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Research Article

BIOEFFICACY OF ESSENTIAL OILS OF *LANTANA CAMARA ACULEATA*, AGAINST *AEDES AEGYPTI*, *ANOPHELES STEPHENSI* AND *CULEX QUINQUEFASCIATUS*

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ABSTRACT

Plant essential oils may act as alternatives to conventional pesticides for malaria, dengue and filariasis vector. The aim of this study was to evaluate the larvicidal activity of oil extract of *Lantana aculeata* against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. Essential oil from *L. aculeata* was hydrodistillated and their bio components were determined by GC-MS analysis. *L. aculeata* essential oil was most effective against *A. aegypti* with LC_{50} 11.01 ppm and LC $_{90}$ 34.12 ppm, when compared to *A. stephensi* (LC_{50} 11.18 ppm and LC $_{90}$ 36.44 ppm) and *C. quinquefasciatus* (LC_{50} 12.85 ppm and LC_{90} 39.42 ppm). The results showed that the leaf essential oil and its bio constituents can be considered as a potent source for the production of natural larvicides.

Keywords: *Lantana camara aculeata*, GC-MS, *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*, Larvicidal activity.

INTRODUCTION

Ecologically, mosquitoes are important components of aquatic and terrestrial food chains, being a food source for a number of animals, such as fish and birds (Knio *et al*., 2008). Mosquitoes are transmitters of several of the world most serious human diseases (Cheng *et al*., 2009a) including malaria, dengue fever, yellow fever and filariasis (Prajapathi *et al*., 2005).

Mosquito borne diseases have an economic impact, including loss in commercial and labour output particularly in countries with tropical and subtropical climates; however no part of the world is free from vector-borne diseases (Fradin and Day, 2002).

Aedes aegypti, a vector of dengue is widely distributed in the trophical and subtrophical zones. Dengue fever incidence has increased fourfold since 1970 and nearly half the world's population is now at risk. In 1990, almost 30% of the world population and 1.5 billion people lived in regions where the estimated risk of dengue transmission was greater than 50% (Hales *et al*., 2002).

Anopheles stephensi is the major malaria vector in India. With an annual incidence of 300-500 million clinically manifested cases and a death toll of 1.1-2.7 million, malaria is still one of the most important communicable diseases. Currently about 40% of the world's population lives in areas where malaria is endemic (Wernsdorfer and Wernsdorfer, 2003).

Culex quinquefasciatus, a vector of lymphatic filariasis is widely distributed with around 120 million people infected worldwide and 44 million people have common chronic manifestation (Bernhard *et al.*, 2003).

Chikungunya virus, a member of the alpha virus genus is of considerable public health concern in Southeast Asian and African countries (Pastorino *et al*., 2005).

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To prevent mosquito-borne diseases and improve public health, it is necessary to control them. But in recent years, mosquito control programmes have been suffering from failures because of the ever-increasing insecticides resistance (WHO, 1992).

Besides insecticide resistance in arthropod vectors of tropical diseases, the increased costs of insecticides and increased public concern over environmental pollution have necessitated a continued search for alternative vector control methods, which would be environmentally safer and specific in their action (Coats, 1994).

The constant use of chemical insecticides for the vector control has often led to the disruption of natural biological control systems and outbreaks of insect species (Chaithong *et al*., 2006). Moreover, the adverse effects of these insecticides on the environment, and the undesirable effects on the non-target population and human beings are creating further problems (Lee *et al*., 2001).

All over the world there is a need to find alternatives to synthetic insecticides. From this point of view, botanical pesticides are promising since they are effective, environmentally friendly easily biodegradable and often inexpensive. Essential oils are well known for their antibacterial, antifungal, antitermite and over all insecticidal activities (Cheng *et al*., 2003).

Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vector plants are considered as a rich source of bioactive chemicals (Wink, 1993), and they may be an alternative source of mosquito control agents. Natural products are generally preferred because of their less harmful nature to non-target organisms and due to their innate biodegradability.

Plant-derived materials are also potentially suitable for use in the continuation of integrated mosquito control programs (Alkofahi *et al*., 1989) because they minimize the continuation of harmful residues in the environment.

Several phytochemicals, extracted from various botanical sources, have been reported to have detrimental effects on mosquitoes (Ansari *et al*., 2000). Plant essential oils in general have been recognized as an important natural source of insecticides (Prajapati *et al*., 2005). Their use as mosquito control agents has been shown to minimize the impact that most pesticidal compounds impose on the environment (Cheng *et al*., 2009b). Botanical pesticides are promising in that they are effective, environment friendly, easily biodegradable and also inexpensive (Dharmagadda *et al*., 2005).

Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and ovipositor attractant and have different activities as observed by many researches (Babu and Murugan, 1998). Phytochemicals on account of minimal hazardous effect on the environment and wide range of availability offer promises in future mosquito control programmes. They have revolutionized the fields of vector control as they possess different bioactive components and can be used as general toxicants against various larval stages of the mosquito (Sharma *et al*., 2004).

The family *Verbenacea* includes many ornamental and medicinal plants (Tripathi *et al*., 2003). *Lantana aculeata* (=*camara*) belongs to the family *Verbenacea,* is cultivated as a garden ornamental garden plant (Kumar *et al*., 2006). All parts of *L. aculeata* have been used traditionally for ailments throughout the world. Roots of *L. aculeata* are used for the treatment of malaria, rheumatism and skin rashes (Chharba *et al*., 1993). Several tri-terpenoids, flavonoids, alkaloids and glycosides isolated from this plant are known to exert diverse biological activities (Sharma *et al*., 1998).

The essential oil of *L. aculeata* showed a wide spectrum of antibacterial, antimicrobial and antifungal activity (Siddiqui *et al*., 1995). *L. aculeata* (L) contains triterpenoids related compounds such as lantandene C, reduced lantanolic and lantic acid with potent antimicrobial activity. Since very long time *L. aculeata* Linn, has been reported to be used in traditional medicine system for itches, cuts, ulcers, swelling, bilious, fever, tetanus, cold,

headache, uterine haemorrhage, chickenpox, eye injurious, whooping cough, asthma (Ross, 1999) bronchitis and arterial hypertension (Rastogi *et al*., 1995). In the present study the larvicidal activity of the oil extract of *L. aculeata* leaves were investigated against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*.

MATERIAL AND METHODS

Lantana aculeata plant leaves were collected from Walaja, Vellore District, Tamil Nadu, India during the month of January, 2013. *L. aculeata* was identified by (Voucher No: 2158) Prof. P. Jayaraman, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai-600 045.

Distillation of essential oils

Fresh leaves of *L. aculeata* were subjected to hydrodistillation using a modified Clevenger-type apparatus for 3 hours (Cheng *et al*., 2005). The yield was averaged over four experiments and calculated according to dry weight of the plant material. Essential oil was stored in an air-tight container prior to analysis by Gas Chromatography Mass Spectrometry (GC-MS).

GC-MS Analysis

The composition of the essential oil was determined using an Agilent 7890A Gas Chromatography Mass Spectroscopy instrument. Oxygen-free nitrogen was used as a carrier gas and hydrogen was used for the flame. The GC conditions used were as follows: capillary column: fused silica (Polydimethylsiloxane 0.25 µm film thickness); temperature program: 70ºC (2 min¹), 70-230º C (3 min¹), 230-240ºC (5 min¹), 270ºC (5 min¹); carrier gas, held at 5 bar, linear velocity of 20 cm min¹; injection port split less at 250ºC; injection volume, 0.1 µL. The MS conditions were as follows: ionization EI at 70 eV; m/z range, 30-300ºC; scan rate 1 sec ¹; ionization chamber at 180ºC; and transfer line at 280ºC. The identification of the essential oil constituents was done based on a comparison of their retention times and these constituents were further identified and authenticated using MS data compared to the NIST mass spectral library.

Selection and identification of mosquito species

The important vector species of mosquitoes such as *A. ageypti*, *A. stephensi* and *C quinquefasciatus* were selected and identified in the Zonal Entomological Research Centre, Vellore, Tamil Nadu, India.

Procurement of eggs and rearing of mosquito larvae

The egg rafts of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* were procured from the Entomology Research Institute (ERI), Loyola College, Chennai, India. The eggs were collected and brought to the laboratory (Presidency College, Chennai) and kept in a tray containing tap water (as culture medium) at laboratory condition (26 ± 2ºC). The next day, eggs were observed to hatch out into first instar larvae. Appropriate amount of nutrients (yeast powder and glucose) were added to the culture medium. On the third day after hatching, the first instar larvae moulted into second instar larvae. On the fifth day, third instar larvae were observed which moulted into fourth instar larvae on the seventh day. The durations of first to fourth instar larval periods of *C. quinquefasciatus* were observed to be similar to that of *A. aegypti* and *A. stephensi*. The fourth instar larvae which moulted on the seventh day were allowed to grow in the medium up to eighth day. The fourth instar larvae of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* were used throughout the experiments.

Bioassays and larval mortality

Fourth instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quiquefasciatus* were exposed to test concentrations of 5, 10, 15, 20, 25 ppm of essential oil for 24 hours according to standard methods described by (WHO, 1981). In the control setup, ethanol was applied in the water (1%) and the numbers of dead larvae were counted after 24 h and 48 h of exposure and the percentage of mortality were analyzed from the average of five replicates. The lethal concentration (LC_{50} and LC_{90}) were calculated by probit analysis (Finney, 1971).

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit and Chi-square values were calculated using the SPSS 11.5 (Statistical Package of Social Sciences) software. Results with $P<0.05$ were considered to be statistically significant.

RESULTS

The results of GC-MS characterization of *L. aculeata* are presented in (Table-1; Fig- 1). In the essential oil of *L. aculeata* 55 components are present. Some major components observed are Longifolene, 1,4,7,-Cycloundecatriene, 1,6, Cycloprope, Gamma- muurolene, Beta-Bisabolene, Caryophyllene oxide, Phytol, Copaene, IH-Cyclopropa naphthalene etc.

The regression equation of the oil extract against 4th instar larvae of *A. stephensi*, *A. aegypti and C. quinquefasciatus* after 24 hrs of exposure is represented in (Table-2; Fig-2). The results clearly indicate that the leaf oil extracts of *L. aculeata* at very low concentration was toxic against all the three mosquito species tested. The oil extract was found to be potent against *A. aegypti* with LC_{50} and LC_{90} value of 11.01ppm and 34.12ppm when compared to *A. stephensi* (11.18ppm and 36.44ppm) and C. *quinquefasciatus* with LC_{50} and LC_{90} (12.85ppm and 39.42ppm) respectively.

Table 1. Gas Chromatography Mass Spectrometry of Essential oil from the leaves of *Lantana aculeata.*

S. No.	Retention Time	Area %	Compounds	Molecular Formula	Molecular Weight
1.	3.99	0.08	2,6,6- Trimethlbicyclo (3.1.1) hept-2-ene	$C_{10}H_{16}$	136.23
2.	4.69	0.40	Beta phellandrene	$C_{10}H_{16}$	136.24
3.	4.80	0.11	Beta-Pinene	$C_{10}H_{16}$	136.23
4.	4.95	0.04	Beta-Myrcene	$C_{10}H_{16}$	136.23
5.	5.64	0.13	p-Cymene	$C_{10}H_{14}$	134.21
6.	5.72	0.08	D-Limonene	$C_{10}H_{16}$	136.24
7.	5.99	0.06	1,3,6-Octatriene, 3,7-dimethyl	$C_{10}H_{16}$	136.23
8.	6.23	0.35	Gamma-Terpinene	$C_{10}H_{16}$	136.23
9.	6.98	6.98	1,6-Octadien-3-ol, 3,7-dimethyl	$C_{10}H_{18}O$	154.24
10.	8.38	0.06	Terpinen-4-ol	$C_{10}H_{18}O$	154.25
11.	10.60	0.25	1,5,5-Trimethyl-6-methylene-cyclohexene	$C_{10}H_{16}$	136.23
12.	10.82	0.12	Alpha-Cubebene	$C_{15}H_{24}$	204.25
13.	11.16	0.03	1,2,4-Metheno-1H-indene,octahydro 1,7a-dimethyl-5-(1-methylethyl)(1s-(1.alpha.,2.alpha.,3a.beta.,4.alpha.,5.alpha.,7a.beta.,8s)	$C_{15}H_{24}$	204.35
14.	11.24	0.67	Copaene	$C_{15}H_{24}$	204.35
15.	11.29	0.07	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl0,(1s-(1.alpha.,2.beta.,4.beta.))	$C_{15}H_{24}$	204.35
16.	11.40	2.20	1H-Cyclopropa(a)naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7,7a-tetramethyl(1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.))	$C_{15}H_{24}$	204.35
17.	11.48	0.05	10s.11s-Himachala-3(12),4-diene	$C_{15}H_{24}$	204.35
18.	11.66	0.12	1H-Cycloprop(e)azulene,1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetrame thyl, (1aR-(1a.alpha.,4. alpha.,4a.alpha.,7b.alpha.))	$C_{15}H_{24}$	204.35
19.	11.86	30.42	Longifolene-(V4)	$C_{15}H_{24}$	204.35
20.	11.98	2.52	1H-Cyclopenta(1,3)cyclopropa(1,2)benzene,octahydro-7-methyl-3-methylene-4-(1-methylethyl),(3aS-	$C_{15}H_{24}$	204.35

			(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS)		
21.	12.18	1.62	(E)-beta-Famesene	$C_{15}H_{24}$	204.36
22.	12.34	14.89	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl,-ZZZ	$C_{15}H_{24}$	204.35
23.	12.54	1.16	Gamma-Muurolene	$C_{15}H_{24}$	204.35
24.	12.65	4.78	1,6-Cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl),(S-(E,E,))	$C_{15}H_{24}$	204.35
25.	12.76	0.82	Cis-Thujopsene	$C_{15}H_{24}$	204.35
26.	12.83	7.27	1H-Cycloprop(e)azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-,[1aR-(1a.alpha.,4.alpha.,4a.beta., 7b.alpha.)]-	$C_{15}H_{24}$	204.35
27.	12.94	4.30	Beta-Bisabolene	C_9H_{14}	122.21
28.	13.10	6.06	Gamma-Muurolene	$C_{15}H_{24}$	204.35
29.	13.33	1.55	Cyclohexene,4-(1,5-dimethyl-1,4-hexdienyl)-1-methyl-1,3,6-Octatriene,3,7-dimethyl-Z)	$C_{15}H_{24}$	186.27
30.	13.47	0.25	Gamma-Muurolene	$C_{15}H_{24}$	204.35
31.	13.55	0.34	Longifolene	$C_{15}H_{24}$	204.36
32.	13.63	0.61	Gamma-Elemene	$C_{15}H_{24}$	204.35
33.	13.85	1.32	1-Hydroxy-1,7-dimethyl-4-isopropyl	$C_{15}H_{26}O$	222.36
34.	13.92	2.88	Caryophyllene oxide	$C_{15}H_{26}$	204.36
35.	14.08	0.85	Naphthalene	$C_{15}H_{24}$	204.35
36.	14.20	0.38	Naphthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)	$C_{15}H_{24}$	204.35
37.	14.26	1.11	3,5-Dimethylcyclohex-1-ene-4-carbo xaldehyde	$C_{10}H_{18}$	138.24
38.	14.36	0.69	Azulene,1,2,3,3a,4,5,6	$C_{15}H_{24}$	123.15
39.	14.45	2.49	Alloaromadendrene	$C_{15}H_{24}$	204.35
40.	14.62	1.29	Bicyclo(4.4.0)dec-1-ene,2-isopropyl-5-methyl-9-mthylene	$C_{15}H_{26}O$	222.36
41.	14.66	2.06	Copaene	$C_{15}H_{24}$	204.36
42.	14.78	1.24	Epiglobulol, alpha-cadinol	$C_{15}H_{26}O$	222.36
43.	14.91	0.30	Alloaromadendrene oxide	$C_{15}H_{24}O$	204.35
44.	14.97	0.66	2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane.	$C_{15}H_{22}$	222.36
45.	15.16	0.86	4-phenyl methanol,4-benzeneamine.	$C_{15}H_{22}$	202.33
46.	15.36	0.25	1,12-Tridecadiene, Alloaromadendrene oxide	$C_{15}H_{22}$	202.33
47.	15.75	0.10	2,3-pyrazinedicarboxamide Tricycloundecane	$C_6H_6N_4O_2$	166.13
48.	16.09	0.11	Benzyl benzoate	$C_{14}H_{12}O_2$	212.24
49.	16.15	0.09	Cycloisolongifolene,8,9,-dehydro-Naphthalene	$C_{15}H_{22}$	202.35
50.	16.25	0.16	1,5,9-undecatriene, 2,6,10-trimethyl	$C_{14}H_{24}$	192.34
51.	16.70	0.09	2-Pentadecanone,6,10,14-trimethyl	$C_{18}H_{36}O$	268.47
52.	18.53	0.09	Nerolidol, Farnesol, methyl ether	$C_{15}H_{26}O$	222.36
53.	19.22	0.03	Butyl 9-tetradecenoate, 6-Octadecenoic acid	$C_{17}H_{28}O$	264.40
54.	19.41	1.33	Phytol	$C_{20}H_{40}O$	296.53
55.	19.60	0.10	Bromoacetic acid, Octadecyl ester	$C_{20}H_{39}BrO_2$	391.42

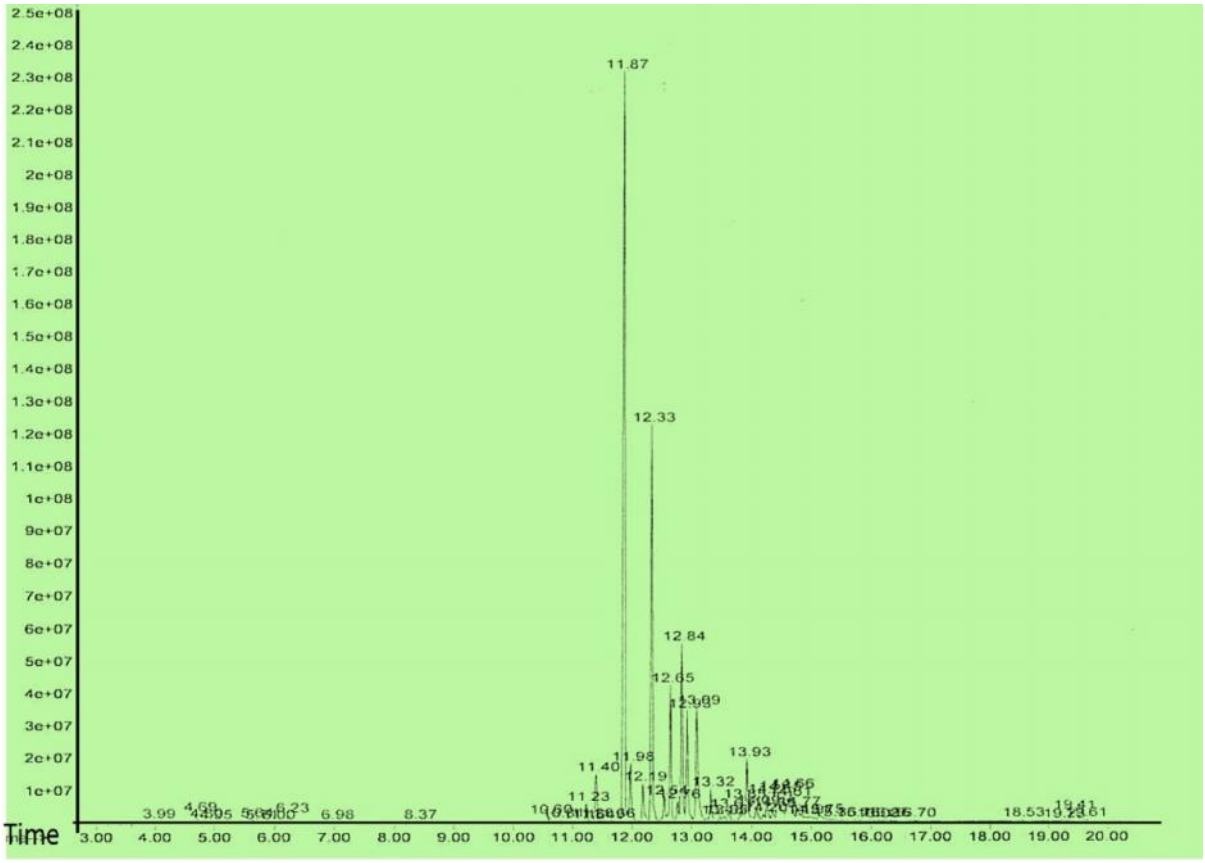

Figure 1. GC-MS analysis of oil leaf extract of *L. aculeata.*

Table 2. Larvicidal activity of oil extract of *Lantana aculeata* against malaria, dengue and filariasis vectors.

Species	Conc. (ppm)	(%) Mortality	LC_{50}	UCL-LCL	LC_{90}	UCL-LCL	Slope	r^2
A. stephensi	25	100	11.18	13.35-9.14	36.44	39.49-32.62	63	0.989
	20	90						
	15	63						
	10	41						
	5	32						
A. aegypti	25	100	11.01	12.96-9.64	34.12	39.19-31.07	39	0.985
	20	100						
	15	64						
	10	39						
	5	27						
C.quinquefasciatus	25	100	12.85	14.21-11.63	39.42	43.17-35.6	48	0.990
	20	83						
	15	48						
	10	33						
	5	24						

Control –Nil Mortality LC_{50}- Lethal concentration of 50%, LC_{90} –Lethal Concentration of 90% LCL-Lower confidence limit, UCL- upper confidence limit.

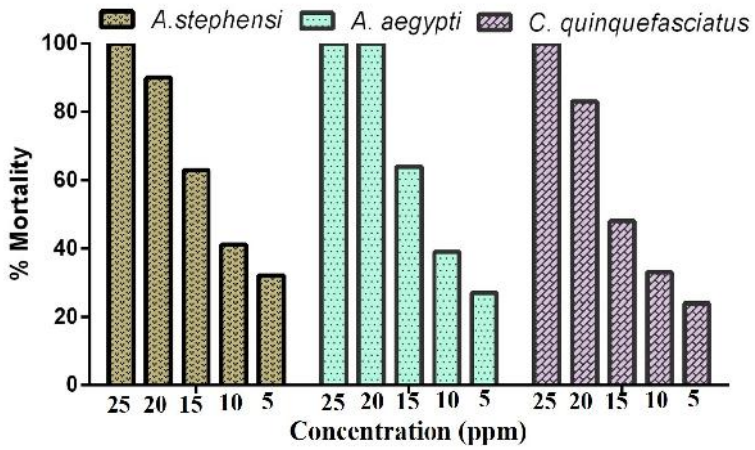

Figure 2. Larvicidal activity of oil extract of *L. aculeata* against malaria, dengue and filariasis vectors.

DISCUSSION

Mosquito control at the larval stage can be an effective procedure due to the low mobility of larvae in their breeding habitats in respect to times (Wiseman *et al*., 2005). Natural pesticides, especially those derived from plants are more promising in their aspect (Amer *et al*., 2006).

Essential oils and plants extracts may be an alternative to synthetic insecticides because they are effective, eco-friendly, easily biodegradable and inexpensive (Choochote *et al*., 2005). The use of plant essential oils in insect control is an alternative pest control method for minimizing the noxious effects of some pesticides compounds on the environment (Fatope *et al*., 1993).

Besides toxic and repellent properties, essential oils have been shown to have a pronounced effect on the developmental period, growth, adult emergence, fecundity, fertility and egg hatching of insects (Elango *et al*., 2010).

GC-MS analysis shows the presence of 55 components. The leaf oil extract was found to be potent against *A. aegypti* when compared to *A. stephensi* and *C. quinquefasciatus.* Extracts of *L. aculeata* against *Plutella xylostells* and *Spodoptera litura* larvae showed antifeeding and repellant effect on tea mosquito bug (Dong *et al*., 2005; Deka *et al.*, 1998).

Major constituents from the *Tagetes patella* essential oil such as limonene, β-ocimene and β-caryophyllene possessed potent larvicidal activity (Rana and Rana, 2012). Similar compound such as limonene and β-caryophyllene present in *L. aculeata* may be responsible for the potent larvicidal activity; these phytocompounds may be responsible for ecdysal failure and mortality (Neraliya and Srivastava, 1996).

All terpenoids, alcohols, ketones and carboxylic esters showed toxicity to mosquito species. Monoterpene alcohols were the most toxic compounds against mosquito species Tiwary *et al*. (2007) reported larvicidal activity of the essential oil extracted from the seeds of *Zanthoxylum armatum* against three species of mosquito vectors, *A. aegypti, A. stephensi* and *C. quinquefasciatus.* Sutthanont *et al*., 2010 investigated the chemical compositions and larvicidal potential of *Citrus hystrix*, *Citrus reticulate*, *Zingiber zerumbet*, *Kaempferia galanya* and *Syzygium aromaticum* against mosquito vectors. They suggested the use of these essential oils from edible herbs as a potentially alternative source for developing novel larvicides to be used in controlling vectors of mosquito-borne diseases.

The essential oils were found to be relatively more toxic to larvae of mosquitoes. Earlier studies involving the essential oils obtained from various plants, viz. *Ocimum lamiifolium, Chenopodium ambrosioides, Mentha spicata, Eucalyptus globules* and *Azadirachta indica* (neem) showed larvicidal activity against the larvae of the *A. gambiae*s mosquito (Massebo *et al*., 2009). Essential oil of *Ocimum americanus* and *O. ratissium* contains *Caryophyllene* as main constituent possessed larvicidal activity against *A. aegypti* (Cavalcanti *et al*., 2004).

Active compounds of *L. aculeata* oil extracts may be responsible for the larvicidal activity. It is evident from the present study that plant oil extracts might have promising larvicidal efficacy and could be useful in producing newer, safer and more effective natural compounds as larvicides.

CONCLUSION

Plant products are emerging as a potential source for mosquito control. From the present study it is evident that the weed extracts of *L. aculeata* have promising larvicidal efficacy. Leaf oil extracts of the plant could be used in stagnant water bodies, which are the breeding grounds for the mosquitoes. Hence the large biomass of the weed *L. aculeata* available in the wastelands of southern India can be used as a bioresource to control the larvae of mosquito.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

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