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ORIGINAL ARTICLE / ÖZGÜN MAKALE

HS-SPME/GC-GC/MS ANALYSIS OF VOLATILE CONSTITUENTS OF *LEONURUS* L.

LEONURUS L.'NİN UÇUCU BİLEŞENLERİNİN HS-SPME/GC-GC/MS ANALİZİ

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ABSTRACT

Objective: *The Leonurus genus is one of the 50 essential herbs used in Chinese medicine. This study investigated the volatile components of four Leonurus species collected from different regions of Türkiye and compared their compositions.*
Material and Method: *Four species of the genus Leonurus were collected from different localities. The essential oils were obtained from aerial parts using microdistillation and analyzed by GC-GC/MS. HS-SPME was also applied for volatile profiling.*
Result and Discussion: *The results demonstrated species-specific differences in chemical composition. However, significant amounts of α-pinene were detected in the essential oils of samples from four species. The findings provide the first contribution to the volatile constituents of Leonurus species from Türkiye.*
Keywords: *Essential oil, GC-GC/MS, HS-SPME, Leonurus, volatile compounds*

ÖZ

Amaç: *Leonurus cinsi, Çin tıbbında kullanılan 50 temel bitkiden biridir. Bu çalışmada, Türkiye'nin farklı bölgelerinden toplanan dört Leonurus türünün uçucu bileşenleri incelenmiş ve bileşimleri karşılaştırılmıştır.*
Gereç ve Yöntem: *Leonurus cinsine ait dört tür farklı bölgelerden toplanmıştır. Uçucu yağlar, mikrodistilasyon yöntemiyle toprak üstü kısımlarından elde edilmiş ve GC-GC/MS ile analiz edilmiştir. Uçucu profillemesi için HS-SPME de uygulanmıştır.*
Sonuç ve Tartışma: *Sonuçlar, türlere özgü kimyasal bileşim farklılıkları olduğunu göstermiştir. Bununla birlikte, dört türe ait örneklerin uçucu yağlarında önemli miktarda α-pinen tespit edilmiştir. Bulgular, Türkiye'deki Leonurus türlerinin uçucu bileşenlerine dair ilk katkıyı sağlamaktadır.*
Anahtar Kelimeler: *GC-GC/MS, HS-SPME, Leonurus, uçucu bileşikler, uçucu yağ*

INTRODUCTION

Volatile organic compounds play a vital role in plant survival, as they serve both ecological and physiological functions, including signaling and defense mechanisms. Characterizing these compounds requires analytical techniques that are capable of detecting even subtle variations in chemical

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composition. A quick, solventless substitute for traditional sample extraction methods is headspace solid phase microextraction (HS-SPME).

HS-SPME provides a representative profile of highly volatile constituents, while still applying to aromatic and medicinal plants with low essential oil yield. The genus *Leonurus* L. (Lamiaceae), commonly referred to as 'Motherwort,' is a group of medicinally valued species that have been used for centuries in traditional medicine. Preparations obtained from these plants are traditionally administered for their calming, cardiotonic, and uterotonic properties [1]. In European and Asian folk medicine, *Leonurus* species are used to treat palpitations, anxiety, menstrual irregularities, and hypertension. *L. cardiaca* L. is particularly notable, being officially recognized in the European Pharmacopoeia and Commission E Monograph, which specifies its flowering tops as the medicinal component. *L. glaucescens*, known as "Boz aslankuyruğu, Öküzguyruğu", is known to have cardiotonic effects among the public and is used in the dressing of inflamed wounds in Türkiye [2,3]. Phytochemical investigations have revealed that *Leonurus* species contain a wide variety of secondary metabolites, including iridoid monoterpenes, labdane diterpenes, triterpenic acids such as ursolic and oleanolic acid, as well as alkaloids like leonurine and stachydrine [4-11]. Furthermore, flavonoids, phenolic acids, phenylpropanoid glycosides, sterols, tannins, and volatile oils have also been reported, highlighting the phytochemical diversity of the genus [1,12]. Previous research conducted in Kazakhstan, Iran, and Lithuania has described differences in the essential oil compositions of *Leonurus* species depending on geographical origin and environmental conditions, with major constituents reported as germacrene D, α-pinene, β-caryophyllene, thymol, and borneol, among others [13,14].

In Türkiye, four species of the genus (*L. cardiaca* L., *L. glaucescens* Bunge, *L. persicus* Boiss., and *L. quinquelobatus* Gilib.) are naturally distributed in the Marmara, Thrace, and Eastern Anatolia regions. Despite their widespread occurrence and long history of use, data on their volatile constituents, especially headspace compositions, remain scarce. To address this gap, the present study aimed to characterize the volatile compounds of Turkish *Leonurus* taxa using complementary techniques—HS-SPME and GC-GC/MS. By applying these methods, this research provides the first comprehensive report on the volatile profiles of all *Leonurus* species native to Türkiye [3].

MATERIAL AND METHOD

Plant Materials

Four species in the genus *Leonurus* were collected from Kocaeli, Edirne, Bursa, Bolu, and Erzurum regions in June-August 2019 and identified by Prof. Dr. Yavuz Bülent Köse and Dr. Nagehan Saltan. Voucher specimens were deposited in the Anadolu University Faculty of Pharmacy Herbarium (ESSE). (*Leonurus cardiaca*: Türkiye: Bursa, İnegöl ESSE 15775, 15776, 15777; *L. cardiaca*: Kocaeli ESSE 15778. *L. cardiaca*: Edirne ESSE 15779; *L. quinquelobatus*: Kocaeli, ESSE 15781; *L. quinquelobatus* Bolu, ESSE 15780; *L. glaucescens* Erzurum ESSE 15783, 15784, 15785; *L. persicus*: Erzurum ESSE 15782).

Analysis of the Essential Oil

Using an Eppendorf Microdistiller®, essential oil was extracted from the aerial parts of *Leonurus* sp (1g). The volatile compounds were analyzed by capillary Gas Chromatography (GC) and Gas Chromatography- Mass Spectrometry (GC/MS) using a Agilent GC-MSD system (Mass Selective Detector-MSD) (HS-SPME analyzed by GC/MS, The injector temperature was set at 250 °C, set for splitless injection [15].

Headspace-Solid Phase Microextraction (HS-SPME) Analysis

60 min sampling (room temperature) was carried out on plant materials (1-1.5 g) using fibre Polydimethylsiloxane-Divinylbenzene (PDMS/DVB - 65µm) - Blue type (supplied by Supelco Bellefonte, USA). The fiber was directly desorbed at 250ºC for 15 min. in GC/MS.

Identification of Compounds

Identification of volatile compounds were performed by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, MassFinder Library and confirmed by comparison of their retention indices.

RESULT AND DISCUSSION

The volatile compositions of different *Leonurus* species were determined using microdistillation (MD) and headspace solid-phase microextraction (HS-SPME), followed by GC and GC/MS analyses. The results revealed marked differences in the chemical profiles depending on both the species and the extraction method. In the MD analysis (Table 1 and 2), the essential oils were dominated by monoterpene hydrocarbons and oxygenated monoterpenes, with notable contributions from sesquiterpenes. For instance, *L. quinquelobatus* (LQ1 and LQ2) contained high levels of p-cymene (18.1–19.4%), 1,8-cineole (12.3–16.3%), and limonene (9.2–9.9%), whereas *L. glaucescens* (LG1 and LG2) exhibited a remarkable abundance of α-pinene (55.4–63.5%). Similarly, *L. cardiaca* samples displayed a more diverse profile, with significant amounts of β-caryophyllene (7.3–24.8%), α-humulene (7.7–24.8%), and oxygenated sesquiterpenes such as caryophyllene oxide (up to 15.2%). Furthermore, α-pinene (48.6%) and trans-verbenol (8.6%) were found to be the main components of *L. persicus*. These findings indicate species-specific chemical fingerprints within the genus *Leonurus*.

The HS-SPME analysis (Table 3 and 4), in contrast, highlighted the predominance of α-pinene, which reached very high proportions (32.5–69.9%) in *L. glaucescens* (LG1, LG2, LG3) and remained consistently high in other species as well. Moreover, the HS-SPME profiles of *L. cardiaca* revealed substantial levels of β-caryophyllene (9.1–44.3%) and α-humulene (9.0–31.5%), which were also detected in the MD samples but at different relative abundances. The primary constituents of *L. persicus* were found to be α-Pinene (34.6%), (Z)-β-ocimene (22.8%), and cis-allocimene (25.1%). Interestingly, sesquiterpene hydrocarbons such as germacrene D were particularly abundant in some HS-SPME samples (up to 33.2%), but were less prominent in the MD extracts.

The comparison of MD and HS-SPME profiles revealed substantial differences, which can be attributed to the selectivity of the extraction methods. While MD provided a broad spectrum of volatiles, including less volatile oxygenated monoterpenes and sesquiterpenes, HS-SPME was more selective for highly volatile constituents such as α-pinene. This methodological variation is consistent with previous studies, where SPME was shown to preferentially extract low molecular weight volatiles, while MD captured compounds of higher boiling points [16,17]. Phytochemically, the results support the existence of species-specific chemical fingerprints within the genus *Leonurus*. The abundance of α-pinene and β-caryophyllene in *L. glaucescens* may be considered potential chemotaxonomic markers, whereas the consistent presence of Germacrene D and β-ylangene in *L. quinquelobatus* differentiates this taxon. *L. cardiaca*, on the other hand, was distinguished by its high sesquiterpene content, particularly caryophyllene oxide, which is in agreement with earlier reports describing this species as rich in sesquiterpenoids [18].

Literature research has revealed a limited number of studies on the volatile compounds of *Leonurus* sp. The study's findings are significant because they report, for the first time, the volatile compounds of all *Leonurus* species distributed in Türkiye. Differences in chemical composition were also identified among taxa collected from different localities, as well as among different species. Beyond taxonomy, these differences may reflect the ecological and adaptive roles of volatiles. Differences in volatile compounds and previous studies may be due to collection time, drying conditions, distillation methods, and geographical and climatic factors. Beyond taxonomy, these differences may reflect ecological and adaptive roles of the volatiles. Overall, the integration of MD and HS-SPME results provides a more comprehensive understanding of *Leonurus* volatile chemistry. While HS-SPME emphasizes the most volatile constituents, MD enables the detection of less volatile yet biologically relevant compounds. Such complementary insights are crucial for chemotaxonomic characterization, ecological interpretation, and the exploration of the pharmacological potential of these medicinal plants.

Table 1. The composition of essential oils of *L. cardiaca* and *L. glaucescens*

RRI	Compounds	% LC1$_{MD}$	% LC2$_{MD}$	% LC3$_{MD}$	% LC4$_{MD}$	% LC5$_{MD}$	% LG1$_{MD}$	% LG2$_{MD}$	% LG3$_{MD}$	IM
1032	α-Pinene	1.5	21.7	1.7	7.7	6.4	63.5	55.4	57.5	t_R, MS
1076	Camphene	-	0.2	-	-	0.6	0.4	-	-	t_R, MS
1093	Hexanal	1.4	0.3	3.8	0.9	tr	0.2	3.6	2.0	t_R, MS
1100	Undecane	-	-	-	1.5	-	-	-	-	t_R, MS
1118	β-Pinene	-	1.5	-	2.2	2.0	2.7	1.5	1.4	t_R, MS
1132	Sabinene	-	0.4	--	-	0.6	0.8	0.5	0.8	t_R, MS
1138	Thuja-2,4 (10)-dien	-	1.3	-	-	-	-	1.0	1.2	MS
1174	Myrcene	-	0.8	-	5.6	2.6	1.4	1.5	1.0	t_R, MS
1188	α-Terpinene	-	0.2	-	0.6	0.5	0.3	-	-	t_R, MS
1200	Dodecane	-	-	0.9	-	-	-	-	-	t_R, MS
1203	Limonene	2.1	1.5	-	7.8	5.7	3.5	3.0	3.4	t_R, MS
1213	1,8-Cineole	2.2	2.2	-	7.6	9.0	4.1	1.9	1.4	t_R, MS
1225	(*Z*)-3-Hexenal	2.1	tr	3.0	1.4	-	-	-	1.3	t_R, MS
1244	Amyl furan	1.2	0.7	3.1	0.9	-	-	1.0	1.0	MS
1255	γ-Terpinene	1.5	1.4	-	6.9	3.5	1.8	1.5	1.5	t_R, MS
1280	*p*-Cymene	4.4	4.3	-	16.9	9.7	5.9	5.4	4.6	t_R, MS
1290	Terpinolene	-	-	-	-	-	tr	-	-	t_R, MS
1300	Tridecane	-	-	1.7	-	-	-	-	-	t_R, MS
1348	6-Methyl-5-hepten-2-one	-	0.3	-	-	-	-	-	-	MS
1400	Nonanal	3.6	0.7	3.2	-	-	-	-	0.5	MS
1406	α-Fenchone	-	0.2	-	-	0.4	-	-	-	MS
1435	γ-Campholene aldehyde	-	0.2	-	-	-	-	-	-	MS
1438	α-Thujone	-	-	-	-	Tr	-	-	-	MS
1452	1-Octen-3-ol	4.4	0.9	7.3	2.8	-	-	-	1.0	t_R, MS
1457	β- Thujone	-	-	-		1.0	-	-	-	MS
1495	Bicycloelemene	-	-	-	2.7	Tr	-	-	-	MS
1499	α-Campholene aldehyde	-	0.6	-	-	-	-	0.7	0.5	MS
1500	Pentadecane	-	-	-	-	-	-	-	0.2	t_R, MS
1503	Dihydroedulane II	-	-	-	-	0.7	-	-	-	MS
1532	Camphor	-	0.7	-	1.8	2.9	1.4	-	0.2	t_R, MS
1535	β-Bourbonene	-	-	1.1	1.4	-	0.3	1.0	1.4	t_R, MS
1535	Dihydroedulane I	-	0.4	-	-	-	-	-	-	MS
1541	Benzaldehyde	-	-	2.6	-	-	-	1.6	0.3	MS
1553	Linalool	-	0.6	1.4	1.2	1.5	0.5	0.7	0.8	t_R, MS
1586	Pinocarvone	-	0.7	-	-	-	-	1.3	-	MS
1600	Hexadecane	1.4	-	-	-	-	-	-	-	t_R, MS
1612	β-Caryophyllene	26.1	12.6	9.3	7.3	15.7	3.8	2.6	3.5	t_R, MS
1648	Myrtenal	-	1.6	-	-	-	-	-	0.4	MS
1662	Pulegone	-	-	-	-	1.6	-	-	0.8	t_R, MS
1664	Ishwarane	-	-	8.9	-	-	-	-	-	MS
1664	Benzene acetaldehyde	1.7	1.5	-	-	-	-	-	-	MS
1686	Methyl chavicol	-	-	-	-	0.4	-	-	0.2	MS
1687	α-Humulene	24.8	17.1	7.7	9.4	14.7	3.9	6.5	5.4	t_R, MS
1690	*trans*-Verbenol	-	3.5	-	-	-	-	3.7	-	MS
1706	α-Terpineol	-	-	-	-	-	-	-	0.2	t_R, MS
1726	Germacrene D	-	-	2.1	3.4	-	3.1	-	2.9	MS
1726	Verbenone	-	1.3	-	-	0.7	-	1.2	-	t_R, MS
1747	*p*-Mentha-1,5-dien-8-ol	-	tr	-	-	-	-	0.2	1.4	MS
1755	Bicyclogermacrene	-	-	-	7.9	1.1	-	-	-	t_R, MS
1783	2-Methyl benzofuran	-	-	-	-	5.4	-	-	-	MS
1802	Cumin aldehyde	-	0.6	-	2.2	1.7	0.6	2.3	0.6	t_R, MS
1868	(E)-Geranyl acetone	-	-	-	-	-	0.8	-	-	t_R, MS
1882	1-Isobutyl 4-isopropyl 3-isopropyl -2,2-dimethyl succinate	2.5	0.7	-	-	0.4	0.6	-	-	MS
1949	Piperitenone	-	-	-	-	0.7	-	-	-	MS
1958	(E)-β-Ionone	-	-	-	-	0.7	-	-	-	MS
2000	Eicosane	-	-	3.2	-	-	-	-	-	t_R, MS
2001	Isocaryophyllene oxide	-	0.5	-	-	-	-	-	-	MS
2008	Caryophyllene oxide	9.1	5.8	15.2	-	4.7	-	-	-	t_R, MS
2016	(E)-9-Eicosene	-	0.2	-	-	-	-	-	-	MS
2071	Humulene epoxide II	5.0	2.6	7.2	-	2.1	-	-	-	MS
2131	Hexahydrofarnesyl acetone	tr	0.3	4.3	-	1.1	-	-	-	t_R, MS

Table 1 (*continue*). The composition of the essential oils of *L. cardiaca* and *L. glaucescens*

RRI	Compounds	% $LC1_{MD}$	% $LC2_{MD}$	% $LC3_{MD}$	% $LC4_{MD}$	% $LC5_{MD}$	% $LG1_{MD}$	% $LG2_{MD}$	% $LG3_{MD}$	IM
2200	Docosane	-	-	3.1	-	-	-	-	-	t_R, MS
2380	Dihydroactinidiolide	-	-	5.0	-	-	-	-	-	MS
2400	Tetracosane	-	-	3.1	-	-	-	-	-	t_R, MS
Monoterpene hydrocarbons		9.5	33.3	1.7	47.7	31.6	80.3	69.8	71.4	
Oxygenated monoterpenes		2.2	12.2	1.4	12.8	19.9	6.6	12.0	6.5	
Sesquiterpene hydrocarbons		50.9	29.7	29.1	32.1	31.5	11.1	10.1	13.2	
Oxygenated sesquiterpenes		14.1	8.9	22.4	-	6.8	-	-	-	
Others		18.3	6.0	44.3	7.5	8.3	1.6	6.2	6.3	
Total identified compounds (%)		95.0	90.1	98.9	100	98.1	99.6	98.1	97.4	

RRI: Relative retention indices experimentally calculated against *n*-alkanes; %: calculated from FID data; tr: Trace (<0.1 %); Identification Method (IM): t_R, Identification based on comparison with co-injected with standards on a HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the libraries. LC1: *Leonurus cardiaca* (İnegöl, Süle village); LC2: *L. cardiaca* (İnegöl, Sarıpınar); LC3: *L. cardiaca* (İnegöl, Turgutalp village); LC4: *L. cardiaca* (Kocaeli, Arıklar village); LC5: *L. cardiaca* (Edirne); LG1: *L. glaucescens* (Erzurum, Geçitköy, Tekman); LG2: *L. glaucescens* (Erzurum, Tekman); LG3: *L. glaucescens* (Erzurum, Tortum)

Table 2. The composition of the essential oils of *L. quinquelobatus* and *L. persicus*

RRI	Compounds	% $LQ1_{MD}$	% $LQ2_{MD}$	% $LP1_{MD}$	IM
1032	α-Pinene	4.7	6.1	48.6	t_R, MS
1035	α-Thujene	1.1	-	-	MS
1076	Camphene	1.1	1.7	-	t_R, MS
1093	Hexanal	1.5	1.0	-	t_R, MS
1118	β-Pinene	3.9	6.1	1.1	t_R, MS
1132	Sabinene	0.9	1.1	-	t_R, MS
1138	Thuja-2,4 (10)-dien	-	-	4.2	MS
1159	δ-3-Carene	-	0.4	-	MS
1174	Myrcene	4.2	5.9	1.1	t_R, MS
1188	α-Terpinene	1.0	0.8	0.4	t_R, MS
1203	Limonene	9.2	9.9	3.1	t_R, MS
1213	1,8-Cineole	12.3	16.3	2.1	t_R, MS
1215	p-mentha-1,3,6-triene	-	-	0.8	MS
1244	Amyl furan (*2-Pentyl furan*)	1.4	-	-	MS
1255	γ-Terpinene	8.1	7.3	2.0	t_R, MS
1280	*p*-Cymene	19.4	18.1	6.9	t_R, MS
1290	Terpinolene	-	tr	tr	t_R, MS
1400	Nonanal	0.9	-	-	MS
1406	α-Fenchone	1.0	0.9	-	MS
1435	γ-Campholene aldehyde	-	-	0.4	MS
1452	1-Octen-3-ol	-	1.2	-	t_R, MS
1452	*p*-Cymenene	-	-	0.4	MS
1457	β- Thujone	-	-	0.1	MS
1499	α-Campholene aldehyde (=Campholenal)	-	-	2.4	MS
1532	Camphor	3.1	3.7	1.2	t_R, MS
1535	Dihydroedulane I	1.0	-	0.6	MS
1541	Benzaldehyde	1.0	-	-	MS
1548	(E)-2-Nonenal	tr	-	-	MS
1553	Linalool	1.3	1.5	0.6	t_R, MS
1586	Pinocarvone	-	-	1.4	MS
1589	β-Ylangene	-	0.6	-	MS
1597	β-Copaene	-	0.6	-	MS
1600	β-Elemene	-	0.8	-	MS
1600	Hexadecane	3.6	-	-	t_R, MS
1612	β-Caryophyllene	-	0.5	-	t_R, MS
1648	Myrtenal	-	-	1.1	MS
1664	Benzene acetaldehyde	1.2	-	-	MS
1670	*trans*-Pinocarveol	-	-	2.2	t_R, MS
1687	α-Humulene	1.2	-	-	t_R, MS
1690	*trans*-Verbenol	-	-	8.6	MS
1726	Germacrene D	1.0	12.4	-	MS
1726	Verbenone	-	-	5.3	t_R, MS
1747	*p*-Mentha-1,5-dien-8-ol	-	-	2.4	MS

Table 2 (*continue*). The composition of the essential oils of *L. quinquelobatus* and *L. persicus*

RRI	Compounds	% LQ1$_{MD}$	% LQ2$_{MD}$	% LP1$_{MD}$	IM
1751	Carvone	-	-	0.3	t_R, MS
1755	Bicyclogermacrene	2.1	-	-	t_R, MS
1802	Cumin aldehyde	-	2.3	0.7	t_R, MS
1804	Myrtenol	-	-	0.5	MS
1845	*trans*-Carveol	-	-	0.5	t_R, MS
2131	Hexahydrofarnesyl acetone	2.8	-	-	t_R, MS
2144	Spathulenol	1.6	-	-	t_R, MS
Monoterpene hydrocarbons		53.6	57.4	68.6	
Oxygenated monoterpenes		17.7	26.5	29.8	
Sesquiterpene hydrocarbons		4.3	14.3	-	
Oxygenated sesquiterpenes		1.6	-	-	
Total identified compounds (%)		90.6	99.2	99.0	

RRI: Relative retention indices experimentally calculated against *n*-alkanes; %: calculated from FID data; tr: Trace (<0.1 %); Identification Method (IM): t_R, Identification based on comparison with co-injected with standards on a HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the libraries. LQ1: *L. quinquelobatus* (Bolu, Taşkesti); LQ2: *L. quinquelobatus* (Kocaeli 1); LP1: *L. persicus* (Erzurum, Tekman)

Table 3. Volatile compounds (HS-SPME) of *L. cardiaca* and *L. glaucescens*

RRI	Compounds	% LC1$_{HS}$	% LC2$_{HS}$	% LC3$_{HS}$	% LC4$_{HS}$	% LC5$_{HS}$	% LG1$_{HS}$	% LG2$_{HS}$	% LG3$_{HS}$	IM
1032	α-Pinene	-	29.2	53.9	56.1	42.0	61.9	69.9	32.5	t_R, MS
1132	Sabinene	-	-	-	-	-	2.6	2.1	-	t_R, MS
1174	Myrcene	-	-	-	-	-	-	-	1.6	t_R, MS
1203	Limonene	-	-	-	-	-	8.2	6.8	2.5	t_R, MS
1246	(*Z*)-β-Ocimene	-	-	-	-	2.3	-	5.7	-	t_R, MS
1266	(E)-β-Ocimene	8.0	-	-	-	-	-	1.1	-	t_R, MS
1280	*p*-Cymene	-	-	-	-	2.6	1.2	-	-	t_R, MS
1300	Tridecane	-	-	-	-	-	-	-	1.7	t_R, MS
1327	(Z)-3-Hexenyl acetate	-	-	-	-	-	-	-	5.0	MS
1382	*cis*-Alloocimene	8.0	-	-	-	2.1	-	4.2	-	MS
1391	(*Z*)-3-Hexenol	1.0	-	-	-	-	-	-	8.5	t_R, MS
1452	1-Octen-3-ol	2.0	-	-	5.1	-	1.7	-	0.6	t_R, MS
1495	Bicycloelemene	-	-	-	8.5	-	-	-	0.5	MS
1496	2-Ethyl hexanol	-	-	-	1.3	-	-	-		MS
1497	α-Copaene	-	-	-	-	-	-	-	0.5	MS
1535	α-Bourbonene	-	-	-	0.9	-	1.6	0.9	3.5	t_R, MS
1538	*trans*-Chrysanthenyl acetate	-	-	-	-	-	-	-	3.7	MS
1541	Benzaldehyde	1.5	-	-	1.7	-	3.1	0.7	-	MS
1589	β-Ylangene	-	-	-	-	-	-	-	3.5	MS
1597	β-Copaene	-	-	-	-	-	-	-	2.1	MS
1600	β-Elemene	-	-	-	-	-	-	-	0.7	MS
1612	β-Caryophyllene	44.3	39.3	26.9	9.1	28.4	8.0	3.2	8.4	t_R, MS
1684	γ-Gurjunene								0.6	MS
1687	α-Humulene	29.5	31.5	19.1	9.0	20.0	7.3	3.0	7.2	t_R, MS
1704	γ-Muurolene	-	-	-	-	-	-	-	0.8	MS
1726	Germacrene D	-	-	-	0.9	-	1.1	0.1	11.5	MS
1726	Verbenone	-	-	-	-	0.4	-	-	-	t_R, MS
1755	Bicyclogermacrene	-	-	-	5.4		-	-	-	t_R, MS
1758	(*E,E*)-α-Farnesene	-	-	-	-	-	-	-	0.4	MS
1773	δ-Cadinene	-	-	-	-	-	-	-	1.1	t_R, MS
1776	γ-Cadinene	-	-	-		-	-	-	0.5	MS
1798	Methyl salicylate	2.3	-	-	-	-	-	-	-	MS
Monoterpene hydrocarbons		16.0	29.2	53.9	56.1	49.0	73.9	89.8	36.6	
Oxygenated monoterpenes		-	-	-	-	0.4	-	-	-	
Sesquiterpene hydrocarbons		73.8	70.8	46.0	33.8	48.4	18.0	7.2	41.3	
Others		6.8			8.1		4.8	0.7	19.5	
Total identified compounds (%)		96.6	100.0	99.9	98.0	97.8	96.7	97.7	97.4	

RRI: Relative retention indices experimentally calculated against *n*-alkanes; %: calculated from FID data; tr: Trace (<0.1 %); Identification Method (IM): t_R, Identification based on comparison with co-injected with standards on a HP Innowax column;

MS, identified on the basis of computer matching of the mass spectra with those of the libraries. LC1: *Leonurus cardiaca* (İnegöl, Süle village); LC2: *L. cardiaca* (İnegöl, Sarıpınar); LC3: *L. cardiaca* (İnegöl, Turgutalp village); LC4: *L. cardiaca* (Kocaeli, Arıklar village); LC5: *L. cardiaca* (Edirne); LG1: *L. glaucescens* (Erzurum, Geçitköy, Tekman); LG2: *L. glaucescens* (Erzurum, Tekman); LG3: *L. glaucescens* (Erzurum, Tortum)

Table 4. Volatile compounds (HS-SPME) of *L. quinquelobatus* and *L. persicus*

RRI	Compounds	% $LQ1_{HS}$	% $LQ2_{HS}$	% $LP1_{HS}$	IM
1032	α-Pinene	-	-	34.6	t_R, MS
1174	Myrcene	-	-	3.5	t_R, MS
1203	Limonene	-	-	7.7	t_R, MS
1246	(*Z*)-β-Ocimene	-	-	22.8	t_R, MS
1266	(E)-β-Ocimene	-	-	3.5	t_R, MS
1280	*p*-Cymene	1.1	-	-	t_R, MS
1300	Tridecane	-	7.3	-	t_R, MS
1382	*cis*-Alloocimene	-	-	25.1	MS
1403	*trans*-Alloocimene	-	-	1.3	MS
1452	1-Octen-3-ol	2.0	3.9	-	t_R, MS
1495	Bicycloelemene	11.2	-	-	MS
1496	2-Ethyl hexanol	0.4	0.7	-	MS
1497	α-Copaene	0.6	1.5	-	MS
1535	β-Bourbonene	1.6	6.8	-	t_R, MS
1538	*trans*-Chrysanthenyl acetate	-	-	1.2	MS
1541	Benzaldehyde	1.0	2.9	-	MS
1562	Octanol	1.4	2.4	-	t_R, MS
1589	β-Ylangene	6.0	10.7	-	MS
1597	β-Copaene	3.7	6.9	-	MS
1600	β-Elemene	1.2	1.1	-	MS
1612	β-Caryophyllene	9.1	-	-	t_R, MS
1628	Aromadendrene	1.8	-	-	MS
1651	γ-Elemene	2.9	-	-	MS
1684	γ-Gurjunene	1.9	-	-	MS
1687	α-Humulene	9.7	2.3	-	t_R, MS
1688	selina-4,11-diene	4.5	-	-	MS
1704	γ-Muurolene	1.6	3.6	-	MS
1726	Germacrene D	18.7	33.2	-	MS
1733	γ-Amorphene		1.6	-	MS
1740	α-Muurolene	0.5	1.0	-	MS
1755	Bicyclogermacrene	9.6	-	-	t_R, MS
1773	δ-Cadinene	2.2	4.8	-	t_R, MS
1776	γ-Cadinene	1.3	2.5	-	MS
1785	7-*epi*-α-Selinene	0.3	-	-	MS
Monoterpene hydrocarbons		1.1	-	98.5	
Sesquiterpene hydrocarbons		88.4	76	-	
Others		4.8	17.2	1.2	
Total identified compounds (%)		94.3	93.2	99.7	

RRI: RRI Relative retention indices experimentally calculated against n-alkanes; %: calculated from FID data; tr: Trace (<0.1 %); Identification Method (IM): tR, Identification based on comparison with co-injected with standards on a HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the libraries. LQ1: *L. quinquelobatus* (Bolu, Taşkesti); LQ2: *L. quinquelobatus* (Kocaeli 1); LP1: *L. persicus* (Erzurum, Tekman)

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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