

SUPPLEMENTARY MATERIAL

The chemical composition of the aerial parts essential oil of *Acinos alpinus* subsp. *nebrodensis* (Lamiaceae) growing in Sicily (Italy).

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Acinos is a small genus of economically important plants belonging to Lamiaceae family whose botanical collocation is quite problematic due to the disagreement among the botanists and the presence in literature of several names and synonyms from different sources. In the present study the chemical composition of the essential oil from aerial parts of *Acinos alpinus* subsp. *nebrodensis* (Strobl) Brullo & Brullo collected in Central Sicily was analyzed by GC-MS. The result showed the presence of large quantity of sesquiterpene hydrocarbons with germacrene D (37.9%) as the most abundant component followed by (*E*)- β -caryophyllene (5.1%). Among the oxygenated monoterpenes thymol (8.3%) was the most abundant; good quantity of hexadecanoic acid (6.8%) was also observed. Chemotaxonomic considerations with respect all the other oils of *Acinos* taxa, studied so far, were carried out.

Keywords: *Acinos alpinus* subsp. *nebrodensis*; Lamiaceae; Essential oils; Germacrene D; Chemotaxonomy

1. Introduction

Acinos alpinus (L.) Moench (Syn. *Clinopodium alpinum* (L.) Kuntze, *Calamintha alpina* (L.) Lam. ssp. *alpina*, *Satureja alpina* (L.) Scheele, *Calamintha alpina* (L.) Lam., *Thymus alpinus* (L.)) is a suffruticous camefita common in most of the mountain areas of southern Europe, which grows between 900 and 2600 m of altitude. It is an aromatic plant with smell of mint, 5-30 cm tall, bristly due to the presence of short hairs. The stems, woody at the base, are first rooting then ascending. The leaves are oblanceolate (5-15 mm long), whilst the inflorescences have a number of flowers between 3 and 8. The corolla, with bilateral symmetry, has a violet colouration. *A. alpinus* subsp. *meridionalis* [Nyman] P.W. Ball (Syn. *A. granatensis* (Boiss. et Reuter) Pignatti subsp. *aetnensis* (Strobl) Pignatti, *Calamintha granatensis* Boiss. et Reuter) is similar to *A. alpinus* but with more lignified stem, leaves with reflected hair (0.1-0.2 mm), 5-6 mm calyx with short teeth and hooked hairs and 10-12 mm corolla. The populations of *A. alpinus* ssp. *nebrodensis* (A. Kern. et Strobl)

Pignatti differ, on the contrary, for the leaves markedly pubescent, with entire margin and acuminate apex and 7-8 mm long calyxes (Pignatti 2018).

3. Experimental

3.1. Plant material

Aerial parts from *Acinos alpinus* subsp. *nebrodensis* (Aan) were collected on Monte Sant'Otiero, a quarzerenic mountainous relief of Madonie (Sicily), at about 1440 m, 37°50'39.17" longitude N and 14°04'12.10" latitude E. One of the samples, identified by Prof. Vincenzo Ilardi, has been stored in the University of Palermo Herbarium (voucher No PAL 109716).

3.2. Isolation of volatile components

Air-dried samples were ground in a Waring blender and then subjected to hydrodistillation for 3 h, according to the standard procedure described in European Pharmacopoeia (2020). The oil was dried over anhydrous sodium sulphate and stored in sealed vial under N₂, at -20 °C, ready for the GC and GC-MS analyses; the sample yielded 0.25% of oil (w/w).

3.3. GC, GC-MS analysis of essential oil

GC-MS analysis was performed using an Agilent 7000C GC system, fitted with a fused silica Agilent HP-5MS capillary column (30 m x 0.25 mm i.d.; 0.25 µm film thickness), coupled to an Agilent triple quadrupole Mass Selective Detector MSD 5973 (ionization voltage 70 eV; electron multiplier energy 2000 V; transfer line temperature, 295 °C Solvent Delay: 4 min). GC analysis was performed with a Shimadzu QP 2010 plus equipped with a AOC-20i autoinjector (Shimadzu, Kyoto, Japan) gas chromatograph equipped with a FID, a capillary column (DB-Wax) 30 m × 0.25 mm i.d., film thickness 0.25 µm and a data processor. The oven program was as follows: temperature increase at 40 °C for 5 min, at a rate of 2 °C/min up to 260 °C, then isothermal for 20 min. Helium was used as carrier gas (1 mL min⁻¹). The injector and detector temperatures were set at 250 °C and 290 °C, respectively. 1 µL of oil solution (3% EO/Hexane v/v) was injected with split mode. Linear retention indices (LRI) were determined by using retention times of *n*-alkanes (C₈-C₄₀) and the peaks were identified by comparison with mass spectra and by comparison of their relative retention indices with WILEY275, NIST 17, ADAMS, and FFNSC2 libraries.

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Table S1. Main compounds (> 3%) of the essential oils from aerial parts of *Acinos* taxa obtained by hydrodistillation.

Taxa	Origin	Main compounds	Ref.
<i>A. alpinus</i>	Mainalon Mt., Greece	germacrene A (32.3), <i>epi</i> -bicyclosesquiphellandrene (15.8), β -elemene (12.5), (<i>E</i>)- β -caryophyllene (12.2), spathulenol (5.4), caryophyllene oxide (4.5), δ -elemene (3.9)	Skaltsa et al. 1999
<i>A. alpinus</i>	Turkey	germacrene D (39.8-3.5), hexadecanoic acid (24.3-3.7), α -terpinyl acetate (13.1-0), thymol (15.3-0), carvacrol (8.3-0), caryophyllene oxide (7.8-0.9), spathulenol (7.5-0.7), geraniol (7.3-0), pulegone (4.9-0), (<i>E</i>)- β -caryophyllene (4.8-1.4), bicyclogermacrene (3.9-0.5), β -bourbonene (3.5-0)	Kaya et al. 1999b
<i>A. alpinus</i> ssp. <i>meridionalis</i>	Jaén, Spain	germacrene D (56.8), (<i>E</i>)- β -caryophyllene (6.2), bicyclogermacrene (5.9), β -bourbonene (4.1), α -phellandrene (3.6), β -elemene (3.3)	Velasco-Negueruela et al. 1993
	Madrid, Spain	germacrene D (43.2), (<i>E</i>)- β -caryophyllene (15.4), bicyclogermacrene (11.7), β -myrcene (5.8)	Velasco-Negueruela et al. 1993
<i>A. arvensis</i>	Canada	germacrene-D (51.4), caryophyllene (7.9), β -bourbonene (4.8), cadinene (3.0)	Kaya et al. 1999a
	Greece	pulegone (51.3), isomenthone (18.1), alloocimene (6.9), menthone (4.2)	Souleles and Katsiotis 1988
	Rtanj, Serbia	germacrene D (47.0), β -bourbonene (7.7), caryophyllene oxide (3.2), (<i>E</i>)- β -caryophyllene (3.0)	Jovanovic et al. 2005
	Kastamonu, Turkey	germacrene-D (14.3), hexadecanoic acid (14.0), β -bourbonene (7.0), caryophyllene oxide (3.2), abietatriene (3.0)	Kaya et al. 1999a
<i>A. graveolens</i>	Fars, Iran	dillapiole (32.7), germacrene D (16.5), δ -cadinene (4.9), terpinen-4-ol (4.1), caryophyllene oxide (3.6), (<i>E</i>)- β -caryophyllene (3.3), α -humulene (3.1), β -selinene (3.1)	Javidnia et al. 2010
	Isfahan, Iran*	germacrene D (56.6), bicyclogermacrene (8.7), caryophyllene oxide (7.5), (<i>E</i>)- β -caryophyllene (4.5), limonene (3.4)	Bidgoli et al. 2019
	Pcinja River, Serbia	germacrene D (58.6), bicyclogermacrene (7.1), acetophenone (3.1)	Golubović et al. 2010
<i>A. hungaricus</i>	Bela Crkva, Serbia	germacrene D (46.7), β -bourbonene (6.6), 1,8-cineol (5.6), caryophyllene oxide (3.5)	Chalchat et al. 2004
	Kurvin, Serbia	caryophyllene oxide (16.8), geraniol (9.7), β -bourbonene (5.7), globulol (5.6), borneol (4.8), spathulenol (4.4), α -cadinol (4.2), 1,8-cineol (3.5)	Jovanovic et al. 2002
<i>A. majoranifolius</i>	Orjen Mt., Serbia	pulegone (97.0), menthone (3.0)	Pavlovic et al. 1984a
<i>A. rotundifolius</i>	Eskişehir, Turkey	germacrene D (73.1), bicyclogermacrene (10.4)	Kaya et al. 1999a

	Eskişehir, Turkey	germacrene D (18.5), hexadecanoic acid (17.5), caryophyllene oxide (3.6), bicyclogermacrene (3.0)	Kaya et al. 1999a
	Susurluk, Turkey	germacrene D (32.6), menthol (23.9)	Kaya et al. 1999a
	Kastamonu, Turkey	hexadecanoic acid (30.2), spathulenol (15.0), germacrene D (14.4), α -cadinol (3.2)	Kaya et al. 1999a
<i>A. suaveolens</i>	Cholomon Mt., Greece	pulegone (69.0), isomenthone (17.0)	Kokkalou 1988
	Dren Mt., Macedonia	pulegone (96.9)	Pavlovic et al. 1984b
	Marmara Island, Turkey	pulegone (80.7), isomenthone (8.8)	Kaya et al. 1999a
	Kaz Mt. (Gürlek), Turkey	pulegone (45.7), isomenthone (45.4)	Kaya et al. 1999a
	Kaz Mt. (Babadağ), Turkey	isomenthone (54.1), pulegone (23.2)	Kaya et al. 1999a
	Dereköy, Turkey	pulegone (37.1), hexadecanoic acid (17.2), (<i>E</i>)-nerolidol (12.6), linalool (10.0), carvacrol (6.0), germacrene D (4.0)	Kaya et al. 1999a
	Kazdağ Mt., Turkey	isomenthone (54.1), pulegone (33.2)	Tümen 1991
	Prespes, Greece	pulegone (67.7), isomenthone (4.9), limonene (4.4), isopulegone (3.0)	Couladis et al. 2002
	Mt. Parnassos, Greece	pulegone (77.7), isomenthone (6.8), isopulegone (3.2)	Couladis et al. 2002
<i>A. troodi</i> ssp. <i>grandiflorus</i>	South-west, Turkey	germacrene D (29.7-14.1), hexadecanoic acid (22.3-15.2), bicyclogermacrene (8.8-8.1), caryophyllene oxide (5.4-0.5), (<i>E</i>)- β -caryophyllene (5.1-0.2), dodecanoic acid (5.0-0.6), manoyl oxide (4.7-4.1), spathulenol (3.9-3.6)	Kaya et al. 1999c
<i>A. troodi</i> ssp. <i>vardaranus</i>	South-west, Turkey	hexadecanoic acid (18.2-7.5), germacrene D (14.5-10.2), manoyl oxide (11.2-5.4), bicyclogermacrene (10.6-7.1), spathulenol (10.3-4.9), caryophyllene oxide (6.1-2.1), dodecanoic acid (3.9-1.0)	Kaya et al. 1999c

*SDE = simultaneous distillation extraction method

Table S2. Composition (%) of the essential oil of *Acinos alpinus* subsp. *nebrodensis* collected in Sicily.

No.	Components ^a	LRI ^b	Area (%)
1	Hexanal	805	0.8
2	Hexanol ^c	871	0.1
3	α -Pinene ^c	937	1.3
4	Camphene ^c	947	0.7
5	β -Pinene ^c	974	1.4
6	Sabinene ^c	980	0.3
7	β -Myrcene ^c	990	tr
8	α -Phellandrene	1003	0.2
9	Octanal	1006	tr
10	α -Terpinene	1017	0.2
11	Limonene ^c	1023	3.8
12	<i>p</i> -Cymene	1025	0.4
13	β -Phellandrene	1032	0.2
14	γ -Terpinene	1065	0.4
15	Terpinolene	1087	0.1
16	Linalool	1097	0.9
17	Camphor ^c	1150	tr
18	Borneol	1171	1.5
19	α -Terpineol	1189	0.8
20	Decanal	1207	0.2
21	Nerol	1225	0.4
22	Geraniol ^c	1232	2.4
23	Linalyl acetate	1259	tr
24	Bornyl acetate	1281	1.0
25	Thymol ^c	1290	8.3
26	Eugenol	1359	2.3
27	α -Copaene	1364	0.6
28	β -Elemene	1372	1.3
29	Geranyl acetate	1381	0.7
30	β -Caryophyllene	1431	5.1
31	α -Humulene	1450	0.5
32	Germacrene D	1486	37.9
33	(<i>E,E</i>)- α -Farnesene	1491	tr
34	Bicyclogermacrene	1508	2.4
35	β -Bisabolene	1510	1.5
36	γ -Cadinene	1511	0.3
37	α -Cadinene	1553	0.9
38	β -Caryophyllene oxide ^c	1568	4.4
39	Dodecanoic acid	1570	0.6
40	τ -Cadinol	1632	0.2
41	α -Cadinol	1680	0.8
42	α -Bisabolol	1698	0.2
43	Tetradecanoic acid	1788	0.5
44	Hexahydrofarnesyl acetone	1832	1.4
45	Hexadecanoic acid	1960	6.8
46	Manool	2027	0.7
47	Phytol ^c	2111	1.1
48	Heptacosane ^c	2700	0.7
	Monoterpene hydrocarbons		9.0
	Oxygenated monoterpenes		18.3
	Sesquiterpene hydrocarbons		50.5
	Oxygenated sesquiterpenes		7.0
	Diterpenes		1.8
	Others		9.7
	Total		96.3

Components listed in order of elution on an HP-5MS column; ^b: LRI = Linear Retention Index determined by retention times on the mentioned column by co-injection of a homologous series of C₈-C₄₀ *n*-alkanes; tr = trace amount (< 0.05%); ^c: Co-injection with authentic standards.