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Short communication

Analysis by gas chromatography–mass spectrometry of the volatile components of *Teucrium lusitanicum* and *Teucrium algarbiensis*

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Abstract

The essential oils from four samples of *Teucrium lusitanicum* and one sample of *Teucrium algarbiensis*, grown in Algarve (southern Portugal) were analyzed by gas chromatography (GC) and gas chromatography–mass spectroscopy (GC–MS). Seventy-one volatile compounds were identified. Major compounds of *T. algarbiensis* oil were α-pinene (8.3%), sabinene (7.2%), β-pinene (10.2%), limonene (11.8%) and germacrene D (7.6%). Concerning *T. lusitanicum*, some quantitative differences were found with regards to the major constituents of the oils from four populations: α-pinene (0.8–8.5%), sabinene (2.1–9.6%), β-pinene (2.5–11.9%), limonene (1.2–11.5%) and elemol (2.6–12.0%).

Keywords: *Teucrium* spp.; Essential oils; Volatile organic compounds

1. Introduction

The genus *Teucrium* L. (Lamiaceae) comprises more than 300 species, 15 of which growing wild in Portugal [1–3]. *Teucrium* species have been used as medicinal plants for more than 2000 years and some of them are still used in folk medicine as antispasmodic, tonic, antipyretic and antiseptic [4,5].

This genus is chemically characterized by high amounts of clerodane-type diterpenoids, some of them having biological relevance, particularly as insect antifeedants [6,7]. Volatile constituents are also characteristic of this genus and the essential oils of many species were studied in the last few years [8–14].

Following the characterization of the essential oils of *Teucrium salviastrum* (section Scorodonia) and *Teucrium capitatum* (section Polium) from Portugal [14,15], the authors now report on the chemical composition of the essential oils of two other section Polium species, *Teucrium lusitanicum* and *Teucrium algarbiensis*, achieved by gas chromatography (GC) and gas chromatography–mass spectroscopy (GC–MS) analysis. *T. lusitanicum* grows in the western and southern Iberian peninsula and northwest Africa, while *T. algarbiensis* is endemic in the southwest Iberian peninsula [3]. In Portugal, these species grow in the Algarve (southern Portugal), although they are scarce, particularly *T. algarbiensis*.

As far as we know, this is the first report on the essential oil composition of *T. algarbiensis*. Concerning *T. lusitanicum*, Velasco-Negueruela and Pérez-Alonso [5] reported the chemical composition of the oil of *T. lusitanicum* subsp. *aureiformis*, endemic from Mijas mountains (Malaga) [16]. This oil is characterized by high amounts of limonene (15.1%), followed by β-pinene (5.2%), α-copaene (4.5%), terpinen-4-ol (3.5%) and α-cadinol (3.3%). In the literature, we did not found any reference on the composition of the essential oil of *T. lusitanicum* from Portugal.

2. Experimental

2.1. Plant material

The aerial parts of *T. lusitanicum* and *T. algarbiensis* were collected at flowering stage, in July 2001, from Algarve. Four samples of *T. lusitanicum* from two different localities were collected: Sagres (samples 1 and 2) and Cabo de S. Vicente (samples 3 and 4). As *T. algarbiensis* is a rare plant, only one sample was collected from Gambelas

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(sample 5). Voucher specimens were deposited in the Herbarium of the Botanical Institute of the University of Coimbra (COI).

2.2. Isolation procedure

Essential oil samples were isolated by water distillation for 3 h from air-dried material, using a Clevenger-type apparatus, according to the procedure described in the European Pharmacopoeia [17]. The essential oils were stored at 4 °C in the dark prior to analysis. The average yields of the essential oil samples of *T. lusitanicum* and *T. algarbiensis* were 0.1 and 0.3% (v/w), respectively.

2.3. Gas chromatography

Analytical GC was carried out in a Hewlett-Packard 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph with a HP GC ChemStation Rev. A.05.04 data handling system, equipped with a single injector and two flame ionization detection (FID) systems. A graphpak divider (Agilent Technologies, part no. 5021-7148) was used for simultaneous sampling to two Supelco (Supelco, Bellefonte, PA, USA) fused silica capillary columns with different stationary phases: SPB-1 (polydimethylsiloxane 30 m × 0.20 mm i.d., film thickness 0.20 μm), and SupelcoWax-10 (polyethyleneglycol 30 m × 0.20 mm i.d., film thickness 0.20 μm). Oven temperature program: 70–220 °C (3 °C/min), 220 °C (15 min); injector temperature: 250 °C; carrier gas: helium, adjusted to a linear velocity of 30 cm/s; splitting ratio 1:40; detectors temperature: 250 °C.

2.4. Gas chromatography–mass spectrometry

Analyses were carried out in a Hewlett-Packard 6890 gas chromatograph fitted with a HP1 fused silica column (polydimethylsiloxane 30 m × 0.25 mm i.d., film thickness 0.25 μm), interfaced with an Hewlett-Packard mass-selective detector 5973 (Agilent Technologies) operated by HP Enhanced ChemStation software, version A.03.00. GC parameters as described earlier; interface temperature: 250 °C; MS source temperature: 230 °C; MS quadrupole temperature: 150 °C; ionization energy: 70 eV; ionization current: 60 μA; scan range: 35–350 units; scans/s: 4.51.

2.5. Qualitative and quantitative analyses

The identity of the components was achieved from their retention indices on both SPB-1 and SupelcoWax-10 columns and from their mass spectra. Retention indices, calculated by linear interpolation relative to retention times of C_8–C_{22} of *n*-alkanes, were compared with those of authentic samples included in our own laboratory database, presently composed by more than 400 volatile natural compounds. Acquired mass spectra were compared with reference spectra from our own library or from literature data [18,19].

Relative amounts of individual components were calculated based on GC peak areas without FID response factor correction.

3. Results and discussion

The quantitative and qualitative composition of the oil samples are given in Table 1, where the components are listed in order to their elution on the SPB-1 column. Seventy-one components representing more than 90% of the volatile oil were identified.

The combination of the retention data acquired in the two different polarity columns and the mass spectra was crucial for the identification of the most of the compounds. Nevertheless, three relevant constituents (>1%) could not be identified. Their mass spectral data are given in Table 2.

Some important differences with regard to the main constituents in the four sample oils of *T. lusitanicum* were observed. Monoterpene hydrocarbons were shown to be the main group of constituents in sample 1 (49.8%), α-pinene (8.2%), sabinene (9.6%), β-pinene (10.5%) and limonene (11.5%) being the major compounds. Sesquiterpenes, either hydrocarbons or oxygenated compounds, were the main group in samples 2 and 3 (61.8 and 61.7%, respectively), elemol (11.2 and 12.0%), germacrene D (5.3 and 6.0%), T-cadinol (5.2 and 5.5%) and δ-cadinene (4.1 and 5.3%) being the major compounds. Similar proportions of monoterpene hydrocarbons and sesquiterpenes (36.2 and 44.9%) were present in sample 4, β-pinene (11.9%), α-pinene (8.5%), sabinene (7.7%), α-cadinol (9.1%) and δ-cadinene (7.3%) being the main constituents. The results obtained showed some differences between the populations, even among those from the same location. Monoterpene hydrocarbons (47.8%) were the main group of constituents in *T. algarbiensis* oil, limonene (11.8%), β-pinene (10.2%), α-pinene (8.3%) and sabinene (7.2%) being the major compounds. Among sesquiterpenes, the hydrocarbons (21.3%) were detected in higher concentration than the oxygenated compounds (14.3%), germacrene D (7.6%) being the most important sesquiterpene hydrocarbon. Other representative sesquiterpenes were T-cadinol (5.8%), α-cadinol (3.9%), δ-cadinene (3.0%) and bicyclogermacrene (3.0%).

The compositions of the oils of *T. lusitanicum* and *T. algarbiensis* are qualitatively similar and relative amounts of monoterpene hydrocarbons, oxygen-containing monoterpenes, sesquiterpene hydrocarbons and oxygen-containing sesquiterpenes are also similar and analogous to those of oils from *T. capitatum* (7.6–43.9, 14.9–33.0, 23.0–32.2 and 7.5–39.7%, respectively), previously reported [15]. Compositional quantitative differences of the oils from *T. lusitanicum*, *T. algarbiensis* and *T. capitatum* are not significant if compared with those observed among different samples of *T. lusitanicum* or *T. capitatum*. Therefore, oil compositions cannot contribute for the distinction between these morphologically similar species, but a correlation between

Table 1
Composition of the essential oils of *T. lusitanicum* (samples 1–4) and *T. algarbiensis* (sample 5)

RI[a]	RI[b]	Compound	Percentage in samples				
			1	2	3	4	5
922	1028	α-Thujene	0.5	t	t	0.4	0.5
930	1030	α-Pinene	8.2	0.8	2.5	8.5	8.3
943	1073	Camphene	–	–	–	–	0.1
945	1131	Verbenene	–	t	0.1	0.1	0.1
959	1444	Oct-1-en-3-ol	0.5	0.6	0.8	0.6	0.5
964	1126	Sabinene	9.6	2.1	3.2	7.7	7.2
970	1116	β-Pinene	10.5	2.5	4.7	11.9	10.2
980	1162	Myrcene	7.3	2.5	2.8	4.1	5.7
997	1168	α-Phellandrene	–	–	–	t	–
1010	1189	α-Terpinene	t	t	t	t	0.4
1011	1273	*p*-Cymene	0.6	0.9	0.5	0.8	1.0
1019	1215	1,8-Cineole	–	1.0	0.5	t	–
1020	1214	β-Phellandrene	0.4	–		–	0.7
1020	1205	Limonene	11.5	3.0	1.2	2.3	11.8
1025	1235	*Z*-β-Ocimene	–	–	–	–	0.2
1035	1253	*E*-β-Ocimene	0.7	–	t	t	0.6
1046	1251	γ-Terpinene	0.5	0.6	0.5	0.4	0.6
1055	1439	*cis*-Linalool oxyde	–	–	t	0.1	–
1079	1393	Nonanal	0.7	1.3	0.8	t	0.6
1082	1542	Linalool	1.6	0.7	1.7	1.2	1.0
1102	1487	α-Campholenal	t	–	–	0.4	–
1117	1515	Camphor	–	–	–	–	0.6
1119	1649	*trans*-Pinocarveol	0.6	1.8	1.0	1.4	0.4
1121	1648	*cis*-Verbenol	t	t	0.2	t	0.5
1124	1672	*trans*-Verbenol	0.6	1.0	0.9	1.1	0.8
1134	1562	Pinocarvone	t	0.6	0.5	0.6	0.5
1144	1665	*iso*-Borneol	–	t	0.1	t	0.1
1157	1845	*p*-Cymene-8-ol	–	–	–	–	2.4
1158	1595	Terpinen-4-ol	1.9	5.5	2.7	2.8	–
1165	1621	Myrtenal	0.5	1.2	0.7	1.0	0.7
1169	1692	α-Terpineol	0.2	0.8	0.7	0.5	0.5
1177	1698	Verbenone	–	t	t	0.5	0.5
1177	1786	Myrtenol	0.4	1.3	0.9	0.7	0.5
1178	1673	*cis*-Piperitol	–	–	–	–	0.1
1196	1830	*trans*-Carveol	t	t	t	0.1	0.5
1211	1727	Carvone	0.5	0.6	0.5	t	0.8
1328	1692	α-Terpinyl acetate	0.5	1.1	0.8	0.5	–
1368	1487	α-Copaene	t	t	t	0.5	0.4
1375	1517	β-Bourbonene	–	–	–	–	t
1379	1536	β-Cubebene	–	–	–	–	t
1381	1584	β-Elemene	0.6	1.1	0.9	0.4	0.5
1401	1524	α-Gurjunene	–	t	t	0.4	t
1407	1590	*E*-Caryophyllene	1.2	2.2	3.9	0.7	1.1
1440	1662	α-Humulene	0.7	1.3	1.0	0.4	0.7
1444	1664	*E*-β-Farnesene	–	0.9	–	–	–
1447	1637	*allo*-Aromadendrene	–	t	0.5	0.6	0.4
1462	1669	n.i. 1[c]	1.4	3.3	2.6	1.1	1.7
1466	1703	Germacrene D	3.1	6.0	5.3	1.0	7.6
1470	1712	β-Selinene	1.6	4.0	2.7	1.0	1.8
1480	1717	α-Selinene	1.5	2.0	1.9	1.0	–
1482	1726	Bicyclogermacrene	–	2.1	2.1	0.6	3.0
1485	1719	α-Muurolene	t	0.7	0.6	1.2	0.4
1490	n.d.	Germacrene A	t	0.2	t	–	–
1496	1752	γ-Cadinene	1.2	2.2	1.8	3.1	1.7
1502	1823	*cis*-Calamelene	–	t	t	t	–
1506	1752	δ-Cadinene	2.0	5.3	4.1	7.3	3.0
1525	2073	Elemol	6.1	12.0	11.2	2.6	2.5
1539	1819	Germacrene B	1.0	0.9	2.7	0.9	0.6
1551	2113	Spathulenol	0.8	1.1	0.6	t	0.8
1554	2041	Germacrene 1(10),5-dien-4-ol	t	0.9	1.6	3.4	–
1557	1969	Caryophyllene oxide	0.5	1.0	0.7	–	0.4

Table 1 (*Continued*)

RI[a]	RI[b]	Compound	Percentage in samples				
			1	2	3	4	5
1561	2063	Globulol	–	–	0.5	–	–
1568	2073	Viridiflorol	0.4	–	0.5	–	–
1596	2091	10-*epi*-γ-Eudesmol	–	–	0.5	–	–
1606	2158	γ-Eudesmol	1.2	1.7	2.2	t	0.5
1615	2176	T-Muurolol	0.6	1.6	1.6	2.8	–
1615	2161	T-Cadinol	5.4	5.5	5.2	6.2	5.8
1619	n.d.	α-Muurolol	t	t	0.6	0.7	–
1622	2208	α-Eudesmol	0.9	1.8	1.9	0.5	0.4
1628	2221	α-Cadinol	4.2	4.7	4.9	9.1	3.9
1628	2216	β-Eudesmol	–	1.5	1.5	–	–
1640	n.d.	n.i. 2	0.6	0.8	1.2	0.4	0.2
1663	n.d.	n.i. 3	–	–	1.4	0.9	0.4
1713	2328	α-Cyperone	0.4	1.1	0.7	0.5	0.5
		Monoterpene hydrocarbons	49.8	12.4	15.7	36.2	47.8
		Oxygen-containing monoterpenes	6.8	15.6	11.0	10.9	9.9
		Sesquiterpene hydrocarbons	12.9	28.9	27.5	19.1	21.3
		Oxygen-containing sesquiterpenes	20.5	32.9	34.2	25.8	14.8
		Other compounds	1.2	1.9	1.6	0.6	1.1
		Total identified	91.2	91.7	90.0	92.6	94.9

Compounds listed in order to their elution on the SPB-1 column; t: traces; n.d.: not determined; n.i.: not identified.

[a] Retention indices on the SPB-1 column relative to C_8–C_{22} *n*-alkanes.

[b] Retention indices on the SupelcoWax-10 column relative to C_8 to C_{22} *n*-alkanes.

[c] Both retention indices propose the identification of γ-gurjunene.

Table 2
Mass spectral data of unidentified compounds

Compound	M^+	*m*/*z* (relative intensity)
n.i. 1	204 (48)	189 (100), 133 (53), 105 (34), 91 (32), 161 (28), 147 (25), 119 (19), 81 (19), 41 (12), 55 (11), 67 (10)
n.i. 2	220 (2)	161 (100), 93 (99), 59 (98), 107 (82), 189 (61), 81 (60), 119 (55), 67 (49), 43 (45), 135 (41), 55 (34), 147 (30), 175 (9)
n.i. 3	222 (8)	84 (100), 161 (57), 109 (46), 121 (39), 69 (36), 55 (35), 93 (34), 41 (34), 79 (23), 137 (21), 204 (18) 189 (10)

the oils composition and the taxonomic position at section level emerges evident. The oils of these three closely related species of section Polium differ from the oils of *T. salviastrum* (section Scorodonia) which are dominated by sesquiterpene hydrocarbons (73.4–85.5%), as previously reported by us [14].

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