

Original Article

Chemical Composition, Larvicidal and Repellency Properties of *Cionura erecta* (L.) Griseb. Against Malaria Vector, *Anopheles stephensi* Liston (Diptera: Culicidae)

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(Received 30 June 2013; accepted 1 Oct 2013)

Abstract

Background: Application of plant derivatives have been suggested as alternative sources for mosquito control.

Methods: The root essential oil and methanol extract of *Cionura erecta* (L.) Griseb was tested under laboratory conditions for larvicidal and skin repellency activities against *Anopheles stephensi*. The chemical compositions of essential oils were analyzed using gas chromatography- mass spectrometry.

Results: Among the five concentrations tested, the 320 ppm of essential oil and 1280 ppm of methanolic extract had the most toxic effects yielding 100% mortality. The LC₅₀ values of *C. erecta* for both essential oil and methanolic extract were 77.30 and 250.38 ppm, respectively. A total of 19 compounds were identified in essential oil of root. The major components were detected in root essential oil including Cedren-9-one (7.89%), alpha cadinol (5.67%), eugenol (4.02%) and alpha muurolene (3.58%). The protection time of 50% solution of essential oil against bites of *An. stephensi* was 2.28 hour on white rabbit and the ED₅₀ and ED₉₀ values of the essential oil were 10.12 and 23.01 ppm respectively.

Conclusion: The findings suggest that *C. erecta* oil has a potential source as larvicidal and repellency properties against *An. stephensi*.

Keywords: *Cionura erecta*, Extract, Essential Oil, *Anopheles stephensi*, Larvicidal Effect, Repellency

Introduction

The mosquitoes are the important vectors of human diseases and can be transmitted malaria, dengue fever, yellow fever and filariasis. They also bother the people both inside and outside places (Lehane 1991). Malaria is one of arthropod-borne disease and approximately 83000 deaths are reported annually in the world (WHO 2012). At the present, this parasitic disease is one of the main health problems in Iran.

Several chemical compounds have been used against malaria vectors as larvicides including organophosphates, insect growth regulator and

microbial derived compounds (Ghosh et al. 2012). Use of synthetic insecticides is causing various problem such as environmental pollution, insecticide resistance and toxic hazards to humans and animals (Aktar et al. 2009, Kunz and Kemp 1994, Vatandoost et al. 2005).

Therefore using plant derivatives have been suggested as alternative sources for mosquito control. They are selective, safe and biodegradable to break down readily in soil and are not stored in plant or animal tissue (Isman 2000, 2006). The various extracts of local plants have been investigated against *An.*

stephensi (HadjiaKhoondi et al. 2000, 2003, 2005, Vatandoost and Vaziri 2004, 2012, Sedaghat et al. 2010, 2011a,b, Vatandoost et al. 2012, Khanavi et al. 2013).

Personal protection using repellents is one of the effective methods for preventing mosquito-borne diseases by reducing man–mosquito contact. The most common insect repellent is DEET (N, N-Diethyl-m-toluamide) moreover, there are several reports about of its toxicity against the skin, nervous and immune systems (Fradin et al. 1998, Katz et al. 2008, Nerio et al. 2010). Insect repellents from natural sources are a good and safe approach for personal protection against the mosquito bites (Fradin et al. 1998). The repellent effect of the plant essential oil has been examined against mosquito species in Iran (Oshaghi et al. 2003, Yaghoobi-Ershadi et al. 2006, Vatandoost and Hanafi-Bojd. 2008, Tavassoli et al. 2011).

Cionura erecta is a woody-based plants 50–100cm high and up to 2m wide perennial with numerous herbaceous rambling stems, often twining at the tips, with copious poisonous milky juice and is widespread in the Mediterranean region which is classified in the family Apocynaceae. This plant known as poisonous composition which traditionally used for killing pest animals, therefore we used of it. Previous studies indicated that safranal, (Z)-3-hexenyl benzoate, heneicosane were obtained as major components from the essential oil of *C. erecta* in Turkey (Myriantopoulos et al. 2007).

This study was aimed at assessing the potential of plant essential oil and methanolic extract for possible use as larvicidal or repellency activities against *An. stephensi* under laboratory conditions and to determine the chemical composition of the essential oil.

Materials and Methods

Mosquito rearing

The tested mosquitoes were the colony of *An. stephensi* which obtained from the Insectary of School of Public Health, Tehran Uni-

versity of Medical Sciences, Iran, and maintained at 29 ± 1 °C with a photoperiod of 12 hours light and 12 hours dark in $60\pm 10\%$ relative humidity. The enriched wheat germ was used as food source. Larvae of *An. stephensi* were continuously available for the larvicidal and repellency experiments. Starved 7 to 10 days old females were used for the repellency tests and the early fourth-instars larvae used for the larval bioassays.

Plant materials

The fresh branch and root of *C. erecta* collected in August 2011 from rural areas located in western part of Ilam Province, Iran ($33^{\circ} 46' N$, $46^{\circ} 11' E$ at elevation 1195m) (Fig. 1). The specimens was identified and authenticated by the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences.

Essential oil isolation

Fresh roots (500g) of *C. erecta* were subjected to hydrodistillation using a modified Clevenger-type (Pyrexfan[®]) apparatus for 3 hour, the oil obtained was separated from water and dried over anhydrous Na_2SO_4 and transferred into airtight vials at 5 °C.

Analysis of essential oils

Chemical composition of *C. erecta* was analyzed using an Agilent 7890–5975 gas chromatography-mass spectrometer. With a HP-5MS (5% Phenyl Methyl Silox) capillary column (30m×0.25mm, film thickness 0.25µm), split ratio, 1: 1, and using a flame ionization detector. The GC was programmed at 50 °C for 2min and then increased at 5 °C/min to 280 °C, and finally held with an isothermal for 3min. The injector temperature was 280 °C. The flow rate of the carrier gas was 1ml/min. The identification of compounds was performed by comparing their retention times and mass spectra with mass spectra from Wiley library. Additional identification was achieved by comparing linear retention indices, relative to n-alkanes, to those from literature (Adams

2001). Details on the identification of volatile compounds were reported in previous paper (Myrianthopoulos et al. 2007).

Methanolic extract of plant

The branch and root of *C. erecta* were air-dried at room temperature and 100g of plant were submitted to percolation separately with methanol (80%) during 3 days, and this procedure were repeated for three times successive and totally last nine day at laboratory temperature (22 to 25 °C). The extracts were next evaporated in a rotary evaporator (Heidolph Persia®).

Larvicidal tests

The essential oil and methanol extract first dissolved in absolute ethanol (99.0%) and methanol (99.0%) respectively. The 400ml glass beakers were used for the treatment or untreated experiments. The third or early fourth instar larvae were exposed to 10, 20, 40, 80 and 160ppm and 40, 80, 160, 320, 640, 1280 ppm of both essential oil and methanolic extract respectively according to standard WHO procedure (WHO 1981).

Repellency tests

The white rabbits (*Oryctolagus cuniculus*) (laboratory reared albino male aged six months) were used to determine both protection time and effective dosage. The 25, 50 percent and pure essential oil of *C. erecta* was prepared using absolute ethanol as well as this solvent used for untreated group against *An. stephensi* on the shaved back of male rabbits with 4 repetitions. The procedure for determination of effective dosages of the repellents was adopted by the standard method of American Society for Testing and Material (ASTM 2000).

The testing kit was made of plexiglas cube at dimension of 4×5×18cm having four rectangular holes 2×3cm. Before starting the test for determination of effective dosage, the abdomen skins of rabbits were cleaned with alcohol and the kit was fixed on the abdomen.

The eligibility of the rabbits for repellency tests was 10 landings or probes within 30 seconds. Each of 4 adjacent cells of kit was provided with 5 female 7–10 days mosquitoes that randomly selected from a cage containing 150 starved mosquitoes. Five circles were drawn on the rabbit's skin. The drawn circles on the abdomen skin's of holded rabbit were treated with 25µl of essential oil diluted with absolute alcohol at 2, 4, 8, 16, 32ppm with 4 repetitions. The serial dilutions were applied on 3 holes as well as the absolute ethanol was applied in remaining control circle. The treated circles were allowed to dry, and then test apparatus containing starved mosquitoes were fixed on the treated skin. The counts of probing and biting were recorded at 1 minute intervals up to 5 minutes. After each test, the mosquitoes were transferred to netted cups and the mortality of mosquitoes was recorded after 24 hours. The ED₅₀ and ED₉₀ values and regression parameters were analyzed using probit 79 program and the regression lines were plotted in Microsoft Excel 2007.

Ethical approval

Animal experiments were performed after obtaining Institutional Animal Ethical Committee's approval from Tehran University of Medical Sciences.

Results

GC-mass analysis

The hydrodistillation of the *C. erecta* root gave oil in 0.16% (w/w) yield on fresh weight material. A total of 19 compounds was 36.4% in roots of *C. erecta* were identified (Table 1). The major components in root oil were cedren-9-one (7.89%), alpha cadinol (5.67%), eugenol (4.02%) and alpha muurolene (3.58%) respectively.

Mosquito larvicidal activity

The larvicidal activities of both essential

oil and methanol extract of the *C. erecta* root against *An. stephensi* larvae under laboratory conditions are shown in Table 2. Among the five concentrations tested, the dosages of 320 ppm and 1280ppm of essential oil and methanolic extract were respectively found to be the most toxic with 100% larval mortality. The essential oil *C. erecta* extracted with root and showed the higher toxicity than methanolic extract against the larvae. The LC₅₀ and LC₉₀ values of *C. erecta* essential oil were 77.30 and 199.58ppm, and for methanolic extract were recorded 250.38 and 490.00ppm, respectively.

Effective doses

The ED₅₀ and ED₉₀ values of *C. erecta* essential oil were 10.12 and 23.01 ppm with confidence intervals ranged, 7.89–13.9 and 16.12–50.37 respectively (Table 4).

Protection time

The 25, 50 percent and pure essential oil *C. erecta* against *An. stephensi* on animal subject were provided 2.0–3.15 hours protection. The repellent failure time was ranged 2.5–4.25 hours (Table 3).

Effective doses

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Protection time

The 25%, 50% and 100% essential oil *C. erecta* against *An. stephensi* on animal subject were provided 2.0–3.15 hours protection. The repellent failure time was ranged 2.5–4.25 hours (Table 3).

Table 1. Chemical constituents of root essential oil from *Cionura erecta*

No.	Compounds	Composition%	RI
1	2,4 decadienal	1.3	1307
2	dimethyl phenyl acetate	1.25	1315
3	Eugenol	4.02	1360
4	beta elemen	0.332	1391
5	alpha humulene	0.21	1457
6	Trans caryophyllene	0.71	1473
7	alpha muurolene	3.58	1483
8	delta cadinene	1.52	1404
9	caryophyllene oxide	1.38	1540
10	Viridiflorol	0.55	1546
11	Silphiperfolenone	0.85	1551
12	Trans cadinene	0.64	1569
13	alpha cadinol	5.67	1577
14	Eudesmol	1.78	1584
15	gama epoxy elemen	0.69	1598
16	Cedren-9-one	7.89	1633
17	Isolongifolene-5-one	2.15	1644
18	Tetradecanol	1.34	1648
19	Cadalene	0.58	1677
		36.44	

*RI: Retention indices determined on HP-5 column



Fig. 1. The plant *C. erecta* in its natural habitat, Ilam Province, west of Iran (original)

Table 2. LC₅₀ and LC₉₀ values of essential oil and methanolic extract of *Cionura erecta* roots against larvae of *An. stephensi*

Type of extraction	a	b ± SE	LC ₅₀ (ppm) ± 95% C.L.	LC ₉₀ (ppm) ± 95% C.L.	² (heterogeneity)	² table (df)	p-Value
Essential oil	-5.87	10.11 ± 0.34	69.28	169.71	5.419 *	11.345 (3)	0.01
			77.30	199.58			
			86.40	244.91			
Methanol extract	4.40	10.54 ± 0.34	229.078	434.03	11.999 *	13.277 (4)	0.01
			250.38	490.00			
			273.66	571.00			

* No heterogeneity

Table 3. Protection and failure times of essential oil of *Cionura erecta* against *Anopheles stephensi* on abdomen of albino rabbits at laboratory condition

Concentration of essential oil	Protection time (h) ± SD	Failure time (h) ± SD
25%	2.01 ± 0.95	2.5 ± 0.5
50%	2.28 ± 15.6	3.25 ± 17
100%	3.15 ± 17.3	4.25 ± 17.3

Tables 4. Effective doses of essential oils *Cionura erecta* (L.) roots against *An. stephensi* on albino rabbits

a	b ± SE	ED ₅₀ (mg/cm ²) ± 95% C.L.	ED ₉₀ (mg/cm ²) ± 95% C.L.	² (heterogeneity)	² table (df)	p-Value
-3.59	3.575 ± 0.784	7.89	16.12	3.048*	13.277 (3)	0.01
		0.01012	0.02301			
		13.89	50.37			

* No heterogeneity

Discussion

Application of larvicides and repellents are generally accepted as the playing a significant role in control of the mosquitoes.

In this study, major constituents of root essential oil of *C. erecta* were evaluated.

Cedren-9-one (7.89%), alpha cadinol (5.67%), eugenol (4.02%) and alpha muurolene (3.58%) were found as main compounds. The chemical ingredients of *C. erecta* essential oil was reported comprised 72 compo-

nents, from which the main one considered as safranal (16.8%), (Z)-3-hexenyl benzoate (6.1%), heneicosane (5.7%) linalool (4.8%) and tricosane (4.4%) (Myrianthopoulos et al. 2007). Some constitute was not found in our study.

According to the larvicidal assay, the essential oil and methanol extract of *C. erecta* were effective against *An. stephensi* with LC₅₀ and LC₉₀ values of 77.30ppm and 250.38 ppm, respectively. The bioassay of different herbal extracts has been studied against *An. stephensi* larvae in Iran. There is a report about the efficacy of the essential oil and methanolic extract of *Eucalyptus camaldulensis* against *An. stephensi* in which, the LC₅₀ and LC₉₀ values were found 89.85ppm and 397.75 ppm, respectively (Sedaghat et al. 2010). The larvicidal activity of *Azadirachta indica* extract against *An. stephensi* were gained 0.35 ppm and 1.81ppm respectively for LC₅₀ and LC₉₀ values (Vatandoost and Vaziri 2004). Also the LC₅₀ and LC₉₀ of *Cupressus arizonica* essential oil have been reported respectively 79.30ppm and 238.89ppm against *An. stephensi* (Sedaghat et al. 2011b). The larvicidal activity of three plants from the family Apiaceae have been studied and the LC₅₀ values of three essential oils ranged from 20.10 to 120.95ppm (Sedaghat et al. 2011a). In the other study, the efficacy of *Kelussia odoratissima* essential oil was evaluated at dose of 10ppm induced 100% larval mortality, against larvae of both *An. stephensi* and *Cx. pipiens* (Vatandoost et al. 2012).

The repellency effect of the *C. erecta* essential oil against *An. stephensi* was first evaluated under laboratory conditions. The mean protection time of 50% essential oil of *C. erecta* provided 2.15 hours protection against *An. stephensi*. The figures for ED₅₀ and ED₉₀ values were 10.12 and 23.01ppm respectively.

The repellency effect of essential oils of many plants has been evaluated against *An. stephensi* (Ansari et al. 2000, Prajapati et al. 2005, Klun et al. 2006, Rajkumar and Jebanesan 2007, Mullai et al. 2008, Pandey et al. 2009,

Govindarajan et al. 2011, Prabhu et al. 2011).

The repellency effect of essential oils of both *Myrtus communis* and *Calendula officinalis* had been reported and the ED₅₀ values were 0.11 and 0.6 mg/cm², respectively on human subjects (Tavassoli et al. 2011). Other laboratory trial revealed the repellency of 3 chemical and herbal repellents against *An. stephensi*. The ED₅₀ value of neem tree's essential oil was 0.191 mg/cm² against field strain of mosquitoes (Vatandoost et al. 2008).

The results indicated both the repellency of essential oil as well as the larvicidal activity of *C. erecta* extract against *An. stephensi*.

Conclusion

Results of this study will provide a clue for possible use of plants for control of mosquito-borne disease.

Acknowledgments

This study was funded and supported by Tehran University of Medical Sciences (TUMS); Grant no. 90-04-27-16617. We are thankful to the staff of Insectary of Culicidae, Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences for the mass production of the mosquitoes for this study. The authors declare that there is no conflict of interests.

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