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Short communication

Analysis of the essential oil composition from the different parts of *Eryngium glaciale* Boiss. from Spain☆

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Abstract

The essential oil from the different parts (inflorescences, stems + leaves and roots) of *Eryngium glaciale* Boiss. gathered in Sierra Nevada (Spain) has been extracted by steam distillation and analysed by gas chromatography and gas chromatography coupled to mass spectrometry. Quantitative but not qualitative differences have been found between the analysed parts. The principal compounds from the inflorescences oil were found to be phyllocladene isomer (43.5%), (*E*)-caryophyllene (15.2%) and valencene (11.5%), while the oil from stems and leaves only showed phyllocladene isomer (41.3%) as main one. The oil from the roots presented phyllocladene isomer (49.4%) and linalool (19.1%) as major constituents. This is the first report on the chemical composition of this species.

Keywords: *Eryngium glaciale*; Apiaceae; Essential oil; Phyllocladene; Linalool; (*E*)-Caryophyllene; Valencene

1. Introduction

The genus *Eryngium* L. belongs to the Apiaceae family and, with about 250 species, is distributed practically all over the world. Fourteen of the 26 species described in Flora Europaea grow in the Iberian Peninsula [1]. Although *Eryngium glaciale* Boiss. has been considered as an endemic species of "Sierra Nevada" (Spain) [2]. It should be a relict taxon that according to Flora Europaea appears also in Northwest Africa and only grows over stony places above 2500 m. Currently in Spain it is included in the red book of vulnerable species and only appears in the Natural and National Park of Sierra Nevada, Granada (Spain) (Fig. 1) [2]. *E. glaciale* is an perennial erect herb with stems of 5–20 cm, basal leaves coriaceous, persistent. Inflorescences bluish, pedunculate, globose and fruits without scales.

The chemistry, genetic diversity and properties of several species of *Eryngium* have been previously studied [3–19], but the essential oils of only few species have been previously reported [20–32]. Table 1 shows the main constituents of the oils of each studied species that have been alphabetically ordered.

In the present work we contribute to the knowledge of the essential oils of *Eryngium* species with the chemical composition of the different parts of *E. glaciale* Boiss. As far as we know this is the first report on the essential oil of this species.

2. Materials and methods

2.1. Plant material

Few specimens of *E. glaciale* were gathered in Sierra Nevada, Granada (Spain) in 27-7-2000. A voucher specimen (MACB-77655) has been lodged at the Herbarium of the Faculty of Biology, Complutense University, Madrid, Spain.

2.2. Isolation of volatile oils

The oils from the different air-dried parts of *E. glaciale* were isolated by steam distillation with cohobation for 8 h according to the method recommended in the Spanish Pharmacopoeia. The

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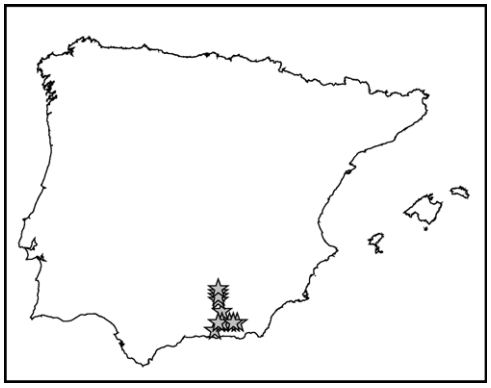

Fig. 1. Distribution of *E. glaciale* Boiss. in the Iberian peninsula.

oils were dried over anhydrous magnesium sulphate and stored at 4 °C in the dark. The yield of the different parts, based on dry weight, appears in Table 2.

2.3. Gas chromatography

Analytical gas chromatography (GC) was carried out on a Varian 3300 gas chromatograph fitted with a fused methyl silicone DB-1 column (50 m × 0.25 mm, 0.25 μm film thickness). Temperature was programmed from 95 to 240 °C at 4 °C min^{-1}. Injection was performed at 250 °C in the split mode (1:100). Nitrogen was used as the carrier gas (1.5 mL min^{-1}). Detection was performed by flame ionisation detection (FID) at 300 °C. Injection volume for all the samples was 0.1 μL of pure oil.

2.4. Gas chromatography–mass spectrometry (GC–MS)

GC–MS was carried out on a Hewlett-Packard 5890 gas chromatograph fitted with a fused Silica SE-30 capillary column (50 m × 0.22 mm, 0.25 μm film thickness), coupled to a HP 5971A mass selective detector. Column temperature was programmed from 70 to 220 °C at 4 °C min^{-1} and helium was used as carrier gas. Mass spectra were recorded in the scan mode at 70 eV. In order to confirm the identification of some compounds, the oil samples were also analysed on a VG Quattro mass spectrometer operating at 70 eV ionisation energy. The GC column used was a DB-wax (60 m × 0.32 mm, 0.25 μm) programmed from 35 to 220 °C at 3 °C min^{-1} with helium as carrier gas.

2.5. Qualitative analyses

Most constituents were tentatively identified by GC by comparison of their retention indices with those of authentic standards available in the author's laboratory or with retention indices from Refs. [33–37]. Further identification was achieved by GC–MS. Other constituents were either synthesised or identified in oils of known composition. The fragmentation patterns

Table 1
Main constituents of the essential oils of *Eryngium* species previously reported

Species	Main components	References
Eryngium billardieri F. Delaroche	α-Muurolene (42.0%) and β-gurjunene (17.0%)	[31]
Eryngium bourgatii Gouan	Inflorescences oil: phyllocladene (37.6%) and bicyclogermacrene (15.1%) Stems and leaves oil: phyllocladene (20.4%), γ-muurolene (11.8%) and (*E*)-caryophyllene (10.1%) Roots oil: γ-muurolene (15.4%) and phyllocladene (15.0%)	[32]
Eryngium expansum F. Muell	7-Epi-α-selinene (38.3%), *cis*-β-guaiene (10.8%), 2,3,6-trimethylbenzaldehyde (8.0%) and (*E*,*E*)-α-farnesene (7.3%)	[28]
Eryngium foetidum L.	(*E*)-2-Dodecenal 2,4,5-Trimethylbenzaldehyde (20.5%) 2,3,6-Trimethylbenzaldehyde (5.5–23.7%) and (*E*)-2-dodecanal	[21,22] [23,24] [25]
Eryngium maritimum L.	Aerial parts: germacrene D (43.1–42.4%) and 9-muurolen-15-aldehyde (22.4–16.4%) Roots: γ-guaiene (40.2%), 2,3,4-trimethyl-benzaldehyde (24.5%) and germacrene D (10.6%)	[26]
Eryngium paniculatum Cav.	(*E*)-Anethole (52.6%) and α-pinene (19.1%)	[27]
Eryngium pandanifolium Cham. & Schlecht.	Aerial parts: bornyl acetate (20.8%), β-selinene (13.8%), α-selinene (11.3%) and α-muurolene (8.0%) Fruit oil: heptanol (11.5%) and β-selinene (9.2%)	[28]
Eryngium rostratum Cav.	Leaf oil: spathulenol (20.0%) and β-bisabolol (8.6%) Fruit oil: β-bisabolol (65.3%)	[28]
Eryngium vesiculosum Labill.	Winter leaves: β-caryophyllene (20.3%), germacrene D (19.2%) and α-humulene (8.8%) Summer leaves: bicyclogermacrene (22.2%), β-caryophyllene (15.6%), germacrene D (15.8%) and α-humulene (8.1%)	[28,29]
Eryngium yuccifolium Michx.	Leaves: germacrene D (18.3%), terpinolene (17.8%), bicyclogermacrene (8.8%) and α-pinene (7.56%) Stalks: germacrene D (38.4%), δ-amorphene (12.2%) and bicyclogermacrene (10.1%) Roots: terpinolene (25.8%), *trans*-(*E*)-bergamotene (18.6%) and 2,3,6-trimethylbenzaldehyde (13.9%)	[30]

Table 2
Oil yield of the different parts of *E. glaciale* Boiss. from Spain

Sample	Voucher details	Yield (%)
E.gl.F.	MACB-77655. Granada: Sierra Nevada.	0.16
E.gl.SL	Grassland in the base of Veleta peak.	0.26
E.gl.R	27-7-2000. 30SVG6605	0.30

Yield based on dried weight. E.gl.: *Eryngium glaciale*; F: inflorescences; SL: stems and leaves; R: roots.

of mass spectra were also compared with those stored in the spectrometer database using the WILEY.L built-in libraries.

3. Results and discussion

Some species of the Apiaceae family are well known for its high yield and characteristic essential oils (*Pimpinella anisum* L., *Foeniculum vulgare* Miller, *Cachrys sicula* L., *Angelica sylvestris* L.). However, according to our results (Table 2) and with those previously reported [20–32] the genus *Eryngium* does not contain great amounts of essential oil. The different parts of *E. glaciale* analysed showed different yields. The part with higher amount was the roots (E.gl.R.), followed by the stems and leaves (E.gl.SL) and finally the inflorescences (E.gl.F). These differences could be explained because the essential oil of this perennial species could be accumulated in the roots during the winter, as other Apiaceae species do, and later distributed throughout the plants during the vegetative season. To confirm this hypothesis a seasonal study of the yield of this species should be done.

The components identified from the different parts of *E. glaciale*, their retention indices and their percentage composition are summarised in Table 3 where all the compounds are arranged in order of their elution on the DB-1 column although the retention indices of compounds confirmed on DB-wax column have been also included. In spite of the yield differences above commented, the chemical composition of different parts share the major constituent that have been identified as phyllocladene isomer, 43.5%, 41.3% and 49.4% for E.gl.F, E.gl.SL and E.gl.R, respectively. However, each sample except E.gl.SL showed other main constituents as linalool (19.1%) in E.gl.R, or (*E*)-caryophyllene (15.2%) and valencene (11.5%) in E.gl.F. Other representative compounds were found to be cryptone (4.6%), β-chamigrene (3.7%), linalool (3.0%), caryophyllene oxide (2.1%) and α-pinene (2.0%) in E.gl.F. The E.gl.SL fraction share also few compounds with the previous one: (*E*)-caryophyllene (7.1%), valencene (6.9%), cryptone (5.9%), linalool (5.9%), caryophyllene oxide (3.4%), β-phellandrene (2.2%) and β-chamigrene (2.2%). The last fraction (E.gl.R) was little bit different with β-phellandrene (7.4%), 6-camphenone (3.8%), α-pinene (2.9%) and *p*-mentha-2,4(8)-diene (2.7%). A total of six compounds could not be identified although their mass spectral fragmentation patterns have been included at the end of Table 3.

It is worth mentioning the presence of a diterpene, phyllocladene isomer, as major compound for all the samples, because the presence of diterpenes as main constituent in essential oils

Table 3
Essential oil composition of the different parts of *E. glaciale* from Spain

Compound	*I*	E.gl.F	E.gl.SL	E.gl.R
Nonane	869	t	t	0.1
α-Pinene	932 (1012)	2.0	1.4	2.9
5-Methyl-3-heptanone	960	t	t	0.1
Sabinene	963 (1113)	t	t	0.4
Myrcene	985 (1160)	t	–	–
δ-3-Carene	1003	0.9	t	0.2
Limonene	1026 (1191)	0.3	0.3	1.0
β-Phellandrene	1027 (1201)	0.1	2.2	7.4
p-Mentha-2,4(8)-diene	1073	0.4	0.8	2.7
trans-Linalool oxide	1077	0.1	0.1	0.4
6-Camphenone	1082	0.5	1.1	3.8
Cryptone	1087 (1669)	4.6	5.9	–
Linalool	1096 (1549)	3.0	5.9	19.1
Undecane	1100	0.2	0.4	1.7
Chrysanthenone	1122	t	t	0.2
Terpinen-1-ol	1130	0.1	0.2	0.7
Lavandulol	1153	t	t	0.2
2,4,5-Trimethylbenzaldehyde	1305 (1896)	t	0.1	0.3
2,4,6-Trimethylbenzaldehyde	1338 (1929)	0.2	–	–
Daucene	1370	t	–	–
β-Elemene	1387 (1587)	0.2	–	–
α-Cedrene	1405 (1669)	0.2	–	–
(*E*)-Caryophyllene	1410 (1594)	15.2	7.1	1.1
α-Humulene	1447 (1667)	1.0	0.4	–
(*E*)-β-Farnesene	1452 (1770)	0.3	0.3	0.3
β-Chamigrene	1470	3.7	2.2	0.4
β-(*E*)-Ionone	1481	t	–	–
Valencene	1485	11.5	6.9	1.0
β-Selinene	1490 (1704)	t	–	–
α-Selinene	1492 (1727)	t	–	–
Bicyclogermacrene	1493 (1750)	t	–	–
β-Bisabolene	1513 (1727)	0.9	0.4	0.2
n.i. 1	1516	0.7	t	0.3
n.i. 2	1517	0.6	–	–
n.i. 3	1559	0.6	–	–
Spathulenol	1576 (2133)	0.4	0.9	0.3
Caryophyllene oxide	1580 (1987)	2.1	3.4	1.6
n.i. 4	1586	2.4	0.2	–
n.i. 5	1587	0.6	1.2	–
n.i. 6	1779	0.2	0.9	–
Phyllocladene isomer	1857	43.5	41.3	49.4
Monoterpene hydrocarbons		3.7	4.7	14.6
Oxygenated monoterpenes		3.2	7.3	24.7
Sesquiterpene hydrocarbons		33.0	17.3	3.0
Oxygenated sesquiterpenes		7.8	10.3	1.9
Diterpene hydrocarbons		43.5	41.3	49.4
Oxygenated diterpenes		0.0	0.0	0.0
Total		90.5	80.8	93.6

I: Kováts retention indices on DB-1 column on DB-wax in parenthesis; t: traces (% <0.1); n.i.: not identified; E.gl.: *Eryngium glaciale*; F: inflorescences; SL: stems and leaves; R: roots. n.i. 1 *I* = 1516, 204[M^+](5), 119(100), 134(60), 91(37), 57(25), 69(23), 105(20), 41(18), 161(15), 189(2); n.i. 2 *I* = 1517, 236[M^+](3), 119 (100), 57(20), 85(19), 134(18), 105(15), 41(10), 221(3); n.i. 3 *I* = 1559, 220[M^+](35), 135(100), 107(88), 159(85), 91(83), 121(81), 177 (79), 81(60), 41(45), 55(40), 137(39), 69(30), 161(23), 205(20), 189(10); n.i. 4 *I* = 1586, 205[M^+](5), 136(100), 91(35), 79(33), 41(30), 69(30), 109(20), 119(17), 159(10), 177(8), 187(7); n.i. 5 *I* = 1587, 220[M^+](5), 143(100), 105(65), 161(47), 85(45), 43(44), 125(30), 71(27), 93(25), 179(15), 187(4), 205(4); n.i. 6 *I* = 1779, 218[M^+](75), 105(100), 91(95), 157(85), 133(65), 77(60), 122(55), 175(48), 41(45), 189(15), 203(10); Phyllocladene isomer *I* = 1857, 272[M^+](1), 91(100), 55(83), 115(78), 117 (70), 129 (55), 159(53), 41(48), 77(47), 103(45), 141(40), 173(15), 187(10), 229(8), 256(5).

is not very common. However, we have previously reported the essential oil composition of *Eryngium bourgatii* that also contained a diterpene as principal compound [32]. As we proposed in that paper, *E. bourgatii* and now *E. glaciale* could use the diterpenes as thermo regulators in winter while in summer still as main constituent because it has high molecular weight that impedes its evaporation to be used as hydro regulator. This is only a hypothesis but the natural habit of both species, mountains exposed to high temperature during the summer and low ones during the winter, could explain the presence of these kind of compounds.

The origin of this genus should be very old according to its diversity and wide distribution, although as far as we know there is not any study about its philogenetic tree. As contribution to that future work, we think that the presence of phyllocladene and derivatives should be studied in detail in other species of this genus. According to our results, *E. bourgatii* and *E. glaciale*, species that grow in the mountains under characteristic climatic conditions, share these principal compounds. If their chemical composition is similar they should be closer in its philogenetic tree while the species whose composition was different should diverge before. Besides, this study should testimony if these species descent from a common chemotype or if they have converged as an adaptation to their habitats. On the other hand, we have also detected in low quantities 2,4,5- and 2,4,6-trimethylbenzaldehyde, compounds that have been described as main or representative constituents in other species of *Erynigum* as *E. foetidum*, *E. maritimum*, *E. expansum* and *E. yuccifolium* and that normally appears in this genus [21–26,29,30]. However, these constituents seem to be so common that they should not be used for chemotaxonomy proposes.

Finally, the distribution of the terpenoid compounds is also different between the samples (Table 3). In the aerial fractions (E.gl.F, E.gl.SL) the distribution is the same, the diterpene amounts are higher with 43.5% and 41.3%, respectively, all of them hydrocarbons. This patter is the same with the sesquiterpene fraction (40.8% and 27.6%) where the hydrocarbons are the majority again (33.0% and 17.3%, respectively). Although the root fraction (E.gl.R) showed also diterpenes in great amount (49.4%) the monoterpene fraction (39.3%) replace the sesquiterpenes and it is the unique fraction where the oxygenated compounds predominate with 24.7%. These differences could be explained as we suggested above because the roots accumulate these compounds during the winter.

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