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Essential oil composition of *Pterospartum tridentatum* grown in Portugal

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Abstract

The essential oils, isolated by hydrodistillation and distillation-extraction, from the aerial parts of different populations of *Pterospartum tridentatum* collected during the flowering phase, at different locations in Portugal, were analysed by GC and GC–MS. All the *P. tridentatum* populations studied afforded a yellowish oil in a yield <0.05% (v/w). *cis*-Theaspirane (2–14%), *trans*-theaspirane (2–17%) and octen-3-ol (2–37%) were, in variable amounts, the dominant components of the oils. Cluster analysis of the essential oil compositions from the nine populations studied, confirmed a major chemical variability.

Keywords: *Pterospartum tridentatum* L. Willk.; Leguminosae; Fabaceae; Essential oil; GC; GC–MS; Theaspiranes

1. Introduction

Pterospartum tridentatum L. Willk. [=*Chamaespartium tridentatum* (L.) P. Gibbs.; *Genistella tridentata* (L.) Samp.] is a European endemic Leguminosae (=Fabaceae) species belonging to the subfamily Papilionoideae (=Faboideae) (Talavera, 1999). This small shrub, growing up to 100 cm, is very common in the mountains of the north of Portugal, showing yellow flowers, alternate branches and coriaceous winged stems (Franco, 1971; Teixeira & Pereira, 2004).

The flowers of *carqueja* or *carqueija*, as they are commonly known in Portugal, are traditionally harvested in spring and either used in traditional medicine or to flavour rice and roast meat (Oliveira & Neiva, 2001; Ribeiro, Monteiro, & Silva, 2000). The dried stems are also used as firewood in traditional ovens because they are highly combustible and impart an enjoyable aroma to bread (Ribeiro et al., 2000; Salgueiro, 2004). The infusion of the dried flowering tops is used as an excellent bechic and emollient but also against liver, bladder, kidney and rheumatism problems (Feijão, 1979; Font Quer, 1981; Oliveira & Neiva, 2001; Ribeiro et al., 2000; Salgueiro, 2004; Tecedeiro, 1996). The flowering stem apices, including both leaves and flowers, of *P. tridentatum,* are also one of the ingredients of several herbal mixtures sold in herbal shops throughout the country for the control of type 2 diabetes (Vitor et al., 2004).

A previous study on the essential oil from the aerial parts of one population of the plant collected during the flowering phase showed *cis*-theaspirane (13%), *trans*-theaspirane (12%) and octen-3-ol (11%) to be the main components (Pereira, Teixeira, Santos, Figueiredo, & Barroso, 2002). The only other phytochemical and pharmacological study on this species reported on the flavonoid composition

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and oxidative injury protective effect of the water extract (Vitor et al., 2004).

In view of the pharmacological potential and commercial value of this species, the purpose of this study was the characterization of the essential oil composition of different populations and different plant parts of *P. tridentatum* grown in Portugal.

2. Materials and method

2.1. Plant material

Samples of the aerial parts of *P. tridentatum* were collected during the full flowering period of the plant from populations growing in Ribatejo and Beira Alta. Sites and years of collection of the different populations, with the corresponding abbreviations, are listed in Table 1. A voucher specimen has been deposited in the Herbarium of the Museu, Laboratório e Jardim Botânico de Lisboa under number LISU 203605.

2.2. Isolation procedure

Each essential oil sample was isolated from deep-frozen (−20 °C) plant material by distillation-extraction for 3 h, using a Likens–Nickerson-type apparatus with distilled *n*-pentane as organic solvent and by hydrodistillation for 3 h, using a Clevenger-type apparatus. The oil samples isolated by hydrodistillation were used to estimate the oil yields, and those isolated by distillation-extraction to determine the percentage composition of the oils, since the chance of artefact formation must be considered smaller when the latter method is used.

2.3. Gas chromatography

GC analyses were done with a Perkin–Elmer 8700 gas chromatograph equipped with two FIDs, a data handling system and a vaporizing injector port into which two columns of different polarities were installed: a DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm; J & W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused-silica column (30 m × 0.25 mm i.d., film thickness 0.15 μm; J & W Scientific Inc.). Oven temperature was programmed, 45–175 °C, at 3 °C/min, subsequently at 15 °C/min up to 300 °C, and then held isothermal for 10 min; injector and detector temperatures, 280 °C and 290 °C, respectively; carrier gas was hydrogen, adjusted to a linear velocity of 30 cm/s.

Samples were injected using the split sampling technique, ratio 1:50. The percentage composition of the oils was computed by the normalization method from the GC peak areas, which were calculated as mean values of two injections of each oil sample, without using response factors.

2.4. Gas chromatography–mass spectrometry

The GC–MS unit consisted of a Perkin–Elmer Autosystem XL gas chromatograph, equipped with DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm; J & W Scientific, Inc.), and interfaced with a Perkin–Elmer Turbomass mass spectrometer (software version 4.1). Injector and oven temperatures were as above; transfer line temperature, 280 °C; ion trap temperature, 220 °C; carrier gas, helium, adjusted to a linear velocity of 30 cm/s; split ratio, 1:40; ionization energy, 70 eV; ionization current, 60 μA; scan range, 40–300 μm; scan time, 1 s.

The identity of the components was assigned by comparison of their retention indices, relative to C_8-C_{25} *n*-alkanes, and GC–MS data were compared with corresponding data of components of reference oils, laboratory-synthesized components and commercially available standards from a home-made library.

2.5. Statistical analysis

The percentage composition of the essential oil samples was used to determine the relationship between the different samples of *P. tridentatum* by cluster analysis using the NTSYS-pc software (version 2.02, Exeter Software, Setauket, New York) (Rohlf, 1998). Correlation coefficients were selected as a measure of similarity among the nine accessions, and the unweighted pair-group

Table 1
Years of collection, plant parts and collection sites of the different populations of *Pterospartum tridentatum* collected during the full flowering phase

Geographic zone	Collection site	Plant part	Year of collection	Abbreviation
Ribatejo	Arneiro das Milhariças, Santarém	Flowers[a]	2002	AMF02
	Arneiro das Milhariças, Santarém	Stems and leaves[a]	2002	AML02
	Arneiro das Milhariças, Santarém	Flowers[a]	2003	AMF03
	Arneiro das Milhariças, Santarém	Stems and leaves[a]	2003	AML03
Beira Alta	Pedra de Altar, Proença-a-Nova	Aerial parts[b]	2004	PAPN
	Póvoa, Sobreira Formosa, Proença-a-Nova	Aerial parts[b]	2004	PSFPNa
	Póvoa, Sobreira Formosa, Proença-a-Nova	Aerial parts[b]	2004	PSFPNb
	Malhada do Corvo, Sarzeda, Castelo Branco	Aerial parts[b]	2004	MCSCB
	Sarzeda, Castelo Branco	Aerial parts[b]	2004	SCB

[a] Flowers were separated from the corresponding stems and leaves.
[b] Flowers plus stems and leaves.

Table 2
Composition (%) of the essential oils, isolated by distillation-extraction, from the aerial parts of *Pterospartum tridentatum,* collected in the flowering phase, in different years and locations

Components	RI	*Pterospartum tridentatum*								
		Flowers		Leaves + Stems		Aerial parts				
		AMF02	AMF03	AML02	AML03	PAPN	PSFPNa	PSFPNb	SCB	MCSCB
trans-2-Hexenal	866	1.6	0.5	*t*	1.6	*t*	*t*	1.7	3.2	*t*
cis-3-Hexen-1-ol	868	1.6	1.2	*t*	5.3	*t*	*t*	0.8	3.0	*t*
cis-2-Hexen-1-ol	882	1.5	1.2	*t*	0.8	*t*	*t*	0.6	1.2	*t*
n-Hexanol	882	0.5	1.6	*t*	1.1	*t*	*t*	1.1	0.7	*t*
n-Heptanal	897	11.8	4.8	*t*	0.5	0.8	*t*	*t*	0.3	*t*
n-Nonane	900	*t*	*t*	*t*	0.2	*t*	*t*	2.3	0.2	*t*
Benzaldehyde	927	0.5	0.8	*t*	0.6	1.0	*t*	0.6	0.1	*t*
α-Pinene	930	*t*	0.3	*t*	0.8	*t*	*t*	0.5	0.1	*t*
n-Heptanol	952	0.5	1.6	*t*	1.5	*t*	*t*	*t*	*t*	1.3
1-Octen-3-ol	961	10.7	21.0	11.5	22.6	1.7	29.7	15.0	25.8	36.8
2-Pentyl furan[a]	972	2.4	1.3	2.5	0.5	*t*	*t*	0.7	2.1	1.4
3-Octanol	974	1.4	1.5	1.9	*t*	*t*	*t*	1.9	0.3	1.5
Benzyl alcohol	996	*t*	*t*	0.3	0.4	*t*	*t*	*t*	0.3	*t*
Benzene acetaldehyde	1002	1.8	1.8	0.3	1.2	*t*	*t*	0.4	1.4	0.6
1,8-Cineole	1005	0.9	1.0	1.1	0.2	*t*	*t*	*t*	*t*	*t*
Limonene	1009	0.9	1.0	1.1	0.2	*t*	*t*	0.3	*t*	*t*
Acetophenone	1017	*t*	1.4	2.1	0.5	*t*	*t*	*t*	*t*	*t*
n-Octanol	1045	0.5	0.4	2.1	0.3	0.6	*t*	*t*	*t*	*t*
Heptanoic acid	1056	0.5	1.2	*t*	0.4	*t*	*t*	*t*	*t*	2.1
Phenyl ethyl alcohol	1064	0.7	1.2	2.0	1.7	*t*	3.6	3.3	3.4	6.3
n-Nonanal	1073	14.5	6.1	4.6	0.9	10.5	4.1	0.2	0.9	1.0
Linalol	1074	2.9	0.5	*t*	2.0	*t*	5.2	*t*	2.3	1.0
cis-Rose oxide	1083	0.6	*t*	3.4	1.5	*t*	*t*	*t*	*t*	*t*
n-Undecane	1100	*t*	*t*	*t*	*t*	*t*	1.0	2.3	0.2	*t*
trans-Rose oxide	1100	*t*	*t*	2.1	0.7	*t*	1.0	*t*	*t*	*t*
trans-Pinocarveol	1106	*t*	*t*	*t*	*t*	*t*	*t*	*t*	0.2	*t*
trans-Verbenol	1114	*t*	*t*	*t*	*t*	*t*	*t*	*t*	*t*	*t*
2- *trans*,6 *cis*-Nonadienal	1106	2.1	0.3	*t*	*t*	*t*	*t*	*t*	*t*	*t*
2- *trans*-Nonen-1-al	1114	0.5	0.4	2.2	0.2	*t*	*t*	*t*	*t*	*t*
Pentyl benzene	1119	1.5	*t*	*t*	0.3	*t*	*t*	*t*	*t*	*t*
Octanoic acid	1156	*t*	0.3	*t*	0.5	*t*	*t*	*t*	*t*	*t*
α-Terpineol	1159	*t*	*t*	*t*	*t*	*t*	1.2	0.8	0.3	*t*
Myrtenol	1168	*t*	*t*	*t*	*t*	*t*	*t*	*t*	*t*	*t*
Safranal	1160	1.4	0.3	*t*	0.5	*t*	*t*	*t*	*t*	*t*
n-Decanal	1180	*t*	0.3	*t*	*t*	*t*	*t*	*t*	*t*	*t*
Geraniol	1236	0.3	1.6	4.0	9.2	3.2	1.0	*t*	1.4	2.8
n-Decanol	1259	0.3	1.6	4.0	0.2	3.2	3.4	2.5	3.2	1.9
Perilla alcohol	1274	*t*	*t*	*t*	3.4	*t*	*t*	*t*	0.6	*t*
Nonanoic acid	1274	*t*	0.3	2.3	*t*	*t*	*t*	*t*	*t*	*t*
cis-Theaspirane	1279	1.6	2.2	12.7	7.1	14.2	5.3	13.2	9.0	6.2
2 *trans*,4 *trans*-Decadienal	1285	0.8	1.3	*t*	0.1	*t*	1.8	*t*	2.0	*t*
trans-Theaspirane	1300	2.4	1.9	12.1	6.8	17.2	6.3	13.6	10.0	5.5
Eugenol	1327	1.4	1.7	3.5	2.6	*t*	3.1	3.0	3.2	3.6
α-Cubebene	1345	*t*	*t*	*t*	*t*	*t*	*t*	*t*	*t*	*t*
α-Copaene	1375	*t*	*t*	*t*	*t*	*t*	0.9	*t*	*t*	*t*
β-Bourbonene	1379	*t*	*t*	*t*	*t*	*t*	1.5	*t*	1.1	*t*
Longifolene	1399	*t*	*t*	*t*	*t*	*t*	1.4	*t*	*t*	*t*
β-Caryophyllene	1414	*t*	0.4	*t*	*t*	*t*	2.7	*t*	2.0	0.9
Geranyl acetone[a]	1434	*t*	3.6	*t*	*t*	*t*	1.2	*t*	0.6	*t*
α-Humulene	1447	*t*	*t*	*t*	*t*	*t*	*t*	*t*	*t*	*t*
γ-Muurolene	1469	*t*	*t*	*t*	*t*	*t*	*t*	*t*	*t*	*t*
Germacrene-D	1474	*t*	0.2	*t*	*t*	9.7	3.3	*t*	0.7	*t*
γ-Cadinene	1500	*t*	3.3	*t*	*t*	*t*	1.2	*t*	1.1	1.9
δ-Cadinene	1505	*t*	2.4	*t*	*t*	*t*	1.6	*t*	2.0	1.9
Dodecanoic acid	1551	3.5	2.1	2.6	0.3	15.0	*t*	*t*	0.9	1.1
β-Caryophyllene oxide	1561	*t*	*t*	*t*	*t*	*t*	1.3	*t*	1.2	2.9
n-Tetradecanal	1596	*t*	*t*	*t*	*t*	*t*	1.1	*t*	2.7	1.5
n-Pentadecanal	1688	*t*	*t*	*t*	*t*	*t*	*t*	*t*	0.8	*t*
n-Tricosane (C23)	2300	*t*	*t*	*t*	*t*	*t*	*t*	*t*	*t*	*t*

(*continued on next page*)

Table 2 (*continued*)

Components	RI	*Pterospartum tridentatum*								
		Flowers		Leaves + Stems		Aerial parts				
		AMF02	AMF03	AML02	AML03	PAPN	PSFPNa	PSFPNb	SCB	MCSCB
n-Pentacosane (C25)	2500	*t*	*t*	*t*	*t*	*t*	*t*	*t*	*t*	*t*
% of identified components		71.8	75.1	78.4	76.8	77.1	82.9	64.8	88.5	82.2
Grouped components										
Monoterpene hydrocarbons		0.9	1.3	1.1	1.0	*t*	*t*	0.8	0.1	*t*
Oxygen-containing monoterpenes		6.2	7.0	10.6	17.5	3.2	9.6	0.8	5.4	3.8
Sesquiterpene hydrocarbons		*t*	6.3	*t*	*t*	9.7	12.6	*t*	6.9	4.7
Oxygen-containing sesquiterpenes		*t*	*t*	*t*	*t*	*t*	1.3	*t*	1.2	2.9
C13 Components (Theaspiranes)		4.0	4.2	24.8	13.9	31.4	11.6	26.8	19.0	11.7
Phenylpropanoids		1.4	1.7	3.5	2.6	*t*	3.1	3.0	3.2	3.6
Others[b]		59.3	54.6	38.4	41.8	32.8	44.7	33.4	52.7	55.5
Oil yield (v/w)		<0.05%	<0.05%	<0.05%	<0.05%	<0.05%	<0.05%	<0.05%	<0.05%	<0.05%

For abbreviations, see Table 1. RI = Retention index relative to C_8–C_{25}*n*-alkanes on the DB-1 column.
t = trace (<0.05%).
[a] Identification based on mass spectra only.
[b] Components that do not fit on the classification of terpenes or phenylpropanoids and which are mainly non-aromatic alcohols, non-aromatic aldehydes, hydrocarbons and fatty acids.

method with arithmetic average (UPGMA) was used for cluster definition. The degree of correlation was evaluated according to Pestana and Gageiro (2000) in: very high (if correlation ranged between 0.9 and 1), high (between 0.7 and 0.89), moderate (between 0.4 and 0.69), low (between 0.2 and 0.3) and very low (if <0.2). The cophenetic correlation values were determined to test the goodness of the fit of the data clustering by the Mantel test (Rohlf, 1998).

3. Results and discussion

All the *P. tridentatum* populations studied afforded a yellowish oil in a yield of <0.05% (v/w). The identified oil components are listed in Table 2 in order of their elution on the DB-1 column. A limited number of components with relative amounts of 0.5–3% each and some trace components could not yet be identified; these are not included in Table 2. Together they cover about 12–35% of the oils.

A fraction, designated as "others" in Table 2, since components were neither terpenes nor phenylpropanoids, and which was mainly composed of non-aromatic alcohols, saturated and unsaturated non-aromatic aldehydes, hydrocarbons and fatty acids (33–59%), dominated the essential oil from all populations studied. The C13 components, theaspiranes, constituted the second main fraction (4–31%) of five of the nine *P. tridentatum* oils studied.

Although not widespread in essential oils, theaspiranes have been identified in the volatile fraction, mostly obtained by solvent extraction, of *Aronia melanocarpa* (black chokeberry), *Camellia sinensis* (black and green tea), *Catharanthus roseus* (periwinkle), *Cydonia oblonga* (quince), *Osmanthus fragrans* (fragrant olive, devilwood), *Passiflora edulis* (passion fruit), *Prunus* spp. (apricot, peach), *Psidium* spp. (guava), *Pyrus* spp. (pear), *Rosa* spp. (rose), *Rubus* spp. (blackberries and raspberries), *Vitis vinifera* (grapes) (Brun, Bassière, Dijoux-Franca, David, & Mariotte, 2001; Full, Winterhalter, Schmidt, Herion, & Schreier, 2005; Riu-Aumatell, Lopez-Tamames, & Buxaderas, 2005; Schmidt, Full, Winterhalter, & Schreier, 1992) and also on Perique tobacco (*Nicotiana tabacum*) (Leffingwell & Alford, 2005). Isomeric theaspiranes can be distinguished as they possess olfactory differences from either (1) weak or (2) fresh camphoraceous note, (3) naphthalene-like or (4) highly attractive, intense fresh-fruit black currant or cassis odour (Schmidt et al., 1992).

Despite the fact that one fraction dominated all the oils, a major chemical variability was clear in all samples studied, which was confirmed by the cluster analysis, with a correlation coefficient varying between 0.38 and 0.97, (Fig. 1). The measure of the goodness of fit between the cophenetic value, obtained from the dendrogram and the correlation matrix, proved very good, with a cophenetic correlation value of 0.90246.

Two groups of samples showed a high degree of correlation in the oil composition. One group was formed by four of the nine oil samples studied (AML03, PSFPNa, MCSCB and SCB, $S_{corr} = 0.91$), Fig. 1, being dominated by 1-octen-3-ol (23–37%) and having both theaspiranes in equivalent amounts, but in the range of 5–10% in each of the samples, (Table 2). AMF03 oil showed a high degree of correlation with this group, $S_{corr} = 0.89$, mainly due to the high amount of 1-octen-3-ol (21%), but showed a lower percentage of each of the theaspiranes (2%).

A second group of oils, that also showed a high degree of correlation (AML02 and PSFPNb, $S_{corr} = 0.91$), (Fig. 1), was dominated by 1-octen-3-ol (12–15%), *cis*-theaspirane (13% in both cases) and *trans*-theaspirane (12–14%) in approximate amounts, (Table 2).

AMF02 and PAPN oils were less correlated ($S_{corr} =$ 0.47 and $S_{corr} = 0.38$, respectively), since they also showed,

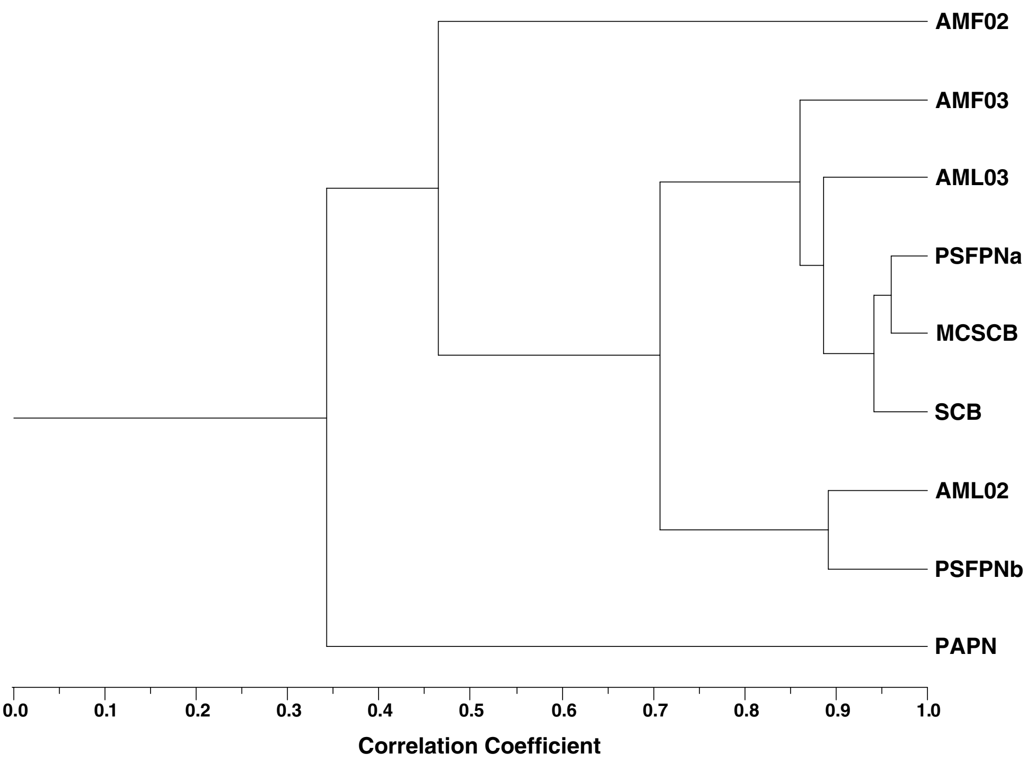

Fig. 1. Dendrogram obtained by cluster analysis of the percentage composition of essential oils from the *Pterospartum tridentatum* samples examined, based on correlation and using the unweighted pair-group method with arithmetic average (UPGMA). For abbreviations, see Table 1.

apart from 1-octen-3-ol, *cis*-theaspirane and *trans*-theaspirane, high relative amounts of *n*-nonanal (15%) and *n*-heptanal (12%) for AMF02 and dodecanoic acid (15%) and *n*-nonanal (11%) in the case of PAPN oil.

Although three year apart samples were studied, as well as different types of plant samples (flowers, stems + flowers and aerial parts in the whole), no particular correlation was found between the oils of any of the different batches analysed. Although some species show quite stable oil composition, independently of the plant part studied or of the collection site (with different altitudes and climatic conditions), the essential oil composition often depends on developmental stage, seasonal variation and altitude distribution, (Figueiredo, Barroso, Pedro, & Scheffer, 1997). Nevertheless, the low correlation between samples of *P. tridentatum* of the same location and year (PSFPNa and PSFPNb) leads us to regard the chemical variability of *P. tridentatum* oils, not as a consequence of climatic factors in different years (AMF02, AMF03, AML02, AML03), but rather due to other genetic and/or environmental factors. In addition, the relative proportions of the different aerial parts on the final batch is also an important factor to consider, particularly for a species that is currently commercialised for household use, such as teas or condiments.

In terms of main components, this study is in agreement with a previous study on a population of *P. tridentatum* (Pereira et al., 2002) but shows a major chemical variability, even in samples from the same collection site. Particularly interesting in these oils is the presence of theaspiranes which are common flavouring agents responsible for the tea, herbal, green and slightly spicy odour of the plant.

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