Original Research Article

Chemical diversity of essential oil composition from five populations of *Dracocephalum Kotschyi* Boiss

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ABSTRACT

Composition and chemical variation in the essential oil yield were studied among five wild growing populations of *Dracocephalum Kotschyi* Boiss. (Lamiaceae) from Iran. The hydrodistilled essential oils were isolated from aerial flowering parts of plant, analyzed by capillary GC-FID (Gas Chromatography Flame Ionization Detector) and GC-MS (Gas Chromatography Mass Spectrometry) were identified. Forty compounds were characterized represented 95.0 – 100 % of the total oil. Oxygenated monoterpenes constituted the main group of compounds (79.9-50.7 %) in the essential oils of all samples followed by monoterpene hydrocarbons (46.1-13.9 %). Perilla aldehyde (49.3 %), limonene (41.6 %), neral (20.9 %) and geranial (22.0 %) were found to be the main compounds. Such great variations in the components of the oils may be ascribed to existence of chemotypes and adaptation of the species to specific habitat. The data obtained contribute to broaden the inventory of wild *Dracocephalum Kotschyi* populations of Iran to plan programs for the selection of chemotypes with new and specific uses in food and drug industries.

***Keywords*:** *Dracocephalum Kotschyi*, perilla aldehyde, essential oil, GC-MS

INTRODUCTION

Dracocephalum L. is a genus of mint family (Lamiaceae) and contains 45 species, mostly in Europe and Asia, but also in North Africa, and the northern United States. Plants of Iran is represented by nine species of this genus, of which two are endemics [1-2]. *Dracocephalum* species are chiefly distributed in north, west, eastern and central parts of Iran which are widely used in Iranian traditional medicine. One of the famous member of this genus, Moldavian dragon's head (*Dracocephalum moldavica* L.) is an annual, herbaceous, essential oil-producing, spicy aromatic plant, which used widely in West Azerbaijan (Iran) folk medicine as a general tonic, stomachic, digestive, antiemetic, sedative and diaphoretic [3]. The decoction of the plant has been used for ages in traditional medicine to treat heart disease, blood pressure, angina, migraine, atherosclerosis, neuralgia, tracheitis, toothache and headache [4,5]. The herbal wild aromatic plant *Dracocephalum kotschyi*, called by the natives as Badrandjboie-Dennaie or Zarrin-giah, grows in the Alborz and Zagros Mountains with interesting pharmacological and biological properties. This species is used for fever decline, joints pain, rheumatism, inflammation against and ulcer betterment [6]. Up to now, the essential oil components of many species of *Dracocephalum* have been reported, for example Sonboli *et al.* in 2011 reported the main components of *D. polychaetum* and *D. surmandinum.* Components like perilla aldehyde (54.3 % and 63.4 %) and limonene (30.1 % and 22.1 %) were the main constituents of *D. surmandinum* and *D. polychaetum* respectively [7].

Many components such as caryophyllene, α-bisabolol, α-bourbonene, δ-elemene, τ-muurolol, γ-muurolene, δ-cadinene, copaene, α-humulene, humulene epoxide II, germacrene D, terpinen-4-ol, carvone, γ-terpinene, linalool, linalool acetate, citronellal, citronellol and nerol were identified from essential oil of *Dracocephalum* species also compounds such as caryophyllene, β-bourbonene, β-elemene, spathulenol, allo-aromadendrene, δ-cadinene, α-humulene, humulene epoxide II, germacrene D, germacrene A, α-terpineol, p-cymene, p-cymen-8-ol, γ-terpinene, neral, cuminaldehyde and α-terpinene were identified from *D. kotschyi* [8-12]. Essential oil components of *D. kotschyi* have been reported in herbs collected from other portions of Iran but until now no comprehensive research has been described with specimens accumulated from climatically diverse natural habitat.

Essential oils of herbs are affected by many agents like genetic and evolution, environmental situations, geographic variations, physiological factors and harvest time [13,14]. So, genetically and climatically different herbs grown in natural habitat can apply as potent sources for detection novel chemotypes, which is of special significance for cosmetic, perfume, pharmacological and food crofts. Knowledge of metabolite variety between plants gathered from different natural habitats allows specify the optimum growing situations for plants cultivation and breeding. The purpose of the present investigation was to specify oil variety among wild-growing populations of *D. Kotschyi*. For this means, chemical constituents in five populations were analyzed by GC-MS to acquire informative chromatographic data.

MATERIALS AND METHODS

Plant Material

Specimenes used in this study were collected from five wild growing populations of the *D. Kotschyi*, natural sites of Dizin, Alborz province, and Damavand, Amol, Mazandaran province, and also Dena in Isfahan, Iran. Voucher specimens (MPH-1414) were deposited in the Herbarium of Medicinal Plants and Drugs Research Institute of Shahid Beheshti University in Iran. After identification the samples were dried in the shadow at room temperature.

Isolation of the Essential Oils

The aerial parts of plants (100-120 g) were air-dried at room temperature in the shadow and hydrodistilled by using a Clevenger-type apparatus for 3 h. The oil was dried over anhydrous Na_2SO_4 and stored in vials at low temperature before analysis.

GC-FID and GC-MS Spectrometry Analysis Conditions

GC-FID analysis of the oil was accomplished using a thermoquest-finnigan appliance equipped with a DB-5 fused silica column (60 m × 0.25 mm). Nitrogen was used as the carrier gas at a stable flow of 1.1 mL/min. The oven temperature was held at 60 ºC for 1 min, then programmed to 250 ºC with acceleration of 4 ºC/min, and then held for 10 min. The injector and detector (FID) temperatures were preserved at 250 ºC and 280 ºC, respectively. GC-MS analysis was carried out on a thermoquest-finnigan trace GC-MS instrument armed with a DB-5 fused silica column (60 m × 0.25 mm).The oven temperature was enhanced from 60 ºC to 250 ºC at a rate of 5 ºC /min, and then held at 250 ºC for 10 min, transfer line temperature was 250 ºC. The quadrupole mass spectrometer was scanned over 45-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 μA.

Identification of the Components

The constituents of the essential oils were identified by computing of their retention indices under temperature-planned conditions for n-alkanes (C_6-C_{24}) and the oil on a DB-5 column under the identical chromatographic conditions. Identification of individual compounds was made by conformity of their mass spectra with those of the internal reference mass spectra library or with reliable compounds and certified by comparison of their retention indices with authentic compounds or with of those offered in the literature [15]. For quantification aim, relative area percentages obtained by FID were used without the utilization of correction factors. Compounds that their percentages exceeded 1.0 % of the total oil composition in at least one species were considered as original variables.

RESULTS

In this study, the essential oils isolated by the hydrodistillation method from five populations of *D. Kotschyi* were analyzed and compared by capillary GC and GC-MS. Volatile compounds, their percentages and Retention Index (RI) are presented in Table 1. Generally, 40 constituents were identified from the essential oil of *D. Kotschyi*, which represented 95.0-100 % of the oil (Table 1).

Table 1. Chemical composition of the oils of five populations of *Dracocephalum Kotschyi*

No.	RI[b]	RI[a]	Compounds	Dizin (1)	Dizin (2)	Damavand (1)	Damavand (2)	Dena
1	937	939	*α*-Pinene	1.9	13.2	2.7	2.2	1.7
2	969	973	Sabinene	-	0.6	-	0.8	0.6
3	980	974	*β*-Pinene	-	0.3	-	-	-
4	988	990	Myrcene	0.3	0.9	-	0.2	0.3
5	1024	1033	Limonene	18.4	30.6	11.0	41.4	41.6
6	1026	1020	p-Cymene	-	0.5	-	0.2	-
7	1059	1054	*γ*-Terpinene	-	-	-	0.2	0.1
8	1067	1074	*cis*-Linalool oxide	-	-	0.4	0.4	-
9	1084	1090	*trans*-Linalool oxide	-	-	0.6	-	-
10	1095	1098	Linalool	0.6	0.4	0.9	-	0.1
11	1122	1129	*α*-Campholenal	1.6	-	2.7	-	-
12	1119	1125	*trans*-*p*-Mentha-2,8-dien-1-ol	0.7	0.4	-	-	0.3
13	1133	1138	*cis*-*p*-Mentha-2,8-dien-1-ol	0.6	0.6	-	0.2	-
14	1137	1142	*cis*-Limonene oxide	2.0	2.5	0.7	-	0.2
15	1139	1137	*trans*-Limonene oxide	-	1.3	-	0.2	0.2
16	1160	1161	Z-Isocitral	-	0.5	1.3	-	-
17	1174	1184	Terpinene-4-ol	0.3	-	1.0	0.3	0.1
18	1177	1179	E-Isocitral	0.5	0.9	1.4	-	-
19	1187	1180	*trans*-*p* -Mentha-1(7),8-dien-2-ol	0.3	-	-	-	-

20	1140	1137	*cis*-Verbenol		0.3	-	-	-
21	1144	1140	*trans*-Verbenol		0.2	-	-	-
22	1186	1196	*α*-Terpineol	1.0	-	1.7	0.6	0.3
23	1204	1216	D-Verbenone	-	-	0.4	-	-
24	1217	1218	*trans*-Carveol	-	-	-	0.2	0.2
25	1228	1232	3Z-Hexenyl 3-methyl butanoate	-	-	0.3	-	-
26	1232	1226	*cis*-Carveol	0.3	-	-	-	-
27	1235	1241	Neral	12.4	9.8	20.9	1.4	1.4
28	1239	1249	Carvone	2.5	-	1.4	1.0	-
29	1242	1243	Carvone	-	2.8	-	-	0.90
30	1264	1269	Greanial	12.4	-	22.0	1.6	-
31	1269	1212	Perilla aldehyde	20.2	4.1	7.6	43.8	49.5
32	1270	1273	Geranial	-	10.5	-	-	1.7
33	1322	1325	methyl Geranate	13.2	9.7	12.0	2.3	0.2
34	1327	1326	Limonene aldehyde	-	-	-	0.9	0.5
35	1359	1359	Neryl acetate	-	6.7	0.6	0.3	-
36	1376	1388	*α*-Copaene	0.4	0.3	0.6	0.1	0.1
37	1379	1379	Geranyl acetate	3.5	-	4.0	-	-
38	1387	1400	*β*-Bourbone	0.5	-	0.8	-	-
39	1388	1389	Limonene-10-yl acetate	-	-	-	0.6	-
40	1484	1496	Germacrene D	1.4	0.3	-	0.4	-
	Monoterpene Hydrocarbons			21.0	46.1	13.9	45.0	44.3
	Oxygenated Monoterpens			71.9	50.7	79.9	53.7	55.6
	Sesquiterpene Hydrocarbons			2.3	0.6	1.2	0.4	0.1
	Oxygenated Sesquiterpenes			-	-	-	-	-
	Diterpene			-	-	-	-	-
	Total			95.0	97.4	95.0	99.1	100

[a] Retention Index (RI) calculated by using *n*-alkane series from C_6 to C_{24}; [b] Retention Index (RI).

In total, 40 mono- and sesquiterpenes were detected with a predominance of perilla aldehyde, limonene, neral and greanial, covering 95.0-100 % of the total essential oils. Oxygenated monoterpens 50.7 %-79.9 % constituted the main compounds in the essential oils of all populations. After that monoterpene hydrocarbons 13.9 %-46.1 % constituted the main compounds in the essential oils. The concentration of these ingredients varied greatly among the essential oils, highlighting the presence of many monoterpenic chemotypes. As it is shown in the Table 1, perilla aldehyde and limonene were determined as two of the main essential oil compounds in *D. Kotschyi* samples. The percentage of perilla aldehyde in essential oils ranged from 7.6 to 49.3, with maximum values recorded in Dena (49.3 %) and Damavand (2) (43.8 %) samples. The percentage of limonene in essential oils ranged from 11.0 % to 41.6 %, with maximum values obtained in Dena (41.6 %) and Damavand (2) (41.4 %) populations. Greanial was determined as the other terpenoid in *D. Kotschyi* samples, varying from 10.5 % to 22.0 %. Plant samples collected from

Dmavand (1) and Dizin (1) showed the maximum geranial amounts. Neral varied from 1.4 to 20.9 % in Dena and Damavand (1) samples, respectively. Other chemical compositions of plant based on population are shown in Table 1. Sesquiterpene hydrocarbons (0.1-2.3 %) were minor part of essential oils.

Table 2. Summary of present study and previous published data on the essential oil main compounds of *Dracocephalum* species

Samples	Compounds				References
Dizin (1)	Limonene 18.4	Perilla aldehyde 20.2	Neral 12.4	Greanial 12.4	present study
Dizin (2)	Limonene 30.6	α-Pinene 13.2	Geranial 10.5	Neral 9.8	present study
Damavand (1)	Limonene 11.0	Perilla aldehyde 7.6	Neral 20.9	Greanial 22.0	present study
Damavand (2)	Limonene 41.4	Perilla aldehyde 43.8	methyl Geranate 2.3	α-Pinene 2.2	present study
Dena	Limonene 41.6	Perilla aldehyde 49.3	α-Pinene 1.7	Neral 1.4	present study
D. nutans	Decahydro–1,1,7–trimethyl–4–Methylene 14.2	Caryophyllene oxide 11.3	1,6–Octadien–3–ol,3,7–dimethyl 7.7	Myrtenylacetate 5.7	[25]
D. subcapitatum	Geranial 63.4	Limonene 23.4	p-Menth-1-en-9-ol 4.4	Caryophyllene E 4.3	[26]
D. moldavica	Geranyl acetate 36.6	Geraniol 24.3	Neral 16.3	Geranial 11.2	[27]
D. kotschyi	Limonene 30.6	Perilla aldehyde 26.0	Methyl geranate 10.8	Geranial 6.4	[24]

D. speciosum	*trans*-Pinocarveol 3.5	Camphor 3.2	*trans*-Pinocarvyl acetate 60.5	*cis*-Pinocarvyl acetate 5.7	[28]
D. heterophyllum (cultivated)	Citronellal 2.5	Citronellol 54.3	Citronellyl formate 21.6	Neryl acetate 11.7	[12]
D. heterophyllum (wild)	*cis*-Rose oxide 1.6	Citronellal 6.7	Citronellol 74.9	Citronellyl formate 6.7	[12]
D. subcapitatum	Limonene 23.4	Geranial 63.4	*p*-Menth-1-en-9-ol 4.4	β-Caryophyllene 4.3	[26]
D. surmandinum	α-Pinene 2.4	Limonene 30.1	Perilla aldehyde 54.3	Carvone 2.2	[29]
D. polychaetum	Limonene 16.6	Perilla aldehyde 69.6	Perilla alcohol 7.2	Limonene-10-yl-acetate 5.4	[30]
D. multicaule	Limonene 28.1	Perilla aldehyde 71.5	-	-	[31]
D. aucheri Boiss	α-Thujene 5.5	Sabinene 55.2	Germacrene B 3.6	(E,E)-Germacrone 9.9	[32]
D. moldavica	Neral 6.0	Greanial 8.8	Geraniol 33.1	Geranyl acetate27.5	[33]
D. moldavica	Neral 32.1	Greanial 21.6	Geraniol 17.6	Geranyl acetate 19.9	[2]

DISCUSSION

Formerly, the essential oils of *D. Kotschyi* aerial parts collected from Aladagh mountains (near Bojnourd in the province of Khorasan in northeastern Iran) were analyzed. Yaghmai *et al.* in 1988 reported that the main components of the essential oils were citral (29.3 %), *β*-caryophellene (21.5 %), terpinyl acetate (12.2 %), myrcene (7.1 %) and menthone (6.8 %) [10]. In other study by Javidnia *et al.* in 2005, the main constituents of the essential oil from *D. Kotschyi* collected from the Muteh protected region in Isfahan province were *α*-pinene (10.5 %), caryophyllene oxide (9.2 %), terpinen-4-ol (5.7 %) and germacrene D (5.6 %) [16]. In addition, Monsef-Esfahani *et al.* in 2007 reported that the main constituents of the essential oil from *D. Kotschyi* collected from natural population in Siahbisheh region on the north slope of the Alborz Mountains in Iran were obtained from three different methods. The main components in the hydrodistillate oil were *α*-pinene (12.1 %), methyl geranate (11.2 %), *β*-ocimene (8.6 %), and limonene (7.2 %). Also, *α*-Pinene (15.0 %), methyl geranate (14.5 %), limonene (11.2 %), and *β*-ocimene (8.4 %) were the most abundant components in the steam distillate oil. Geraniol (13.0 %), trans-verbenol (11.6 %), and terpinen-4-ol (11.2 %) were the principal ingredients in the hydrolate oil [17]. Saeidnia *et al.* in 2007 reported that geranial (35.8 %), limonene-10-al (26.6 %), limonene (15.8 %) and 1,1-dimethoxy decane (14.5 %)were the major components of the essential oil extracted from Tochal Mountain near to Tehran [18]. For comparison with five studied samples, essential components of some species of *Dracocephalum* listed in Table 2. It could be concluded that there is notable variations in the main marker ingredients of the essential oils within *Dracocephalum* species and also high variation of the oil composition among *D. Kotschyi* populations accumulated from different bioclimatic zones of Iran.

The essential oil components and their biological properties were found to be influenced by ecological factors. However, genetic agents also influence terpene biosynthesis pathways and consequently the principal characteristic ingredients and their percentages. These data permit an inventory, broadening of the variability of essential oil profiles of *D. Kotschyi* populations in Iran. Generally, this discrepancy in the major components of the essential oils of these plants may be attributed to differences in enviromental factors (climatic conditions, seasonal and geographic conditions, edaphic, elevation and topography, handling method, harvest period) and genetic (species, subspecies, ecotype, cultivar, chemotype), and their intraction effects are very effective on the type and percentage of components of essential oils [19,20].

The relevance between the geographical distribution and the essential oil composition was shown. Further investigation of the composition of the essential oils from more populations of the Iran is necessary for the identification of possible chemotypes, while molecular analysis is needed to show the significance of terpenes as taxonomical markers in *D. Kotschyi* Boiss.

CONCLUSION

The dissimilarity in essential oil profile within the species offers encouragement for the prospects of breeding homogeneous plant material of *D. Kotschyi* with favorable commercial characteristics for particular uses. To verify this presumption, furthers studies under controlled conditions should be conducted in order to select interesting *D. Kotschyi* genotypes. *D. Kotschyi* essential oils exhibited a substantial antihyperlipidemic, immunomodulatory, antinociceptive and cytotoxic activity indicating their potential use as natural products in several fields such as cosmetic, pharmaceutical and food industries [21-24]. This selection is surely linked to its future success as industrial crop. This work should be made jointly with selection of suitable plants with high percentage of the desired compounds.

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