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Determination of *Cyclotrichium niveum* Essential Oil and Its Components at Different Altitudes

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Abstract

Cyclotrichium niveum (Boiss.) Manden. & Scheng. is a perennial species of Lamiaceae family of high importance due to essential oils compounds. Essential oil rates and essential oil components were determined in plants of this species collected from different altitudes. In order to have specific data, the plants were harvested from their natural growing area; the samples were picked up during full flowering period, from three different altitudes (890 m, 1239 m and 1605 m) in order to enhance that essential oil rates are directly influenced by this aspect. The analysis of essential oil extracted included 42 components and pulegone was the main component among these. Whereas the lowest pulegone rate was determined as 59.9% at 890 m altitude, the highest value (68.12%) was determined at 1605 m. It was also determined that as the altitude increased, the rate of this particular component in essential oil also increased, while the rate of other important components (such as isomenthone, limonene, 1,8-cineole, isopulegone) decreased.

Keywords: *Cyclotrichium niveum,* elevation, pulegone, secondary metabolites

Introduction

It is known that several internal and extrinsic factors are efficient on the secondary metabolites in medicinal and aromatic plants (Chalchat *et al*., 1997; Hudaip *et al*., 2002; Kofidis and Bosabilidis, 2008; Panou-Filotheou *et al*., 2001; Takaloo *et al*., 2012). Essential oils included among the secondary metabolites group produced by the plants have been intensely used in food industry due to their aromatic and protective properties (Smith-Palmer *et al*., 2001). Structures of the secretory hairs and the amount of secreted substances existing on the surface of leaves of plants growing at different altitudes can be different. It has been reported by some researchers that however the number of epidermal tissues on the surface of leaf and the density of hair on leaf surface, the amount of secreted essential oil increases with the altitude (Bosabalidis, 2002; Kofidis and Bosabilidis, 2008). Similarly, the rate of essential oil and essential oil components are affected by different altitudes. It has also been determined in several studies that have been carried out with different plants, that as the altitude increases, the rate of essential oil decreases (Delazar *et al*., 2011; Gulbaba *et al*., 2002; Haider *et al*., 2009), which is in agreement with our conclusions as well.

Cyclotrichium niveum (Bois.) Mandan. & Scheng. a member of the family Lamiaceae is a perennial species endemic for Turkey, which have a significant release of essential oil (Başer *et al*., 1994; Dirmenci *et al*., 2010; Sanon *et al*. 2007; Tel, 2009). Essential oils are complex compounds and their chemical composition and concentration of elements show great changes. Essential oils, terpenoids, phenylpropanoids and benzenoids, existing in the plants as the secondary metabolites, can be present as fatty acid derivatives and amino acid derivatives (Lodhia *et al*., 2009).

In dried leaves of *C. niveum,* essential oil exists at a rate of 5.6% (Baytop, 1984). Depending upon the picking period of the plant and the used sample organ, this amount of essential oil can vary between 1.5-2.1% (Alim *et al*., 2009; Başer, 2002; Cetinus *et al*., 2007; Goze *et al*., 2010). The main components of this essential oil are pulegone and isomenthone (Başer, 2002). It has been determined that in the essential oil samples taken out from different organs of plants, the rate of pulegone varies between 32.5-56.4% and the rate of isomenthone varies between 34.2-35.4% (Başer *et al*., 1994). It has been reported that 76.84% pulegone and 6.65% isomenthone exists in the essential oil extracted from the upper section of plants (Cetinus *et al*., 2007). Alim *et al*. (2009) reported that they determined 26 leading components in *C. niveum* essential oil from plants picked in full blossoming period; the rate of pulegone was determined as 50.46%. the rate of isomenthone was determined as 34.53%, while limonene (% 2.11), 1,8-cineole (% 1.91), Y-element (% 1.53), gama-pentene (% 1.46) were the subsequent.

Studies related to essential oil components existing in different organs of *C. niveum* species have been carried out and reported in speciality literature. This study aimed to determine the effect of different altitudes upon the rate of essential oil and essential oil components in *C. niveum* plants growing naturally.

Materials and methods

Plant Material

C. niveum plants were collected from three different altitudes upon the road of Adıyaman/ Kahta/ Değirmenbaşı - Malatya (West of Mount Nemrut). The dates on which plants were picked up, the coordination and pick-up altitudes are presented in Tab. 1. The determinations of plants collected in full-flowering period was conducted by Ahmet Zafer Tel (Adıyaman University, Faculty of Arts and Science, Department of Biology) and their herbariums were recorded in the aforementioned faculty (Voucher No: Tel 8881). Because blossoming was earlier at lower altitudes, the dates for collection of the plants differ. Whereas flowering in low altitudes was at the beginning of July, it occurred towards the end of July as the altitude increased. After the plants were picked up, they were naturally dried in a shady, breezy area, and stalk-leaf separation was performed.

Tab. 1. Collecting dates, coordination and altitudes of sample picking locations for *Cyclotrichium niveum* (Bois.) Mandan & Scheng plants

Location	Collected Date	Coordination	Altitude (m)
Location 1 (L_1)	04.07.2013	37°57'44" North 38°40'20" East	890
Location 2 (L_2)	12.07.2013	38°01'08" North 38°43'51" East	1239
Location 3 (L_3)	25.07.2013	38°00'30" North 38°45'36" East	1605

Essential Oil Analysis

The leaves separated from stalks were boiled in Clevenger type steam distillation device for 3 hours, with 3 replications from the samples taken out of each location. The averages determined with 3 replications were found and compounded oils were put into bottles for GC/MS analysis and stored at +4 °C temperature.

Gas Chromatography Mass Spectrometry (GC/MS) Analysis

The analysis of essential oils of *C. niveum* was carried out by using Thermo Scientific Focus Gas Chromatograph equipped with MS, auto sampler and TR-5MS (0.25 mm x 60 m i.d, film thickness 0.25). The carrier gas was helium (99.9%) at a flow rate of 1 mL/min; ionization energy was 70 eV. Mass range m/z 50-650 amu. Data acquisition was scan mode. MS transfer line temperature was 250 °C, MS ionization source temperature was 220 °C, the injection port temperature was 220 °C. The samples were injected with 250 split ratio. The injection volume was 1 μL. Oven temperature was programmed in the range of 50 to 220 °C at 3 °C /min. The structure of each compound was identified by comparison with their mass spectrum (FFNSC13 and Wiley9 library). The data were handled using Xcalibur software program.

Soil and Temperature

Soil samples from the experimental locations contained low organic matter (0.32%), salt content of 0.017%, pH of 7.90% and the texture was clay (Celik, 2012). According to the long-term meteorological record, the average temperature values in July were 26.8 °C in the research area.

Results and discussion

Essential Oil

The rate of essential oil can be affected by several factors. Some studies have revealed that the altitude negatively affects the rate of essential oil and yield (Delazar *et al*., 2011; Gulbaba *et al*., 2002; Haider *et al*., 2009). In this study that we carried out on *C. niveum*, we determined that as the altitude increased, the rate of essential oil obtained from plants decreased. In accordance to this, the rate of essential oil in plants picked up from L_1 (890 m), L_2 (1239 m) and L_3 (1605 m) locations were 5.5%, 4.8% and 4.1%, respectively (Tab. 2). These determined data were higher than the results reported by other researchers (Alim *et al*., 2009; Başer, 2002; Cetinus *et al*., 2007; Goze *et al*., 2010). This difference might probably be resulted from the section of plant, climate, geographical position, soil structure, etc.

Chemical Composition of the Essential Oil

The components of essential oil obtained from *C. niveum* plants are presented in Tab. 2, while the changes of significant components, at different altitudes of the 3 locations, are shown in Fig. 1.

As a result of GC/MS analysis, specifically 42 compounds were determined as leading in *C. niveum* essential oil. Total rate of components in essential oil extracted from samples collected at the lowest altitude was 95.12%, 96.87% for the second location, and 96.13% for the highest altitude location. Pulegone, isomenthone, 1,8-cineole and limonene components included almost 86% of the total essential oil structure. According to the average obtained for the three locations, pulegone was the main component, having almost 65% of the essential oil composition.

Puleone amount increased with the altitude. In accordince to this, whereas the lowest pulegone value was obtained from L_1 point as 59.9%, the rate of pulegone in plants picked up from L_2 and L_3 was 65.23% and 68.12%, respectively. These values we determined for pulegone component are higher than the values of some researchers (Alim *et al*., 2009; Başer *et al*., 1994), Cetinus *et al*. (2007); they have reported lower values than ours (50.46%). This situation can be caused by different locations and altitudes.

As the altitude increased, the rates of some components in essential oil, such as limonene, 1,8- cineole, isomenthone, isopulegone, exo-isocamphonone, were determined to decrease, in contrast to pulegone. A similar case was also observed for some other constituents, while components such as verbenone, citronellol, ocimenone, spathulenol, selinene, and naphthalenone were determined to increase in rate, once with the altitude.

Tab. 2. Essential oil rates and components obtained from *C. niveum* plants collected from different altitudes (%)

			Location		
			L_1	L_2	L_3
		Essential Oil (%)	5.5	4.8	4.1
No	Retention Time	Components (%)			
1	9.43	2-hexenal	0.05	0.04	0.05
2	10.92	Cyclopentane	-	0.05	0.05
3	11.6	α- pinene	0.86	0.67	0.59
4	11.77	1-octene,3-ethyl	-	0,04	0.04
5	12.79	Sabinene	0.44	0.21	0.24
6	13.05	β- pinene	1.72	1.01	1.06
7	13.77	Psedulimonene	0.06	0.05	0.05
8	14.44	α- terpinene	0.04	-	-
9	14.54	limonene	3.26	2.57	2.17
10	14.72	1,8- cineole	4.49	1.71	1,31
11	15.87	trans-sabinene	0.06	-	-
12	16.27	Terpinolene	0.06	0.05	0.05
13	16.56	Linalool	0.18	0.12	0.22
14	16.96	Myrtanal	0.05	0.04	0.04
15	17.97	iso-carveol	0.04	-	-
16	18.33	Cyclobutene	0.23	0.12	0.16
17	18.63	Menthone	0.13	0.16	0.13
18	18.95	isomenthone	10.58	7.23	6.53
19	19.13	carvenone	0.77	0.19	0.05
20	19.26	isopulegone	2.03	1.59	1.64
21	19.84	terpineol	0.17	0.09	0.11
22	19.94	carvone	0.15	0.09	-
23	20.25	verbenone	-	0.21	0.31
24	20.41	citronellol	-	0.04	0.05
25	20.48	cuminaldehyde	0.1	0.25	0.05
26	21.32	pulegone	59.9	65.23	68.12
27	22.35	thymol	0.18	0.06	0.08
28	22.54	pamabel	0.07	0.04	0.06
29	22.96	azido-2-phenylethene	0.09	0.07	-
30	23.22	trans-carvyl acetate	0.04	0.22	0.04
31	24.08	ocimenone	0.76	0.85	0.88
32	24.23	eugenol	0.07	0.28	-
33	26.02	β- elemene	0.12	0.09	0.08
34	26.65	citronellic acid	0.21	0.45	0.14
35	27.26	exo-isocamphonone	11.03	10.02	5.47
36	27.69	germacrene	0.52	0.58	0.44
37	28.07	bicyclogermacrene	1.21	1.49	1.23
38	28.29	eugenyl acetate	0.04	0.43	-
39	30.18	spathulenol	0.17	0.19	0.22
40	31.61	napthy-1-ol	0.04	0.05	0.05
41	34.09	selinene	0.04	0.07	0.11
42	36.42	naphthalenone	0.22	0.36	0.61
Total			95,12	96,87	96,13

The results we determined related to isomenthone component, varying from 6.53 for L_3 to 10.58 for L_1 ,had lower values than the results reported in previous studies (Alim *et al*., 2009; Başer *et al*., 1994).

Some of the components could not be determined in essential oils of the plants collected from the lowest altitude (cyclopentane, verbenone, citronellol) nor at the highest altitude (components such as α- terpinene, trans-sabinene, iso-carveol, carvone) (Tab. 2).

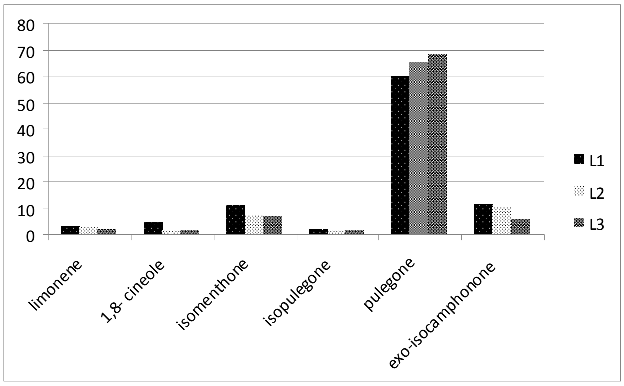

Fig 1. Changes of important components of *C. niveum* essential oil extracted from samples from different altitudes

On the other hand, several components, such as limonene, 1,8-cineole obtained from plants picked up from the lowest altitude (L_1) were determined to be higher in comparison with the data recorder for higher altitudes.

There has been no literature related data for exo-isocamphonone component, in studies carried out on essential oil extracted from plants. Whereas in the current study, this component was determined with 11.03% at the lowest altitude, while its rate at the highest altitude decreased to 5.47%.

These changes in essential oil composition are probably caused by photoperiod and air temperature at different altitudes.

Conclusion

All important components determined at high rate in *C. niveum* essential oil were negatively or positively affected by altitude. Even more, the data determined that as the altitude increased, the essential oil in *C. niveum* plants decreased proportionally. The highest rate of pulegone, as one of the most important components of the essential oil, was determined at high altitudes, but some other important components, such as isomenthone, 1,8-cineole, limonene, isopulegone decreased in rate as the altitude increased.

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