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Gas chromatography mass spectrometry analysis and larvicidal activity of leaf essential oil extract of *Leucas aspera* against dengue, malaria and filariasis vectors

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Abstract

Background: Insect-transmitted diseases cause high morbidity but they also include deadly diseases that cause high mortality rates among infected individuals. Several mosquito species including *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* are vectors. *Ae. aegypti*, a vector of yellow fever, dengue, and chikungunya is widely distributed in the tropical and subtropical zones. *An. stephensi* is the primary vector of malaria in India and other west Asian countries. *C. quinquefasciatus* is a vector of lymphatic filariasis and it is widely distributed tropical diseases with around 120 million people infected worldwide.

Results: In the view of an increasing interest in developing plant-derived insecticides as an alternative to chemical insecticides, this study was undertaken to assess the larvicidal potential of essential oil extracted from *Leucas aspera* leaves against three medically important species of mosquito vectors, *Ae. aegypti*, *An. stephensi*, and *C. quinquefasciatus*. The oil extract was found to be potent against *Ae. Aegypti* LC_{50} and LC_{90} (lethal concentration LC) value of 15.59 ppm and 46.77 ppm when compared to *An. stephensi* (17.10 ppm and 51.20 ppm) and *C. quinquefasciatus* with LC_{50} and LC_{90} (16.19 ppm and 47.79 ppm) respectively. Gas chromatography mass spectrometry analysis of the essential oil extract of *L. aspera* was done to identify the major phytocompounds.

Conclusion: The results showed that the oil extract of *L. aspera* and its effective constituents can be considered as potent source for the production of natural larvicides.

Keywords: Vector control, *L. aspera*, GC-MS, *Ae. aegypti*, *An. stephensi* and *C. quinquefasciatus*

Background

Mosquitoes are one of the most medically significant vectors, and they transmits parasites and pathogens, which continue to have devastating impact on human beings; the vector-borne disease caused by mosquitoes are one of the major health problems in many countries. Malaria, dengue, yellow fever, and filariasis are few of the deadly disease spread by mosquitoes (Maheswaran, Kingsley, & Ignacimuthu, 2008). The mosquito is the principal vector of the vector-borne disease affecting human beings and other animals; several mosquito species including *Aedes aegypti, Anopheles stephensi*, and *Culex quinquefasciatus* are vectors for the pathogens of various diseases (Karthikeyan, Sivakumar, Aishwarya, & Mohanasundram, 2012).

An. stephensi (Liston) is the primary vector of malaria in India and other West Asian countries; malaria remains one of the most prevalent diseases in the tropical world. With 200 million to 450 million infections annually worldwide, it causes up to 2.7 million deaths (World Health Organization, 2010).

C. quinquefasciatus (Say.) acts as a vector for filariasis in India. Lymphatic filariasis caused by *Wuchereria*

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bancrofti and transmitted by mosquito *C. quinquefasciatus* is found to be more endemic in the Indian subcontinent. It is reported that *C. quinquefasciatus* infects more than 100 million individuals worldwide annually (Govindarajan, Mathivanan, Elumalai, Krishnappa, & Anandan, 2011).

The control of mosquito larvae worldwide depends primarily on continued applications of synthetic insecticides including organophosphates and chlorinated hydrocarbons (e.g., DDT); however, heavy wide use of these insecticides has caused several environmental and health concerons (Chen, Chao, Ligang, & Zhi, 2013).

Botanical pesticides have the advantage of providing novel modes of actions against insects that can reduce the risk of cross resistance as well as offering new designs for specific molecule targets. During the screening program for new agrochemicals from Chinese medical herbs and wild plants, essential oil of *Ageratum conyzoides L.* aerial parts at flowering stage was found to possess strong insecticidal toxicity against the Asian tiger mosquito *Aedes albopictus* (Liu & Liu, 2014).

Essential oils play important role in controlling several mosquito species. In general, essential oils from plants have been considered important natural resources to act as insecticides (Gbolade, Dyedele, Sosan, Adewayin, & Soylea, 2012); they are effective, environmentally friendly, and easily biodegradable in nature. It is suggested that many compounds derived from various essential oils can cause toxic activity against mosquito species (Bhat & Kempraj, 2009).

The use of herbal products is one of the best alternatives for mosquito control. Many researchers have been reported on the larvicidal properties of plant essential oils against *Anopheles* mosquitoes. Essential oils extracted from *Azadirachta indica* (Okumu, Knols, & Fillinger, 2007) and leaves and rhizomes of *Curcuma longa* (Molodchik, 2013). *Plectranthus amboinicus*, *Zanthoxylum aratum*, *Eucalyptus tereticornis*, and *Tagetes patula* demonstrated larvicidal activity against *Anopheles stephensi* (Dharmagadda, Naik, Mittal, & Asudevan, 2014). Larvicidal activity of essential oils from *Blumea mollis* and *Zingifer officinalis* (Pushpanathan, Jebanesan, & Govindarajan, 2008) has been reported against *C. quinquefasciatus.*

Larvicidal activity of essential oils from *Melaleuca leucadendron*, *Litsea cubeba* and *Listea salicifolia*, *Ocimum suave*, and *O. kilimandshricum* (Kweka, Mosha, Lowassa, Mahande, Kitau, Matowo, Mahande, Massenga, Tenu, Feston, Lyatuu, Mboya, Meneme, Chuwa, & Temu, 2008) have been reported against *Anopheles arabiensis*, *An. gambiae*, and *C. quinquefasciatus.* Larvicidal activity of essential oils from *Zanthoxylum armatum* (Tiwary, Naik, Tewary, Mittal, & Yadav, 2007) and *Ocimum canum* (Singh, Kumari, & Chauhan, 2003) have been reported against *C. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi.* Essential oils derived from various plants not only exhibit inhibitory activity against bacteria, fungi, and termites but also show strong mosquito repellent larvicidal activities; the present study was aimed to assess the larvicidal and knockdown activities of the essential oils from various plants against *C. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi* (Cheng, Liu, Tsai, Chen, & Chang, 2004).

Leucas aspera (*Wild.*) Link of the family *Lamiaceae* is an annual, branched herb that commonly grows in grassland. It is distributed throughout India from the Himalaya to Ceylon. In traditional medicine, this plant is used as an antipyretic and insecticide, and diverse biological activities such as antioxidant, antimicrobial, hepatoprotective, antinociceptive cytotoxic, and anthelminthic have been reported. The major volatile constituents are alpha-farnesene, alpha-thujene, and menthol leaves, while namely propionate and isoamyl propionate from flowers of *L. aspera* have been reported from India (Mangathayatu, Amitabha, Rajeev, & Kaushik, 2006). In another report from Nepal, the main constituents from the essential oil of aerial parts of *L. aspera* were identified as1-octen-3-ol, caryophyllene, and caryophyllene oxide; the essential oil of the seeds of *L. aspera* has larvicidal properties against the mosquito *Aedes aegypti* (Joshi 2013). In the present study, the larvicidal activity of the oil extract of *L. aspera* leaves were investigated against *Ae. aegypti*, *An. stephensi*, and *C. quinquefasciatus.*

Methods

Collection of plant

Leucas aspera plant leaves were collected from Kancheepuram District, Tamil Nadu, India during the month of January, 2016. *L. aspera* (Fig. 1) was identified by (Voucher. No. 2110; Flora of South India by G.S. Gamble—

Fig. 1 *Leucas aspera*

Volume-II) Prof. P. Jayaraman, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai-600045.

Distillation of essential oils

Fresh leaves of *L. aspera* were subjected to hydrodistillation using a modified clevenger type apparatus for 3 h (Cheng, Liu, Tsai, Chen, & Chang, 2004). 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).

Gas chromatography mass spectrometry analysis

The composition of the essential oil was determined using an Agilent 7890 GC-MS 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: 70 °C (2 min^1), 70–230 °C (3 min^1), 230–240 °C (5 min^1), 270 °C (5 min^1); carrier gas, held at 5 bar, linear velocity of 20 cm min^1; injection port splitless at 250 °C; injection volume, 0.1 μL. The MS conditions were as follows: ionization EIat 70 eV; m/z range, 30–300 °C; scan rate 1 s^1; ionization chamber at 180 °C; and transfer line at 280 °C. 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 mass spectrophotometry (MS) data compared to the NIST mass spectral library.

Selection and identification of mosquito species

The important vector species of mosquitoes such as *Ae. aegypti*, *An. stephensi*, and *C. quinquefasciatus* were selected and identified in the Zonal Entomological Research Centre, Vellore, Tamil Nadu, India. *An. stephensi* is vector of malaria in India and larvae of these species are generally found in distinctly different habitat. *Ae. aegypti* is a vector for transmitting several tropical fevers such as dengue fever, chikungunya, yellow fever, and other diseases. *C. quinquefasciatus* (Say.) acts as a vector for filariasis in India. *C. quinquefasciatus* is the vector of West Nile which causes encephalitis or meningitis affecting the brain tissue resulting in permanent neurological damage.

Bioassays and larval mortality

Fourth instar larvae of *Ae. aegypti*, *An. stephensi*, and *C. quinquefasciatus* were exposed to test concentrations of 5, 10, 15, 20, and 25 ppm of essential oil for 24 h according to standard methods described by the World Health Organization (WHO, 1981). In the control setup, ethanol was applied in the water (1%) and the numbers of dead larvae were counted after 24 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

Table 1 Larvicidal activity of oil extract of *Leucas aspera* against malaria, dengue, and filariasis vectors

Species	Concentration (ppm)	24 h % Mortality	LC_{50} (UCL–LCL) (ppm)	LC_{90} (UCL–LCL) (ppm)	r^2
An. stephensi	50	100	17.10 (20.56–14.21)	51.20 (58.49–47.19)	0.983
	30	82			
	30	70			
	20	56			
	10	43			
Ae. aegypti	50	100	15.59 (18.74–13.14)	46.77 (49.90–42.09)	0.944
	30	93			
	30	74			
	20	63			
	10	49			
C. quinquefasciatus	50	100	16.19 (19.70–12.18)	47.79 (45.91–40.05)	0.942
	30	92			
	30	71			
	20	60			
	10	44			

Control—nil mortality
LC50—lethal concentration of 50%
LC 90—lethal concentration of 90%
LCL lower confidence limit, *UCL* upper confidence limit

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.

Result and discussion

The regression equations of the oil extract against fourth instar larvae of *Ae. aegypti*, *An. stephensi*, and *C. quinquefasciatus* after 24 h of exposure is represented in (Table 1). The results clearly indicate that the leaf oil extracts of *L. aspera* at very low concentration was toxic against all the three mosquito species tested. The oil extract was found to be potent against *Ae. aegypti* with LC_{50} and LC_{90} value of 15.59 ppm and 46.77 ppm when compared to *An. stephensi* (17.10 ppm and 51.20 ppm) and *C. quinquefasciatus* with LC_{50} and LC_{90} (16.19 ppm and 47.79 ppm) respectively. The essential oils were found to be relatively more toxic to the 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 *Anopheles* gambiae mosquito (Massebo, Tadesse, Bekele, Balkew, & Michael, 2009). 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, Ibrahim, & Takeda, 1993).

The results of GC-MS characterization of *L. aspera* are presented in (Table 2). In the essential oil of *L. aspera*, 30 components are present. Some major components observed are Longifolene, 1,4,7,-caryophyllene

Table 2 Gas chromatography mass spectrometry of essential oil from the leaves of *L. aspera*

S. No	RT	Name of the compound	Molecular formula	Mol. weight (g/mol)	Peak area
1	9.767	2H-1-1Benzopyran	$C_{13}H_{22}O$	194.31	0.06
2	10.55	Alpha-Cubebene	$C_{15} H_{24}$	204.35	0.04
3	11.13	Cyclohexane, 1-ethenyl-1-methyl	$C_{12}H_{20}$	164.28	1.40
4	11.36	Isocaryophillene	$C_{15} H_{24}$	204.35	1.63
5	11.59	Longifolene	$C_{15}H_{24}$	204.36	42.69
6	11.69	Bicyclo	C_9H_{16}	124.22	0.63
7	11.98	1,4,7,-Cyclooundecatriene	$C_{15}H_{24}$	204.35	6.19
8	12.23	1H-Cyclopropa(a) naphthalene	$C_{11}H_8$	140.181	1.34
9	12.35	Spiro	C_9H_4O	138.20	0.84
10	12.39	Naphthalene	$C_{10}H_8$	138.17	2.62
11	13.19	Caryophyllene oxide	$C_{15}H_{24}$	204.36	0.31
12	13.74	Beta-Santalol	$C_{15}H_{24}O$	220.35	0.14
13	14.05	Magastigma-4,6(E),8(Z)-triene	$C_{13}H_{20}$	176.29	0.21
14	14.11	Alloaromadendrene oxide-(1)	$C_{15}H_{24} O$	220.35	0.17
15	14.51	8-Heptadecene	$C_{17}H_{34}$	238.45	0.90
16	14.93	Pentadecanal	$C_{15}H_{30}O$	226.39	0.26
17	16.30	2-Pentadecanone,6,10,14-trimethyl	$C_{18}H_{36}$	268.47	0.49
18	16.60	10,10-Dimethyl-2,6-dimethylenebicy	$C_{15}H_{24}O$	220.35	0.31
19	16.76	13-Tetradece-11-yn-1-ol	$C_{14}H_{24} O$	208.33	0.15
20	16.82	3-Tetradecen-5-yne	$C_{14}H_{24}$	192.34	0.49
21	17.04	Octadecanal	$C_{18}H_{38}O$	270.49	0.29
22	17.59	*n*-Hexadecanoic acid	$C_{16}H_{32} O_2$	256.42	4.68
23	17.77	5-Eicosene	$C_{20}H_{40}$	280.53	0.38
24	17.81	13-Ctadecadien-1-ol	$C_{18}H_{34}O$	266.46	0.41
25	19.59	1-Docosene	$C_{22}H_{44}$	308.58	0.41
26	20.50	Heptacosane,1-chloro	$C_{27}H_{56}$	380.73	4.33
27	22.12	Octadecane	$C_{18}H_{38}$	254.44	0.11
28	22.53	Phytol	$C_{20}H_{40}O$	296.53	1.11
29	23.62	Heptacosane	$C_{26}H_{54}$	366.70	1.08
30	22.53	Tetracosane	$C_{24}H_{50}$	338.65	0.54

Table 3 Activity of phyto-components identified in medicine of essential oil from the leaves of *L. aspera*

Sl. No	Name of the compound	Compound medicinal activities
1	Alpha-Cubebene	Antimicrobial activity, antioxidants, anti-inflammatory, anti-cancer
2	Isocaryophillene	Anti-inflammatory agents that are non-steroidal in nature. In addition to anti-inflammatory actions, they have analgesic, antipyretic, and platelet-inhibitory actions.
3	Longifolene	The antitumor activity of compounds
4	Spiro	Microbial infections like cancer and tubercular, viral, HIV, bacterial, and fungal infections
5	Naphthalene	The antimicrobial and antifungal activity
6	Caryophyllene oxide	Antiedemic, antifeedant, anti-inflammatory, antitumor, calcium antagonist, fungicide, insecticide, pesticide
7	8-Heptadecene	Pulmonary edema, irritation, tetany, diarrhea, anemia, respiratory failure
8	Pentadecanone,6,10, 14-trimethyl	Food additives and flavoring agents
9	Phytol	Antinociceptive activity and antioxidant activity
10	5-Eicosene	Anticancer activity and ntibacterial activity

oxide, 1,6,-hexadecanoic acid, 8-heptadecene, naphthalene, heptacosane,1-chloro, phytol, 5-eicosene, IH-Cyclopropa naphthalene, pentadecanal, etc. GC-MS analysis shows the presence of 30 components (Table 3, Fig. 2). The essential oil from the leaf extract was found to be potent against *Ae. aegypti* when compared to *An. stephensi* and *C. quinquefasciatus.* Extracts of *Lantana aculeata* against *Plutella xylostells* and *Spodoptera litura* larvae showed antifeeding and repellant effect on tea mosquito bug (Deka & Handique, 1998). Essential oil of *Ocimum americanus* and *Ocimum ratissium* contains Caryophyllene as main constituent which possessed larvicidal activity against *Ae. aegypti* (Cavalcanti, Demorais, Lima, & Santana, 2004).

Major constituents from the *Tagetes patella* essential oil such as limonene, β-ocimene, and β-caryophyllene

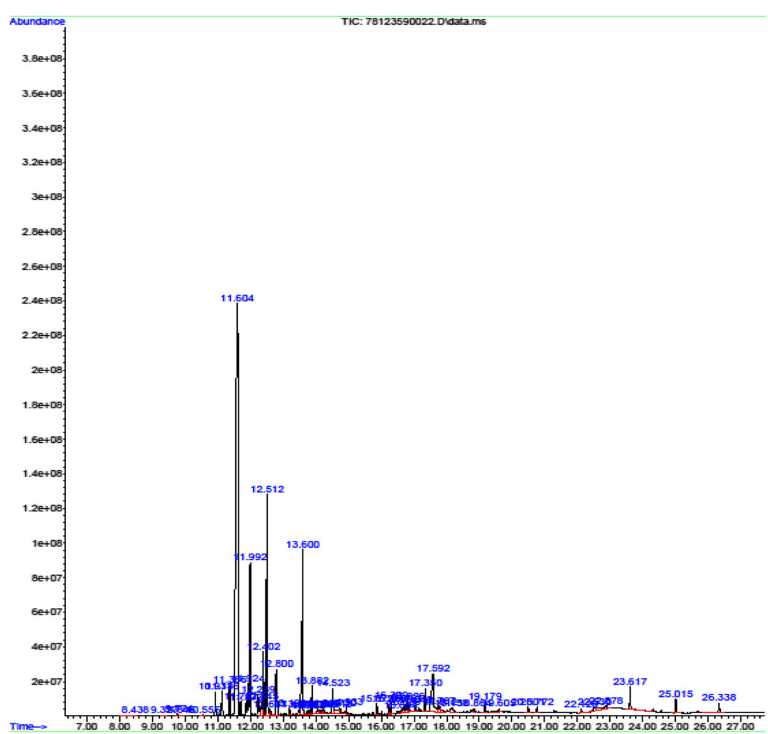

Fig. 2 GC-MS analysis of oil leaf extract of *L. aspera*

possessed potent larvicidal activity (Rana & Rana, 2012). Similar compound such as limonene and β-caryophyllene present in *L. aspera* may be responsible for the potent larvicidal activity; these phytocompounds may be responsible for ecdysal failure and mortality (Hemalatha, Elumalai, Vignesh, Murugesan, & Kaleena, 2014). 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, Rahuman, Kamaraj, Zahir, & Bagavan, 2010).

All terpenoids, alcohols, ketones, and carboxylic esters showed toxicity to mosquito species. Monoterpene alcohols have been reported to be toxic against mosquito species Tiwary, Naik, Tewary, Mittal and Yadav (2007) reported larvicidal activity of the essential oil extracted from the seeds of *Zanthoxylum armatum* against three species of mosquito vectors, *Ae. aegypti*, *An. stephensi*, and *C. quinquefasciatus*. Sutthanont, Choochote, Tuetun, Junkum, Jitpakdi and Chaithong (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. Active compounds of *L. aspera* 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 essential oil leaf extracts of *L. aspera* 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. The mode of action and larvicidal efficiency of the *L. aspera* oil extract under the field conditions should be scrutinized and determined. Besides, further investigation regarding the effect on non-target organism is extremely important and imperative in the near future.

Abbreviations

BHC: Benzene hexa chloride; DDT: Dichloro diphenyl trichloroethane; LC: Lethal concentration; MS: Mass spectrophotometry; PARC: Plant anatomy research center; SPSS: Statistical package of social sciences; WHO: World health organization

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

The work was framed and executed by DE. DE and MK supervised the first author. The manuscript edition (calculation and language) was done by PKK and DE. AV and MK served as sample collectors during the experimental period. All authors read and approved the final manuscript.

Ethics approval

We declare that we do not need an ethics approval regarding our work on the mosquito control.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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