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Flower Colour and Essential Oil Composition in *Erica australis* L. Grown in Portugal

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Abstract: The chemical composition of the essential oils isolated from the flowering aerial parts of *Erica australis* L. (Ericaceae), collected in Portugal, were studied by gas-chromatography (GC) and by gas-chromatography/mass spectrometry (GC-MS). In order to evaluate if the flower colour was related to different volatiles composition, the dried flowering aerials parts of *E. australis* were separated into flowers showing light pink, medium pink and dark pink colour, and assessed separately. Forty-three components were identified in each sample showing the dominance of 1-octen-3-ol (33-38 %), the aldehyde *n*-nonanal (8-11 %), and the alcohol *n*-octanol (6-7 %). Other alcohols were also present, although in minor amounts, like *n*-heptanol (4 %), *cis*-3-hexen-1-ol (2-5 %), and 2-octen-1-ol (2-3 %). The aldehydes 2-*trans*, 4-*trans*-decadienal (2-4 %), and 2-*trans*-decenal (2 %), and nonanoic acid (2 %) were also identified in the analysed samples. No remarkable differences were observed on their chemical composition, suggesting that colour polymorphism does not influence *E. australis* essential oil yield or composition.

Key words: *Erica australis*, Ericaceae, heath, essential oil, GC, GC-MS.

Introduction

The genus *Erica* belongs to the Ericaceae, also known as the heath family. *Erica* genus is represented by more than 700 species in the world, mainly found in the South Africa, furthermore in Mediterranean and West Europe ¹. *Erica* (heath), and the closely related *Calluna* (heather), present a quite homogeneous habit of small-mid size shrubs with narrow ‘ericoid’ leaves, which contrasts with a strongly diversified floral morphology ². Many *Erica* species have a large variety of colour morphs, as shown, for example, by *E. coccinea* and *E. longifolia* ³. However, most populations are monomorphic for one of the colours, which is a result of unknown factors and appears

to be influenced by pollination processes, although the determinants of colour polymorphism are obscure ³.

E. australis L. is endemic to the Iberian Peninsula and North West Africa ⁴, and is known in some regions of Portugal as “queiró”, “urgueira” or “urze-vermelha” ⁵. Studies involving the chemical characterization and biological activities of aqueous and methanol extracts from *E. australis* collected in Algarve (Portugal) were recently reported ⁶⁻⁸.

Flower colour and volatile profile have major importance in pollinators flower discrimination. Nevertheless, to the best of our knowledge no previous study evaluated the volatile composition

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of *E. australis*, nor checked for the relation between flower colour polymorphism and volatiles composition. Aiming at determining whether flower colour polymorphism and essential oil composition were correlated, the present study evaluated the chemical composition of the essential oils isolated from the flowering aerial parts of *E. australis* with distinct flower colours.

Material and methods

Plant material

E. australis L. flowering aerial parts [500 g (d.w.) of stems, leaf and flowers as commonly used by locals in the herbal teas] were collected from wild populations in the Tarouca region, Montemuro Mountain border (Portugal). The plant was identified by Prof. Ana Isabel Vasconcelos Correia, from the Herbarium of Jardim Botânico, Faculdade de Ciências da Universidade de Lisboa, where a voucher specimen (LISU 236833) is deposited.

Isolation of essential oils

The EOs were obtained from the dried flowering aerial parts of plants by hydrodistillation, for 3 h, using a Clevenger-type apparatus⁹. Each EO extraction was performed with about 500 g (d.w.) of plant material having three different flower colours: light pink (Ea-lp), medium pink (Ea-mp) and, dark pink (Ea-dp) tonalities, Figure 1. Given the essential oil low yield, the EO was recovered from the graduated tube of the Clevenger appara-

tus after rinsing with distilled *n*-pentane (*n*-pentane ≥ 99 % purity, HPLC grade, is in lab distilled prior to use, to remove stabilizers that may contaminate the sample, particularly low essential oil yield samples) when the distillation procedure was over, and allowed to settle for about 10-15 min. For this procedure, the tap was open anti-clockwise, so that the water flowed out of the connecting tube until just below the filling funnel. Distilled *n*-pentane was introduced in the filling funnel followed by water, so that pentane evaporated, with the residual heat of the distillation flask, and then condensed, and dissolved the EO, over the aqueous phase in the graduated tube. The tap was then open clockwise, in order to recover the mixture of distilled *n*-pentane and EO in an appropriate vial. The mixture was then concentrated to a minimum volume of about 200 μ L, at room temperature under nitrogen flux, using a blow-down evaporator system. After extraction and until analysis, the EO samples were stored at -20°C in the dark.

Chemical analysis of the essential oils

Gas Chromatography (GC-FID)

Gas chromatographic analyses were performed using a Perkin Elmer Autosystem XL gas chromatograph (Perkin Elmer, Shelton, CT, USA) 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 col-

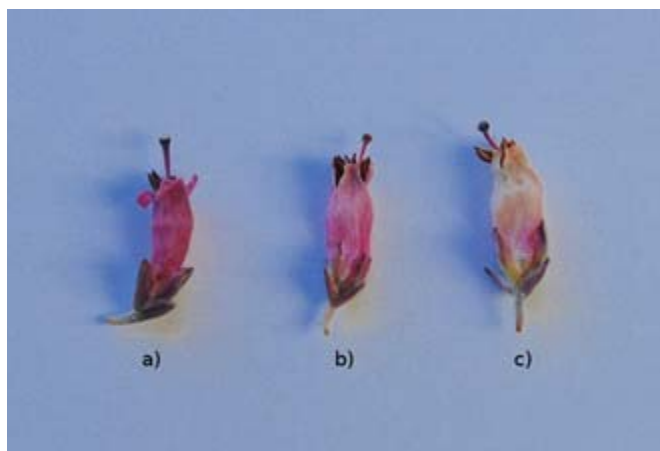


Figure 1. Different colour tonalities of *Erica australis* L. flowers: dark pink (a), medium pink (b) and light pink (c)

umn (30 m x 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 x 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 300°C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of 30 cm/s. The samples were injected using split sampling technique, ratio 1:50. The volume of injection was 0.1 μL of a pentane-essential oil solution. The percentage composition of the volatiles was computed, by the normalization method from the GC peak areas, calculated as the mean value of two injections, from each sample, without response factors.

Gas chromatography-mass spectrometry (GC-MS)

The GC-MS unit consisted on a Perkin Elmer Autosystem XL gas chromatograph, equipped with DB-1 fused-silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm ; J & W Scientific, Inc.), and interfaced with Perkin-Elmer Turbo-mass mass spectrometer (software version 4.1, Perkin Elmer, Shelton, CT, USA). 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; split ratio, 1:40; ionization energy, 70 eV; scan range, 40-300 u; scan time, 1 s.

The identification of the components was assigned by comparison of their retention indices, relative to $\text{C}_8\text{-C}_{25}$ *n*-alkane and GC-MS spectra from a lab-made library, created with reference essential oils, laboratory-synthesized components and laboratory isolated compounds, and commercial available standards.

Results and discussion

Regardless of the flower colour, the hydro-distillation of the flowering aerial parts of *E. australis* yielded always <0.05 % (0.001 %, 0.004 % and 0.008 % for *Ea-lp*, *Ea-mp* and *Ea-dp*, respectively, that is, 0.004 % in average, v/d.w.) of pale yellowish essential oils.

Forty-three compounds were identified in *E. australis* samples essential oils, covering 81-86 % of the total. Identified essential oil components are listed in Table 1 in the elution order on the GC-FID DB-1 column and the corresponding GC-MS profile provided in Figure 2.

The alcohol 1-octen-3-ol (33-38 %) was present in all samples and dominated among the main constituents (≥ 5 %), followed by the aldehyde *n*-nonanal (8-11 %), and the alcohol *n*-octanol (6-7 %). Other alcohols like *n*-heptanol (4 %), *cis*-3-hexen-1-ol (2-5 %), and 2-octen-1-ol (2-3%) were also present in the three samples, although in a minor proportion. The aldehydes 2-*trans*, 4-*trans*-decadienal (2-4 %), and 2-*trans*-decenal (2 %), and nonanoic acid (2 %) were also identified in the three samples. Only two sesquiterpene hydrocarbons, *trans*, *trans*- α -farnesene (0.2-0.8 %), and *cis*-bourbonene (traces-0.3 %), and three oxy-

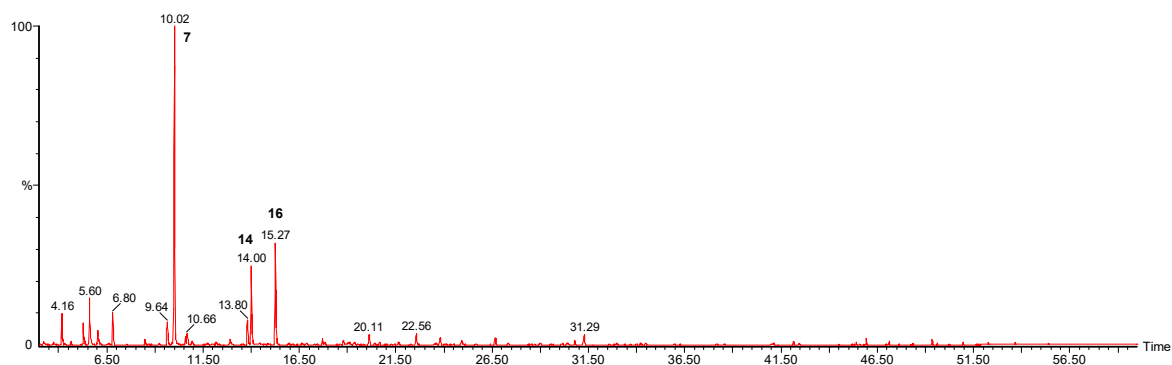


Figure 2. Gas chromatography-mass spectrometry profile of the essential oil isolated from *Erica australis*, showing medium pink-flowers (*Ea_mp*).

7 = 1-Octen-3-ol, 14 = *n*-octanol, and 16 = *n*-nonanal

Table 1. Percentage composition of the essential oil isolated by hydrodistillation from the dried flowering aerals parts of *Erica australis*, showing light pink, medium pink and dark pink flowers (*Ea_lp*, *Ea_mp* and *Ea_dp*, respectively).

No.	Compounds	RI	RI ^a	<i>Erica australis</i>		
				Light pink	Medium pink	Dark pink
1	2- <i>trans</i> -Hexenal	866	827	t	t	t
2	<i>cis</i> -3-Hexen-1-ol	868	834	2.2	3.8	5.4
3	<i>n</i> -Hexanol	882	854	t	t	t
4	<i>n</i> -Heptanal	897	865	1.2	1.1	1.1
5	Benzaldehyde	927	961	t	t	t
6	<i>n</i> -Heptanol	952	969	3.8	4.0	3.6
7	1-Octen-3-ol	961	983	32.8	34.8	38.3
8	2-Pentyl furan	973	975	0.9	1.5	1.2
9	<i>n</i> -Octanal	973	983	1.0	1.1	0.9
10	Benzene acetaldehyde	1002	1004	t	t	t
11	2,6,6-Trimethyl cyclohexanone	1003	1016	0.3	0.6	0.4
12	Acetophenone	1017	1027	t	0.4	0.4
13	2-Octen-1-ol	1024	1045	2.1	2.6	2.8
14	<i>n</i> -Octanol	1045	1052	5.8	7.0	7.3
15	2-Nonanone	1058	1070	t	0.1	t
16	<i>n</i> -Nonanal	1073	1084	8.3	11.0	8.4
17	<i>trans</i> -Pinocarveol	1106	1120	0.4	0.5	0.1
18	2- <i>trans</i> -Nonenal	1124	1135	0.2	0.9	0.7
19	<i>n</i> -Nonanol	1151	1152	t	0.7	0.5
20	Octanoic acid	1152	1157	0.9	0.7	0.8
21	α -Terpineol	1159	1157	1.3	0.7	0.7
22	<i>n</i> -Decanal	1180	1190	1.2	1.2	0.8
23	Octanol acetate	1187	1193	t	0.2	0.2
24	2- <i>trans</i> -Decenal	1224	1239	2.2	1.7	1.7
25	Nonanoic acid	1273	1279	2.1	2.3	1.6
26	2-Undecanone	1275	1277	t	t	t
27	2- <i>trans</i> ,4- <i>trans</i> -Decadienal	1286	1287	2.1	3.6	1.9
28	<i>trans</i> -2-Undecenal	1323	1345	0.5	0.4	0.4
29	Decanoic acid	1350	1390	t	t	t
30	<i>cis</i> -Bourbonene	1379	1385	t	t	0.3
31	2-Pentadecanone	1390		0.6	0.5	0.1
32	<i>n</i> -Tetradecane	1400		0.6	0.2	0.6
33	Geranyl acetone	1434	1430	1.7	1.5	1.1
34	<i>trans</i> , <i>trans</i> - α -Farnesene	1500	1494	0.8	0.5	0.2
35	3- <i>cis</i> -Hexenyl benzoate	1533		t	t	0.1
36	<i>n</i> -Tetradecanal	1596	1589	0.3	0.2	0.2
37	<i>n</i> -Hexadecane	1600		1.0	0.1	0.3
38	<i>n</i> -Pentadecanal	1688	1687	1.7	0.7	0.9
39	<i>n</i> -Octadecane	1800		t	t	t
40	Palmitic acid	1980	1973	2.6	0.6	0.6

table 1. (continued).

No.	Compounds	RI	RI ^a	<i>Erica australis</i>		
				Light pink	Medium pink	Dark pink
41	<i>n</i> -Eicosane	2000		0.8	0.1	0.2
42	<i>n</i> -Heneicosane	2100		1.6	0.3	0.6
43	<i>n</i> -Docosanal	2426	2426	0.3	t	0.1
	% of identification			81.3	85.6	84.5
	Grouped components					
	Oxygen-containing monoterpenes			3.4	2.7	1.9
	Sesquiterpene hydrocarbons			0.8	0.5	0.5
	Others			77.1	82.4	82.1
	Yield (%; v/d.w.)			<0.05	0.004	0.008

RI: In lab retention index calculated relative to C₈-C₂₅ *n*-alkanes on the DB-1column;

RI^a: (Linstrom and Mallard, 2015)¹⁵ - reported literature retention indices on DB-1 or similar phase column (100 % Dimethylpolysiloxane) not from the authors lab.

t: trace (<0.05 %).

gen-containing monoterpenes, *trans*-pinocarveol (0.1-0.5 %), α -terpineol (1 %), and geranyl acetone (1-2 %) were detected in all samples.

Despite the differences in the flower colour, no remarkable differences were observed between the three samples in volatile components, although there was a slight predominance of oxygen-containing monoterpenes, sesquiterpene hydrocarbons, *n*-pentadecanal, palmitic acid, *n*-eicosane, and *n*-heneicosane, in the Ea-lp sample. Greater amounts of the aldehydes *n*-nonanal, 2-*trans*-nonenal, and 2-*trans*, 4-*trans*-decadienal were detected in the Ea-mp sample, while the Ea-dp was richer in the alcohols 1-octen-3-ol, *n*-octanol and *cis*-3-hexen-1-ol (Table 1).

To the best of our knowledge, this is the first report on the chemical composition of *E. australis* flowering aerial parts essential oil. Tzitsa and co-workers¹⁰ investigated the essential oil composition of *E. manipuliflora* Salisb. collected in two different regions from Greece, and one of the analysed samples showed some components common to *E. australis* essential oil, like 1-octen-3-ol, *n*-nonanal, and *n*-octanol. The essential oil composition of *E. arborea* L. leaves from Algeria was recently reported¹¹, being palmitic acid its main component (33 %), while in present study

samples volatiles it was detected in minor amounts (1-3 %).

1-Octen-3-ol, the major constituent identified in *E. australis* essential oil, is known as “mushroom alcohol” because it has been isolated from almost every fungal species. It is widely used as a food and flavouring agent as well as a component of perfumes¹². Moreover, this compound was reported for its antifungal, insecticidal and herbicidal properties¹³.

Many factors influence the yield and the composition of an essential oil. Physiological, environmental and geographic variations, genetic factors and evolution, amount of plant material, and agricultural practices, among others¹⁴, are some factors that can explain the chemical variability in essential oil composition and yield. Nevertheless, in the present study no relationship was detected between flower colour and volatile composition.

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