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Chemical Composition of Volatiles in Sardinian Myrtle (*Myrtus communis* L.) Alcoholic Extracts and Essential Oils

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The chemical composition of the volatile fraction of myrtle (*Myrtus communis* L.) alcoholic extracts and essential oils from leaves and berries collected in different places in Sardinia (Italy) was studied. A simple and rapid liquid−liquid extraction method was used to isolate volatile compounds from myrtle alcoholic extracts followed by GC and GC-MS analysis allowing the detection of 24 compounds. The volatile fraction was characterized by the terpenes fraction corresponding to that of the essential oils and by a fatty acid ethyl esters fraction. The variation during extraction of the volatile fraction in alcoholic extracts of berries and leaves was evaluated. Essential oils were obtained by hydrodistillation, and the yields were on average 0.52 ± 0.03% (v/w dried weight) and 0.02 ± 0.00% for leaves and berries, respectively. The essential oils were analyzed by GC and GC-MS, and a total of 27 components were detected, accounting for 90.6−98.7% of the total essential oil composition. Strong chemical variability depending on the origin of the samples was observed. The major compounds in the essential oils were α-pinene (30.0 and 28.5%), 1,8-cineole (28.8 and 15.3%), and limonene (17.5 and 24.1%) in leaves and berries, respectively, and were characterized by the lack of myrtenyl acetate.

KEYWORDS: ***Myrtus communis* L.; alcoholic extract; volatile compounds; essential oil**

INTRODUCTION

Myrtle (*Myrtus communis* L.) is an evergreen shrub belonging to the family of Mirtaceae that grows spontaneously throughout the Mediterranean area. In Italy it grows along the coast and in the internal hills, and it is spread in the islands, where it is one of the most characteristic species (*1*). The essential oil obtained by hydrodistillation from leaves and, sometimes, flowers and berries has been employed for its antimicrobial, tonic, and balsamic properties (*2*−*4*), and it is used in the flavor and fragrance industries (*5*). Myrtle berries and leaves are mostly employed for the industrial formulation of sweet liquors with digestive properties (*6*). The most renowned Sardinian liquor is obtained by alcoholic extraction of berries (red myrtle) or leaves (white myrtle) (*5*).

Myrtle leaf and berry essential oils from the Mediterranean regions have been the subject of a number of studies (*7*−*18*). Only a few papers have reported on the chemical composition of Sardinian myrtle essential oils (*19*, *20*) and on the volatile fraction of myrtle alcoholic extracts (*21*, *22*). Moreover, little is known about the chemical variability of the essential oils obtained from different naturally grown stations.

The aims of the present paper were (a) to implement a simple and rapid method for the isolation of volatile compounds by myrtle alcoholic extracts, (b) to investigate the chemical composition and the changes of the volatile fraction from myrtle berry and leaf alcoholic extracts during maceration, and (c) to thoroughly investigate the chemical composition and variability of Sardinian myrtle essential oils from leaves and berries.

Table 1. Sample Sites and Essential Oil Yields[a]

sample	site	berry color	yield (%, v/w ± SD) berries	yield (%, v/w ± SD) leaves
1	Villacidro[b,c]	red	0.02 ± 0.00	
2	Mix Sardegna[b]	red	0.02 ± 0.00	
3	S. Lussurgiu	white	0.02 ± 0.00	0.80 ± 0.02
4	S. Lussurgiu	red	0.02 ± 0.00	0.22 ± 0.02
5	Iglesias	red	0.02 ± 0.00	0.42 ± 0.05
6	Capoterra	red	0.03 ± 0.00	0.53 ± 0.01
7	Palmas Arborea	red	0.02 ± 0.00	
8	Ingurtosu	red		0.58 ± 0.02
9	Sinnai[b,c]	red		0.30 ± 0.04
10	Villamassargia	red	0.03 ± 0.00	0.90 ± 0.03
11	Paulilatino[b]	red		0.44 ± 0.01

[a] Expressed on dried weight. [b] Samples from which were obtained the alcoholic extracts. [c] Samples from which were evaluated the changes of the aromatic fraction.

MATERIALS AND METHODS

Plant Material. Wild samples were collected in different sites of Sardinia (Italy) between November and December 2004, at industrial ripening grade. The specimens were identified and deposited in the Herbarium of the Department of Toxicology of the University of

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Table 2. Comparison (Relative Percentage) of the Alcoholic Extract (AE) and Essential Oil (EO) Terpenic Fraction of *M. communis* L.

			sample[a]							
compound[b]	RI[c]	ID method[d]	1(AE)	1(EO)	2(AE)	2(EO)	9(AE)	9(EO)	11(AE)	11(EO)
α-thujene	924	MS, RI, std	3.1	1.1	2.7	0.7	0.7	0.2	0.7	1.1
α-pinene	931	MS, RI, std	19.1	18.9	34.1	26.4*	65.6	59.5*	53.1	50.0*
β-pinene	975	MS, RI, std	2.6	0.9	2.6	0.7*	0.6	0.3	0.8	0.6*
myrcene	988	MS, RI, std	1.9	0.8	1.1	0.2	0.1	0.1	0.4	0.0
α-phellandrene	1007	MS, RI, std	2.1	1.3	2.7	2.2	0.2	0.1	0.2	0.0
δ-3-carene	1009	MS, RI, std	2.8	2.7	0.9	0.0	0.1	0.1	0.3	0.0
α-terpinene	1016	MS, RI, std	nd	0.0	nd	0.3	nd	0.0	nd	0.0
p-cymene	1023	MS, RI, std	3.5	5.1	3.3	4.2	0.3	0.2	0.3	0.3
limonene	1028	MS, RI, std	43.4	44.2	5.7	6.8	9.8	6.2*	8.0	6.6
1,8-cineole	1031	MS, RI, std	5.9	8.7*	20.7	20.0	12.8	20.9*	26.6	30.4*
γ-terpinene	1056	MS, RI, std	2.3	2.7	2.1	2.4	0.3	0.2	0.5	0.0
terpinolene	1083	MS, RI, std	2.0	2.7	0.9	1.8	0.1	0.1	0.2	0.3
linalool	1101	MS, RI, std	1.4	1.0	2.0	2.2	0.1	0.7	0.5	0.2
terpinen-4-ol	1180	MS, RI, std	nd	0.0	nd	0.6	nd	0.1	nd	0.0
α-terpineol	1190	MS, RI, std	0.9	0.9	2.0	1.9	0.3	1.6	0.4	3.3*
linalyl acetate	1256	MS, RI, std	1.7	0.5	2.6	2.0	2.2	0.9	0.9	1.8
terpenyl acetate	1314	MS, RI, std	1.1	0.4	2.4	4.9	0.4	0.3	0.8	0.0
neryl acetate	1366	MS, RI, std	nd	0.0	nd	0.3	nd	0.1	nd	0.0
geranyl acetate	1380	MS, RI, std	1.7	0.9	8.2	10.8	2.1	2.0	2.3	1.9
methyl eugenol	1398	MS, RI, std	1.1	3.1	1.6	2.2	0.3	1.3	0.4	0.5
β-caryophyllene	1403	MS, RI, std	1.1	0.4	1.5	1.2	1.0	0.0	2.1	0.0
α-humulene	1450	MS, RI, std	1.2	0.2	1.4	0.5	2.7	0.2	0.9	0.0
allo-aromadendrene	1455	MS, RI, std	nd	0.3	nd	0.2	nd	0.0	nd	0.0
β-selinene[e]	1482	MS, RI	nd	0.3	nd	0.2	nd	0.3	nd	0.0
germacrene B[e]	1555	MS, RI	nd	0.2	nd	1.0	nd	0.0	nd	0.0
spathulenol[e]	1574	MS, RI	nd	0.3	nd	0.3	nd	0.0	nd	0.0
caryophyllene oxide	1580	MS, RI, std	1.2	0.7	1.4	1.4	0.3	0.1	0.4	0.8
monoterpenes			88.7	89.1	76.8	65.4	90.6	87.9	87.9	89.3
alcohols			2.3	1.9	4.0	4.1	0.4	2.3	0.9	3.5
esters			4.5	1.8	13.2	17.7	4.7	3.2	4.0	3.7
ethers			1.1	3.1	1.6	2.2	0.3	1.3	0.4	0.5
sesquiterpenes			3.5	1.3	4.3	3.1	4.0	0.3	3.4	0.8
oxides			1.2	0.7	1.4	1.4	0.3	0.1	0.4	0.8

[a] AE, alcoholic extract (%); EO, essential oil (%). Values within a column marked with an asterisk for each extract are significantly different from each other, using Tukey's LSD test ($P < 0.05$). nd, not detected (<0.05%). [b] Compounds are listed in order of their elution from a DB-5MS column. [c] Retention indices as determined on a DB-5MS column using a homologous series of *n*-alkanes. [d] Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; std, by injection of an authentic sample. [e] Tentatively identified according to the mass spectrum (MS) and by comparison of RI with the literature (RI).

Cagliari. The harvest involved a random sampling with three different samples (2.5 kg) collected in each area. **Table 1** shows the sampling sites and the essential oil yields (%, v/w) from the different parts of the plant. The "Mix Sardegna" sample was a homogeneous sample from 10 different localities of southern Sardinia supplied by the liquor industry. Berries and leaves were selected and cleaned of impurities in the laboratory and processed immediately after harvest.

Alcoholic Extracts Processing. According to the industrial process (*5*), the alcoholic extracts from myrtle berries and from leaves (samples 9 and 11) were prepared on a laboratory scale with the following methodology: 25.5% of matrix (berries or leaves), 72.7% of ethanol (95%, v/v), and 1.8% of water. Each sample was stored for 5 weeks in the dark at 20 °C.

Isolation of Volatile Compounds. A 10 mL aliquot of alcoholic extract was transferred in a 20 mL glass test tube; 0.7 g of NaCl, 2 mL of diethyl ether/*n*-hexane mixture (50:50, v/v), and 5 mL of deionized water were added. The tube was then agitated for 1 h in a rotatory shaker. The phases were allowed to separate. Fifty microliters of the internal standard was added to 450 μL of the extraction solvent and injected for the GC analysis.

Recovery Assay. Preliminary tests were carried out to assess the recovery of the extraction method. A sample of the myrtle alcoholic extract was fortified with standard solutions of representative components of the extract such as α-pinene, limonene, 1,8-cineole, ethyl palmitate, and ethyl linolenate, to reach concentrations of 1, 10, and 100 mg/kg. Prior to the extraction step, the fortified samples were allowed to settle for 30 min. Afterward, they were processed according to the above extraction procedure. Three replicates for each concentration were analyzed, and recoveries were 96.5 ± 1.5% on average.

Essential Oil Distillation. An aliquot of 200 or 100 g of matrix (berries and leaves, respectively) was hydrodistilled in triplicate with a Clevenger-type apparatus according to the Italian Official Pharmacopoeia XI (*23*). The essential oils were stored with anhydrous sodium sulfate in dark vials at 4 °C and dissolved to reach a concentration of 1% (v/v) in *n*-hexane before GC analysis.

GC-FID Analysis. A gas chromatograph Trace (Thermo Finningan, Rodano, Milan, Italy) equipped with a FID detector, an AS 800 autosampler, and a split−splitless injector was used. The capillary column was a fused silica DB5 (30 m, 0.25 mm i.d.; 0.25 μm film thickness) (J&W Scientific, Fisons, Folsom, CA). The injector and the detector were operated at 150, and 280 °C, respectively. One microliter of sample was injected in split mode (1:20). The oven was programmed as follows: 60 °C, raised to 180 °C (3 °C/min), and from 180 to 250 °C, and isothermally held for 2 min. Helium was used as carrier gas and nitrogen as makeup gas at 120 and 80 kPa, respectively.

GC-MS Analysis. A Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with an MS detector HP 5971 A, an HP 7673 autosampler, a split−splitless injector, and an MS ChemStation HP v. C.00.07 was used. The column was a fused silica capillary DB-5MS (5% phenylmethylpolysyloxane, 30 m, 0.25 mm i.d.; 0.25 μm film thickness; J&W Scientific, Fisons). The injector and interface were operated at 150 and 280 °C, respectively. The oven temperature was programmed as follows: from 60 to 180 °C (3 °C/min) and then from 180 to 250 °C and isothermally held for 2 min. Helium was the carrier gas at 0.9 mL/min; the sample (1 μL) was injected in the split mode (1:20). MS conditions were as follows: ionization voltage, 70 eV; scan rate, 1.6 scan/s; mass range, 50−500 amu; ion source temperature, 180 °C. The oil components were

Table 3. Constituents (Milligrams per Kilogram) of the Berry Alcoholic Extract of *M. communis* L.

			week[a]				
compound[b]	RI[c]	ID method[d]	1	2	3	4	5
α-thujene	924	MS, RI, std	4.0ab	3.5a	3.6a	4.6b	3.7a
α-pinene	931	MS, RI, std	25.0ab	31.8ab	23.7a	37.7b	27.3ab
β-pinene	975	MS, RI, std	3.4a	2.7a	3.0a	3.1a	2.9a
myrcene	988	MS, RI, std	2.4bc	1.6ab	1.8abc	2.7c	1.3a
α-phellandrene	1007	MS, RI, std	2.7a	2.4a	2.3a	4.0a	3.6a
δ-3-carene	1009	MS, RI, std	3.7c	2.9b	3.1b	1.1a	1.1a
α-terpinene	1016	MS, RI, std	nd	nd	nd	nd	nd
p-cymene	1023	MS, RI, std	4.6a	3.5a	3.9a	4.1a	4.0a
limonene	1028	MS, RI, std	56.8a	54.3a	42.5a	59.7a	44.7a
1,8-cineole	1031	MS, RI, std	7.7a	7.3a	6.2a	8.5a	8.3a
γ-terpinene	1056	MS, RI, std	3.0b	2.2a	2.6ab	2.5ab	2.3ab
terpinolene	1083	MS, RI, std	2.6b	1.6a	2.2ab	1.4a	1.9ab
linalool	1101	MS, RI, std	1.9b	1.6a	1.8ab	1.6a	1.6a
terpinen-4-ol	1180	MS, RI, std	nd	nd	nd	nd	nd
α-terpineol	1190	MS, RI, std	1.2a	1.2a	1.2a	1.2a	1.2a
linalyl acetate	1256	MS, RI, std	2.3a	2.2a	2.4a	2.2a	2.2a
terpenyl acetate	1314	MS, RI, std	1.4a	1.2a	1.5a	1.2a	1.3a
neryl acetate	1366	MS, RI, std	nd	nd	nd	nd	nd
geranyl acetate	1380	MS, RI, std	2.3a	2.3a	2.3a	2.0a	1.6a
methyleugenol	1398	MS, RI, std	1.4a	1.4a	1.6a	1.4a	1.4a
β-caryophyllene	1403	MS, RI, std	1.5a	1.4a	1.4a	1.4a	1.4a
α-humulene	1450	MS, RI, std	1.6a	1.6a	1.6a	1.6a	1.6a
allo-aromadendrene	1455	MS, RI, std	nd	nd	nd	nd	nd
β-selinene[e]	1482	MS, RI	nd	nd	nd	nd	nd
germacrene B[e]	1555	MS, RI	nd	nd	nd	nd	nd
spathulenol[e]	1574	MS, RI	nd	nd	nd	nd	nd
caryophyllene oxide	1580	MS, RI, std	1.6a	1.5a	1.7a	1.6a	1.5a
ethyl palmitate	1990	MS, RI, std	4.1a	6.7a	6.8a	9.1ab	16.3b
ethyl linoleate	2063	MS, RI, std	2.8a	2.6a	3.3a	6.6ab	9.8b
ethyl linolenate	2110	MS, RI, std	5.9a	3.7a	8.6a	12.8ab	22.5b
ethyl stearate	2191	MS, RI, std	2.3a	2.7ab	2.3ab	4.2c	3.7bc
monoterpenes			115.9	113.8	94.9	129.4	101.1
alcohols			3.1	2.8	3.0	2.8	2.8
esters			6.0	5.7	6.2	5.4	5.1
ethers			1.4	1.4	1.6	1.4	1.4
sesquiterpenes			4.7	4.5	4.7	4.6	4.5
oxides			1.6	1.5	1.7	1.6	1.5
total terpenes			131.0	128.2	110.5	143.6	114.8
ethyl esters			15.1	15.8	21.1	32.8	52.3
identified compounds			146.1	144.0	131.5	176.3	167.2

[a] Values within a column for each sampling period having different letters are significantly different from each other, using Tukey's LSD test ($P < 0.05$). nd, not detected (<0.5 mg/kg). [b] Compounds are listed in order of their elution from a DB-5MS column. [c] Retention indices as determined on a DB-5MS column using a homologous series of *n*-alkanes. [d] Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; std, by injection of an authentic sample. [e] Tentatively identified according to the mass spectrum (MS) and by comparison of RI with the literature (RI).

identified by comparison of their relative retention times with those of authentic samples or by comparison of their retention index (RI) relative to a series of *n*-hydrocarbons determined with C_7-C_{26} alkane standards as reference. Computer matching against commercial (Adams, Nist 98) (*24*, *25*) and homemade library mass spectra made up of pure substances and components of known oils, as well as MS literature data, was also used for the identification. The RI calculated were in agreement with those reported by Adams (*24*).

Quantitative Analysis. Calibration graphs were constructed according to the internal standard method by measuring peak height versus concentration. The concentrations of the compounds were expressed in milligrams per kilogram. Good linearity for all compounds was achieved, and correlation coefficients ranged between 0.9991 and 0.9998.

The percentage composition of the essential oils was calculated from GC peak areas without using correction factors.

Three replicates were performed for each sample. The average of these three values and the standard deviation were determined for each compound identified.

Chemicals. α-Thujene, α-pinene, β-pinene, myrcene, α-phellandrene, δ-3-carene, α-terpinene, *p*-cymene, limonene, 1,8-cineole, γ-terpinene, terpinolene, linalool, terpinene-4-ol, α-terpineol, linalyl acetate, terpenyl acetate, neryl acetate, geranyl acetate, methyleugenol, β-caryophyllene, α-humulene, *allo*-aromadendrene, caryophyllene oxide, ethyl palmitate, ethyl linoleate, ethyl linolenate, and ethyl stearate were from Aldrich, Acros, Fluka (Milan, Italy), and Extrasynthese (Genay, France). All compounds were of analytical standard grade. β-Selinene, germacrene B, and spathulenol were not available on the market. The internal standard was 2,6-dimethylphenol (Sigma-Aldrich, Milan, Italy).

n-Hexane and diethyl ether were analytical grade solvents; Na_2SO_4 and NaCl were of analytical reagent grade from Carlo Erba (Milan, Italy). Ethanol (95% v/v) for food use was from Silvio Carta srl (Baratili S. Pietro, Oristano, Italy).

Statistical Analysis. Analysis of variance (ANOVA) was carried out, and the average values have been compared with the Tukey's LSD test at $P < 0.05$ using GenStat v. 7.1 software (VSN International Ltd., Herts, U.K.).

RESULTS AND DISCUSSION

Table 1 reports essential oil yields (v/w, dried weight) and the sites of harvest. Sample 3 is a peculiar myrtle variety with white berries. The alcoholic extracts were carried out on three samples (samples 1, 9, and 11) harvested in the most typical myrtle collection areas and on the sample 2 supplied from the liquor industry. Samples 1 and 9 were also used to evaluate the changes of volatile compounds in myrtle alcoholic extracts

Table 4. Constituents (Milligrams per Kilogram) of the Leaf Alcoholic Extract of *M. communis* L.

			week[a]				
compound[b]	RI[c]	ID method[d]	1	2	3	4	5
α-thujene	924	MS, RI, std	8.3b	7.5ab	7.5ab	7.8a	7.0a
α-pinene	931	MS, RI, std	812.3b	815.3ab	811.0ab	727.8a	740.0ab
β-pinene	975	MS, RI, std	8.0a	8.4a	8.3a	7.8a	8.0a
myrcene	988	MS, RI, std	1.8a	1.2a	1.2a	1.5a	1.2a
α-phellandrene	1007	MS, RI, std	2.9b	1.8a	1.8a	4.4d	3.5c
δ-3-carene	1009	MS, RI, std	1.8a	1.1a	1.1a	1.1a	4.1b
α-terpinene	1016	MS, RI, std	nd	nd	nd	nd	nd
p-cymene	1023	MS, RI, std	3.6a	4.8b	4.8b	5.0bc	5.3c
limonene	1028	MS, RI, std	121.7c	104.8b	91.4a	93.7a	92.4a
1,8-cineole	1031	MS, RI, std	158.9b	124.4a	113.0a	118.5a	122.3a
γ-terpinene	1056	MS, RI, std	3.1a	3.7a	2.8a	3.5a	3.3a
terpinolene	1083	MS, RI, std	1.0a	1.0a	1.0a	1.9a	1.3a
linalool	1101	MS, RI, std	1.6a	1.6a	1.6a	8.7b	8.7 b
terpinen-4-ol	1180	MS, RI, std	nd	nd	nd	nd	nd
α-terpineol	1190	MS, RI, std	3.7b	4.9c	1.2a	5.4c	4.9c
linalyl acetate	1256	MS, RI, std	27.1a	26.6a	25.0a	26.2a	26.4a
terpenyl acetate	1314	MS, RI, std	4.9a	6.7b	6.5b	7.0b	6.6b
neryl acetate	1366	MS, RI, std	nd	nd	nd	nd	nd
geranyl acetate	1380	MS, RI, std	25.5a	26.9a	25.3a	27.9a	24.9a
methyleugenol	1398	MS, RI, std	3.7a	4.5a	6.4a	6.3a	6.0a
β-caryophyllene	1403	MS, RI, std	12.0a	15.9a	14.9a	16.5a	15.7a
α-humulene	1450	MS, RI, std	33.6a	39.1a	39.9a	44.6a	41.8a
allo-aromadendrene	1455	MS, RI, std	nd	nd	nd	nd	nd
β-selinene[e]	1482	MS, RI	nd	nd	nd	nd	nd
germacrene B[e]	1555	MS, RI	nd	nd	nd	nd	nd
spathulenol[e]	1574	MS, RI	nd	nd	nd	nd	nd
caryophyllene oxide	1580	MS, RI, std	3.2a	4.8b	5.0b	4.6ab	4.6ab
ethyl palmitate	1990	MS, RI, std	4.2a	8.0b	10.7c	13.5d	13.6d
ethyl linoleate	2063	MS, RI, std	2.8a	6.3b	8.5b	13.1c	12.8c
ethyl linolenate	2110	MS, RI, std	12.4a	23.7b	36.6c	62.3d	68.1e
ethyl stearate	2191	MS, RI, std	2.0a	2.0a	5.8b	4.4b	7.7c
monoterpenes			1121.6	1074.0	1043.9	973.0	988.4
alcohols			5.3	6.5	2.8	14.1	13.6
esters			57.5	60.2	56.8	61.1	57.9
ethers			3.7	4.5	6.4	6.3	6.0
sesquiterpenes			48.8	55.0	54.8	61.1	57.5
oxides			3.2	4.8	5.0	4.6	4.6
total terpenes			1240.1	1205.0	1169.7	1120.2	1128.0
ethyl esters			21.4	40.0	61.6	93.3	102.2
identified compounds			1261.5	1245.0	1231.3	1213.5	1230.2

[a] Values within a column for each sampling period having different letters are significantly different from each other, using Tukey's LSD test ($P < 0.05$). nd, not detected (<0.5 mg/kg). [b] Compounds are listed in order of their elution from a DB-5MS column. [c] Retention indices as determined on a DB-5MS column using a homologous series of *n*-alkanes. [d] Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; std, by injection of an authentic sample. [e] Compound tentatively identified according to the mass spectrum (MS) and by comparison of RI with the literature (RI).

during maceration. Great differences in essential oil yields from different parts of plant were observed. Leaves showed always the highest yields, from 10- to 30- or 40-fold higher (samples 10 and 3, respectively) than the berries.

Composition and Changes of Myrtle Alcoholic Extracts. GC-MS analysis of the volatile fraction of the alcoholic extracts was characterized by terpenes and ethyl esters of fatty acids. Twenty-four compounds were identified: 20 were terpenoids and 4 were ethyl esters of fatty acids. **Table 2** reports the comparison of the percentage composition of the terpene fraction of myrtle alcoholic extract and essential oil from the same samples. A qualitative and quantitative correspondence was assessed; only a few statistically significant differences were found. The alcoholic extracts are characterized by the presence of the ethyl esters of fatty acids, ethyl palmitate, ethyl linoleate, ethyl linolenate, and ethyl stearate. Fatty acids derive from leaves and berries (*26*) and in the presence of ethanol form esters. The synthesis of these esters showed a constant trend to a maximum within the end of the maceration period (**Tables 3** and **4**). This can be tentatively explained considering that the esterification process is affected by the amount of free fatty acids and by the diffusion processes of the hydroalcoholic solution through the plant material.

The ethyl esters increased especially in the leaf alcoholic extracts from 21.4 to 102.2 mg/kg versus from 15.1 to 52.3 mg/kg in the berry alcoholic extract. Some of the latter components increased by a factor of 3 (ethyl palmitate and ethyl stearate in the leaves, ethyl linoleate in the berries), whereas some others increased by a factor of 4 (ethyl linoleate in the leaves and ethyl palmitate and ethyl linolenate in the berries); ethyl linolenate in the leaf extract increased 5-fold. Only ethyl stearate did not increase in the berry extract. As shown in **Tables 3** and **4** significant differences in the amounts of the ethyl esters were found.

The total amount of volatile terpene compounds in leaf alcoholic extracts was higher than in berry extracts, ranging on average between 1172.6 and 125.6, respectively (**Tables 3** and **4)**. This is in accordance with the higher essential oil yields obtained from the leaves. Terpenes generally did not change from the first to the fifth week of extraction, which can be explained by considering that they are easily extracted from the matrix and stable in the hydroalcoholic solution.

Table 5. Constituents (Area Percent ± SD) of the Berry Essential Oil of *M. communis* L.

			sample[a]							
compound[b]	RI[c]	ID method[d]	1	2	3	4	5	6	7	10
α-thujene	924	MS, RI, std	1.1 ± 0.6	0.7 ± 0.0	0.3 ± 0.1	1.0 ± 0.1	2.1 ± 0.6	1.0 ± 0.1	0.4 ± 0.1	1.9 ± 0.2
α-pinene	931	MS, RI, std	18.9 ± 1.0	26.4 ± 0.5	30.9 ± 0.7	22.4 ± 3.0	27.7 ± 4.8	26.5 ± 2.6	18.2 ± 2.4	38.9 ± 0.9
β-pinene	975	MS, RI, std	0.9 ± 0.0	0.7 ± 0.0	0.9 ± 0.1	0.3 ± 0.0	0.9 ± 0.2	0.4 ± 0.0	0.5 ± 0.2	0.8 ± 0.0
myrcene	988	MS, RI, std	0.8 ± 0.7	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.3	0.4 ± 0.0	nd	0.5 ± 0.2	0.2 ± 0.0
α-phellandrene	1007	MS, RI, std	1.3 ± 0.1	nd	nd	1.1 ± 0.3	1.5 ± 1.3	0.8 ± 0.1	0.1 ± 0.2	1.1 ± 0.0
δ-3-carene	1009	MS, RI, std	2.7 ± 0.3	2.2. ± 0.1	nd	2.6 ± 0.3	6.1 ± 1.9	1.8 ± 0.1	1.9 ± 0.5	4.6 ± 0.1
α-terpinene	1016	MS, RI, std	nd	0.3 ± 0.0	nd	0.5 ± 0.1	0.7 ± 0.1	0.4 ± 0.0	0.2 ± 0.2	nd
p-cymene	1023	MS, RI, std	5.1 ± 0.3	4.2 ± 0.3	0.1 ± 0.1	2.3 ± 0.2	10.3 ± 0.8	2.3 ± 0.2	3.4 ± 0.8	5.5 ± 0.5
limonene	1028	MS, RI, std	44.2 ± 2.7	6.8 ± 0.5	10.2 ± 2.1	27.7 ± 2.5	5.0 ± 0.4	23.7 ± 1.5	44.5 ± 4.3	3.7 ± 1.2
1,8-cineole	1031	MS, RI, std	8.7 ± 0.7	20.0 ± 6.3	24.8 ± 3.1	11.1 ± 2.0	5.8 ± 1.4	12.3 ± 1.4	9.5 ± 0.7	17.7 ± 3.1
γ-terpinene	1056	MS, RI, std	2.7 ± 0.3	2.4 ± 0.1	0.5 ± 0.3	4.9 ± 1.1	5.8 ± 0.3	3.0 ± 0.2	2.4 ± 0.6	0.6 ± 0.1
terpinolene	1083	MS, RI, std	2.7 ± 0.2	1.8 ± 0.1	nd	4.0 ± 0.7	5.9 ± 0.7	2.9 ± 0.3	1.4 ± 0.5	5.5 ± 0.6
linalool	1101	MS, RI, std	1.0 ± 0.1	2.2 ± 0.3	0.7 ± 0.2	1.5 ± 0.1	0.4 ± 0.0	14.7 ± 1.5	0.8 ± 0.3	4.9 ± 0.6
terpinen-4-ol	1180	MS, RI, std	nd	0.6 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	nd
α-terpineol	1190	MS, RI, std	0.9 ± 0.8	1.9 ± 0.3	2.6 ± 0.5	1.0 ± 0.3	0.7 ± 0.1	0.7 ± 0.6	0.6 ± 0.1	0.5 ± 0.3
linalyl acetate	1256	MS, RI, std	0.5 ± 0.1	2.0 ± 0.5	0.5 ± 0.2	0.3 ± 0.1	0.5 ± 0.1	nd	0.6 ± 0.1	0.4 ± 0.0
terpenyl acetate	1314	MS, RI, std	0.4 ± 0.1	4.9 ± 0.9	5.4 ± 1.1	0.2 ± 0.0	2.3 ± 0.6	0.1 ± 0.1	2.9 ± 0.1	1.0 ± 0.2
neryl acetate	1366	MS, RI, std	nd	0.3 ± 0.0	0.2 ± 0.0	nd	0.2 ± 0.0	nd	0.1 ± 0.1	nd
geranyl acetate	1380	MS, RI, std	0.9 ± 0.1	10.8 ± 1.5	13.0 ± 3.2	5.7 ± 1.3	8.2 ± 2.6	4.7 ± 1.0	5.7 ± 0.0	0.2 ± 0.0
methyleugenol	1398	MS, RI, std	3.1 ± 0.2	2.2 ± 0.2	1.4 ± 0.1	2.6 ± 0.3	0.9 ± 0.2	0.9 ± 0.2	0.7 ± 0.1	3.8 ± 1.0
β-caryophyllene	1403	MS, RI, std	0.4 ± 0.0	1.2 ± 0.6	0.1 ± 0.0	1.0 ± 0.1	0.9 ± 0.4	0.8 ± 0.2	0.4 ± 0.1	0.7 ± 0.1
α-humulene	1450	MS, RI, std	0.2 ± 0.0	0.5 ± 0.3	nd	0.4 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	nd	0.7 ± 0.1
allo-aromadendrene	1455	MS, RI, std	0.3 ± 0.0	0.2 ± 0.2	0.5 ± 0.2	0.4 ± 0.0	0.6 ± 0.0	nd	0.3 ± 0.1	0.2 ± 0.1
β-selinene[e]	1482	MS, RI	0.3 ± 0.0	0.2 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.0	nd	nd	nd
germacrene B[e]	1555	MS, RI	0.2 ± 0.0	1.0 ± 0.6	1.2 ± 0.5	0.3 ± 0.0	0.7 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
spathulenol[e]	1574	MS, RI	0.3 ± 0.0	0.3 ± 0.1	nd	0.4 ± 0.0	0.7 ± 0.2	nd	0.2 ± 0.0	0.4 ± 0.0
caryophyllene oxide	1580	MS, RI, std	0.7 ± 0.0	1.4 ± 0.5	0.6 ± 0.1	0.9 ± 0.0	0.8 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.1 ± 0.2
monoterpenes			89.1	65.7	67.9	78.0	72.2	75.1	83.0	80.5
alcohols			1.9	1.2	3.6	3.0	1.7	15.8	1.7	5.4
esters			1.8	1.8	19.1	6.2	11.2	4.8	9.3	1.6
ethers			3.1	2.2	1.4	2.6	0.9	0.9	0.7	3.8
sesquiterpenes			2.4	4.8	5.0	3.9	4.5	2.0	1.9	2.3
oxides			0.7	1.4	0.6	0.9	0.8	0.7	0.8	0.1
identified compounds			98.4	95.3	95.1	93.7	90.6	98.6	96.6	93.5

[a] See **Table 1** for sample corresponding number. nd, not detected (<0.05%). [b] Compounds are listed in order of their elution from a DB-5MS column. [c] Retention indices as determined on a DB-5MS column using a homologous series of *n*-alkanes. [d] Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; std, by injection of an authentic sample. [e] Compound tentatively identified according to the mass spectrum (MS) and by comparison of RI with the literature (RI).

Essential Oil Composition. Twenty-seven components were identified accounting for 90.6−98.7% of the total essential oil composition. The chemical composition of individual samples exhibited small qualitative differences. Nevertheless, large variations depending on the origin of the samples were observed in the concentration of the main constituents.

Essential Oil from Berries. Although the content of monoterpenes represented 65.7−89.1% of the entire oils, some of them were remarkably different in the individual samples (**Table 5**).

For example, α-pinene ranged from 18.2% (sample 7) to 38.9% (sample 10); δ-3-carene ranged from 0.0 to 6.1% (samples 5 and 3, respectively). *p*-Cymene ranged from 0.1% (sample 3) to 10.3% (sample 5); limonene widely ranged from 3.7% (sample 10) to 44.5% (sample 7). 1,8-Cineole ranged from 5.8% (sample 5) to 24.8% (sample 3); γ-terpinene ranged from 0.5% (sample 3) to 5.8% (sample 5); terpinolene ranged from 0.0% (sample 3) to 5.9% (sample 5). Linalool widely ranged from 0.4% (sample 5) to 14.7% (sample 6); terpenyl acetate ranged from 0.1% (sample 6) to 5.4% (sample 3); and geranyl acetate ranged from 0.2% (sample 10) to 13.0% (sample 3).

The berries showed a moderate amount of sesquiterpenes representing 5.0% of the entire oil at the most.

Essential Oil from Leaves. The content of monoterpenes represents 72.1−92.6% of the entire oils. Some of them (δ-3-carene, *p*-cymene, γ-terpinene, terpinolene, and terpenyl acetate) are in lower amount with respect to berry essential oils (**Table 6**).

Generally, α-pinene was 30.0% of each sample except for sample 9, in which the content was 2-fold higher (59.5%). Limonene ranged from 5.2% (sample 5) to 29.8% (sample 4); 1,8-cineole ranged from 15.9% (sample 4) to 41.7% (sample 3). Linalool ranged from 0.2% (samples 10 11) to 16.7% (sample 6). α-Terpineol ranged from 1.3% (sample 4) to 4.8% (sample 3), and geranyl acetate ranged from 0.4% (sample 10) to 7.2% (sample 3). Methyleugenol ranged from 0.5% (sample 11) to 3.5% (sample 4).

The leaves showed a low amount of sesquiterpenes, accounting for 1.6% of the entire oil.

From the results it appeared that the chemical compositions of the myrtle essential oils from leaves and berries were similar except for the contents of α-terpinene, *allo*-aromadendrene, germacrene B, and spathulenol that were not detected in the leaves. Generally, the major components of the essential oils obtained from both leaves and berries were α-pinene, 1,8-cineole, and limonene.

Tables 5 and **6** show a low variability of monoterpenes (65.7−89.1%, berries; 72.1−92.6%, leaves) with respect to alcohols (1.7−15.8%, berries; 1.8−20.6%, leaves), esters (1.6−19.1%, berries; 1.9−9.5%, leaves), and sesquiterpenes (0.0−1.6%, berries; 1.9−5.0%, leaves).

The differences among samples can be tentatively ascribed not only to the geographical origin (*27*, *28*) but also to the variety. For instance, essential oils from sample 3 (the only one

Table 6. Constituents (Area Percent ± SD) of the Leaf Essential Oil of *M. communis* L.

			sample[a]							
compound[b]	RI[c]	ID methods[d]	3	4	5	6	8	9	10	11
α-thujene	924	MS, RI, std	0.2 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.5 ± 0.0	1.1 ± 0.1
α-pinene	931	MS, RI, std	30.0 ± 0.6	32.3 ± 0.7	39.5 ± 1.8	31.8 ± 2.2	35.8 ± 0.9	59.5 ± 5.0	58.9 ± 2.1	50.0 ± 1.9
β-pinene	975	MS, RI, std	0.5 ± 0.0	0.2 ± 0.0	0.6 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.6 ± 0.0
myrcene	988	MS, RI, std	0.1 ± 0.1	nd	0.1 ± 0.1	nd	0.1 ± 0.0	0.1 ± 0.0	nd	nd
α-phellandrene	1007	MS, RI, std	nd	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	nd	nd
δ-3-carene	1009	MS, RI, std	nd	0.6 ± 0.0	0.8 ± 0.0	nd	0.3 ± 0.1	0.1 ± 0.0	0.6 ± 0.0	nd
α-terpinene	1016	MS, RI, std	nd	0.1 ± 0.0	0.1 ± 0.1	nd	nd	nd	nd	nd
p-cymene	1023	MS, RI, std	0.5 ± 0.1	1.1 ± 0.1	2.1 ± 0.2	0.4 ± 0.0	0.7 ± 0.1	0.2 ± 0.0	0.8 ± 0.0	0.3 ± 0.0
limonene	1028	MS, RI, std	7.3 ± 0.1	29.8 ± 1.2	5.2 ± 0.2	12.5 ± 0.5	8.4 ± 0.7	6.2 ± 0.7	5.4 ± 0.2	6.6 ± 0.2
1,8-cineole	1031	MS, RI, std	41.7 ± 0.8	15.9 ± 0.1	28.1 ± 1.1	25.8 ± 1.3	25.2 ± 1.2	20.9 ± 2.8	25.4 ± 1.5	30.4 ± 1.4
γ-terpinene	1056	MS, RI, std	0.2 ± 0.0	1.1 ± 0.1	1.0 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	0.2 ± 0.0	nd	nd
terpinolene	1083	MS, RI, std	nd	0.7 ± 0.0	0.7 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.1 ± 0.0	0.6 ± 0.0	0.3 ± 0.0
linalool	1101	MS, RI, std	1.5 ± 0.1	4.6 ± 0.2	1.5 ± 0.2	16.7 ± 2.1	7.9 ± 0.7	0.7 ± 0.6	0.2 ± 0.2	0.2 ± 0.1
terpinen-4-ol	1180	MS, RI, std	0.4 ± 0.0	0.4 ± 0.1	0.8 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	0.1 ± 0.0	nd	nd
α-terpineol	1190	MS, RI, std	4.8 ± 0.0	1.3 ± 0.3	3.2 ± 0.2	3.5 ± 0.2	3.5 ± 0.2	1.6 ± 0.3	1.6 ± 0.1	3.3 ± 0.0
linalyl acetate	1256	MS, RI, std	0.5 ± 0.0	0.5 ± 0.2	0.6 ± 0.1	0.2 ± 0.2	1.7 ± 0.5	0.9 ± 0.1	1.5 ± 0.3	1.8 ± 0.1
terpenyl acetate	1314	MS, RI, std	1.7 ± 0.0	0.1 ± 0.1	1.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.1	0.3 ± 0.1	nd	nd
neryl acetate	1366	MS, RI, std	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	nd	0.2 ± 0.0	0.1 ± 0.0	nd	nd
geranyl acetate	1380	MS, RI, std	7.2 ± 0.4	2.0 ± 0.1	6.3 ± 0.6	3.9 ± 0.2	4.2 ± 0.4	2.0 ± 0.3	0.4 ± 0.0	1.9 ± 0.0
methyleugenol	1398	MS, RI, std	1.5 ± 0.0	3.5 ± 0.3	2.4 ± 0.2	0.7 ± 0.2	1.8 ± 0.5	1.3 ± 0.2	1.0 ± 0.1	0.5 ± 0.1
β-caryophyllene	1403	MS, RI, std	nd	0.2 ± 0.0	nd	0.4 ± 0.0	0.4 ± 0.0	nd	nd	nd
α-humulene	1450	MS, RI, std	nd	nd	nd	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	nd	nd
allo-aromadendrene	1455	MS, RI, std	nd	nd	nd	nd	nd	nd	nd	nd
β-selinene[e]	1482	MS, RI	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	0.5 ± 0.1	0.3 ± 0.0	nd	nd
germacrene B[e]	1555	MS, RI	nd	nd	nd	nd	nd	nd	nd	nd
spathulenol[e]	1574	MS, RI	nd	nd	0.2 ± 0.0	nd	nd	nd	nd	nd
caryophyllene oxide	1580	MS, RI, std	nd	0.5 ± 0.0	0.4 ± 0.0	0.2 ± 0.1	0.5 ± 0.1	0.1 ± 0.2	nd	0.8 ± 0.1
monoterpenes			80	82.6	79.1	72.1	72.6	87.9	92.6	89
alcohols			6.7	6.3	5.5	20.6	11.8	2.4	1.8	3.5
esters			9.5	2.7	8.4	4.6	6.5	3.3	1.9	3.7
ethers			1.5	3.5	2.4	0.7	1.8	1.3	1.0	0.5
sesquiterpenes			0.2	0.9	1.1	0.8	1.6	0.6	0.0	0.8
oxides			0.0	0.5	0.4	0.2	0.5	0.1	0.0	0.8
identified compounds			97.8	95.9	96.4	98.7	94.2	95.4	97.3	97.8

[a] See **Table 1** for sample corresponding number. nd, not detected (<0.05%). [b] Compounds are listed in order of their elution from a DB-5MS column. [c] Retention indices as determined on a DB-5MS column using a homologous series of *n*-alkanes. [d] Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; std, by injection of an authentic sample. [e] Compound tentatively identified according to the mass spectrum (MS) and by comparison of RI with the literature (RI).

with white berries) show the highest amount of 1,8-cineole and total esters, especially geranyl acetate.

The results of the essential oil composition of leaves are in accordance with Tateo and Picci (*19*) and Pirisino et al. (*20*), suggesting that the Sardinian myrtle oils, even though from berries, belong to the α-pinene, 1,8-cineole, limonene chemotype and are characterized by the lack of myrtenyl acetate. This ester was found by various authors. Jamoussi et al. (*8*) reported the high content of α-pinene (52.3%), 1,8-cineole (19.0%), and limonene (9.1%) and the presence of myrtenyl acetate in low amount (0.2%) in Tunisian myrtle leaf essential oil, which was not in accordance with the data reported by Chalchat et al. (*9*), who analyzed myrtle leaf essential oil from seven Mediterranean regions and reported the strong chemical variability and the presence of myrtenyl acetate in some samples (Morocco, Yugoslavia, Spain, Albania) and the lack of it in some others (Tunisia, Lebanon, Corsica). Many other authors evidenced the presence of myrtenyl acetate in the essential oils from myrtle leaves and berries: Saviking-Fodulovic et al. (*10*) found myrtenyl acetate (13.4%) from Yugoslavian myrtle leaves; Jierkovic et al. (*11*) found myrtenyl acetate in Croatian myrtle leaf (13.5−30.7%) and berry (12.2−33.2%) essential oils. Similar results are reported by Özek et al. (*12*) in Turkish myrtle leaves essential oil, which was characterized by the presence of myrtenyl acetate ranging between 14.5 and 10.8%; Asllani et al. (*13*), in the myrtle leaf and berry essential oil from Albania, found myrtenyl acetate but in different concentrations (11.3 and 17.7%, respectively). Data from Boelens et al. (*14*, *15*) showed that myrtenyl acetate was the most important constituent occurring in high concentration (≈30%) in Spanish essential oil from leaves. Koukos et al. (*16*) reported that myrtle leaf essential oil from Greece was dominated by α-pinene (18,0%), limonene (21.8%), linalyl acetate (31.4%), and geranyl acetate (6.5%), but neither myrtenyl acetate nor 1,8-cineole were detected. In a paper reporting the chemical composition of myrtle leaf oil from Corsica, Bradesi et al. (*17*) reported that the essential oil was characterized by high contents of α-pinene and 1,8-cineole and by the lack of myrtenyl acetate. Recently, Flamini et al. (*18*) reported the chemical composition of the essential oils from leaves and berries of *M. communis* from Liguria (Italy). They found α-pinene, 1,8-cineole, and limonene as the major compounds, and noteworthy is that *trans*-myrtanol acetate was evidenced but not the corresponding saturated derivative myrtenyl acetate.

In conclusion, a strong chemical variability in myrtle leaf and berry essential oils depending on the origin of the samples was observed. The Sardinian myrtle oils were similar to the Corsican ones, even though with a higher content of limonene, and were characterized by the lack of myrtenyl acetate. The method used in this study to isolate volatile compounds from myrtle alcoholic extracts was simple and rapid. After 1 week of extraction, all terpenes showed the maximum concentration in the alcoholic extracts, whereas ethyl esters increased during the extraction process, as in grapes (*5*).

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