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Lipid and essential oil constituents of *Cota hamzaoglui* Özbek & Vural (Asteraceae)

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Abstract: In the present work, lipids and essential oil constituents of endemic *Cota hamzaoglui* Özbek & Vural were investigated with GC-FID/MS techniques. The fatty acids fraction was isolated with liquid-liquid extraction from the herb with Folch method and then methylated with BF_3 reagent. Linolenic, linoleic, oleic, and hexadecanoic acids were found to be the main fatty acids. The unsaturated fatty acids (66.0%) prevailed upon saturated (33.6%) ones. The essential oil was characterized with high percentage of the fatty acids (34.7%), alkanes (14.0%) and aliphatic aldehydes (8.3%). The present study is the first report on chemical composition of *Cota hamzaoglui Özbek & Vural* lipids and essential oil.

Keywords: *Cota hamzaoglui Özbek & Vural*; essential oil, lipids, GC-FID/MS.

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INTRODUCTION

The Asteraceae family contains the largest number of described species, approximately 25,000 distributed in over 2,200 genera (1-3). The plants of Asteraceae family have been found to be the most commonly used families in the traditional medical treatments in Turkey. Ethnomedicinal aspects of potency of Asteraceae plants have recently been reported by Altundag *et al.* (4). Importance of the essential oils of the Anthemideae plants has been discussed in recently reported paper by Silva (5). Many genera have been approved for applying in treatment of a number of diseases, *Tanacetum* (6), *Silybum* (7), *Matricaria* (8), *Achillea* (9), *Artemisia* (10) and *Anthemis* (11). The genus *Cota* J. Gay is represented by 63 taxa which are mainly distributed in Europe (excluding northern Europe), North Africa, Caucasia and Central Asia. In Turkey, the genus consists of 22 taxa, nine of which are endemic (1, 2). Earlier, *Cota* was recorded as a section in the genus *Anthemis* L. in Flora of Turkey (12). Recently, the *Anthemis* section *Cota* has been accepted as a generic name, *Cota* (13, 14). The genus *Cota* morphologically resembles *Anthemis*, however differs by achenes (2). Representatives of the genus *Cota* have economic importance because of their uses for various purposes such as obtaining drug, food and dye (15).

Today there is increasing demand for cheap, safe, and scientifically approved botanicals from domestic sources. However, there are still species have not been investigated for phytochemical and biological potentials. The plants of the genus *Cota* are among less-investigated species. In literature, there is information that the flowers of the genus *Cota* were used as antiseptic and healing herbs. The main components are natural flavonoids and

essential oils (Table 1), which are widely used as antiinflammatory, antibacterial, antispasmodic, and sedative agents (16). To the best of our knowledge, there is no previous information about chemical composition and biological activity of *C. hamzaoglui*.

Table 1. Chemical composition of the essential oils of *Cota* species (literature survey)

***Cota* species**	**Plant part**	**Compound, (%)**	**Ref.**
C. altissima (L.) J. Gay (syn. *Anthemis altissima* L.)	AP	α-Pinene (4.0), benzaldehyde (27.1), Δ^2 -carene (4.2), linalool (4.6), β-caryophyllene (7.6)	(17)
C. altissima (L.) J. Gay (syn. *Anthemis altissima* L.)	Fl	Decanoic acid (6.1), β-caryophyllene (25.3), α-humulene (5.2), germacrene D (6.9), spathulenol (5.4), caryophyllene oxide (6.5)	(18)
	L	Carvacrol (3.5), β-caryophyllene (17.2), spathulenol (17.4), caryophyllene oxide (9.6)	(18)
	St	*trans*-β-Farnesene (2.6), pentadecanoic acid (3.1), palmitic acid (39.6), linoleic acid (36.2)	(18)
Cota palestina Kotschy (syn. *A. melanolepis* Boiss.; *Anthemis palestina* (Reut. Ex. Kotschy) Boiss.)	AP	Benzaldehyde (0.3-13.8), *p*-cymene (4.2-11.2), chrysanthenol (3.3-4.4), benzyl alcohol (0-26.9), 2-phenyl-1-ethanol 33.6), *trans*-verbenol (3.6-10.0), caryophyllene oxide (1.5-5.7)	(17)
Cota triumfetti (L.) J. Gay (syn. *Anthemis triumfetti* (L.) DC; *A. talyshensis* A. Fedor.)	AP	α-Eudesmol (18.2), borneol (13.3), hexadecanoic acid (9.5), γ-eudesmol (8.6%), elemol (7.6)	(19)
Cota tinctoria (L.) J. Gay (syn. *A. tinctoria* L.)	Fl	1,8-Cineole (7.9), β-pinene (7.3), decanoic acid (5.4), α-pinene (4.4)	(20)
Cota triumfetti (L.) J. Gay (syn. *A. triumfetti* (L.) DC.)	AP	β-Pinene (16.9), camphor (15.0), α-pinene (14.4), 1,8-cineole (5.8)	(21)

AP: aerial parts; Fl: flowers; L: leaves; St: steams; syn: synonymous

Recently, a new species *Cota hamzaoglui* Özbek & Vural in Anthemideae tribe has been described in Turkey. Information given on the new species includes comments on the species' affinity to *Cota oxylepis* Boiss. and *C. fulvida* (Grierson) Holub (2). Several aspects on chemical and pharmacological potent of the genus *Anthemis* have recently been reported by Siasar-Karbasky *et al.* (22). A previous phytochemical studies on *Anthemis* species resulted with polyphenols (23, 24), mono- and sesquiterpenes, fatty acids (25). Biological activity investigations of *Anthemis* species encompasses antibacterial (26), antioxidant (27), cytotoxic (28), antiproliferative (29), antidiabetic (30), antiinflammatory (31) and lipoxygenase inhibition (32) potentials.

In scope of the present work, we attempted to investigate chemical composition of the essential oil as well as fatty acid compositions of *C. hamzaoglui.* We have extracted the fatty acids with Folch method (33) for subsequent analysis of their composition after methylation with boron trifluoride reagent (BF_3). So, the present work is the first comprehensive investigation of the lipids and essential oil constituents from aerial parts of *C. hamzaoglui.*

MATERIALS AND METHODS

Chemicals

Boron trifluoride reagent (BF_3), hydrochloric acid, *n*-hexane (Sigma-Aldrich, Germany), calcium chloride, anhydrous sodium sulfate (Fluka, Germany), diethyl ether (JT Baker, Holland), chloroform (Sigma-Aldrich, France), methanol (Sigma-Aldrich, Poland) were of analytical grade. A C_8–C_{40} *n*-alkane standard solution was purchased from Fluka (Buchs, Switzerland).

Instrumentation

Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey) was equipped with the HP-Innowax FSC column (60 m × 0.25 mm id with 0.25 μm film thickness, Agilent, USA). The GC-FID analysis was carried out with capillary GC using an Agilent 6890N GC system (SEM Ltd., Istanbul, Turkey).

Plant Material

The aerial parts of C*. hamzaoglui* were collected on Bursa: Uludağ, above hotels, between cable cars, and near an old tungsten mine, 2050-2100 m, 31.07.2009, U. Özbek 2812 & M. Vural, and dried under the shade. Botanical identification was performed by Dr. M. U. Özbek. The voucher specimen is keep in the Herbarium of Gazi University, under herbarium code GAZI.

Hydrodistillation of Essential Oil
The flowers and the herb of *C. hamzaoglui* were subjected to hydrodistillation (3 h) to yield essential oils in Clevenger-type apparatus according to European Pharmacopeia (34). The oil was dried over anhydrous sodium sulfate and stored in sealed vial in refrigerator (4 °C), until GC-FID and GC/MS analyses. The oil was dissolved in *n*-hexane (10 %, v/v) to conduct chromatographic determination of its composition.

Isolation of Fatty Acids and Derivatization
The ground plant material was subjected to maceration with chloroform: methanol (2:1) at room temperature for 24 h. The extract was filtered and the residue material was macerated twice (for 30 min) more with new portions of the solvent. All filtrates were combined and half of the solvent was evaporated under vacuum in a rotary evaporator. Then, half amount of chloroform was added into the extract. The obtained extract was washed (three times) with $CaCl_2$ solution (0.4%) in a separatory funnel. At the end of the procedure, the chloroform extract was filtered through anhydrous sodium sulfate to remove moisture, and then chloroform was removed under vacuum. The dried extract was subjected to saponification. To do this, the crude extract was boiled in KOH-H_2O-MeOH (1:1:8) solution for 2 hours in a refluxing system. After the saponification process, 1-2 mL of *n*-hexane was added to remove non-saponified compounds. The fatty acids were extracted with diethyl ether after acidification of the extract with HCl (15% solution) (33). The methylation of the free fatty acids was performed using BF_3 reagent (35). The fatty acids methyl esters were subjected to analysis with GC/MS and GC-FID techniques.

Gas-Chromatography - Mass Spectrometry (GC/MS)
The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey). HP-Innowax FSC column (60 m × 0.25 mm, 0.25 □m film thickness, Agilent, USA) was used with a helium carrier gas at 0.8 mL/min. GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, kept constant for 10 min at 220 °C, and then programmed to increase at a rate of 1 °C/min to 240 °C. The oils were analyzed with a split ratio of 40:1. The injector temperature was 250 °C. Mass spectra were taken at 70 eV and the mass range was from m/z 35 to 450.

Gas Chromatography – Flame Ionization Detection (GC-FID)
The GC-FID analysis was carried out with capillary GC using an Agilent 6890N GC system (SEM Ltd., Istanbul, Turkey). Flame ionization detector (FID) temperature was set at 300 °C in order to obtain the same elution order with GC/MS. Simultaneous injection was performed using the same column and appropriate operational conditions.

Identification and Quantification of Compounds
Identification of the volatile constituents was based on the following: (i) comparison of GC/MS Relative Retention Indices (RRI) of the compounds on polar column determined relative to the retention times of a series of *n*-alkanes (C_8-C_{40}), with those of authentic compounds or literature data; (ii) computer matching with commercial mass spectral libraries: MassFinder software 4.0, Adams Library, Wiley GC/MS Library (Wiley, New York, NY, USA) and Nist Library, and comparison of the recorded spectra with literature data. Confirmation was also achieved using the in-house Başer Library of Essential Oil Constituents database, obtained from chromatographic runs of pure compounds performed with the same equipment and conditions (Table 2).

RESULTS AND DISCUSSION

In literature it could be found highlighting promising phytochemical properties and biological activities of diverse *Anthemis* species. However, there is no information about phytochemistry of endemic species *C. hamzaoglui Özbek & Vural*. The main goal of the present work was to evaluate chemical composition of *C. hamzaoglui Özbek & Vural* lipids and volatile metabolites.

Essential oil composition
In the present work, the essential oil of *C. hamzaoglui Özbek & Vural* has been hydrodistilled from aerial parts and chemical profile is investigated for the first time. The hydrodistillation of the flower and herb of *C. hamzaoglui* resulted with yellowish essential oil (0.04 % yield) with specific odor. Gas-chromatographic profile of *C. hamzaoglui Özbek & Vural* oil is presented on Figure 1.

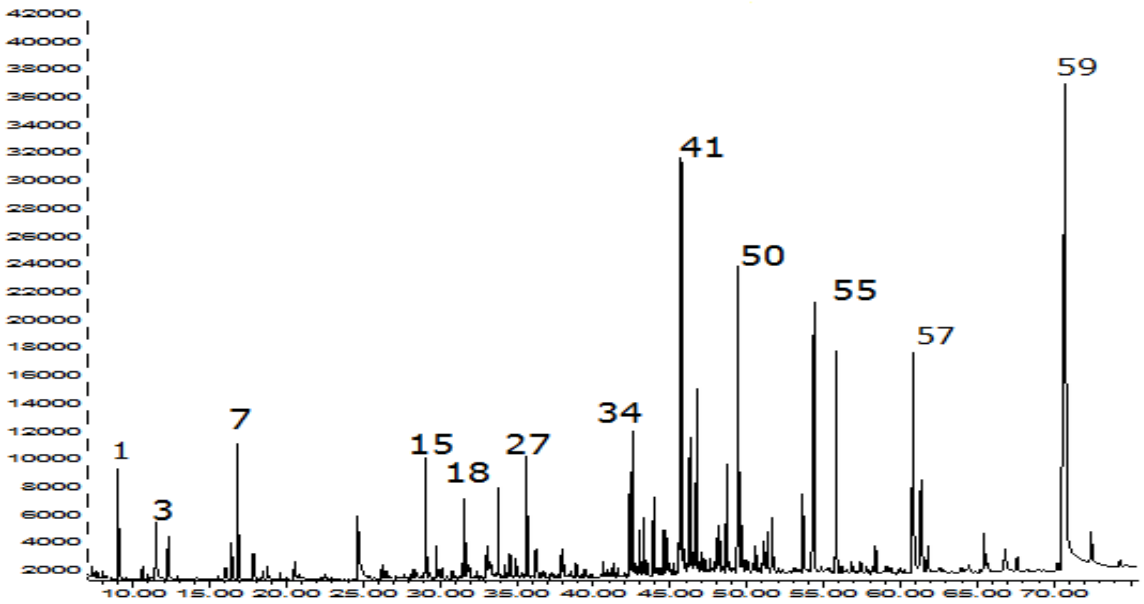

Figure 1. Gas-chromatographic profile of the essential oil of *C. hamzaoglui Özbek & Vural*. Numeration of the peaks is depicted according to list of the detected compounds in Table 2.

The list of detected compounds with their relative retention indices, relative percentages and method of identification is given in Table 2 in order of their elution on the HP-Innowax FSC column.

Table 2. Chemical composition of *C. hamzaoglui Özbek & Vural* essential oil.

No	RRI[a)]	RRI[b)]	Compound	%[c)]	Identification method
1	1032	1032(36)	α-Pinene	1.5	d,e,f
2	1076	1076 (36)	Camphene	0.2	d,e,f
3	1093	1091 (36)	Hexanal	1.1	d,e,f
4	1118	1118(36)	β-Pinene	0.7	d,e,f
5	1194	1194 (36)	Heptanal	0.4	d,e,f
6	1203	1203 (37)	Limonene	0.7	d,e,f
7	1213	1211 (38)	1,8-Cineole	1.2	d,e,f
8	1244	1236 (39)	Amyl furan	0.5	d,e,f
9	1255	1255(36)	γ-Terpinene	t	d,e,f
10	1266	1267 (36)	(*E*)-β-Ocimene	0.3	d,e,f
11	1280	1280 (36)	*p*-Cymene	t	d,e,f
12	1282	1287 (36)	Octanal	0.3	d,e,f
13	1400	1400 (36)	Nonanal	2.0	d,e,f
14	1438	1438 (36)	Dimethyl tetradecane	t	e,f
15	1532	1532 (40)	Camphor	2.0	d,e,f
16	1548	1531 (41)	(*E*)-2-Nonenal	t	d,e,f
17	1611	1611 (36)	Terpinen-4-ol	0.6	d,e,f
18	1612	1612(36)	β-Caryophyllene	1.3	d,e,f
19	1655	1655 (36)	(*E*)-2-Decenal	0.4	d,e,f
20	1661	1661 (36)	Alloaromadendrene	0.3	e,f
21	1671	1680 (36)	Benzeneacetaldehyde	0.4	e,f
22	1687	1689(36)	α-Humulene	1.4	d,e,f
23	1706	1706(36)	α-Terpineol	0.3	d,e,f
24	1708	1709 (42)	Ledene	0.3	d,e,f
25	1719	1719 (36)	Borneol	0.3	d,e,f
26	1726	1726 (36)	Germacrene D	0.6	d,e,f
27	1755	1757 (36)	Bicyclogermacrene	1.7	d,e,f
28	1764	1762 (36)	*(E)*-2-Undecenal	0.5	d,e,f
29	1773	1774(36)	δ-Cadinene	0.4	d,e,f
30	1776	1776(36)	γ-Cadinene	0.4	d,e,f

No	RRI[a)]	RRI[b)]	Compound	%[c)]	Identification method
31	1827	1827 (36)	(*E,E*)-2,4-Decadienal	0.3	d,e,f
32	1838	1838 (36)	(*E*)-β-Damascenone	t	e,f
33	1868	1868 (36)	(*E*)-Geranyl acetone	t	d,e,f
34	2008	2008 (36)	Caryophyllene oxide	2.1	d,e,f
35	2026	2024 (36)	Humulene epoxide II	1.3	e,f
36	2041	2041 (36)	Pentadecanal	1.1	d,e,f
37	2084	2089 (36)	Octanoic acid	0.2	d,e,f
38	2098	2098 (36)	Globulol	0.7	d,e,f
39	2104	2104 (36)	Viridiflorol	0.5	d,e,f
40	2131	2131 (43)	Hexahydrofarnesyl acetone	2.0	d,e,f
41	2144	2133 (44)	Spathulenol	5.4	d,e,f
42	2155	-	Hexadecanal	1.0	d,e,f
43	2187	2185 (40)	T-Cadinol	2.5	d,e,f
44	2192	-	Nonanoic acid	2.1	d,e,f
45	2206	-	Alismol (= 6,10(14)Guaiadien-4-β-ol)	0.4	e,f
46	2247	2247 (45)	*trans*-α-Bergamotol	0.4	d,e,f
47	2255	2255(42)	α-Cadinol	0.7	d,e,f
48	2259	-	Heptadecanal	0.5	d,e,f
49	2298	2296 (36)	Decanoic acid	1.3	d,e,f
50	2300	2300 (42)	Tricosane	5.8	d,e,f
51	2316	-	Caryophylla-2(12),6(13)-dien-5α-ol	0.7	d,e,f
52	2338	2347 (42)	Octadecanal	0.7	d,e,f
53	2392	-	Caryophylla-2(12),6-dien-5β-ol	1.0	d,e,f
54	2400	2400 (42)	Tetracosane	1.0	d,e,f
55	2500	2500 (42)	Pentacosane	7.2	d,e,f
56	2503	2503 (36)	Dodecanoic acid	1.4	d,e,f
57	2670	2670 (36)	Tetradecanoic acid	5.8	d,e,f
58	2822	2822 (36)	Pentadecanoic acid	0.9	d,e,f
59	2931	2931 (42)	Hexadecanoic acid	26.6	d,e,f
			Total	**93.4**	

a) **RRI**: Relative Retention Indices calculated against *n*-alkanes (C_9-C_{40}) on HP-Innowax column; **b)** RRI values obtained on polar column and reported in literature**; c)** **%** calculated from FID data; **d)** Identification based on retention index of genuine compounds on the HP-Innowax column; **e)** Identification on the basis of computer matching of the mass spectra from Başer Library; **f)** Tentative identified on the basis of computer matching of the mass spectra from Adams, MassFinder, Wiley and NIST libraries; ***t*** : Trace (< 0.1 %).

Gas-chromatographic analysis of the oil resulted with 59 compounds (total) which belong to diverse phytochemical groups, namely, fatty acids, mono- and sesquiterpene hydrocarbons and their oxygenated forms, aliphatic aldehydes and alkanes. Distribution of the main compounds groups detected in *C. hamzaoglui Özbek & Vural* oils is presented on Figure 2.

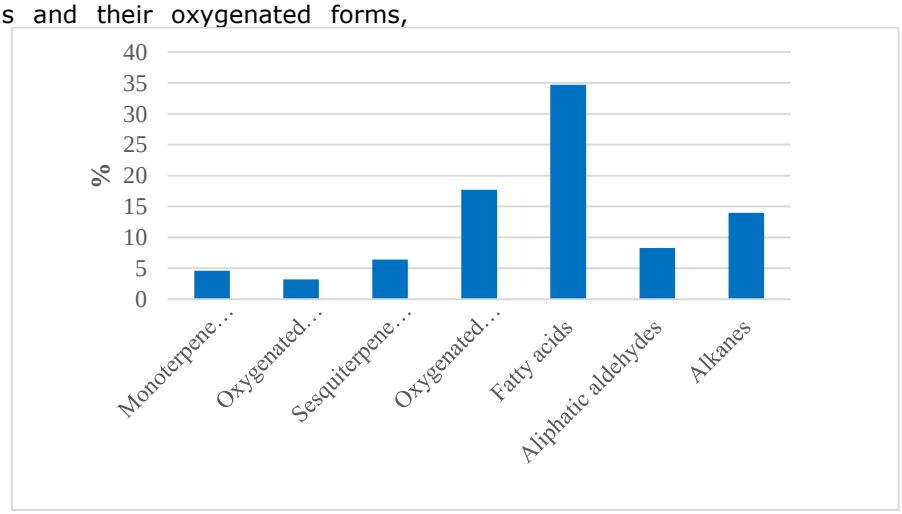

Figure 2. Distribution of the main compound groups detected in the essential oil of *C. hamzaoglui Özbek & Vural.*

In general, the essential oil was characterized with high abundance of the fatty acids (34.7%). Hexadecanoic acid (26.6%) was found as predominant fatty acid in the essential oil. It is noteworthy to mark that the oxygenated sesquiterpenes (17.7%) with spathulenol (5.4%) and caryophyllene oxide (2.1%) as the major constituents were the second important group (after fatty acids) that made contribution into volatile profile of *Cota hamzaoglui Özbek & Vural*. Camphor (2.0%) and 1,8-cineole (1.2%) were the major representatives of this compound class. The next noteworthy of mention compound's group was found to be the alkanes (14.0 %) with pentacosane (7.2%) and tricosane (5.8%) as the main representatives. Aliphatic aldehydes (8.3%) were comprised mostly by nonanal (2.0%), hexanal (1.1%), pentadecanal (1.1%) and hexadecanal (1.0%). □-Caryophyllene (1.3%), □-humulene (1.4%) and bicyclogermacrene (1.7%) comprised the sesquiterpene hydrocarbons (6.4 %) group.

It was interesting to compare the chemical profile of *Cota hamzaoglui Özbek & Vural* essential oil with those reported earlier for different Cota and *Anthemis* species. Actually, there are several reports in the literature dealing with the essential oils of diverse *Cota* or *Anthemis* species. We have recently reported about essential oil of *C. fulvida* (Grierson) Holub (46), that was characterized with hexadecanoic acid (25.6%), camphor (6.1%), caryophyllene oxide (5.3%), 1,8-cineole (4.9%) and humulene epoxide (3.9%). The fatty acids and especially hexadecanoic acid have earlier been observed to be the major constituents in previously studied essential oils obtained from aerial parts of *A. dipsacea* Bornm. (13.5%), *A. pseudocotula* Boiss. (9.5%) (47), *A. altissima* L. (39.6%) (48)*, A. ruthenica* M. Bieb. (9.9%) and *A. arvensis* L. (21.2%) (49). Camphor was reported as main volatile constituent in *A. cretica* subsp. *leucanthemoides* (Boiss.) Grierson (19.4%) (50), *A. tenuisecta* Ball. (17.5%) (51), *A. triumfetti* (L.) DC. (15.0%) (52), *A. hyalina* DC. (11.6%) (53) and *A. pseudocotula* Boiss. (9.4%) (54). 1,8-Cineole was mentioned as a major constituent in the oils of *A. pseudocotula* (39.4%) (54), *A. xylopoda* O. Schwarz (16.7%) (55), *A. widemanniana* (8.9%) (56) and *A. segetalis* Ten (6.1%) (57). Observation of the main constituents detected in different Anthemis species, it can be concluded that the oil of *Cota hamzaoglui Özbek & Vural* was found to be similar to many *Anthemis* species.

Fatty acids composition

The literature search revealed limited information about fatty acids profile of *Anthemis* or *Cota* species. In the present work, the lipid constituents of *C. hamzaoglui Özbek & Vural* isolated with liquid-liquid extraction technique from aerial part were chromatographically separated with GC-FID/MS after methylation process. To best of our knowledge, the present work is the first report about *C. hamzaoglui Özbek & Vural* lipid constituents. Gas-chromatographic analysis of *C. hamzaoglui Özbek & Vural* fatty acid methyl esters resulted with 15 compounds representing 99.6% of the plant lipids. All detected lipid constituents were classified as total saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. Palmitic acid (22.2 %) was found to be the main representative of SFAs, followed by stearic acid (6.3%). Other six saturated fatty acids, C10:0, C12:0, C14:0, C15:0, C20:0 and C22:0 were found in lower percentages (0.2-1.5%). MUFA group was presented by oleic acid (20.9 %). Linoleic (26.9 %) and linolenic (13.2 %) acids from PUFA fraction were found in high amount. Table 3 summarizes the fatty acid content of *C. hamzaoglui Özbek & Vural*.

Table 3. Fatty acid composition of *Cota hamzaoglui*

No	Carbon number: double bonds number	Compound	%
1	C10:0	Methyl decanoate (=*Methyl caproate*)	1.5
2	C12:0	Methyl dodecanoate (=*Methyl laurate*)	0.2
3	C14:0	Methyl tetradecanoate (=*Methyl myristate*)	0.9
4	C15:0	Methyl pentadecanoate	0.2
5	C16:0	Methyl hexadecanoate (=*Methyl palmitate*)	22.2
6	C16:1 D-7 *cis*	(*Z*)-7-Methyl hexadecenoate	0.1
7	C16:1 D-9 *cis*	(*Z*)-9-Methylhexadecenoate (= *Methyl palmitoleate*)	0.2
8	C18:0	Methyl octadecanoate (=*Methyl stearate*)	6.3

9	C18:1 D-9 *cis*	(*Z*)-9-Methyl octadecenoate (=*Methyl oleate*)	20.9
10	C18:1 D-7 *cis*	(*Z*)-7-Methyl octadecenoate	2.1
11	C18:2 D-9,12 *cis*	(*Z,Z*)-9,12-Methyl octadecadienoate (=*Methyl linoleate*)	26.9
12	C18:1 D-9 *cis*	Ethyl linoleate	2.6
13	C18:3 D-9,12,15 *cis*	Methyl linolenate	13.2
14	C20:0	Methyl eicosanoate (=*Methyl arachidate*)	1.2
15	C22:0	Methyl docosanoate (=*Methyl behenate*)	1.1
		∑saturated	33.6
		∑unsaturated	66.0

It was interesting to compare the fatty acids composition with other *Anthemis* or *Cota* species. The scarce reports about fatty acid's profile of *Anthemis* or *Cota* species revealed that hexadecanoic (16:0) (28.8%), (*E,E*)-9,12-octadecadienoic (18:2) (8.6%), (*Z,Z*)-9,12-octadecadienoic (13.2%), 9,12,15-octadecatrienioc (18:3) (16.3%), octadecanoic (18:0) (7.0%) were detected in *A. triumfetti* (L.) DC. [syn.: *Cota triumfetti* (L.) Gay] (58). Butyric acid (C4:0), arachidic acid (C20:0) and palmitoleic acid (C16:1) were found to be the major fatty acids in *A. wiedemanniana* Fisch. & Mey. (59). SFA constituents appeared in higher percentages than MUFA and PUFAs. SFAs were determined as 63.17%, UFAs as 20.89% and PuFAs as 15.95%. Approximately similar amounts (36% and 39%) of palmitic and linoleic acids constituted the main compounds of the stem oil of *A. altissima* L. grown in Iran. In Turkey, *A. dipsacea* Bornm., *A. pseudoculata* Boiss., *A. pectinata* Boiss.& Reute var. *pectinata* oils have been characterized with high percentage of the fatty acids with hexadecanoic acid as main representative of this compounds class (60). *A. arvensis* growing in Serbia showed a high content of palmitic (21.2%) and linoleic (6.5%) acids (61). *A. tinctoria* seed oil contained saturated (2.0%), 18:1 (18.0%), 18:2 (73.0%), 18:2 conjugated (2.0%), 18:3 (0.4%) (62) fatty acids.

CONCLUSION

We herein disclose the first report on chemical composition of the volatile and lipids profiles of endemic species *C. hamzaoglui Özbek & Vural.* This species can be considered as a source of valuable metabolites; the oil is rich with diverse fatty acids, mono- and sesquiterpenes.

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