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Chemical constituents of essential oil of *Dracocephalum moldavica* L. and *Dracocephalum kotschyi* Boiss. from Iran

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ABSTRACT

Dracocephalum moldavica L. and *Dracocephalum kotschyi* Boiss. are aromatic plants belonging to Lamiaceae family. The aim of this study was to identify the chemical components of *D. kotschyi* and *D. moldavica* from Iran. The aerial parts of *D. kotschyi* were collected from (Kamu Mountain) Isfahan province and the aerial parts of *D. moldavica* were collected from Sari (Mazandaran province) North of Iran, during 2014. The essential oil was extracted by a Clevenger approach and analyzed using GC/MS. In total, 32 and 24 compounds were identified in the essential oil from the aerial parts *D. kotschyi* and *D. moldavica*, respectively. The results obtained in our study indicated that the major components in the oil *D. kotschyi* were limonene (23.56 %), carvacrol (14.65 %), γ-terpinene (12.99 %), α -pinene (12.62 %), 2-methyl-1-octen-3-yne (9.73 %), camphene (4.66 %), myrcene (3.65 %) and α -terpinene (3.12 %). The major constituents of the oil *D. moldavica* were geranyl acetate (36.62 %), geraniol (24.31 %), neral (16.25 %) and geranial (11.21 %). *D. kotschyi* is one of the important sources of limonene and *D. moldavica* is one of the important sources of geranyl acetate.

Key words: *Dracocephalum moldavica* L., *Dracocephalum kotschyi* Boiss., chemical constitutes of essential oils

IZVLEČEK

KEMIJSKA SESTAVA ETERIČNIH OLJ V DVEH VRSTAH KAČJEGLAVKE (*Dracocephalum moldavica* L., *Dracocephalum kotschyi* Boiss.) IZ IRANA

Vrsti kačjeglavk *Dracocephalum moldavica* L. in *Dracocephalum kotschyi* Boiss.sta aromatični rastlini iz družine ustnatic (Lamiaceae**).** V raziskavi je bila preučevana kemijska sestava obeh vrst iz Irana. Nadzemni deli vrste *D. kotschyi* so bili nabrani na gori Kamu v provinci Isfahan, nadzemni deli vrste *D. moldavica* pa v Sariju, provinci Mazandaran, v severnem Iranu, v sezoni 2014. Eterična olja so bila ekstrahirana po Clevengerjem postopku in analizirana z GC/MS. Celukupno so v nadzemnih delih vrst določili 32, oziroma 24 sestavin eterečnih olj za vrsti *D. kotschyi* in *D. moldavica*. Rezultati te raziskave so pokazali, da so glavne sestavine eteričnih olj pri vrsti *D. kotschyi* limonen (23.56 %), karvakrol (14.65 %), γ-terpinen (12.99 %), α -pinen (12.62 %), 2-metil-1-okten-3-ine (9.73 %), kamfene (4.66 %), mircen (3.65 %) in α -terpinene (3.12 %). Glavne sestavine eteričnega olja vrste *D. moldavica* so bile geranil acetat (36.62 %), geraniol (24.31 %), neral (16.25 %) in geranial (11.21 %). Vrsta *D. kotschyi* je eden izmed pomembnih virov limonene, vrsta *D. moldavica* pa geranil acetata.

Ključne besede: *Dracocephalum moldavica* L., *Dracocephalum kotschyi* Boiss., sestava eteričnih olj

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1 INTRODUCTION

Dracocephalum moldavica L. and *Dracocephalum kotschyi* Boiss. are aromatic plants belonging to Lamiaceae family. *D. moldavica* known as Moldavian balm or Moldavian dragonhead is an annual herb and it is native to central Asia and is naturalized in eastern and central Europe. There is 45 species of herbaceous and shrub's dragonhead in the world and there is 8 species of annual and perennial fragrant herb in Iran, from which three are endemic to Iran (Rechinger, 1986; Mozaffarian, 2008). *D. kotschyi* is an herbaceous plant, endemic in Iran and known as Badrandjboie-Dennaie and Zarrin-Giah. Its oil has been used in folk medicine as an antispasmodic agent. Aerial parts of *D. kotschyi* plants are sources of valuable flavonoids and essential oils (Fattahi et al., 2013). Chromosome number of 2n=2x=10 and 2n=2x=20 are for *D. moldavica* and *D. kotschyi*, respectively (Salehi et al., 2014). *Dracocephalum* is used in folk medicine as painkiller and for treatments of kidney complaints, against toothache and colds as well as antirheumatism, antitumor (Chachoyan and Oganesyan, 1996), antimutagens, antioxidant, antiseptic and stimulant properties (Kakasy et al., 2006; Dastmalchi et al., 2007). Recent findings indicated that some of the medicinal plant characteristics can be affected by genetic and ecological factors, including precipitation, temperature and plant competition. Since essential oils are the product of a predominantly biological process further studies are needed to evaluate if the reported characteristics of each population are maintained at the level of individual plants and along the breeding and selection program when grown under climatic conditions (Ghasemi Pirbalouti and Mohammadi, 2013). Maham et al., (2013) reported the major components of *D. moldavica* essential oil collected from Maragheh of East Azerbaijan province as follows: citral (31.14 %), 3,7- dimethyl -2,6 octadienal (21.43 %), cis-geraniol (17.08 %), neral (9.63 %) and neryl acetate (4.03 %). The major constituents of the essential oil of *D. moldavica* collected from North Iran were limonene (19.8 %), α-pinene (14.4 %), methyl geranate (8.5 %), geranyl acetate (7.9 %), carvacrol (7.8 %) and geranial (5.4 %) (Morteza-Semnani et al., 2007). In studies Saeidnia et al., (2014) reported the folowing main components of the oil of *D. kotschyi* collected from Iran: geranial (37.2 %), limonene-10-al (28.5 %), limonene (20.1 %) and 1,1-dimethoxy decane (14.5 %). Javidnia et al. (2005) reported the main components of the oil of *D. kotschyi* as α-pinene, caryophyllene oxide, terpinen-4-ol and germacrene. Golshani et al., (2004), and also Yaghmai and Tafazzoli (1988) reported citral, myrcene, β-caryophyllene and terpinyl acetate as the main constituents of *D. kotschyi* from northeast mountains. The aim of this study was to identify of the chemical components of *Dracocephalum moldavica* L and *Dracocephalum kotschyi* Boiss from Iran.

2 MATERIALS AND METHODS

2.1 Plant material

The aerial parts of the plant samples of *Dracocephalum kotschyi* Boiss were collected from Kamu Mountain, Isfahan province. Kamu is a city in Qamsar district, Kashan County, Isfahan province, in center Iran (33°, 36′ N and 51°, 14′ E) and the aerial parts of the plant samples of *Dracocephalum moldavica* L. were collected from Sari (Mazandaran province), North of Iran (36°, 39′ N, and 53°, 4′ E) , during 2014. The samples of the plants were identified by regional floras and authors with floristic and taxonomic references, and voucher specimens were deposited at the Herbarium of Agriculture Researches Islamic Azad University, Isfahan (Khorasgan), Iran.

2.2 Essential oil extraction

The fresh aerial part of *D. kotschyi* and *D. moldavica* were dried inside for six days at room temperature (25 ± 5 °C), and ground to fine powder using Moulinex food processor. The essential oil was extracted from 50 g of ground tissue in 1 L of water contained in a 2 L flask and heated by heating jacket at 100 °C for 3 h in a Clevenger–type apparatus, according to producers outlined British Pharmacopoeia. The collected essential oil

was dried over anhydrous sodium sulphate and stored at 4 °C until analyzed.

2.3 GC/MS analysis

Compositions of the essential oils were determined by GC–MS. The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. HP-5MS column (30 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas with flow rate of 1.0 mL/min. The oven temperature was kept 20 °C at 50 °C for 4 min and programmed to 280 °C at a rate of 5 °C /min, and kept 20 °C constant at 280 °C for 5 min, at split mode. The injector temperature was at 20 °C at 280 °C. Transfer 20 line temperatures 280 °C. MS were taken at 70 eV. Mass range was from *m/z* 35 to 450. Identification of the essential oil components was accomplished based on comparison of retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (Adams 2007).

3 RESULTS AND DISCUSSION

3.1 Compositions of the essential oils

Qualitative and quantitative analysis of the essential oils volatile profile are listed in Table 1. In total, 32 and 24 compounds were identified in the essential oil from the aerial parts *D. kotschyi* and *D. moldavica*, respectively. The results obtained in our study indicated that the major components in the oil *D. kotschyi* were limonene (23.56 %), carvacrol (14.65 %), γ-terpinene (12.99 %), α -pinene (12.62 %), 2-methyl-1-octen-3-yne (9.73 %), camphene (4.66 %), myrcene (3.65 %) and α -terpinene (3.12 %) (Figure 1). The major constituents of the oil *D. moldavica* were geranyl acetate (36.62 %), geraniol (24.31 %), neral (16.25 %) and geranial (11.21 %) (Figure 2).

Structural identification of a number of monoterpene synthases has indicated that they all have similar properties (like molecular mass, a divalent metal ion and neutral pH optimum requirements). Interestingly, a terpene synthase is able to form multiple products (Rajaonarivony et al., 1992; Bohlmann et al., 1998), the pinene synthases (from sage and grand fir) can catalyze the production of both α- and β-pinene (Bohlmann et al., 1997).

The biosynthesis of secondary metabolites, although controlled genetically, is strongly affected by the environmental influences of a particular growing region, and also by the agronomic conditions, harvesting time and the type of processing. In addition, for maximum oil production, long days and high light intensities are required during the maturation period (Thompson, 2003; Golparvar et al., 2015).

For example, Hashemian Ahmadi and Hadipanah (2014) reported that the highest oil content (0.065 %) of *D. moldavica* is obtained at the first sowing date (June 12) and the highest oil content (0.058 %) was obtained at the 30 cm planting density. Davazdahemami (2008) showed that of five major components neral, geraniol, geranial, neryl acetate and geranyl acetate in oil of *D. moldavica* were 92 % and 64 % in spring and summer sowing date and maximum change was seen in geranyl acetate from 35.3 % in spring to 14 % in summer. In studies of Omidbaigi (2010), the highest amount of geranyl acetate (50.10 %), geranial (25.27 %), neral (19.34 %) and geraniol (28.80 %), were obtained from the plants sown on 5th of May, 5th June and 20th of March, respectively. Alaei and Mahna (2013) showed that thirty six and twenty one components were identified from *D. moldavica* in field and greenhouse conditions, respectively. The major constituents of the oil of *D. moldavica* were found as geranyl acetate (46.72 %), geraniol (15.87 %), geranial (8.36 %), neral (5.8 %), cedroxyde (3.39 %), neryl acetate (2.57 %) and hinesol (2.39 %) (totally, 88.49 %) in field condition and geranyl acetate (39 %), geraniol (27.30 %), methyl citronellate (12.92 %) and neral (9.32 %) in greenhouse condition. Aziz et al. (2010) stated that the essential oil of the dragonhead plant grown in newly reclaimed land in Egypt was generally characterized by a high percentage of oxygenated compounds and the major constituents under all agricultural sulfur and ammonium sulfate treatments were geraniol (29.11–42.56 %), geranial (14.08–30.94 %), geranyl acetate (15.08–23.51 %) and neral (10.96–15.35 %).

In the study of Abd-El-Baky and El-Baroty (2007) on the dragonhead they found that 44 combination of essential oils was obtained which consist 97.18 % of essential oil and 90 % of them was combined with oxygenated monoterpenes and consisted less than one percent of the weight of the plant which include compounds such as: gerannyl acetate, neryl acetate, geranial, geraniol, neral, nerol, linalool.

Table 1: Chemical compositions of essential oils of *Dracocephalum moldavica* L. and *Dracocephalum kotschyi* Boiss

NO	Compound	RI	*D. kotschyi* %	*D. moldavica* %
1	α-thujene	923	1.71	-
2	α - pinene	928	12.62	-
3	Camphene	944	4.66	-
4	Sabinene	974	1.83	0.42
5	β-pinene	978	-	0.86
6	Myrcene	992	3.65	0.04
7	α -phellandrene	1008	0.31	-
8	α -Terpinene	1016	3.12	-
9	*p*-Cymene	1021	-	0.92
10	limonene	1035	23.56	1.35
11	Cis-β-ocimene	1041	1.03	-
12	γ-Terpinene	1056	12.99	0.17
13	Trans-sabinene hydrate	1066	0.81	-
14	Linalool oxide	1076	-	0.64
15	α -Terpinolene	1091	0.26	-
16	Linalool	1101	-	0.81
17	Cis-sabinene hydrate	1104	0.21	-
18	E,Z-alloocimene	1137	0.57	-
19	Camphor	1149	0.22	-
20	Cis chrysanthenol	1162	-	0.48
21	Borneol	1170	2.22	-
22	R-terpinen-4-ol	1180	0.44	-
23	α -Terpineol	1193	0.13	0.36
24	3-Methylene-1,5,5-trimethylcyclohexene	1198	0.21	-
25	2-Methyl-1-octen-3-yne	1206	9.73	-
26	Nerol	1221	-	0.35
27	Neral	1236	0.83	16.25
28	Carvone	1241	-	1.14
29	Geraniol	1257	0.81	24.31
30	Geranial	1270	-	11.21
31	Bornyl acetate	1287	0.41	-
32	Thymol	1300	0.23	1.41
33	Carvacrol	1311	14.65	-
34	Neryl acetate	1360	-	0.91
35	Geranyl acetate	1379	-	36.62
36	α -copaene	1378	0.54	0.12
37	β-bourbonene	1387	0.61	-
38	Beta- elemene	1392	-	0.15
39	2-Cyclohexen-1-ol, 2-methyl-5-(1-m ethylethenyl)	1415	0.14	-
40	β-caryophyllene	1422	0.95	0.51
41	γ -muurolene	1479	0.11	-
42	Germacrene-D	1482	-	0.47
43	γ -cadinene	1517	0.21	-
44	delta-cadinene	1526	0.17	-
45	Caryophyllene oxide	1580	-	0.17
46	Viridiflorol	1590	-	0.02
	Total		99.9	99.6

RI: Retention indices determined on HP-5MS capillary column.

Terpene synthases have been cloned from different species and also the phylogenetic distances among them have been well documented. *D. moldavica* contains 0.06–0.92 % essential oil, with the maximal level during flowering. Its lemon-like scented essential oil consists mainly of oxygenated acyclic monoterpenes, geraniol, geranyl acetate, geranial, neral and nerylacetate (Kakasy et al., 2006).

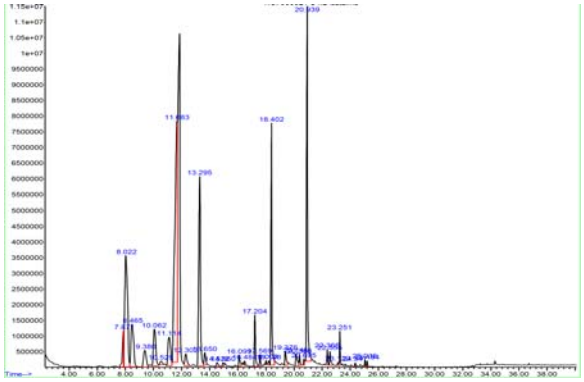

Figure 1: The chromatograms found in essential oils of *D. kotschyi.*

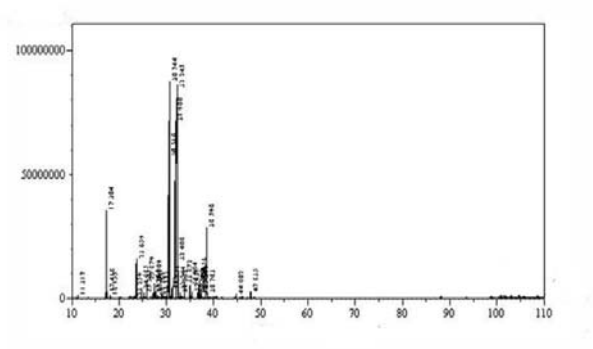

Figure 2: The chromatograms found in essential oils of *D. moldavica*.

Chu et al., (2011) reported that the major constituents of the flowering aerial parts essential oil of *D. moldavica* from Beijing, China were 1,8-cineol (31.25 %) and 4-terpineol (22.82 %), cumin alcohol (4.29 %), α-terpineol (4.21 %) and sabinene (3.62 %). Also in Egypt, Aziz and El-Sherbeny (2003) observed that the essential oil of *D. moldavica* was characterized by a high percentage of oxygenated monoterpenes (81.84 %–96.05 %), with the major compounds being geranial (22.82 %–55.83 %), geranyl acetate (9.75 %–31.48 %), neral (16.08 %–22.02 %) and geraniol (0.42 %–16.59 %). In China, *D. moldavica* essential oil from Xinjiang autonomous region contained citral (31.43 %), n-hexadecanoic acid (16.48 %), and geraniol ester (9.02 %) (Tian et al., 2009). The major constituents of the oil of *D. moldavica* extracted by hydro distillation were found to be geranyl acetate, geranial, neryl acetate, geraniol, neral and nerol (Li, 2001). Hawthorne et al., (1993) identified geranyl acetate (65.8 %), carvacrol (14.9 %) and thymol (7 %) as the major components of the oil of *D. moldavica*. But Shatar and Altantseg (2000) introduced linalool (67 %) and carvone (5.9 %) as the main components of the oil of *D. moldavica*.

4 CONCLUSION

In conclusion, the results obtained in our study indicated that the major components of oil of *D. kotschyi* were limonene, carvacrol, γ-terpinene and α –pinene. The major components of oil of *D. moldavica* were geranyl acetate, geraniol, neral and geranial. A comparison of our results with different reports ibndicates that differences in the volatile oil composition of the plants could be attributed to genetic (genus, species, and ecotype), chemotype, distinct environmental and climatic conditions, seasonal sampling periods, geographic origins, plant populations, vegetative plant phases, and extraction and quantification methods.

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