ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2015; 2 (2): 01-08

Received: 03-12-2014
Accepted: 19-01-2015

Tamilselvan Pratheeba
Natural Drug Research Laboratory, Department of Biotechnology, Periyar University, Salem – 636 011, Tamil Nadu, India.

Chinnasamy Ragavendran
Natural Drug Research Laboratory, Department of Biotechnology, Periyar University, Salem – 636 011, Tamil Nadu, India.

Devarajan Natarajan
Natural Drug Research Laboratory, Department of Biotechnology, Periyar University, Salem – 636 011, Tamil Nadu, India.

For Correspondence:
Devarajan Natarajan
Natural Drug Research Laboratory, Department of Biotechnology, Periyar University, Salem – 636 011, Tamil Nadu, India.

Larvicidal, pupicidal and adulticidal potential of *Ocimum gratissimum* plant leaf extracts against filariasis inducing vector

Tamilselvan Pratheeba, Chinnasamy Ragavendran and Devarajan Natarajan

Abstract
The current study was aimed to investigate larvicidal, pupicidal and adulticidal activities of acetone, hexane and chloroform extracts of *Ocimum gratissimum* against filariasis mosquito vector *Culex quinquefasciatus* Chloroform extract exhibited better mortality rate than other extracts with the LC_{50} and LC_{90} values of 2.8916 mg/ml and 5.4521 mg/ml at 24 hrs. The better pupal activity of mosquito was noticed in the same extract exposure at 24 hrs and the LC_{50} and LC_{90} values were of 2.6916 mg/ml and 4.6521 mg/ml. Adulticidal activity of extracts showed varying percentage of mortality at different time intervals. HPLC analysis showed 3 major peaks with the maximum peak area of 72% (at RT of 9.888 minutes). The GC - MS results reveals the presence of 33 compounds, out of them, 5 are identified as bioactive compounds. Major functional groups of bioactive extracts were identified by FT-IR analysis. Results suggested that *O. gratissimum* chloroform extract was an excellent agent for controlling filarial vector, *Cx. quinquefasciatus*.

Keywords: *Cx. quinquefasciatus*; *Ocimum gratissimum*; Phytochemical; TLC; HPLC; GC-MS; FTIR

1. Introduction
Mosquitoes are the major vector and play an important role in spreading of vector-borne diseases like malaria, dengue, chikungunya, filariasis and Japanese encephalitis which leads to cause thousands of deaths per year [37]. *Cx. quinquefasciatus* is a vector of lymphatic filariasis, (tropical disease) which accounted 120 million people infected worldwide, and 44 million people having common chronic manifestation [6]. According to World Health Organization (WHO) estimates, globally about 90 million people are infected with *W. bancrofti* and ten times more peoples are at the risk of being infected. So far, 25 million people harboring microfilaria and 19 million people suffering from filarial disease manifestations were recorded from India [19, 16].

The controlling of mosquito populations using chemical insecticides had some advantages viz. their speedy action and easy application. Whereas, with the continuous use of chemical insecticides, the mosquitoes are becoming resistant. Alternatively, natural pesticides (especially from plants), are act as more potential agents for mosquito vectors. Aromatic plants and their essential oils are best sources of many active compounds for multipurpose uses. Plant based phytochemicals having mosquitocidal properties are now recognized as potent alternative insecticides to replace synthetic insecticides [1]. Previously, many naturally occurring chemicals have been reported to influence mosquito oviposition [17, 20, 10], act as general toxicant, growth and reproductive inhibitors, repellents and oviposition-deterrent [33], essential oils isolated from *Cinnamomum zeylanicum, Zingiber officinale* and *Rosmarinus officinalis* showed oviposition-deterrent and repellent activities against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* [21]. Another plant *Cipadessa baccifera* extract showed adulticidal activity against selected three mosquito vectors [23]. The present investigation was chosen to study the mosquitocidal activities of *Ocimum gratissimum* (commonly known as Ram Tulsi, wild basil (in English), Elumichathulasi and Peruntulasi (In Tamil), which is a traditional medicinal plant and worldwide distribution. The essential oils obtained from leaves and stems of this plant was used as remedy for several health problems like fever, throat inflammations, ears or eyes,

stomach pain, diarrhea, skin diseases. The whole plant is used as stomachic and in treating sunstroke, headache and influenza. Leaves are used for abdominal pains, sore eyes, ear troubles, coughs and blocked noses. An infusion of the leaves was used as a disinfectant and act as best insecticidal agents [15]. Considering the above information's the present investigation deals with the use of *Ocimum gratissimum* plant extracts for controlling *Culex quinquefasciatus* mosquito vector (Diptera: Culicidae).

2. Materials and Methods

2.1 Mosquito rearing

Culex quinquefasciatus mosquito eggs were collected from National Centre for Disease Control (NCDC), Mettupalayam, Tamil Nadu, India. The eggs were kept in plastic trays contain tap water and were maintained at 27±2 ºC and 75–85% relative humidity under 14:10 light and dark photoperiod cycles. Mosquito larvae were reared into tap water in plastic trays and provided with dog biscuit and yeast powder in the ratio of 3:1as a larval food. The adults were emerged and transferred to mosquito rearing cages, holding 10% sugar solution, a food source for adults.

2.2 Plant materials

The fresh and healthy leaves of *Ocimum gratissimum* L. (Fig.1) were collected from Yercaud Hills ,Salem District (latittude – 11.7794ºN, longitude – 78.2034ºE), a part of Eastern Ghats of Tamilnadu, harbor rich in floral biodiversity and indigenous populations.

Fig 1: *Ocimum gratissimum* L.

2.3 Preparation of plant extracts

The leaves were shade-dried for 7–10 days, and powdered mechanically using commercial electrical stainless steel blender. The powdered leaves (300 g) were extracted with chloroform, acetone and hexane (300 ml) in a Soxhlet apparatus (boiling point range 50–80 ºC) for 8 h. The extract was concentrated under reduced pressure (22–26 mm Hg) at 45 ºC. The obtained residue was stored at room temperature.

2.4 Larval and pupal toxicity test

The larvicidal and pupacidal activity of the plant crude extracts were tested as per the modified method of World Health Organization (2005) [35]. About 25 fourth instar larvae were transferred to small disposable paper cups, containing 249 ml of water and 1.0 ml of the desired plant extract at different concentrations (0.1, 0.3, 0.5, 1 and 2 mg/ml). The equal number of controls was maintained simultaneously using tap water. The numbers of dead larvae were counted after 24 h of exposure and percentage mortality was recorded from the average of three replicates. The LC_{50} (lethal concentration that kills 50% of the exposed larvae) and LC_{90} (lethal concentration that kills 90% of the exposed larvae) values were calculated (after 24 h observation) by probit analysis [9].

2.5 Adulticidal bioassay

Mosquito coil preparation

Mosquito coils were prepared as per the modified methods of Ramkumar *et al.,* (2014) [23] and Saini *et al.,* (1986) [26]. The mosquito coils were prepared using shade dried plant crude extract (4 g) and 2 g of saw dust and 2 g of coconut shell as a burning materials. All the materials were mixed thoroughly in distilled water to form a semi solid paste and the paste was prepared 0.5 cm thickness mosquito coils. Coils were shade-dried and used for further experiment.

Smoke toxicity test

Smoke toxicity experiment was conducted in a glass chamber measuring 60 x 40 x 35 cm with mid bottom of the chamber. Fifty blood fed adult mosquitoes were released to the chamber and the mosquitoes were exposed to the smoke of burning coils for 40 mins and the mortality data were recorded after every 10min. The smoke toxicity was compared with the commercially available mosquito coil as tested above [30]. The commercial mosquito coil active ingredients contain extract allethrin as active ingredient.

2.6 Phytochemical analysis

The Phytochemical analysis of various solvents extracts from the leaves of *O. gratissimum* was carried out as per the method of Satheesh et al., (2012) [28]; Thamaraiselvi *et al.,* (2012) [35].

2.7 Thin Layer Chromatography (TLC)

TLC experiment was performed (on analytical plates over silica gel) to isolate the principle components. The different solvent systems (having different polarities) were prepared and TLC studies were carried out to select optimum solvent system showing better resolution. The above prepared plant extracts were applied on pre-coated TLC plates by using capillary tubes and developed in a TLC chamber using suitable mobile phase. The developed TLC plates were air-dried and observed under ultra violet light at 254 nm and 366 nm. Later, the plant spot was sprayed with different spraying reagents and placed in an iodine vapour chamber for 1 min for the development of color in separated bands. The retention factor (Rf) values were calculated based on the movement of samples in TLC plate [27].

$$Rf = \frac{\text{Distance travel by solute}}{\text{Distance travel by solvent}}$$

2.8 High performance Liquid Chromatography analysis

The HPLC analysis of bioactive compound from *O. gratissimum* was performed using Waters 600 pump and Waters 2487 dual detector set to 270 nm. The separation was carried out with an isocratic elution program (60% CH_3CN, 40% H_2O), waters Nova-Pack C18 column (4 Km, 3.9 x 150 mm) adapted to waters Nova-Pack C18 60, a guard column (3.9 x 20 mm) and flow rate of 1ml/min. Acetonitrile (CH_3CN) was HPLC grade from Merck (Darmstadt, Germany); Distilled

water (H_2O) was purified by a Milli-Q system (Millipore, Bedford, MA, USA). The analyses were carried out at room temperature (25 ºC) and with volume of 20 µL and three injections. Peaks were identified by comparison of their retention times (Rt). Cariphenone A was quantified by a calibration curve with at least five data points covering the concentration range of 5 - 1400 mg/ml.

2.9 GC–MS analysis

The bioactive extract of *O. gratissimum* was analyzed by gas liquid chromatography (Polaris Q Ion Trap GC/FID) and mass spectrometry (Perkin Elmer Q-700 equipment) (Cheng *et al.,* (2009) [3] Column temperature programme was 35 °C for 2 min, increased to 180 °C at 4 °C/min, and 280 °C at 20 °C/min. Helium was used as the carrier gas at 0.9 ml/min. The mass spectrum was obtained at 70eV ionization voltage. The identification of individual compound 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 several authentic reference compounds were compared with isolated compounds for identification.

2.10 Fourier transmission-infrared spectroscopy

FT-IR spectra were measured on Arid Zone FT-IR spectrometer (ABB MB-Series, Houston, TX) equipped with a DTGS detector. Liquid derivatives were pressed between two NaCl discs (25 mm x 5 mm) to provide thin transparent oil films for analysis by FT-IR spectrometry. An absorbance spectrum was acquired at $4cm^{-1}$ resolution and signal-averaged over 32 scans. Interferograms were fourier transformed using cosine apodization for optimum linear response. Spectra were baseline corrected, scaled for mass differences and normalized to the methylene peak at 2927 cm^{-1}.

Statistical analysis

The average values of larvae, pupae and adult mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistical parameters i.e. at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL) values, and chi-square test were calculated using the SPSS (Statistical Package of Social Sciences) software version 16.0.

3. Results and Discussion

3.1 Bioassay Tests

The bioassay activity result of present study show that higher mortality was observed in chloroform extract against larvae, pupae and adult *Cx. quinquefasciatus* mosquitos compared with other solvents. The better larvicidal mortality was recorded at LC_{50} (2.8 mg/L) and LC_{90} (5.4 mg/L) respectively (Table 1). Pupicidal activity of the mosquito was also found in chloroform extracts, with LC_{50} and LC_{90} recorded as 2.6 mg/L and 4.6 mg/L (in 24 hours) respectively (Table 2). The highest adulticidal activity was observed in chloroform extract (after 40 minutes of observation). The maximum mortality rate was recorded in chloroform extract i.e. 80.66% and mean of mortality (91.64 ± 0.86) (Table.3).

Table 1: Larvicidal activity of *O. gratissimum* extracts against *Cx. quinquefasciatus*

Extracts	n[a]	LC_{50} (LCL-UCL) (95% confidence limit) mg/L	LC_{90} (LCL-UCL) (95% confidence limit) mg/L	χ^2	*Df*
Acetone	375	3.0511 (2.5818-6.0224)	7.6326 (5.2354-9.2541)	0.9351	3
Hexane	375	3.5407 (2.6891-5.8678)	8.012 (5.8745-9.8521)	0.9477	3
Chloroform	375	2.8916 (1.8961-4.5868)	5.4521 (3.8911-6.8941)	0.0128	3

LCL lower confidence limits, *UCL* upper confidence limits, *χ2* chi-square, *df* degrees of freedom

Table 2: Pupicidal activity of *O. gratissimum* extracts against *Cx. quinquefasciatus*

Extracts	n[a]	LC_{50} (LCL-UCL) (95% confidence limit) mg/L	LC_{90} (LCL-UCL) (95% confidence limit) mg/L	χ^2	*Df*
Acetone	375	3.1511 (2.3818-5.9224)	7.8326 (6.2354-9.6541)	0.9851	3
Hexane	375	4.1407 (2.6891-5.8678)	8.012 (5.6745-7.8521)	1.0477	3
Chloroform	375	2.6916 (1.8961-4.5868)	4.6521 (3.1911-5.8941)	0.2128	3

LCL lower confidence limits, *UCL* upper confidence limits, *χ2* chi-square, *df* degrees of freedom

Table 3: Smoke Toxicity of *O. gratissimum* plant extracts against mosquito vectors

Observation in min	Percentage of Mortality (%)	Mean Mortality	commercial mosquito coil	*Df*
10	22	19.66±0.82	78%	2
20	33.86	36.33±0.55	90%	2
30	69.33	80.66±0.21	100%	2
40	80.66	91.64±0.86	100%	2

% - Percentage of mortality, *df* degrees of freedom

3.2 Preliminary phytochemical analysis

The result of preliminary phytochemical test indicates that the chloroform extract harbor the presence of phenols, flavonoids, tannins. The proteins and amino acids were also present in hexane and chloroform extracts. The phenols, flavonoids and tannins may be the bioactive compounds and act as potential

insecticidal agents against *Cx. quinquefasciatus* mosquito vectors (Table 4).

Table 4: Preliminary phytochemical analysis of *O. gratissimum*

Phytochemicals test	Name of the test	Hexane	Chloroform	Acetone
Phenols	$FeCl_2$	+	+	+
Flavonoids	NaOH	+	+	+
Alkaloids	Wanger's	–	–	+
Saponins	Foam	+	+	+
Tannins	Braymer's	+	+	+
Glycosides	Keller Killiani	–	-	–
Proteins	Biuret	+	+	–
Amino Acid	Ninhydrin	+	+	–
Quinones	Quinone test	+	–	–
Carbohydrates	Fehlings	-	+	–

+ = Present, – = Absent

3.3 Thin Layer Chromatography (TLC)

The chloroform extract of *O. gratissimum* was used to perform the TLC 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 was calculated (Fraction1. 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 identified as phenolic group of compounds.

3.4 High Performance Liquid Chromatography (HPLC)

The qualitative HPLC were performed in the chloroform extract of *O. gratissimum*. Three major peaks were observed in the different time intervals (UV detector at 270 nm). The highest peak value was obtained in chloroform extract *i.e.* 9.888 (mV) and lowest peak value has 3.337(mV). The fractions obtained from the HPLC system was performed and observed similar bands of phenolic groups were confirmed in the chloroform extract (Table 5 and Figure 2).

Table 5: Peak area of bio-compounds in chloroform extracts of *O. gratissimum*

S. No	Ret. Time	Area	Height	Area %	Height %
1	3.337	13466	268	11.767	12.730
2	9.888	72563	1338	63.410	63.571
3	12.577	28405	499	24.822	23.699
Total		11443	2104	100.00	100.00

3.5 Fourier transmission infrared (FT-IR)

Chloroform extract of *O. gratissimum* was observed by FT-IR, the highest peak value indicates the presence of functional groups. The major oxygenated constituents and functional groups were depicted. The amines, imines (N – H), alkanes (-CH_3), disubstituted alkenes (R_1 CH=CHR_2), Nitrates (O – NO_2 v) stretching, wagging, bending of oxygenated bonding (O – H) were considered as major functional groups of bioactive compounds (Table 6; Figure 3).

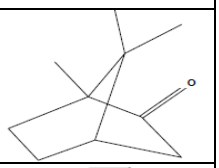
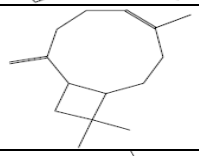
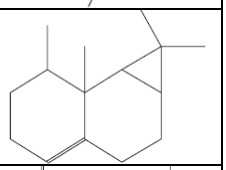
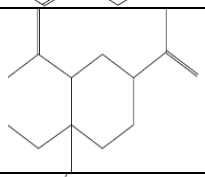
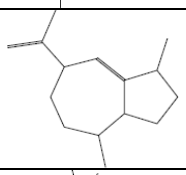
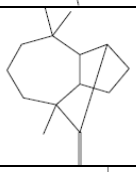
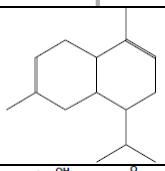
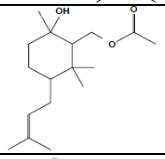
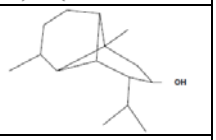
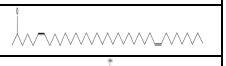
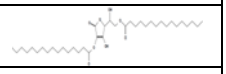
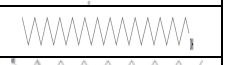
Table 6: FT- IR results from chloroform extract of *O. gratissimum*

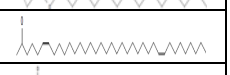
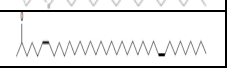
Peak values	Functional group	Bonding pattern
3395.79	Amines, Imines associated	N – H str
2947.54	Alkanes (-CH_3)	C - H str
2837.65	Alkanes (-CH_3)	C – H str
2527.88	Phosphorus / organo sulfur compounds	O – H / S – H str
2049.46	Deuterated Alkanes	C - D str
1571.67	Alpha –Halogenonitro Compound	NO_2str
1404.07	Phenols, Tert, Alcohols	O – H def
1262.51	Nitrates	O – NO_2 v
1118.69	Secondary Alcohols	C – OH str
1024.5	Primary Alcohols	C – OH str
756.11	Benzene ring with three hydrogen H atoms	C – H def
695.60	Haloids	C – Clstr
652.97	Disubstituted Alkenes (R_1 CH=CHR_2) Cis	C – H def

3.6 Gas chromatography mass spectroscopy (GCMS)

The GC-MS chromatogram of chloroform extract of *O. gratissimum* (Figure 4) showed 33 peaks, which reflects the presence of thirty three phytochemical constituents. Out of them, five were identified as major compounds (Tables 7).

Table 7: Identification of some bioactive compounds, molecular formula and library structure using GC MS analysis

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	$C_{16}H_{22}O_4$	

4. Discussion

Plant extracts and phytochemicals are good source for controlling mosquitoes due to their efficiency, easy biodegradability, development of less to nontoxic products, and may be applied to mosquito breeding places [33, 12]. Many plant extracts and essential oils possess better larvicidal activity against many mosquito species [13, 18, 5, 33, 12]. Sukumar *et al.,* (1991) [33] has pointed out most promising botanical mosquito control agents of selected plant families namely A*steraceae*, *Cladophoraceae*, *Lamiaceae*, *Meliaceae*, *Oocystaceae*, and *Rutaceae*. The present study reported mosquitocidal property (larvicidal, pupicidal and adulticidal) of three different solvent extracts obtained from *O. gratissimum* against *C. quinquefasciatus* mosquito vectors. The highest mortality was observed in chloroform extract.

Similarly, Govindarajan *et al.,* (2008) [11] reported that methanolic leaf extract of *Cassia fistula* was potential larvicidal agents against *Cx. quinquefasciatus* and *An. stephensi* with LC_{50} values of 17.97 and 20.57 mg/L. Previously, many authors reported that acetone extracts of various plants have highest larvicidal and adulticidal activity against *A. stephensi* and *Cx. quinquefasciatus* [11, 23]. 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 extract may be depends on collection and season of plant materials [32, 34]. On the other hand, Sosan *et al.,* (2001) [31] reported larvicidal activities of essential oils obtained from *Ocimum gratissimum Cymbopogon citrus*, and *Ageratum conyzoides* against *Aedes aegypti* and achieved 100% mortality at 120, 200, and 300 ppm concentrations.

The preliminary phytochemical analysis of extracts from *O. gratissimum* was found to have presence of alkaloids, flavonoid, tannins and phenolic compounds in the acetone, chloroform and hexane extracts. Similarly, Shafqatullah *et al.,* (2013) [29] reported the presence of various phytochemicals viz., alkaloids, glycosides, flavonoids, tannins, terpenoids and saponins in *O. santum* stem and leaves extract. Several, researchers have contributed the presence of various phytochemicals from various *Ocimum* species [25, 7, 24].

Chromatographic methods were widely employed for the separation of phytochemicals present in the plant crude extracts. In our study, presence of phytochemicals was separated from TLC (using different solvent systems like chloroform and methanol in the ratio of 7:3). Totally 3 fractions were identified. Similarly, Qureshi *et al.,* (2011) [22] reported the separation of essential oils from the *O. sanctum* and *O. americanum* using chromatographic techniques. Alam *et al.,* (2012) [2] separated the Eugenol from *O. sanctum* using HPTLC method. Based on Peak values of HPLC, the three major peaks were identified and come under phenolic groups. Likewise, Jamal *et al.,* (2002) [14] separated and identified the bioactive compound (rosmanic acid) from *O. basilicum* plant extract.

The gas chromatography and mass spectroscopic analysis of *O. gratissimum* chloroform extract has shown the presence of 33 major peaks including 5 potential bioactive compounds. Similarly, Amvam Zollo *et al.,* (1998) [4] identified about 72 bioactive compounds from *O. basilicum* and *O. cannum*. The functional group analysis (FT-IR) of the chloroform extract revealed the presence of major amines, imines (N – H str), Alkanes ($-CH_3$), disubstituted alkenes (R1 $CH{=}CHR_2$), Nitrates ($O–NO_2$ v) stretching, wagging, bending of oxygenated bonding (O–H), C-N stretching alcohols, carboxylic acids, ethers and esters. Previously, Mallikarjuna *et al.,* (2011) [8] recorded O-H stretch carboxylic acid in the extracts of *Ocimum* species.

5. Conclusion

The outcome of present results concluded that mosquitocidal property of three solvents (acetone, hexane and chloroform) extracts of *O. gratissimum*. Among them, chloroform extract exhibits highest mortality rate compared with other solvents. The major phytochemicals (phenolic groups) identified in the crude extracts may be responsible for the highest mortality rate against mosquito. The HPLC analysis showed 3 major peaks with the maximum peak area of 72% at RT of 9.888 minutes. The GC - MS results reveals the presence of 33 major and minor compounds and 5 compounds were identified as bioactive compounds. Further, the functional groups of bioactive chloroform extract were identified by performing FT-IR analysis. The overall results suggest the chloroform extracts of *O. gratissimum* contain potential insecticidal agent and may be used for controlling *Cx. quinquefasciatus* mosquito vectors.

6. Acknowledgements

We would like to thank the Department of Biotechnology, Periyar University, Salem, for providing the infrastructural facility for carrying out this research work and we also thank the St. Joseph's College, Trichy for HPLC, FT-IR and Vellore Institute of Technology, Vellore for GC-MS analysis. The first authors acknowledged the financial assistance by Tamilnadu State Council for Science and Technology, Chennai, India, under student project scheme, 2013-2014 (TNSCST/SPS/TD/2013-2014/).

7. References

1. Ahn YJ, Won MK, Park HM, Han CG. Potent insecticidal activity of Ginkgo bilab-derived trilactone terpenes against *Nilaparvata lugens*. 90 – 105, in Phytochemicals for pest control, Eds. P.A. Hedin, R.M. Hollingworth, E.P. Masler, J. Miyamoto and D.G. Thompson. Am. Chem. Symp. Ser. 658, American Chemical Society, Washington DC, 1997.
2. Alam P, Gupta J, Firdouse S, Firdouse A, Afshan J. HPTLC method for qualitative and quantitative estimation of eugenol from *Ocimum sanctum* linn in polyherbal

formulation. International Journal of Comprehensive Pharmacy 2012; 3:07-10.
3. Cheng SS, Chua MT, Chang EH, Huang CG, Chen WJ, Chang ST. Variations in insecticidal activity and chemical compositions of leaf essential oils from *Cryptomeria japonica* at different ages. Bioresource Technology 2009; 100:465–470.
4. Amvam ZPH, Biyiti L, Tchoumbougnang F, Menut C, Lamaty G, Bouchet P. Aromatic Plants of Tropical Central Africa. Chemical Composition and Antifungal Activity of Thirteen Essential Oils from Aromatic Plants of Cameroon. Flav & fragrance journal 1998; 13:107–114.
5. Berenbaum MR. North American ethnobotanicals as sources of novel plant-based insecticides. In: Arnason JT, Philogene BJR, Morand P, Insecticides of plant origin. American Chemical Society, 1989, 11–24.
6. Bernhard L, Bernhard P, Magnussen P. Management of patients with lymphoedema caused by filariasis in North-eastern Tanzania: alternative approaches. *Physiotherapy* 2003; 89:743–749.
7. Devendran G, Balasubramanian U. Qualitative phytochemical screening and GC-MS analysis of *Ocimum sanctum L*. leaves. Asian Journal of Plant Science & Research, 2011, 44-48.
8. Mallikarjunaa K, Narasimha G, Dillipa G, Praveen B, Shreedhar B, Sree LC *et al*. Green synthesis of silver nanoparticles using *Ocimum* leaf extract and their characterization. Digest J Nanomaterials Biostruct 2011; 6:181–186.
9. Finney DJ. Probit analysis. Cambridge Univ. Press, Cambridge, 1971, 333.
10. Geetha I, Paily KP, Padmanaban V, Balaraman K. Oviposition response of the mosquito, *Culex quinquefasciatus* to the secondary metabolite(s) of the fungus, Trichoderma viride. Mem Inst Oswaldo Cruz 2003; 98:223–226.
11. Govindarajan M, Jebanesan A, Pushpanathan T. Larvicidal and ovicidal activity of *Cassia fistula* Linn. Leaf extract against filarial and malarial vector mosquitoes. Parasitology Research 2008; 102:289–292.
12. Hostettmann K, Potterat O. Strategy for the isolation and analysis of antifungal, molluscicidal and larvicidal agents from tropical plants. Phytochemicals for pest control, 1997, 1426.
13. Jacobson M. Botanical pesticides: past, present, and future In: Insecticides of plant origin, BJ.R. (Eds. J.T. Amason Philogne and P. Morand) Am. Chem. Symp. Ser. 387, American Chemical Society, Washington DC, 1989, 110.
14. Jamal J, Khalighi A, Kashi A, Bais HP, Vivanco JM. Chemical Characterization of Basil (*Ocimum basilicum* L.) Found in Local Accessions and Used in Traditional Medicines in Iran. Journal of Agricultural Food Chemistry 2002; 50:5878-5883.
15. Kokwaro JO. Medicinal plants of East Africa Nairobi, Kenia. East Africa Publishing Bureau, 1980, 111.
16. Kovendan K, Murugan K, Thiyagarajan P, Naresh K, Abirami, D, Asaikkutti A, Impact of climate change on the filarial vector, *Culex quinquefasciatus* Say Diptera: Culicidae). In: International Congress of Global Warming on Biodiversity of Insects: Management and Conservation, 9–12 February Tamil Nadu, India, 2009, 62.
17. Millar JG, Chaney JD, Mulla MS. Identification of oviposition Attractants for *Culex quinquefasciatus* from fermented Bermuda grass infusion. Journal of American Mosquito Control Association 1992; 8:11–17.
18. Miyakado M, Nakayama I, Ohno N. Insecticidal unsaturated isobutylamides from natural productst agrochemical leads, In J.T. Arnason, B.J.R. Philoge`ne, & P. Morand(Eds), Insecticides of plant origin. American Chemical Society Symposium Series, 1989, 387.
19. NICD National Institute of Communicable Diseases (1990). Proceedings of the National Seminar on operation research on vector control in filariasis. New Delhi, 1990.
20. Olagbemiro TO, Birkett MA, Mordue AJ, Pickett JA. Production of (5R, 6S)-6-acetoxy-5- hexadecanolide, the mosquito oviposition pheromone, from the seed oil of the summer Cypress plant, Kochia scoparia (Chenopodiaceae). Journal of Agricultural Food Chemistry 1999; 47:3411–3415.
21. Prajapati V, Tripathi AK, Aggarwal KK, Khanuja SPS. Insecticidal, repellent and oviposition- deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Bioresources Technology 2005; 96:1749–1757.
22. Qureshi R, Maqsood M, Arshad M, Chaudhry AK. Ethnomedicinal uses of plants by the People of Kadhi Areas of Khushab, Punjab, Pakistan. Pakistan Journal of Botany, 2011, 121-133.
23. Ramkumar G, Karthi S, Muthusamy R, Natarajan D, Shivakumar MS. Adulticidal and smoke toxicity of *Cipadessa baccifera* (Roth) plant extracts against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*. Parasitology Research 2014; 41:73-5.
24. Sai KSD, Venkata SBA. Pharmacognostic and phytochemical studies of *Ocimum americanum*. Journal of Chemical Pharmaceutical Research, 2011, 337-347.
25. Saiprasanna BS, Manohar BY, Roja R, Prasanta KC. Hepatoprotective Effects of *Ocimum canum Sims* Hydro-alcoholic Leaf Extract in I/R Induced Hepatic Injury in Rats JGHR 2012; 211(10):266-273.
26. Saini HK, Sharma RM, Bami HL, Sidhu KS. Preliminary study on constituents of mosquito coil smoke. Pestic 1986; 20:15-18.
27. Sanjay R, Biradar BD. Extraction of some secondary metabolites and thin layer Chromatography from different parts of *Centella asiatica* L. (URB). American Journal of Life Science 2013; 6:243-247.
28. Satheesh KB, Suchetha KN, Vadisha S, Sharmila KP, Mahesh Prasad B. Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole Fruit and seeds nujhs 2012, 2249-7110.
29. Shafqatullah, Muhammad K, Asadullah, Khaliqurrehman FA. Comparative Analyses of *Ocimum santum* Stem and Leaves for Phytochemicals and Inorganic Constituents. Middle-East Journal of Science Research 2013; 13:236-240.
30. Singha, Utpal Adhikari, Goutam Chandra. Smoke repellency and mosquito larvicidal potentiality of *Mesua ferra* L. leaf extract against filarial vector *Culex quinquefasciatus* Say. Asian Pac J Trop Biomed, 2011, 119-123.
31. Sosan MB, Adewoyin FB, Adewunmi CO. Larvicidal properties of three indigenous plant Oils on the mosquito *Aedes aegypti*. Productive Medicine 2001; 5:30–33.
32. Sujatha CH, Vasuki V, Mariappan T, Kalyanasundaram M, Das PK. Evaluation of plant Extracts from biological activity against mosquitoes. International Pest Control

1998; 30:122–124.
33. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: a review. Journal of American Mosquito Control Association 1991; 7:210–237.
34. Tawatsin A, Asavadachanukorn P, Thavara U, Wongsinkongman P, Bansidhi J, Boonruad T *et al*. Repellency Of essential oils extracted from plants in Thailand against four mosquito vectors (Diptera: Culicidae) and oviposition deterrent effects against *Aedes aegypti* (Diptera: Culicidae). Southeast Asian Journal of Tropical Medicine Public Health 2006; 37:915–931.
35. Thamaraiselvi P, Lalitha P, Jayanthi. Preliminary studies on phytochemicals and Antimicrobial activity of solvent extracts of *Eichhornia crassipes* (Mart.) Solms. Asian Plant Science Research 2012; 2:115-122.
36. WHO Guidelines for laboratory and field testing of mosquito larvicides. Communicable disease control, Prevention and eradication, WHO pesticide evaluation scheme, 2005.
37. WHO Combating waterborne diseases at the household level Part1. WHO, Geneva, 2007.