



## Chemotaxonomic value of the essential oil compounds in species of *Teucrium pumilum* aggregate

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Received 2 May 2000; received in revised form 7 August 2000

### Abstract

Volatile compounds from the aerial parts of *Teucrium lepicephalum* Pau and *Teucrium carolipau* C. Vicioso ex Pau, both belonging to the *Teucrium pumilum* aggregate, were analysed by capillary GC and GC–MS. From the common and specific compounds (mono- and sesquiterpenes) of the two taxa, chemical characterization was carried out and taxonomic relationships were assessed. Application of the cluster and *k*-means discriminant analysis shows a differential chemical relationship between *T. lepicephalum* and *Teucrium carolipau*. © 2000 Published by Elsevier Science Ltd.

**Keywords:** *Teucrium lepicephalum*; *Teucrium carolipau*; Lamiaceae; Essential oils; Terpenes; Discriminant Analysis; Chemotaxonomy

### 1. Introduction

Studies on the chemical composition of the Mediterranean *Teucrium* species show relationships between the chemical composition and morphological characteristics at the infraspecific taxonomic level (Velasco Negueruela and Pérez Alonso, 1990; El-Oualidi et al., 1996).

The *polium* section of the genus *Teucrium* includes scarcely differentiated taxa (El-Oualidi et al., 1999). The *Teucrium pumilum* L. species, endemic to the central and southeastern Iberian Peninsula, presents a pronounced morphological variability related to both climatic and edaphic characteristics (Rivas Goday, 1956). This variability has made it possible to establish several taxonomic categories at the subspecies level, and an aggregate of species based on morphological characteristics has been proposed.

The latest studies published do not coincide on the systematic and nomenclatural aspects of this aggregate. In one of them (Rivas-Martínez, 1974) the author proposes five species based on inflorescence variability:

*T. pumilum* L., *T. libanitis* Schreber, *T. lepicephalum* Pau, *T. carolipau* C. Vicioso ex Pau and *T. turredanum* Losa & Rivas Goday, all included in the subsection *pumilum* (*Polium* section). Other authors (Bolós and Vigo, 1995) propose three taxa at the subspecies level for the stirpes with opposed leaves: *T. pumilum* subsp. *pumilum*; subsp. *lepicephalum* (Pau) O.de Bolós et J. Vigo and subsp. *carolipau* (C. Vic. ex Pau) D. Wood, while others recognize only two subspecies (Tutin et al., 1980) or other combinations (Fernández-Casas, 1972; Rigual, 1972).

Biochemical studies have also contributed to the taxonomy of some sections of the genus *Teucrium*. The essential oils have been analysed to establish the relationships within the *Scorodonia* section (Velasco Negueruela and Pérez Alonso, 1990). Flavonoids have also provided biochemical information on the systematics of the *Polium* section (Miller) Schreber, which includes *T. libanitis*, *T. carolipau* and *T. pumilum* (Harborne et al., 1986).

However, although studies on the neoclerodane diterpenoids (Savona et al., 1986) have been carried out in *T. pumilum* aggregate, the composition of the essential oils has not been examined.

The present study analyses the essential oil of *T. carolipau* and *T. lepicephalum* for the first time to determine both the phytochemical relationships between taxa

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and the taxonomic value of the main essential oil compounds and to clarify the systematic arrangement.

## 2. Results and discussion

A comparative study of the essential oils of *T. carolipau* and *T. lepicephalum* was carried out. The yields of essential oils ranged from 0.18% for sample 1 (*T. lepicephalum*) to 3.09% for sample 4 (*T. carolipau*). It is interesting to note the low yield obtained for *T. lepicephalum* in comparison to the *T. carolipau*.

The qualitative and quantitative composition of the essential oils were very different. The hydrocarbon fraction, which makes up more than 94% in *T. lepicephalum*, is present in *T. carolipau* at low levels. GC and GC–MS revealed the occurrence of sabinene and  $\alpha$ -pinene rich oils in *T. lepicephalum*. The quantitative prevalence of both compounds can be considered the most exceptional chemical characteristic of the essential oil of *T. lepicephalum* herbs growing wild in Alicante (Spain), which can therefore be defined as a specific taxon within the *Polium* section. In the literature we found only one reference (Arnold et al., 1991) to oils with a significant amount (21.2%) of sabinene: *T. cyprium* Boiss. subsp. *cyprium* in the *Polium* section.

The phenolic compounds, thymol and carvacrol and their phenolic precursor *p*-cymene were not detected in the *T. lepicephalum* plants analysed, but are present in *T. carolipau* as well as in other species of the *Polium* section (Arnold et al., 1991).

Differences in the oxygenated monoterpenes were also observed between sites and between species plants. With the exception of linalool and  $\alpha$ -terpineol, which were found in the two analysed species, the oxygenated monoterpenes found in *T. carolipau* were in general not present in *T. lepicephalum*.

A substantial difference is observed in the oxygenated sesquiterpenes. Only caryophyllene oxide and  $\alpha$ -cadinol were found in all the analysed samples. *T. carolipau* is characterized by a large amount of the sesquiterpene alcohols *tau*-cadinol, T-murolol and  $\beta$ -eudesmol, which were not present in *T. lepicephalum*.

On the other hand, three of the four analyzed samples of *T. carolipau* present as the main compound the oxygenated sesquiterpene with *m/z* 202(100), 159(93), 59(57), 145(56), 43(49), 91(46), 187(44), 131(41), 41(37), 105(32), not found in *T. lepicephalum*.

Statistical data processing of the phytochemical relationships established between samples was carried out by cluster analysis from the data in Table 1. Metric distance is the Pearson coefficient correlation and single linkage (nearest-neighbour).

The distance (0.92) between *T. carolipau* and *T. lepicephalum* (Fig. 1) shows these as two independent groups.

The taxonomic indicator value of the common compounds was determined by *k*-means discriminant analysis. For each compound, *k*-means cluster analysis compares the between-cluster mean square (Between SS/df) with the within-cluster mean square (Within SS/df) and reports the *F*-ratio. Because of the limited number of data, the *F*-ratio is not used to test significance. Instead, these statistics are used to characterize relative discrimination. The compounds are plotted according to the *F* value. Those having a higher *F*-ratio are those with a greater differential value for separating *T. carolipau* from *T. lepicephalum*.

Characteristic compounds are distributed according to the means value within each group related to general mean (Fig. 2). Relative distances to axis of general mean is calculated by equation  $d = (X_G - X_i)/SD$ , ( $X_G$ , general mean;  $X_i$ , mean within group; SD, standard deviation).

T-murolol ( $F=1825.2$ ),  $\gamma$ -eudesmol ( $F=89.5$ ),  $\alpha$ -cadinol ( $F=83.7$ ),  $\beta$ -eudesmol ( $F=71.0$ ) and caryophyllene oxide ( $F=39.8$ ), are the characteristic compounds with the highest differential value for *T. carolipau* (Fig. 2A). Although  $\alpha$ -thujene,  $\alpha$ -terpinene and *p*-cymene are present only in this group, they do not appear to be differential compounds because of their scarce presence and relatively variable *F* values.

In the *T. lepicephalum* cluster, the compounds that best define the species chemically are those present only on the right of the central axis:  $\alpha$ -pinene ( $F=14063.8$ ),  $\beta$ -myrcene ( $F=1427.4$ ), sabinene ( $F=461.1$ ), bornyl acetate ( $F=182.8$ ) and limonene ( $F=90.9$ ) (Fig. 2B). Sabinene,  $\alpha$ -pinene, limonene and *tau*-cadinol also constitute a group of differential compounds between the *Polium* and *Scorodonia* sections (Velasco Negueruela and Pérez Alonso, 1990).

The characteristic compounds of cluster A (*T. carolipau*), have mean values above the general average, and are plotted on the graph according to the values falling on the right of Fig. 2A. This reasoning is also valid for the cluster B (*T. lepicephalum*; Fig. 2B).

Finally, the application of the ANOVA test in the yield of the essential oils showed significative differences between the samples (mean *T. lepicephalum* = 0.28 SD = 0.095; mean *T. carolipau* = 1.413 SD = 1.194;  $P \leq 0.05$ ).

According to the phytochemical results *T. carolipau* and *T. lepicephalum* should be considered two different taxa at the species level.

## 3. Material and methods

### 3.1. Plant material

Randomised individual plants from the *T. carolipau* and *T. lepicephalum* populations (Alicante, Spain) were taken for chemometric purposes. The aerial parts of

Table 1  
Volatile compounds identified in the essential oils of *T. lepicephalum* and *T. carolipau*<sup>a</sup>

Sample	RR <sub>t</sub> <sup>b</sup>	RI <sup>c</sup>	<i>T. lepicephalum</i> (T. lep)			<i>T. carolipau</i> (T. car)			
			1	2	3	4	5	6	7
Yield % v/w			0.18	0.37	0.29	3.09	0.78	1.18	0.6
Compound			%			%			
$\alpha$ -Thujene	2.43	310	—	—	—	—	0.02	0.16	0.07
$\alpha$ -Pinene*	2.52	316	19.14	19.25	19.35	—	0.08	0.58	0.18
Camphene*	2.70	340	0.40	0.37	0.35	—	0.05	0.23	0.07
Sabinene	3.23	379	47.54	57.43	54.43	—	0.12	0.87	0.22
$\beta$ -Myrcene*	3.17	413	1.20	1.10	1.22	—	—	—	—
$\alpha$ -Terpinene	4.28	455	—	—	—	—	0.05	0.24	0.08
<i>p</i> -Cymene*	4.53	468	—	—	—	—	0.26	1.84	0.48
Limonene*	4.65	476	8.31	7.43	7.11	—	0.36	2.44	0.79
$\beta$ -Phellandrene*	5.17		0.03	0.02	—	—	—	—	—
$\gamma$ -Terpinene*	5.50	547	0.08	0.05	0.06	—	—	0.04	0.16
<i>Trans</i> -linalool-oxide*	5.77	585	—	—	—	—	0.11	0.64	—
Terpinolene*	6.49	605	0.32	0.21	0.30	—	0.03	0.08	—
Linalool*	7.69	644	0.18	0.19	0.16	1.47	0.30	1.36	0.42
$\beta$ -Thujone	8.12	665	—	—	—	—	0.05	0.25	0.03
Isoborneol	9.80	768	0.10	0.20	0.10	—	—	—	—
Terpinen-4-ol*	10.46	819	—	—	—	—	0.55	1.24	0.31
<i>p</i> -Cymen-8-ol	10.93		—	—	—	—	0.19	0.15	0.06
$\alpha$ -Terpineol*	11.06		0.19	0.20	—	—	0.05	0.10	0.04
Myrtenal	11.09	857	0.19	0.28	0.10	—	—	—	—
Carvone	12.90		—	—	—	—	0.07	0.04	0.04
Bornyl acetate	14.29	1088	0.54	0.45	0.40	—	—	—	—
Thymol	14.80		—	—	—	—	0.11	0.07	—
Carvacrol	14.91		—	—	—	—	0.03	0.04	0.06
$\delta$ -Elemene	16.16	1263	0.01	—	—	0.60	0.07	0.36	0.05
$\alpha$ -Cubebene*	16.31	1262	0.01	—	—	—	0.10	0.08	0.02
$\alpha$ -Copaene*	16.87	1338	0.06	0.02	0.07	2.76	1.17	1.28	4.33
$\beta$ -Bourbonene	17.13	1360	0.67	0.32	0.43	0.15	0.02	0.07	0.02
$\beta$ -Elemene	17.46	1376	—	—	—	0.32	0.11	0.05	0.12
$\beta$ -Caryophyllene	18.19	1442	4.65	3.19	1.66	0.26	1.91	0.99	2.25
$\beta$ -Gurjunene	18.40	1481	0.10	0.07	0.06	—	—	—	—
Aromadendrene*	18.62	1504	0.03	—	0.03	—	—	—	—
$\alpha$ -Humulene*	18.97	1529	0.06	—	0.03	0.06	0.50	0.64	0.31
<i>Allo</i> -aromadendrene	19.15	1550	0.04	0.45	0.03	0.03	0.38	0.06	0.08
Germacrene-D	19.38	1597	9.90	4.98	5.30	0.02	0.11	0.04	—
Germacrene-B	20.19	1645	1.54	0.86	3.70	0.01	0.10	0.04	0.03
$\alpha$ -Murolene	20.37	1657	0.08	0.02	0.09	0.03	0.20	0.08	0.05
$\beta$ -Bisabolene	20.69	1683	0.24	—	0.21	—	—	—	—
$\gamma$ -Cadinene	20.81	1689	0.12	0.08	0.11	4.54	0.33	2.57	0.55
$\delta$ -Cadinene	21.11	1716	0.40	0.12	0.27	3.87	7.59	6.21	7.04
Caryophyllene oxide	22.37	1864	0.29	0.24	0.59	1.44	1.19	1.12	1.08
$\gamma$ -Eudesmol	23.69	1949	—	—	—	1.14	1.74	1.69	1.63
<i>tau</i> -Cadinol	24.05	1974	—	—	—	17.00	6.53	11.18	5.05
T-Murolol	24.41	1976	—	—	—	12.13	12.30	11.28	11.62
$\beta$ -Eudesmol	24.43	1991	—	—	—	8.35	10.08	6.17	9.37
$\alpha$ -Cadinol	24.49	2002	0.21	0.41	0.75	8.07	12.32	11.45	11.72
Sesquiterpene oxygenated	25.00	2082	—	—	—	7.69	23.93	17.18	23.02

<sup>a</sup> (\*) Identified by authenticated samples.

<sup>b</sup> Relative retention times on a OV-17 column.

<sup>c</sup> Kovat's retention index on a DB-5 column,  $t < 0.010$ .

*Teucrium* species were collected from plants growing in Orxeta (sample 1, 38°34'42"N; 00°15'20"W), Finestrat (sample 2, 38°34'42"N; 00°14'52"W) and Bello Horizonte (sample 3, 38°36'22"N; 00°05'30"W) (Alicante, Spain)

for *T. lepicephalum*; Santa Pola (sample 4), Villajoyosa (sample 5, 38°32'03"N; 00°14'43"W and sample 6, 38°32'17"N; 00°12'46"W) and Finestrat (sample 8, 38°34'13"N; 00°13'19"W) for *T. carolipau*.

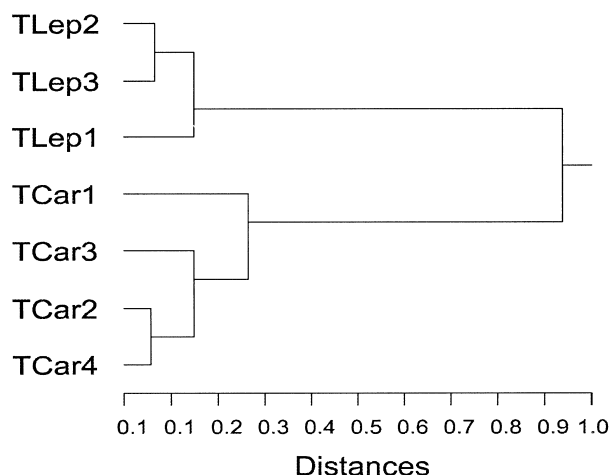


Fig. 1. Cluster tree using Pearson correlations as the basis for the dissimilarities between *T. lepicephalum* (T. lep) and *T. carolipau* (T. car).

Leaves and inflorescence of ten plants for each population were cut up and blended to obtain the essential oils. Harvesting of the material was done by hand in mid-July 1999 when the plants were in full flower. Voucher specimens were deposited in the Laboratorio de Botánica, Universidad Politécnica de Valencia (Spain).

The fresh plant material was subjected to hydro-distillation in a Clevenger-type apparatus for 2.5 h and yielded after three distillations (v/w)%: 0.18 (1); 0.37 (2); 0.29 (3); 3.09 (4); 0.78 (5); 1.18 (6) and 0.6 for the sample of *T. carolipau* collected in Finestrat. The distillate was dried over anhydrous sodium sulphate and stored at 4–6°C.

### 3.2. Gas chromatography

GC analyses were performed using a Konic gas chromatograph (model 2000 C) equipped with a flame ionisation detector and a Spectra Physic (model 4290) electronic integrator. An OV-17 (25 m×0.25 mm i.d., film thickness 0.25 mm) capillary column was employed. The oven temperature was programmed at 60°C for 6 min, then increased by 5°C/min to 150°C and held isothermally for 10 min; injector and detector temperatures were 225 and 250°C respectively. The carrier gas was hydrogen and the samples were injected using the splitless technique. The percentages of the components were calculated from the GC peak areas, using the normalization method.

### 3.3. Gas chromatography–mass spectrometry

The GC–MS analyses were done on a Thermo mass spectrometer (model Trio 1000), combined with a Thermo gas chromatograph (model 8000) using an OV-17 column (25 m×0.25 mm i.d., film thickness 0.25 mm).

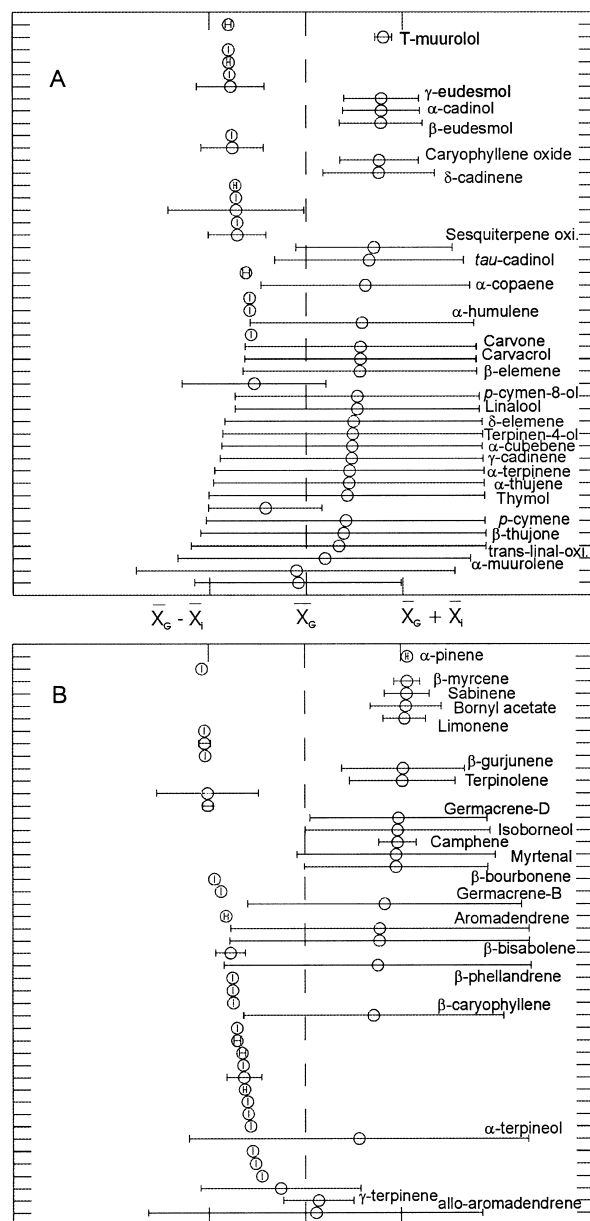


Fig. 2. Range of characteristic compounds for *T. carolipau* (A) and *T. lepicephalum* (B), in a cluster profile plots. Central line represents general standardized mean value ( $X_G$ ). The differential compounds for both species have mean values within the cluster greater than general mean ( $X_G + x_i$ ) (O = standardized values; horizontal lines = SE). Compounds are plotted according to decrescent  $F$  value.

The same working conditions used for GC were employed except that the carrier gas was He at 1 ml/min (splitless mode). The quadruple mass spectrometer was scanned over the 28–400 a.m.u. range at 1 scan  $s^{-1}$ , with an ionising voltage of 70 eV and an ionisation current of 150  $\mu A$ . For the determination of the retention indices a Varian instrument (GC model Star 3400; MS model Saturn II) and a DB-5 column were used. The Kovat retention indices (RI) were calculated using co-chromatographed standard hydrocarbons. The identity

of the components was assigned by comparison of their RIs, relative to C<sub>8</sub>–C<sub>19</sub> *n*-alkanes, and by the mass spectra and retention times of authentic samples or with the data available in the literature (Cornu and Massot, 1975; Noever de Brauw et al., 1979; Stenhagen et al., 1974).

### 3.4. Statistical studies

Statistical procedures (Hierarchical Cluster and *k*-means Discriminant Analysis) were performed using the SYSTAT 8.0, package 1998, Statistical Software SPSS, Inc. Chicago.

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