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Variation in essential oil composition of *Mentha longifolia* var. *chlorodichtya* Rech.f. and *Ziziphora clinopodiodes* Lam. growing in different habitats

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Essential oils obtained by hydrodistillation of the aerial parts of *Mentha longifolia* (L.) Hudson var. *chlorodictya* Rech. f. collected from two different locations in Iran and three populations of *Ziziphora clinopodiodes* Lam. were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). 98.47 and 96.99% of total oils in *M. longifolia* were identified. Cis-piperitone oxide (36.4 and 40.5%), piperitenone oxide (22.5 and 37.3%) and caryophyllene oxide (13.65 and 7.43%) were found as the major components. In *Z. clinopodiodes*, 99.81, 98.23, and 97.63% of the oils were identified of which P-mentha-3-en-8-ol (7.00, 24.71, and 12.41%), menthol (12.09, 4.24, and 14.54%), pulegone (7.29, 11.44, and 3.90%), piperitone (11.50, 6.53, and 2.58%) and 1,8-cineole (2.74, 4.50, and 12.46%) were found as the main components of the oils, respectively. Interestingly, a high amount of neo-menthol (30.48 and 25.09%) was identified only in the oils of two samples of *Z. clinopodiodes*.

Key words: *Mentha longifolia* var. *chlorodictya*, *Ziziphora clinopodiodes*, essential oil, cis-piperitone oxide, piperitenone oxide, P-mentha-3-en-8-ol, neomenthol.

INTRODUCTION

The Labiatae family is widespread around the world and is rich in essential oil. The members of this family have medical properties and some of them are commercially important products. The Labiatae family has great diversity and distribution in Iran with 46 genera, 410 species and subspecies from which 124 are endemic (Rechinger, 1982; Zargari, 1995). Among this rich array of plants yielding essential oils, the genus *Mentha*, distributed worldwide, is very important, including 20 species with considerable economic values. There are several reports on the essential oil composition of *Mentha* species (Venskutonis, 1996; Kokkini et al., 1998; Hendriks, 1998; Hajiakhoondi et al., 2000; Monfared et al., 2002; Lorenzo et al., 2002; Agnihotri et al., 2005; Stoyanova et al., 2005). *Mentha longifolia* (L.) Hudson, commonly known as "wild mint" is a perennial herb common in wet places with creeping rhizomes. All plant parts are highly aromatic with a typical mint smell. It is widely used in pharmaceutical, perfumery, food, confectionary and cosmetic industries. The aerial part of the plant is used in folk medicine for the treatment of cold, cough, asthma and chest inflammations. It is believed to be a diaphoretic and has spasmolytic effect on the smooth muscle of the digestive tract, hence, useful for cramp-like complaints of the gastro-intestinal tract, gall bladder and the biliary tract. It is also used to treat wounds and glands (Watt

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et al., 1962). Many biological activities such as: antimicrobial (Hendriks et al., 1998; Rasooli and Rezaei, 2002; Viljoen et al., 2006; Eidla et al., 2009; Hajloui et al., 2010), antifungal (Singh et al., 2006) and antioxidant (Dzamic et al., 2010) have also been reported from different species of *Mentha* spp.

The genus *Ziziphora* L. (Labiatae), consists of four species: *Ziziphora clinopodiodes* Lam., *Ziziphora capitata* L., *Ziziphora persica* Bunge and *Ziziphora tenuior* L., distributed in Iran, Iraq, Afghanistan and Turkey. *Z. clinopodiodes* Lam. commonly known as "Kakuti-e kuhi" in Iran is a valuable medicinal plant. *Ziziphora* species have been used as infusion for various purposes such as sedative, stomachache and carminative. It is also used as anti-spasmodic and as antiseptic in wound healing (Ozturk et al., 1995).

A literature survey showed that the oil of *Ziziphora* spp. is rich in pulegone. The main constituents in the oil of *Ziziphora vichodceviana* and *Z. persica* from Kazakhistan were pulegone (57.5 to 66.0%) and isomenthone (5.1 to 15.7%) (Dembistikii et al., 1995). In the oil of *Z. tenuior*, pulegone (87.1%) has been reported (Sezik et al., 1991). The Turkish endemic *Ziziphora taurica* subsp. *clenioides* showed pulegone (81.9%) as the main component in the essential oil (Meral et al., 2002). Pulegone (6.4 to 22.3%), 1,8-cineole (6.3 to 29.6%), and neomenthol (0.9 to 10.8%) were found in the oil of *Z. clinopodiodes* subsp. *rigida* from Iran, as the main compounds (Dehghan et al., 2010). Another report of the same species which was collected from a different habitat in Iran, showed pulegone (45.8%), piperitenone (14.7%) and p-mentha-3-en-8-ol (12.5%) as the major components of the oil (Salehi et al., 2005).

Several biological activities such as antibacterial (Chitsaz et al., 2005; Gozde et al., 2006; Soltani, 2012), antioxidant (Salehi et al., 2005; Meral et al., 2002), antifungal (Khosravi et al., 2011) and also larvicidal activity (Verdian Rizi, 2008) were reported for the essential oil and various extracts of *Ziziphora* species.

In this study, we aim to compare the essential oil composition of *M. longifolia* var .*chlorodictya* and *Z. clinopodiodes* collected from different localities and evaluate the effect of ecological condition on essential oil yield in this species and find out about the existence of different chemotypes in these taxa. Thus, we collected our materials from different geographical locations which have not been the subject for previous works. In this study, we show the dependency of chemical composition of the essential oils to the environmental conditions and habitats that these species grow.

MATERIALS AND METHODS

Plant samples

The aerial parts of *M. longifolia* were collected from Dailaman and Namak-dareh (two high altitude localities in Gilan and Mazandaran Provinces, North of Iran), during flowering stage in June 2010. Three populations of *Z. clinopodiodes* were collected from Malard, Dizin and Garmabdareh in Tehran province, Iran in July 2010. The samples were authenticated and voucher specimens were deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

Isolation of the volatile oils

The aerial parts of the plants were dried in the dark. Then 79,145 g of Dailaman and Namak-Dareh specimens, also 65, 93, and 96 g of the Malard, Dizin and Garmabdareh specimens, were separately hydrodistilled using a Clevenger-type apparatus for 3 h. Finally, the oils were dried over anhydrous sodium sulfate.

Gas chromatography (GC)

GC analysis was performed on a Shimadzu GC-15A equipped with a split/splitless injector (250°C). N_2 was used as carrier gas (1 ml/min). The DB-5 (50 m × 0.2 mm, film thickness 0.32 µm) capillary column was used. The column temperature was kept at 60°C for 3 min. and then heated to 220°C with a 5°C min^{-1} rate. Relative percentage amount of the constituents was calculated from peak area using a Shimadzu C-R4A Chromatopac without using the correction factors.

Gas chromatography-mass spectroscopy (GC/MS)

GC-MS analysis was performed using a mass selective detector (Agilent 5973) coupled with a gas chromatograph (Agilent 6890), equipped with a capillary column (HP-5MS) (30 × 0.25 mm, film thickness 0.25 µm). The column temperature was kept at 50°C for 5 min and programmed to 240°C at a rate of 3°C min^{-1}, and then the temperature was increased to 300°C at the rate of 15°C min^{-1} and was kept constant at 300°C for 3 min. The injector temperature was 290°C and the flow rate of helium as carrier gas was 0.8 ml/min. The MS operating parameters were as follows: ionization energy at 70 ev; ion source temperature at 220°C.

Identification of constituents

The constituents of each oil were identified by comparing their retention indices (RRI) relative to n-alkanes, computer matching with the Wiley library, and confirmed by comparing their mass spectra with those of authentic samples or with data already available in the literature (Adams, 2000). The percentage composition of the identified compounds was computed from the GC peak area without any correction factor.

RESULTS AND DISCUSSION

The yields of colorless mint oils isolated from the aerial part of *Mentha* are 0.2% v/w for both Dailaman and Namak-Dareh samples. Seventeen and nineteen compounds were identified in these oils, these accounted for over 97.0 and 98.5% of the total oils composition, respectively. The identified compounds are shown in Table 1, in which the percentage and retention indices are also given.

Both samples showed cis-piperitone oxide (40.51 and 36.41%), piperitenone oxide (37.33 and 22.58%), caryo-

Table 1. Essential oil composition of two specimens of *Mentha longifolia* var. *chlorodictya*.

Compound	RI	A (%)	B (%)
α-Pinene	939	0.31	0.12
β-Pinene	980	0.60	-
3-octanol	993	-	0.76
1,8-cineol	1033	4.74	0.63
3-Octanyl acetate	1124	-	0.38
Dihydro linalool	1134	-	o.26
cis-Piperitone oxide	1268	36.43	40.51
trans-Carvone oxide	1277	0.23	-
Thymol	1290	3.19	3.98
Dihydro carveol acetate	1325	1.93	-
Piperitenone oxide	1363	22.58	37.33
β-Bourbonene	1384	1.07	2.74
trans-Caryophyllene	1418	8.02	0.79
α-Humulene	1454	0.54	-
trans-β-Farnesene	1458	0.81	0.34
Germacrene D	1480	2.31	-
Caryophyllene oxide	1581	13.65	7.43
Humulene epoxide	1606	0.80	0.47
Cedr-8(15)-en-9-α-ol	1644	0.16	0.17
Khusinol	1674	0.88	0.73
2-Pentadecanone,6,10,14trimethyl	1844	0.20	0.18
Phytol	1949	0.20	0.17
Total	-	98.47	96.99

RI: Retention indices relative to n-alkanes; A, B: essential oils of Namak-Dareh and Dailaman specimens.

phyllene oxide (7.43 and 13.65%) and thymol (3.98 and 3.19%) as the major component. Other main components in Namak-Dareh specimen were: trans-caryophyllene (8.02%), 1,8-cineole (4.74%), while in Dailaman specimen, a low amount of these two compounds were identified and germacrene D (2.13%) which was not seen in the oil of Dailaman specimen is present in the oil of Namak-Dareh specimen.

The oil compositions of Dailaman and Namak-Dareh specimens were found to be characterized by monoterpenes (83.59 and 70.01%). Sesquiterpenes were the smaller fraction of both oils (12.84 and 28.26% respectively).

M. longifolia has been the subject of many previous investigations and different chemotypes of the species have been reported (Monfared et al., 2002; Ghoulami et al., 2001; Maffei, 1988). The major compounds identified in our samples have been reported as the main constituent in *M. longifolia* from different countries. Piperitenone oxide was identified in *M. longifolia* from Moraco, Croatia, and Lithuania (Ghoulami et al., 2001; Mastelic and Jerkovic, 2002; Venskutonis, 1996). Cis-piperitone oxide has been identified as the main component in the oil of Croatia and South Africa specimens (Mastelic and Jerkovic, 2002; Viljoen et al., 2006). Caryophyllene oxide, another main component of our samples has also been reported previously (Mastelic and Jerkovic, 2002).

The chemical constituents of *M. longifolia* var. *chlorodictya* collected from two different habitats in Gorgan (North of Iran) were reported (Mazandarani and Rezaee, 2003). The major constituents for their sample-1 were p-menth-1-en-9-ol (62.1%), β-caryophyllene (6.3%) and carvacrole (4.8%). The major constituents for their sample-2 were p-menth-1-en-9-ol (36.1%), 1,8-cineole (14.4%), piperitone (9.7%), carvacrol (9.3%) and germacrene D (9.1%). Comparing their results with ours show major differences, with the exception of germacrene D and β-caryophyllene, the other main components reported by them were not present in our samples.

In another study on the same taxon from three different localities in Iran (Rezaei et al., 2000) with different percentage of piperitenone oxide (33.91%), isopiperitenone (11.98%) and piperitone (8.40%) in their sample-1; isopiperitenone (57.96%), piperitone oxide (19.99%) and 1,8-cineol (5.49%) in their sample-2; piperitone (43.96%), 1,8-cineol (13.73%), trans-piperitol (12.92%) and cis-piperitol (9.34%) were the main components in their sample-3. Among these major components which were reported by Rezaei et al. (2000), the following compounds: isopiperitenone, piperitone and cis, trans-piperitol, have not been identified in the essential oil composition of our samples.

The results of our work revealed that there are major differences among the compositions of our samples and those reported earlier. It is known that the different chemotypes, based on qualitative differences within a taxon is a common feature in most *Mentha* spp. and hybrids. The differences of major compounds in the previous studies and the present study are shown in Table 3.

The hydrodistillation of aerial parts of three populations of *Z. clinopodiodes* Lam., gave oils in 0.01, 0.01 and 0.02% v/w yield, based on dry weight of the plants. Fifteen, twenty seven and thirty seven compounds were identified in the oils obtained from Malard, Dizin and Garmabdareh specimens, representing 99.81, 98.23 and 97.66% of total compounds. The results are shown in Table 2.

The results show that the essential oils were rich in monoterpenes (83.56, 87.39, and 42.93% in three samples, respectively). The sesquiterpenes were also present in the oil but with less quantity (10.94, 6.21 and 12.88%). Non-terpenoids were low in oil composition (5.31, 4.63 and 1.85% in total oils). Oxygenated compounds were predominated in the oils as well. A high amount of neo-menthol (30.48 and 25.09%) was found in the oils of Malard and Dizin specimens, while it was not seen in the oil obtained from Garmab-Dareh sample. Menthone (7.70%) was another major compound which was found only in the oil of Malard specimen. P-mentha-3-en-8-ol (7.00, 24.71 and 12.41%), menthol (12.09, 4.24 and 14.54%), pulegone (7.29, 11.44 and 3.90%), piperitone

Table 2. Essential oil composition of three populations of *Ziziphora clinopodiodes.*

Compound	RI	A (%)	B (%)	C (%)
α-Thujene	931	-	-	0.32
α-Pinene	939	-	0.46	1.90
Camphene	953	-	0.20	1.42
Sabinene	976	-	-	5.00
β-pinene	980	-	1.17	-
Myrcene	991	-	-	0.62
α-Terpinene	1018	-	0.24	2.08
1,8-Cineole	1033	2.74	4.50	12.46
β-Ocimene	1050	-	-	0.13
γ-Terpinene	1062	-	0.50	3.60
Cis-Sabinene hydrate	1068	-	-	2.00
p-Mentha-3,8-diene	1072	-	1.83	-
α-Terpinolene	1088	-	0.56	1.04
Terpin-1-ol	1134	-	-	0.92
p-Mentha-3-en-8-ol	1149	7.00	24.71	12.41
Menthone	1154	7.70	-	-
Neomenthol	1165	30.48	25.09	-
Menthol	1173	12.09	4.24	14.54
Terpin-4-ol	1177	-	2.17	7.50
Isomenthol	1182	-	-	2.47
trans-Piperitol	1205	-	-	0.32
trans-pulegol	1213	-	1.00	0.18
Isobornyl formate	1233	-	-	0.50
Pulegone	1237	7.29	11.44	3.90
Piperitone	1252	11.55	6.53	2.58
Bornyl acetate	1285	1.15	-	0.44
Isobornyl acetate	1285	-	0.90	4.30
Menthyl acetate	1294	3.56	0.79	2.26
Isomenthyl acetate	1306	-	0.62	-
p- methoxy acetophenone	1348	4.32	3.89	1.62
Eugenol	1356	-	0.41	-
β-Bourbonene	1384	0.97	0.79	1.13
β-Elemene	1391	-	-	0.44
trans-Caryophyllene	1418	-	0.46	1.12
β-Gurjunene	1432	-	-	0.25
trans-β-Farnesene	1458	-	-	0.76
Germacrene D	1480	4.75	2.76	5.10
Bicyclogermacrene	1494	1.17	0.53	1.86
Spathulenol	1576	4.05	1.08	1.66
α-Cadinol	1653	-	-	0.27
Khusinol	1674	-	0.42	0.33
Bisabolone	1744	-	0.23	-
6,10,14-trimethyl-2-penthadecanone	1847	-	0.42	0.11
Dibuthyl phtalate	1922	0.99	0.32	0.12
total	-	99.81	98.23	97.66

RI: Retention indices relative to n-alkanes; A,B,C; essential oils of Malard, Dizin, and Garmabdareh specimens.

(11.50, 6.53 and 2.58%) and 1,8-cineole (2.74, 4.50 and 12.46%) were found as the major components in all three oils, respectively.

There are some reports on the essential oil composition of *Z. clinopodiodes* collected from different areas. The essential oil composition of *Z. clinopodiodes* collected from two different locations of Iran was reported by Aghajani et al. (2008). Their results showed thymol (53.6%), p-cymene (10.5%), carvacrol (8.7%), γ-terpinene (6.7%) and 1,8-cineole (5.4%) in their sample-1. In the oil of their sample-2, 1,8-cineole (21.6%), terpinene-4-ol (18.2%), linalool (7.9%), pulegone (7.7%) and α-terpineol (5.3%) were identified as the main compounds. Based on the reports from *Z. clinopodiodes*, it is understood that the characteristic compound of the oil from *Z. clinopodiodes* is pulegone, but not thymol and carvacrol, the compounds that are characteristic of different genera, that is, *Thymus* and *Origanum.* Regarding to morphological characteristics of *Thymus* and their similarities to *Z. clinopodiodes*, it is probable that the results for their sample-1 probably belong to a *Thymus* species and it should be a misidentification of the plant material. Another report of the same species collected from North of Iran, showed pulegone (36.45%), piperitenone (19.12%), menth-2-en-1-ol (5.31%), carvacrol (5.10%) and neo-menthol (4.46%) as the main components (Verdian-Rizi, 2008).

The chemical composition of *Z. clinopodiodes* collected from Lar (an area around Tehran), Iran, have been investigated (Chitsaz et al., 2005). Pulegone (29.28%), P-mentha-3-en-8-ol (19.2%), neo-menthol (11.58%) and piperitenone (9.43%) have been identified as the main components. In the other report of the same plant collected from Khoram-Abad in Lorestan province, West Iran, the influence of growth phase on the essential oil composition was studied and the main components found were pulegone, thymol, p-mentha-3-en-8-ol, piperitenone and iso-menthone with some differences in chemical composition and percentage of components (Amiri, 2009). Pulegone (34.4%), piperitenone (15.1%), I,8-cineole (6.5%) and neo-menthol (5.7%) were the main components of the oil in *Z. clinopodiodes* collected from Baft in Kerman province, South Iran, (Soltani, 2012).

Among these compounds, pulegone, γ-terpinene, 1,8-cineole, terpine-4-ol, p-mentha-3-en-8-ol and neo-menthol were also identified in our samples, but with different percentages. There are some differences among our samples and those reported earlier. *Z. clinopodiodes* is a perennial species with high morphological variations that has been the reason for describing several varieties and even species in its complex (Rechinger, 1982). The differences of major compounds in the previous studies and the present study are presented in Table 4.

Regarding to the existing morphological variation which could be the effects of ecological conditions, it is probable that the studied specimens belong to different varieties and consequently their essential oils composition have been different. However, environmental conditions such as geographical distribution, habitat conditions, collecting

Table 3. Comparison of major compounds in the essential oil of *Mentha longifolia* var. *chlorodichtya* in the previous studies and the present study.

Compound	A-1 (%)	A-2 (%)	B-1 (%)	B-2 (%)	C-1 (%)	C-2 (%)	C-3 (%)
Cis-piperitone oxide	36.43	40.51	-	-	-	-	-
Piperitenone oxide	22.58	37.33	-	-	33.91	19.99	0.31
Caryophyllene oxide	13.65	7.43	-	-	-	-	-
Trans- caryophyllene	8.02	0.79	6.3	-	-	-	-
1,8-cineole	4.74	0.63	-	14.4	2.36	5.49	13.73
Thymol	3.19	3.98	-	-	-	-	-
p-menth-1-en-9-ol	-	-	62.1	36.1	-	-	-
Carvacrol	-	-	4.8	9.3	-	-	-
Piperitone	-	-	-	9.7	8.40	-	43.96
Germacrene D	2.31	-	-	9.1	2.59	2.04	2.40
Iso- piperitenone	-	-	-	-	11.98	57.96	0.95
Trans- piperitol	-	-	-	-	4.23	0.23	12.92
Cis- piperitol	-	-	-	-	0.02	0.03	9.34

A -1, A-2: Two samples of our study; B-1, B-2: Mazandarani et al. (2003); C-1, C-2, C-3: Reza et al. (2000).

Table 4. Comparison of major compounds in the essential oil of *Ziziphora clinopodiodes* in previous studies and the present study.

Compound	A-1 (%)	A-2 (%)	A-3 (%)	B-1 (%)	B-2 (%)	C (%)	D (%)	E (%)	F-1 (%)	F-2 (%)	F-3 (%)
P-Mentha-3-en-8-ol	7.00	24.71	12.41	-	-	-	19.12	-	12.9	10.5	11.0
Neo-menthol	30.48	25.09	-	-	-	4.46	11.58	5.7	-	-	-
Menthol	12.09	4.24	14.54	-	-	-	1.06	34.4	-	-	-
Pulegone	7.29	11.44	3.90	-	7.7	36.45	29.28	-	30.1	44.6	41.3
Piperitenone	11.54	6.53	2.58	-	-	-	2.43	-	-	-	-
1,8-cineol	2.74	4.50	12.46	5.4	21.6	-	4.48	6.5	-	10.4	-
Terpin-4-ol	-	2.17	7.50	-	18.2	-	-	-	-	-	-
Thymol	-	-	-	53.6	-	-	-	-	21.3	6.7	5.8
p-cymene	-	-	-	10.5	-	-	-	-		-	-
Carvacrol	-	-	-	8.7	-	5.10	-	-		-	-
Γ-terpinene	-	0.50	3.60	6.7	-	-	-	-	-	-	-
Linalool	-	-	-	-	7.9	-	-	-	-	-	-
α-terpineol	-	-	-	-	5.3	-	-	-	-	-	-
Piperitenone	-	-	-	-	-	19.12	9.43	15.1	9.3	8.7	-
Menth-2-en-1-ol	-	-	-	-	-	5.31	-	-	-	-	-
Iso-menthone	-	-	-	-	-	-	-	-	-	-	11.6
p-mentha-3,8-diene	-	-	-	-	-	-	-	-	-	-	7.2

A-1, A-2, A-3: Three samples of our study; B-1, B-2: Aghajani et al. (2008); C: Verdian Rizi et al. (2008); D: Chitsaz et al. (2005); E: Soltani Nejad et al. (2012); F-1, F-2, F-3: Amiri et al. 2(009).

time, drying conditions, mode of distillation and climate factors are known to have significant influence on the essential oil composition of the plants.

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