_2$	
16	20.005	2.762	Methyl 8,11,14-Heptadecatrienoate	278	$C_{18}H_{30}O_2$	
17	20.195	0.990	Chloroacetic Acid, Tetradecyl Ester	290	$C_{16}H_{31}O2Cl$	
18	20.375	1.067	Z,Z-6,28-Heptatriactontadien-2-One	530	$C_{37}H_{70}O$	
19	21.886	0.566	Chloroacetic Acid, Tetradecyl Ester	290	$C_{16}H_{31}O_2Cl$	
20	23.037	1.037	1,2-Benzenedicarboxylic Acid, Mono(2-Ethylhexyl) Ester	278	$C_{16}H_{22}O_4$	
21	25.057	--	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-, (All-E)-	410	$C_{30}H_{50}$	

22	25.638	--	Hentriacontane	436	$C_{31}H_{64}$	
23	26.313	--	Hentriacontane	436	$C_{31}H_{64}$	
25	27.068	--	Hentriacontane	436	$C_{31}H_{64}$	
26	27.403	--	2h-1-Benzopyran-6-Ol, 3,4-Dihydro-2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)-, Acetate, [2r-[	472	$C_{31}H_{52}O_3$	
27	27.924	--	Hentriacontane	436	$C_{31}H_{64}$	
28	28.429	--	5,7-Dihydroxy-3,6,8-Trimethoxyflavone	344	$C_{18}H_{16}O_7$	
29	28.684	--	Stigmasterol	412	$C_{29}H_{48}O$	
30	28.939	--	Hentriacontane	436	$C_{31}H_{64}$	
31	29.334	--	Gamma.-Sitosterol	414	$C_{29}H_{50}O$	
32	29.794	--	Hentriacontane	436	$C_{31}H_{64}$	
33	30.119	--	Hentriacontane	436	$C_{31}H_{64}$	
34	30.475	--	2r-Acetoxymethyl-1,3,3-Trimethyl-4t-(3-Methyl-2-Buten-1-Yl)-1t-Cyclohexanol	282	$C_{17}H_{30}O_3$	
35	31.535	--	Hentriacontane	436	$C_{31}H_{64}$	

DISCUSSIONS

Plants are store house of phytochemicals, which are widely used in the place of synthetic insecticides. The continuous use of synthetic insecticides causes side effects to non-target organisms and insecticide resistance against mosquitoes (Shaalan *et al*., 2005). The results of the present study show the potential mosquitocidal activities of *O. gratissimum.* The crude extracts of different solvents exhibit the potent larvicidal, pupicidal and adulticidal activities against *A. aegypti*. The results of present study highlights chloroform extracts having higher potential mortality rates against tested mosquito. After 24hrs, the least mortality was observed in the chloroform extract against the tested vector. The findings of the present study were positively correlated with the earlier reports of Mohamed Anees (2008), Amer and Mehlhorn (2006) and Eveline Solon *et al*., (2004) showed higher larvicidal, pupicidal and adulticidal activity of *O. sanctum* against *A. aegypti*. Veena Prajapati *et al*., (2005) reported the essential oils of *Ocimum* species having potential larvicidal, adulticidal, ovicidal, oviposition-deterrent and repellent activities against the *A. aegypti* vectors. Likewise, Senthil Kumar *et al*., (2009) reported adulticidal properties of certain medicinal plants (Lamiaceae) against malarial vectors, and Kalaivani *et al*., (2012) worked on larvicidal, adulticidal properties of *Ocimum* species against *A. aegypti* vectors, the extracts of *O. basilicum* showed potential insecticidal activity with least ppm values. Sosan *et al*., (2001) reported larvicidal activities of essential oils obtained from *O. gratissimum*, *Cymbopogon citratus*, and *Ageratum conyzoides* against *Aedes aegypti* as they achieved 100% mortality in 120, 200, and 300 ppm concentrations respectively. The varying results obtained in lethal concentration and lethal time are probably due to the differences in levels of toxicity among the insecticidal ingredients of each plant and the effect of plant extracts are varied with the time of collection and season (Sujatha *et al*., 1998). Thin Layer Chromatography techniques were performed for separation of bioactive compounds. Three different Rf values were observed in the chloroform extracts of *O. gratissimum.* Previous report suggested that the Rf values of some bioactive compounds from *O. gratissimum*, *O. sanctum* and *O. canum* were (0.513, 0.40, 0.58, 0.38 and 0.82) referred as Lutein Pheophytin Xanthophyll Oil Chlorophyll b and β - carotene, *O. sanctum* (0.445, 0.59, 0.74, 0.934) and *O. gratissimum* 21(0.431, 0.573, 0.78) 20 (Quereshi *et al*., 2011) and Eugenol and Methyleugenol from the petroleum ether extracts of *O. sanctum* (Nasare, 2013). The GC-MS results of chloroform extracts of *O. gratissimum*, reported 35 chemical peaks. Many reports stated the identification of the bioactive compounds using GCMS analysis in different species belonging to the Lamiaceae family i.e, *Origanum dictamnus*, *Teucrium polium* and *Lavandula vera* (Proestos *et al*., 2006) and *Thymus comosus* (Pavela *et al*., 2009). So far, several bioactive compounds were identified in the *Ocimum* species using chromatography and mass spectral analysis (Amvam Zollo *et al*., (1998); Eva Klimankova *et al*., (2008) and Bhanu Prakash et al., (2011); and Syeda Khair-ul-Bariyah 2013). The current study exhibits chloroform extracts of *O. gratissimum* was found better lethal (LC_{50}) effect against the *Aedes* vector and the extract having higher larvicidal, pupicidal and adulticidal activity. The outcome of present investigation is highlighting that mosquitoes control property is directly correlated with the increasing concentrations of the crude extracts. The higher concentrations of chloroform extract of plant exhibit maximum larvicidal, pupicidal, adulticidal activities. Further steps pertaining to the structural elucidation of bioactive compounds are in progress.

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