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Original article

Seasonal variation of mono- and sesquiterpenoid components in the essential oil of *Dracocephalum kotschyi* Boiss.

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Abstract

Background and objectives: *Dracocephalum kotschyi* is a plant which belongs to the Lamiaceae family and exists mostly in south-west Asian countries, including Iran. This plant is used as antispasmodic, analgesic and anti-inflammatory to treat rheumatoid diseases. **Methods**: In order to investigate the impact of the harvesting time changes on the quantity and quality of mono- and sesquiterpenoid components of *D. kotschyi* aerial parts, ten samples were collected from cultivated plants from 19 April to 27 August 2013. Also samples of flower and root were harvested in order to investigate their essential oil components. The essential oils were obtained through hydrodistillation method. The components were studied and identified by GC and GC ⁄ MS systems. **Results**: The highest yield of the essential oil was obtained on 3 May (1.10% V.W) and the lowest on 28 July (0.29% V.W). Totally 55 compounds were identified in the essential oil while the highest percentage belonged to monoterpenes especially the oxygenated ones. Most variations were observed in geraniol (1.40-15.34%), geranyl acetate (trace-14.41%) and neryl acetate (0.62-17.51%). The major value in most cases belonged to geranial. **Conclusion:** the results of this study indicate that the harvesting time of plant is an effective factor in the quality and quantity of the essential oil of *Dracocephalum kotschyi*.

Keywords: *Dracocephalum kotschyi,* essential oil, geranial, Lamiaceae, seasonal variations

Introduction

Dracocephalum kotschyi is a plant which belongs to the Lamiaceae family and exists mostly in south-west Asian countries, including Iran; North Khorasan. Lamiaceae is one of the plant families which has 46 genera and 410 species and subspecies in Iran [1]. *D. kotschyi* is used as antispasmodic and analgesic in Iranian Traditional Medicine [2] while its analgesic effect has been demonstrated in recent researches to be due to the presence of limonene and terpineol [3]. Also this herb is used to treat rheumatoid diseases and possesses inhibitory effect of lymphocytes due to the compound called calycopterin [4]. In addition, the species contains compounds such as verbenone, caryophyllene and geraniol which have shown various biological effects [3,5]. Also in a previous study, the antiproliferative activity of

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the flavonoids of *D. kotschyi* has been demonstrated [6].

Large amounts of volatile oils are produced and used as therapeutic and flavoring agents or raw materials for the synthesis of other chemicals [7]. Factors such as plant harvesting time, weather, temperature, humidity, rainfall and type of chemicals used in soil are among environmental factors affecting the quantity and quality of the essential oils [8]. It has been shown that the plant collection time is effective on the essential oil compounds [9]. For example, a study on *Salvia officinalis* has showed that the highest percentage of pharmacologically effective material in this plant (alpha-thujune) has been obtained in October-September [10]. Also the content and amount of volatile oil compounds of chamomile depends on the plant age and significant decrease can be observed with increase in the age of the plant [11]. An investigation on *Curcuma longa* L. has indicated that the best time to collect the rhizome of this plant is in the age of 7.5-8 month, since in this age most of the plant's volatile oil is allocated to turmeric compounds, thus volatile oil constituents such as monoterpenes (1,8-cineole and alpha-phellandrene) decrease and sesquiterpene compounds (ar-turmerone and turmerone) increase. Also the amount of monoterpene compounds in the early stages of plant growth had reduced [12]. Considering that the quality and quantity of compounds found in plants are affected by factors such as harvesting time, in the present study the effect of seasonal changes on the quality and quantity of the essential oil of *D. kotschyi* has been investigated.

Experimental

Plant material

The herb was cultivated in a village named Hojat Abad near the Zayandeh-Rud dam, west of Isfahan. From the beginning of the flowering season (19 April 2013), ten samples of the aerial parts of the plant were collected randomly in different times and dried in shade. The root and flower of the plant were collected from the same location.

Essential oil extraction

Plant aerial parts were powdered with electric mill and prepared for the analysis of compounds. Also samples of the flower and root were analyzed to investigate the compounds of their essential oil. The essential oils were obtained by hydrodistillation [13] and then quantitative and qualitative identifying of the plant volatile compounds was performed by GC and GC/MS.

Gas chromatography (GC)

Gas chromatography was performed with Agilent 7890A model and helium was used as the carrier gas. Capillary column HP-5MS (length 30 m, inner diameter 0.25 mm, thickness of stationary phase layer 0.25 micrometer) was used. Column temperature was programmed as follows: initial temperature of 60 °C, the final temperature of 280 °C, the temperature increasing rate of 4 °C per minute, the temperature of the injector and detector 280 °C. The rate was adjusted to 2 mL/min.

Gas chromatography–mass spectrometry (GC/MS)

Gas chromatography–mass spectrometry model Agilent 5975 C mass Selective Detector with ionization energy of 70 e.V and EI ionization model and ionization source temperature of 200 °C was used. The organized materials of volatile oils were separated by gas chromatography and then mass spectrum of each separated compound was obtained in mass spectrometry device. The connected computer compared the obtained spectra with its database and introduced three combinations for each spectrum. The retention indices (RI were calculated using a homologous series of *n*-alkanes injected in conditions equal to the samples.

Results and Discussion

Table 1 presents the amount and type of compositions in the essential oil at different harvesting times. As could be seen, the type and amount of the essential oil components vary at different times. In a similar study on the volatile oil obtained from *Laurus nobilis*, it has also been shown that volatile oil components may vary from month to month and the results have suggested that the amount of the volatile oil and the amount of 1,8-cineol reach was highest in July-August [14].

Totally 53 compounds including 40 monoterpenes, 10 sesquiterpenes, two phenylpropanoids derivatives and one fatty acid have been identified while the highest value belonged to geraniol in the sample of 27 August, neryl acetate on 14 June and geranial in the rest of the samples. In another study on *D. kotshyi* essential oil from different areas of Mazandaran province, the highest percentages belonged to alpha-pinene and limonene [15]. This difference could be due to geographical variations. The highest amount (19.63%) belonged to the geranial composition in the sample of 3 May and it seemed to be the main component of the plant. Compounds that showed the largest change in harvesting times include geraniol (1.40- 15.34 %), geranyl acetate (trace-14.41%) and neryl acetate (0.62-17.51%) while other compounds did not show much altrations. In a similar study on *Pycnocycla spinosa* Decne. ex Boiss. It has been revealed that the greatest changes belonged to beta-eudesmol composition (1.9-9.17%), alpha-cadinol (0-5.59%) and caryophyllene oxide (0-5.88%) [16]. It seems that the amount of these compounds might vary due to the environmental factors during the growing period. A similar report of these changes in one of the species of yarrow on its essential oil compounds especially chamazulene (formed from matricin) has been reported [17]. Some compounds were found only in one of the harvesting times of the plant: alpha- thujene, citronellal, geraniol formate, methyl eugenol, caryophyllene oxide and farnesol. This is in accordance with the results of a research about *Eucalyptus maculata* essential oil where compounds such as alpha-humulene, beta-selinene, alpha-selinene, beta-elemene and geranyl acetate were observed only in one season which could be due to the seasonal variations and environmental stresses [18]. A number of compounds such as alpha-pinene, limonene, alpha-campholene-aldehyde, carveol, neral, geraniol, geranial, methyl geranate and neryl acetate were observed in all harvesting times. The harvesting times have been planned in spring and summer however, some terpene compounds including camphene, alpha-phellandrene, alpha-terpinene, gamma-terpinene, sabinene hydrate, 1,4,8-menthatriene, verbenone, nerol, eugenol, jasmone, caryophyllene, bicyclogermacrene, delta cadinene and spathulenol were only observed in summer. It seems that the amount and intensity of light or the temperature of the environment could be the main factors for these changes. Similar changes in the essential oil of a species of peppermint regarding menthol and menthone composition has been reported before [19]. Terpene compounds which were found to be present in amounts of less than one percent were camphene, verbenene, sabinene, beta-pinene, alpha-phellandrene, alpha-terpinene, para-cymene, 1,8-cineol, gamma-terpinene, sabinene hydrate, linalool oxide, 1,4,8-menthatriene, pinocarvone, verbenone, nerol, bornylacetate, eugenol, alpha-copaene, jasmone, caryophyllene, alloaromadendrene, bicyclogermacrene, delta cadinene, spathulenol and widdrol.

In general, the present investigation about the qualitative changes in this plant indicated that some compounds could be present or absent depending on various harvesting times.

As demonstrated in table 2, the essential oil of the plant showed its maximum amount on 3 May (1.10%) and the lowest amount on 28 July (0.29%). According to table 1, the highest amount of the essential oil terpene compounds in all samples belonged to monoterpenes especially the oxygenated monoterpenes which have demonstratedthe highest percentage on 3 May (91.38%) and the lowest on 17 May (71.38%).

The highest total percentage of sesquiterpenes was on 27 August (3.75%) and the

Table 1. Chemical composition of *D. kotschyi* aerial parts essential oil during different growing period.

components	RT	RI	percentage									
			19 April	3 May	17 May	31 May	14 June	18 July	28 July	7 August	17 August	27 August
Alpha-Thujene	3.68	0930	t*	t	t	0.79	t	t	t	t	t	t
Alpha-pinene	3.88	0939	8.44	9.01	3.96	10.78	6.85	9.96	9.03	7.49	4.88	3.48
Camphene	4.09	0954	t	t	t	t	t	0.14	0.15	0.13	0.11	0.05
Verbenene	4.17	0968	0.16	t	0.22	t	0.35	0.15	0.16	0.19	0.16	0.23
Sabinene	4.55	0975	0.28	t	t	t	t	0.80	0.57	0.63	0.85	0.18
Beta-pinene	4.62	0979	0.32	t	0.12	t	t	0.45	0.44	0.40	t	0.19
Myrcene	4.89	0991	0.45	t	t	t	t	1.41	1.16	1.02	1.35	0.35
Alpha-phellandrene	5.21	1003	t	t	t	t	t	0.24	0.18	0.20	0.17	0.08
Alpha-terpinene	5.49	1017	t	t	t	t	t	0.25	0.21	0.22	0.12	0.20
Para-cymene	5.69	1025	0.47	0.64	0.60	t	0.65	0.35	0.33	0.36	t	0.33
Limonene	5.78	1029	7.93	4.91	3.10	7.08	2.94	8.97	6.93	6.18	5.07	2.07
1,8-cineol	5.89	1031	t	0.60	0.66	t	0.47	t	t	t	t	0.32
Ocimene	5.99	1037	0.40	1.88	t	t	t	0.74	0.65	0.61	0.48	0.19
Gamma-terpinene	6.53	1060	t	t	t	t	t	0.44	0.36	0.37	0.35	0.43
Sabinene hydrate	6.74	1070	t	t	t	t	t	t	t	0.29	0.09	0.31
Linalool oxide	6.90	1087	0.32	t	t	t	t	0.20	0.25	0.26	0.34	0.31
Terpinolene	7.30	1089	1.00	t	t	t	2.00	0.84	0.52	0.51	0.79	0.31
Linalool	7.65	1097	t	t	1.21	2.49	t	1.67	1.79	1.72	2.17	2.51
1,4,8-menthatriene	8.25	1110	t	t	t	t	t	0.29	0.31	t	t	t
Alpha-campholene aldehyde	8.44	1126	2.71	3.77	4.14	5.45	4.85	1.71	2.01	2.30	2.33	2.09
Pinocarveol	8.73	1139	1.10	t	0.93	t	0.81	1.08	t	1.35	t	1.43
Sabinol	8.79	1143	t	0.96	t	t	t	t	1.24	t	1.64	t
Verbenol	9.00	1145	1.76	t	t	t	t	t	1.99	2.23	3.28	2.91
Citronellal	9.20	1153	t	t	t	t	t	t	t	t	t	0.24
Pinocarvone	9.47	1165	t	t	0.20	t	t	t	0.18	0.15	t	0.14
Terpineol	9.92	1177	0.93	t	0.70	t	0.55	1.68	t	1.64	1.69	1.53
Cryptone	10.20	1186	t	t	0.73	t	0.75	1.05	1.14	1.26	1.24	t
Myrtenol	10.50	1196	1.30	t	1.31	t	1.21	t	1.01	1.06	1.46	1.35
Verbenone	10.94	1205	t	t	t	t	t	t	t	t	0.58	0.67
Carveol	11.39	1217	1.49	2.04	1.56	2.12	2.36	1.44	1.80	1.67	1.58	1.81
Nerol	11.66	1230	t	t	t	t	t	0.81	0.96	0.75	t	t
Neral	12.13	1238	12.67	16.31	13.13	8.62	9.92	10.72	11.88	12.95	12.54	8.61
Geraniol	12.41	1253	7.05	9.69	1.40	5.00	5.86	10.01	10.31	6.09	8.32	15.34
Geranial	13.20	1267	15.60	19.63	17.30	13.72	12.71	12.49	13.71	14.67	14.16	8.36
Bornyl acetate	13.37	1289	0.48	t	0.63	t	0.91	0.41	0.42	0.43	t	0.76
Geraniol formate	13.91	1298	t	t	t	t	t	t	t	t	t	0.16
Methyl geranate	14.67	1325	7.75	11.00	7.62	8.45	11.87	6.13	6.63	6.79	7.23	8.59
Eugenol	15.55	1359	t	t	t	t	t	t	0.24	0.17	0.22	0.62
Neryl acetate	15.80	1362	0.73	10.94	0.62	10.54	17.51	0.83	0.81	1.08	1.35	0.79
Alpha-copaene	16.06	1377	0.54	t	0.91	t	t	0.35	0.40	0.32	0.33	0.32
Geranyl acetate	16.59	1381	14.41	t	11.24	t	t	10.01	9.05	9.58	10.18	8.42
Jasmone	16.95	1391	t	t	t	t	t	t	t	t	0.12	0.65
Methyl eugenol	17.07	1404	t	t	t	t	t	t	t	t	0.10	t
Caryophyllene	18.15	1419	t	t	t	t	t	t	t	t	0.08	0.14
Alloaromadendrene	18.62	1460	0.54	t	0.47	t	0.54	0.30	0.39	0.33	0.40	0.56
Germacrene-D	19.23	1485	0.41	t	t	t	t	1.19	1.21	1.11	0.98	1.72
Bicyclo germacrene	19.69	1500	t	t	t	t	t	t	t	t	0.12	0.12
Delte-cadinene	20.49	1523	t	t	t	t	t	t	t	t	0.16	0.18
Spathulenol	22.03	1578	t	t	t	t	t	t	0.19	0.18	0.25	0.42
Caryophyllene oxide	22.19	1583	t	t	t	t	t	t	t	t	t	0.14
Widdrol	24.16	1599	0.28	t	0.36	t	t	t	t	t	t	0.15
Farnesol	24.65	1701	t	t	t	t	t	t	t	t	0.11	t
Palmitic acid	32.35	1971	t	t	1.30	t	t	t	t	t	0.15	0.24
Hydrocarbonated Monoterpenes			8.09	14.33	18.31	21	25.03	12.79	18.65	8	16.44	19.45
Oxygenated			67.30	70.30	66.27	65.18	60.24	69.78	56.39	63.38	74.94	68.3

components	RT	RI	percentage									
			19 April	3 May	17 May	31 May	14 June	18 July	28 July	7 August	17 August	27 August
Monoterpenes												
Total Monoterpenes			75.39	84.63	84.58	86.18	85.27	82.57	75.04	71.38	91.38	87.75
Hydrocarbonated Sesquiterpenes			3.04	2.07	1.76	2	1.84	0.54	t	1.38	t*	1.49
Oxygenated Sesquiterpenes			0.71	0.36	0.18	0.19	t	t	t	0.36	t	0.28
Total Sesquiterpenes			3.75	2.43	1.94	2.19	1.84	0.54	t	1.74	t	1.77

* t = trace

Table 2. The yield of the essential oil of D. kotschyi in different harvesting times

Harvesting time	19 April	3 May	17 May	31 May	14 June	18 July	28 July	7 August	17 August	27 August
Essential oil % (v/w)	0.74	1.10	0.4	0.48	0.6	0.97	0.29	0.80	0.55	0.48

lowest (trace) on 31 May, while in a similar study on *Ocimum basilicum* it has been revealed that the maximum amount of winter samples belonged to the oxygen-containing monoterpenes while the summer samples mostly contained hydrocarbonated sesquiterpenes [20]. The amount of the essential oil in the flower has been found to be 0.48%, nearly double the amount of the essential oil in root (0.29%).

Table 3 shows available compounds in the flower essential oil including: alpha-pinene, limonene, linalool, alpha-campholene aldehyde, verbenol, alpha-phellandrene-8-ol, carveol, nerol, neral, geraniol, geranial, methyl geranate, eugenol, alpha-copaene, geranyl acetate, alloaromadendrene, myristicin, spathulenol and hexahydro farnesyl acetone.

As could be observed, except for the three compounds (α-phellandrene-8-ol, myristicin and hexahydro farnesyl acetone), the rest of terpene compounds are in common between the essential oil of the flower and the aerial parts.

Table 4 demonstrates available compounds in the root essential oil including: alpha-pinene, limonene, delta-carene, neral, geraniol, geranial, methyl geranate, neryl acetate, alloaromadendrene, germacrene-D, beta-selinene, alpha-selinene and widdrene. Except for delta-carene, beta-selinene, alpha-selinene and widdrene, rest of the terpene compounds were in common between root and aerial parts essential oils .According to table 3 and 4, common terpene compounds in the flower and root essential oils are alpha-pinene, limonene, neral, geranial, geraniol, methyl geranate and alloaromadendrene.

Table 3. The essential oil composition in the flower of *D. kotschyi*.

components	RT	RI	%
Alpha-pinene	3.79	0939	1.18
Pseudocumene	4.95	1026	1.48
Limonene	5.74	1029	1.04
Linalool	7.61	1097	0.93
Alpha campholene aldehyde	8.36	1126	1.74
Verbenol	8.91	1145	0.86
Alpha-phellandrene-8-ol	9.56	1170	0.49
Carveol	11.15	1217	1.40
Nerol	11.46	1230	0.73
Neral	11.86	1238	4.84
Geraniol	12.29	1253	2.82
Geranial	12.80	1267	7.81
Methyl geranate	14.47	1325	8.85
Eugenol	15.47	1359	1.07
Alpha-copaene	16.00	1377	0.64
Geranyl acetate	16.33	1381	5.62
Alloaromadendrene	18.56	1460	0.80
Myristicin	20.42	1519	0.47
Spathulenol	22.00	1578	0.40
Decanoic acid	27.22	1760	2.01
Hexahydro farnesyl acetone	29.21	1833	1.33
Benzyl salicylate	29.70	1866	1.34
Palmitic acid	32.33	1971	12.84
Ethyl linoleolate	36.24	2133	5.03
pentacosane	50.66	2500	1.12
Hydrocarbonated monoterpenes			2.22
Oxygenated monoterpenes			36.09
Total Monoterpenes			38.31
Hydrocarbonated sesquiterpenes			1.44
Oxygenated sesquiterpenes			1.73
Total sesquiterpenes			3.17

Table 4. Essential oil composition in the root of *D. kotschyi*

components	RT	RI	%
Alpha-pinene	3.80	0939	1.42
Pseudocumene	4.95	1026	1.48
Limonene	5.75	1029	3.08
Delta-carene	11.46	1031	4.81
Neral	11.86	1238	1.08
Geraniol	12.33	1253	20.32
Geranial	12.79	1267	1.70
Methyl geranate	14.46	1325	2.74
Neryl acetate	16.35	1362	12.83
Alloaromadendrene	18.56	1460	t*
Germacrene-D	19.18	1485	1.50
Beta-selinene	24.03	1490	2.64
Alpha-selinene	24.15	1498	3.40
Widdrene	24.70	1680	25.10
Allyl ionone	27.88	1780	2.04
Oleic acid	32.15	1967	1.62
Linoleic acid	35.96	2133	t
Hydrocarbonated monoterpenes			9.31
Oxygenated monoterpenes			38.67
Total Monoterpenes			47.98
Hydrocarbonated sesquiterpenes			32.64
Oxygenated sesquiterpenes			t*
Total sesquiterpenes			32.64

* t = trace

According to the results presented in table 4 and 5, the highest percentage of terpene compounds in flower and root belonged to the monoterpenes especially the oxygenated ones. However, the percentage of sesquiterpenes in the root and flower were found to be 32.64% and 3.17%, respectively. The highest percentage of the essential oil of the flower belonged to the oxygenated monoterpene, methyl geranate (8.85%), and in the root related to widdrene sesquiterpene (25.10%).

Finally, it could be concluded that the seasonal changes and harvesting time can affect the quality and quantity of *D. kotschyi* essential oil compounds. Regarding the results of the present study, for achieving the more desired components of the essential oil of *D. Kotschyi*, the best harvesting time could be predictable.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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