Journal of the Saudi Society of Agricultural Sciences (2015) xxx, xxx–xxx

King Saud University

Journal of the Saudi Society of Agricultural Sciences

www.ksu.edu.sa
www.sciencedirect.com

FULL LENGTH ARTICLE

Larvicidal activity and GC–MS analysis of *Leucas aspera* against *Aedes aegypti Anopheles stephensi* and *Culex quinquefasciatus*

D. Elumalai, P. Hemalatha, P.K. Kaleena *

Department of Zoology, Presidency College (Autonomous), Chennai 600005, Tamil Nadu, India

Received 14 August 2015; revised 16 October 2015; accepted 18 October 2015

KEYWORDS

Leucas aspera;
GC–MS analysis;
Larvicidal activity;
Aedes aegypti;
Anopheles stephensi;
Culex quinquefasciatus

Abstract The mosquitocidal activity of aqueous, ethanol, methanol, chloroform and petroleum ether plant extracts of *Leucas aspera* against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* was analyzed. The larval mortality of fourth instar larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* after 24 h and 48 h of treatment was observed separately in control 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 ppm concentrations. The plant extracts were screened to identify the phytochemical bioactive compounds. *Ae. aegypti* was found to be most susceptible than the other species. Based on probit analysis the 24 h and 48 h methanol extracts of *L. aspera* showed pronounced larvicidal activity when compared with the other extracts. An LC_{50} and LC_{90} value of methanol extracts against *Cx. quinquefasciatus* was found to be 37.649 ppm and 27.855 ppm (24 h), 79.150 ppm and 73.284 ppm (48 h) respectively. LC_{50} and LC_{90} values were 35.624 ppm and 20.897 ppm (24 h), 64.260 ppm and 60.096 ppm (48 h) against *Ae. aegypti*. The 24 h and 48 h LC_{50} and LC_{90} values of ethanol extracts of *L. aspera* were found to be 40.877 ppm and 34.359 ppm, 72.903 ppm and 67.355 ppm against *An. stephensi*. The extracts of this plant showed potent larvicidal efficacy and can be considered for further investigation.

1. Introduction

Mosquitoes play a predominant role in the transmission of malaria, dengue fever, yellow fever, filariasis and several diseases which are today among the greatest health problems in the world. Mosquitoes are one of the most medically significant vectors, and they transmit parasites and pathogens, which continue to have a devastating impact on human beings and other animals (Elumalai et al., 2013a,b). Several mosquito species belonging to genera *Anopheles*, *Aedes* and *Culex* are the vectors for the pathogens of various diseases and contribute

* Corresponding author. Tel.: +91 9840152600.
E-mail addresses: pkkaleena@yahoo.co.in, drpkklab@gmil.com (P.K. Kaleena).
Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jssas.2015.10.003
1658-077X © 2015 Production and hosting by Elsevier B.V. on behalf of King Saud University.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Please cite this article in press as: Elumalai, D. et al., Larvicidal actiL. aspera –>vity and GC–MS analysis of *Leucas aspera* against *Aedes aegypti Anopheles stephensi* and *Culex quinquefasciatus*. Journal of the Saudi Society of Agricultural Sciences (2015), http://dx.doi.org/10.1016/j.jssas.2015.10.003

Table 1 Phytochemical screening of plant extracts of *L. aspera*.

S. No	Secondary metabolite	Aqueous extract	Chloroform extract	Ethanol extract	Petroleum ether extract	Methanol extract
1	Carbohydrates	+ + +	+ + +	+ + +	–	+ + +
2	Tannins	+ + +	+ + +	+ + +	+ + +	+ + +
3	Saponin	–	–	–	+ + +	+ + +
4	Flavonoids	+ + +	–	+ + +	–	+ + +
5	Alkaloids	–	–	+ + +	+ + +	+ + +
6	Quinones	+ +	+ + +	+ + +	+ +	+ + +
7	Glycosides	–	–	–	–	–
8	Terpenoids	+ +	+ + +	+ + +	+ + +	+ + +
9	Triterpenoids	–	–	+ + +	+ + +	+ + +
10	Phenols	+ + +	–	+ + +	+ +	+ + +
11	Coumarins	+ + +	+ +	+ + +	–	+ + +
12	Acids	–	+	+ + +	+ + +	–
13	Proteins	+ + +	–	–	–	+ +
14	Cyanin	+ +	+ + +	+ + +	+ +	–
15	Cardiac glycosides	+ + +	+ +	+ + +	+ +	+ + +

+ + +: Strongly positive. + +: Positive.
+: Trace. –: Not detected.

significantly to poverty and social debility in tropical countries (Jiang et al., 2009).

An. stephensi (L) is the primary vector of malaria in India and other West Asian countries (Mittal and Subbarao, 2003). Larvae of the *Anopheles* species are generally found in distinctly different habitat and are nocturnal, crepuscular in nature and also transmit the filarial worm causing filariasis (Dean, 2001).

Ae. aegypti (L) the yellow fever mosquito spreads dengue fever, chikungunya and yellow fever, viruses and other diseases. It is a vector for transmitting several tropical fever and only the female bites for blood which she needs to mature her eggs (Hahn et al., 2001).

Cx. quinquefasciatus (S) is the predominant house-reaching mosquito in many tropical countries. It is an important vector of filariasis and breeds in polluted waters. Lymphatic filariasis is probably the fastest spreading insect-borne disease of man in the tropics, affecting about 146 million people (Elumalai et al., 2013a,b).

One of the approaches for controlling mosquitoes borne diseases is the interruption of disease transmission either by killing, preventing mosquito bite by using repellents or by causing larval mortality in a large scale at the breeding centers of the vector. The control of mosquito larvae worldwide depends on continued application of organophosphates and insect growth regulators (Rahuman et al., 2009). These problems have highlighted the need for new strategies for mosquito larvae control.

Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and has led to

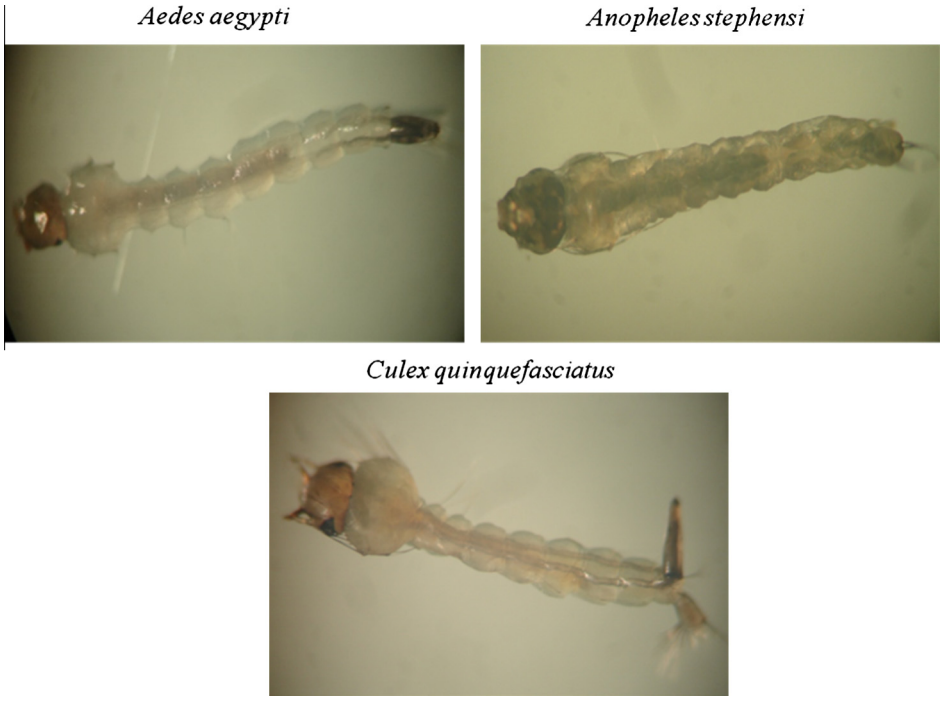

Figure 1 Mosquito fourth instar larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*.

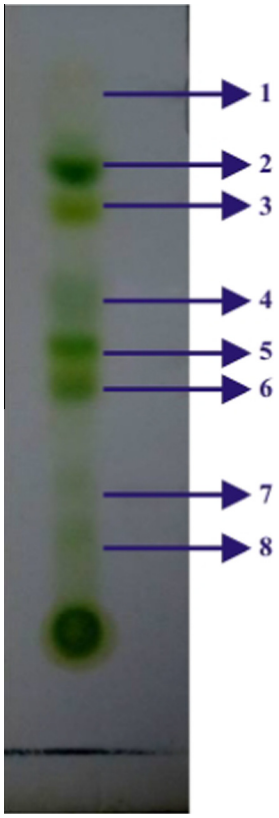

Figure 2 Separation of bioactive compound from the whole plant methanol extracts of *L. aspera* using TLC. **TLC profile of methanol extract**: Eight major bands were observed in long UV 372 nm. R_f was calculated as distance traveled by solute/distance traveled by solvent. **Methanol extracts R_f values**: Band-1: R_f 0.95 – Triterpenoids and steroid, Band-2: R_f 0.81 – Phenolic compound and catechin, Band-3: R_f 0.69 – Flavonoids-c-glycosides and mentione, Band-4: R_f 0.58 – Saponin, Band-5: R_f 0.46 – Terpene alcohols and quercertin, Band-6: R_f 0.38 – Sterols, Band-7: R_f 0.06 – Polyines and Band-4: R_f 0.04 – Unknown compound.

resurgences in mosquito population. It has also resulted in the development of resistance, ecological imbalance, harm to human and animals and undesirable effects on non-target organisms (Kamaraj et al., 2008).

One such possibility is the use of botanicals which are readily biodegradable, nontoxic and show broad spectrum target specific activity (Sharma et al., 2005). The mosquito control at the larval stage of development with phytochemicals that occur in the oils, leaves, and roots of plants is one of the techniques which affords a cheaper and environment-friendly method of mosquito larval control (Shyamapada Mandal, 2011).

Phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides for replacing synthetic insecticides in mosquito control programs due to their excellent larvicidal, pupicidal and adulticidal properties. Certain natural plant compounds are not only a source of new insecticides and insect repellents but are also botanical chemical derivatives which are environmentally friendly than synthetic chemicals (Cantrell et al., 2005).

Leucas aspera (wild) belonging to *Lamiaceae* family is known for its medicinal properties and the leaves are used in traditional medicine for treating dyspepsia, cough, cold, painful swelling, fevers, ulcers and chronic skin eruptions (Chopra et al., 2002). The leaves are used as insecticide and mosquito repellent in rural areas (Kirtikar and Basu, 1990; Reddy et al., 1993; Sadhu et al., 2003; Maheswaran et al., 2008) and as a natural pesticide against *An. stephensi* (Karunamoorthi and Bekele, 2009) and also exhibit larvicidal activity against *Cx. quinquefasciatus* (Arivoli et al., 1999).

In recent years, much effect has been focussed on the exploration of bioactive, chemical compounds from indigenous plants for mosquito control in India. In this study, whole plant extracts of the weed *L. aspera* were tested against the larvae of mosquito species, *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* larvae. The results of the present study would be useful in promoting research aiming toward the development of new agents for mosquito control based on bioactive chemical compounds from indigenous plant sources.

2. Materials and methods

2.1. Collection of plant

L. aspera plant was collected from the natural population in and around Chennai, and identified in the Department of Botany, Government Arts College, Nandanam, Chennai.

Table 2 Phytocompounds identified from the whole plant methanol extract of *L. aspera* by GC–MS.

S. No	RT	Name of the compound	Molecular formula	Mol. weight (g/mol)
1	15.73	1-Hexadecanol, 2-methyl	$C_{17}H_{36}O$	256.4671
2	16.23	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.5310
3	17.22	Catechin	$C_{15}H_{14}O_6$	290.03
4	18.87	9,12-Octadecadienoic acid(zz)-methyl ester	$C_{19}H_{34}O_2$	294.4721
5	19	9,12,15- Octadecatrienoic acid, methyl ester, (zzz)-	$C_{19}H_{32}O_2$	292.456
6	19.17	Heptadecanoic acid, 9-methyl-, methyl ester	$C_{18}H_{36}O_2$	284.47
7	20.98	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	362.
8	21.1	Cholestan-3 ol, 2-methylene, (3a, 5a)	$C_{28}H_{48}O$	400
9	23.03	Aspidospermidne-17 ol, 1-acetyl, 19, 21-epoxy-15, 16- dimethoxy	$C_{22}H_{26}N_2O_4$	354.610
10	25.5	Tetradecane,2,6,10-trimethyl-	$C_{17}H_{36}$	240.4677
11	26.1	Oxiraneundecanoic acid, 3-pentyl, methyl ester	$C_{19}H_{36}O_3$	312.487
12	28.12	2, 6, 10, 14, 18, 22-Tetracosahexane, 2, 6, 10, 15, 19, 23-hexamethyl	$C_{30}H_5O$	410.72

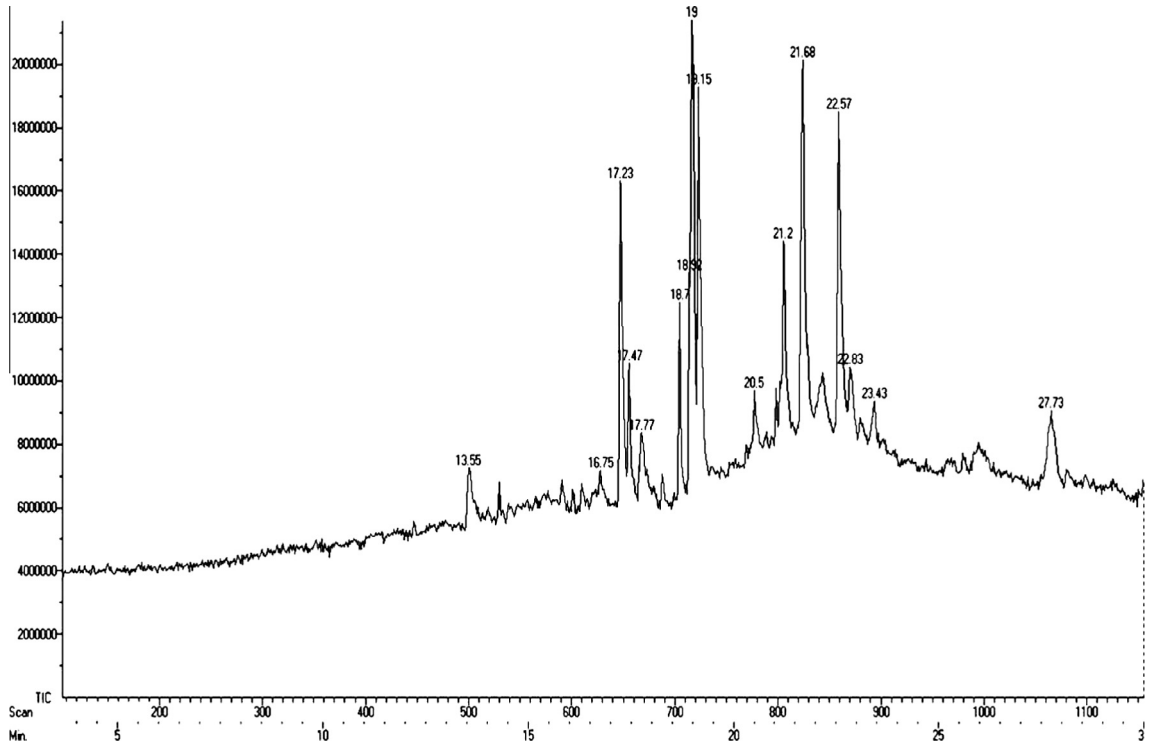

Figure 3 GC–MS chromatogram of whole plant methanol extract of *L. aspera*.

Table 3 Mortality percentage of fourth instar larvae of three mosquito species exposed for 24 h and 48 h to different concentration of plant extract of *Leucas aspera*.

Solvents	Exposure (h)	Control	20	40	60	80	100	120
Concentration of extract (ppm) Aedes aegypti								
Aqueous extract	24	0	26	48	59	70	95	98
	48	0	38	57	60	94	100	100
Chloroform extract	24	0	25	46	68	77	100	100
	48	0	39	51	72	90	95	100
Ethanol extract	24	0	36	49	64	85	97	100
	48	0	40	52	70	96	99	100
Petroleum extract	24	0	25	48	68	80	90	95
	48	0	39	54	65	92	100	100
Methanol extract	24	0	40	58	60	100	100	100
	48	0	42	60	78	100	100	100
Concentration of extract (ppm) Anopheles stephensi								
Aqueous extract	24	0	24	54	73	90	100	100
	48	0	35	60	87	100	100	100
Chloroform extract	24	0	33	51	85	97	100	100
	48	0	47	65	89	99	100	100
Ethanol extract	24	0	41	58	86	98	100	100
	48	0	48	65	89	100	100	100
Petroleum extract	24	0	20	52	85	92	98	100
	48	0	46	67	87	96	100	100
Methanol extract	24	0	49	60	83	95	100	100
	48	0	50	66	90	100	100	100
Concentration of extract (ppm) Culex quinquefasciatus								
Aqueous extract	24	0	13	24	58	72	92	97
	48	0	15	58	80	92	95	100
Chloroform extract	24	0	17	50	66	91	95	98
	48	0	30	52	80	93	98	100
Ethanol extract	24	0	25	45	68	95	100	100
	48	0	35	60	85	100	100	100
Petroleum extract	24	0	34	47	68	76	88	100
	48	0	40	55	82	95	100	100
Methanol extract	24	0	35	58	87	97	100	100
	48	0	42	60	90	100	100	100

Please cite this article in press as: Elumalai, D. et al., Larvicidal actiL. aspera –>vity and GC–MS analysis of *Leucas aspera* against *Aedes aegypti Anopheles stephensi* and *Culex quinquefasciatus*. Journal of the Saudi Society of Agricultural Sciences (2015), http://dx.doi.org/10.1016/j.jssas.2015.10.003

The whole plant dried under shade at room temperature for 20 days.

2.2. Preparation of plant extracts

The dried plant was powdered and sieved to get fine powder using an electric blender. 70 g of the plant powder was filled in the thimble and extracted successively with aqueous, chloroform, ethanol, petroleum ether and methanol using soxhlet extractor for 10 h. All the extracts were concentrated using rotary flash evaporator and preserved at 5 °C in airtight bottle until further use.

2.3. Phytochemical screening

The phytochemical screening was carried out as described by Nazer et al., (2009); Senthil kumar and Reetha, (2009). By this analysis, the presence of several phytochemical listed in Table 1 was tested.

2.4. Separation of bioactive compounds using TLC

2.4.1. Preparation of extract

10 mg/ml of the extract in ethanol solvent was used for TLC examination. The same procedure was followed for methanol and chloroform extract preparation.

2.4.2. TLC plate preparation

The silica gel 60 F 254 coated aluminum sheets were cut in size 1.5 × 5.5 cm and the prepared ethanol extract was loaded on silica plate and air-dried.

2.4.3. Mobile phase preparation

The extracts were standardized in ethyl acetate with acetone and finally chloroform:methanol (9:1) ratio showed separated bands (Serker and Nagar, 2011).

The movement of the analytes is expressed by its retardation factor, R_f such that:

$$R_f = \frac{\text{Distance moved by analytes from origin}}{\text{Distance moved by solvent front from origin}}$$

2.5. GC–MS analysis

GC–MS analysis of the crude extracts of whole plants was carried out on Agilent technologies (6890 N), JEOL GCMATE II which comprised of an auto sampler and gas chromatography interfaced to a mass spectrometer (GC–MS) instrument employing the following condition: capillary column – 624 ms (30 m × 0.32 mm × 1.8 m) operating in an electron mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1.491 ml/min and injection volume of 1.0 ml, injector temperature of 140 °C; ion source temperature of 200 °C. The oven temperature was programmed for 45 °C. Mass spectra were taken at 70 eV.

2.6. Selection of mosquito species and culture

All tests were carried out using laboratory reared vector mosquitoes viz., *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* (Fig. 1) free of exposure to insecticides and pathogens. Cyclic generations of vector mosquitoes were maintained at 25–29 °C insectariums. Larvae were fed on larval food powdered dog biscuit and yeast in the ratio 3:1 and adult mosquitoes on 10% glucose solution (Arivoli and Samuel, 2011).

2.7. Larvicidal bioassay

A total of three trials were carried out with five replicates per trial against vector mosquitoes. Stock solution (1000 ppm) was prepared by dissolving 100 mg of crude extract in 1 ml acetone and volume raised to 100 ml with distilled water. From the

Table 4 Lethal concentration of whole plant extracts of *Leucas aspera* against fourth instar larvae of *Aedes aegypti*.

Solvents	Exposure (h)	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	95% Confidence limits				Chi-square	*P* value
					UCL (ppm)		LCL (ppm)			
					LC_{50} (ppm)	LC_{90} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)		
Aqueous	24	44.774	91.220	$Y = -2.741 + 0.062X$	45.603	105.954	41.784	84.600	04.41	0.040
	48	37.997	78.791	$Y = -2.703 + 0.071X$	39.922	84.074	35.314	75.710	07.44	0.059
Chloroform	24	43.915	90.743	$Y = -1.912 + 0.046X$	41.371	100.231	37.428	85.513	07.30	0.009
	48	36.195	78.135	$Y = -2.498 + 0.069X$	38.404	81.576	32.907	75.813	11.52	0.007
Ethanol	24	42.588	81.872	$Y = -1.536 + 0.037X$	43.586	87.456	35.779	78.530	01.76	0.012
	48	34.692	72.966	$Y = -1.446 + 0.045X$	35.754	75.618	23.530	71.105	04.79	0.000
Petroleum ether	24	43.797	85.202	$Y = -1.896 + 0.043X$	46.437	94.256	40.794	80.554	04.44	0.000
	48	34.359	75.969	$Y = -2.060 + 0.063X$	35.308	76.099	27.126	70.861	06.88	0.017
Methanol	24	40.877	72.903	$Y = -1.671 + 0.038X$	40.758	76.015	38.183	70.794	09.21	0.000
	48	32.256	67.355	$Y = -2.104 + 0.061X$	37.105	69.531	29.870	65.567	07.37	0.001

Control – nil mortality.
Significant at $p < 0.05$ level.
LC_{50} – lethal concentration that kills 50% of the exposed larvae; LC_{90} – that kills 90% of the exposed larvae.
UCL – upper confidence limit; LCL – lower confidence limit.

stock solution different dilutions of 25 ppm, 50 ppm, 75 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm were prepared in 200 ml deionized water, 25 fourth instar larvae were released and mortality was scored after 24 h and 48 h. The beakers were kept in a temperature control room at 28 °C ± 2° and the larvae exposed 0.1 ml of acetone served as control. Each treatment was replicated five times (Tonk et al., 2006).

2.8. Larval susceptibility tests

The larval susceptibility tests were carried out according to the standard WHO procedure (WHO, 2005). The fourth instar larva of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* was placed in each test solution to study the larvicidal property as per the following procedure. 25 fourth instar larvae were released in 200 ml of the extract solution and control experiments without extract were run in parallel.

The larvae in each solution were then left for 24 h and 48 h, the number of dead larvae was counted after 24 h and 48 h of exposure, and the percentage mortality was reported from the average of five replicates. Mortality was recorded when control mortality ranged from 5% to 20%, and it was corrected by Abbott's (1925) formula. Based on the percent mortality values, LC_{50} and LC_{90} values of plant extract of *L. aspera* against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* were recorded by calculating the regression line employing probit analysis of Finney (1971) as described by Busvin (1971).

2.9. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% fiducial 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.

3. Results

The preliminary phytochemical screening of whole plant extracts revealed the presence of flavonoids, tannins, alkaloids, quinones, saponins, phenol, terpenoids, triterpenoids, coumarins and carbohydrates in the plant extracts (Table 1). Methanol extracts showed the strong presence of various phytocompounds and subjected to TLC.

TLC of methanolic extracts showed 8 major bands with R_f values of 0.95, 0.81, 0.69, 0.58, 0.46, 0.38, 0.06 and 0.04 which corresponds to major compounds such as triterpenoids and steroids, phenolic compound and catechin, flavonoids-c-glycosides and mentione, saponin, terpene alcohols and quercetin, sterols and polyines (Fig. 2).

The composition and identification of the main compounds present in the methanol extracts of *L. aspera* are shown in Table 2. Twelve compounds were identified by GC–MS. The main compounds were tetracosahexane, 2, 6, 10, 15, 19, 23-hexamethyl, oxiraneundecanoic acid, 3-pentyl methylester, tetradecane 2,6,10- trimethyl, catechin, 1-hexadeconol, 2-methyl, 3,7,11,15 tetramethyl-2-hexadec-1-ol, 9,12-octadecadienoic acid- methyl ester, eicosanoic acid and methylester (Fig. 3).

The results clearly indicate that the whole plant methanol extracts of *L. aspera* exhibited potent lethality against all the three mosquito species tested. Methanol extract of *L. aspera* was found to be more potent and showed 100% mortality at 80 ppm whereas other extracts showed 100% mortality against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* at 100 ppm and 120 ppm (Table 3). Methanol extracts at a lowest concentration of 20 ppm killed 35% to 40% larval population, when exposed for 24 h and 50% when exposed for 48 h. Methanol extract of *L. aspera* was found to be a potent larvicidal agent when compared to the other extracts.

Based on probit analysis between the concentration of plant extract against fourth instar larvae of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* are represented in Tables 4 and 5.

Table 5 Lethal concentration of whole plant extracts of *Leucas aspera* against fourth instar larvae of *Anopheles stephensi*.

Solvents	Exposure (h)	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	95% Confidence limits				Chi-square	*P* value
					UCL (ppm)		LCL (ppm)			
					LC_{50} (ppm)	LC_{90} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)		
Aqueous	24	37.671	74.174	$Y = -1.618 + 0.042X$	40.281	82.126	34 .360	70.309	04.57	0.000
	48	23.629	66.945	$Y = -1.185 + 0.050X$	28.188	70.437	14.377	64.634	06.91	0.005
Chloroform	24	39.609	67.844	$Y = -1.604 + 0.040X$	42.542	71.275	36.545	65.576	05.45	0.000
	48	27.281	63.479	$Y = -2.210 + 0.081X$	29.844	66.926	23.218	60.586	13.11	0.035
Ethanol	24	37.076	65.137	$Y = -1.821 + 0.049X$	39.373	68.244	34.125	62.900	08.88	0.019
	48	24.980	64.563	$Y = -1.230 + 0.058X$	25.838	67.628	11.184	60.261	09.21	0.095
Petroleum ether	24	38.076	69.917	$Y = -1.721 + 0.049X$	39.373	68.245	34.125	66.040	11.02	0.003
	48	22.986	65.063	$Y = -1.230 + 0.058X$	52.838	69.628	11.838	61.061	16.31	0.000
Methanol	24	35.624	64.260	$Y = -1.407 + 0.039X$	38.502	75.405	31.129	62.505	03.87	0.000
	48	20.897	60.096	$Y = -1.717 + 0.068X$	28.308	64.528	18.918	56.954	14.97	0.005

Control – nil mortality.
Significant at $p < 0.05$ level.
LC_{50} – lethal concentration that kills 50% of the exposed larvae; LC_{90} – that kills 90% of the exposed larvae.
UCL – upper confidence limit; LCL – lower confidence limit.

Please cite this article in press as: Elumalai, D. et al., Larvicidal actiL. aspera –>vity and GC–MS analysis of *Leucas aspera* against *Aedes aegypti Anopheles stephensi* and *Culex quinquefasciatus*. Journal of the Saudi Society of Agricultural Sciences (2015), http://dx.doi.org/10.1016/j.jssas.2015.10.003

The LC_{50} and LC_{90} for methanol extract of *L. aspera* against 4th instar larvae of *An. stephensi* after 24 h post treatment were 35.624 ppm and 64.260 ppm respectively and after 48 h exposure were 20.897 ppm and 60.096 ppm (Table 6). Ethanol extracts were also found to be effective against fourth instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* with LC_{50} and LC_{90} values of 40.877 ppm and 72.903 ppm for *Ae. aegypti*, 37.640 ppm and 27.855 ppm for *Cx. quinquefasciatus* (Table 6). All the other extracts (aqueous, chloroform, petroleum ether) were also effective against all the tested mosquitoes species but at a slightly higher concentration.

4. Discussion

Today environmental safety of an insecticide is considered to be of paramount importance and should not cause mortality on non-target organism in order to be acceptable (Kabaru and Gichia, 2001).

Mosquito larval control using larvicidal agents is a major component in the control of vector borne diseases. Plant as potential larvicides is considered as viable and preferred alternative in the control of the mosquito species at the community level. Phytochemicals derived from plants act as general toxicants against adult as well as against larval stages of mosquitoes, while some act as growth inhibitors or as chemosterilant or act as repellant or attractants.

A large number of plant extracts have been reported to have mosquitocidal or repellent activities against mosquito vectors, but very few plant products have shown practical utility for mosquito control (Sun et al., 2006).

In the present study methanol, ethanol extracts of *L. aspera* showed enhanced larvicidal activity against all the three mosquito species studied. The results obtained are in accordance with the observation of Mwangi and Rembold (1988). Murugan and Jayabalan (1999) reported that 90% mortality was exhibited at 4% concentration of *L. aspera* leaf extract against fourth instar larvae of *An. stephensi*. Sakthivadivel and Daniel (2008) reported that the petroleum ether extract of *L. aspera* showed LC_{50} value between 100 and 200 ppm against the larvae of *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi*.

Phytochemicals derived from plant sources act as larvicides, insect growth regulators, repellent, ovipositor attractant and have different activities which have been observed by many researchers (Venketachalam and Jebasan, 2010). Triterpenoids are generally credited with mosquito larvicidal activities (Gbolade, 2000). The potent larvicidal activity of *L. aspera* could be attributed to the strong presences of terpenoids, triterpenoids and alkaloids.

It may be concluded that natural product as extracts from parts of plants of insecticidal and medicinal values has higher efficiency in reducing mosquito menace due to their larvicidal toxicity. The crude leaf extracts of *L. aspera* showed effective larvicidal properties against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*.

The findings of the present investigation revealed that *L. aspera* has potent larvicidal activity against *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi*. Further studies on the screening, isolation and purification of bioactive phytochemical constituents/compounds followed by in depth laboratory and field bioassay are needed as the present study shows that there is scope to use *L. aspera* to control the immature stages of vector mosquitoes. Further investigations are currently underway to study their mode of action and to isolate the bioactive compounds.

5. Conclusion

It is evident from the present study that crude extracts from *L. aspera* have promising larvicidal efficacy. Crude extract or isolated bioactive compounds from the plant could be used in stagnant water bodies which are known to be the breeding grounds for the mosquitoes. Screening, purification and identification of effective compounds available in this species will certainly bring more success toward the control of mosquitoes. The extract could be used for spraying in stagnant water bodies which are known to be the breeding grounds for

Table 6 Lethal concentration of whole plant extracts of *Leucas aspera* against fourth instar larvae of *Culex quinquefasciatus*.

Solvents	Exposure (h)	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	95% Confidence limits				Chi-square	*P* value
					UCL (ppm)		LCL (ppm)			
					LC_{50} (ppm)	LC_{90} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)		
Aqueous	24	42.738	89.142	$Y = -1.668 + 0.039X$	45.610	152.353	39.006	79.638	08.19	0.009
	48	32.978	79.150	$Y = -2.887 + 0.057X$	35.275	75.523	29.481	69.381	22.88	0.026
Chloroform	24	40.915	76.737	$Y = -1.912 + 0.046X$	43.371	82.065	37.428	73.708	07.30	0.042
	48	36.195	73.234	$Y = -2.498 + 0.069X$	38.404	76.939	32.907	70 .859	11.52	0.000
Ethanol	24	44.018	73.239	$Y = -1.856 + 0.042X$	46.750	77.534	40.969	70.620	06.18	0.053
	48	32.946	71.036	$Y = -2.229 + 0.067X$	35.736	78.911	28.421	69.816	05.84	0.000
Petroleum ether	24	48.242	102.618	$Y = -2.104 + 0.043X$	51.779	121.799	45.665	93.614	07.78	0.058
	48	30.248	78.702	$Y = -1.592 + 0.052X$	34.248	86.603	22.596	74.890	10.35	0.009
Methanol	24	37.640	79.150	$Y = -1.136 + 0.030X$	41.679	76.699	28.132	71.042	05.75	0.000
	48	27.855	73.284	$Y = -1.596 + 0.057X$	32.233	90.018	19.363	74.709	10.11	0.040

Control – nil mortality.
Significant at $p < 0.05$ level.
LC_{50} – lethal concentration that kills 50% of the exposed larvae; LC_{90} – that kills 90% of the exposed larvae.
UCL – upper confidence limit; LCL – lower confidence limit.

mosquitoes acting as vector for a multitude of infectious diseases. Hence the large biomass of the weed *L. aspera* available in the wastelands of Southern India can be used as a bioresource to commercially produce mosquito larvicides.

Conflict of interest

No conflict of interest.

Acknowledgments

The authors are grateful to University Grants Commission – India (F.No.35-69/2008 (SR) dated 20.03.2009 for providing financial assistance to carry out the present investigations. Our Thanks are also extended to The Principal, Presidency College, Chennai, for providing infrastructure and research facilities.

References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267.

Arivoli, S., Narendran, T., Ignacimuthu, S., 1999. Larvicidal activity of some botanicals against *Culex quinquefasciatus* (Say). J. Adv. Zool. 20, 19–23.

Arivoli, S., Samuel, T., 2011. Bioefficacy of *Citrullus colocynthis* (L.) Schrad (cucurbitaceae) whole plant extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Dipters: Culicidae). Int. J. Curr. Res. 3, 296–304.

Busvin, R.J., 1971. A Critical Review of the Techniques for Testing Insecticides. Commonwealth Agric. Bureau, Lond, pp. 263–288.

Cantrell, C.L., Pridgeon, J., Fronczek, F.R., Becnel, J., 2005. Structure–activity relationship studies on natural cudesmanolides from *Inula helenium* as toxicants against *Aedes aegypti* larvae and adults. Chem. Biod. 7, 1681–1697.

Chopra, R.N., Nair, S,L., Chopra, I.C., 2002. Glossary of Indian Medicinal Plants. CSIR, New Delhi, 23.

Dean, M., 2001. Lymphatic Filariasis, the Quest to Eliminate, Bulletin a 4000- Year Old Disease. Publishing Company, Hollis N.H..

Elumalai, D., Kaleena, P.K., Fathima, M., Muttapan, M., 2013a. Evaluation of Biological activity of *Hyptis suaveolens* (L) Poit and *Leucas aspera* (Wild) aganist *Culex quinquefasciatus*. Int. J. Biosci. Res. 2, 1–6.

Elumalai, D., Kaleena, P.K., fathima, Mujeera, Naresh kumar, C., 2013b. Phytochemical screening and larvicidal activity of *Tridax procumbens* (L) against *Anopheles stephensi* (Liston), *Aedes aegypti* (L) and *Culex quinquefasciatus* (say). Int. J. Biosci. Res. 2, 1–14.

Finney, D.J., 1971. In: Probit Analysis. Cambridge University Press, London, pp. 68–72.

Gbolade, A., 2000. Plant derived insecticides in the control of malaria vector. In: Adewunmi, C.O., Adesina, S.K. (Eds.), Phytomedicines in Malaria and Sexually Transmitted Diseases: Challenges for new Millennium, Drug research and Production Unit, Faculty of Pharmacy. Obafemi, Awolowo University, lle-fe, Nigeria, pp. 48–50.

Hahn, C.S., French, O.G., Foleg, P., Martin, E.N., Taylor, R.P., 2001. Biospecific monoclonal antibodies of dengue virus to erythrocytes in a monkey model of positive viremia. J. Immunol. 66, 1057–1065.

Jiang, J., Oberdorster, G., Biswas, P., 2009. Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies. J. Nano. Res. 11, 77–89.

Kabaru, J.M., Gichia, L., 2001. Insecticidal activity of extracts derived from different parts of the mangrove tree *Rhizophora mucronata* (Rhizophoraceae) Lam. Against three arthropods. Afr. J. Sci. Technol. 2, 44–49.

Kamaraj, C., Rahuman, A.A., Bagavan, A., 2008. Antifeedant and larvicidal effect of plant extracts against *Spodoptera litura* (F), *Aedes aegypti* L. and *Culex quinquefasciatus* say. Parasitol. Res. 103, 325–331.

Karunamoorthi, K., Bekele, M., 2009. Prevalence of malaria from peripheral blood smears examination: a 1-year retrospective study from the Serbo Health Center, Kersa Woreda, Ethiopia. J. Infec. Public. Heal. 2, 171–176.

Kirtikar, K.R., Basu, B.D., 1990. Indian Medicinal Plants. In: Bidtter, E., Caius, J.F., Mhaskar, K.S. (Eds.). Periodical Expert's Book Company, 2019.

Maheswaran, R., Kingsley, S., Ignacimuthu, S., 2008. Larvicidal and repellent activity of *Clerodendron phlomides* against *Culex quinquefasciatus* Say (Diptera: Culicidae). Proc. Recent Trend Insect Pest Manage., 240–243

Mittal, P.K., Subbarao, S.K., 2003. Prospectus of using herbal products in mosquito control. ICMR Bull. 33, 1–10.

Murugan, K., Jayabalan, D., 1999. Effect of certain plant extracts the mosquito, *Anopheles stephensi* Liston. Curr. Sci. 76, 631–633.

Mwangi, R.W., Rembold, H., 1988. Growth inhibiting and larvicidal effects of Melia volkensii extract on *Aedes aegypti* larvae. Entomol. Exp. Appl. 46, 103–108.

Nazer, S.S., Ravikumar, G.P., Williams, M., Syed, A., Suganthi, P., 2009. Screening of Indian coastal plant extracts for larvicidal. J. Med. Ento. 43, 258.

Rahuman, A.A., Bagavan, A., Kamarai, C., Saravanan, E., Zahir, A. A., Elango, G., 2009. Efficacy of the larvicidal botanical extracts against *Culex quinquefasciatus Say* (*Dipetera; Culicidae*). Parasitol. Res. 104, 1365–1372.

Reddy, K.M., Viswanathan, S., Thirugnana sabmantham, D., Santa, R., Lalitha, K., 1993. Analgesic activity of *L. aspera*. Fitoterapia 64, 151–154.

Sadhu, S.K., Okuyama, E., Fujimoto, H., Ishibashi, M., 2003. Separation of *Leucas aspera*, a medicinal plant of Bangladesh, guided by prostaglandin inhibitory and antioxidant activities. Chem. Pharm. Bull. 51, 595–598.

Sakthivadivel, M., Daniel, T., 2008. Evaluation of certain insecticidal plants for the control of vector of vector mosquitoes viz., *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*. Appl. Entomol. Zool. 43, 57–63.

Senthil kumar, P.K., Reetha, D., 2009. Screening of antimicrobial properties of certain Indian medicinal plants. J. Phytol. 1, 193–198.

Serker, S.D., Nagar, L., 2011. Natural production isolation. In: Methods and Protocols, third ed. Humana Press, New Yourk, 202.

Sharma, P., Mohan, L., Srivastava, C.N., 2005. Larvicidal potential of *Nerium indicm* and *Thiya orientelis* extracts against Malaria and Japanese Encephalitis vector. J. Environ. Biol. 26, 67–70.

Shyamapada mandal, 2011. Effect of *Azardirachta india*, *A. Juss* (Meliaceae) seed oil and extract against *Culex quinquefasciatus* say (Diptera: Culicidae) larval susceptibility of Indian subcontinent Macedonian. J. Med. Sci. 15, 5–11.

Sun, Lixin, Dong, Huiqin, Guo, Chongxia, Qian, Jin, Sun, Jing, Ma, Lei, Zhu, Changliang, 2006. Larvicidal activity of extracts of *Ginkgo biloba* exocarp for three different strains of *Culex pipiens* pallens. J. Med. Entomol. 43, 258–261.

Tonk, S., Bartarya, R., Maharaj Kumari, K., Bhatnagar, V.P., Srivastava, S.S., 2006. Effective method for extraction of larvicidal component from leaves of *Azadirachta indica* and *Artemisia annua* Linn. J. Environ. Biol. 27, 103–105.

Venketachalam, M.R., Jebasan, A., 2010. Larvicidal activity of *Hydrocotyl javanica thumb* (Apjaceal) extract against *Culex quinquefasciatus*. J. Expt. Zool. India 4, 99–101.

WHO, 2005. Communicable Disease Tool kit, Sudan. World Health Organization, WHO/CDS/2005.26.