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Abstract

Background: *Kundmannia anatolica* Hub.-Mor. is an endemic specie of Apiaceae diversified in Turkey. Several parts of the plant may contain essential oils in different quantity which can be influenced by environmental factors, mainly altitude. The aim of this study was to test whether there is any altitude effect on volatile chemical constituents of essential oil obtained from the fruits of *K. anatolica* growing spontaneously in different altitudes of Lakes Region in Turkey.

Materials and Methods: *K. anatolica* was collected in 2015 at different altitudes (400, 820, 1002 and 1560 m) of Lakes Region Turkey. The fruits of the plants were distilled for 3 h using a Clevenger type apparatus according to the British Pharmacopiea (1980). Essential oils of the fruits were collected using hydro distillation method and analyzed by GC-MS/FID.

Results: Essential oil contents of fruits increased by corresponding increase in altitude level. Predominant compounds were α -Pinene (27.87-61.94%) and β -Pinene (24.92-36.46%) of the total oil of *K. anatolica*. Other important compounds were α -Thujene (2.66-8.15%), 1-Limonene (1.83-8.23%), α -Phellandrene (1.85-5.01%) and these compounds were higher in low altitudes.

Conclusion: Altitude change affected the terpenoid biosynthesis and oxygenated monoterpenes generally and were greatest when low; while sesquiterpene constituents were greatest at high altitudes. The influence of altitude seems to be an important factor for yielding the chemical profile of *K. anatolica* essential oils. Thus, the location of the plant must be taken into account depending on the intended use.

Key words: Altitude, essential oil composition, *Kundmannia anatolica* Hub.-Mor.

Introduction

Apiaceae (Umbelliferae) is one of the largest families of flowering plants with more than 3500 species spread across 455 genera (Arbizu et al., 2014). In Turkey, it is represented by 101 genera belonging to 485 species included in 511 taxa and endemism rate of the family is about 37.3% with 181 species (Özhatay et al., 2009; Baser and Kırmır, 2014). Lake District of Turkey located on the boundary between Mediterranean and Iran-Turan geographic regions is very rich in the number of plant species, particularly in Apiaceae family. It was reported that more than 70 taxa included in the Apiaceae family has been found in the floristic studies conducted in the region (Davis, 1988; Sağıroğlu and Duman, 2006; Yıldırım and Selvi, 2006; Pimenov and Leonov, 2014). Members of the family Apiaceae are usually comprised of aromatic plants having a distinctive flavour attributed to high concentration of volatile terpenic compounds (Kılıc, 2014a, b).

Kundmannia is a typical genus of the Apiaceae and are well represented in Spain, France, Italy, Turkey, Syria and North Africa. The genus *Kundmannia* consists of five species; *K. anatolica*, *K. syriaca*, *K. insulana*, *K. pastinacifolia* and *K. sicula* (Djarri et al., 2008). The genus *Kundmannia* is represented by *K. anatolica* and *K. syriaca* in Turkey with 100% endemism (Özhatay et al., 2009). *Kundmannia anatolica* Hub.-Mor. is located at elevations between 550-650 m in Southeast Anatolia region of Turkey (Davis et al., 1988; Çinbilgel and Gökçeoğlu, 2010). However, our recent field observations in the region showed the species spread at elevations between 400-1560 m in Lakes Region of Turkey in C3 Antalya, Isparta and Burdur (Karadogan et al., 2016). Similar to the other taxa of the genus *Kundmannia*, *K. anatolica* produces essential oils, which can be extracted from different parts of the plant, including the fruit, root and flower as well as stem and leaves, with significant diversity in yield and composition. In general, the relative composition of essential oils varies remarkably with geographical position, climate conditions and several other factors (Arruda et al., 2012). There are many studies related to the essential oils in Apiaceae species. To our best knowledge, there is no study performed on the chemical composition of fruit from *K. anatolica*, but essential oil compounds and antimicrobial activity of aerial parts of the specie has been recently reported by Paksoy et al. (2016). Some studies on the essential oil composition of fruit and aerial parts from *K. sicula* growing in Algeria and Tunisia have been carried out (Djarri et al., 2008; Abderrahim et al., 2013; Faidi et al., 2014).

However, this study was aimed at testing possible altitudinal effect on volatile chemical constituents of essential oil obtained from the fruits of *K. anatolica* growing spontaneously in different altitudes of Lakes Region in Turkey. This is the first report on the composition of essential oils from *K. anatolica* fruits.

Materials and Methods

Plant Material

K. anatolica was collected in 2015 at different altitudes of Lakes Region Turkey (Table 1). All samples were collected at full flowering stage for species identification and fruit maturing stage for essential oil analyses. Plant materials were identified by Dr. Hasan ÖZÇELİK of the Department of Biology at Süleyman Demirel University, Isparta according to “Flora of Turkey” (Davis et al., 1988) and voucher specimens (63.36.1.1) and deposited in the Herbarium GUL, at Suleyman Demirel University, Turkey. As a description of *K. anatolica*: Erect, branched, glabrous perennial with terete very finely ridged stems, up to 100 cm. Basal leaves ovate-triangular, 10–30 × 5–10 cm, 1–2-pinnate; ultimate segments ovate, pinnatifid or irregularly toothed, 2–3 × 1.5–3 cm, petiole 8–15 cm. Upper cauline leaves 1-pinnate with linear-oblong to linear segments 30 × 1.5–2 mm and broad sheathing petioles. Umbel rays 20–30, subequal, 1.5–3.5 cm. Bracts up to 12, narrow oblong-setaceous, 2–5 mm long. Umbellules 20–25 flowered; pedicels 1.5–4 mm. Bracteoles 3–6, linear, 1–5 mm. Petals yellow. Fruit oblong-cylindrical, 3.5–4.5 × 1–1.5 mm, not attenuate towards apex, glabrous, ridges of mericarps broadly filiform, vittae numerous, irregularly arranged. Stylopodium conical.

Table 1: Location of the analyzed populations of *K.anatolica* Hub.-Mor.

Localities		Habitat	Latitude	Longitude	Altitude (m)
Sütçüler District - Çandır Village	(A)	Macchie and red pine forest	37°45' 58"7	30°94'87"5	400
Altınyayla District	(B)	Macchie and red pine forest	37°06' 09"6	29°53'31"3	820
Atabey District	(C)	Pastures and open forests.	37°94' 62"2	30°63'60"8	1002
Eğirdir District-Barla Village	(D)	Pine forest opens	38°08' 92"7	30°78'97"7	1560

Essential oil analysis

Seeds of the plants were distilled for 3 h using a Clevenger type apparatus according to the British Pharmacopiea (1980). The oil obtained was stored at -4°C in a sealed vial until chemical analyses.

Gas chromatography–mass spectrometry

GC-MS analysis was performed on QP5050 GC-MS equipped with FID detector. The GC was equipped with CP-Wax 52 CB capillary column (50 m x 0.32 mm; film thickness = 0.25 µm) and helium was used carrier gas with flow rate of 1 mL/min. The GC oven was heated from 60°C to 230°C at a rate of 3°C/min, the final temperature was then maintained during 20 min. The injector was maintained at a temperature of 250°C. Injection volume 0.1 mL of 1% solution prepared in n-hexane; split ratio 20:1. The mass spectrometer was operating in EI mode at 70 eV with mass scan range of 40– 450 amu. Identification of constituents was done on the basis of RI (determined with reference to homologous series of n-alkanes C8–C25, under identical experimental condition), MS library search (NIST 08MS Library (Version 2.0 f) and Wiley MS 9th edition), and by comparison with MS literature data (Adams, 2007). The relative amounts of individual components were calculated based on GC peak area (FID response) without using correction factor.

Data analyses

The data was statistically analyzed using a completely randomized design (CRD) using SPSS (19.0) software. Means of the main constituents of the essential oils were compared by Duncan’s multiple range test at %5 of significance level.

Results and Discussion

Fresh fruits of *K. anatolica* afforded yellowish oils, with yields (mean of four replicates) 0.7% (A), 0.9% (B), 1.0% (C) and 1.2% (D) (v/w), respectively. The essential oil components of *K. anatolica* growing at different localities

are given in Table 2. In the results of GC/FID; 38, 46, 31 and 35 components were identified and accounted for 98.89%, 98.51%, 97.17% and 98.28% of the total essential oils for A, B, C and D localities, respectively.

The essential oil consists mainly of monoterpene hydrocarbons (88.44–95.58%) followed by sesquiterpene hydrocarbons (0.42–4.89%) and oxygenated monoterpenes (0.45-3.08%). Moreover, levels of phenolic compounds in A essential oil were also detected in a higher percentage (1.5%) than other localities (0.17-0.22%). Essential oils from A and B localities contain higher oxygenated monoterpenes (2.15% for A oil and 3.08% for B oil) and sesquiterpene hydrocarbons (1.96% A oil and 4.89% for B oil). The predominant compounds were α -Pinene accounting for 27.87% (B) to 61.94% (C) and β -Pinene 24.92% (C) to 36.46 % (A) of the total oil of *K. anatolica*. Other major compounds were α -Thujene (2.66 to 8.15%), l-Limonene (1.83 to 8.23%), α -Phellandrene (1.85 to 5.01%) and camphene (1.02 to 1.87%). The results indicated that there were significant differences between the contents of main components: α -Pinene ($p \leq 0.01$), β -Pinene ($p \leq 0.05$), α -Phellandrene ($p \leq 0.05$), α -Thujene ($p \leq 0.01$) and l-Limonene ($p \leq 0.01$) in oils (Table 3).

Among the monoterpene hydrocarbons α -Pinene predominates in the oil from C (61.94%) and D (59.05%) and it is also present at high concentrations in A oils (31.91%) and B oils (27.87%). On the other hand, essential oils from A and B have higher concentration of β -Pinene (36.46%, 33.07%, respectively) than C and D (24.92%, 26.98%, respectively) (Table 2). Essential oils from the populations of A and B have a high level of α -Thujene (8.15%, 8.02%), l-Limonene (7.31%, 8.23%) and α -Phellandrene (5.01%, 4.7%), which are about two to three-fold as high compared with the same class of compounds identified in the oil from C and D. Concerning the content of sesquiterpene hydrocarbons, the proportion of the compounds changes drastically. In fact, α -Muurolole content was higher in B (3.77%) than other localities (0.07%) although all localities contain similar compounds (Table 2).

Table 2: Percentage composition of the essential oil of the fruits of *K.anatolica* Hub.-Mor.

No	RI	Chemical Component	%			
			A	B	C	D
1	899	n-Nonane	-	0.05	-	-
2	925	Tricyclene	0.24	0.25	-	0.07
3	929	α-Thujene	8,15	8,02	2,66	3,46
4	938	α-Pinene	31,91	27,87	61,94	59,05
5	955	Camphene	1,61	1,87	1,02	1,16
6	959	Verbenone	-	0,04	-	-
7	949	α-Phellandrene	5,01	4,7	1,85	2,08
8	970	β-Pinene	36,46	33,07	24,92	26,98
9	980	2-Amylfuran	-	0,07	-	-
10	986	β -Myrcene	1,17	2,41	0,2	0,26
11	993	Heptanol<2,6-dimethyl-2->	-	-	0,06	0,05
12	1005	l-Phellandrene	0,23	0,24	0,06	0,06
13	1007	α -Terpinene	0,06	0,07	-	-
14	1011	ρ -Cymene	0,16	0,49	0,04	0,04
15	1025	l-Limonene	7,31	8,23	1,83	2,35
16	1033	cis-Ocimene	0.06	0.1	-	0.02
17	1041	1,5-Heptadiene	0.16	0.07	0.11	0.07
18	1055	γ -Terpinene	0.16	0.82	0.04	0.05
19	1062	Octilin	-	0.5	0.4	0.08
20	1082	<i>trans</i> -2-carene-4-ol	-	0.09	-	-
21	1088	α -Terpinolene	0.12	0.25	-	-
22	1115	α -Campholenealdehyde	0.24	0.26	0.05	0.05
23	1142	<i>trans</i> -Pinocarveole	0.25	0.28	0.06	-
24	1149	Verbenol	0.36	0.37	-	-
25	1164	Pinocarvone	0.13	0.16	-	-
26	1175	ρ -Mentha-1,5-dien-8-ol	0.05	0.07	-	-
27	1186	α -Terpineol	0.19	0.27	-	0.04

28	1192	Myrtenal	0.1	0.14	-	-
29	1195	Linalylpropionate	0.13	0.22	-	0.04
30	1198	p-Allylanisole	-	0.14	-	-
31	1200	trans-Myrtenol	0.2	0.18	-	-
32	1207	3-Tetradecene, (Z)	0.12	0.14	0.25	0.2
33	1213	n-Octylacetate	-	-	0.28	-
34	1217	trans-Carveol	0.05	0.07	-	-
35	1221	trans-3-Caren -2-ol	0.05	0.06	-	-
36	1236	d-Carvone	-	0.4	0.01	-
37	1242	Thymolmethylether	0.03	0.17	0.09	0.05
38	1261	(E)-Caryophyllene	-	-	0.06	-
39	1265	α -Muurolene	0.07	3.77	0.07	0.06
40	1278	Endobornylacetate	0.13	0.2	-	0.04
41	1322	Methylgeranate	0.26	0.42	0.1	0.22
42	1441	α -Humulene	0.1	0.54	-	0.11
43	1457	Limoneneoxide	0.24	0.13	0.08	0.09
44	1464	Octylbutyrate	-	0.5	0.06	-
45	1485	Germacrene-D	-	0.1	0.11	0.03
46	1501	α -Farnesene	0.07	-	-	0.51
47	1504	β -Bisabolene	1.66	0.21	0.16	0.26
48	1516	Myristicin	1.47	-	0.09	0.13
49	1520	δ -Cadinene	-	0.26	-	0.06
50	1524	2-Methyldodecan-1-ol	-	-	-	0.05
51	1532	(-)- α -Santalal	-	-	0.05	0.07
52	1539	α -cadinene	-	-	-	0.07
53	1584	3-Hexadecene, (Z)	0.11	0.12	0.27	0.25
54	1623	γ -epoxy-elemene	-	-	0.04	-
55	1786	9-Octadecene, (E)	0.07	0.12	0.21	0.17
Total identified			98.89	98.51	97.17	98.28
Monoterpene hydrocarbons			92.65	88.44	94.56	95.58
Oxygenated monoterpenes			2.15	3.08	0.67	0.45
Sesquiterpene hydrocarbons			1.96	4.89	0.42	1.11
Phenolic compounds			1.5	0.17	0.18	0.22
Fatty acid methyl esters, Alcohols, Alkenes and Alkanes			0.3	0.88	0.79	0.67
Others			0.63	1.93	1.34	0.92
Total			31	38	46	35

Note: The percentages are based on GC peak areas. RI, retention index

It is interestingly noticeable that some of the compounds, such as α -Thujene, α -Phellandrene, β -Pinene, β -Myrcene, 1-Limonene and γ -Terpinene decreased in quantity with altitudinal increase, while constituents like α -Pinene increase in quantity with a decrease in altitude. Interestingly, several sesquiterpene constituents like Heptanol <2,6-dimethyl-2->, (-)- α -Santalal and α -cadinene, which were observed at high altitude, were not observed in low altitude. In contrast, some oxygenated monoterpenes such as Verbenol, Pinocarvone, p-Mentha-1,5-dien-8-ol, Myrtenal, trans-Myrtenol, trans-Carveol and trans-3-Caren -2-ol were only detected at low altitudes (Table 2).

To our knowledge, there are few reports on essential oil compounds of *Kundmannia* species (Djarri et al., 2008; Faïdi et al., 2014; Paksoy et al., 2016). Essential oil compositions of *K. sicula* from Algerian were analyzed previously by Djarri et al (2008) and those from Tunisian by Faïdi et al (2014). Essential oil compositions and antimicrobial activity of *K. syriaca* and *K. anatolica* from Turkey have been reported for the first time by Paksoy et al

(2016). However, Paksoy et al (2016) identified the essential oil constituents from the aerial parts of *K. anatolica* and reported that spathulenol (17.4%), curdione (4.6%), apritone (4.4%) and germacrene-d (4.5%) were the major components. Faidi et al (2014) reported that the major compounds of essential oils from the inflorescences with mature seeds of *K. Sicula* growing in Tunisia were identified as isocurcumenol (10.1%), hexadecanoic acid (10.9%), spathulenol (3.4%), 10-epi-geudesmol (.5%), α -cubebene (6.1%) and trans-dihydro occidenalol (6.6%).

Although spathulenol was the major compound in *K. anatolica*, *K. syriaca* and *K. sicula* collected from different localities, it was not detected in essential oils of seed samples of *K. anatolica* in the present study. Taking into account previous studies and comparing present results with those previously reported in relation to the analysis of *K. anatolica* essential oils from Turkey; we noticed a clear variation in major components present in essential oils from different plant parts and populations of *K. anatolica*. Thus, spathulenol detected as the main constituent in the essential oils derived from aerial parts of the species growing in Turkey, whereas α -Pinene and β -Pinene considered as one of the prominent chemical constituents of the seed essential oils in our study. Additionally, it was found that differences between major compounds of *Kundmannia* species when compared to each other. These variations can be explained by the differences of mainly part of plant utilized, genotype, the plant ecotype, geographic origins or adaptive process to some particular and local ecological conditions (Chalchat et al., 1995; Djarri et al., 2008; Faidi et al., 2014). The origin of changes should be sought mainly in the differences in the nature of soil on the one hand and solar radiation on the other. Both factors involve the activation or inactivation of certain enzymatic groups, leading to the predominance of a particular biosynthetic pathway (Arruda et al., 2012).

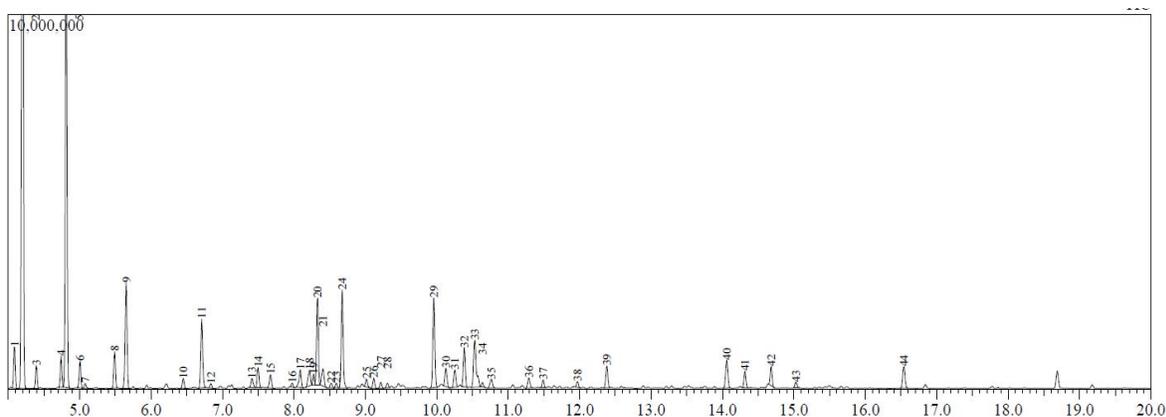


Figure 1: Gas Chromatogram with mass spectral data for location A essential oil components. On the chromatogram x-axis is retention time (RT) in minutes and on the y-axis is abundance in arbitrary units.

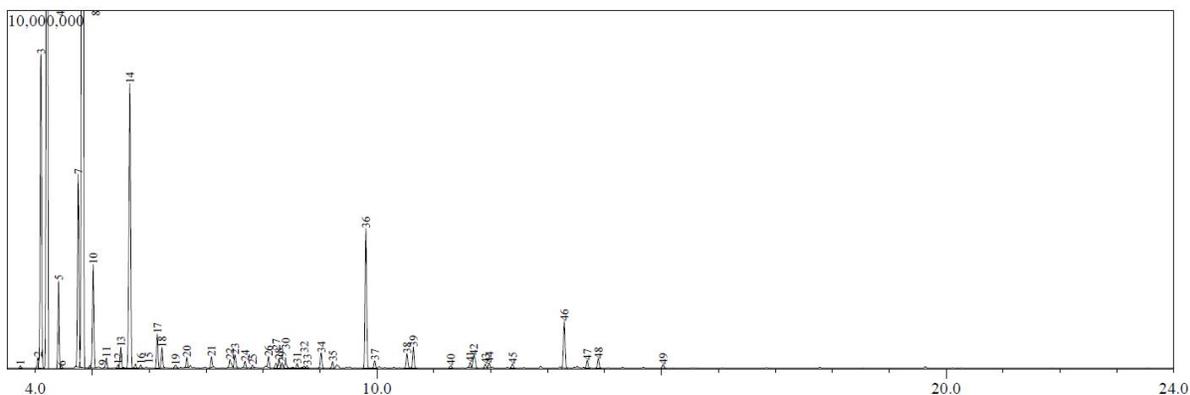


Figure 2: Gas Chromatogram with mass spectral data for location B essential oil components. On the chromatogram x-axis is retention time (RT) in minutes and on the y-axis is abundance in arbitrary units.

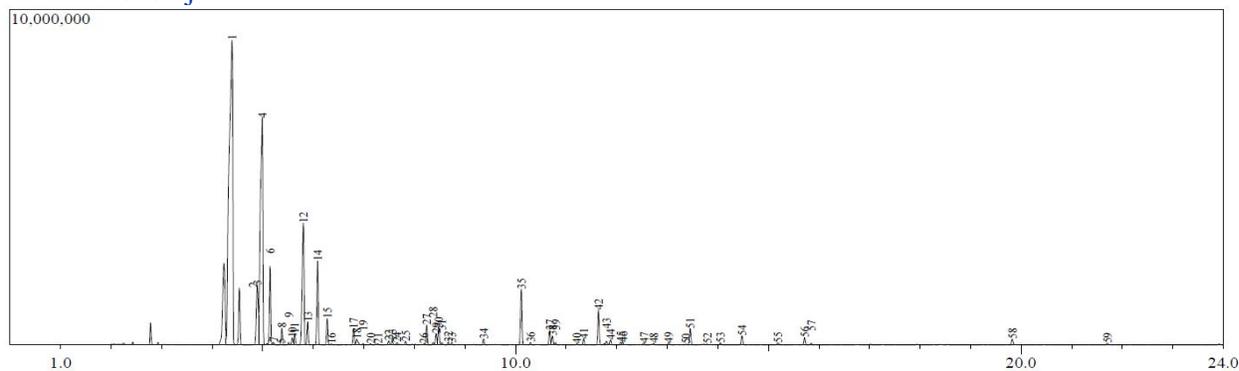


Figure 3: Gas Chromatogram with mass spectral data for location C essential oil components. On the chromatogram x-axis is retention time (RT) in minutes and on the y-axis is abundance in arbitrary units.

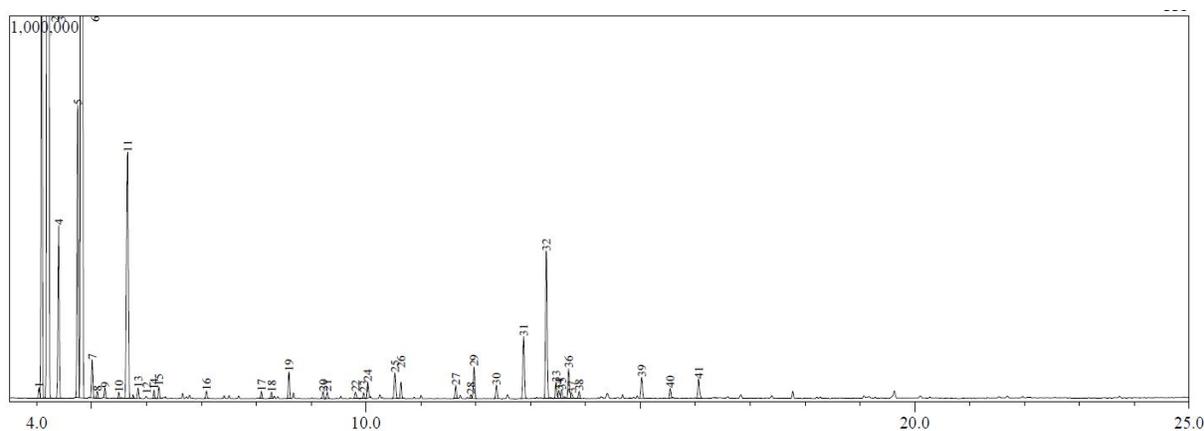


Figure 4: Gas Chromatogram with mass spectral data for location D essential oil components. On the chromatogram x-axis is retention time (RT) in minutes and on the y-axis is abundance in arbitrary units.

Table 3: The main components of essential oil of *K. anatolica* Hub.-Mor. from four altitudes

Component	A	B	C	D	Anova
α -Thujene	8,15 \pm 0,30 a	8,02 \pm 0,37 a	2,66 \pm 0,22 c	3,46 \pm 0,27 b	**
α -Pinene	31,91 \pm 3,28 b	27,87 \pm 3,25 b	61,94 \pm 2,47 a	59,05 \pm 1,10 a	**
α -Phellandrene	5,01 \pm 1,37 a	4,7 \pm 1,10 a	1,85 \pm 0,26 b	2,08 \pm 0,31 b	*
β -Pinene	36,46 \pm 1,98 a	33,07 \pm 2,09 a	24,92 \pm 2,54 b	26,98 \pm 1,14 b	*
1-Limonene	7,31 \pm 1,32 a	8,23 \pm 1,40 a	1,83 \pm 0,21 b	2,35 \pm 0,51 b	**

Values of major compounds are given as means \pm SD. * at $p \leq 0.05$; **: significant at $p \leq 0.01$.

Altitudinal changes sea level is an important factor influencing terrestrial ecosystems. Thus, significant change in altitude level can bring about corresponding changes in temperature, relative humid, wind speed, available water, and radiation rate. As a result, changes in environmental conditions will alter many eco-physiological reactions in plant bodies. Secondary metabolites of *K. anatolica* are strongly influenced by edaphic factors, climate and geologic formation. The study area is located in transition area of Mediterranean climate and Mediterranean-Steppe climate. Climatic characteristics can show significant variations at short distances due to topographic structure. Sample areas at high altitudes located in Mediterranean-Steppe climate have calcareous soil textures with semi dry cool and semi mountainous humid climates. Low altitudes sample areas are located in Mediterranean climate with in general red and decalcified soils and very humid climates (Fakir et al., 2015). Hence, it is expected that changing ecological niches will alter the essential oil compositions and its components, as seen in the present study especially in the case of the constituents like: α -Pinene, β -Pinene, α -Phellandrene, α -Thujene and 1-Limonene.

Conclusion

Essential oils of fruits from *K. anatolica* collected in Lakes Region of Turkey contain high percentage monoterpene-rich volatile fractions, α -Pinene and β -Pinene. These compounds used as base compound in many areas including pharmaceuticals, foods and medicine. On the other hand, altitude affected the terpenoid biosynthesis and oxygenated monoterpenes generally and were greater at low altitude, while sesquiterpene constituents were greater at high altitudes. In conclusion, the influence of altitude seems to be an important factor for improved yield in chemical profile of *K. anatolica* essential oils, and thus the location of the plant must be taken into account depending on the intended use. The results of the study revealed that this specie might be evaluated for its rich content of α -Pinene and β -Pinene by different industries.

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