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Micromorphology of trichomes and composition of essential oil of *Teucrium capitatum*

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ABSTRACT: The morphology and distribution of the glandular trichomes of *Teucrium capitatum* L., as well as the chemical composition of the essential oils, were studied. Important differences were found with regard to the major constituents of the essential oils of five populations of *T. capitatum* grown in Portugal. The oil isolated from one population was characterized by a high content of oxygenated monoterpenes (33.0%), isomenthone (7.7%) being the major constituent. Another oil from a population collected from the same region was dominated by monoterpene and sesquiterpene hydrocarbons (43.9% and 23.2%, respectively), α-pinene (7.7%), sabinene (11.2%) and β-pinene (10.3%) being the main compounds. The oils from the other three populations were characterized by a high content of both sesquiterpene hydrocarbons (23.0%, 32.2% and 33.2%) and oxygenated sesquiterpenes (39.7%, 23.4% and 20.4%). T-cadinol (24.1%) and α-cadinol (9.8%) were the major compounds in the oil from one population, whereas δ-cadinene (7.5% and 9.8%) and *E*-caryophyllene (5.4%) or α-muurolol (6.0%) were the major constituents in the other samples. The indumentum of the vegetative and reproductive ograns from the five populations of *T. capitatum*, observed under scanning electron microscopy, showed the same type and distribution of glandular and non-glandular trichomes. Since the ecological and edaphic features of the collecting sites were quite similar, the chemical polymorphism observed seems to be due to genetic factors. Copyright © 2004 John Wiley & Sons, Ltd.

KEY WORDS: *Teucrium capitatum*; trichomes; histochemistry; essential oils; GC; GC–MS

Introduction

The section *Polium*, which includes *Teucrium capitatum* L. [= T. *polium* L. ssp. *capitatum* (L.) Arcangeli], contains more than half of the *Teucrium* spp. and is the largest and most morphologically diverse section of the genus.[1,2] *Teucrium capitatum*, family Lamiaceae, is a Mediterranean dwarf shrub up to 45 cm tall, that grows wild in central and south-east Portugal. As in other Lamiaceae described in the literature, the aerial organs of *Teucrium* spp. are covered by an indumentum of glandular and non-glandular trichomes.

Some work on the composition of the volatile oils of *Teucrium polium* s.l. (subspecies not referred) were reported in the literature,[3–6] and some chemical differences, probably related to the different subspecies and/or to the geographical origin of the plants, were also described. As far as we know, only one single study on the essential oil from *T. polium* mentions the subspecies (*T. polium* subsp. *capitatum* = *T. capitatum*).[7] Thus, continuing our research on the micromorphology of trichomes and on the composition of the essential oils of the Portuguese *Teucrium* taxa, we now report on the results obtained with *Teucrium capitatum* L. The purpose of this work is to investigate the composition and chemical polymorphism of the essential oil from this species, as well as the micromorphology and histochemistry of the trichomes occurring on the leaves and flowers.

Materials and Methods

Plant Material

Aerial parts of the plants were collected at the flowering stage in June 1999 and 2000, in the centre of Portugal: Fonte Coberta; Serra do Sicó (sample 1); Rabaçal, Serra do Sicó (sample 2); Portunhos, Cantanhede (samples 3 and 4); and Covão do Feto, Serra D′Aire (sample 5). All

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the collecting sites are on limestone (calcareous). Voucher specimens were deposited in the Herbarium of the Botanical Institute of the University of Coimbra (COI).

Scanning Electron Microscopy

For scanning electron microscopy SEM, specimens from different plant parts were fixed for 3 h in a solution containing 3% glutaraldehyde in o.1 M cacodylate buffer (pH 7.1). The specimens were subsequently dehydrated in a graded ethanol series and dried by the critical point drying method, using a Polaron E 3500. The dried specimens were sputter-coated with gold in a Polaron E 5350, and then observed on a JEOL JSM T220 scanning electron microscope at 10 kV.

Histochemistry

The histochemical tests were carried out using fresh plant material. Total lipids were detected using Sudan Black[8] and Nile blue A.[9] The controls were tested simultaneously. The autofluorescence of secreted material was studied using a light epifluorescence microscope.

Essential Oil Analysis

The essential oil content of the air-dried plant material was determined according to the *European Pharmacopoeia* method.[10] Analysis of volatile oils obtained by water distillation for 3 h were carried out by GC and GC–MS, using fused silica capillary columns with two different stationary phases: SPB-1 (polydimethylsiloxane, 30 m × 0.20 mm i.d., film thickness 0.20 μm), and Supelco Wax 10 (polyethyleneglycol, 30 m × 0.20 mm i.d., film thickness 0.20 μm); oven temperature programme, 70 °C to 220 °C (at 3 °C/min), 220 °C (15 min); injector temperature, 250 °C; carrier gas, helium, adjusted to a linear velocity of 30 m/s; split ratio, 1:40; detector temperature, 250 °C.

GC–MS was performed with a HP1 fused silica column (polydimethylsiloxane 30 m × 0.25 mm i.d., film thickness 0.25 μm), interfaced with a mass selective detector. GC parameters were as above; 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 u; scans/s, 4.51.

The identity of the components was achieved from their retention indices, calculated by linear interpolation relative to retention times of a series of *n*-alkanes, and their mass spectra, which were compared with those from our own library and from literature data.[11,12]

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

Results and Discussion

Leaf, calyx and corolla specimens from the five populations of *T. capitatum* were studied using SEM. As in other *Teucrium* spp.[13–15] vegetative and reproductive organs are covered by a very dense indumentum. The leaves showed the revolute form characteristic of the section *Polium*, to which this species belongs. The pluricellular non-glandular trichomes with an arborescent form, observed on the leaves (Figures 1, 2 and 3) and the calyx (Figure 7), make the observation of the glandular trichomes more difficult. Non-glandular trichomes with a similar shape were also observed on *T. heterophyllum*, an endemic species from Madeira.[14] This type of trichome is found in only 16% of the 127 genera of the Lamiaceae studied.[16] In contrast, the corolla indumentum (Figure 8) showed unbranched non-glandular trichomes, mainly located on the upper part of the adaxial surface.

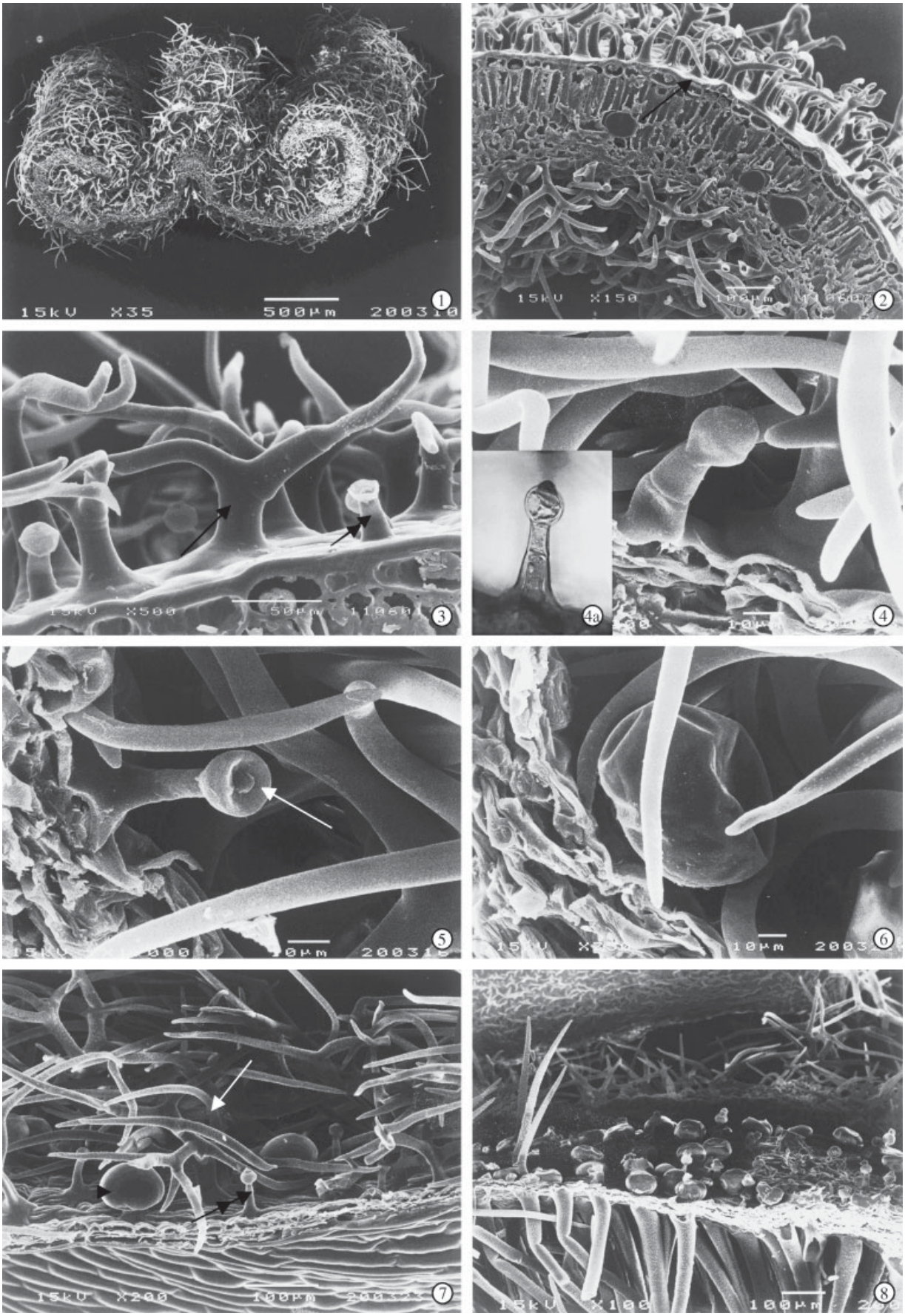
Only transverse sections of the organs under study allowed up to recognise capitate and peltate glandular trichomes similar to those already reported for *Teucrium* species.[17,18] The capitate trichomes possess one basal epidermal cell, two stalk cells and a glandular head cell (Figures 4 and 5), whereas the peltate ones are composed of a basal epidermal cell, a very short stalk cell and a multicellular head (Figure 6).

We did not recognize any particular pattern in the morphology and distribution of non-glandular and glandular trichomes in the five populations studied.

The capitate trichomes are uniformly distributed on the abaxial and adaxial surfaces of the leaves, while the peltate ones occur only on the abaxial surface (Figure 2). It is within the peltate glands that most of the essential oil is believed to be synthesized.[19] The calyx indumentum of the abaxial side (Figure 7) is very similar to that of the leaves.

At the abaxial surface of the corolla, the glandular trichomes, capitate and peltate, are observed (Figure 8), the peltates being more abundant.

The apical cell of a capitate trichome shows a protuberance that corresponds to an elevation of the cuticle, formed by the secreted material accumulated in the subcuticular space. The content of this protuberance stains with Nile Blue (Figure 4A) and Sudan black, revealing the lipophilic nature of the secreted material. Similarly to *T. salviastrum*,[18] the apical cell of capitate trichomes from *T. capitatum* assumes a cup-shape after the secretion release (Figure 5). Based on studies carried out with *Salvia blepharophylla*, BISIO *et al*.[20] suggested two possible methods of release of secreted material:



(a) breakage of the cuticle; or (b) via pores in the cuticle. As we could not observe any cuticle rupture on either peltate or capitate trichomes, it seems plausible that the secretion release via cuticle pores may operate in the glandular trichomes of *T. capitatum*.

The volatile oils of the air-dried aerial plant material were obtained with an average yield of 0.15 (v/w). In total, 103 compounds were identified in the five samples, which are listed in Table 1 in order of their elution on a polydimethylsiloxane column. Some important differences were found regarding the main constituents of the oils. One sample form Rabaçal, Serra do Sicó (sample 1) was characterized by a high content of oxygen-containing monoterpenes (33.0%), isomenthone (7.7%) being the main compound. This constituent, as well as menthol (2.1%) and pulegone (1.4%), were not detected in any of other four samples. The oil isolated from the other sample collected from the same region (Serra do Sicó: sample 2), was dominated by monoterpene and sesquiterpene hydrocarbons (43.9% and 23.2%, respectively), α-pinene (7.7%), sabinene (11.2%) and β-pinene (10.3%) being the main compounds. In contrast, the oils from the two samples collected at Portunhos (samples 3 and 4), and the sample from Serra D'Aire (sample 5), were characterized by high contents of both sesquiterpene hydrocarbons (23.0%, 32.2% and 33.2%, respectively) and oxygen-containing sesquiterpenes (39.7%, 23.4% and 20.4%, respectively). T-cadinol (24.1%) and α-cadinol (9.8%) were the major constituents of the oil from sample 3; δ-cadinene (7.5%) and *E*-caryophyllene (5.4%) the major components in sample 4; and δ-cadinene (9.8%) and α-muurolol (6.0%) the main constituents in sample 5.

Figures 1–8. Scanning electron micrographs of the leaf and flower surfaces of *Teucrium capitatum*. (1) Transverse leaf section showing its revolute margins and a dense indumentum. The glandular trichomes are obscured by the density of the non-glandular ones. (2) Transverse leaf section showing a very dense cuticle (arrow) and very abundant branched trichomes covering both the abaxial and adaxial surfaces. (3) Detail of Figure 2. Showing both non-glandular (arrow) and two capitate glandular trichomes (double arrow). (4) Aspect of a capitate trichome, observed in the middle of branched non-glandular trichomes. (a: Inset). The secreted material located in the subcuticular space reacts positively to Nile blue reagent. (5) Detail of a capitate trichome, showing a depression on the apical cell (arrow). (6) Detail of the abaxial leaf surface, showing a peltate trichome intermingled with non-glandular ones. (7) Transverse section of a sepal. The indumentum of the abaxial surface is similar to that observed on the leaves; non-glandular (arrows), capitate (double arrows) and peltate (arrow heads) trichomes are clearly seen. (8) Transverse section of calyx and corolla. On the abaxial surface of the corolla, the pelate trichomes are very abundant. On the adaxial surface the trichomes are non-glandular and unbranched.

Table 1. Composition of the essential oils of *Teucrium capitatum* from Portugal

		% in samples				
RI*	Compound	1	2	3	4	5
923	α-Thujene	0.2	1.5	—	—	0.2
930	α-Pinene	0.9	7.7	0.6	0.7	2.9
941	Camphene	—	0.3	—	t	—
942	Verbenene	—	0.2	—	t	t
959	Oct-1-en-3-ol	0.3	0.6	0.4	0.5	0.3
964	Sabinene	2.1	11.2	1.3	1.1	3.1
970	β-Pinene	2.6	10.3	1.3	1.9	5.0
981	Myrcene	1.1	3.5	1.3	0.8	2.2
1010	α-Terpinene	0.3	t	0.1	0.1	0.3
1012	*p*-Cymene	0.9	2.8	0.9	1.6	1.0
1020	β-Phellandrene	0.4	0.7	0.2	0.3	—
1020	1,8-Cineole	—	—	—	—	1.0
1020	Limonene	1.0	3.1	0.7	0.6	1.2
1025	*Z*-β-Ocimene	0.1	0.1	t	t	—
1036	*E*-β-Ocimene	0.2	0.3	t	t	0.2
1047	γ-Terpinene	1.0	1.7	0.6	0.3	0.7
1051	*Z*-Sabinene hydrate	—	—	0.3	—	—
1056	*Z*-Linalool oxide	0.1	—	0.2	—	—
1066	*E*-Linalool oxide	0.2	—	—	—	—
1066	2,5-Dimethylstyrene	—	0.2	—	t	—
1066	Fenchone	—	0.1	0.6	—	—
1079	Terpinolene	0.4	0.5	0.3	0.2	0.2
1081	Linalool	1.5	1.1	1.5	0.6	0.8
1081	Nonanal	—	—	—	—	0.4
1089	β-Thujone	0.1	—	0.3	t	—
1104	α-Campholenal	1.3	0.3	1.3	t	0.1
1105	Nopinone	—	—	—	—	0.3
1106	*cis*-*p*-Menth-2-en-1-ol	—	0.6	—	—	—
1121	Camphor	—	0.1	—	—	0.1
1122	*E*-Pinocarveol	2.2	1.3	2.4	1.8	0.8
1125	*Z*-Verbenol	2.5	1.1	3.5	—	—
1126	Sabina ketone	—	—	—	2.1	—
1129	*E*-Verbenol	—	—	—	—	0.2
1129	*trans*-*p*-Menth-2-en-ol	—	—	—	—	0.4
1135	Pinocarvone	0.5	0.6	0.9	0.6	0.3
1139	Isomenthone	7.7	—	—	—	—
1141	*p*-Menth-1,5-dien-8-ol	—	—	—	—	0.3
1144	Borneol	0.6	1.0	0.8	1.1	0.4
1158	*p*-Cymene-8-ol	0.4	0.2	0.5	0.9	0.1
1158	Terpineol-4	3.5	2.4	2.6	3.0	1.4
1165	Menthol	2.1	—	—	—	—
1165	Myrtenal	1.3	0.8	0.6	1.4	0.5
1169	α-Terpineol	0.3	0.3	0.4	0.7	0.3
1176	Verbenone	—	—	—	—	0.2
1176	Myrtenol	2.3	0.8	2.0	1.3	0.4
1192	*E*-Carveol	—	—	—	—	0.1
1209	Cuminaldehyde	0.7	0.5	0.9	0.9	0.3
1211	Pulegone	1.4	—	—	—	—
1212	Carvone	0.5	—	t	—	—
1223	Piperitone	0.1	—	—	—	—
1233	Geraniol	—	—	—	0.3	—
1256	*E*-Anethol	0.3	—	—	—	—
1260	Cuminic alcohol	0.3	—	0.4	1.4	—
1266	Bornyl acetate	0.6	—	0.4	—	0.2
1267	Thymol	—	2.3	—	1.5	0.1
1272	Carvacrol	0.4	0.4	t	0.9	3.0
1301	Myrtenyl acetate	0.9	—	—	—	0.5
1329	α-Terpinyl acetate	1.3	0.9	—	1.3	0.7
1342	α-Cubebene	0.3	0.2	0.5	0.7	0.6
1360	Cyclosativene	—	0.4	—	1.1	0.3
1369	α-Copaene	0.3	0.6	1.3	1.2	1.3
1376	β-Bourbonene	0.3	—	—	—	0.1
1381	β-Cubebene	1.1	1.2	3.3	2.8	3.0
1381	β-Elemene	—	—	—	—	0.2
1402	α-Gurjunene	—	t	0.5	0.4	0.4
1409	*E*-Caryophyllene	4.8	3.8	3.3	5.4	4.6
1418	*Z*-α-Bergamotene	—	—	—	0.1	—

Table 1. (*Continued*)

RI* Compound	% in samples				
	1	2	3	4	5
1428 *E*-α-Bergamotene	—	0.6	—	—	0.3
1428 α-Guaiene	0.5	—	0.5	—	—
1441 α-Humulene	2.0	1.8	2.0	2.7	2.6
1446 *E*-β-Farnesene	0.6	0.6	—	—	0.1
1448 *allo*-Aromadendrene	—	—	0.5	0.5	0.1
1466 γ-Muurolene	—	—	0.6	—	t
1466 γ-Gurjunene	—	—	—	—	0.4
1467 Germacrene D	3.5	3.6	—	3.5	2.6
1479 α-Selinene	—	—	—	—	0.6
1482 Bicyclogermacrene	1.9	1.9	—	0.7	2.3
1487 α-Muurolene	0.6	—	0.4	0.8	0.6
1498 γ-Cadinene	1.3	—	5.5	1.1	1.9
1497 β-Bisabolene	—	—	—	0.8	—
1502 *Z*-Calamelene	—	—	—	0.3	0.2
1503 7-*epi*-α-Selinene	0.8	1.9	—	—	—
1508 δ-Cadinene	3.0	4.9	3.7	7.5	9.8
1517 Cadina-1,4-diene	—	—	—	—	0.2
1521 α-Cadinene	0.8	0.4	—	0.8	—
1526 Elemol	1.8	—	—	1.8	—
1531 α-Bisabolene	—	0.6	—	—	0.4
1540 Germacrene B	0.5	0.4	—	—	0.5
1542 *E*-Nerolidol	—	0.6	1.7	0.9	0.3
1552 Spathulenol	1.1	—	—	2.5	0.5
1554 Germacrene-1(10),5-dien-4-ol	—	—	—	—	1.3
1558 Caryophyllene oxide	1.4	0.6	3.1	1.4	0.8
1568 Viridiflorol	0.3	—	—	1.1	—
1596 δ-Selinene	1.9	0.7	0.8	2.0	0.4
1606 γ-Eudesmol	—	—	—	2.1	—
1616 T-Cadinol	5.5	1.6	24.1	3.0	3.2
1616 α-Muurolol	—	—	—	—	6.0
1620 β-Eudesmol	0.0	0.4	—	2.7	—
1628 T-Muurorol	1.5	1.2	1.0	1.7	—
1628 α-Cadinol	3.2	1.6	9.8	4.2	4.6
1632 7-*epi*-α-Eudesmol	—	—	—	2.0	—
1659 α-Bisabolol	—	1.5	—	—	3.7
2098 Phytol	0.1	—	—	0.2	—
Monoterpene hydrocarbons	11.2	43.9	7.3	7.6	17.0
Oxygen-containing monoterpenes	33.0	14.9	19.8	19.7	12.5
Sesquiterpene hydrocarbons	24.3	23.2	23.0	32.2	33.2
Oxygen-containing sesquiterpenes	14.9	7.5	39.7	23.4	20.4
Others	0.4	1.2	0.4	0.8	1.0
Total identified	83.8	90.7	90.0	83.7	84.1

* Compounds listed in order of their elution from the polymethylsiloxane column. $t < 0.05\%$.

The chemical composition of our oil sample 2 (Serra do Sicó, Rabaçal) is quite similar to that reported for *T. polium* ssp. *capitatum* grown on Sierra Helada, Spain, which possessed considerable amounts of α-pinene (11.6%), β-pinene (18.1%) and sabinene (13.0%).[7]

Our results showed important differences among the essential oils obtained from plants collected at the same developmental stage, even among those from the same collection sites or very close localities with similar ecological and edaphic features. This fact indicates that the chemical polymorphism of the volatile oil of *T. capitatum* might be due, in part, to genetic characters.

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