Mosquitocidal properties of *Ocimum canum* Sims (Lamiaceae) leaf extracts against dengue vector *Aedes aegypti L.* (Diptera: Culicidae)

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Abstract

The *Ocimum* plant was traditionally used for mosquitoes repellent and control in India especially in Tamil Nadu. In this research, deals with the larvicidal, pupicidal and adulticidal potential of three different solvent extracts of *O. canum* against *Aedes aegypti.* The overall result highlights that the chloroform extracts of *O. canum* were shown significant larvicidal (15.027 mg/L) activity at 24 h of exposure. The pupicidal and adulticidal activity of this plant exhibits highest mortality against *A. aegypti* within 24 h at the dose ranges of 89.773 mg/mL, 41.912 mg/mL respectively. The chloroform extracts contain major phyto-constituents like phenol, alkaloids, protein and tannins. Thin layer chromatography profiles also provide a database for the presence of active components. GC-MS analysis of bioactive chloroform extract revealed that a total of seventeen compounds, six were considered as major and the remaining as minor compounds. The spectral studies of FT-IR denoted the functional groups of bioactive components like alkenes, ketone, hydroxyl and others. Based on the outcome of results show that *Ocimum* canum have found to potent ability for controlling the mosquitoes, it can be used as an ideal eco-friendly agent for arresting dengue fever in future.

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Key words: Mosquitoes repellent activity; Ocimum canum; GC-MS analysis; *Aedes aegypti*.

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Introduction

Mosquitoes are the most important single group of insects in terms of public health importance, which transmit variety of diseases like malaria, filariasis, dengue and Japanese encephalitis. It causing millions of deaths every year (Brown, 1986). Mosquito-borne diseases contribute to a larger proportion of health problems in developing countries. Repetition of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It also resulted in the development of resistance, undesirable effects on non-target organisms, and fostered environmental and human health concern (Thomas *et al*., 2004). The recent research focusing for herbal preparations that do not produce any adverse side effects in the non-target organisms and easily biodegradable (Kant *et al*., 1996). In general, plant derived compounds (phytopesticides) have been recognized as an important natural resources of insecticides (Gbolade *et al.,* 2000). Several phytochemicals have been reported to exhibit harmful effect against mosquito larvae and insecticides, reproduction of inhibitors, repellent potential, ovicidal and oviposition deterrent (Prajapati *et al.,* 2005; Pushpanathan *et al*., 2006) properties. One of the method available for the control of mosquito population is over and injudicious applications of persistent synthetic insecticides, resulting undesirable effect of synthetic insecticides. Previous research has been proved the effectiveness of plant derived secondary compounds, such as saphonine (Chowdhury *et al.,* 2008), steroids (Ghosh *et al.,* 2008), isoflavonoids (Josep *et al.,* 2004), essential oil (Cavalcanti *et al.,* 2004), alkaloids and tannins (Khanna *et al.,* 2007) reported as mosquitocidal agents.

Dengue fever is considered as a serious public health problem in the world. In tropical countries, where the favorable environmental conditions are responsible for the proliferation of vector *Aedes aegypti*. Among the arbovirus in India, distribution of all the dengue virus type is continuously expanding. Remarkably the reemergence of Chikungunya virus (CHIK) since 2005 is posing an additional concurrent diseases burden in the country. *Aedes aegypti* (L) (Diptera: Culicidae) is a fresh water breeding mosquito it is very difficult to control during rainy season.Approximately 2500 million people, two fifths of the world's population, are now at risk from Dengue fever (Fulmali *et al*., 2008; Kumar *et al*., 2008). The WHO currently estimates there may be 50 million cases of Dengue fever worldwide every year (WHO, 2011).

Lamiaceae have been traditionally used in developing countries for their insecticidal and repellent property against several insect

species (Ngamo *et al*., 2007). For example, *Rosmarinus officinalis* and *Lavandulaan gustifolia* showed moderate larvicidal activity (Conti *et al*., 2010). Prajapati *et al*. (2005) reported the essential oils from selected plants as noticeable repellent and ovicidal properties, *Hyptissu aveolens* has useful insecticidal (Amusan *et al*., 2005; Jaenson *et al*., 2006) and control many stored product pests (Peerzada, 1997; Othira *et al*., 2009; Conti *et al*., 2011). Moreover, its chemical composition and biological activity might be changed due to the function origin and collection time of plants (Noudjou *et al*., 2007).

Ocimum canum (Lamiaceae) is a pubescent erect much branched herb, 15-60 cm high with sub-quadrangular striate branches. Leaves are elliptic-lanceolate, glabrous and gland dotted strongly aromatic herb, widely distributed in throughout India (especially in fields of waste lands, plains and lower hills). It contain several volatile oils include methyl cinnamate, methylheptenone, methylnonylketone, d-camphor, citral, ocimin, methylchavicol, linalool, nevadensin, salvigenin, beta-sitosterol, betulinic, ursolic, oleanolic acids, flavanoids, pectolinarigenin-7-methylether and nevadensin, respectively. Polysaccharides composed of xylose, arabinose, rhamnose and galacturonic acids (Rastogi and Mehrotra, 1993). The main biological properties of *O. canum* reported as antimicrobial, antioxidant, anthelmintic and anti-diabetic agents (Bhattacharjee, 2001; Chopra, 1956). The present study was focused on to perform the mosquitocidal activity and identifying the potential bioactive compounds from *O. canum* against Dengue vector *Aedes aegypti.*

Materials and methods

Collection of plant materials

The fresh leaves of *O. canum* were collected (during the month of November and December 2013) from Pottaneri village, Mettur (Tk), Salem District, Tamil Nadu. The plant specimen was authenticated by Dr. D. Natarajan, Assistant Professor, Department of Biotechnology, Periyar University, Salem and also cross-checked with available books and herbarium records. The voucher specimen was deposited in the NDRL for further reference. The collected plants leaves were washed with tap water to remove unwanted solid dust particles and shade - dried at room temperature for 10 days. The dried plant material was powdered separately using commercial electrical blender.

Preparation of extracts

The processed plant materials (500 g) were sequentially extracted by hot extraction method in a soxhlet apparatus using three organic solvents (acetone, chloroform and hexane) for 48 to 72 h until the influx solvent changed into 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

Aedes aegypti (larva, pupa and adult) mosquitos were collected from NCDC, Connoor, Tamil Nadu, India. It was maintained at 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 h light and dark. Larvae were fed with diet of yeast, dog biscuits and sugar solution for adult mosquitos.

Bioassay tests

Larvicidal bioassay

The larvicidal activities of selected plant crude extracts were assessed as per WHO protocol (1981). Briefly, in a container 25 fourth instar larvae were 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 1 mL of DMSO served as negative control. After 24 h exposure, the dead larvae were counted and corrected using Abbott's formula and the percentage mortality was recorded from the average of three replicates. The average mortality percentages of three replicates were used to carry out lethal concentrations (LC_{50} and LC_{90}) by Probit analysis.

Pupicidal bioassay

Pupicidal activities of crude extracts was evaluated as per the modified method of Modify as Krishnappa *et al.* (2012). For the bioassay in a container, 25 pupae were kept in 249 mL of distilled water with 1 mL of extract at different concentrations (100, 200, 300, 400 and 500 mg/L) in DMSO. The container received 1 mL of DMSO served as negative control. All containers were maintained at room temperature (28±2) with naturally prevailing photoperiod (12:12 h/L:D) in the laboratory. Any pupa was considered to be dead if did not move when prodded 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

A total of 15 adult female mosquitoes (3-5 days old) were treated with different concentrations (100, 200, 300, 400 and 500 mg/L) of plant crude extracts impregnated with filter papers (WHOm 1981). The mosquitoes were allowed to acclimatize in the holding tube for 1 h and then exposed to test paper for 1 h. Mortality was recorded every 10 min throughout the exposure period. Mortality of mosquitoes was determined at the end of 24 h recovery period. Percentage of mortality was corrected by Abbott's formula. LC_{50} and LC_{90} with 95% confidence limits were determined using Probit analysis (Finney, 1971).

Statistical analysis

The average larval (adult) mortality data were subjected to probit analysis for calculating LC_{50} and LC_{90} statistics at 95 % confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL) values and chi square test were calculated using the SPSS 14.0.

Phytochemical analysis

The qualitative phytochemicals (phenol, alkaloids, quinones, glycosides, flavanoids, amino acids, tannins, proteins, cabhohdrate and saponin) analysis of three different extracts (acetone, chloroform, and hexane) were performed as per the methods of Behera *et al.* (2012).

Thin layer chromatography

Thin layer chromatography was performed as per the method of Bishnu *et al.* (2011). Silica powder was added to distilled water and mixed with magnetic stirring continuously. The slurry was poured into a clean and dried slide scattered all over the slide to make a thin film. The silica plates were activated by heating them in hot air oven at 120°C for 3 h. After 3 h, the silica plates were allowed to cool at room temperature and marked about 1cm from the bottom. The extracts were loaded at the bottom center of the slide.The beaker was saturated with suitable solvent system [methanol: chloroform (7:3)].The final solvent front was marked and the plate was dried. The developed TLC plates

were dried and visually observed for various bands. The Rf (Retension frequency) value was calculated as follows:

$$Rf = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent}}$$

Spot visualization

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

High performance liquid chromatography

The HPLC system was used to quantify the bioactive compounds from chloroform extracts of *O. canum*. The chromatographic separation was performed on a C18 column (5 mm, 250×4.6 mm i.d.) with the column temperature of 35°C. Linear gradient elution was collected (with methanol and aqueous) and the procedure as follows (v/v):5 to 25% (0 to 8 min), 25 to 55% (8 to 20 min) and 55 to 100% (20 to 30 min). The UV detector is used as 280 nm (Deo *et al*., 2011).

GC-MS analysis

The GC-MS analyses of chloroform extract of plant was done by Perkin Elmer Q-700 equipment. Column temperature was programmed at 35°C-180°C for 2 min and simultaneously, increased about 4°C-20°C/min. Helium was used as the carrier gas at 0.9 mL/min. The mass spectrum was obtained at 70 eV ionization voltage. The identification of individual compound was done using 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 Cheng *et al*. (2009).

Fourier transform infrared spectroscopy

An arid Zone FT-IR equipped with DTGS detector was used to measure the spectrum developed in chloroform extracts of *O. canum.* FT-IR spectrum was analyzed a thin transparent oil films were made by pressing two NaCl discs (25×5 mm) of the liquid derivatives. Absorption spectra were acquired at 4 cm^{-1} resolution and signal-averaged over 32 scans. Interferegrams 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}.

Results and discussion

Larvicidal activity

Mortality percentage was calculated for its toxicity effect of plant crude extracts (acetone, hexane and chloroform) against fourth instar larvae of *A. aegypti.* The mortality percentage of larvae was calculated 24hrs of exposure. The highest larval mortality found in choloroform extract of *O. canum* plant against IVth instar larvae of *Ades agypti* with the LC_{50} and LC_{90} values of 15.027 and 353.49 mg/L. Whereas the acetone extract exhibits moderate activity with LC_{50} and LC_{90} values of 58.873 and 134.47 mg/L respectively (Table 1). Recently, the researcher reported that *Ocimum* plant extract shows better larvicidal activity against larval and adults of mosquito species (Pratheeba *et al*., 2015; Murugan *et al*., 2016).

Pupicidal activity

The pupal mortality of *Ades agypti* (after treatment of *O. canum* plant extract) was observed at various concentrations (Table 1). The

Table 1. Mosquitocidal activity of *O. canum* leaf extracts against *A. agypti*.

O. canum Extracts	Larvicidal				Pupicidal				Adulticidal			
	Con. Mg/L	% Mortality	LC_{50}	LC_{90}	Slope	Con. Mg/L	% Mortality	LC_{50}	LC_{90}	Slope	Con. Mg/L	% Mortality	LC_{50}	LC_{90}	Slope
Acetone	100	42	58.873	134.47	-6.323	100	44	41.912	204.25	-3.023	100	42	160.71	419.27	-6.789
	200	48				200	48				200	43			
	300	52				300	48				300	48			
	400	58				400	52				400	53			
	500	66				500	88				500	59			
Chloroform	100	56	15.027	353.49	-1.1	100	55	72.265	119.38	-10.93	100	70	22.662	156.43	-2.070
	200	68				200	72				200	76			
	300	78				300	76				300	88			
	400	91				400	80				400	100			
	500	97				500	80				500	100			
Hexane	100	12	190.50	341.47	-11.58	100	53	92.265	159.28	-7.93	100	12	108.38	842.32	-1.379
	200	27				200	56				200	32			
	300	42				300	73				300	56			
	400	55				400	73				400	68			
	500	57				500	77				500	61			

Con., concentration; LC_{50}, lethal concentration 50; LC_{90}, lethal concentration 90.

mortality rate was increased on the basis of concentration/dose (low to high) of the extracts. The highest pupal mortality was observed in acetone extract with low LC_{50} value 41.912 mg/L respectively. Similarly, the above result was supported by various scientists like Selvakumar *et al.* (2015) reported that the better pupicidal activity of different solvents extracts of *Annona reticulata* and Gokulakrishnan *et al*., (2013) were identified essential oils from *Pogostemon cablin* and evaluated the pupicidal activity against various mosquitoes including *A. aegypti.*

Adulticidal activity

The result of adulticidal activity of chloroform leaves extract of *O. canum* show maximum adulticidal property with very low LC_{50} values (22.662 mg/L) compared with other solvents like acetone and hexane (Table 1). The results highlights the leaf and flower of *O. sanctum* were tested against fourth instar larvae of *Aedes aegypti* and they determined the LC_{50} values at various concentrations of extract like 425.94, 150.40, 350.78, 575.26 and 175.67 mg/L (Mohamed *et al.*, 2008). Similarly, Govindarajan *et al*. (2014) reported the larvicidal, and adulticidal potential of the chloroform extract from the *Erythrina indica* tested against *A. aegypti.* The highest larval mortality and adulticidal activity were noticed in methanol extract of *E. indica*. Hexane leaves extracts of *Citrus sinesis* against the early fourth instars and female adult of *Aedes aegypti*. The hexane extract from *C. sinensis* leaves are proved to be reasonably larvicidal but remarkably irritant against dengue vector (LC_{50} and LC_{90} values of 446.84 and 1370.96 mg/L respectively) was noticed after 24 h exposure (Radhika *et al*., 2011). Methanolic leaf extract of *S. campanulata* have the potential to be used as an ideal eco-friendly approach the control of mosquitoes especially *A. aegypti* (Karthika Devi *et al.,* 2013). Ethanolic and petroleum ether extracts from various parts of *R. nasutus, D.elliptica, T. reidioides, H. aromatica, S. tuberose* and *A. calamusi* were tested for their larvicidal activity potential against *A. aegypti* mosquitoes (Naruman Komalamisra *et al*., 2005). The larval toxicity and smoke repellent potential of *Albizzia amara* and *O. basilicum* at different concentrations against *Aedes aegypti,* resultedthat the *A. amara* was more effective against *A. aegypti* than *O. basilicum* (Murugan *et al*., 2007). The adulticidal and repellent activities of crude hexane, chloroform, benzene, acetone and methanol extracts of the leaf of *Cassia tora* leaves against *A. aegypti*. Among them, methanol extract was showed significant activity (Duraisamy Amerasan *et al.,* 2012). Kamaraj *et al*. (2008) reported high larval mortality in methanol extracts of *Cryptocoryne auriculata* and *Solanum torvum* against the larvae of *An. subpictus* (LC_{50} 44.21, 44.69, 53.16, 41.07, 35.32, 28.90 and 44.40 ppm; LC_{90} 187.31, 188.29, 233.18, 142.66, 151.60, 121.05, 192.11 ppm, respectively) and *Cx. tritaeniorhynchus* (LC_{50} 69.83, 51.29, 81.24, 71.79, 44.42, 84.47 and 65.35 ppm; LC_{50} 335.26, 245.63, 300.45, 361.83, 185.09, 351.41 and 302.42 ppm, respectively). Bioefficacy of plant extracts differ from species to species of plants. The changes in adulticidal activity of these extracts is probably due to variation in the types and levels of active ingredients that depend not only on the genetic characteristics of the plant species but also the conditions under which they were grown and harvested (Tawatsin *et al*., 2006).

Phytochemical analysis

Phytochemical analysis of *O. canum* reveals that the acetone extracts indicate the presence of phenol, saponins, tannins and proteins (Table 2). Chloroform extract show the presence of phenol, flavonoids, saponins, protein and carbohydrates and the hexane extracts showed the presence of flavonoids, saponins, glycosedes and proteins. Saponins and proteins are presented in all the tested extracts. Similarly, the results were reported that the presence of volatile oils, flavonoids, carbohydrates, phytosterols, tannins and fixed oils from the leaves extracts of *O. americanum* (Sarma *et al.,* 2011). The results revealed that the presence of phytochemicals *viz*., alkaloids, saponins, tannins, steroids, phlobatannin, terpenoids, flavinoids and cardiac glycosides in the *Ocimum*speceis (Muhammad Neem Abbas *et al*., 2013). The phytochemical in the peels of *R. sativus*contain most important phyto-constituents like tannins, saponins, flavonoids, amino acids, terpenoids, cardiac glycosides and chalcones (Safia Janjua *et al.,* 2014) and also the results were suggested in *O. sanctum* (Himal Paudel Chetri *et al.,* 2008).

Thin layer chromatography profile for chloroform extracts of *O. canum*

The results of thin layer chromatography profile show different band formations (Figure 1) based on the solvent systems (methanol and chloroform) percentage of 5%, 10%, 20% and 25%, which shows different Rf value. The Rf values are 0.038, 0.192, 0.615, 0.803 and 1cm. Based on the Rf value suggested and assumed some bioactive components using confirmation test. The qualitative and quantitative analysis of major constituents from *O. sanctum* includes eugenol, qurcetine and some others by TLC (Rawat *et al.,* 2011) and ursolic acid (Kedar Kumar Rout *et al.,*2012). *O. gratissimum*, *O. sanctum* and *O. canum* leaves extracts were showed the better band formation with the Rf values of *O. canum* (0.513, 0.40, 0.58, 0.38 and 0.82) referred to Lutein Pheophytin Xanthophyll Oil Chlorophyll b and -carotene, *O. sanctum* (0.445, 0.59, 0.74, 0.934) and *O. gratissimum* (0.431, 0.573, 0.78) (Quereshi *et al*., 2011). Eugenol and Methyl eugenol from the petroleum ether extracts of *O. sanctum* (Nasare, 2013).

High performance liquid chromatography analysis of *O. canum*

The HPLC analysis of *O. canum* leaf crude chloroform extracts results show major peaks (more concentration of components) at the retention times (min.) of 3.482, 5.459, 9.960 and12.688 (at wavelength of 254 nm) (Table 3 and Figure 2). The previous studies of HPLC analysis were reported that the presence ofeugenol (Joshi *et al*., 2011) and phenolic compounds and terpenes (Shanmuga Sundaram *et al*., 2011) identified from the leaves of *O. sanctum.* Similarly, the total content of bioactive compounds from the species of lamiaceae family plants, flavonoids derivatives were identified from the *Thymus* species (Kulevanova *et al.,* 2001). Flavonoid content from the methanolic and aqueous extracts of *O. sanctum* and *O. kilimandsacharicum* (Deo *et al.,* 2011), the flavonoid and total phenolic contents of methanolic extract was higher than aqueous extract of *Stachys inflata* (Sayyed Mehdy *et al*., 2011), The content of rosmarinic acid was quantified from the methanol crude extract of *Perilla frutescens* (Jing Liu, 2013).

Table 2. Phytochemical analysis of crude extract of *O. canum*.

Phytochemical test	Acetone	Hexane	Chloroform
Phenol	+	-	+
Quinons	-	-	-
Flavonoids	-	+	+
Alkaloids	-	-	-
Amino acids	-	-	-
Saponins	+	+	+
Glycosides	-	+	-
Proteins	+	+	+
Tannins	+	-	-
Carbohydrates	-	-	+

-, absence;+, presence.

Fourier transform infrared spectroscopy analysis of chloroform extracts of *O. canum*

FT-IR analysis of chloroform extracts show the presence of some functional groups with corresponding intensity peaks O-H stretching or H bending for alcohols or phenols groups of active compounds,C-H stretching which was correspond to alkenes, N-H bend for 1° amines, C-C stretching was denoted the aromatic ring (Table 4). C-H(-CH_2X) which is assumed alkyl halides groups of bioactive compounds then C-N stretching, which may indicates aliphatic amines. C-Cl stretching which may denoted for alkyl halides groups of component, C-H *oop* for aromatics groups of bioactive component, C-Cl stretching for corresponds to alkyl halides, C-Br stretching for alkyl halides groups and -C=C-H or CH bending denoted for alkynes (Figure 3). The results of FT-

Table 3. High-performance liquid chromatography analysis of crude extracts of chloroform leaf extract of *O. canum*.

Peak	Ret. Tim	Area	Height	Area%	Height %
1.	3.482	19975	365	8.170	8.284
2.	5.459	4502	144	1.841	3.255
3.	9.960	133262	2438	54.504	55.264
4.	12.688	86759	1465	35.485	33.198

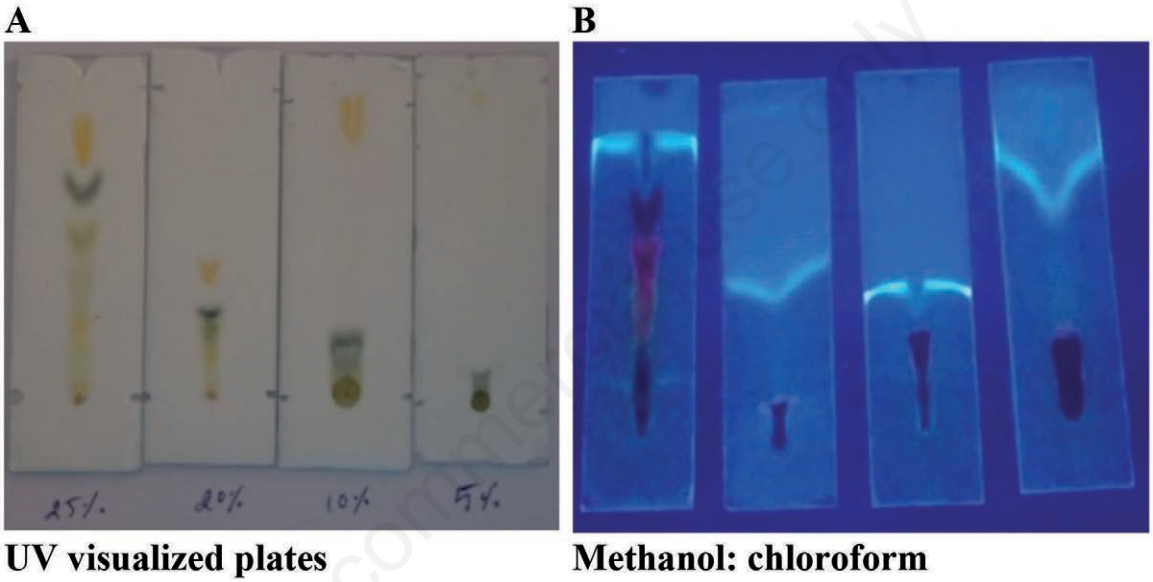


Figure 1. Thin layer chromatography profile for chloroform extracts of *O. canum*.

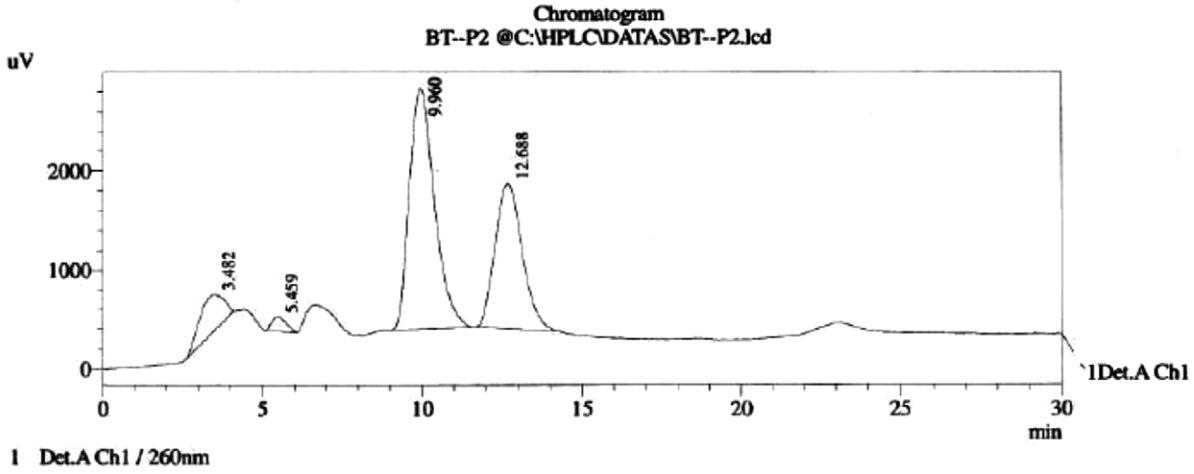


Figure 2. High-performance liquid chromatogram of chloroform extracts of *O. canum*.

IR highlights the bioactive phenyl propanoid from *O. sanctum* (Upadhyaya *et al.*, 2014) and crude extracts of *O. bacillicum* (Gabi Baba *et al.,* 2012) which showed active functional groups.

GC-MS analysis of chloroform extracts of *O. canum*

GC-MS analysis of crude chloroform extracts of *O. canum* were identified as contain seventeen bioactive compounds (Figure 4 and 5). Among them, six (Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl-,(IS), Azulene,1,2,3,3A,4,5,6,7-Octahydro-1,4-dimethyl-7-ci-methylethenyz)-,[Ir-(1.alpha.,3A.beta,4 alp., methyl8,11,14-heptadeca-trienoate,2,6,10,14,8, 22- Tetracosahexaene,2,6,10,15,19,23-hexamethyl(ALL-E)-, eicosane, hentriacontane)were considered as major and remaining minor compounds (caryophyllene,Z,Z-6,28-heptatriactontadien-2-one, Z,Z-6,28-Heptatriactontadien-2-ONE, Phytol, CIS-1-chloro-9-octadecene, 3,7,11,15-tetramethyl-2-hexadecen-1-OL, Hentriacontane, 5-Acetoxy methyl-2,6,10-trimrthyl-2,9-undecadien-6-OL, 2H-1-Benzopyran-6-OL, 3,4-Dihydro-2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)-,Acetate,[2R-[Gamma.-sitosterol) based on the retention time, peaks, molecular weight and percentage of area.Similar kind of work was done by several researchers with different lamiaceae plant species *i.e*., *Stachys oblique* (Harmandar *et al*., 1997), *Origanum dictamnus*, *Teucrium polium* and *Lavandula vera* (Proestos *et al*., 2006), *Teucrium marum* subsp. *marum* (Ricci *et al*., 2005), *Nepeta argolica* (Skaltsa *et al*., 2000) and *Thymus comosus* (Pavel *et al*., 2009) and supports the findings of the present study.

Table 4. Fourier transform infrared spectroscopy analysis of chlorofom leaf extract of *O. canum*.

S. No	Peak range	Types and functional group	Bonding pattern
1	3377.12	Alcohols, phenols	O-H str (s),H band (b)
2	2930.77	Alkanes	C-H stretching
3	1572.21	1° Amines	N-H (bend)
4	1403.82	Aromatics	C-C (str) (in ring)
5	1260.71	Alkyl halides	C-H (-CH_2X)
6	1122.19	Aliphatic amines	C-N (str)
7	1031.51	Aliphatic amines	C-N (str)
8	923.13	Carboxylic acid	O-H (bend)
9	831.98	Alkyl halides	C-Cl (str)
10	757.23	Aromatics	C-H 'OOP'
11	696.11	Alkyl halides	C-Cl str
12	651.77	Alkyl halides	C-Br str
13	618.90	Alkynes	-C=C-H;CH bend

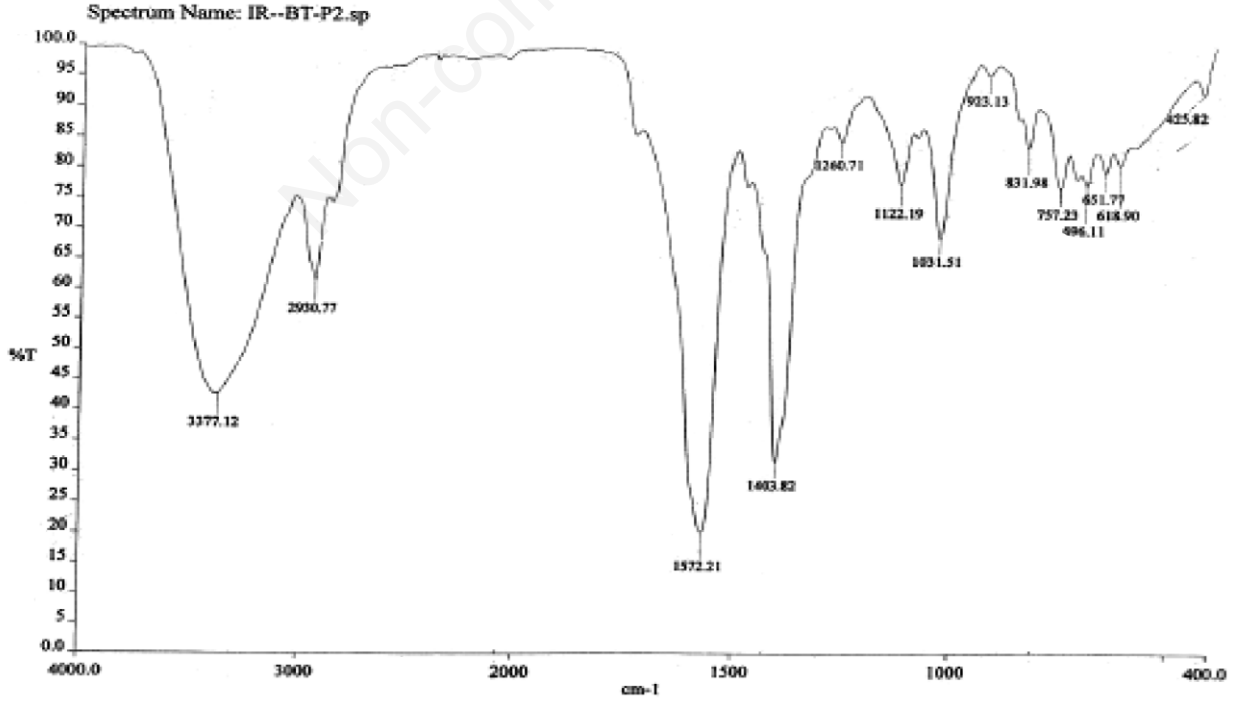


Figure 3. Fourier transform infrared spectroscopy analysis of chloroform extract of *O. canum*.

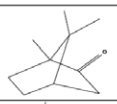
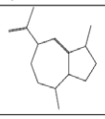
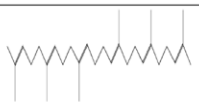
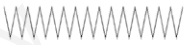
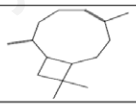
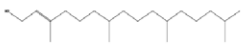
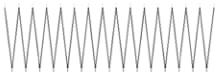
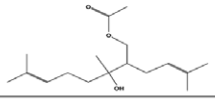
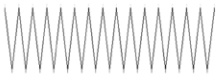
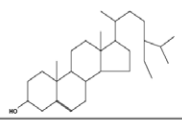
S. No	Retention time	% of peak area	Name of the compounds	Molecular weight	Molecular formula	Structure
1	8.176	16.638	Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl-,(is)	152	$C_{10}H_{16}O$	
2	19.870	9.279	Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-ci-methylethenyz)-,[ir-(1.alpha.,3a.beta.,4-alp.	204	$C_{15}H_{24}$	
3	13.083	8.437	Methyl8,11,14-heptadeca-trienoate	278	$C_{18}H_{30}O_2$	
4	28.944	7.097	2,6,10,14,8,22-tetracosahexaene,2,6,10,15,19,23-hexamethyl(all-e)-	410	$C_{30}H_{50}$	
5	27.929	3.093	Eicosane	282	$C_{20}H_{42}$	
6	12.993	6.172	Hentriacontane	436	$C_{31}H_{64}$	
7	12.067	2.907	Caryoohyllene	204	$C_{15}H_{24}$	
8	16.799	5.696	Z,Z-6,28-heptatriactontadien-2-one	530	$C_{37}H_{70}O$	
9	17.254	1.331	Z,Z-6,28-heptatriactontadien-2-one	530	$C_{37}H_{70}O$	
10	19.480	1.981	Phytol	296	$C_{20}H_{40}O$	
11	20.195	1.124	Cis-1-chloro-9-octadecene	286	$C_{18}H_{35}CL$	
12	20.375	1.032	3,7,11,15-tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$	
13	25.643	1.061	Hentriacontane	436	$C_{31}H_{64}$	
14	25.883	1.034	5-acetoxymethyl-2,6,10-trimethyl-2,9-undecadien-6-ol	282	$C_{17}H_{30}O_3$	
15	27.073	3.706	Hentriacontane	436	$C_{31}H_{64}$	
16	27.413	-	2h-1-benzopyran-6-ol,3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-,acetate,[2r-[	472	$C_{31}H_{52}O_3$	
17	29.349	-	Gamma-sitosterol.	414	$C_{29}H_{50}O$	

Figure 4. GC-MS analysis of chloroform extracts of *O. canum*.

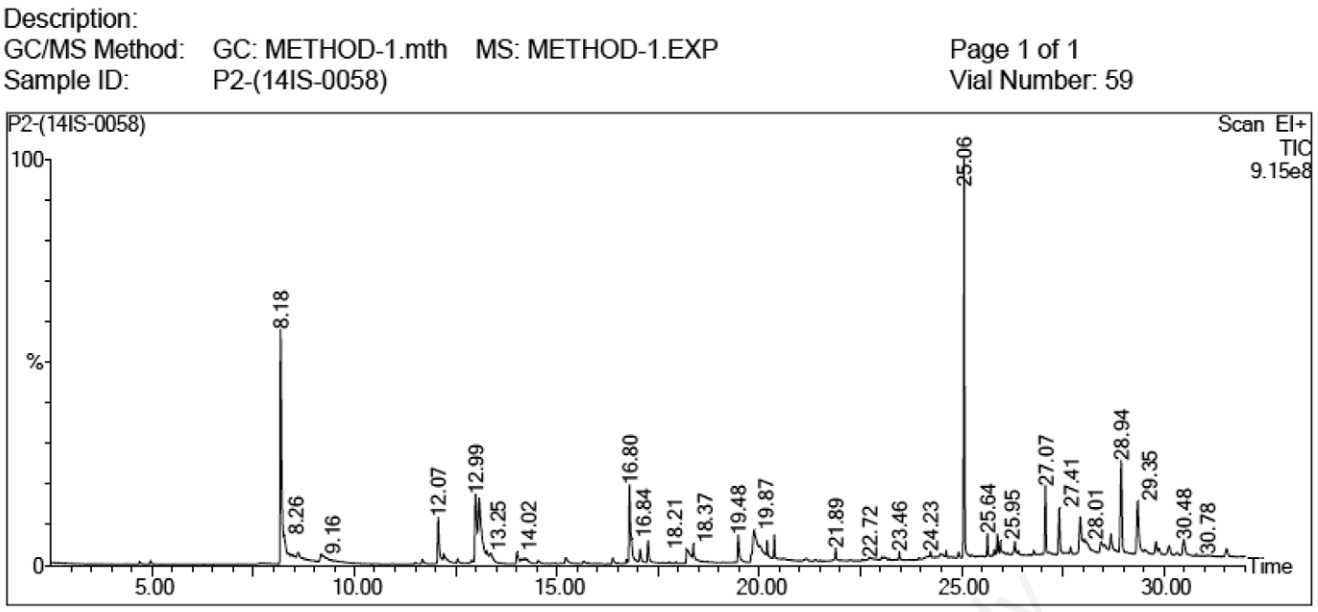


Figure 5. GC-MS chromatogram of *O. canum*.

Conclusions

The chloroform extracts of *Ocimum canum* showed highest mortality against *A. aegypti* vector in all stages. Presence of potential phytoconstituents was identified using preliminary screening test and the functional groups of phytoconstituents were characterized by FT-IR and HPLC. The structural derivation of the identified compounds was carried out by GC-MS analysis. The overall results concluded that the chloroform extracts contain potential bioactive compounds for the mosquitoes repellent, which can be useful for development of green based mosquitocidal agents in future for the control of vector borne diseases especially dengue fever.

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