Research Journal of Pharmacology 2 (2): 17-19, 2008
ISSN: 1815-9362

Essential Oil Composition and Biological Activity of *Ziziphora clinopodioides* Lam. from Iran

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Abstract: The chemical composition of the essential oil obtained by hydrodistillation from the aerial parts of *Ziziphora clinopodioides* Lam. growing in Iran was analysed by GC and GC/MS. Twenty-six components accounting to 97.62% of the total oil were identified. The major components were pulegone (36.45%), piperitenone (19.12%), Menth-2-en-1-ol (5.31%), carvacrol (5.10%), neomenthol (4.78) and menthone (4.46%). The essential oil was tested against *Anopheles stephensi* and *Culex pipiens* larvae. The results obtained show that the essential oil could be considered as natural larvicidal agents.

Key words: *Ziziphora clinopodioides* Lam., essential oil, GC/MS, biological activity

INTRODUCTION

The genus *Ziziphora L.* belongs to the family Labiatae consists of four species (*Z. clinopodioides* Lam.., *Z. capitata* L., *Z. persica* BUNGE. and *Z. tenuior* L.) that widespread all over Iran. *Z. clinopodioides* Lam. with the common Persian name "*kakuti-e kuhi*" is an endemic species, grows wild in Iran and also Afghanistan, Iraq and Talish. *Ziziphora clinopodioides* Lam. is an edible medicinal plant that leaves, flowers and stems are frequently used as wild vegetable or additive in foods to offer aroma and flavor (Zargari,1995). In Iranian and Turkish folk medicine, *Ziziphora* species have been used as infusion for various purposes such as sedative, stomachache and carminative. In Iranian folklore, the dried aerial parts of this plant have been frequently used as culinary and also in cold and cough treatments. *Ziziphora* species has been also used to treat various ailments such as antiseptic and wound healing (Ozturk *et al*., 1995).

A literature survey showed that the oil of *Ziziphora* species has been found to be rich in pulegone. The main constituents found in the oil of *Z. vychodceviana* and *Z. persica* collected from Kazakhestan were pulegone (57.5-66%) and isomenthone (5.1-15.7%) (Dembistikii *et al*., 1995). The major constituent found in the oil of *Z. tenuior* L. has been reported to be pulegone (87.1%) (Sezik *et al*., 1991). The essential oil of Turkish endemic *Z. taurica* subsp. *clenioides* was found to contain pulegone (81.9%), limonene (4.5%) and piperitenone (2.3%) (Meral *et al*., 2002). The chemical composition of the essential oil of *Z. clinopodioides* from Turkey has been studied by GLC (Belyaev and Demeubaeva, 1999).

In the present study we report the larvicidal activity of the essential oil extracted from the aerial parts of *Z. clinopodioides* subsp. *rigida* against two species of mosquito vectors, *A. stephensi* and *C. pipiens*. The results of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive chemical compounds from indigenous plant source.

MATERIALS AND METHODS

Plant material: The aerial parts of *Z. clinopodioides* subsp. *rigida* were collected during its flowering stage in July 2005 from the north of Iran and identified. A voucher specimen was deposited in the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences.

Mosquitoes: The third instar larvae of *A. stephensi* and *C. pipiens* were obtained from laboratory bred culture maintained at ambient rearing conditions. All the bioassays were conducted at 26±1°C, 60.0±5% RH and 12 h light and 12 h dark photoperiod. Yeast suspension (5%) was used as food source.

Isolation of the essential oil: Air-dried plant material (100 g) was hydrodistilled for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous Na_2SO_4 and then was kept in a sealed vial at 4°C until analysis.

Gas Chromatography/Mass Spectrometry (GC-MS): The analyses of the volatile compounds were carried out on a Hewlet Packard GC/MS system (GC 5890 Series II; MSD 5971A). The fused silica HP-20 M polyethylene glycol column (50 m×0.2 mm, 0.2 μm film thickness) was directly coupled to the mass spectrometer. The carrier gas was helium (1 mL min^{-1}). The programme used was 4 min isothermal at 70°C, then 70 -180°C at a rate of 4°C min^{-1} then held isothermal for 10 min. The injection port temperature was 250°C. The ionization of the sample components was performed in the E.I. mode (70 eV).

Identification of components: The linear retention indices for all the compounds were determined by coinjection of the sample with a solution containing the homologous series of C8-C22 *n*-alkanes (Van Den Dool and Kratz, 1963). The individual constituents were identified by their identical retention indices, referring to known compounds from the literature (Adams, 1995) and also by comparing their mass spectra with either the known compounds or with the Wiley mass spectral database.

Larvicidal bioassay: Bioassays were performed according to the WHO protocol. A series of concentrations ranging from 2-100 μg mL^{-1} of the dissolved oil (in DMSO) was prepared and 5 replicates were run for each concentration. Control tests were carried out in parallel, using DMSO and water for comparison. Malathion, a conventional insecticide was used as positive control sample. The number of dead larvae were counted after 24 h of exposure and the percentage mortality is reported from the average of 5 replicates. Observations were also made on the behaviour of larvae.

Statistical analysis: Probit analysis was conducted on the mortality rate to determine the LC_{50} and LC_{90} representing the concentrations in μg mL^{-1} that caused 50 and 90% mortality along with 95% confidence limits.

RESULTS AND DISCUSSION

The hydrodistillation of aerial parts of *Z. clinopodioides* Lam. gave an oil in 0.9% (w/w) yield, based on the dry weight of the plant. Twenty-six components were identified representing 97.62% of the total oil. The qualitative and quantitative essential oil compositions are presented in Table 1, where compounds are listed in order of their elution on the DB-1 column. The major constituents of the oil were pulegone (36.45%), piperitenone (19.12%), Menth-2-en-1-ol (5.31%), carvacrol (5.10%), neomenthol (4.78) and menthone (4.46%). The result of this research is in accordance with other earlier studies on *Ziziphora* species that all found to be rich in pulegone (Dembistikii *et al*., 1995; Sezik *et al*., 1991; Meral *et al*., 2002). Compared to the other *Ziziphora* species, pulegone content of the essential oil of *Z. clinopodioides* Lam. (36.45%) was lower than those of *Z. tenuior* (87.1%), *Z. taurica* subsp. *cleonioides* (81.9%), *Z. persica* (66%) and *Z. vychodceviana* (57.5%) (Ozturk *et al*., 1995) . Piperitenone (19.12%) as the second major oil component of *Z. clinopodioides* Lam. was only found in the oil of *Z. taurica* subsp. *cleonioides* (2.3%), while the other major compound was only reported from *Z. tenuior* essential oil.

The essential oil was subjected to laboratory bioassay studies against *A. stephensi* and *C. pipiens* larvae. The tested essential oil demonstrated significant larvicidal activity on both the vector species. Table 2 summarizes the LC_{50} and LC_{90} values for the essential oil. Pulegone alone did not show promising activity in the dose re-sponse bioassay against any of the test larvae (mortality >50% was observed only at the highest testdose). Malathion (used as positive control) caused 100% mortality against all the larvae at very low test dose (>0.625 mg L^{-1}).

Table 1: The chemical omposition of the essential oil from Z *clinopodioides* Lam.

No.	Components	RI	%
1	β-Pinene	977	0.21
2	1,8-Cineole	1023	3.50
3	γ-Terpinene	1059	1.27
4	Terpinolene	1075	0.21
5	Linalool	1083	0.42
6	Menth-2-en-1-ol	1131	5.31
7	Menthone	1141	4.46
8	Menthofuran	1145	0.21
9	Neomenthol	1149	4.78
10	4- Terpineol	1159	0.42
11	Menthol	1172	3.18
12	Isomenthol	1179	1.11
13	Verbenone	1192	2.28
14	Cuminyl acetate	1196	1.80
15	Pulegone	1218	36.45
16	Isomenthone	1222	0.26
17	Piperitenone	1226	1.70
18	Carvone	1236	0.79
19	Thymol	1265	2.23
20	Carvacrol	1282	5.10
21	Isomenthyl acetate	1296	0.26
22	Piperitone	1310	19.12
23	Eugenol	1324	0.37
24	β-Bourbonene	1382	0.21
25	Germacrene D	1433	1.16
26	Spathulenol	1561	0.63

Table 2: Larvicidal activity of essential oil from *Ziziphora clinopodioides* Lam. against *Anopheles stephensi* and *Culex pipiens*

Species	LC_{50} (μg mL^{-1})	LC_{90} (μg mL^{-1})	Regression equation	RP
A. stephensi	14.9	22.3	y = 3.17*x*-2.69	0.076
C. pipiens	16.5	28.6	y = 3.49*x*-2.83	0.078

All means are statistically significant (p<0.05), Numbers in parenthesis are 95% cl values, RP-Relative potency (LC_{50} standard/LC_{50} test substance)

CONCLUSION

The present study indicated that the essential oil from aerial parts of *Ziziphora clinopodioides* Lam. possessed remarkable larvicidal properties and compared favorably with the commercially available insecticide malathion. The results could be useful in search of newer, safer and more effective natural compounds as larvicides. Further studies are needed to devise a formulation using the oil and the compounds of this plant for use as larvicides in mosquito control programs.

ACKNOWLEDGEMENT

We are grateful to acknowledgment the Faculty of Pharmacy, Tehran University of Medical Sciences, for the financial support of this investigation.

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