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Phytochemical typologies in some populations of *Myrtus communis* L. on Caprione Promontory (East Liguria, Italy)

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Abstract

The composition of the essential oils of leaves and fruits of *Myrtus communis* from Italy has been studied. Differences between plants collected in different habitats have been educed. The main diversities in the composition of the essential oils of the leaves between the two habitats centered on α-pinene and limonene, which were identified in greater amounts in plants living on calcareous soil, while linalool, linalyl acetate and *trans*-myrtanol acetate were detected in higher percentages on siliceous soil. Furthermore, the different olfactory perceptions noted for plants growing in the two habitats were clarified by mean of SPME. Among the volatiles emitted in vivo by the plants, the percentages of α-pinene, limonene and 1,8-cineole were considerably lower in the leaves of plants growing on siliceous soil than to those emitted by the leaves of plants from calcareous soil. On the other hand, β-caryophyllene duplicated its percentage and β-elemene passed from 0 to 17.2% in plant growing on calcareous soil. Also the volatiles emitted in vivo by the fruits showed very similar patterns. In all the samples, *trans*-myrtanol acetate has been identified for the first time as a natural constituent of an essential oil of *Myrtus*.

Keywords: *Myrtus communis*; Myrtaceae; Essential oil composition; SPME; Different habitats; α-Pinene/1,8-cineole chemotype; *trans*-Myrtanol acetate

1. Introduction

Myrtus communis L. is a stenomediterranean species; in Italy it grows wild in almost all the coastal areas, in Sicily and Sardinia and in many minor islands (Tutin et al., 1964–1980; Pignatti, 1982). It is an evergreen shrub or a small tree that adapts to many kinds of soil (Valsecchi & Camarda, 1990).

In Italy, especially in Sardinia, it is used to prepare two very celebrated liquors: the macerated fruits yield ''Mirto Rosso'', while the macerated leaves produce the ''Mirto Bianco''.

This species is also widely used in folk medicine because of its astringent and balsamic properties (Benigni, Capra, & Cattorini, 1964; Gastaldo, 1997; Negri, 1979). *M. communis* has also been widely employed in Italian folk medicine. In past times, ripe fruits were used as food integrators because of their high vitamin contents. The fruit decoction was used to bath new-borns with reddened skin, while the decoction of leaves and fruits was useful for sore washing. The decoction of the leaves is still used for vaginal lavage, enemas and against respiratory diseases (Maccioni, Tomei, & Rizzo, 1994–1995; Marchini & Maccioni, 1998).

The essential oil obtained from this species has been widely investigated. Its composition is quite variable (Lawrence, 1976–1977, 1979–1980, 1990, 1993a, 1993b, 1996). One of the main constituents of myrtle essential oil is 1,8-cineole (Bradesi, Casanova, Costa, & Bernardini, 1997). These oils can be separated into two groups, according to the amounts of myrtenyl acetate. Each group can be further divided into two subgroups, according to the relative ratio of α-pinene to myrtenyl acetate or α-pinene to cineole. There is also a good correlation between these groups and the geographical origin of the plants (Bradesi et al., 1997).

This investigation was carried out as phytochemical studies on the flora of Promontorio del Caprione (Flamini, Cioni, Morelli, Maccioni, & Tomei, 1994;

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Maccioni, Flamini, Cioni, & Tomei, 1992) (La Spezia province, East Liguria, Italy), an area of great naturalistic importance, part of the Regional Park of Montemarcello-Magra (Cardelli, Di Tommaso, & Signorini, 2000; Maccioni, 1991; Maccioni and Tomei, 1988; Monti & Maccioni, 1996).

Because of its situation, between La Spezia Gulf and the plain of the Magra River, the western slope, facing the sea, is characterized by more thermophilous and xerophilous environmental conditions than the eastern slope facing the river, which benefits from a greater humidity throughout the year. These conditions are strengthened also by the different soil types, prevalently calcareous in the northern and in the western sectors and siliceous in the southern and eastern areas (Cardelli et al., 2000; Maccioni & Tomei, 1988). Here, *M. communis* grows on the entire promontory. On the sea slope, on calcareous soil, it is present as a component of Mediterranean scrub, but also in the underwood of ilex grove and *Pinus halepensis* Miller pinewood, starting from about 50 m above the sea level up to 400 m, on the top of the hills. On the riverside, on siliceous soil, it is present from 100 to 400 m, mainly in *P. pinaster* Aiton pinewood but, occasionally, also, in mixed deciduous wood.

This paper deals with the composition of the essential oils of two populations of *M. communis* growing in two different habitats characterized also by different soil types. Furthermore, we have examined the relationships between the different plant odour and the volatiles emitted in vivo by the plants.

2. Materials and methods

The aerial parts of *M. communis* L. (15–20 cm long) were collected at the end of October, during the fructification period, in Monte Murlo (calcareous soil, station 1) and Cima Marrana (siliceous soil, station 2). Monte Murlo station is situated at 360 m and it is characterized by Mediterranean ''macchia'', constituted mainly of *Phillyrea latifolia* L., *Pistacia lentiscus* L., *Smilax aspera* L., *Cistus salvifolius* L. and *Clematis flammula* L. Here, *M. communis* lives in full light; they are shrubs about 1.60 m high, with many small leaves. Cima Marrana (Montemarcello municipality) is at about 220 m and the plants were collected near the edge of a *P. pinaster* Aiton pine wood; other species present were *Arbutus unedo* L., *Erica arborea* L. and *Cistus salvifolius* L. Here, *M. communis* plants live in dim light and are shrubs about 80 cm high, with bigger leaves. There were some olfactory differences between the plants of the two stations, in particular the plants which grow in Monte Murlo had a stronger scent than individuals that grow in Cima Marrana, which had a sweeter aroma.

The plant samples (100 g of terminal leafy twigs and 100 g of fruits) were separately water distilled the next day in a Clevenger-type apparatus for 2 h.

The GC analyses were accomplished with a HP-5890 Series II instrument equipped with HP-WAX and DB-5 capillary columns (30 m×0.25 mm, 0.25 μm film thickness), working with the following temperature programme: 60–240 °C at 3 °C/min; injector and detector temperatures 220 °C; carrier gas nitrogen (2 ml/min); detector dual FID; split ratio 1:30; injection of 0.5 μl). The identification of the components was performed, for both the columns, by comparison of their retention times with those of pure authentic samples and by mean of their linear retention indices (lri) relative to the series of *n*-hydrocarbons.

The relative proportions of the essential oil constituents were percentages obtained by FID peak-area normalization, all relative response factors being taken as one (mean value of three analyses).

GC/EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m×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 and 240 °C, respectively; oven temperature programmed from 60 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 a 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, Abrahamsson, & McLafferty, 1974; Swigar & Silverstein, 1981). Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using MeOH as CI ionizing gas.

Volatiles emitted in vivo were sampled by mean of SPME using a Supelco SPME device coated with polydimethylsiloxane (PDMS, 100 μm) for sampling the headspace of five living leaves (about 0.38 g and 0.54 g for stations 1 and 2, respectively) or 10 fruits (about 4.06 and 4.82 g, respectively) inserted into a 50-ml glass conical flask and allowed to equilibrate for 20 min. The flask was closed by means of a pierceable stopper with the inner part covered by Teflon film. After the equilibration time, the fibre was exposed to the headspace for 15 min at room temperature. Once sampling was finished, the fibre was withdrawn into the needle and transferred to the injection port of the GC and GC/MS system, operating under the same conditions as above, for both quantification and identification of the constituents, except that

Table 1
SPME analyses[a] of volatiles emitted in vivo by plants of *Myrtus communis* collected in Monte Murlo and Cima Marrana

Constituents	l.r.i.[b]	l.r.i.[c]	Monte Murlo		Cima Marrana	
			Fruits	Leaves	Fruits	Leaves
α-Thujene	933	1017	0.9	tr[d]	0.3	1.1
α-Pinene	940	1029	47.2	19.9	24.7	9.5
Sabinene	977	1126	tr	0.9	1.2	0.7
β-Pinene	981	1112	0.5	tr	0.3	tr
Myrcene	992	1168	0.4	tr	0.9	1.0
Mesitylene	996		tr	–	–	–
α-Phellandrene	1007	1176	tr	–	tr	2.9
3-Carene	1012	1150	2.0	–	–	2.7
α-Terpinene	1020	1183	0.2	–	–	0.9
p-Cymene	1027	1272	1.6	tr	0.3	1.8
Limonene	1032	1196	8.5	6.0	3.8	3.0
1,8-Cineole	1035	1218	14.6	52.7	8.0	33.6
(*E*)-Ocimene	1051	1251	0.2	tr	0.2	0.5
γ-Terpinene	1063	1253	1.5	tr	0.3	3.6
cis-Linalool oxide (furanoid)	1076	1555	–	–	tr	–
Terpinolene	1089	1286	1.6	–	tr	5.0
p-Cymenene	1091		tr	–	–	tr
Linalool	1099	1547	0.4	–	1.0	1.8
Isoamyl 2-methylbutyrate	1102		0.8	tr	–	–
Nonanal	1104	1385	–	–	tr	tr
4-Terpineol	1179	1630	0.2	tr	0.3	tr
p-Cymen-8-ol	1185	1845	tr	–	tr	–
(*Z*)-3-Hexenyl butyrate	1187		–	tr	–	tr
α-Terpineol	1191	1724	0.8	1.4	3.2	tr
n-Dodecane	1200	1200	–	–	–	tr
Methyl chavicol	1196		tr	–	–	–
Decanal	1205	1484	–	–	–	tr
Linalyl acetate	1258		3.2	1.2	3.9	1.7
δ-Elemene	1340		–	–	–	tr
α-Terpinyl acetate	1351		1.7	1.4	3.8	1.2
Neryl acetate	1361		tr	–	–	–
α-Copaene	1377	1521	tr	–	2.7	0.6
β-Bourbonene	1383		–	–	1.5	–
trans-Myrtanol acetate	1386		1.4	tr	2.0	tr
β-Elemene	1391	1588	–	–	17.2	7.3
n-Tetradecane	1400	1400	–	tr	tr	tr
Methyl eugenol	1402		1.0	–	0.6	–
β-Caryophyllene	1420	1604	7.9	6.0	14.4	11.7
γ-Elemene	1434		0.5	4.2	–	3.8
α-Guaiene	1440		–	–	0.6	–
α-Humulene	1455	1676	1.1	0.9	2.0	1.6
Alloaromadendrene	1462	1640	–	1.5	0.3	0.7
β-Chamigrene	1476		–	–	–	tr
Germacrene D	1482	1722	–	–	0.5	tr
β-Selinene	1488	1705	–	–	1.4	tr
Bicyclogermacrene	1496		–	tr	–	0.9
Viridiflorene	1497	1704	–	–	1.6	–
α-Bulnesene	1503		–	–	tr	–
(*E,E*)-α-Farnesene	1507	1748	–	–	0.3	–
cis-γ-Cadinene	1511	1752	–	–	tr	–
δ-Cadinene	1524	1744	–	–	0.3	tr
Caryophyllene oxide	1582	2071	0.4	–	tr	–
Total identified (%)			98.6	96.1	97.6	97.6

[a] Percentages (mean of three analyses) obtained by FID peak-area normalization, all relative response factors being taken as one (DB-5 column).
[b] Linear retention indices (DB-5 column).
[c] Linear retention indices (HP-WAX column).
[d] tr < 0.1%.

Table 2
Compositions[a] of the essential oils obtained from fruits and leaves of *Myrtus communis* collected in Monte Murlo and Cima Marrana

Constituents	l.r.i.[b]	l.r.i.[c]	Monte Murlo		Cima Marrana	
			Fruits	Leaves	Fruits	Leaves
(*E,Z*)-2,4-Hexadienal	841		–	tr[d]	–	–
(*E*)-2-Hexenal	855	1220	–	0.4	–	0.6
2-Methylbutanol acetate	877		–	tr	–	–
Propyl butyrate	916	1248	0.3	0.7	–	–
α-Thujene	933	1017	0.3	0.3	1.0	0.5
α-Pinene	940	1029	51.1	41.6	21.4	28.9
α-Fenchene	953		–	tr	tr	tr
Camphene	956	1072	tr	tr	tr	tr
Thuja-2,4(10)-diene	959		–	tr	–	–
Sabinene	977	1126	tr	0.2	0.3	tr
β-Pinene	981	1112	0.7	0.7	0.8	0.7
Myrcene	992	1168	0.2	0.3	0.6	0.4
1-Decene	993		–	tr	–	tr
Butyl butyrate	1002		0.6	1.3	–	–
Pseudolimonene	1005		–	–	tr	–
α-Phellandrene	1007	1176	tr	–	1.2	0.3
3-Carene	1012	1150	–	–	1.7	0.5
α-Terpinene	1020	1183	tr	–	0.5	0.1
p-Cymene	1027	1272	0.1	0.2	1.4	0.8
Limonene	1032	1196	7.9	9.5	5.6	5.2
1,8-Cineole	1035	1218	23.1	25.5	25.4	24.2
(*E*)-Ocimene	1051	1251	0.1	0.2	0.2	0.3
γ-Terpinene	1063	1253	0.2	0.3	3.0	0.8
cis-Linalool oxide (furanoid)	1076	1555	–	–	–	tr
Terpinolene	1089	1286	0.2	0.1	3.4	0.7
p-Cymenene	1091		–	–	tr	tr
Linalool	1099	1547	2.1	2.9	6.2	11.7
Isoamyl 2-methylbutyrate	1102		1.2	1.4	0.4	0.9
exo-Fenchol	1120		tr	tr	–	–
trans-*p*-Menth-2,8-dien-1-ol	1122		–	tr	–	–
cis-*p*-Menth-2-en-1-ol	1124		–	tr	tr	–
α-Campholenal	1127		tr	tr	–	–
trans-Pinocarveol	1141		tr	0.2	–	0.1
trans-*p*-Menth-2-en-1-ol	1142		–	–	tr	–
Camphene hydrate	1150	1518	–	tr	–	–
Pinocarvone	1165		tr	tr	–	–
Borneol	1168	1706	tr	tr	–	–
4-Terpineol	1179	1630	0.3	0.5	0.7	0.6
p-Cymen-8-ol	1184	1845			tr	tr
α-Terpineol	1191	1724	1.6	2.8	1.7	3.6
Methyl chavicol	1196		0.2	0.2	0.1	0.2
trans-Piperitol	1208		–	–	tr	–
trans-Carveol	1219	1835	–	tr	–	–
endo-Fenchyl acetate	1221		tr	tr	–	–
Hexyl 2-methyl butyrate	1236	1585	tr	–	tr	–
Geraniol	1255	1851	tr	0.4	tr	0.7
Linalyl acetate	1258		0.5	0.7	1.6	2.9
Isobornyl acetate	1286		tr	tr	tr	–
trans-Pinocarvyl acetate	1298		tr	tr	–	–
α-Terpinyl acetate	1351		1.2	1.1	3.1	1.6
Neryl acetate	1361		0.1	0.1	0.2	0.4
α-Copaene	1377	1521	–	–	tr	tr
trans-Myrtanol acetate	1386		3.7	4.2	6.0	5.2
Benzyl valerate	1388		tr	tr	–	–
β-Elemene	1391	1588	–	–	0.7	0.2
n-Tetradecane	1400	1400	–	–	0.9	–
Methyl eugenol	1402		0.6	0.8	–	1.1
β-Caryophyllene	1420	1604	0.7	0.3	2.1	1.0
γ-Elemene	1434		–	–	tr	–

Table 2 (*continued*)

Constituents	l.r.i.[b]	l.r.i.[c]	Monte Murlo		Cima Marrana	
			Fruits	Leaves	Fruits	Leaves
α-Guaiene	1440		–	–	tr	–
Aromadendrene	1441		tr	–	tr	–
α-Humulene	1455	1676	0.2	0.2	0.7	0.4
Alloaromadendrene	1462	1640	tr	–	tr	–
β-Chamigrene	1476		–	–	0.1	–
β-Selinene	1488	1705	–	–	0.5	0.2
(*E*)-Methyl isoeugenol	1494		0.1	tr	–	–
Bicyclogermacrene	1496		tr	tr	–	–
Viridiflorene	1497	1704	–	–	0.6	0.3
β-Bisabolene	1508	1710	–	tr	–	–
δ-Cadinene	1524	1764	–	–	tr	tr
Spathulenol	1577	2106	tr	tr	0.3	0.1
Caryophyllene oxide	1582	2071	0.2	0.6	0.4	0.6
Globulol	1584		–	–	0.1	–
Humulene epoxide II	1607		0.1	0.2	0.1	0.3
Humulane-1,6-dien-3-ol	1619		–	–	0.3	–
α-Cadinol	1654		0.1			
Selin-11-en-4-α-ol (=kongol)	1660				1.3	0.5
Juniper camphor	1692				0.2	
Total identified (%)			97.7	97.9	94.8	96.6

[a] Percentages (mean of three analyses) obtained by FID peak-area normalization, all relative response factors being taken as one (HP-5 column).
[b] Linear retention indices (DB-5 column).
[c] Linear retention indices (HP-WAX column).
[d] tr <0.1%.

splitless injection mode was used and the injector temperature was 250 °C. The optimal desorption time for these samples was 60 s.

3. Results and discussion

The results of all the analyses are shown in Tables 1 and 2. Fifty-one compounds were identified in the headspace analyses and 82 in the essential oils, which accounted for 96.1–98.6% and 94.8–98.6% of the total compositions, respectively.

Among the volatiles emitted in vivo by the plants, it must be pointed out that the percentages of α-pinene, limonene and 1,8-cineole were considerably lower in the leaves of plants growing in Cima Marrana than in the leaves of plants from Monte Murlo. On the other hand, β-caryophyllene duplicated its percentage and β-elemene passed from 0 to 17.2% in plant growing in Monte Murlo. Also, the volatiles emitted in vivo by the fruits showed very similar patterns. Even if minor compounds can contribute to the perception of a smell, which results from a stimulus containing much information, the differences noted in the odours of the two samples could be sufficiently correlated with the different emission patterns of volatiles and, consequently, with the different pedological and climatic conditions of the two collection places.

The essential oil obtained from the leaves of plants growing in Monte Murlo was characterized by more than 90% of monoterpenes (32 compounds), while sesquiterpenes were less represented, both numerically (seven derivatives) and as a percentage (1.3%). Other chemicals identified in this essential oil were non-terpenic alcohols, aldehydes, hydrocarbons, esters and some phenylpropanoid derivatives. The main compounds identified in this essential oil were α-pinene (41.6%), 1,8-cineole (25.5%), limonene (9.5%) and *trans*-myrtanol acetate (4.2%). The essential oil of the fruits collected from the same plants showed a close composition. It was constituted by 93.4% of monoterpenes and 1.3% of sesquiterpenes. The most representative components were the same as above, but in different amounts: α-pinene (51.1%), 1,8-cineole (23.1%), limonene (7.9%) and *trans*-myrtanol acetate (3.7%).

Monoterpenes were also the main constituents of the essential oil of the leaves of plants collected in Cima Marrana: 28 derivatives were identified, representing almost 90% of the whole oil, while the 11 sesquiterpenes detected accounted for a further 3.6%. The essential oil of the fruits collected from these plants contained more monoterpenes (31 compounds), but they accounted for a lesser percentage (about 86%). Also, sesquiterpenes were observed in greater number (19 derivatives), which represented 5.4% of the oil. The main compounds of the essential oil of the leaves were α-pinene (28.9%),

1,8-cineole (24.2%), linalool (11.7%), limonene (5.2%), *trans*-myrtanol acetate (5.2%) and α-terpineol (3.6%), while in the oil of the fruits they were 1,8-cineole (25.4%), α-pinene (21.4%), linalool (6.2%), *trans*-myrtanol acetate (6.0%) and limonene (5.6%).

The main differences between the composition of the essential oils of the leaves in the two stations centered on the terpene hydrocarbons, α-pinene and limonene, which were identified in greater amounts in plants growing in Monte Murlo, while the oxygenated derivatives, linalool, linalyl acetate and *trans*-myrtanol acetate, were detected in higher percentages in Cima Marrana. Furthermore, α-phellandrene, 3-carene, α-terpinene, β-elemene, β-selinene, viridiflorene and selin-11-en-4-α-ol were detected, even if in small amounts, only in plants collected in Cima Marrana, while propyl butyrate and butyl butyrate were exclusive to plants growing in Monte Murlo.

In the essential oil of the fruits of plants collected in Monte Murlo, similarly to the oil of the leaves, α-pinene and limonene were in greater amounts than in plants growing in Cima Marrana, which instead were richer in linalool, linalyl acetate and *trans*-myrtanol acetate. Propyl butyrate, butyl butyrate, methyleugenol, (*E*)-methylisoeugenol and α-cadinol were found only in plants growing in Monte Murlo, while 3-carene, β-elemene, tetradecane, β-selinene, viridiflorene, globulol and selin-11-en-4-α-ol were identified only in the fruits of plants growing in Cima Marrana.

Comparison of our results with literature data allows our samples, particularly the Monte Murlo one, to be assigned to the chemotype α-pinene/1,8-cineole (Bradesi et al., 1997; Weyerstahl, Marschall, & Rustaiyan, 1994) because of the high content of these two compounds. Other papers (Asllani, 2000; Boelens & Jimenez, 1992; Chalchat, Garry, & Michet, 1998; Ozek, Demirci, & Baser, 2000) reported, among the constituents of the essential oil of leaves and fruits of *M. communis*, the presence of myrtenol, myrtenal and myrtenyl acetate. This latter compound has been identified in percentages between 0.1 and 33%. On the other hand neither of these compounds were identified in our four samples. Noteworthy instead, was the presence, in all the samples, of *trans*-myrtanol acetate (the corresponding saturated derivative of myrtenyl acetate), for the first time identified as a constituent of an essential oil of *M. communis* in this study.

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