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SHORT COMMUNICATION

Chemical constituents and larvicidal potential of *Feronia limonia* leaf essential oil against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*

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Abstract In the present investigation, the leaf essential oil of *Feronia limonia* was evaluated for chemical constituents and mosquito larvicidal activity against the larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. GC and GC–MS analyses revealed that the essential oil contain 51 compounds. Estragole (34.69 %) and β-pinene (23.59 %) were identified as the major constituents followed by methyl (*Z*)-caryophyllene (11.05 %), eugenol (6.50 %), linalool (3.97 %), phytol (3.27 %), sabinene (2.41 %) and limonene (2.27 %). Larval mortality was observed after 12 and 24 h of exposure period. The oil showed remarkable larvicidal activity against *A. stephensi* (LC_{50}=38.93 and LC_{90}=108.64 ppm (after 12 h); LC_{50}=15.03 and LC_{90}= 36.69 ppm (after 24 h)), *A. aegypti* (LC_{50}=37.60 and LC_{90}=104.69 ppm (after 12 h); LC_{50}=11.59 and LC_{90}= 42.95 ppm (after 24 h)) and *C. quinquefasciatus* (LC_{50}= 52.08 and LC_{90}=124.33 ppm (after 12 h); LC_{50}=22.49 and LC_{90}=60.90 ppm (after 24 h)). Based on the results, the essential oil of *F. limonia* can be considered as a new source of larvicide for the control of vector mosquitoes.

Introduction

The vector-borne diseases caused by mosquitoes are one of the major health problems in tropical and sub-tropical countries. Malaria, dengue, chikungunya and filariasis are some of the deadly diseases spread by mosquitoes. Malaria is a deadly disease resulted in 216 million cases and about 655,000 deaths in 2010 (WHO 2011). In India, *Anopheles stephensi* is responsible for malaria transmission in urban areas (Senthilkumar et al. 2009). Dengue fever has in recent years seen a great resurgence in tropical climates and spreading to new areas transmitted predominantly by one of the vectors, *Aedes aegypti* (WHO 2012a). It is now estimated that over 100 million infections with dengue virus occur annually throughout the world and 25,000 resulting in death (Wilder-Smith and Schwartz 2005). Lymphatic filariasis is the most important vector-borne disease in India (Babu et al. 2002) caused by *Wuchereria bancrofti* and transmitted by the tropical house mosquito, *Culex quinquefasciatus*. Over 120 million people are currently infected and more than 1.3 billion people in 72 countries worldwide are threatened by lymphatic filariasis (WHO 2012b). Filariasis causes long-term suffering and morbitity as well as high social and economic burden to individuals and communities (Ramaiah et al. 2000). So, the transmission of the above-mentioned vector-borne diseases can be controlled by vector control measures because of the spread of resistance in malaria parasite, challenges in the development of vaccine against dengue and socio-economic burden caused by lymphatic filariasis. Management of disease vector using synthetic chemicals has facing a threat due to the emergence of resistance, effect on non target organisms and environmental pollution. Hence, the search of plant-based insecticides/larvicides (especially essential oils) will be a bequest to overcome the resistance problem. Previous investigations have indicated that various plant essential oils display larvicidal and repellant effects on *Culex*, *Aedes* and *Anopheles* mosquitoes (Amer and Mehlhorn 2006a,b,c; Evergetis et al. 2009; Liu et al. 2012). Moreover, the resistance by vectors against plant-derived insecticides has not been reported so far (Kannathasan et al. 2011).

Feronia limonia (L.) Swingle (Syn. *Feronia elephantum*) that belongs to the family Rutaceae is a small deciduous tree with short, erect, cylindrical stem, bearing thorny branches. Leaves are pinnate, 7–10 cm long, with small ovate or

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obovate leaflets. Fruits are large, globose or oblate with hard, rough and woody pericarp. Previous phytochemical studies from the leaf essential oil of *F. limonia* showed *trans*-anethole and methyl chavicol (Pande et al. 2010), Eudesma-4 (14) 11-dine, carvacrol and1,5-cyclodecandine (Senthil Kumar et al. 2010), beta-pinene, *Z*-anethole, methyl chavicol and *E*-anethole (Joshi et al. 2011) and Caryophyllene oxide (Thirugnanasampandan and David 2012) as the major contituents. Moreover, these studies were focused only on antimicrobial, antioxidant and cytotoxic activities. To best of our knowlegde, the essential oil of leaf of *F. limonia* has not been studied for mosquito larvicidal activity. So, the present study was made to analyse the chemical constituents and to study the mosquito larvicidal activity of *F. limonia* leaf essential oil against the larvae of *A. stephensi*, *A. aegypti* and *C. quinquefasciatus*.

Materials and methods

Plant materials and extraction of essential oil

The leaves of *F. limonia* were collected from Keerapalayam [11°26′03 N, 079°39′02 E (elevation: 16 m)], Cuddalore district, Tamil Nadu, India during September, 2010. The voucher specimen (no. AUBOT# 209) is deposited at the herbarium, Department of Botany, Annamalai University. The fresh leaves were cut in to small pieces and subjected to hydro distillation using Clevenger-type apparatus for 4 h. The essential oil was dried over anhydrous sodium sulphate and the purified essential oil was stored at +4 °C until further use.

GC and GC–MS analysis

Gas chromatography (GC) analysis was carried out using Varian 3800 gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatograph was fitted with DB 5 MS capillary column (30 m×0.25 mm i.d., film thickness 0.25 μm). The injector temperature was set at 280 °C, and the oven temperature was initially at 45 °C then programmed to 300 °C at the rate of 10 °C/min and finally held at 200 °C for 5 min. Helium was used as a carrier gas with the flow rate of 1.0 ml/min. One microlitre of the sample (diluted with acetone 1:10) was injected in the split mode in the ratio of 1:100. The percentage of composition of the essential oil was calculated by the GC peak areas.

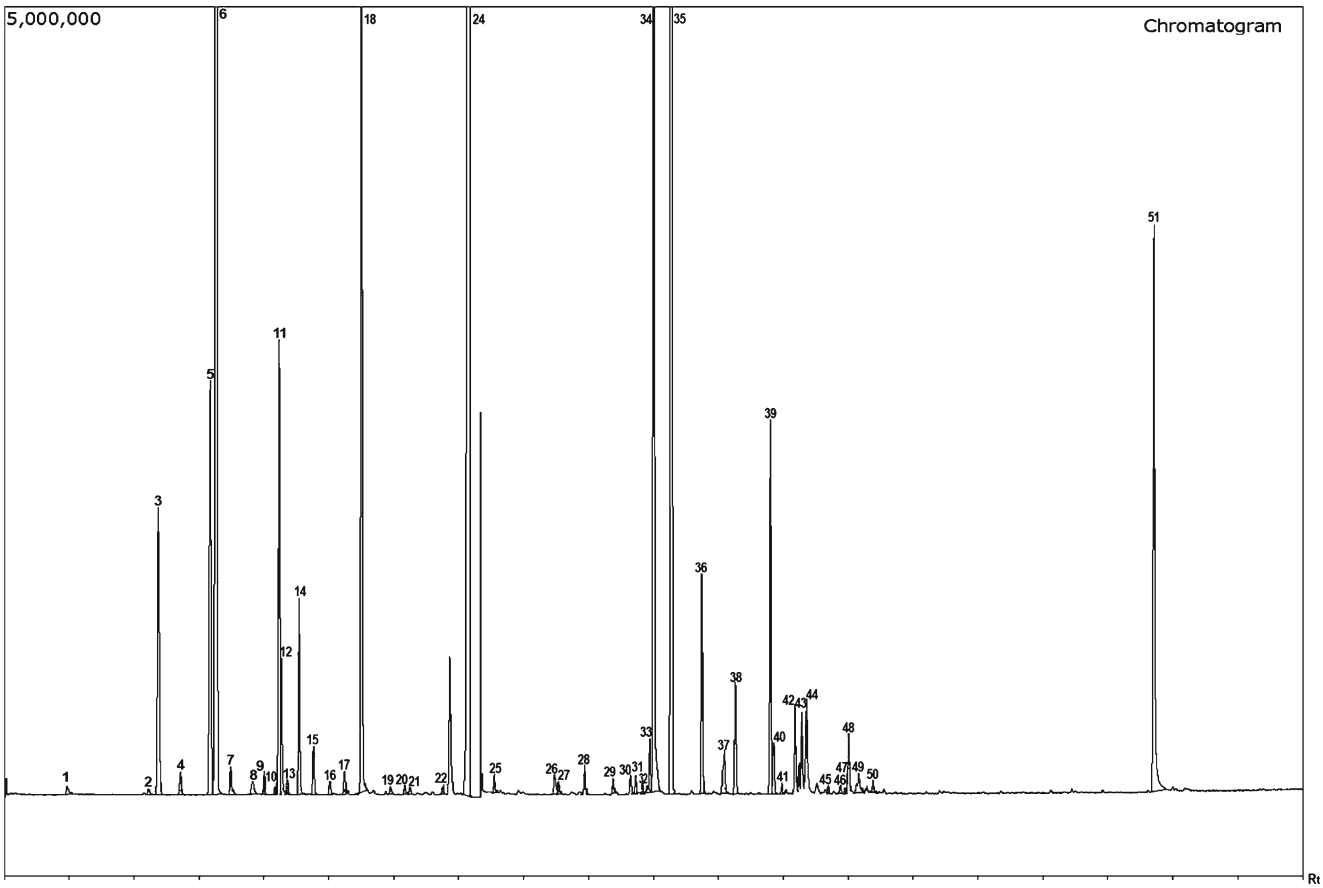

Fig. 1 Gas chromatogram of *F. limonia* leaf essential oil

Table 1 Chemical composition of the essential oil of the leaves of *F. limonia*

Peak no.	R_t	RI	Chemical compound	Percentage
1	3.996	801	Hexanal	0.07
2	5.226	924	α-Thujene	0.04
3	5.373	932	α-Pinene	1.75
4	5.716	946	Camphene	0.14
5	6.172	969	Sabinene	2.41
6	6.267	974	β-Pinene	23.59
7	6.486	988	Myrcene	0.17
8	6.829	1,004	(3*E*)-3-hexenyl acetate	0.10
9	7.007	1,014	α-Terpipene	0.10
10	7.168	1,020	ρ-Cymene	0.03
11	7.236	1,024	Limonene	2.27
12	7.271	1,025	β-Phellandrene	0.55
13	7.354	1,032	β-(*Z*)-ocimene	0.07
14	7.541	1,044	β-(*E*)-ocimene	0.90
15	7.764	1,054	γ-Terpinene	0.23
16	8.015	1,078	Camphenilone	0.08
17	8.240	1,084	Terpinolene	0.11
18	8.502	1,095	Linalool	3.97
19	8.948	1,119	Myrcenol	0.04
20	9.170	1,128	*allo*-Ocimene	0.05
21	9.250	1,138	Geijerene	0.05
22	9.756	1,156	*cis*-Dihydro-β-terpineol	0.03
23	9.862	1,165	Borneol	0.79
24	10.156	1,195	Estragole	34.69
25	10.549	1,199	γ-Terpineol	0.09
26	11.478	1,239	*o*-Anisaldehyde	0.12
27	11.540	1,249	(*Z*)-anethole	0.07
28	11.941	1,271	Citronellyl formate	0.12
29	12.378	1,274	Pregeijerene B	0.07
30	12.645	1,361	(*Z*)-β-damascenone	0.11
31	12.726	1,379	Geranyl acetate	0.08
32	12.830	1,389	β-Elemene	0.04
33	12.946	1,392	(*Z*)-jasmone	0.30
34	13.005	1,403	Methyl eugenol	6.50
35	13.275	1,408	(*Z*)-Caryophyllene	11.05
36	13.744	1,484	Germacrene D	1.05
37	14.091	1,498	α-Selinene	0.33
38	14.262	1,505	α-(*E*,*E*)-farnesene	0.55
39	14.800	1,548	Elemol	1.77
40	14.853	1,555	Elemicin	0.24
41	14.977	1,565	(3*Z*)-hexenyl benzoate	0.05
42	15.179	1,569	γ-Undecalactone	0.42
43	15.283	1,576	Santalenone	0.36
44	15.359	1,582	Caryophyllene oxide	0.47
45	15.693	1,608	Humulene epoxide II	0.04
46	15.878	1,632	(3*Z*)-Hexenyl phenyl acetate	0.05
47	15.967	1,645	Cubenol	0.10
48	16.009	1,652	α-Eudesmol	0.28
49	16.161	1,678	(*Z*)-Methyl epi-jasmonate	0.18
50	16.379	1,713	Longifolol	0.06
51	20.705	1,942	Phytol	3.27
Total				100

RI retention indices

GC–mass spectrometry (GC–MS) analysis of essential oil was performed using Varian 3800 gas chromatography equipped with Varian 1,200-L single quadrupole mass spectrometer. GC conditions were the same as reported for GC analysis and the same column was used. The mass spectrometer was operated in the electron impact mode at 70 eV. Ion source and transfer line temperature was kept at 250 °C. The mass spectra were obtained by centroid scan of the mass range from 40 to 1000 amu. The compounds were identified based on the comparison of their retention indices (RI), retention time (RT) and mass spectra of Wiley, NIST library data of the GC–MS system and literature data (Adams 2009).

Mosquito larvicidal assay

The eggs of *A. stephensi* and *A. aegypti* were received from the Field Station, Centre for Research in Medical Entomology (ICMR-Government of India), Viruthachalam and the eggrafts of *C. quinquefasciatus* were collected from drainage of local residential area of Annamalai Nagar and reared in the laboratory. The larvae were fed with Brewer's yeast/dog biscuit (1:3). The larvicidal activity was observed as per the standard procedures recommended by the World Health Organisation (1981). The essential oil was dissolved in 1 ml of acetone and prepared into different concentrations viz., 3.125, 6.25, 12.5, 25, 50 and 100 ppm with distilled water. Twenty larvae (in a 100-ml beaker) of early fourth instar stage were used for larvicidal assay and five replicates were maintained for each concentration. During this experiment, no food was offered to the larvae. The larval mortality was calculated after 12 and 24 h of the exposure period. The lethal concentrations, LC_{50} and LC_{90} and their 95 % confidence limit of upper and lower confidence levels were calculated by profit analysis (SPSS, version 11.5).

Results and discussion

The leaves of *F. limonia* yielded 0.53 % (*v*/*w*) of colourless essential oil. The GC chromatogram is presented in Fig. 1. Totally, 51 (Table 1) chemical constituents were identified from the essential oil and the major constituents were estragole

[methyl chavicol] (34.69 %) followed by β-pinene (23.59 %), methyl (Z)-caryophyllene (11.05 %), eugenol (6.50 %), linalool (3.97 %), phytol (3.27 %), sabinene (2.41 %) and limonene (2.27 %). Previous studies (Pande et al. 2010; Senthil Kumar et al. 2010; Joshi et al. 2011; Thirugnanasampandan and David 2012) reported various constituents as the major ones. Variation, presence and absence of chemical constituents in the essential oil may be due to various factors (Daferera et al. 2000).

The essential oil extracted from the leaves of *F. limonia* had remarkable larvicidal activity (Table 2) against *A. stephensi* (LC_{50}=38.93 and LC_{90}=108.64 ppm (after 12 h); LC_{50}=15.03 and LC_{90}=36.69 ppm (after 24 h)), *A. aegypti* (LC_{50}=37.60 and LC_{90}=104.69 ppm (after 12 h); LC_{50}=11.59 and LC_{90}=42.95 ppm (after 24 h)) and *C. quinquefasciatus* (LC_{50}=52.08 and LC_{90}=124.33 ppm (after 12 h); LC_{50}=22.49 and LC_{90}=60.90 ppm (after 24 h)). The oil showed 100 % larval mortality against *A. stephensi* and *A. aegypti* at

Table 2 Larvicidal potential of *F. limonia* leaf essential oil against the larvae of *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* after 12 and 24 h of exposure period

Name of the mosquito species	Time	Concentration (ppm)	% of Mortality±SE	LC_{50} (LCL–UCL) [a]	LC_{90} (LCL–UCL) [a]	χ^2 (df=4)[b]
A. stephensi	After 12 h	3.125	14±0.37	38.93 (16.49–73.79)	108.64 (73.78–261.08)	24.31
		6.25	21±0.48			
		12.5	36±0.73			
		25	51±0.86			
		50	69±0.66			
		100	80±0.31			
	After 24 h	3.125	19±0.37	15.03 (10.76–19.60)	36.69 (29.79–49.90)	7.41
		6.25	27±0.50			
		12.5	52±0.87			
		25	76±0.73			
		50	96±1.06			
		100	100±0.0			
A. aegypti	After 12 h	3.125	17±0.50	37.60 (24.38–54.28)	104.69 (79.73–163.04)	10.58
		6.25	26±1.16			
		12.5	32±0.74			
		25	49±0.91			
		50	66±0.48			
		100	84±1.0			
	After 24 h	3.125	26±0.37	11.59 (3.67–17.78)	42.95 (33.10–65.71)	8.64
		6.25	45±0.70			
		12.5	59±1.31			
		25	73±0.81			
		50	92±1.02			
		100	100±0.0			
C. quinquefasciatus	After 12 h	3.125	10±0.44	52.08 (34.22–85.15)	124.33 (89.33–235.00)	16.76
		6.25	17±0.50			
		12.5	26±1.15			
		25	41±0.73			
		50	58±1.32			
		100	74±0.66			
	After 24 h	3.125	12±0.40	22.49 (1.36–49.63)	60.90 (39.66–198.79)	38.185
		6.25	23±0.63			
		12.5	47±1.12			
		25	68±0.81			
		50	83±0.92			
		100	97±0.40			

LCL lower confidence level, *UCL* upper confidence level

[a] 95 % Confidence interval

[b] Degrees of freedom

100 ppm. The larvicidal activity of the essential oil may be due to the presence of the major chemical constituents such as estragole and β-pinene. Estragole (methyl chavicol) is a bioactive compound present in the plant essential oils. Some of the essential oil rich in methyl chavicol showed larvicidal activity against mosquito larvae. Essential oil from *Tagetes filifolia* showed the strongest larvicidal activity against the third instar larvae of *A. aegypti* with the LC_{50} value of 47.7 ppm (Ruiz et al. 2011). Conti et al. (2010) studied *Foeniculum vulgare* essential oil for larvicidal activity against fourth instar larvae of *Aedes albopictus* and the oil showed larvicidal activity with an IC_{50} value of 142.9 ppm. Estragole present in the *Foeniculum vulgare* essential oil showed insecticidal activity against *Liposcelis bostrychophila* adults (Zhao et al. 2012). Moreover, estragole exhibited contact and fumigant toxicity against fruit flies (*Ceratitis capitata*, *Bactrocera dorsalis* and *Bactrocera cucurbitae*) (Chang et al. 2009), house dust mites (Lee 2004), and various stored product insects (Kim and Ahn 2001; Wang et al. 2011). β-pinene, another major chemical constituent present in the *F. limonia* essential oil may also be responsible for the larvicidal activity (Conti et al. 2010). Santos et al. (2012) studied β-pinene-rich essential oil from red and pink variants of *Alpinia purpurata* showed good larvicidal activity against fourth instar larvae of *A. aegypti* with the LC_{50} values of 80.7 and 71.5 ppm respectively. It is also possible that minor components present in the essential oil may also be responsible for the larvicidal activity (Silva et al. 2008).

Control of vector mosquito larvae frequently depends on continued application of organophosphates and insect growth regulators (Yang et al. 2002) and these synthetic chemical agents have been favourable so far, because of their speedy action and easy application. However, in recent years, the mosquito control programme using synthetic chemicals have failed because of the ever-increasing insecticide resistance (WHO 1992). So, use of natural products, especially essential oil will be an answer to the questions regarding the insecticide resistance. In that way, the essential oil of *F. limonia* can be considered as a new source of larvicide for the control of vector mosquitoes.

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