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Research article/Araştırma makalesi

Essential oil characterization of *Cousinia sivasica* Hub.-Mor. (Asteraceae)

Mehmet TEKİN *[1], Gülmira ÖZEK[2], Esra MARTİN[3],Temel ÖZEK[2,4], Gülden YILMAZ[5],Kemal Hüsnü Can BAŞER [2,6]

[1] Trakya University, Department of Pharmaceutical Botany, Faculty of Pharmacy, 22030, Edirne, Turkey
[2]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470, Eskişehir, Turkey
[3]Necmettin Erbakan University, Faculty of Science, Department of Biotechnology, 42090, Konya, Turkey
[4]Anadolu University, Medicinal Plant Drug and Scientific Research Center, 26470, Eskişehir, Turkey
[5]Trakya University, Faculty of Science, Department of Biology, Balkan Campus, 22030, Edirne, Turkey
[6]Near East University, Faculty of Pharmacy, Department of Pharmacognosy, Lefkoşe, Nord Cyprus

Abstract

In this study, essential oil properties of an endemic plant of Turkey, *Coucinia sivasica* Hub.-Mor. were studied for the first time. Essential oil of *C. sivasica* was obtained by using Clevenger apparatus with hydrodistillation technique. The essential oil was analyzed by GC-FID and GC/MS techniques on a polar column and total 44 compounds were identified representing 98.3%. Fatty acids, their esters, methyl-branched carboxylic acids and aromatic acid esters predominated (66.3%) in the oil with hexadecanoic acid (42.8%), 1-isobutyl 4-isopropyl 3-isopropyl-2,2-dimethyl succinate (12.2%) and methyl salicylate (7.1%) as major constituents.

Key words: Compositae, *Cousinia,* endemic, essential oil, GC/MS, Turkey

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***Cousinia sivasica* Hub.-Mor. (Asteraceae)'nın uçucu yağ karakterizasyonu**

Özet

Bu çalışmada Türkiye için endemik bir bitki olan *Coucinia sivasica* Hub.-Mor.'nın uçucu yağı ilk defa çalışılmıştır. *C. sivasica*'nın uçucu yağı hidrodistilasyon yöntemi ile Clevenger apareyi kullanılarak elde edilmiştir. Elde edilen uçucu yağın analizi GC-FID and GC/MS teknikleri kullanılarak polar kolonda gerçekleştirilmiş ve yağın %98.3'ünü oluşturan 44 bileşik tespit edilmiştir. Yağ asitleri, onların esterleri, metil bağlı karboksilik asitler ve aromatic asit esterleri yağın %66.3'ünü oluştururken ana bileşikler olarak hekzadekanoik asit (%42.8), 1-izobütil 4-izopropil 3-izopropil-2,2-dimetil süksinat (%12.2) ve metil salisilat (%7.1) bulunmuştur.

Anahtar kelimeler: Compositae, *Cousinia,* endemik, uçucu yağ, GC/MS, Türkiye

1. Introduction

Asteraceae family is represented by 23.600 species, 1620 genera and 12 subfamilies in the world. Members of Asteraceae family are distributed naturally in temperate, sub-tropical and tropical climates, and they can be used for ornamental, vegetable and some of them also used in pharmaceuticals due to the their aromatic and medicinal property (Stevens, 2001). This family is the largest family of flowering plants in Turkey, with a total of 1209 recorded species. From these species, 447 are endemic, with an endemism ratio of 37% (Dogan et al., 2009). The genus *Cousinia* Cass. includes about 600-700 species throughout the world and nearly all of them grown in the central and southwestern Asia (Attar and Djavadi, 2010; Attar and Maroofi, 2010). In Turkey, *Cousinia* genus is represented with 39 species from which 26 are endemic with an endemism ratio of 66% (Güner et al., 2012). There are some natural hybrids for Turkish flora such as *Cousinia x kurubasgecidiensis* Ilcim & H. Ozcelik (Asteraceae) (İlçim et al., 2013). Especially, in Asia where *Cousinia* genus has been densely distributed, some species are used traditionally by local people against various

* *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +903462191010; Fax.: +903462191634; E-mail: mtekin2280@gmail.com
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diseases. For this aim, an infusion and decoction of the aboveground and underground parts of some *Cousinia* species are used for treatment of stomach and gastrointestinal ulcers and hypoxia in mountainous conditions and also to prevent tumor growth. Therefore, number of chemical studies on *Cousinia* genus were carried out (Plekhanova et al., 1983; Turdumambetov et al., 2007). In Pakistan, whole plant of *Cousinia stocksii* C. Winkl. is used for diuretic and antiseptic properties. The fresh juice of the plant is used for the dropsy, hematuria, vomiting and also used for chronic diarrhea, dysentery, asthyma and liver complaints (Tareen et al., 2010).

Cousinia sivasica Hub.-Mor. is one of the endemic species in Flora of Turkey which belonging to sect. *Cousinia* and is spread only in a very few localities from Sivas and Kahramanmaraş provinces of Turkey. Ekim et al. (2000) reported that the threat category of *Cousinia sivasica* is ‘‘Vulnerable’’ (VU) at the basis of IUCN threat category (Anonymous, 1994). The aim of this study is to reveal the essential oil characteristics of *Cousinia sivasica*.

2. Materials and methods

Plant materials: As a plant material, flowering aerial and underground parts of *C. sivasica* were collected in 2012-2015 years from the following localities. *Locality 1.* **B6** Sivas: Gürün to Darende, 2. km roadside, 1302 m, 38° 43' 07,2″ N, 37° 18' 38,5″ E, M. Tekin 1262, 21 June 2012 ibid. Tekin 1556, 05 June 2014; *Locality 2.* **B6** Sivas: Crossroads of Sivas-Kangal-Gürün, roadside, 1520 m, 39° 07' 52,4″ N, 37° 14' 33,3″ E, M. Tekin 1488, 19 July 2015 (Figure 1).

Figure 1. A. General view of *Cousinia sivasica* in natural habitat; **B.** Capitula and flowers of *Cousinia sivasica*

Essential oil characterization methods:

Hydrodistillation:

Hydrodistillation was performed according to the method described in the European Pharmacopoeia (Eu.Ph.). Air-dried aerial parts of *C. sivasica* (50.0 g) were ground and then hydrodistilled for 3 h using a Clevenger-type apparatus. The oil yield was calculated on a dry weight basis. The essential oil was dried over anhydrous sodium sulfate and stored in sealed vials in the dark, at 4°C. The oil dissolved in *n*-hexane (10%, v/v) before the chromatographic determination of its composition was subsequently subjected to GC/FID and GC/MS analyses.

Gas chromatography–mass spectrometry (GC/MS):

The gas chromatography–mass spectrometry (GC/MS) analysis of the oil was carried out with an Agilent 5975 GC/MSD system as reported previously (Abbas et al., 2015; Özek et al., 2010). An Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) was used with Helium as the carrier gas (0.9 mL/min). GC oven temperature was kept at 60°C for 10 min, increased to 220°C at a rate of 4°C/min, kept constant at 220°C for 10 min, and then increased to 240°C at a rate of 1°C/min. The split ratio was adjusted to 40:1, and the injector temperature was at 250°C. MS were taken at 70 eV. Mass range was from *m/z* 35 to 450.

Gas chromatography (GC):

The volatiles were analyzed by capillary GC using an Agilent 6890N GC system (SEM A.Ş., 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:

The components of the essential oil were identified by coinjection with standards (wherever possible) which were purchased from commercial sources and/or isolated from natural sources. Confirmation was also achieved using our *in-house* "Başer Library of Essential Oil Constituents" database, which was obtained from chromatographic runs of pure compounds performed with the same equipment and conditions together with comparison of their retention indices. Also, compounds were confirmed by comparison of their mass spectra with those in Wiley GC/MS Library (Wiley, New York, NY, USA), MassFinder software 4.0 (Dr. Hochmuth Scientific Consulting, Hamburg, Germany), Adams Library, and NIST Library. A C_8–C_{40} *n*-alkane standard solution (Fluka, Buchs, Switzerland) was used to spike the samples for the determination of relative retention indices (RRI). Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

3. Results

Hydrodistillation of the aerial parts of *C. sivasica* gave yellowish oil with a specific odor in 0.012% yield (moisture free basis). The list of detected compounds with their relative percentages, relative retention indices (RRI), and percentages of compound classes is given in Table 1 in the order of their elution on the HP-Innowax FSC column. A total of 44 volatile compounds accounting 98.3% of the oil were identified and quantified. Among the detected volatile constituents aldehydes (11 compound), fatty acids, fatty acid esters (3 compound), esters (5 compound), sesquiterpenes (5 compound), phenylpropanoids (2 compound), nor-isoprenoids (5 compound), alkanes (4 compound) were found. Percentage distribution of the main compound classes in the essential oil and chromatographic profile of *C. sivasica* volatiles are presented in Figure 2 and Figure 3.

Mostly the oil of *C. sivasica* consisted of fatty acids and their esters (46.1%), among which were hexadecanoic, pentadecanoic and tetradecanoic acids, methyl and ethyl esters. In addition, methyl-branched carboxylic acid and aromatic acid esters were detected. Nonacosane (7.0%) and heptacosane (4.5%) were the major alkane type compounds detected in the oil of *C. sivasica*.

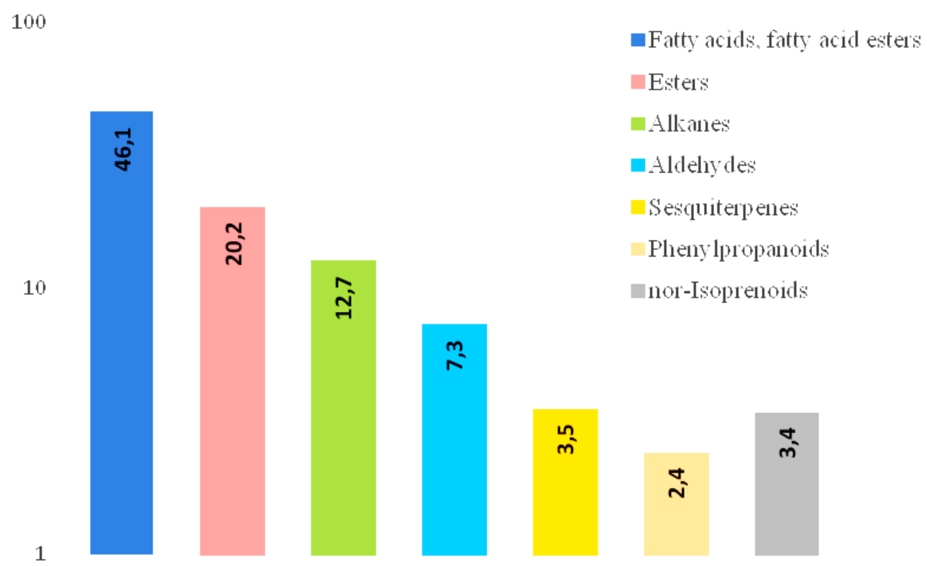

Figure 2. Percentage distribution of main compound classes in the essential oil of *C. sivasica*

Table 1. Chemical composition of volatile compounds of *C. sivasica*

No	RRI[a]	Compound class	Compound	%[b]	ID method
1	1496	alcohol	2-Ethyl hexanol	0.2	c, d
2	1506	aldehyde	Decanal	t	c, d
3	1533	nor-isoprenoid	Theaspirane A	0.8	c, d
4	1560	nor-isoprenoid	Theaspirane B	0.5	c, d
5	1599	aldehyde	(*E,Z*)-2,6-Nonadienal	t	c, d
6	1600	alkane	Hexadecane	t	c, d
7	1611	monoterpene	Terpinen-4-ol	0.1	c, d
8	1618	aldehyde	Undecanal	0.5	c, d
9	1638	monoterpene	□-Cyclocitral	t	c, d
10	1655	aldehyde	(*E*)-2-Decenal	0.4	c, d
11	1715	aldehyde	(*E,E*)-2,4-Nonadienal	0.3	c, d
12	1722	aldehyde	Dodecanal	0.5	c, d
13	1764	aldehyde	*(E)*-2-undecenal	0.6	c, d
14	1779	aldehyde	(*E,Z*)-2,4-Decadienal	0.3	c, d
15	1786	sesquiterpene	*ar*-Curcumene	t	c, d
16	1798	aromatic acid ester	**Methyl salicylate**	**7.1**	c, d
17	1822	ester	Ethyl salicylate	0.6	c, d
18	1827	aldehyde	(*E,E*)-2,4-Decadienal	2.2	c, d
19	1845	phenylpropanoid	(*E*)-Anethole	1.0	c, d
20	1868	sesquiterpene	(*E*)-Geranyl acetone	1.0	c, d
21	1880	ester	**1-Isobutyl 4-isopropyl 3-isopropyl-2,2-dimethyl succinate (= *2,2,4-trimethyl-3-carboxy isopropyl-isobutyl pentanoate*)**	**12.2**	e
22	1882	fatty acid ester	1-Methylethyldodecanoate (= *Isopropyl laurate*)	t	c, d
23	1940	nor-isoprenoid	(*E*)-□□-Ionone	1.3	c, d
24	1973	alcohol	Dodecanol	t	c, d
25	2008	sesquiterpene	Caryophyllene oxide	0.5	c, d
26	2009	nor-isoprenoid	*trans*-□-Ionone-5,6-epoxide	0.5	c, d
27	2041	aldehyde	Pentadecanal	1.1	c, d
28	2131	sesquiterpene	Hexahydrofarnesyl acetone	1.8	c, d
29	2179	furanone	3,4-Dimethyl-5-pentylidene-2(5H)-furanone (= *Bovolide*)	0.8	c, d
30	2186	Phenylpropanoid	Eugenol	1.4	c, d
31	2228	furanone	3,4-Dimethyl-5-pentyl-5H-furan-2-one	0.6	c, d
32	2232	sesquiterpene	□-Bisabolol	0.2	c, d
33	2242	aldehyde	Heptadecanal	1.1	c, d
34	2237	nor-isoprenoid	2,6-Diisopropylnaphthalene	0.3	f
35	2262	fatty acid ester	Ethyl hexadecanate (=*Ethyl palmitate*)	0.3	c, d
36	2300	alkane	Tricosane	0.3	c, d
37	2480	aromatic ketone	Benzophenone	0.6	c, d
38	2500	alkane	Pentacosane	0.9	c, d
39	2670	Fatty acid	Tetradecanoic acid (= *Myristic acid*)	2.3	c, d
40	2700	alkane	Heptacosane	4.5	c, d
41	2822	Fatty acid	Pentadecanoic acid	1.0	c, d
42	2874	furanone	□-Palmitolactone (= *4-Hexadecanolide*)	0.7	c, d
43	2900	alkane	**Nonacosane**	**7.0**	c, d
44	2931	Fatty acid	**Hexadecanoic acid**	**42.8**	c, d
			Total	**98.3**	
			Aldehydes (11 compound)	7.3	
			Fatty acids, fatty acid esters (3 compound)	46.1	
			Esters (5 compound)	20.2	
			Sesquiterpenes (5 compound)	3.5	
			Phenylpropanoid (2 compound)	2.4	
			nor-Isoprenoids (5 compound)	3.4	
			Alkanes (4 compound)	12.7	
			Others	2.7	

[a] Relative retention indices calculated against n-alkanes (C_8-C_{40}) on HP-Innowax column; [b] Percentage calculated from FID data; [c] Identification based on co-injection and retention index of genuine compounds on the HP-Innowax column; [d] Identification on the basis of computer matching of the mass spectra from Başer, Adams, Mass Finder, Wiley, and NIST libraries; [e] Identification based on comparison of RRI and spectrum of compound isolated from other natural sources; [f] tentatively identified using Wiley mass spectra library. t, trace (<0.1%).

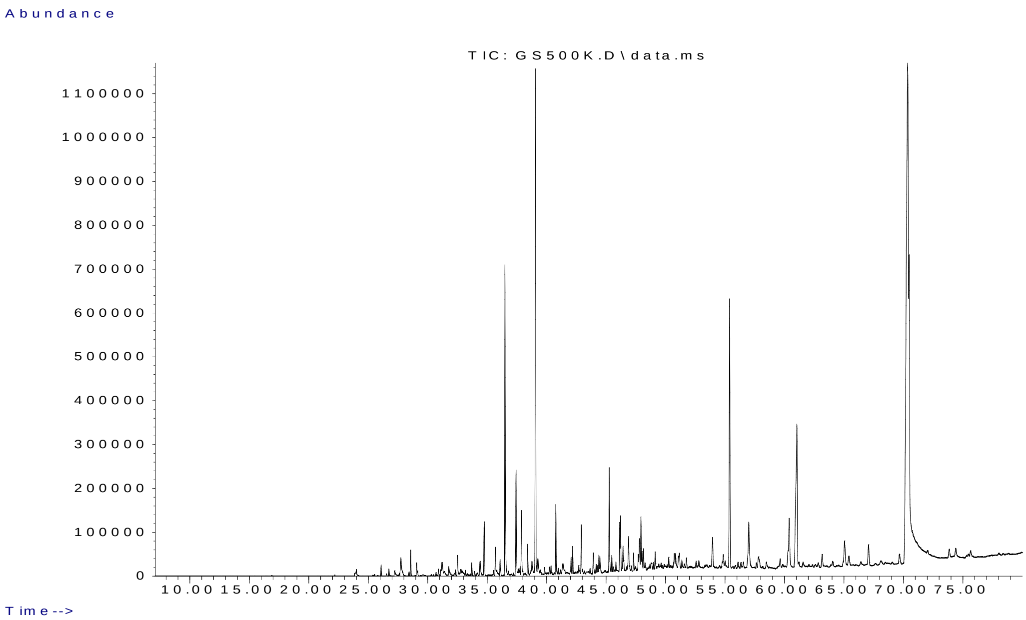

Figure 3. Chromatographic profile of *C. sivasica* volatiles

4. Conclusions and discussion

The present work is the first report on the composition of volatiles of endemic *C. sivasica*. The volatile compounds were isolated by hydrodistillation and investigated by GC-FID and GC/MS techniques on a polar column. Fatty acids, their esters and methyl-branched carboxylic acids (66.3%) predominated in the oil with hexadecanoic acid (42.8%), 1-isobutyl 4-isopropyl 3-isopropyl-2,2-dimethyl succinate (12.2%), methyl salicylate (7.1%) as main constituents. Hexadecanoic acid is known to be as common constituent in the oils of a number of Asteraceae plants: *Helichrysum* spp. (Öztürk et al., 2014), *Centaurea* spp. (Senatore et al., 2005), *Tripleurospermum callosum* (Boiss. & Heldr) E. Hossain (Yaşar et al., 2005).

1-Isobutyl 4-isopropyl 3-isopropyl-2,2-dimethyl succinate (or 2,2,4-trimethyl-3-carboxyisopropyl-isobutyl pentanoate) has earlier been detected in many natural sources like fungus *Polyporus sulphureus* (Bull.) Fr. (Wu et al., 2005), *Allomyrina dichotoma* larvae (Youn et al., 2012), cabbage and lettuce (Barry-Ryan et al., 2009). It was interesting to found aromatic acid ester, methyl salicylate (7.1%) in the oil of *C. sivasica*. Methyl salicylate is known to be the main constituent of wintergreen oil (*Gaultheria procumbens*). Also it was found in several Asteraceae plants, *Tanacetum* spp., *Centaurea* (Christensen et al., 1999; Formisano et al., 2008). This constituent was reported as an important allelopathic agent (De Feo et al., 2002) and repellent (Hardie et al., 1994).

Methyl salicylate has also an acaricidal activity on *Varroa jacobsoni* (Lindberg et al., 2000). It should be noted that spiro-ethers theaspirane A and B were detected in the oil of *C. sivasica*. Previously these rare nor-isoprenoids were found in several representatives of Asteraceae family, *Helichrysum* spp. (Öztürk et al., 2014), *Telekia speciosa* (Schreb.) Baumg. (Radulovic et al. 2010), *Centaurea luschaniana* Heimerl (Köse et al., 2008). In addition, other 13-carbon constituents like (*E*)-□-ionone and *trans*-□□ionone-5,6-epoxide were detected in the oil of *C. sivasica* (Table 1, Figure 2). The essential oil of *C. sivasica* has unique composition due to containing rare constituents like theaspiranes, aromatic acid esters and methyl-branched carboxylic acids.

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