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ESSENTIAL OILS COMPOSITION OF *MELITTIS MELISSOPHYLLUM* L. AND *OENANTHE PIMPINELLOIDES* L. (LIGURIA, ITALY)

Abstract - The chemical composition and the yield of the essential oils of the flowering parts of *Melittis melissophyllum* L. (Lamiaceae), collected from plants growing on calcareous soil, and *Oenanthe pimpinelloides* L. (Apiaceae), collected on metamorphic rocks and cavernous limestone, in Montemarcello (Eastern Liguria, Italy), have been investigated. Few data concerning the essential oils of the two species is available in the literature. The most important feature of the essential oil of *Melittis melissophyllum* L. is the very similar percentages of terpene derivatives (47.6%), and non-terpene ones (47.5%). The former are mainly constituted by mono- and sesquiterpene hydrocarbons, while the latter are mostly oxygenated compounds. The main constituents are 1-octen-3-ol (29.7%), germacrene D (10.2%), β-caryophyllene (9.0%) and *(E)*-3-hexen-1-ol (8.2%). The differences between this sample and *M. melissophyllum* L. subsp. *albida* Guss. from Greece support the separation of the *taxa* in two different species, *M. melissophyllum* L. and *M. albida* Guss., as proposed by some authors. As regards *Oenanthe pimpinelloides* L., the main constituents of the essential oil are *(Z)*-ocimene (12.1%-20.2%), *(E)*-ocimene (21.2%-22.9%), germacrene D (8.4%-11.6%) and β-caryophyllene (8.1%-5.7%). The different composition of the essential oil of *O. pimpinelloides* L. from Greece allows to hypothesize the presence of two different chemotypes.

Key words - *Melittis melissophyllum*, Lamiaceae, *Oenanthe pimpinelloides*, Apiaceae, essential oil.

Riassunto - *La composizione degli oli essenziali di* Melittis melissophyllum *L. e di* Oenanthe pimpinelloides *L. (Liguria, Italia).* Sono state studiate la composizione chimica e la resa dell'olio essenziale delle sommità fiorite di *Melittis melissophyllum* L. (Lamiaceae), raccolte su suolo calcareo, e di *Oenanthe pimpinelloides* L. (Apiaceae), provenienti da suolo metamorfico e da calcare cavernoso, entrambe raccolte a Montemarcello (Liguria, Italia). In letteratura i dati disponibili sugli oli essenziali di queste due specie sono pochi. La caratteristica più importante dell'olio essenziale di *Melittis melissophyllum* L. è la pressoché identica percentuale di derivati terpenici (47,6%) e non terpenici (47,5%). I primi sono costituiti principalmente da idrocarburi mono- e sesquiterpenici, mentre i secondi sono prevalentemente composti ossigenati. I costituenti principali risultano essere 1-octen-3-olo (29,7%), germacrene D (10,2%), β-caryophyllene (9,0%) e *(E)*-3-hexen-1-olo (8,2%). Le differenze tra questo campione e *M. melissophyllum* L. subsp. *albida* Guss. della Grecia sostengono la separazione dei *taxa* in due diverse specie, *M. melissophyllum* L. e *M. albida* Guss., come proposto da alcuni autori. Per quanto riguarda *Oenanthe pimpinelloides* L., i costituenti principali dell'olio essenziale sono *(Z)*-ocimene (12,1%-20,2%), *(E)*-ocimene (21,2%-22,9%), germacrene D (8,4%-11,6%) e β-caryophyllene (8,1%-5,7%). La diversa composizione dell'olio essenziale di *O. pimpinelloides* L. della Grecia permette di ipotizzare la presenza di 2 differenti chemotipi.

Parole chiave - *Melittis melissophyllum*, Lamiaceae, *Oenanthe pimpinelloides*, Apiaceae, olio essenziale.

Introduction

A research project on the chemistry of plants from Caprione Promontory has been going on for several years (Flamini *et al*., 1994, 2004, 2007; Maccioni *et al*., 1992, 2007a, 2007b). Caprione Promontory is located within Montemarcello-Magra Natural Regional Park in province of La Spezia, Eastern Liguria, and has rich and diverse flora and vegetation (Cardelli *et al*., 2000; Maccioni, 1991; Maccioni *et al.*, 2003; Maccioni & Cardelli, 2002; Maccioni & Tomei, 1988).
This paper reports the composition of the essential oils obtained from the flowering parts of *Melittis melissophyllum* L. and *Oenanthe pimpinelloides* L. growing in this area, for both of which few published data concerning the essential oil is available in the literature.
In Italy, the genus *Melittis* (Lamiaceae) is represented by two species, *M. melissophyllum* L. and *M. albida* Guss. (Pignatti, 1982), which mainly differ because of the presence of glandular hairs in the former only. According to other authors, *M. albida* Guss. should be considered as a subspecies of *M. melissophyllum* L. (Tutin *et al.*, 1972).
M. melissophyllum L. is widely distributed in Central, Southern and Western Europe; in Italy it is common in Central-Northern regions, rare in the Southern ones; it is completely absent in Calabria, Sicily and Sardinia (Pignatti, 1982; Tutin *et al*., 1972). The plant is used as antispasmodic, urinary antiseptic, diuretic and sympaticolitic (Benigni *et al*., 1964; Gastaldo, 1987; Negri, 1979).
The essential oil of this species has received little attention from researchers; only a paper about the oil of *M. melissophyllum* L. subsp. *albida* Guss. from Greece and one about the oil of *M. melissophyllum* L. subsp. *melissophyllum* from Central Italy are present in the literature (Maggi *et al*., 2009; Skaltsa *et al*., 1991).
The genus *Oenanthe* is represented by numerous species, mainly distributed in humid habitats and on riverbanks. Among them, *O. pimpinelloides* L. also lives in different environments, but still characterized by high humidity percentages (Pignatti, 1982; Tutin *et al*., 1972). This species is very common throughout

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Western, Eastern and Southern Europe. In Italy it can be found in Liguria, in the peninsular part, in Sicily and Sardinia; it is quite rare in the northern part of the country (Pignatti, 1982; Tutin *et al*., 1972).
Another species, *O. aquatica* (L.) Poir., is used in medicine for its sedative and expectorant properties (Benigni *et al*., 1964; Gastaldo, 1987; Negri, 1979).
Some species of *Oenanthe* are employed as food, mainly in Asiatic countries, i.e. *O. stolonifera* Wall. is used for various cooked vegetable dishes and kimchi, a fermented vegetable dish (Park & Kim, 1996), even if it is easily contaminated by *Salmonella* sp. (Awang Salleh *et al.*, 2003). The leaves of *O. javanica* DC. are also used as a seasoning in soups (Facciola, 1990; Reid, 1977); the flavour is reminiscent of carrots or parsley (Larkcom, 1991). Also *O. pimpinelloides* L., in some areas, is much esteemed as a food; it has a starchy root with a flavour somewhat like filberts (Facciola, 1990). There is only a recent paper about the oil of *O. pimpinelloides* L. from Greece present in the literature (Evergetis *et al*., 2009).

Materials and methods

The flowering parts of *Melittis melissophyllum* L. were collected at the end of May 2003 in the Botanic Garden of Montemarcello (Monte Murlo, La Spezia province, Italy). Here the plant grows in a mixed oak-wood, on calcareous soil (AA.VV., 2001), facing East, at 360 m above sea level. The tree species are mainly *Quercus cerris* L. and *Q. pubescens* Willd., together with *Fraxinus ornus* L., *Crataegus monogyna* Jacq. and *Arbutus unedo* L. In the understorey, *Asparagus acutifolius* L., *Cephalanthera longifolia* (Hudson) Fritsch, *Cyclamen hederifolium* Aiton, *Erica arborea* L., *Geranium robertianum* L., *Lonicera etrusca* Santi, *Prunus spinosa* L., *Rosa sempervirens* L., *Rubia peregrina* L., *Stachys officinalis* (L.) Trevisan, *Tamus communis* L., *Tanacetum corymbosum* (L.) Sch.-Bip. var. *corymbosum* and *Viburnum tinus* L. can be found.
A voucher specimen of *M. melissophyllum* L. is deposited in PI (Nuove Acquisizioni N. 7261 *Melittis melissophyllum*/15).
The flowering parts of *Oenanthe pimpinelloides* L. have been collected at the end of May 2003 in two different places:

1. Cima Terroni, on the metamorphic unit of Punta Bianca, the Monte Marcello layer (AA.VV., 2001) facing South-South West, at 250 m above sea level, on the edge of an olive-grove. This place is very sunny and directly exposed to marine winds. Other species living together with *O. pimpinelloides* L. are *Anthoxanthum odoratum* L., *Aster sedifolius* L., *Dianthus balbisii* Ser. subsp. *balbisii*, *Bromus sterilis* L., *Cistus salvifolius* L., *Rubia peregrina* L., *Scabiosa argentea* L., *Smilax aspera* L. A voucher specimen of *O. pimpinelloides* L. is deposited in PI (Nuove Acquisizioni N. 6046 *Oenanthe pimpinelloides*/19).
2. La Valle, on cavernous calcareous soil (AA.VV., 2001) facing East-North East, at 180 m above sea level, in an olive-grove. It is a little valley, scarcely sunny, very moist and protected from wind. Other species present in this station are *Bellis perennis* L., *Bromus sterilis* L., *Inula viscosa* L., *Linaria vulgaris* Miller, *Scabiosa argentea* L. A voucher specimen of *O. pimpinelloides* L. is deposited in PI (Nuove Acquisizioni N. 6046 *Oenanthe pimpinelloides*/20).

The fresh plant material of *Melittis melissophyllum* L., about 150 g, and the dried plant material of *Oenanthe pimpinelloides* L., about 100 g, were coarsely cut and hydrodistilled in a Clevenger-type apparatus for two hours.
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. Analytical conditions: injector and transfer line temperatures 220°C and 240°C respectively; oven temperature programmed from 60°C to 240°C at 3°C/min; carrier gas helium at 1 ml/min; injection of 0.2 μl (10% 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 the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra built up from pure substances and components of known oils and MS literature data (Adams, 1995; Davies, 1990; Jennings & Shibamoto, 1980; Massada, 1976; Stenhagen *et al*., 1974; Swigar & Silverstein, 1981). Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using MeOH as CI ionizing gas.

Results and discussion

For *Melittis melissophyllum* the essential oil yield was 0.2% (w/w) and its composition is reported in Table 1. Fifty-nine compounds were identified in the essential oil, accounting for 95.8% of the whole oil. Among them 19 are monoterpenes (13 hydrocarbons and 6 oxygenated ones) representing 18.7% of the whole oil, and 19 are sesquiterpenes (15 hydrocarbons and 4 oxygenated ones) representing 28.2% of the whole oil. The oxygenated derivatives account for 2.0% and 1.3%, respectively. Furthermore, three diterpenes have been identified in small amounts: phyllocladene, epi-13-manoyl oxide and abietatriene. The peculiarity of this essential oil is the very similar percentage of terpene derivatives (47.6%) and non-terpene ones (47.5%). The former are mainly constituted by mono- and sesquiterpene hydrocarbons, while the latter are mostly oxygenated compounds. The main constituent is an unsatured non-terpenic aliphatic alcohol, 1-octen-3-ol (29.7%), followed by germacrene D (10.2%), β-caryophyllene (9.0%), (E)-3-hexen-1-ol (8.2%) and sabinene (6.5%). Also in the essential oil of *M. melissophyllum* L. subsp. *melissophyllum* from Central Italy (Maggi *et al*., 2009) the main component is 1-octen-3-ol (43.6-54.2%), followed by hexadecanoic acid (11.3-9.0%) and phytol (4.6-3.8%).

Tab. 1 - Composition and yield of the essential oil of the flowering parts of *Melittis melissophyllum* L.

Constituents	**l.r.i.***	**%**
(E)-3-hexen-1-ol	853	8.2
(E)-2-hexen-1-ol	861	0.3
heptanal	900	0.8
(E,E)-2,4-hexadienal	911	tr†
α-thujene	933	0.5
α-pinene	941	4.4
camphene	955	tr
(Z)-2-heptenal	964	tr
benzaldehyde	966	tr
sabinene	978	6.5
1-octen-3-ol	980	29.7
3-octanone	989	2.9
myrcene	993	0.7
3-octanol	995	1.0
(E)-3-hexen-1-ol acetate	1004	1.2
α-phellandrene	1006	0.5
α-terpinene	1020	0.1
p-cymene	1027	tr
limonene	1032	1.0
β-phellandrene	1033	0.1
(Z)-ocimene	1041	tr
phenylacetaldehyde	1045	0.9
γ-terpinene	1064	0.3
cis-sabinene hydrate	1069	0.3
terpinolene	1089	2.7
linalool	1101	1.1
nonanal	1103	2.1
(E)-2-nonenal	1164	tr
4-terpineol	1179	0.3
p-cymen-8-ol	1185	tr
α-terpineol	1190	0.3
2-methyl-2-nonen-4-one	1215	tr
β-cyclocitral	1223	0.1
isobornyl acetate	1286	tr
(E,E)-2,4-decadienal	1316	tr
α-cubebene	1351	tr
α-copaene	1377	0.3
β-bourbonene	1385	0.3
β-cubebene	1390	0.2
β-caryophyllene	1420	9.0
coumarin	1429	0.7
α-humulene	1459	3.6
farnesane	1462	0.6
γ-muurolene	1477	0.1
germacrene D	1483	10.2
viridiflorene	1495	0.1
bicyclogermacrene	1496	0.3
α-muurolene	1499	0.1
(E,E)- α-farnesene	1509	1.1
δ-cadinene	1524	0.7
β-sesquiphellandrene	1527	0.3
caryophyllene oxide	1583	0.6
viridiflorol	1592	0.3
humulene epoxide II	1608	0.3
α-cadinol	1656	0.1
phyllocladene	2011	0.4
epi-13-manoyl oxide	2014	0.4
abietatriene	2055	tr
heneicosane	2100	0.1
Total identified		95.8
Yield (% w/w)		0.2

* Linear Retention Indices (HP-5 column); † tr < 0.1%

The essential oil of *M. melissophyllum* L. subsp. *albida* Guss. from Greece (Skaltsa *et al*., 1991) contains as main components chrysanthenyl acetate (12.3%), caryophyllene oxide (10.8%), α-terpineol (7.8%) and 6,10,14-trimethylpentadecan-2-one (6.9%). These differences (Tab. 2) could support the separation of the two *taxa* in two different species, *M. melissophyllum* L. and *M. albida* Guss., as suggested by some authors (Pignatti, 1982).

For *Oenanthe pimpinelloides* L. the essential oils yields were 0.03% (w/w) for both stations and their composition is reported in Table 3. Thirty-four compounds were identified in the two essential oils, accounting for 94.2% and 92.2% of the whole oils from Cima Terroni and La Valle stations, respectively.

Tab. 2 - Comparison between the essential oil of *Melittis melissophyllum* L. (A) and *M. melissophyllum* L. subsp. *albida* Guss. (B; Skaltsa *et al*., 1991).

Constituents	% A	% B
(E)-3-hexen-1-ol	8.2	–
sabinene	6.5	–
1-octen-3-ol	29.7	–
β-caryophyllene	9.0	1.63
germacrene D	10.2	–
chrysanthenyl acetate	–	12.33
caryophyllene oxide	0.6	10.8
α-terpineol	–	7.8
6,10,14-trimethylpentadecan-2-one	–	6.9

Tab. 3 - Composition and yields of the essential oil of the flowering parts of *Oenanthe pimpinelloides* L. from two different habitats.

Constituents	**l.r.i.***	**Cima Terroni**	**La Valle**
heptanal	901	1.1	2.4
α-pinene	940	1.0	1.5
sabinene	978	tr†	0.4
β-pinene	981	0.7	1.2
myrcene	992	3.2	0.8
octanal	1003	0.6	0.7
p-cymene	1028	–‡	tr
limonene	1033	0.4	–
β-phellandrene	1035	0.6	1.0
(Z)-ocimene	1040	12.1	20.2
(E)-ocimene	1051	21.2	22.9
γ-terpinene	1064	tr	tr
linalool	1101	0.3	0.4
nonanal	1104	0.4	0.4
camphor	1145	1.1	–
borneol	1175	0.9	–
4-terpineol	1182	–	tr
α-terpineol	1192	0.5	tr
isobornyl acetate	1286	0.7	–
α-terpinyl acetate	1351	–	0.7
α-copaene	1377	0.6	0.3
methyl eugenol	1403	–	0.9
β-caryophyllene	1420	8.1	5.7
γ-elemene	1432	0.3	–
trans-α-bergamotene	1439	0.3	–
α-humulene	1456	0.4	tr
(E)-β-farnesene	1458	9.3	8.0
germacrene D	1482	8.4	11.6
bicyclogermacrene	1496	11.1	5.1
α-muurolene	1499	0.3	–
(E,E)-α-farnesene	1509	0.5	0.9
δ-cadinene	1525	1.5	0.8
spathulenol	1578	5.6	3.9
caryophyllene oxide	1583	3.0	1.7
Total identified		94.2	92.2
Yield (% w/w)		0.03	0.03

* Linear Retention Indices (HP-5 column); † tr < 0.1%; ‡ not identified

In the essential oil of plants from La Valle, monoterpenes reach almost 50% of the whole oil, but also sesquiterpenes are abundant (38.0%). Among monoterpenes, the hydrocarbons *(E)*- and *(Z)*-ocimene (43.1% altogether), are the main constituents of the oil. Among sesquiterpenes, the most distinctive compounds are the hydrocarbons germacrene D (11.6%), *(E)*-β-farnesene (8.0%), bicyclogermacrene (5.1%), β-caryophyllene (5.7%) and the alcohol spathulenol (3.9%). Other constituents (4.4%) identified are straight chain non-terpenic aldehydes and the phenylpropanoid derivative methyleugenol. Oxygenated compounds are scarcely represented with respect to mono- and sesquiterpene hydrocarbons.

The essential oil obtained from plants collected at Cima Terroni contains the same constituents, even if in different percentages. Sesquiterpenes are present in greater amounts (49.4%) than monoterpenes (42.7%). This essential oil contains a lesser quantity, about a half, of *(Z)*-ocimene (12.1%) than the oil of La Valle, but a greater amount of bicyclogermacrene (11.1%), *(E)*-β-farnesene (9.3%), β-caryophyllene (8.1%), spathulenol (5.6%) and caryophyllene oxide (3.0%).
It can be hypothesized that these quantitative differences could be mainly attributable to the different environmental conditions of the collecting places, which may influence the production of plant secondary metabolites (Figueiredo *et al*., 2008; Issaoui *et al*., 2010; Tholl & Pichersky, 1996).
On the contrary, in the essential oil of *O. pimpinelloides* L. from Greece (Evergetis *et al*., 2009), besides quantitative differences, substantial qualitative differences were evidenced. In particular, γ-terpinene (43.42%), O-cymene (17.75%), β-sesquiphellandrene (8.25%) and β-pinene (6.79%) (Tab. 4) were detected as main components. Consequently, at least two different chemotypes may be hypothesized.

Tab. 4 - Comparison between the essential oil of *Oenanthe pimpinelloides* L. from Montemarcello (A = calcareous soil; B = siliceous soil) and from Greece (C; Evergetis *et al*., 2009).

Constituents	**% A**	**% B**	**% C**
β-pinene	0.7	1.2	6.79
myrcene	3.2	0.8	2.7
o-cymene	–	–	17.75
(Z)-ocimene	12.1	20.2	–
(E)-ocimene	21.2	22.9	–
trans-β-ocimene	–	–	2.84
γ-terpinene	tr	tr	43.42
β-caryophyllene	8.1	5.7	1.33
(E)-β-farnesene	9.3	8.0	–
germacrene D	8.4	11.6	2.14
bicyclogermacrene	11.1	5.1	0.56
spathulenol	5.6	3.9	–
β-sesquiphellandrene	–	–	8.25
caryophyllene oxide	3.0	1.7	0.07

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