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Essential oil composition of leaves of *Pistacia lentiscus* L. growing wild in Valencia (Spain)

Juan A. Llorens-Molina[1*], Sandra Vacas González[2] and Josep Sabater Martínez[3]

[1] Mediterranean Agroforestry Institute, Universitat Politècnica de València, Camí de Vera s/n, 46022, Valencia SPAIN
[2] Centre for Agricultural Chemical Ecology, (Mediterranean Agroforestry Institute), Universitat Politècnica de València.
[3] School of Agricultural Engineering and Environment, Universitat Politècnica de València.

*Corresponding author. Email: juallom2@qim.upv.es

Abstract

Pistacia lentiscus L. grows wild in a wide range of habitats in Mediterranean countries. The essential oil (EO) composition of its aerial parts has been extensively studied showing noticeable differences according to plant organ (leaves and twigs or fruits), geographical origin and harvest time. In this study, EO composition of leaves of samples coming from two representative habitats from Valencia (Spain) -siliceous (Segart) and calcicolous (Xeraco) soils-were determined by GC/MS and GC/FID. Monoterpene hydrocarbons were found as the major fraction (57.5 %) in calcicolous soil, whereas sesquiterpene hydrocarbons were in siliceous soil (39.9 %). Oxygenated monoterpenes reached a significantly higher amount in leaves from siliceous location (11.2 %) as against the calcicolous one (6.7 %). The rate of oxygenated sesquiterpenes was also higher in siliceous soil (15.2 % in contrast to 1.6 % in calcicolous one. These results lead to consider soil parameters as hypothetical source of EO composition variability being the target for further researches.

Keywords: *Pistacia lentiscus*, essential oil, leaves, siliceous soil, calcicolous soil

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Introduction

Pistacia lentiscus L is a termophilous evergreen shrub growing wild in a wide range of habitats throughout the Mediterranean countries and Canary Islands. It is abundant in sunny scrublands of Eastern and Southern Iberian Peninsula, sharing their habitats with species such as *Chamaerops humilis* L., *Quercus coccifera* L., *Rhamnus lycioides* L., etc., being adapted to a varied range of substrates (Del Campo et al., 2015). It has been used in folk medicine as antihypertensive, while its resin from stems (gum mastic) is used for the treatment of digestive diseases (Vidrich et al., 2004). More recently, antimicrobial, antifungicidal, antioxidant and other pharmacological activities have been reported (Nahida et al., 2012)

The essential oil (EO) composition of its aerial parts (leaves and twigs, fruits and galls) has been extensively studied. Recent data from many studies carried out in different Mediterranean countries, detailing the major components for each type of terpenic components, are displayed in Table 1. A wide range of chemical variability affecting both major compounds and total amounts of terpene classes can be noted. Generally, monoterpene hydrocarbons is the major fraction, mainly in ripe fruits (96.3 – 98.8 %) as reported by Boelens & Jiménez, (1991) and Vidrich et al., (2004).

Table 1. Chemical composition of *Pistacia lentiscus* EO (leaves, twigs and fruits) from literature

References. **Plant material and harvest stage**	**Sampling location**	**Major compounds (> 5 %) by hydrodistillation (% peaks area)**	**Monoterpenes**		**Sesquiterpenes**	
			No oxig.	**Oxig.**	**No oxig.**	**Oxig.**
Boelens & Jiménez (1991), October (fruiting)	Spain (Sevilla)	Leaves: α-pinene (11.20 %), β-myrcene (19.25 %), terpinen-4-ol (8.41 %), α-terpineol (6.68 %), β-caryophyllene (8.60 %), germacrene-D (6.35 %)	50.3 %	19.41 %	24.54 %	1.65 %
		Unripe fruits: α-pinene (21.65 %), β-myrcene (54.46 %)	90.0 %	1.83 %	2.39 %	0.07 %
		Ripe fruits: α-pinene (10.50 %), β-myrcene (72.25 %), limonene (6.70 %)	96.3 %	0.68 %	1.01 %	0.03 %
Fernández et al. (2000). Air-dried aerial parts. Fruiting	Spain (Jaén)	α-pinene (13.0 %), limonene, β-phellandrene (5.4 %), β-caryophyllene (6.9 %)	33.4 %	7.3 %	24.8 %	13.3 %
Zrira et al. (2003), Air dried aerial parts (leaves and twigs). Monthly sampling, december to june	Morocco[a]					
	Mehdia	Terpinen-4-ol (4.8-7.5 %), limonene (6.7-8.1 %), α-pinene (4.8-7.5 %), caryophyllene oxide (6.5-10.3 %), sabinene (3.3-6.2 %), γ-terpinene (5.1-6.0 %)	26.8-41.2 %	19.1-25.2 %	6.1-11.8 %	6.7-10.8 %
	Chaouen	Terpinen-4-ol (32.7-43.8 %), α-pinene (7.1-13.5 %), bornyl acetate (6.8-10.3 %), β-caryophyllene (4.3-5.2 %), sabinene (4.2-5.4 %)	20.0-45.6 %	39.5-55.0 %	9.0-17.0 %	1.2-1.6 %
	Oulmes	α-pinene (16.5-38.5 %), β–myrcene (10.2-11.5 %), limonene (6.8-9.8 %)	41.9-75.8 %	0.8-3.1 %	4.4-8.8 %	0.1-0.6 %
Vidrich et al.(2004) Seasonal sampling: Winter, spring, summer, autumn (fruiting) over 2 years	Italy (Tuscany)[a]	Leaves: α-pinene (16.1-25.3 %), germacrene-D (9.6-14.3 %), terpinen-4-ol (7.6-12.7 %), β-caryophyllene (5.2-8.7 %), β-myrcene (4.6-8.3 %), β-pinene (4.2-6.8 %), δ-cadinene (2.7-5.2 %), β-phellandrene (2.4-5.0 %)	56.9-64.4 %	7.8-12.9 %	21.0-29.1 %	-
		Fruits: α-pinene (7.5-11.2 %), β-myrcene (68.2-71.2 %), limonene (9.6-19.7 %)	97.8-98.8 %	0.2-1.3 %	0.7 %	-
Kivçak et al. (2004) Twigs and leaves, separately. February	Çeşme-Ardiç (Turkey)	Twigs: Sabinene (23.2 %); α-pinene (19.4 %); germacrene-D (14.1 %); limonene (6.9 %); β-phellandrene (6.5 %); terpinen-4-ol (5.7 %)	49.0 %	6,6 %	21.4 %	4.9 %
		Leaves: terpinen-4-ol (29.2 %); *p*-cymene (7.1 %)	12.8 %	36.0 %	15.0 %	15.20 %
Ben Douissa et al. (2005), fresh leaves	Tunisia (Zaghouan)	α-pinene (16.8 %), 4-terpineol (11.9 %), β-phellandrene (8.9 %), sabinene (5.7 %), γ-terpinene (5.5 %),	59.5 %	16.4 %	12.7 %	-
Tahar Dob et al. (2006). Air dried aerial parts	Algeria					
	Algiers	Longifolene (12.8 %), γ-cadinene (6.2 %), (*E*)-β-terpineol (5.0 %)	13.6 %	20.0 %	35.5 %	14.9 %
	Tizi-Ozou	Longifolene (16.4 %), (*E*)-β-terpineol (15.6 %), terpinen-4-ol (7.0 %), γ-muurolene (5.7 %)	13.5 %	3.6 %	29.9 %	5.1 %
	Oran	α–pinene (19.0 %), (*E*)-β-terpineol (13.1 %), sabinene (12.6 %), β-pinene (6.5 %), (*E*)-β-ocimene (5.5 %), longifolene (5.2 %)	56.3 %	23.1 %	10.9 %	3-0 %
Barra et al. (2007) Fresh aerial parts. Full flowering	Sardinia (Italy)					
	Torre delle Stele	α-pinene (20.40 %), β-myrcene (18.29 %), p-cymene (14.79 %), terpinen-4-ol (14.17 %)	70.01 %	20.33 %	4.99 %	t
	Orroli	α-pinene (19.15 %), β-myrcene (19.36 %), terpinen-4-ol (15.07 %) p-cymene (9.92 %)	68.54 %	21.46 %	6.81 %	t
	Villaputzu	Terpinen-4-ol (19.74 %), α-pinene (14.81 %), p-cymene (14.79 %), β-myrcene (10.84 %), γ-				

		terpinene (6.56 %), α-terpinene (5.12 %),	57.24 %	24.2 %	12.87 %	2.75 %
	Oristano	α-pinene (21.55 %), terpinen-4-ol (19.84 %), p-cymene (14.79 %), α–phellandrene (10.84) %, β–phellandrene (5.39 %), γ-terpinene (5.15 %)	67.42 %	23.73 %	6.30 %	t
	Alghero	Terpinen-4-ol (28.29 %), α-pinene (22.59 %), sabinene (8.13 %), *p*-cymene (16.22 %)	58.88 %	34.31 %	3.26 %	0.36 %
Gardeli et al. (2008) Air dried leaves. Before flowering, flowering, fruiting	Greece (Zakynthos)[a]	α-pinene (9.4-24.9 %), myrcene (0.1-33.1 %), sabinene (1.0-6.7 %), β-pinene (2.0-6.9 %), limonene (9.0-17.8 %), terpinen-4-ol (2.7-13.5 %), germacrene D (2.7-13.5%)	45.0-68.3%	13.3-23.1 %	9.2-28.1%	0.5-1.6 %
Lo Presti et al. (2008) Leaves and twigs, Vegetative stage	Italy (Sicily)	Leaves: germacrene-D (18.61 %), β-caryiophyllene (14.68 %), δ-cadinene (11.13 %), epi-α-muurolol (5.79 %), α-cadinol (5.36 %)	3.05 %	0.97 %	67.08 %	15.61 %
		Twigs: germacrene-D (16.77 %), sabinene (12.19 %), α-phellandrene (7.56 %)	34.7 %	0.81 %	38.20 %	10.75 %
Mecherara-Idjeri et al. (2008) Leaves and fruits (air dried for 2-3 days). September-november	Algeria[b] Leaves (17 locations)	Leaves: limonene (0.1-43.8 %), α-pinene (0.5-34.2 %), myrcene (0.1-33.1 %), β-pinene (0.2-21.8 %), α-phellandrene (tr-14.1 %), sabinene (0.1-11.7 %), p-cymene (0.1-7.7 %), camphene (tr-7.1 %), terpinen-4-ol (0.2-7.0 %), germacrene D (2.7-13.5%), germacrene-D (0.0 – 10.3 %), β-caryophyllene (0.7-2.4 %).	2.1-76.1 %	0.7-12.5 %	6.5-45.4 %	1.8-25.9 %
	Fruits (9 locations)	Fruits: myrcene (0.9-69.7 %), α-pinene (9.4-51.5 %), limonene (0.8-24.1 %), sabinene (0.1-11.6 %), β-pinene (2.8-6.8 %), camphene (0.5-5.8 %)	70.4-94.0 %	1.1-12.2 %	1.7-9.4 %	0.4-4.7 %
Amhamdi et al. (2009). Leaves. February	Morocco (Tafoghalt)	β-myrcene (39.2 %), limonene (10.3 %), β-gurgujene (7.8 %)	57.7 %	2.6 %	26.1%	1.6 %
Djenane et al. (2011) Shopping precedence	France (biological cultive)	β-myrcene (15.18 %), 1,8-cineol (15.02 %), terpinen-4-ol (6.41 %), α-pinene (5.54 %), β-pinene (5.10 %)	40,21 %	33,42 %	8,10 %	-
Ismail et al. (2012) Fresh leaves. October	Tunisia (Korbous)	α–pinene (20.6 %), limonene (15.3 %), β-pinene (9.6 %), germacrene-D (8.4 %), terpinen-4-ol (8.2 %)	63.9 %	11.7 %	19.6 %	3.7 %
Hafsé et al. (2013) Dried leaves and twigs	Morocco (Taounate)	Leaves: myrcene (25.3 %), limonene (15.7 %), terpinen-4-ol (9.2 %)	57.6 %	16.8%	10.8%	1.3%
		Twigs: myrcene (34.1 %), limonene (9.6 %), β-gurgujene (9.6 %), terpinen-4-ol (9.2 %)	60.0 %	14.4 %	26.4 %	2.9 %
Haloui et al., (2015) Fresh leaves and twigs. May (flowering stage)	Morocco (Taounate)	Leaves: Tricyclene (7.71 %), terpinen-4-ol (7.44 %), sabinene (6.96 %), β-caryophyllene (6.62 %), caryophyllene oxide (6.05 %), *p*-cymene (5.04 %)	33.52 %	16.12 %	18.93 %	8.77 %
		Twigs: α-pinene (19.24 %), tricyclene (8.16 %), (*E*)-β-ocimene (6.90 %), β-caryophyllene (6.18 %), germacrene-D (5.17), 3-carene (5.18 %).	48.12 %	4.53 %	24.48 %	6.97 %

[a] Values range over sampling period is displayed

[b] Values range from sampling location

The major part of studies displayed in Table 1 are focused on fresh or dried leaves and twigs. Monoterpene hydrocarbons are considered as the predominant fraction in some studies (20.0 – 75.8 %) (Boelens & Jiménez, 1991; Fernandez, Camacho, Fernández, Altarejos & Pérez, 2000; Zrira et al., 2003; Vidrich et al., 2004; Ben Douissa et al., 2005; Dob, Dahmane & Chelghoum (2006); Barra, Coroneo, Dessi, Cabras & Angioni (2007).; Gardeli et al., 2008; Amhamdi, Aouinti, Wathelet & Elbachiri, (2009); Djenane, Yangüela, Montañés, Djerbal & Roncalés (2011); Ismail, Lamia, Mohsen & Bassem, (2012); Hafsé, Benbrahim, Saidi, & Farah, (2013); Haloui et al., 2015). Nevertheless, strong variability can be observed according to the harvest time (Zrira, Elamrani & Benjilali, (2003); Vidrich et al., 2004; Gardeli, Vassiliki, Athanasios, Kibouris & Komaitis, (2008) and the place of sampling, even when it is carried out in close locations in the same country (Zrira et al., 2003; Dob et al., 2006; Mecherara-Idjeri, Hassani, Castola & Casanova, 2008a). Indeed, samples containing up to 82.69 % of sesquiterpene fraction have been reported (Lo Presti et al., 2008) and oxygen-containing monoterpenes in leaves fluctuate between 0.97 % (Lo Presti et al., 2008) and 55.0 % (Zrira et al., 2003). Small amounts of diterpenes (dimyrcene isomers) have also been reported by Boelens & Jiménez (1991) (0.47 %, 0.53 %, 0.81 % in leaves, unripe and ripe fruits, respectively) and Lo Presti et al. (2008) (0.16 % in twigs). This strong chemical variability agrees with the high degree of morphological and physiological plasticity related to its environmental tolerance and therefore, its wide range of habitats in which it grows (Nahum, Inbar, Ne'eman & Ben-Shlomo, (2008).

The aim of this work is to extend the knowledge of EO profiles of *Pistacia lentiscus* leaves coming from Eastern Iberian Peninsula. Given that no references about type of soil are available in the above mentioned literature, a first approach to this source of variability may be interesting. For this purpose, two different sampling areas according to their geological features were selected. These ones, with similar climatic conditions, are some of the most representative habitats concerning soil composition (calcicolous and siliceous) in Valencia (Spain) natural environment.

Materials and Methods

Plant material

Three samples each one coming from ten individuals randomly selected were collected within a 10 ha land (approximately) in two different locations distant from each other by 90 km, approximately. Both sampling zones (Segart and Xeraco in Valencia, Spain) were Southeast-oriented and their climatic and geological features are detailed in Table 2.

Table 2. Climatic and geological features of sampling areas

Location	**Altitude (m a.s.l.)**	**Average yearly precipitation[a]**	**Average yearly temperature[a]**	**Thermicity Index[b]**	**Coordinates (centre of sampling zone)**	**Geological features[c]**
Segart	300 m	469 mm	15,8 ºC	341	39º 40’ 57’’ N; 0º 22´38 ” W	Silicious (argillites, sandstone)
Xeraco	250 m	485 mm	18,0 ºC	405	39º 01’ 18’’ N; 0º 15’ 26’’ W	Calcicolous (limestone lagoon, limestone with dolomite and orbitoline)

[a]Meteorological data from the nearest climatic station, [b]Thermicity index calculated as described by Martinez (1983)

[c]Source: **terrasit**.gva.es/

The leaves and fruits were harvested all around the shrub. A voucher specimen was placed in the Herbarium of Mediterranean Agroforestry Institute at the *Universitat Politècnica de València* (Spain) (VALA 9570-9571). After removing damaged and yellowish material, each whole sample was dried in a dry and shady place at room temperature for 20 days just before essential oil extraction.

Essential oil extraction

After homogenizing each sample, 50 g were subjected to hydrodistillation for 2.5 h in a Clevenger type apparatus (fruits and leaves were crushed with a domestic grinder immediately before extraction). The essential oil was swept away with 2 mL of dichloromethane (DCM). After removing aqueous phase, the essential oil extract was dried over anhydrous sodium sulphate. The solvent was removed by N_2 stream. A solution containing 10 μL EO/1 mL of DCM was kept in the dark at -18ºC in sealed vials until analysis.

GC analysis

The analysis of samples was carried out by gas chromatography with flame ionization detector (GC-FID) in a Clarus 500 GC (Perkin-Elmer Inc.) chromatograph equipped with a capillary column ZB-5 (30 m x 0.25 mm i.d. x 0.25 mm film thickness; Phenomenex Inc.).The injection volume was 1 μL. The GC oven temperature was programmed from 50ºC to 250°C at a rate of 3°C min^{-1}. Helium was the carrier gas (1.2 mL min^{-1}). Detector temperature was set at 250ºC. The percentage composition of the essential oil was computed from GC peak areas without correction factors by means of the software Total Chrom 6.2 (Perkin-Elmer Inc.).

Analysis by GC-MS was carried out using a Clarus 500 GC-MS (Perkin-Elmer Inc.) equipped with the same column mentioned above. Ionization source temperature was set at 200˚C and 70 eV electron impact mode was employed. MS spectra were obtained by means of total ion scan (TIC) mode (mass range m/z 45-500 uma). The total ion chromatograms and mass spectra were processed with the software Turbomass 5.4 (Perkin-Elmer Inc.).

Retention indices were determined by injection of C8–C25 *n*-alkanes standard (Supelco®) under the same GC conditions. The essential oil components were identified by comparison of their mass spectra with those of computer library NIST MS Search 2.0 and available data in the literature. The identification of the following compounds was confirmed by comparison of their experimental RI with those of authentic reference standards (Sigma-Aldrich®): α-pinene, β-pinene, camphene, myrcene, camphor, terpinolene, borneol, terpinen-4-ol, bornyl acetate and linalool.

Statistical analysis

The statistical analyses were carried out by means of one-way ANOVA using Statgraphics 5.1. Software. It was applied to the main compounds, as well as to the terpene classes. As the raw data were expressed as % peak areas, they were subjected to arcsin [square root (%/100)] transformation and previous homocedasticity test and the means were compared by Fisher's least significant difference (LSD).

Results and Discussion

Oil yields (calculated as oil V/wt. of air dried leaves) were 0.13 ± 0.03 % and 0.12 ± 0.02 % for samples from Xeraco and Segart, respectively. The average of triplicate values (± standard deviation) of EO composition are displayed in Table 3. 77 compounds, which represented 94.4-98.7 % were identified. Two main EO profiles could be distinguished according the rate of monoterpene/sesquiterpene composition in leaf samples. A predominance of monoterpenoid over sesquiterpenoid fraction was noted in samples collected from Xeraco (calcicolous soil) (64.2 %-33.8 %) whereas the opposite tendency was found in Segart (siliceous soil) (31.6 %-55.1 %). These different profiles agree with those referred in the literature. Indeed, data from other Spanish locations (Boelens & Jiménez, 1991; Fernández et al., 2000), Morocco (Amhamdi et al., 2009; Hafsé *et al.*, 2013; Haloui et al., 2015) and Tunisia (Ismail *et al.*, 2012) are quite similar to those that were found in calcicolous location. On the contrary, profiles as those reported by Dob et al. (2006) in Algiers (Algeria) and Lo Presti et al. (2008) in Sicily show a clear predominance of sesquiterpenes over monoterpenes, being closer to siliceous location. It is worth to mention the data reported by Mecherara-Idjeri et al. (2008) coming from Algeria, in which very wide range of variation concerning both terpene classes and main components was found.

Significant differences were found within these fractions. Monoterpene hydrocarbons rate was higher for calcicolous soil (57.5 %), as against 20.4 % in siliceous one. On the contrary, oxygenated monoterpenes and sesquiterpenes were also significantly higher for siliceous soil (11.2 – 6.7 % and 15.2 - 1.6 %, respectively). No significant differences were observed for sesquiterpene hydrocarbons fraction.

With regard to main compounds, EO from leaves showed significant differences concerning the main monoterpene hydrocarbons. α-pinene (19.0 %) and limonene (8.5 %), together with δ-3-carene, β-pinene and sabinene reach the higher levels in calcicolous soil. In the same way, samples rich in α-pinene, limonene and some of the aforementioned compounds are also reported by Fernández et al. (2000), Ben Douissa et al. (2005), Dob et al. (2006), Barra et al. (2007) in samples from Alghero and Gardeli et al. (2008). However, myrcene is the predominant monoterpene hydrocarbon in the siliceous location. This profile is also reported by Boelens & Jiménez (1991), Amhamdi et al. (2009) and Djenane et al. (2011). Other data from literature show more equilibrate rates between myrcene and the rest of monoterpene hydrocarbons (Zrira et al., 2003, in Oulmes (Morocco) and Barra et al. 2007).

In agreement with previous studies, terpinen-4-ol is the main oxygenated monoterpene accounting for 5.1-6.7 %, without significant difference between locations. It is worth mentioning that this compound reaches high rates in *P. lentiscus* EO from several origins being reported as major component by Zrira et al. (2003) in samples from Mehdia (Morocco), Kivçak *et al*. (2004) and Barra et al. (2007) –samples from Alghero-.

In respect of sesquiterpene components, germacrene-D and β-caryophyllene are the major components as referred to by the majority of previous researches. Nevertheless, noticeable amounts of other sesquiterpene hydrocarbons such as longifolene (Dob et al., 2006), δ-cadinene (Lo Presti et al., 2008; Mecherara-Idjeri et al. 2008, in two locations) or β-gurjunene (Amhamdi et al., 2009) are also reported. Within the sesquiterpene fraction, oxygenated sesquiterpenes are very significant higher in siliceous location, which agrees with results from Turkey (Kivçak et al., 2004) and Sicily (Lo Presti et al., 2008). Moreover, this agreement also concerns to major compounds as epi-⍰-cadinol or epi-α-muurolol. It is worth to mention the occurrence of diterpenic fraction in siliceous location (5.9 %). This type of compounds are not reported in *P. lentiscus* aerial parts in the majority of previous studies except by Boelens & Jiménez (1991) and Lo Presti et al. (2008).

Table 3. Constituents (% peak areas) of EO of *Pistacia lentiscus* leaves from each sampling location.

			% peaks area[a]	
	RI	**Identification**	**Segart**	**Xeraco**
tricyclene[b]	925[c]	MS, RI[d]	0.1 ± 0.1	0.3 ± 0.1
α-thujene	929	MS, RI, St.	tr[e]	0.2 ± 0.1
α-pinene	**937**	MS, RI	**2.4 ± 1.3a**	**19.0 ± 5.7b**
camphene	953	MS, RI, St.	0.5 ± 0.4	1.5 ± 0.4
sabinene	975	MS, RI	**0.9 ± 0.2a**	**6.1 ± 1.3b**
β-pinene	979	MS, RI, St.	**0.7 ± 0.5a**	**6.4 ± 1.1b**
myrcene	996	MS, RI, St.	**11.0 ± 8.3a**	**1.1 ± 0.0b**
δ-3-carene	1010	MS, RI	**1.4 ± 1.1a**	**7.2 ± 0.4b**
α-terpinene	1019	MS, RI, St.	0.4 ± 0.7	1.7 ± 0.5
p-cymene	1027	MS, RI, St.	0.2 ± 0.4	1.1 ± 0.4
limonene	**1032**	**MS, RI, St.**	**1.3 ± 1.6a**	**8.5 ± 0.4b**
(*Z*)-β-ocimene	1040	MS, RI	-[f]	tr
(*E*)-β-ocimene	1052	MS, RI	0.2 ± 0.3	0.3 ± 0.0
γ-terpinene	1061	MS, RI, St.	1.0 ± 1.2	3.0 ± 0.7
terpinolene	1086	MS, RI, St.	0.4 ± 0.6	0.9 ± 0.2
p-cimenene	1090	MS, RI	tr	tr
monoterpenes			**20.4 ± 16.6a**	**57.5 ± 11.3b**
linalool	1097	MS, RI, St.	tr	-
menhta-2-en-1-ol <*Z*-p>	1125	MS, RI	tr	0.1 ± 0.0
3-terpinen-1-ol	1134	MS, RI	tr	tr
menta-2-en-1-ol <*E*-p>	1141	MS, RI	-	tr
camphor	1148	MS, RI, St.	0.1 ± 0.1	0.0 ± 0.0
(*Z*)-β-terpineol	1152	MS, RI	tr	-
borneol	1173	MS, RI, St.	0.1 ± 0.2	-
terpinen-4-ol	**1181**	**MS, RI, St.**	**6.7 ± 1.6a**	**5.1 ± 0.5a**
α-terpineol	1194	MS, RI	2.8 ± 0.6	1.0 ± 0.3
γ-terpineol	1199	MS, RI	tr	-
β-cyclocitral	1223	MS, RI	0.0 ± 0.1	-
bornyl acetate	1283	MS, RI, St.	1.4 ± 1.1	0.5 ± 0.1
verbenone	1204	MS, RI, St.	tr	-
(*E*)-piperitol	1205	MS, RI	-	tr
carvacrol	1298	MS, RI, St.	tr	tr
Oxygenated monoterpenes			**11.2 ± 3.8a**	**6.7 ± 0.9b**
δ-elemene	1338	MS, RI	-	0.1 ± 0.0
α-copaene	1373	MS, RI	-	0.3 ± 0.1
Ylangene	1375	MS, RI	tr	tr
β-elemene	1386	MS, RI	0.4 ± 0.1	-
β-bourbonene	1380	MS, RI	-	0.8 ± 0.2

β-caryophyllene	**1415**	**MS, RI**	**12.8 ± 2.6a**	**11.1 ± 2.1a**
β-copaene	1432	MS, RI	-	tr
(*Z*)-muurola-3,5-diene	1450	MS, RI	tr	tr
α-humulene	1451	MS, RI	1.5 ± 0.2	1.3 ± 0.3
aromadendrene <allo>	1456	MS, RI	-	0.2 ± 0.0
γ-gurgujene	1472	MS, RI	-	0.3 ± 0.0
germacrene-D	**1485**	MS, RI	**17.5 ± 5.4a**	**10.8 ± 3.1a**
β-selinene	1488	MS, RI	-	-
bicyclogermacrene	1490	MS, RI	1.6 ± 0.5	0.9 ± 0.3
(*E*)-muurola-4(14),5-diene	1494	MS, RI	0.1 ± 0.1	0.4 ± 0.1
α-muurolene	1500	MS, RI	0.9 ± 0.2	0.9 ± 0.2
β-cadinene	1501	MS, RI	-	0.1 ± 0.0
β-bisabolene	1504	MS, RI	-	0.1 ± 0.1
(E,E)-α-farnesene	1506	MS, RI	0.4 ± 0.4	0.2 ± 0.1
γ-cadinene	1508	MS, RI	0.6 ± 0.2	0.5 ± 0.0
δ-cadinene	1514	MS, RI	4.0 ± 1.4	3.6 ± 0.8
(*E*)-cadina-1,4-diene	1528	MS, RI	-	0.1 ± 0.0
zonarene	1530	MS, RI	-	0.3 ± 0.1
(*E*)-γ-bisabolene	1531	MS, RI	0.1 ± 0.2	0.1 ± 0.1
α-cadinene	1533	MS, RI	-	0.1 ± 0.0
Sesquiterpenes			**39.9 ± 11.5a**	**32.2 ± 7.6a**
elemol	1550	MS, RI	tr	0.1 ± 0.1
(*E*)-nerolidol	1563	MS, RI	-	0.1 ± 0.0
caryophyllene oxide	1576	MS, RI	0.9 ± 0.3	0.1 ± 0.1
globulol	1585	MS, RI	1.1 ± 0.4	0.1 ± 0.1
viridiflorol	1593	MS, RI	0.8 ± 0.4	0.1 ± 0.1
rosifoliol	1599	MS, RI	0.1 ± 0.1	-
junenol	1605	MS, RI	tr	-
cubenol <1,10-di-epi>	1622	MS, RI	0.1 ± 0.2	tr
γ-eudesmol	1632	MS, RI	0.5 ± 0.2	0.2 ± 0.1
cadinol-epi-α	**1638**	**MS, RI**	**4.9 ± 3.4a**	**0.6 ± 0.2b**
muurolol-epi-α	**1640**	**MS, RI**	**9.1 ± 4.8a**	**0.8 ± 0.5b**
α-muurolol	1642	MS, RI	0.5 ± 0.5	-
Oxygenated sesquiterpenes			**15.2 ± 9.2a**	**1.6 ± 0.9b**
unidentified diterpene ($C_{20}H_{32}$) [g]	1949	MS, RI	4.8 ± 2.2	-
manoyl oxide	1991	MS, RI	1.1 ± 0.8	-
diterpenoids			**5.9 ± 3.0**	**0**
3-hexen-1-ol	854	MS, RI	tr	tr
2-hexenal	855	MS, RI	-	tr
nonanal	1105	MS, RI	0.2 ± 0.3	tr
2-nonanone	1092	MS, RI	tr	tr
isopentyl isovalerate	1107	MS, RI	0.1 ± 0.1	0.1 ± 0.0
2-methyl butyl hexanoate	1253	MS, RI	0.1 ± 0.1	0.1 ± 0.0

2-undecanone	1292	MS, RI	1.2 ± 1.5	0.3 ± 0.2
(*Z*)-3-hexenyl n-hexanoate	1379	MS, RI	-	0.1 ± 0.0
3-methyl-1-butyl benzoate	1441	MS, RI	0.3 ± 0.2	0.2 ± 0.1
Other			**1.8 ± 2.2**	**0.7 ± 0.4**
Total identified			**94,4**	**98,7**

[a] Values within a row for each compound having different letters are significantly different from each other according Fisher's least significant difference (LSD) test ($P<0.05$). [b] Elution order as determined on ZB-5 column.[c] Retention indices as determined on ZB-5 column using homologous series of n-alkanes. [d] Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; St., by injection of an authentic sample. [e] t: traces (<0.1%).f -: no detected

[g] (*m/z*: 55 (45), 67 (64), 77 (40), 79 (60), 91 (100), 93 (65), 105 (60), 107 (42), 109 (45), 119 (59)

In summary, taking into account the very similar climatic conditions of both locations, soil features could be expected the main environmental source of variability of EO chemical profiles. Nevertheless, the high standard deviation of some results coming from the same location leads to consider genotypic diversity as another relevant factor of chemodiversity. For this reason, further studies based on matching chemical data of EO from individual samples and soil parameters could be advisable.

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