Journal of Entomology and Zoology Studies
Available online at www.entomoljournal.com

ISSN 2320-7078
JEZS 2014; 2 (5): 20-24

Received: 11-07-2014
Accepted: 27-08-2014

Xin Chao Liu
Department of Entomology, China Agricultural University, Haidian District, Beijing 100193, China

Guo Hua Jiang
Analytic and Testing Center, Beijing Normal University, Haidian District, Beijing 100875, China

Zhi Long Liu
Department of Entomology, China Agricultural University, Haidian District, Beijing 100193, China

Correspondence:
Zhi Long Liu
Department of Entomology, China Agricultural University, Haidian District, Beijing 100193, China

GC-MS analysis of larvicidal essential oil of *Cirsium pendulum* Fisch. aerial parts against the *Aedes albopictus* mosquito

Xin Chao Liu, Guo Hua Jiang and Zhi Long Liu

Abstract
During the screening program for new agrochemicals from local wild plants, the essential oil of *Cirsium pendulum* Fisch. aerial parts (Compositae) at flowering stage was found to possess strong larvicidal activity against *Aedes albopictus*. Water-distilled essential oil from *C. pendulum* aerial parts was analyzed by gas chromatography-mass spectrometry (GC-MS). Forty-three compounds, accounting for 97.15% of the oil, were identified and β-eudesmol (9.67%), caryophyllene (8.14%), 1,8-cineole (6.80%) and caryophyllene oxide (6.42%) are the four main components of the essential oil followed by camphor (4.46%), δ-cadinene (4.37%) and spathulenol (4.01%). The essential oil of *C. pendulum* exhibited larvicidal activity against *A. albopictus* with a median lethal concentration (LC_{50}) value of 46.77 μg/ml. These findings suggested that the essential oil of *C. pendulum* has potential to be developed as a new natural larvicide for the control of mosquitoes.

Keywords: *Cirsium pendulum*; *Aedes albopictus*; larvicidal activity; essential oil.

1. Introduction

Cirsium pendulum Fisch. (syn. *C. falcatum* Turcz. ex DC.) is a perennial plant (1-3 m tall) of the Compositae family. It is mainly distributed in Gansu, Hebei, Heilongjiang, Henan, Jilin, Liaoning, Inner Mongol, Shaanxi, Shanxi, and Yunnan Province of China as well as Japan, Korea, Mongolia, and Russia. It usually grows in grasslands in mountain valleys and on mountain slopes, forests, forest margins, streamsides, near villages and rock crevices between about 300-2300 m above sea level [1]. In some areas of China, the aerial parts or roots of *C. pendulum* were used instead of *C. japonicum* (Herba Cirsii Japonici/Radix Cirsii Japonici) as an antihemorrhagic agent and uretic in Chinese traditional medicine [2]. Previous phytochemical studies on *C. pendulum* resulted in the identification of several phenolics, flavonoids, triterpenoids, and flavone glucosides [4-13]. However, a literature survey has shown that there is no report on chemical composition of the essential oil of *C. pendulum* and larvicidal activity of the essential oil. Thus we decided to investigate the chemical constituents and larvicidal activity of the essential oil of *C. pendulum* aerial parts against the mosquitoes for the first time.

The Asian tiger mosquito (*Aedes albopictus* Skuse) is one of the two main species of mosquito responsible for dengue fever and malaria in China. The control of mosquito larvae worldwide depends primarily on continued applications of synthetic insecticides including organophosphates such as temephos and fenthion and insect growth regulators such as diflubenzuron and methoprene [14]. However, heavy and wide use of these synthetic insecticides has caused several environmental and health concerns [15]. Thus, there is urgent need to look for new natural strategies for mosquito control. From this point of view, botanical pesticides, including essential oils, are promising since they are effective, environmentally friendly, easily biodegradable and often inexpensive [16]. Many essential oils and constituent compounds derived from various essential oils can exert toxic activity against mosquito species [17-22]. During our mass screening program for new agrochemicals from wild plants and Chinese medicinal herbs, the essential oil of *C. pendulum* aerial parts at flowering stage was found to possess larvicidal activity against the Asian tiger mosquito, *A. albopictus*.

2. Materials and Methods

2.1. Plant material

Fresh aerial parts (10 kg of leaves, stems and flowers) of *C. pendulum* were harvested in August 2013 from Xiaolongmeng National Forest Park (39.48° N and 115.25° E, Mentougou District, Beijing 102300). The herb was air-dried and identified by Dr. Liu QR (College of Life Sciences, Beijing Normal University, Beijing, China), and a voucher specimen (ENTCAU-Compositae-Yanguanji-10018) was deposited at the herbarium of Department of Entomology, China Agricultural University (Haidian District, Beijing, China).

2.2. Essential oil extraction

The dried samples were grinded to powdered form using a grinding mill. Each 600 g portion of powder was mixed in 1,800 ml of distilled water and soaked for 3 h. The mixture was then boiled in a round-bottom flask and steam distilled for 6-8 h. Volatile essential oil from distillation was collected in a flask. Separation of the essential oil from the aqueous layer was done in a separate funnel using the non-polar solvent, n-hexane. The solvent was evaporated using a vacuum rotary evaporator (BUCHI Rotavapor R-124, Switzerland). The sample was dried over anhydrous Na_2SO_4 and kept in a refrigerator (4 °C) for subsequent experiments.

2.3. GC-MS analysis

The essential oil of *C. pendulum* aerial parts was subjected to GC-MS analysis on an Agilent system consisting of a model 6890N gas chromatograph, a model 5973N mass selective detector (EIMS, electron energy, 70 eV) and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a 5% phenyl-methylpolysiloxane stationary phase, film thickness of 0.25 µm, a length of 30 m and an internal diameter of 0.25 mm. The GC settings were as follows: the initial oven temperature was held at 60 °C for 1 min and increased at 10 °C min^{-1} to 180 °C for 1 min and then increased at 20 °C min^{-1} to 280 °C for 15 min. The injector temperature was maintained at 270 °C. The samples (1 µl, diluted to 1:100 with acetone) were injected with a split ratio of 1: 10. The carrier gas was helium at flow rate of 1.0 ml min^{-1}. Spectra were scanned from 20 to 550 m/z at 2 scans s^{-1}. Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of n-alkanes (C_8–C_{24}) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature [23]. Component relative percentages were calculated based on GC peak areas without using correction factors.

2.4. Insect cultures and rearing conditions

Mosquito eggs of *A. albopictus* utilized in bioassays were obtained from a laboratory colony maintained in the Department of Vector Biology and Control, Institute for Infectious Disease Control and Prevention, Chinese Center for Disease Control and Prevention. The dehydrated eggs were placed on a plastic tray containing tap water at 24-26 °C under natural summer photoperiod to hatch and yeast pellets served as food for the emerging larvae. The newly emerged larvae were then isolated in groups of ten specimens in 100 ml tubes with mineral water and a small amount of dog food. Larvae were daily controlled until they reached the fourth instar stage when they were utilized for bioassay (within 12 h).

2.5. Larvicidal bioassay

Range-finding studies were run to determine the appropriate testing concentrations. Concentrations of 200, 100, 50, 25, and 12.5 µg/ml of essential oil were tested. The larval mortality bioassay was carried out according to the test method for larval susceptibility proposed by the World Health Organization (WHO) [24]. Twenty larvae were placed in glass beaker with 250 ml of aqueous suspension of tested material at various concentrations and an emulsifier dimethyl sulfoxide (DMSO) was added in the final test solution (< 0.05%). Five replicates per concentration were run simultaneously and with each experiment, a set of controls using 0.05% DMSO and untreated sets of larvae in tap water were also run for comparison. For comparison, commercial chlorpyrifos (purchased from National Center of Pesticide Standards, Tiexi District, Shenyang, China) was used as positive control. The toxicity of chlorpyrifos was determined at concentrations of 5, 2.5, 1.25, 0.6, and 0.3 µg/ml. The assay was carried out in a growth chamber (L16:D9, 26-27 °C, 78-80% relative humidity). Mortality was recorded after 24 h of exposure and the larvae were starved of food over this period.

2.6. Statistical analysis

Percent mortality was corrected for control mortality using Abbott's formula [25]. Results from all replicates for the pure compounds/oil were subjected to probit analysis using PriProbit Program V1.6.3 to determine LC_{50} values and their 95% confidence intervals [27]. Samples for which the 95% fiducial limits did not overlap were considered to be significantly different.

3. Results and Discussion

3.1. Chemical composition of the essential oil

The steam distillation for 3 h of aerial parts at flowering stage of *C. pendulum* afforded essential oil (yellow) with a yield of 0.09% (v/w) and the density of the concentrated essential oil was determined to be 0.92 g/ml. The GC and GC–MS analysis of the essential oils of the aerial parts of *C. pendulum* led to the identification and quantification of a total of 43 major components accounting for 97.15% of the total components present (Table 1). The main components found in the essential oil were β-eudesmol (9.67%), caryophyllene (8.14%), 1,8-cineole (6.80%) and caryophyllene oxide (6.42%) are the four main components of the essential oil followed by camphor (4.46%), δ-cadinene (4.37%) and spathulenol (4.01%) (Table 1). Sesquiterpenoids represented 22 of the 43 compounds, corresponding to 61.00% of the whole oil while 18 of the 43 constituents were sesquiterpenoids (30.82% of the crude essential oil). This is the first attempt to determine the chemical composition of the essential oil of *C. pendulum* aerial parts at flowering stage. Chemical composition of the essential oil of *C. pendulum* was quite different from those of the plants in the same Genus [27-30]. For example, the main constituents in the essential oil of C. *creticum* aerial parts were 4-ethyl guaiacol (15%), hexadecanoic acid (10.6%), (E)-β-damascenone (7.8%), dihydroactinidiolide (6.0%) and 4-vinyl guaiacol (4.5%) [29] while the essential oil from *C. japonicum* rhizomes mainly contained palmitic acid (14.42%), caryophyllene oxide (12.57%), khusinol (6.31%), pentadecanoic acid (6.28%) and myristic acid (4.66%) [28].

Table 1: Chemical constituents of the essential oil of *Cirsium pendulum* aerial parts

Peak No	Compound	RI[a]	Percent Composition
1	α-Pinene[b]	939	0.44
2	β-Pinene[b]	981	0.48
3	β-Myrcene[b]	991	0.91
4	ρ-Cymene	1025	0.08
5	Limonene[b]	1029	0.11
6	1,8-Cineole[b]	1031	6.80
7	γ-Terpinene	1059	1.21
8	*cis*-Linalool oxide	1067	0.68
9	Linalool[b]	1094	3.28
10	2,6-Dimethylcyclohexanol	1110	0.32
11	Camphor[b]	1146	4.46
12	Borneol[b]	1174	1.52
13	4-Terpineol[b]	1177	1.05
14	3,9-Epoxy-1-p-menthene	1178	3.74
15	p-Cymen-8-ol	1182	1.15
16	α-Terpineol[b]	1188	0.78
17	Pulegone	1236	1.32
18	Carvone[b]	1243	0.45
19	Piperitone	1257	2.36
20	Longipinene	1351	1.83
21	Cyclosativene	1362	1.51
22	Copaene	1374	1.12
23	*trans*-β-Damascenone	1382	2.08
24	β-Cubebene	1387	2.64
25	Caryophyllene[b]	1420	8.14
26	α-Caryophyllene	1454	1.31
27	Seychellene	1458	1.16
28	γ-Muurolene	1473	1.19
29	β-Ionone	1487	3.85
30	β-Selinene	1490	0.85
31	β-Himachalene	1493	0.55
32	δ-Cadinene	1524	4.37
33	Dihydroactinolide	1538	1.16
34	Spathulenol	1578	4.01
35	Caryophyllene oxide[b]	1583	6.42
36	Eremophyllene	1586	1.51
37	Carotol	1593	1.49
38	Epiglobulol	1629	2.78
39	τ-Muurolol	1642	2.15
40	β-Eudesmol[b]	1648	9.67
41	α-Cadinol	1654	1.23
42	Apiol	1682	3.58
43	Nootkanone	1812	1.41
	Total identified		97.15
	Monoterpenoids		30.82
	Sesquiterpenoids		61.00
	others		5.33

[a] RI, retention index as determined on a HP-5MS column using the homologous series of *n*-hydrocarbons; [b] Identification based on comparison of RI and spectra with authentic standards.

3.2. Larvicidal activity of the essential oil

The essential oil of *C. pendulum* aerial parts possessed strong larvicidal activity against the 4th instar larvae of *A. albopictus* with a LC_{50} value of 46.77 µg/ml (Table 2). The commercial insecticide, chlorpyrifos exhibited larvicidal activity against the mosquitoes with a LC_{50} value of 1.86 µg/ml, thus the essential oil of *C. pendulum* was 25 times less toxic to *A. albopictus* larvae compared with chlorpyrifos. However, compared with the other essential oils using the same bioassay in the literature, the essential oil of *C. pendulum* exhibited the same level of or stronger larvicidal activity against *A. albopictus* larvae, e.g., essential oils of Salvia elegans and S. splendens (LC_{50} = 46.4 ppm and LC_{50} = 59.2 ppm, respectively) [31]; *Zanthoxylum avicennae* leaves and stems (LC_{50} =48.79 µg/ml) [20]; *Cryptomeria japonica* leaves (LC_{50} = 51.2 µg/ml) [32]; *Toddalia asiatica* roots (LC_{50} = 69.09 µg/ml) [18]; *Allium macrostemon* (LC_{50} = 72.86 µg/ml) [22]; *Eucalyptus urophylla* (LC_{50} = 95.5 µg/ml) [33]; and essential oils of *Achillea millefolium* (LC_{50} = 211.3 µg/ml), *Helichrysum italicum* (LC_{50} = 178.1 µg/ml) and *Foeniculum vulgare* (LC_{50} = 142.9 µg/ml) [34]. However, the essential oil of *C. pendulum* possessed weaker larvicidal activity against *A. albopictus* larvae than essential oils of *Saussurea lappa* roots (LC_{50} = 12.41 µg/ml) [19]; *Eucalyptus camaldulensis* (LC_{50} = 31.0 µg/ml) [33] and *Clinopodium gracile* aerial parts (LC_{50} = 31.0 µg/ml) [17].

In previous reports, one of the main constituent compounds of the essential oil, β-eudesmol, possessed contact toxicity and ovicidal activity against diamondback moth, *Plutella xylostella* [35] and contact toxicity against *Drosophila melanogaster* [36]. However, β-eudesmol has not been evaluated for larvicidal activity against mosquitoes so far. Another constituent compound, β-caryophyllene showed weak larvicidal activity against *Aedes aegypti* (LC_{50} = 1038 ppm) [37]. 1.8-Cineole was found to exhibit contact and fumigant toxicities against several insects [38, 39] and possessed feeding and ovipositional repellent action against mosquitoes [40]. Moreover, 1,8-cineole induced 100% larval mortality of *A. aegypti* after 1 day with a dosage of 100 mg/l [41] and had a LC_{50} value of 47.9 µg/ml against the third-instar larvae of *A. aegypti* [42]. However, in other reports, 1.8-cineole was found to exhibit weak larvicidal activity against *A. aegypti* (LC_{50} = 1419 ppm) or no toxicity to larval *Culex pipiens* [43, 44]. Thus, the isolation and identification of the bioactive compounds in the essential oil of *C. pendulum* aerial parts at flowering stage are of utmost importance to determine if their potential application in controlling mosquito pests can be fully exploited.

Considering that the currently used larvicides are synthetic insecticides, larvicidal activity of the crude essential oil is quite promising and it shows its potential for use in the control of *A. albopictus* larvae and could be useful in the search for newer, safer and more effective natural compounds as larvicides. For the actual use of *C. pendulum* aerial parts essential oil and its constituents as a novel larvicide or insecticide to be realized, further research is needed to establish their human safety and environmental safety. Additionally, their larvicide modes of action have to be established, and formulations for improving larvicidal potency and stability need to be developed.

Table 2: Larvicidal activity of *Cirsium pendulum* essential oil against fourth-instar larvae of *Aedes albopictus*

Treatment	LC_{50} (µg/ml) (95% FL)	LC_{95} (µg/ml) (95% FL)	Slope ± SD	Chi-square value (χ^2)
C. pendulum Mean Range	46.77 (42.08-51.38)	143.54 (128.73-157.87)	1.86 ± 0.16	13.09
Chlorpyrifos Mean Range	1.86 (1.71-2.05)	6.65 (6.21-7.48)	0.87 ± 0.01	3.13

FL: Fiducial limits

4. Conclusions

Chemical composition of the essential oil of *C. pendulum* aerial parts was determined for the first time and the essential oil demonstrated some larvicidal activity against the mosquito. It showed potential to be developed as a possible natural larvicide for control of the mosquito but needs to be further evaluated for safety in humans and to enhance its activity.

5. Acknowledgments

This work was funded by Special Fund for Agro-scientific Research in the Public Interest (Grant No. 201003058). The authors thank Dr. QR Liu from College of Life Sciences, Beijing Normal University, Beijing 100875, for the identification of the investigated plant.

6. References

1. Wu CY, Li HW. Flora Reipublicae Popularis Sinicae, Vol 65, Science Press, Beijing, 1977, 2, 352-354. http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200023684.
2. Jiangsu New Medical College. Dictionary of Traditional Chinese Materia Medica. Shanghai Science and Technology Press, 1977, 115-117.
3. Yun HS, Chang IM. Separation and identification of cirsimarin from *Cirsium pendulum* Fisch. Saengyak Hakhoechi 1978; 9:145-147.
4. Syrchina AI, Semenov AA, Zinchenko SV. Chemical investigation of *Cirsium setosum*. Chem Nat Compds 1997; 33: 212.
5. Chen GL, Li XL, Shi LG, Zhang SJ. Chemical constituents from *Cirsium pendulum* Fisch. ex DC. J Chin Med Mater 2007; 30: 291-294 (in Chinese with English abstract).
6. Jeong DM, Jung HA, Choi JS. Comparative antioxidant activity and HPLC profiles of some selected Korean thistles. Arch Pharm Res 2008; 31:28-33.
7. Thao NTP, Cuong TD, Hung TM, Lee JH, Na MK, Son JK *et al*. Simultaneous determination of bioactive flavonoids in some selected Korean thistles by high-performance liquid chromatography. Arch Pharm Res 2011; 34:455-461.
8. Han BC, Li N, Li X. Isolation and identification of chemical constituents from Cirsium setosum (Willd.) MB. J Shenyang Pharm Univ 2008; 25:793-795.
9. Ke R, Zhu EY, Chou GX. A new phenylpropanoid glycoside from *Cirsium setosum*. Acta Pharm Sin 2010; 45:879-882.
10. Li LL, Sun Z, Shang XY, Li JJ, Wang R, Zhu J *et al*. Triterpene compounds from *Cirsium setosum*. China J Chin Materia Med 2012; 37:951-955.
11. Sun Q, Chang L, Ren YP, Cao L, Sun YG, Du YF *et al*. Simultaneous analysis of 11 main active components in *Cirsium setosum* based on HPLC-ESI-MS/MS and combined with statistical methods. J Separation Sci 2012; 35:2897-2907.
12. Jiang H, Meng YH, Yang L, Wang QH, Yang BY, Liu C *et al*. A new megastigmane glycoside from the aerial parts of *Cirsium setosum*. Chin J Nat Med 2013; 11:534-537.
13. Yuan ZZ, Duan HM, Xu YY, Wang A, Gan LS, Li JJ *et al*. α-Tocospiro C, a novel cytotoxic α-tocopheroid from Cirsium setosum. Phytochem Lett 2014; 8:116-120.
14. Liu ZL, Liu QZ, Du SS, Deng ZW. Mosquito larvicidal activity of alkaloids and limonoids derived from *Evodia rutaecarpa* unripe fruits against *Aedes albopictus* (Diptera: Culicidae). Parasitol Res 2012; 111:991-996.
15. Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 2006; 51:45-66.
16. Regnault-Roger C, Vincent C, Arnason JT. Essential oils in insect control: Low-risk products in a high-stakes world. Annu Rev Entomol 2012; 57:405-424.
17. Chen XB, Liu XC, Zhou L, Liu ZL. Essential oil composition and larvicidal activity of *Clinopodium gracile* (Benth.) Matsum (Labiatae) aerial parts against the *Aedes albopictus* mosquito. Trop J Pharm Res 2013; 12:799-804.

18. Liu XC, Dong HW, Zhou L, Du SS, Liu ZL. Essential oil composition and larvicidal activity of *Toddalia asiatica* roots against the mosquito *Aedes albopictus* (Diptera: Culicidae). Parasitol Res 2012; 112:1197-1203.
19. Liu ZL, He Q, Chu SS, Wang CF, Du SS, Deng ZW *et al*. Essential oil composition and larvicidal activity of *Saussurea lappa* roots against the mosquito *Aedes albopictus* (Diptera: Culicidae). Parasitol Res 2012; 110:2125-2130.
20. Liu XC, Liu QR, Zhou L, Liu QR, Liu ZL. Chemical composition of *Zanthoxylum avicennae* essential oil and its larvicidal activity on *Aedes albopictus* Skuse. Trop J Pharm Res 2014; 13:399-404.
21. He Q, Liu XC, Sun RQ, Deng ZW, Du SS, Liu ZL *et al*. Mosquito larvicidal constituents from the ethanol extract of *Inula racemosa* Hook.f. Roots against *Aedes albopictus*. J Chem 2014.
22. Liu XC, Liu QY, Zhou L, Liu ZL. Evaluation of larvicidal activity of the essential oil of *Allium macrostemon* Bunge and its selected major constituent compounds against *Aedes albopictus* (Diptera: Culicidae). Parasite Vector 2014; 7:184.
23. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy; Allured: Carol Stream, IL, USA. 2007.
24. World Health Organization. Instruction for determining the susceptibility or resistance of mosquito larvae to insecticide 1981.
25. Finney DJ. Probit analysis. Edn 3, Cambridge University, London, UK, 1971.
26. Sakuma M. Probit analysis of preference data. Appl Entomol Zool 1998; 33:339-347.
27. Nazaruk J, Gora J, Majda T, Gudej J. Investigation of essential oil from *Cirsium rivulare* (Jacq.) All flowers Herba Polonica 2002; 48:193-197.
28. Miyazawa M, Yamafuji C, Kurose K, Ishikawa Y. Volatile components of the rhizomes of *Cirsium japonicum* DC. Flav Fragr J 2003; 18:15-17.
29. Formisano C, Rigano D, Senatore F, De Feo V, Bruno M, Rosselli S *et al*. Composition and allelopathic effect of essential oils of two thistles: *Cirsium creticum* (Lam.) D.'Urv. ssp. *triumfetti* (Lacaita) Werner and *Carduus nutans* L. J Plant Interactions 2007; 2: 115-120.
30. Nazaruk J, Karna E, Kalemba D. The chemical composition of the essential oils of *Cirsium palustre* and *C. rivulare* and their antiproliferative effect. Nat Prod Commun 2012; 7:269-272.
31. Mathew J, Thoppil JE. Chemical composition and mosquito larvicidal activities of *Salvia* essential oils. Pharm Biol (London) 2011; 49:456-463.
32. Cheng SS, Chua MT, Chang EH, Huang CG, Chen WJ, Chang ST. Variations in insecticidal activity and chemical compositions of leaf essential oils from *Cryptomeria japonica* at different ages. Bioresour Technol 2009; 100: 465-470.
33. Cheng SS, Huang CG, Chen YJ, Yu JJ, Chen WJ, Chang ST *et al*. Chemical compositions and larvicidal activities of leaf essential oils from two eucalyptus species. Bioresour Technol 2009; 100:452-456.
34. Conti B, Canale A, Bertoli A, Gozzini F, Pistelli L. Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedes albopictus* (Diptera: Culicidae). Parasitol Res 2010; 107:1455-1461.
35. Dayrit FM, Trono CM, Morallo-Rejesus B, Maini H. Anti-pest compounds from the volatile oil of *Vitex negundo* L. Philippine J Sci 1995; 124:15-27.
36. Chu SS, Jiang GH, Liu ZL. Insecticidal compounds from the essential oil of Chinese medicinal herb, *Atractylodes chinensis*. Pest Manag Sci 2011; 67:1253-1257.
37. Doria GAA, Silva WJ, Carvalho GA, Alves PB, Cavalcanti SCH. A study of the larvicidal activity of two Croton species from northeastern Brazil against *Aedes aegypti*. Pharm Biol (London) 2010; 48:615-620.
38. Liu ZL, Du SS. Fumigant components from the essential oil of Evodia rutaecarpa Hort unripe fruits. E-J Chem 2011; 8:1937-1943.
39. Liu ZL, Jiang GH, Zhou L, Liu QZ. Analysis of the essential oil of *Dipsacus japonicus* flowering aerial parts and its insecticidal activity against *Sitophilus zeamais and Tribolium castaneum*. Z Naturforsch 2013; 68C:13-18.
40. Klocke JA, Darlington MV, Balandrin MF. Biologically active constituents of North American plants. Part III. 1,8-Cineole (eucalyptol), a mosquito feeding and ovipositional repellent from volatile oil of *Hemizonia fitchii* (Asteraceae). J Chem Ecol 1987; 13:2131-2141.
41. Araujo ECC, Silveira ER, Lima MAS, Neto MA, Andrade ILD, Lima MAA *et al*. Insecticidal activity and chemical composition of volatile oils from *Hyptis martiusii* Benth. J Agric Food Chem 2003; 51:3760-3762.
42. Lima MAA, De-Oliveira FFM, Gomes GA, Lavor PL, Santiago GMP, Nagao-Dias AT *et al*. Evaluation of larvicidal activity of the essential oils of plants species from Brazil against *Aedes aegypti* (Diptera: Culicidae). Afr J Biotechnol 2011; 10:11716-11720.
43. Santos SRL, Silva VB, Melo MA, Barbosa JDF, Santos RLC, de Sousa DP *et al*. Toxic effects on and structure-toxicity relationships of phenylpropanoids, terpenes, and related compounds in *Aedes aegypti* larvae. Vector Borne and Zoonotic Diseases (Larchmont, N.Y.) 2010; 10:1049-1054.
44. Koliopoulos G, Pitarokili D, Kioulos E, Michaelakis A, Tzakou O. Chemical composition and larvicidal evaluation of Mentha, Salvia, and Melissa essential oils against the West Nile virus mosquito *Culex pipiens*. Parasitol Res 2010; 107:327-335.