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Chemical composition and larvicidal activity of leaf essential oil from *Clausena anisata* (Willd.) Hook. f. ex Benth (Rutaceae) against three mosquito species

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ABSTRACT

Objective: To determine the mosquito larvicidal activity of leaf essential oil and their chemical constituents from *Clausena anisata(C. anisata)* (Willd.) Hook. f. ex Benth. against *Culex quinquefasciatus, Aedes aegypti* and *Anopheles Stephensi*. **Methods:** Essential oil was obtained by hydro-distillation and the chemical composition of the leaf essential oil was analyzed using gas chromatography-mass spectrometry. The mosquitoes were reared in the vector control laboratory and twenty late III instar larvae of three mosquito species were exposed to based on the wide range and narrow range tests, essential oil was tested at 50, 100, 150, 200 and 250 ppm and each compound was tested at various concentration (5-75 ppm) and were assayed in the laboratory by using the protocol of WHO 2005; the 24 h LC_{50} values of the *C. anisata* leaf essential oil and their major compounds were determined following Probit analysis. **Results:** The oil contained were mainly β-pinene (32.8%), sabinene (28.3%), germacrene-D (12.7%), estragole (6.4%) and linalool (5.9%). The essential oil from the leaves of *C. anisata* exhibited significant larvicidal activity, with 24 h LC_{50} values of 140.96, 130.19 and 119.59 ppm, respectively. The five pure constituents extracted from the *C. anisata* leaf essential oil were also tested individually against three mosquito larvae. The LC_{50} values of β-pinene, sabinene, germacrene-D, estragole and linalool appeared to be most effective against *Anopheles stephensi* (LC_{50}-23.17, 19.67, 16.95, 11.01, 35.17 ppm) followed by *Aedes aegypti* (LC_{50}-27.69, 21.20, 18.76, 12.70, 38.64 ppm) and *Culex quinquefasciatus* (LC_{50}-32.23, 25.01, 21.28, 14.01, 42.28). **Conclusions:** The essential oil of *C. anisata* contains five major compounds and has remarkable larvicidal properties, which may be considered as a potent source for the production of natural larvicides.

1. Introduction

Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, and Japanese encephalitis (JE)[1]. Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions such as angioedema[2]. *Aedes aegypti (Ae. aegypti)*, a vector of dengue, is widely distributed in tropical and subtropical 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, 1.5 billion people, lived in regions where the estimated risk of dengue transmission was greater than 50%[3]. An outbreak of chikungunya virus infection emerged in the southwest Indian Ocean islands in 2005, spread out to India, and resulted in an ongoing outbreak that has involved >1.5 million patients, including travelers who have visited these areas[4]. *Anopheles stephensi (An. stephensi)* are major malaria vectors 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[5]. *Culex quinquefasciatus (Cx. quinquefasciatus)*, a vector of lymphatic filariasis, is widely distributed in tropical zones with around 120 million people infected worldwide and 44 million people having common chronic manifestation[6].

Continued use of synthetic insecticides for vector control has resulted in resistance in mosquitoes. In addition, synthetic insecticides are toxic and adversely affect the environment by contaminating soil, water, and air[7]. Essential oils and plant extracts may be an alternative

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to synthetic insecticides because they are effective, eco-friendly, easily biodegradable and inexpensive[8]. The essential oils and extracts of edible and medicinal plants, herbs, and spices constitute a class of very potent natural bioactive compounds used by cosmetics, pharmaceutical, and food industries. Thus, many researchers were intrigued to exploit essential oils as a potential source for the identification of novel natural pest control agents[9], with a strong focal point on mosquito control[10]. Previous literature findings have indicated that the essential oils of the Rutaceae family plants constitute efficient natural mosquito control agents[11].

Essential oils provide a rich source of biologically active monoterpenes and are well documented for bioactivities against insect pests. Some of the essential oils with promising mosquito control potential are plant from genus *Tagetes* spp.[12], *Ocimum* spp.[13], *Cymbopogon* spp.[14], and *Mentha* spp.[15] etc. Further, essential oils of cassia, camphor, wintergreen, pine, and *eucalyptus* are already being used in several commercial products for mosquito control[16]. The essential oils are generally considered nontoxic to human beings[17] apart from their uses in flavoring, pharmaceuticals, and confectionary industries. In this study, *Clausena anisata (C.anisata)* leaf essential oil and its major compounds were tested as larvicides against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* larvae.

2. Materials and methods

2.1. Plant material and extraction of essential oil

The leaves of *C. anisata* were collected from the Sirumalai hills, Dindugal District, India. It was authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen is deposited at the herbarium of plant phytochemistry division, Department of Zoology, Annamalai University. Essential oil was obtained by the hydro- distillation of 3 kg fresh leaves in a Clevenger apparatus for 4 h. The oil layer was separated from the aqueous phase using a separating funnel. The resulting essential oil was dried over anhydrous sodium sulphate and stored in an amber-coloured bottle at 8 ℃ for analysis.

2.2. Gas chromatography analysis

Analysis was carried on a varian-gas chromatograph equipped with a flame ionization detector and a BPI (100 % dimethyl polysiloxane) capillary column. Helium at a flow rate of 1.0 mL min-1 and 8 psi inlet pressure was employed as a carrier gas. Temperature was programmed from 60 to 220 ℃ at 5 ℃ min^{-1} with a final hold time of 6 min. The injector and detector temperatures were maintained at 250 and 300 ℃ , respectively. The sample (0.2 μL) was injected with 1:20 split ratio.

2.3. Gas chromatography -mass spectrometry analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was performed on an Agilent 6890 GC equipped with 5973 N mass selective detector and an HP-5(5% phynyl methylpolysiloxane) capillary column. The oven temperature was programmed from 50 to 280 ℃ at the rate of 4 ℃ min^{-1} and held at this temperature for 5 min. The inlet and interface temperatures were 250 and 280 ℃ respectively. The carrier gas was helium at a flow rate of 1.0 mL min^{-1} (constant flow). The sample (0.2 μL) was injected with a split of 20:1. Electron impact mass spectrometry was carried out at 70 eV. Ion source and quadrupole temperatures were maintained at 230 and 150 ℃ respectively.

2.4. Mosquitoes

The mosquitoes, *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were reared in the vector control laboratory, Department of zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at (28±2)℃, 70 - 85 % relative humidity (RH), with a photo period of 14 h light, 10 h dark.

2.5. Larvicidal activity

Larvicidal activity of the essential oil and its five major compounds (β -pinene (32.8%), sabinene (28.3%), germacrene-D (12.7%), estragole (6.4%) and linalool (5.9%)) isolated from *C. anisata* leaves were evaluated according to WHO protocol[18]. Based on the wide range and narrow range tests, essential oil was tested at 50, 100, 150, 200 and 250 ppm and each compound was tested at various concentrations (5-75 ppm). Essential oil or/and individual compounds were dissolved in 1 mL DMSO, then diluted in 249 mL of filtered tap water to obtain each of the desired concentrations. The control was prepared using 1 mL of DMSO in 249 mL of water. Twenty late third instar larvae were then introduced into each solution. For each concentration, five replicates were performed, for a total of 100 larvae. Larval mortality was recorded at 24 h after exposure, during which no food was given to the larvae. The lethal concentrations (LC_{50} and LC_{90}) were calculated by probit analysis[19].

2.6. 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 SPSS12.0 (Statistical Package of Social Sciences) software. Results with $P<0.05$ were considered to be statistically significant.

3. Results

The yield of *C. anisata* leaf essential oil was 4.8 mL/kg fresh weight. The oil was a pale yellow colour. Table 1 shows the constituents of the essential oil, their percentage composition and their Kovats Index (KI) values listed in order of elution. A total of 18 compounds representing 98.2% of the essential oil were identied. The major constituents of this oil were β -pinene(32.8%), sabinene (28.3%), germacrene-D (12.7%), estragole (6.4%) and linalool (5.9%). The percentage compositions of remaining thirteen compounds ranged from 0.2% to 1.8%. The 24 h larvicidal results of essential oil and its five major compounds against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* larvae are presented in Tables 2 and 3. The essential oil from the

leaves of *C. anisata* exhibited significant larvicidal activity, with 24 h LC_{50} values of 140.96, 130.19 and 119.59 ppm, respectively. The five pure constituents extracted from the *C.anisata* leaf essential oil were also tested individually against three mosquito larvae. The LC_{50} values of β-pinene, sabinene, germacrene-D, estragole and linalool appeared to be most effective against *An. stephensi* (LC_{50} - 23.17, 19.67, 16.95, 11.01, 35.17 ppm) followed by *Ae. aegypti* (LC_{50} - 27.69, 21.20, 18.76, 12.70, 38.64 ppm) and *Cx. quinquefasciatus* (LC_{50} - 32.23, 25.01, 21.28, 14.01, 42.28 ppm). Chi-square values of the essential oil and its five compounds show signicant larvicidal activity.

Table 1
Chemical composition of leaf essential oil from *C. anisata*.

Peak	Compounds	Retention time (Kovats index)	Concentration (%)
1	α-Pinene	940	1.2
2	Sabinene	974	28.3
3	β-Pinene	977	32.8
4	Myrcene	992	1.6
5	α-Phellandrene	1004	0.2
6	p-Cymene	1026	0.2
7	Limonene	1029	1.2
8	1,8-cineole	1031	0.8
9	γ-Terpinene	1067	0.3
10	Linalool	1100	5.9
11	Estragole	1192	6.4
12	β-Elemene	1395	0.4
13	β-Caryophyllene	1425	1.3
14	α-Caryophyllene	1459	1.5
15	α-Humulene	1458	1.8
16	Germacrene-D	1487	12.7
17	Germacrene-A	1517	0.9
18	Caryophyllene oxide	1587	0.7

4. Discussion

Different parts of plants contain a complex of chemicals with unique biological activity[20] which is thought to be due to toxins and secondary metabolites, which act as attractants or deterrents[21]. In my result showed that essential oil of the leaf of *C. anisata* has significant larvicidal activity. This result is also comparable to earlier reports of Singh *et al*[22] who observed the larvicidal activity of *Ocimum canum* oil against vector mosquitoes namely, *Ae. aegypti* and *Cx. quinquefasciatus* (LC_{50} 301 ppm) and *An. stephensi* (LC_{50} 234 ppm). Traboulsi et al[23] reported that the larvicidal activity of essential oils of *Citrus sinensis*, *Eucalyptus* spp., *Ferrula hermonis*, *Laurus nobilis*, and *Pinus pinea against Cx. pipiens*. LC_{50} values were 60.0, 120.0, 44.0, 117.0, and 75.0 ppm, respectively. Cheng *et al*[24, 25] examined plant essential oils against *Ae. aegypti* larvae with LC_{50} values ranging from 36.0 to 86.8 μg/mL. In other investigation, Cavalcanti et al[26] reported that the larvicidal activity of essential oils from Brazilian plants with LC_{50} values ranging from 60 to 69 μg/mL against *Ae. aegypti* larvae. Rahuman *et al*[27] also found that n-hexadecanoic acid in *Feronia limonia* dried leaves was effective against fourth-instar larvae of *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* with LC_{50} values of 129.24, 79.58, and 57.23 μg/mL, respectively.

In conclusion, this study reveals that the essential oil of *C. anisata* has remarkable larvicidal properties. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. Our findings suggested that the essential oil from *C. anisata* leaves and its effective constituents may be explored as a potential environmental-benign larvicide. Further investigations for the mode of the constituents' actions, effects on non-target organisms and eld evaluation are necessary. These results obtained are useful in search of more selective, biodegradable and naturally produced larvicidal compounds.

Table 2
Larvicidal activity of essential oil from *C. anisata* against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*.

Mosquito	Concentration	24 h mortality	LC_{50} (ppm)	95%Confidence limits(ppm)		LC_{90} (ppm)	χ^2
				Lower	Upper		
Cx. quinquefasciatus	Control	0.0±0.0	140.96	119.33	163.65	243.19	9.549*
	50	17.2±1.2					
	100	35.4±0.8					
	150	52.8±0.8					
	200	71.4±1.6					
	250	93.6±1.8					
Ae. aegypti	Control	0.0±0.0	130.19	104.70	156.18	227.80	13.395*
	50	23.6±0.6					
	100	35.2±0.8					
	150	55.2±0.6					
	200	79.6±1.2					
	250	96.4±1.6					
An. stephensi	Control	0.0±0.0	119.59	89.48	149.25	209.96	18.924*
	50	25.8±1.2					
	100	41.6±1.6					
	150	58.6±0.8					
	200	84.2±0.6					
	250	100.0±1.4					

*Significant at $P<0.05$ level.

Table 3

Larvicidal activity of five major compounds based on the concentration (%) from essential oil of *C. anisata* against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* larvae.

Compounds	Mosquitoes	LC_{50} (ppm)	95%Confidence limits(ppm)		LC_{90}(ppm)	χ^2
			lower	upper		
Sabinene	*Cx. quinquefasciatus*	25.01	20.14	29.83	45.15	11.591*
	Ae. aegypti	21.20	15.81	26.19	39.22	14.660*
	An. stephensi	19.67	13.94	24.87	36.45	17.057*
β – Pinene	*Cx. quinquefasciatus*	32.23	26.82	37.75	56.58	10.237*
	Ae. aegypti	27.69	22.37	32.81	49.91	10.519*
	An. stephensi	23.17	15.91	29.68	43.39	18.327*
Linalool	*Cx. quinquefasciatus*	42.28	35.64	49.25	73.13	9.874*
	Ae. aegypti	38.64	30.42	46.97	69.08	14.407*
	An. stephensi	35.17	24.78	45.23	63.45	22.317*
Estragole	*Cx. quinquefasciatus*	14.01	11.68	16.45	24.41	10.668*
	Ae. aegypti	12.70	10.15	15.26	22.32	13.462*
	An. stephensi	11.01	8.26	13.62	19.79	16.140*
Germacrene– D	*Cx. quinquefasciatus*	21.28	17.66	24.95	37.04	10.647*
	Ae. aegypti	18.76	15.01	22.39	33.37	11.904*
	An. stephensi	16.95	12.22	21.36	30.95	17.865*

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