Eucalyptus from Mata Experimental do Escaroupim (Portugal): Evaluation of the Essential Oil Composition from Sixteen Species

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Abstract

The essential oils isolated from the vegetative aerial parts (mature branches with leaves) of 16 *Eucalyptus* species, grown in Mata Experimental do Escaroupim (Salvaterra de Magos, Portugal) were studied. The essential oils from *E. bosistoana* F. Muell., *E. botryoides* Sm., *E. camaldulensis* Dehnh., *E. cinerea* F. Muell., *E. citriodora* Hook., *E. cordieri* Trabut, *E. dives* Schauer, *E. ficifolia* F. Muell., *E. globulus* Labill., *E. pauciflora* Sieber ex Spreng., *E. polyanthemos* Schauer, *E. radiata* Sieber ex DC, *E. saligna* Sm., *E. smithii* R.T. Baker, *E. urophylla* S.T. Blake and *E. viminalis* Labill. were analyzed by GC and GC-MS, and the percentage composition of the volatiles was used to determine the relationship between the different oil samples by cluster analysis. Cluster analysis showed a high correlation (S_{corr}≥0.80) among 11 species (*E. bosistoana*, *E. botryoides*, *E. camaldulensis*, *E. cinerea*, *E. cordieri*, *E. globulus*, *E. polyanthemos*, *E. radiata*, *E. saligna*, *E. smithii* and *E. viminalis*), mainly due to their richness in 1,8-cineole (27-83%). The remaining 5 species were dominated by citronellal (36%, *E. citriodora*), piperitone (40%, *E. dives*), limonene and α-pinene (41 and 44%, respectively, *E. ficifolia*), α-pinene (82%, *E. pauciflora*) and α-phellandrene (45%, *E. urophylla*).

INTRODUCTION

Eucalyptus genus, which includes more than 700 species, is mainly used for the production of timber, firewood (as well as charcoal), pulp, and for its essential oils, employed in the pharmaceutical and perfumery industries (Brophy and Southwell, 2002). *Eucalyptus* essential oils are chiefly characterized by their 1,8-cineole (eucalyptol) content, important for the pharmaceutical industry, or by its aroma, in perfumery. It is also valued for its antimicrobial and antiseptic properties, among others (Batish et al., 2008), and is commonly used to alleviate breathing afflictions.

The introduction of eucalyptus in Portugal seems to have been part of a general movement, by the mid-nineteenth century, of ordering exotic plants to embellish parks and gardens. Nevertheless, given the favourable edaphoclimatic conditions, *Eucalyptus globulus* was fast in becoming an unavoidable element of the Portuguese forest (Pereira, 2007; Radich, 2007).

At present, the eucalyptus is the third most representative forest species in Portugal, following cork oak (*Quercus suber* L.) and maritime pine (*Pinus pinaster* Aiton). Portugal, with a paper manufacturing industry since the end of the 14th century, was the first country to manufacture chemical pulp from eucalyptus. The pulp and paper sectors contribute strongly for the Portuguese economy, being the 4th largest net exporter, after textile, leather and wood industries (CELPA, 2008).

In addition to the use of raw *Eucalyptus globulus* wood material in pulp and paper industry, its fresh, or dried, leaves are traditionally used in Portugal, in the treatment of several ailments (Salgueiro, 2004), and the dried leaves sole or as one of the ingredients of herbal mixtures are sold in herbal shops throughout the country. The infusion is used externally in the treatment of hair problems, cutaneous ulcers, measles and all-purposes baths. Internally, the infusion is used against round-worms, diabetes, and urinary disorders. Syrup containing eucalyptus infusion, sweetened with honey, is used against cough. Gargles with the leaf infusion water heal mouth wounds, sore throat and bronchial inflammation. Cigarettes made of mashed partially toasted leaves are considered good for respiratory conditions (Salgueiro, 2004).

The Mata Experimental do Escaroupim (Salvaterra de Magos, Portugal), is an area of protected forest tutored by Autoridade Florestal Nacional, which includes an arboretum with an identified, and documented, collection of 125 different eucalyptus species, considered to be the most complete in Europe (Goes, 1977).

Aiming at performing a comprehensive characterization of the essential oils of the existing species in Mata Experimental do Escaroupim, we herewith report a preliminary study on the essential oils isolated from 16 *Eucalyptus* species grown in this arboretum.

MATERIAL AND METHODS

Plant Material

The vegetative aerial parts (mature branches with leaves, from one sample per species) of *E. bosistoana* F. Muell., *E. botryoides* Sm., *E. camaldulensis* Dehnh., *E. cinerea* F. Muell., *E. citriodora* Hook., *E. cordieri* Trabut, *E. dives* Schauer, *E. ficifolia* F. Muell., *E. globulus* Labill., *E. pauciflora* Sieber ex Spreng., *E. polyanthemos* Schauer, *E. radiata* Sieber ex DC, *E. saligna* Sm., *E. smithii* R.T. Baker, *E. urophylla* S.T. Blake and *E. viminalis* Labill. were collected at Mata Experimental do Escaroupim (Salvaterra de Magos, Portugal), in the spring of 2009 and stored at -20°C until extraction.

Isolation of the Essential Oils

The essential oils were isolated by hydrodistillation for 3 h using a Clevenger-type apparatus according to the European Pharmacopoeia method (Council of Europe, 2007). The isolation procedure was run at a distillation rate of 3 ml min^{-1}, and the essential oils were stored at -20°C in the dark until analysis.

Gas Chromatography

Gas chromatographic analyses were performed using a Perkin Elmer Autosystem XL gas chromatograph (Perkin Elmer, Shelton, CT) equipped with two flame ionization detectors (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^{-1}, subsequently at 15°C min^{-1} up to 300°C, and then held isothermal for 10 min; injector and detector temperatures, 280 and 300°C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of 30 cm s^{-1}. The samples were injected using a split sampling technique, ratio 1:50. The volume of injection was 0.1 μl of a distilled *n*-pentane-oil solution (1:1). The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as a mean value of two injections from each oil, without response factors.

Gas Chromatography-Mass Spectrometry

The GC-MS unit consisted on 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 Perkin-Elmer Turbomass mass spectrometer (software version 4.1, Perkin Elmer, Shelton, CT). Injector and oven temperatures were as above; transfer line temperature, 280°C; ion source temperature, 220°C; carrier gas, helium, adjusted to a linear velocity of 30 cm s^{-1}; split ratio, 1:40; ionization energy, 70 eV; scan range, 40-300 u; scan time, 1 s. The identity of the components was assigned by comparison of their retention indices, relative to C_9-C_{17} *n*-alkane indices, and GC-MS spectra from a laboratory made library, based upon the analyses of reference oils, laboratory-synthesized components, and commercial available standards.

Statistical Analysis

The percentage composition of the isolated essential oils was used to determine the relationship between the different samples by cluster analysis using Numerical Taxonomy Multivariate Analysis System (NTSYS-pc software, version 2.2, Exeter Software, Setauket, New York) (Rohlf, 2000). For cluster analysis, correlation coefficient was selected as a measure of similarity among all accessions, and the Unweighted Pair Group Method with Arithmetical Averages (UPGMA) was used for cluster definition. The degree of correlation was evaluated according to Pestana and Gageiro (2000) and classified as very high (0.9-1), high (0.7-0.89), moderate (0.4-0.69), low (0.2-0.39) and very low (<0.2).

RESULTS AND DISCUSSION

The essential oil yields of the *Eucalyptus* species studied varied between 0.4% (v/f.w.) in *E. ficifolia* and *E. polyanthemos*, and 5.6% (v/f.w.) in *E. radiata* (Table 1).

The monoterpene fraction was dominant in all the oils analysed (≥64%). In variable amounts, monoterpene hydrocarbons were dominant (54-95%) in *E. botryoides*, *E. dives*, *E. ficifolia*, *E. pauciflora* and *E. urophylla* (Table 1). Oxygen-containing monoterpenes (51-87%) dominated the oils of *E. bosistoana*, *E. camaldulensis*, *E. cinerea*, *E. citriodora*, *E. cordieri*, *E. globulus*, *E. radiata*, *E. saligna*, *E. smithii* and *E. viminalis*. In *E. polyanthemos* the monoterpene hydrocarbons and the oxygen-containing monoterpenes occurred in similar relative amounts (34:30%).

With the exception of *E. polyanthemos* essential oil, in which the sesquiterpene fraction attained 32%, this fraction was ≤11% in the remaining oils (Table 1).

Essential oil cluster analysis showed a high correlation (S_{corr}≥0.80) among 11 species (*E. bosistoana*, *E. botryoides*, *E. camaldulensis*, *E. cinerea*, *E. cordieri*, *E. globulus*, *E. polyanthemos*, *E. radiata*, *E. saligna*, *E. smithii* and *E. viminalis*), mainly due to their high content in 1,8-cineole (27-83%), (Fig. 1). The essential oils isolated from this group of species showed low correlation with the remaining ones (S_{corr}≤0.44) (Fig. 1). *E. pauciflora* and *E. ficifolia* formed another highly correlated cluster (S_{corr}≥0.74), given the high relative amount of α-pinene in both species (82 and 44%, respectively) and the low amount of 1,8-cineole. The essential oils from the remaining three species were dominated by citronellal (36%, *E. citriodora*), piperitone (40%, *E. dives*), and α-phellandrene (45%, *E. urophylla*).

In general, the yield and the essential oil composition, regarding the main components, reported herewith agree with those published by Brophy and Southwell (2002), for the same species. Nevertheless, some differences were noticed, such as the high percentages of *p*-cymene and α-phellandrene in *E. radiata* and *E. urophylla* oils, respectively, detected in the present study. The differences found may reflect the high flexibility of the chemical pathways leading to terpene synthesis reported by Keszei et al. (2008) for the genus *Eucalyptus* and/or the particular environmental growth conditions.

The sole source of eucalyptus essential oil produced in Portugal is *E. globulus*, which has been planted to meet the demands of the large pulp and paper industry. Portuguese eucalyptus oil export rates have progressively decreased due to the increase of labour costs and the severe competition with the low-priced Chinese eucalyptus oil. It is, therefore, fundamental to improve oil quality, by increasing 1,8-cineole content and decreasing α-phellandrene content, as well as to find alternative essential oil sources, in order to attract industries other than the pharmaceutical, namely perfumery- and arising industries such as that of industrial solvents (Brophy and Southwell, 2002).

Within the species studied, *Eucalyptus smithii* was particularly interesting, as it showed both a high oil yield (2.8%) and was 1,8-cineole-rich (83%) and α-phellandrene free. *E cordieri* and *E. globulus* showed a similar essential oil composition but with lower yields.

E. pauciflora essential oil showed potential for use in the solvents industry given the large relative amount of α-pinene (≥80%). *E. dives* showed a high yield (3.3%), and was relatively rich in the oxygen-containing monoterpene, piperitone (40%), which is the main raw material for the production of synthetic menthol and thymol. *E. citriodora* oil showed a rather high citronellal relative amount (36%) and demonstrated the characteristic lemon-scented fresh fragrance of this species, which is commonly used as ingredient of commercial cleaning products.

Essential oil yield and composition screening of other species of the genus *Eucalyptus* from Mata Experimental do Escaroupim is in progress and this knowledge could be used to support models of development of interesting and high-yield producing species that are economically profitable, simultaneously promoting an ecologically responsible and sustainable management of Portuguese forest.

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Tables

Table 1. Percentage composition of the main essential oils components (present in a relative amount ≥5%, in at least one sample), isolated from the aerial parts of 16 *Eucalyptus* species, grown in Mata Experimental do Escaroupim (Portugal). *E. bosistoana* (Ebos), *E. botryoides* (Ebot), *E. camaldulensis* (Eca), *E. cinerea* (Ecin), *E. citriodora* (Ecit), *E. cordieri* (Eco), *E. dives* (Ed), *E. ficifolia* (Ef), *E. globulus* (Eg), *E. pauciflora* (Epa), *E. polyanthemos* (Epo), *E. radiata* (Er), *E. saligna* (Esa), *E. smithii* (Esm), *E. urophylla* (Eu) and *E. viminalis* (Ev).

Components	*Eucalyptus* species																
	RI	*Ebos*	*Ebot*	*Eca*	*Ecin*	*Ecit*	*Eco*	*Ed*	*Ef*	*Eg*	*Epa*	*Epo*	*Er*	*Esa*	*Esm*	*Eu*	*Ev*
α-Thujene	924	t	0.5	t				5.8	t		t	2.2	3.0			1.2	t
α-Pinene	930	14.2	43.2	31.6	7.7	1.2	6.3	1.0	43.5	15.5	82.2	4.2	2.6	40.2	6.7	2.0	12.5
β-Pinene	963	0.4	2.8	0.4	t	1.6	0.1	t	8.8	0.2	0.2	0.2	0.7	0.2	0.1	2.6	0.2
α-Phellandrene	995		5.7	0.8	t		0.1	19.3	t		t	7.8	4.2			45.3	0.1
p-Cymene	1003	0.1	0.6	0.5	0.1	t	1.1	18.5	t	0.7	0.3	6.4	12.8	0.1	0.8	4.0	6.0
1,8-Cineole	1005	59.3	35.0	50.8	67.4	11.4	71.9	t	2.0	70.3	1.4	27.2	47.9	47.9	83.3	22.6	46.4
β-Phellandrene	1005		t					3.5	t			9.1	t			t	
Limonene	1009	4.5	4.3	3.3	5.3	0.2	6.3	0.7	41.1	2.5	3.0	1.3	4.8	3.1	4.0	3.0	5.1
γ-Terpinene	1035	0.1	0.2	t	t	0.1	0.4	0.6	1.7	t		0.8	1.9	t	t	7.9	11.5
Isopulegol	1116					13.4											
Citronellal	1121					35.8											
neo-Isopulegol	1121					5.8											
Terpinen-4-ol	1148	0.3	0.4	t	0.1	0.1	0.5	4.3	t	t	0.1	2.0	9.1	t	0.1	1.3	1.2
α-Terpineol	1159	6.9	1.6	1.2	3.4	0.4	4.4	0.6	0.2	0.6	2.7	0.4	6.3	1.4	1.5	0.3	2.5
Citronellol	1207					12.4					t						
Piperitone	1211							40.2		t	t		0.5				
α-Terpinyl acetate	1334				10.0		4.8			0.7						1.5	
trans β-Caryophyllene	1414	0.1		t	0.6	6.1			0.1	0.2	t	4.8				1.9	
γ-Eudesmol	1609	0.1		1.2			0.2				0.5	6.4			0.1	0.2	
β-Eudesmol	1620	0.1	0.1	2.5		t	0.4		0.1		0.6	7.7			1.1	0.1	0.1
α-Eudesmol	1634		0.1	1.2			0.2		0.1		0.7	5.5			0.2		t
% of Identification		98.1	98.1	99.6	99.0	95.8	99.5	99.9	99.9	98.6	98.4	96.0	99.3	99.9	99.9	99.3	97.2
Grouped components																	
Monoterpene hydrocarbons		19.9	58.0	37.3	13.2	3.3	14.5	53.5	95.4	19.0	86.9	33.5	32.2	44.1	11.7	67.5	36.1
Oxygen-containing monoterpenes		68.5	37.7	56.2	83.7	84.3	82.9	45.3	2.4	77.0	8.5	30.3	66.4	55.7	86.8	25.9	50.5
Sesquiterpene hydrocarbons		5.6	1.2	0.5	1.4	7.0	0.8	0.8	0.4	0.9	0.6	8.1	0.3	t	t	3.9	5.0
Oxygen-containing sesquiterpenes		4.0	1.2	5.6	0.7	1.1	1.3	0.3	1.7	1.7	2.4	24.1	0.4	0.1	1.4	2.0	5.6
Others		0.1	t	t	t	0.1	t	t	t	t	t	t	t	t	t	t	t
Oil yield (%, v/f.w.)		1.8	1.2	1.4	1.6	0.9	1.1	3.3	0.4	1.3	0.8	0.4	5.6	1.0	2.8	0.9	1.1

RI: Retention index relative to C_9-C_{17} *n*-alkanes on the DB-1 column; t: traces (<0.05%).

Figures

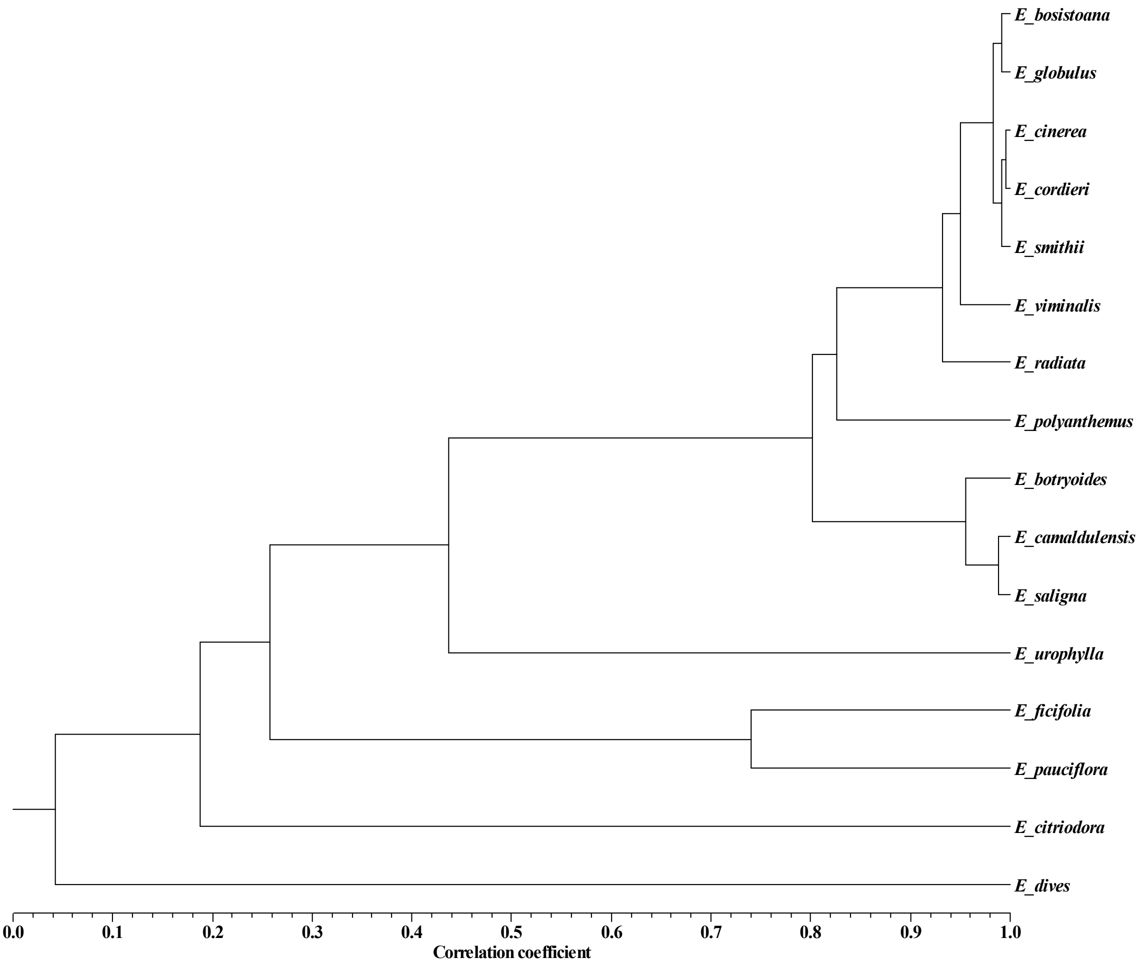

Fig. 1. Dendrogram obtained by cluster analysis of the percentage composition of essential oils from the sixteen *Eucalyptus* species based on correlation and using unweighted pair-group method with arithmetic average (UPGMA).