Original Article

Chemical Composition, Larvicidal and Repellent Properties of Cionura erecta (L.) Griseb. Against Malaria Vector, Anopheles stephensi Liston (Diptera: Culicidae) Under Laboratory Conditions

Ehsan Mozaffari 1, Mohammad Reza Abai 1, Mahnaz Khanavi 2, *Hassan Vatandoost 1, Mohammad Mehdi Sedaghat 1, Alireza Sanei-Dehkordi 1, Abbas Moridnia 1, Mahsa Saber-Navaei 2, Fatemeh Rafi 1

1Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

(Received 30 June 2013; accepted 1 Oct 2013)

Abstract
Background: the using 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 examined under laboratory conditions for larvicidal and skin repellent activities against Anopheles stephensi. The chemical compositions of essential oils were analyzed using gas chromatography- mass spectrometry.
Results: Among the five concentrations tested, the dosage of 320ppm of essential oil and 1280 ppm of methanol extract had the most toxic effect yielding 100% mortality. The LC50 values of C. erecta for both essential oil and methanol extract were 77.30 and 250.38 ppm, respectively. A total of 19 compounds amounting to 37% in roots of C. erecta were identified. The major components in root oil were Cedren-9-one (7.89%), alpha cadinol (5.67%), eugeno (4.02%) and alpha muurolene (3.58%). The protection time of 50% solution of essential oil from C. erecta against bites of An. stephensi on white rabbit was 2.28 hour and also the ED50 and ED90 value of the C. erecta essential oil were 10.12 and 23.01 ppm respectively.
Conclusion: The findings suggest that C. erecta oil is a potential source of larvicidal and repellent compounds.

Keywords: Malaria, Cionura erecta, Vector, Anopheles stephensi, repellents- larvicides and plant derivatives

Introduction

The mosquitoes (family Culicidae) are important in medical entomology research because can threaten the human health by transmitting diseases of malaria, dengue fever, yellow fever and filariasis, They are also causative agents for nuisance of human in both inside and outside places (Lehane 1991). Malaria is the most important one among arthropod-borne diseases and responsible for approximately one million deaths annually and has affected nearly half of the world’s population (WHO 2008). At the present, this parasitic disease is one of the main health problems in Iran.

At present, several chemical compounds have been used against malaria vectors include organophosphates, insect growth regulator and microbial larvicides (Coluzzi 1992). Use of synthetic insecticides is causing various problem such as environmental pollution, insecticide resistance and toxic hazards to humans and animals (Mellanby 1967, WHO 1985, Kunz and Kemp 1994, Alloway and Ayres 1997, Vatandoost et al. 2005, Davari et al. 2006, 2007).

Therefore using plant derivatives have been suggested as alternative sources for mosquito
control. They are selective, safe and biodegrade 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 classified in the family Apocynaceae, subfamily Asclepiadoideae, tribe Marsdenieae. This plant is a woody-based 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 (Tutin et al. 1976). 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 (Myrianthopoulos et al. 2007).

This study was aimed at assessing the potential of plant essential oil and methanolic extract for possible use as larvicide or repellent 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 University of Medical Sciences, Iran, and maintained at 27 °C with a photoperiod of 12 hours light and 12 hours dark in 80±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-instar 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° 46' N, 46° 11' E, elevation 1195) (Fig. 1), and 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 Na₂SO₄ and transferred into airtight vials at 5 ºC.

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 0.25 m), split ratio, 1: 1, and using a flame ionization detector. The GC was programmed at 50 ºC for
2 min and then increased at 5 °C/min to 280 °C, and finally held with an isothermal for 3 min. The injector temperature was 280 °C. The flow rate of the carrier gas was 1 ml/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).

**Methanol 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®).

**Bioassays and larval mortality**

The essential oil and methanol extract first dissolved in ethanol (99.0%) and methanol (99.0%) respectively. The 400ml glass beakers were used for the treatment or untreated experiments. The 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 methanol extract respectively according to standard WHO procedure (WHO 1981).

**Repellency tests**

The white rabbits (*Oryctolagus cuniculus*) (laboratory reared albino females/ sex month) were used to determine both protection time and effective dosage. The 25%, 50% and 100% of essential oil from *C. erecta* was prepared using absolute ethanol as well as this solvent used for untreated group against *An. stephensi* on the shaved back of female rabbits with 4 repeat. 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 five circles with 29mm diameters. 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 criterion for selection of rabbits for repellency tests was 10 landings or probes within 30 seconds. Each of 5 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 rabbit’s skin were treated with 25µl of essential oil diluted with absolute alcohol at 2, 4, 8, 16, 32ppm with 4 repeat. The serial dilutions were applied on 4 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 maintenance for 24 hours. The ED_{50} and ED_{90} 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, Fig. 2). 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 methanol 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 methanol extract against the larvae. The LC50 and LC90 values of *C. erecta* essential oil were 77.30 and 199.58ppm, and for methanol 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 23.01ppm with confidence interval ranged, 7.89–13.9 and 16.12–50.37 respectively (Table 4).

**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).

![Fig. 1. The plant *C. erecta* (original)](image)

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Compounds</th>
<th>Composition%</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.4 decadienal</td>
<td>1.3</td>
<td>1307</td>
</tr>
<tr>
<td>2</td>
<td>dimethyl phenyl acetate</td>
<td>1.25</td>
<td>1315</td>
</tr>
<tr>
<td>3</td>
<td>Eugenol</td>
<td>4.02</td>
<td>1360</td>
</tr>
<tr>
<td>4</td>
<td>Beta elemen</td>
<td>.332</td>
<td>1391</td>
</tr>
<tr>
<td>5</td>
<td>Trans caryophyllon</td>
<td>.21</td>
<td>1417</td>
</tr>
<tr>
<td>6</td>
<td>Trans caryophyllene</td>
<td>.71</td>
<td>1473</td>
</tr>
<tr>
<td>7</td>
<td>alpha muurolene</td>
<td>3.58</td>
<td>1483</td>
</tr>
<tr>
<td>8</td>
<td>delta cadinene</td>
<td>1.52</td>
<td>1404</td>
</tr>
<tr>
<td>9</td>
<td>caryophyllene oxide</td>
<td>1.38</td>
<td>1540</td>
</tr>
<tr>
<td>10</td>
<td>Viridiflorol</td>
<td>.55</td>
<td>1546</td>
</tr>
<tr>
<td>11</td>
<td>Silphiperfoleneone</td>
<td>.85</td>
<td>1551</td>
</tr>
<tr>
<td>12</td>
<td>Trans cadinene</td>
<td>.64</td>
<td>1569</td>
</tr>
<tr>
<td>13</td>
<td>alpha cadinol</td>
<td>5.67</td>
<td>1577</td>
</tr>
<tr>
<td>14</td>
<td>Eudesmol</td>
<td>1.78</td>
<td>1584</td>
</tr>
<tr>
<td>15</td>
<td>Gama epoxy elemen</td>
<td>.69</td>
<td>1598</td>
</tr>
<tr>
<td>16</td>
<td>Cedren-9-one</td>
<td>7.89</td>
<td>1633</td>
</tr>
<tr>
<td>17</td>
<td>isolongifolene-5-one</td>
<td>2.15</td>
<td>1644</td>
</tr>
<tr>
<td>18</td>
<td>Tetradecanol</td>
<td>1.34</td>
<td>1648</td>
</tr>
<tr>
<td>19</td>
<td>Cadalene</td>
<td>.58</td>
<td>1677</td>
</tr>
</tbody>
</table>

| 36.44 |                |

*RI: Retention indices determined on HP-5 column*
Fig. 2. GC-MS chromatogram of essential oil of C. erecta

Table 2. LC$_{50}$ and LC$_{90}$ values of different concentrations from essential oil and methanol extract of Cionura erecta roots against larvae of An. stephensi

<table>
<thead>
<tr>
<th>Type of extraction</th>
<th>a</th>
<th>b ± SE</th>
<th>LC$_{50}$ (ppm) ± 95% C.L.</th>
<th>LC$_{90}$ (ppm) ± 95% C.L.</th>
<th>$\chi^2$ (heterogeneity)</th>
<th>$\chi^2$ table (df)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential oil</td>
<td>-5.87</td>
<td>10.11 ± 0.34</td>
<td>69.28 ± 169.71</td>
<td>77.30 ± 199.58</td>
<td>86.40 ± 244.91</td>
<td>5.419 *</td>
<td>11.345 (3)</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>4.40</td>
<td>10.54 ± 0.34</td>
<td>229.078 ± 434.03</td>
<td>250.38 ± 490.00</td>
<td>273.66 ± 571.00</td>
<td>11.999 *</td>
<td>13.277 (4)</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Concentration of essential oil</th>
<th>Protection time (h) ± SD</th>
<th>Failure time (h) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>25%</td>
<td>2.01 ± 0.95</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>50%</td>
<td>2.28 ± 1.56</td>
<td>3.25 ± 1.7</td>
</tr>
<tr>
<td>100%</td>
<td>3.15 ± 1.73</td>
<td>4.25 ± 1.73</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>a</th>
<th>b ± SE</th>
<th>ED$_{50}$ (mg/cm$^2$) ± 95% C.L.</th>
<th>ED$_{90}$ (mg/cm$^2$) ± 95% C.L.</th>
<th>$\chi^2$ (heterogeneity)</th>
<th>$\chi^2$ table (df)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3.59</td>
<td>3.575 ± 0.784</td>
<td>0.01012 ± 0.02301</td>
<td>0.01012 ± 0.02301</td>
<td>3.048 *</td>
<td>13.277 (3)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* No heterogeneity
Discussion

Application of Larvicides and repellents are generally accepted as the playing a significant role in preventing mosquito-borne diseases.

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 components, 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. 2011). 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 LC_{50} and LC_{90} values of 77.30ppm and 250.38ppm, 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 LC_{50} and LC_{90} values were found 89.85ppm and 397.75ppm, 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 LC_{50} and LC_{90} values (Vatandoost and Vaziri 2004). Also the LC_{50} and LC_{90} of *Cupressus arizonica* essential oil have been reported respectively 79.30ppm and 238.89ppm against *An. stephensi* (Sedaghat et al. 2011a). The larvicidal activity of three plant from family Apiaceae have been studied and the LC_{50} values of three essential oils ranged from 20.10 to 120.95ppm (Sedaghat et al. 2011b). In the other study, the efficacy of *Kelussia odoratissima* essential oil was evaluated at dose of 10 ppm 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 for ED_{50} and ED_{90} values were 10.12 and 23.01ppm respectively.


The repellency effect of essential oils of both *Myrtus communis* and *Calendula officinalis* had been reported and the ED_{50} 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 ED_{50} value of neem tree’ s essential oil was 0.191mg/cm² against field strain of mosquitoes (Vatandoost et al. 2008).

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

Acknowledgments

This work was supported by a grant (90-04-27-16617) from Tehran University of Medical Sciences, Tehran, Iran. 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.

References

Mosq Control Assoc. 3: 302–303.
Alloway B, Ayres DC (1997) Chemical principles of environmental pollution. CRC.
Finney DJ (1947) Probit analysis, a statistical treatment of the sigmoid response curve.


Tavassoli M, Shayegehi M, Vatandoost H, Khoobdel M, Sarali M, Ghaderi A, Rafi F (2011) Repellency Effects of Essential Oils of Myrtle (Myrtus communis), Marigold (Calendula officinalis) Com-
pared with DEET against *Anopheles stephensi* on Human Volunteers. Iran J Arthropod-Borne Dis. 5: 10–22.


WHO (1981) Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides. World Health Organization-VBC.

WHO (1985) Resistance of vectors of disease to pesticides. 10th report of the expert committee on vector Control.
