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GC-MS Analysis of the Essential Oils of Ripe Fruits, Roots and Flowering Aerial Parts of *Elaeoselinum asclepium* subsp. *meoides* growing in Sicily

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The compositions of the essential oils obtained by hydrodistillation of the ripe fruits, flowering aerial parts and roots of *Elaeoselinum asclepium* (L.) Bertol subsp. *meoides* (Desf.) Fiori (Apiaceae) were determined by GC-MS analysis. All the analyzed parts were very rich in α-pinene (77.1%, 92.2% and 60.8%, respectively). Other compounds detected in appreciable amounts were β-pinene (4.3%, 4.0% and 8.2%), and sabinene (11.9% in ripe fruits; 12.8% in aerial parts). The plant synthesizes different classes of monoterpenes, including pinane, camphane, thujane, menthane, and fenchane derivatives, besides some acyclic monoterpenes.Trace amounts of sesquiterpenes and diterpenes were also detected.

Keywords: Essential oil, *Elaeoselinum asclepium* subsp. *meoides*, Apiaceae, GC, MS, biodiversity.

The genus *Elaeoselinum* W. D. J. Koch ex DC. (Umbelliferae) is represented in the European flora by four species: *E. asclepium* (L.) Bertol, *E. foetidum* (L.) Boiss, *E. gummiferum* (Desf.) Tutin, and *E. tenuifolium* (Lag.) Lange [1]. The last of these is now classified as *Distichoselinum tenuifolium* (Lag.) Garcia Martin & Silvestre [2], with some subspecies. *E. fontanesii* Boiss is the unique non-European species, growing in north-west Africa [2].

In Italy, *E. asclepium* comprises two subspecies [3]: *E. asclepium* subsp. *asclepium* (syn. *E. hispanicum* (Lange) Pau), and *E. asclepium* subsp. *meoides* (Desf.) Fiori (syn, *E. meoides* (Desf.) Koch ex DC). The former is distributed in eastern parts of Spain, Ibiza, southern parts of Italy, Sicily, Greece and adjacent islands [2], while the latter grows in southern Italy, Sardinia, Sicily, Algeria and Morocco [2]. *E. asclepium* subsp. *meoides* differs from the other subspecies by the presence of narrowly winged fruits and the presence of a few bracts and bracteoles [1].

The genus *Elaeoselinum* belongs to the Apioideae subfamily (Laserpitieae tribe). This tribe is considered to be typical of the Old World, and includes the genera *Laserpitium*, *Siler*, *Melanoselinum*, *Guillonea*, *Rouya*, *Polylophium*, *Thapsia* and *Elaeoselinum* [4,5].

It is reported that the leaves of *E. asclepium* subsp. *meoides* are used as bulk plugs to filter milk; during its passage through the plant material, the milk acquires a characteristic pleasant aroma [6]. In addition, *E. asclepium* is the host of a much sought after mushroom, *Pleurotus eryngii* var. *elaeoselini* [7].

Diterpenes (beyerane, kaurane and atisane derivatives), some with gibberellin-like activity, were reported from the neutral and acid fractions of the benzene extract of the roots of *E. asclepium* [8,9]. Kaurane flavonoids have also been detected, such as luteolin in *E. foetidum* and *E. meoides*, quercetin in *E. gummiferum*, and kaempferol in *E. tenuifolium* [4]. Recently, in the acetone fraction of the aerial parts of *E. asclepium* subsp. *meoides*, meoidic acid and some known diterpenes were identified and tested as cytotoxic agents against rat glioma C6 cells [10]. A recent study reported the larvicidal effectiveness against *Culex pipiens* L. of the essential oil of *E. asclepium* growing in Greece. This activity was attributed mainly to the non-oxygenated monoterpene fraction [11].

In this paper we report the composition of the essential oils obtained from ripe fruits, flowering aerial parts and roots of *E. asclepium* subsp. *meoides*, and the results are compared with oils from other *Elaeoselinum* species and subspecies.

The highest essential oil yield was obtained from the ripe fruits (3.8%), followed by the roots (2.2%) and the aerial parts (0.95%). Globally, 46 constituents were identified, accounting for 92.2-99.1% of the whole oils (Table1). All the plant parts produced essential oils dominated by monoterpenes (>92%). Among them, monoterpene hydrocarbons were the main chemical class (86.4-98.8%), while oxygenated monoterpenes were less represented, varying from 0 in the roots to 0.9% in ripe fruits and 5.8% in the aerial parts. The essential oil of the roots also contained small amounts of sesquiterpene hydrocarbons (0.2%) and diterpenes (0.1%). These compounds were either absent or detected in trace amounts in the other oils. Among the monoterpenes, the most representative constituents were α-pinene (60.8, 77.1, and 92.2% for aerial parts, ripe fruits and roots, respectively), followed by sabinene (12.8, 11.9 and 0.3%) and β-pinene (8.2, 4.3 and 4.0%). It was noted that the decrease in the sabinene content of the roots was compensated by a higher percentage of α-pinene, in harmony with the essential oils of other *Elaeoselinum* species [18-21], as summarized in Table 2. Either α-pinene or α+β-pinene as the main constituent is a common character of all the species, with the exception of *E. tenuifolium* fruits (where myrcene (47.9%) and sabinene (24.3%) were the main components), and one sample of *E. fontanesii* fruits, in which limonene (32.9%) was the principal compound. The sum α+β-pinene > sabinene was found for all the species, with the exception of *E. tenuifolium* (Table 2). Furthermore, the sum α+β- pinene > sabinene > limonene is a common characteristic of all the species, with exception of *E. tenuifolium* and one sample of *E. fontanesii* (Table 2).

E. asclepium subsp. *meoides* synthesizes various classes of monoterpenes, with pinane, camphane, thujane, menthane, and fenchane derivatives, together with some acyclic monoterpenes. Some of the volatile constituents, such as chrysanthenone, α-campholenal, *trans*-pinocarveol, *cis*-verbenol, pinocarvone, *p*-mentha-1,5-dien-8-ol, verbenone, *trans*-carveol, thymol methyl ether, carvone, cyclosativene, β-elemene and γ-cadinene are recorded for the first time for the genus.

Some authors have revised the chemistry and biological activity of the genus evidencing the ability of these plants to synthesize kauranes, beyeranes and artisane tetracyclic diterpenes, which derive from pimarane precursors [22].

Table1: Composition of the essential oils of *Elaeoselinum asclepium* subsp. *meoides* obtained from different plant parts.

Constituents	l.r.i^a	l.r.i^b	Ripe fruits	Roots	Aerial parts
α-Thujene	932	1018	0.2	tr	0.6
α-Pinene	942	1027	77.1	92.2	60.8
Camphene	955	1067	0.3	0.3	0.4
Sabinene	978	1116	11.9	0.3	12.8
β-Pinene	982	1108	4.3	4.0	8.2
Myrcene	992	1166	1.4	1.4	0.9
α-Phellandrene	1008	1167	tr	-	tr
δ-3-Carene	1013	1144	0.3	tr	0.3
α-Terpinene	1020	1180	tr	-	0.3
ρ-Cymene	1028	1242	0.2	tr	0.2
Limonene	1032	1198	0.6	0.6	0.8
β-Phellandrene	1033	1205	1.2	tr	0.3
(*Z*)-Ocimene	1040	1240	0.2	-	-
(*E*)-Ocimene	1051	1255	tr	-	tr
γ-Terpinene	1064	1246	0.2	tr	0.5
cis-Sabinene hydrate	1070	1462	tr	-	0.5
Terpinolene	1088	1288	tr	tr	0.1
trans-Sabinene hydrate	1103	1457	tr	-	0.5
cis-*p*-Menth-2-en-1-ol	1123	1585	-	-	tr
Chrysanthenone	1125	1506	tr	-	-
α-Campholenal	1127	1507	0.2	-	0.8
trans-Pinocarveol	1141	1635	0.2	-	0.8
Pinocarvone	1164	1548	0.1	-	0.5
p-Mentha-1,5-dien-8-ol	1166	-	-	-	0.2
4-Terpineol	1179	1607	tr	-	0.9
ρ-Cimene-8-ol	1185	1833	-	-	tr
Myrtenal	1195	1622	tr	-	0.8
Verbenone	1206	1716	tr	-	0.4
trans-carveol	1218	1872	-	-	0.4
endo-Fenchyl acetate	1220	1479	tr	-	-
Thymol methyl ether	1235	1593	tr	-	-
Carvone	1244	1742	-	-	tr
Isobornyl acetate	1287	1582	-	-	tr
Carvacrol	1299	2219	tr	-	-
α-Cubebene	1351	1461	-	tr	-
Cyclosativene	1370	1480	-	tr	-
α-Copaene	1376	1477	tr	0.2	-
β-Elemene	1392	1593	-	tr	-
β-Caryophyllene	1419	1598	tr	tr	-
Germacrene D	1481	1695	tr	-	-
γ-Cadinene	1511	1750	-	tr	-
δ-Cadinene	1524	1734	tr	tr	-
epi-13-Manoyl oxide	2010	-	tr	tr	tr
Kaurene	2034	2427	-	0.1	tr
Monoterpene hydrocarbons			**97.9**	**98.8**	**86.4**
Oxygenated monoterpenes			**0.9**	**-**	**5.8**
Sesquiterpene hydrocarbons			**-**	**0.2**	**-**
Diterpenes			**-**	**0.1**	**-**
Total identified			**98.8**	**99.1**	**92.2**

l.r.i ^{a} Linear retention indexes, relative to *n*-alkane series on a HB-5 column
l.r.i ^{b} Linear retention indexes, relative to *n*-alkane series on a DB-WAX column
tr = trace amount (<0.1%)

The analysis of the essential oil of *E. asclepium* subsp. *meoides* revealed the presence of kaurene and *epi*-13-manoyl oxide in very small amounts. The presence of manoyl oxide in *E. gummiferum* [19] confirms the ability of this genus to synthesize labdane diterpenes. However, because of their very low volatility, the small percentage of diterpenes detected in the essential oil does not reflect the real percentages of these compounds, especially the oxygenated ones produced by the plant. So, the presence of small amounts of diterpenes in the essential oils could be a good chemical indicator of plants with potentially higher contents of these compounds.

Table 2: Main components of *Elaeoselinum asclepium* ssp. *meoides* and the other *Elaeoselinum* species from literature.

Plant name Main components References	***1*** % **[18]**	***2*** % **[18]**	***3*** % **[18]**	***4*** % **[18]**	***5*** % **[18]**	***6*** % **[19]**	***7*** % **[18]**	***8*** % **[20]**	***9*** % **[11]**	***10*** % **[21]**	***11*** %	***12*** %	***13*** %
α-Pinene						37.4		67.0	27.4	81.4	77.1	92.2	60.8
β-Pinene						14.0		21.4	6.3	8.9	4.3	4.0	8.2
(α+β)-Pinene *	3.6	94.6	28.8	60.0	54		75.4						
Sabinene	24.3	1.0	10.8	20.0	16.3		15.1	5.4	35.3		11.9		12.8
α-fenchene						10.4							
Myrcene	47.9	1.1	25.0	2.5	1.3	22.8	1.4	1.4	2.7		1.4		
Limonene	7.1	1.1	32.9	9.0									
α-Terpinene	4.0												
γ-Terpinene	2.1			1	1.5								
β-Phellandrene						7.10			1.6		1.2		
Terpinen 4-ol	4.0			2.3	2.8		1.3		4.2				
α-Terpineol						2.5							
trans-β-Farnesene									4.7				
Germacrene-D									5.0				

* as cited on the original resource

1 *Elaeoselinum tenuifolium* fruits, Spain; ***2*** *E. foetidum* fruits, Spain; ***3*** *E. fontanesii* fruits, Morocco sample1 "Safi site"; ***4*** *E. fontanesii* fruits, Morocco sample 2 "Chichaoua and Taliouine sites"; ***5*** *E. gummiferum* fruits, Spain; ***6*** *E. gummiferum* aerial parts, Spain (Toledo); ***7*** *E. asclepium* ssp *asclepium* fruits, Spain; ***8*** *E. asclepium* ssp *asclepium* fruits, Spain (Alicante); ***9*** *E. asclepium* aerial parts, Greece; ***10*** *E. asclepium* ssp *millefolium* fruits, Spain (Malaga); ***11*** *E. asclepium* ssp *meoides* fruits Sicily; ***12*** *E. asclepium* ssp meoides roots Sicily; ***13*** *E. asclepium* ssp *meoides* aerial parts Sicily.

Experimental

Plant material: Flowering aerial parts of *E. asclepium* subsp. *meoides* were collected in Sicily at Randazzo (Catania Province) in July, while the ripe fruits and the roots were collected in September at the same site. The plant was identified by Professor Francesco Maria Raimondo, Department of Botanical Sciences, University of Palermo, Italy. A voucher specimen was deposited in the Herbarium of the Laboratory of Phytochemistry and Pharmacognosy at Al-Zaytoonah Private University of Jordan.

After air drying at room temperature, the plant material was ground, and 100 g of each sample was submitted to hydrodistillation in a Clevenger-like apparatus for 2 h. The essential oils were stored in sealed vials under refrigeration prior to analysis.

The GC analyses were accomplished using a HP- 5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m x 0.25 mm,0.25 μm film thickness; working conditions are as reported previously [23]. GC-EIMS analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Injector and transfer line temperatures were 220 and 240°C, respectively; oven temperature was programmed from 60°C to 240°C at 3°C/min; carrier gas helium at 1 mL/min; injection of 0.2 mL (10% *n*-hexane solution); split ratio 1:30.

Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their Linear Retention Indices relative to a series of *n*-hydrocarbons, and by computer matching against commercial [12] and home-made library MS built up from pure substances and components of known essential oils and MS literature data [12-18]. Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

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