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ORIGINAL PAPER

Diversity in chemical compositions of essential oil of myrtle leaves from various natural habitats in south and southwest Iran

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Abstract Myrtle, *Myrtus communis* L. (Myrtaceae), an evergreen shrub also known as wild myrtle, has a history of use as a culinary and medicinal plant. To determine the diversity within the species, plant leaves of myrtle were collected in 12 natural habitats in Iran for investigation of chemical constituents in the essential oil. Extraction of the essential oils produced yields ranging from 0.7 to 1.5 mL per 100 g dry tissue. An analysis of the oils by GC and GC/MS revealed 40 compounds, constituting 90.1–99.9 % of the essential oils. Chemical constituents varied with the site of sample origin, although the principal essential oil components from all populations, were α-pinene (17.5–37.1 %), 1,8-cineole (9.9–29.8 %), linalool (7.0–23.1 %), and α-terpineol (5.3–8.3 %). Limonene (tr, 22.7 %) was a major constituent in three populations. Characterized chemotypes included Chemotype I: α-pinene/1,8-cineole/linalool, Chemotype II: α-pinene/linalool, Chemotype III: α-pinene/1,8-cineole, and Chemotype IV: α-pinene/1,8-cineole/limonene. The main source of variability in chemical composition and oil yield appeared to be differences in environmental conditions and chemotypes as plant populations collected from close geographical areas could be classified in a cluster.

Keywords Chemotypes · *Myrtus communis* · Limonene · Linalool · 1,8-Cineole · α-Pinene · Variation

Introduction

Myrtle (*Myrtus communis* L., Myrtaceae) is a wild evergreen shrub that grows primarily in Mediterranean climates. Although the plant is cultivated in Iran, the species, which is commonly known as "Mord or Mort," grows wild throughout the Zagros Mountainous Range (Ghasemi Pirbalouti 2009). Due to the high essential oil content in leaves, flowers, and fruit, myrtle is an important medicinal and aromatic plant. Leaves and berries are sources of essential oil with medicinal properties, including activity as an antimicrobial (Atzei 2003; Zanetti et al. 2010; Mahboubi and Ghazian Bidgoli 2010; Djenane et al. 2011; Messaoud et al. 2012; Bajalan and Ghasemi Pirbalouti 2014; Ghasemi Pirbalouti et al. 2014), antioxidant and antimutagenic (Hayder et al. 2008; Messaoud et al. 2012), astringent, antiseptic, anti-hyperglycemic (Elfellah et al. 1984; Djenane et al. 2011; Messaoud et al. 2012), analgesic (Levesque and Lafont 2000), and anti-inflammatory (Rossi et al. 2009). The plant is also used as insecticide (Tavassoli et al. 2011; Motazedian et al. 2012), and nematicide (Barbosa et al. 2010; Oka et al. 2012). Leaf decoctions of myrtle are frequently employed in Iranian folk medicine as a treatment for skin and digestive disorders, as an astringent, as a bronchodilator, and as a hair conditioner (Zargari 1982–1992; Ghasemi Pirbalouti 2009).

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The essential oil obtained from myrtle has been widely investigated and is known to vary in composition (Lawrence 1996), although 1,8-cineole is a major constituent (Bradesi et al. 1997). Depending on the amount of myrtenyl acetate, myrtle essential oils can be separated into two groups that can be further divided into two subgroups, depending on the relative ratios of α-pinene to myrtenyl acetate and α-pinene to 1,8-cineole (Flamini et al. 2004). A strong correlation exists between these groups and subgroups and the geographical origin of the plants (Bradesi et al. 1997).

Materials and methods

Plant material

Wild populations of myrtle (*Myrtus communis* L.) were used in this study. Leaves (0.5 kg of terminal leafy twigs) were collected during the early flowering stage (10 June–5 July 2012) from myrtle plants growing in 12 localities in five provinces (Fars, Kohgelouyeh va Boyerahmad, Chaharmahal va Bakhtiari, Lorestan and Khuzestan) located in south and southwest Iran (Table 1; Fig. 1). The collection areas were geographically separate and included areas in which physical differences in plant characteristics were observed. Each collected sample was labeled and the location was recorded using a global positioning system (GPS, Vista Garmin) receiver. Plant identity was confirmed by H.A. Shirmardi, Ph.D., and voucher specimens (No. 231) were placed in the Herbarium of Islamic Azad University, Shahrekord Branch, Iran.

Environmental

The physical and chemical characteristics of the soil, including pH, electrical conductivity (EC), organic carbon (%OC), and texture at the sample collection sites, were measured along with climatic conditions as recorded at the nearest meteorology station (Table 1).

Essential oil extraction

For essential oil extraction, collected fresh myrtle leaves were dried for 1 week at room temperature (water content was approximately 75 % of plant fresh weight). The dried leaves were ground to a fine powder using a Moulinex food processor, and 100 g powdered leaf tissue was distillated with 1 L of water for 3 h using a Clevenger-type apparatus, following the method recommended in the European Pharmacopoeia (Council of Europe 1997). The essential oil yields were expressed in mL/100 g dry weight of plant material.

Oil constituency

GC and GC/MS analysis were used to determine the composition of the essential oils, using an Agilent Technologies 7890 gas chromatograph equipped with flame ionization detector (FID) and a HP-5MS 5 % capillary

Table 1 Environment conditions of various habitats of myrtle in south and southwest Iran

Region	Province	Altitude (m)	Latitude	Longitude	P[a]	T[b]	RH[c]
Ghachsaran	Kohgiluyeh va Boyer-Ahmad	970	30°15′N	50°45′E	400	21.1	42
Dehdasht	Kohgiluyeh va Boyer-Ahmad	800	30°50′N	50°30′E	380	22.9	48
Simakan	Fars	1271	28°81′N	50°45′E	474	17.8	43
Cheshmeh-Ali	Fars	860	29°36′N	51°39′E	315	25.1	45
Seyedan	Fars	1620	29°50′N	52°40′E	400	22.1	44
Chavoni-Andimeshk	Khuzestan	356	32°40′N	48°10′E	400	24	47
Mongere-Andimeshk	Khuzestan	354	32°39′N	48°15′E	400	24	47
Pelazh-Sade Dez	Khuzestan	151	32°23′N	48°35′E	400	23.9	47
Sardasht-Dezful	Khuzestan	970	32°45′N	48°45′E	400	24	47
Dinarvand	Lorestan	1170	33°46′N	48°35′E	500	17.2	46
Tangehaft	Lorestan	1488	33°30′N	49°04′E	500	17.2	46
Madan	Chaharmahal va Bakhtiari	1225	31°41′N	50°52′E	550	15.8	40

Meteorological information was obtained from weather stations located within the study area and the surrounding zone; each value in the mean of 10–15 year data

[a] Average annual precipitation (mm)

[b] Average annual temperature (°C)

[c] Average annual relative humidity (%)

Fig. 1 Sampling locations of wild populations of myrtle in south and southwest Iran

column (30 m × 0.25 mm, 0.25 µm film thicknesses). Oven temperature was programed 60 °C for an initial 4 min, and then raised 4 °C/min to 260 °C. Injector and detector temperatures were set at 290 and 300 °C, respectively. Helium was used as the carrier gas at a flow rate of 2 mL/min, and 0.1 µL samples were injected manually in the split mode. Peak areas were used for quantifying the constituent percentage of total oil.

Constituent identification was confirmed by coupling the gas chromatograph to an Agilent 5975 C (Agilent Technologies, Palo Alto, CA, USA) mass selective detector. Operating parameters for the EI-MS were: ionization voltage, 70 eV; ion source temperature, 200 °C. Retention indices were calculated for all components using a homologous series of *n*-alkanes (C_5–C_{24}) injected in conditions equal to the oil samples. Identification of oil components was accomplished based on comparison of retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILLEY/ChemStation data system) (Adams 2007). The area percent was obtained electronically from the GC–FID response without the use of an internal standard or correction factors.

Statistical analysis

Data were statistically analyzed using a one-way ANOVA using the program SPSS (19.0). Means of the main constituents of the essential oils were compared and separated from each other using Duncan's multiple range test at $p \leq 0.05$ level. Analytical data for Hierarchical cluster analysis were treated by means of the SPSS statistical software.

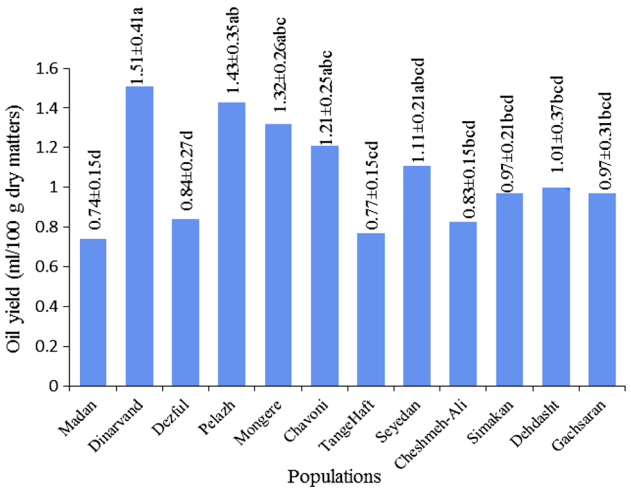

Fig. 2 Effect various populations on the oil yield of myrtle (In each graph, *similar letter* indicates a non significant effect at $p \leq 0.05$)

Table 2 Effect of various populations on chemical compositions of myrtle essential oil

Compound	RI[a]	%[c]					
		1[b]	2	3	4	5	6
Monoterpene hydrocarbons							
α-Thujene	929	0.90 ± 0.70[d]	0.0 ± 0.0	0.6 ± 0.0	0.0 ± 0.0	0.6 ± 0.6	0.5 ± 0.5
α-Pinene	939	20.8 ± 0.9bcd	25.9 ± 2.3abcd	24.6 ± 2.7bcd	18.0 ± 1.2cd	18.9 ± 5.1cd	28.6 ± 7.7abcd
Camphene	951	0.3 ± 0.7	0.1 ± 0.1	Tr	Tr	Tr	Tr
Verbenene	957	0.1 ± 0.1	Tr	0.1 ± 0.0	Tr	Tr	Tr
β-Pinene	978	0.6 ± 0.2	0.0 ± 0.0	0.6 ± 0.1	0.0 ± 0.0	0.5 ± 0.0	0.4 ± 0.1
β-Myrcene	991	0.4 ± 0.1	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0	0.4 ± 0.1	0.3 ± 0.1
α-Phellandrene	1006	0.2 ± 0.1	0.0 ± 0.0	0.4 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.1
γ-3-Carene	1008	0.4 ± 0.1	0.0 ± 0.0	0.9 ± 0.2	0.0 ± 0.0	0.5 ± 0.1	0.4 ± 0.0
α-Terpinene	1012	0.3 ± 0.2	0.0 ± 0.0	0.4 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.1
p-Cymene	1025	0.2 ± 0.3c	1.1 ± 0.9ab	1.4 ± 0.2a	0.0 ± 0.0c	0.8 ± 1.2abc	0.5 ± 0.5bc
Limonene	1031	0.0 ± 0.0c	0.0 ± 0.0c	1.8 ± 1.6c	0.0 ± 0.0c	0.0 ± 0.0c	15.1 ± 7.9ab
(*Z*)-β-Ocimene	1036	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.2 ± 0.0
(*E*)-β-Ocimene	1037	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.0	0.0 ± 0.0	0.3 ± 0.4	0.5 ± 0.2
γ-Terpinene	1056	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.1	0.0 ± 0.0	0.5 ± 0.1	0.7 ± 0.2
Terpinolene	1087	7.4 ± 0.8abc	8.0 ± 0.9ab	5.9 ± 0.1bc	8.3 ± 1.9a	6.2 ± 0.1abc	5.3 ± 0.5c
Total		31.6	36.1	39.1	26.3	29.2	52.9
Oxygenated monoterpenes							
α-Fenchene	950	0.1 ± 0.1	Tr	0.1 ± 0.1	Tr	0.1 ± 0.0	Tr
1,8-Cineole	1033	23.2 ± 8.3abc	25.3 ± 0.6abc	10.5 ± 8.4bc	22.5 ± 4.0abc	26.9 ± 8.7ab	9.9 ± 2.0c
Linalool oxide	1072	5.1 ± 0.6bc	7.4 ± 0.7a	7.1 ± 1.0b	7.1 ± 0.3a	7.8 ± 0.6b	7.4 ± 1.4b
Linalool	1088	15.0 ± .1bcd	16.2 ± 0.4b	15.3 ± 1.8bcd	23.1 ± 0.8a	15.5 ± 1.6bcd	15.6 ± 1.0bcd
Fenchol, exo-	1114	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
α-Campholene aldehyde	1125	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Sabinol	1136	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Nerol oxide	1152	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	Tr	0.0 ± 0.0
Pinocarvone	1160	0.0 ± 0.0c	0.0 ± 0.0c	0.1 ± 0.1c	1.4 ± 0.6a	0.1 ± 0.0	0.1 ± 0.1
Terpinene-4-ol	1174	0.8 ± 0.1	0.0 ± 0.0	0.7 ± 0.1	0.0 ± 0.0	0.8 ± 0.1	0.5 ± 0.1
Myrtenol	1193	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
(*Z*)-Carveol	1215	0.2 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.0 ± 0.0
Nerol	1224	0.4 ± 0.1	0.0 ± 0.0	0.6 ± 0.0	0.0 ± 0.0	0.4 ± 0.1	0.2 ± 0.2
Linalyl acetate	1252	6.1 ± 0.6bc	7.4 ± 0.7b	7.1 ± 1.0b	10.5 ± 0.3a	7.8 ± 0.6b	7.5 ± 1.4b
Thymol	1286	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.2
Carvacrol	1295	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.0
Neryl acetate	1344	0.7 ± 0.2	0.0 ± 0.0	0.9 ± 0.2	0.3 ± 0.6	0.5 ± 0.1	0.5 ± 0.1
Geranyl acetate	1380	2.2 ± 2.1bc	4.1 ± 0.3a	2.0 ± 0.6bc	4.5 ± 0.6a	0.8 ± 0.0c	1.1 ± 0.3bc
Total		54	53.4	44.7	69.4	61	43.5
Phenylpropanoids							
Methyl chaviol	1195	0.4 ± 0.2cd	0.6 ± 1.0bcd	1.2 ± 0.5abcd	0.3 ± 0.3d	0.3 ± 0.1d	1.4 ± 0.2abc
Methyl eugenol	1399	1.6 ± 0.3	0.0 ± 0.0	1.2 ± 0.3	0.6 ± 0.0	0.2 ± 0.0	1.4 ± 0.4
Total		2	0.6	2.4	0.9	0.5	2.8
Sesquiterpenes							
(*Z*)-Caryophyllene	1412	0.8 ± 0.1 cd	1.3 ± 1.1abc	2.2 ± 0.9a	2.0 ± 0.6ab	0.8 ± 0.0 cd	1.0 ± 0.4bcd
α-Humulene	1446	0.2 ± 0.2d	3.9 ± 0.1a	2.1 ± 0.8b	1.2 ± 1.0c	0.0 ± 0.0d	0.6 ± 0.4 cd
Caryophyllene oxide	1574	0.7 ± 0.2	0.0 ± 0.0	0.8 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.2
Total		1.7	5.2	5.1	3.2	0.8	2

Table 2 continued

Compound	RI[a]	%[c]						ANOVA
		7	8	9	10	11	12	
Monoterpene hydrocarbons								
α-Thujene	929	0.3 ± 0.1	0.7 ± 0.5	1.1 ± 1.3	0.9 ± 0.6	0.5 ± 0.6	2.1 ± 0.3	NS
α-Pinene	939	32.6 ± 3.6ab	29.7 ± 9.4abc	26.4 ± 3.5abcd	37.1 ± 5.2a	27.2 ± 5.2abcd	17.5 ± 3.5d	*
Camphene	951	0.2 ± 0.1	Tr	Tr	0.1 ± 0.0	0.1 ± 0.0	0.6 ± 0.1	NS
Verbenene	957	0.1 ± 0.0	Tr	Tr	Tr	Tr	0.4 ± 0.0	NS
β-Pinene	978	0.6 ± 0.1	0.5 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.2	**
β-Myrcene	991	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.6 ± 0.1	**
α-Phellandrene	1006	0.2 ± 0.1	Tr	0.0 ± 0.1	0.2 ± 0.1	0.3 ± 0.0	0.7 ± 0.2	**
γ-3-Carene	1008	0. 5 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.2	**
α-Terpinene	1012	0.2 ± 0.0	0.1 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.6 ± 0.1	NS
p-Cymene	1025	1.0 ± 0.3ab	0.5 ± 0.4bc	0.20 ± 0.3c	0.7 ± 0.1abc	0.4 ± 0.0bc	0.3 ± 0.0c	*
Limonene	1031	0.0 ± 0.0c	0.0 ± 0.0c	12.9 ± 8.6b	2.1 ± 3.6c	22.7 ± 1.6a	0.6 ± 0.0c	**
(*Z*)-β-Ocimene	1036	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.0 ± 0.0	**
(*E*)-β-Ocimene	1037	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	NS
γ-Terpinene	1056	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	NS
Terpinolene	1087	7.3 ± 0.6abc	7.1 ± 1.7abc	7.1 ± 1.1abc	7.5 ± 0.5abc	7.2 ± 2.0abc	7.5 ± 0.8abc	*
Total		43.9	40	49.7	51.7	61	32.9	
Oxygenated monoterpenes								
α-Fenchene	950	Tr	Tr	Tr	Tr	Tr	Tr	NS
1,8-Cineole	1033	29.2 ± 2.2a	29.8 ± 2.4a	23.0 ± 18.0abc	21.9 ± 11.7abcs	14.5 ± 4.7abc	19.9 ± 0.9abc	*
Linalool oxide	1072	2.3 ± 0.5bc	3.9 ± 1.9bc	4.2 ± 0.4bc	3.1 ± 4.1bc	3.2 ± 0.2bc	7.9 ± 1.0 b	*
Linalool	1088	7.0 ± 1.7f	10.7 ± 3.1ed	10.2 ± 2.4fe	10.6 ± 3.7ed	10.9 ± 1.5de	12.1 ± 1.2cde	**
Fenchol, exo-	1114	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	Tr	Tr	0.0 ± 0.0	NS
α-Campholene aldehyde	1125	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS
Sabinol	1136	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS
Nerol oxide	1152	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS
Pinocarvone	1160	1.1 ± 1.1b	0.6 ± 0.2bc	0.3 ± 0.1c	0.2 ± 0.2	0.0 ± 0.0c	0.0 ± 0.0c	**
Terpinene-4-ol	1174	0.5 ± 0.0	0.5 ± 0.2	0.5 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.9 ± 0.1	NS
Myrtenol	1193	Tr	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS
(*Z*)-Carveol	1215	0.4 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS
Nerol	1224	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.0 ± 0.0	0.5 ± 0.0	**
Linalyl acetate	1252	2.3 ± 0.4c	3.9 ± 1.9c	4.2 ± 0.4c	3.2 ± 4.1c	3.2 ± 0.3c	9.9 ± 1.0ab	**
Thymol	1286	0.0 ± 0.0	1.7 ± 2.2	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS
Carvacrol	1295	0.2 ± 0.0	2.2 ± 0.1	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	**
Neryl acetate	1344	0.4 ± 0.1	0.3 ± 0.2	0.4 ± 0.2	1.0 ± 1.0	0.5 ± 0.2	1.1 ± 0.1	N.S
Geranyl acetate	1380	2.2 ± 0.6bc	2.5 ± 1.2b	1.1 ± 0.5bc	1.3 ± 0.4bc	1.3 ± 0.9bc	0.7 ± 0.4c	**
Total		46.1	56.3	44.6	42.1	34.1	53	
Phenylpropanoids								
Methyl chaviol	1195	1.2 ± 0.1abcd	0.9 ± 0.4bcd	2.1 ± 0.9a	0.8 ± 0.1bcd	1.5 ± 0.6ab	0.8 ± 0.1bcd	**
Methyl eugenol	1399	1.2 ± 0.3	0.9 ± 0.3	2.1 ± 0.3	0.7 ± 0.3	1.4 ± 0.3	1.7 ± 0.4	**
Total		2.4	1.8	4.1	0.15	2.9	2.5	
Sesquiterpenes								
(*Z*)-Caryophyllene	1412	0.4 ± 0.2cd	0.4 ± 0.2cd	0.3 ± 0.2cd	0.4 ± 0.2cd	0.3 ± 0.3d	1.3 ± 0.4abc	**
α-Humulene	1446	0.3 ± 0.2d	0.3 ± 0.2d	0.3 ± 0.2d	0.1 ± 0.1d	0.2 ± 0.2d	0.0 ± 0.0d	**

Table 2 continued

Compound	RI[a]	%[c]						ANOVA
		7	8	9	10	11	12	
Caryophyllene oxide	1574	0.6 ± 0.1	0.3 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.1 ± 0.0	0.7 ± 0.0	NS
Total		1.3	1	1	0.8	0.6	2	

[a] *RI* retention indices determined on HP-5MS capillary column, *tr* trace (<0.01 %), *N.S* not significant

[b] *1* Gachsaran, *2* Dehdasth, *3* Simakan, *4* Cheshmeh-Ali, *5* Seyedan, *6* TangeHaft, *7* Chavoni, *8* Mongere, *9* Pelazh, *10* Dezful, *11* Dinarvand, *12* Madan

[c] Calculated from TIC data

[d] Values of major compounds are given as means ± SD, and means with different letter in a row are statistically significant at 5 % level probability

* $p \leq 0.05$; ** $p \leq 0.01$

Results

Essential oil yield

The essential oils extracted from the collected myrtle samples were a clear, light yellow to yellow color. Oil yields ranged from 0.7 to 1.5 mL/100 g of dry tissue and varied with the genotype (ecotype) and the environmental conditions at the collection site (Fig. 2). Oil content differed significantly ($p \leq 0.01$) by geographic location. The 12 sampled populations ranked highest to lowest in terms of oil yield were: Dinarvand > Pelazh > Mongere > Chavoni > Seyedan > Dehdasth > Gachsaran = Simakan > Dezful > Cheshmeh–Ali > TangeHaft > Madan (Fig. 2).

Essential oil composition

The GC and GC-MS analysis identified 40 constituents in the essential oils (Table 2) that constituted the bulk of the oils, ranging from 90.1 to 99.9 % of the total oil. A total of 14 oil constituents were present at >1.0 % and 7 compounds were present at >5.0 % in oil from one or more locations. Some 26 oil constituents were at levels <1.0 %. A total of seven major oil constituents were detected: α-pinene (25.81 ± 7.64 %), 1,8-cineole (20.98 ± 9.59 %), linalool (23.38 ± 4.37 %), α-terpineol (6.99 ± 1.26 %), limonene (4.73 ± 8.52 %), linalyl acetate (5.82 ± 2.85), and geranyl acetate (2.08 ± 1.49). Of the top 12 oil constituents in the myrtle samples, 10 oil constituents varied by population source (Table 2).

Monoterpenes were the main constituents of the essential oil from the leaves of collected plants with 33 derivatives identified, representing 83.8–95.7 % of all oil, three sesquiterpenes accounted for 0.6–5.2 % of the oil. Essential oil composition varied by origin of the samples as evidenced by the highest and lowest concentrations of oxygenated monoterpenes being obtained from the Cheshmeh-Ali population (69.4 %), and the Dinarvand population (34.1 %), while the highest content of monoterpene hydrocarbons was obtained from the Dinarvand population (61.0 %) and the lowest content of monoterpene hydrocarbons (26.3 %) was in the Cheshmeh-Ali population (Table 2).

Cluster analysis

Correlation and cluster analyses of compounds were computed to determine the relationship between chemical components in myrtle essential oil and the environmental conditions of sampled habitats. Compounds, such as α-pinene, 1,8-cineole, linalool, α-terpineol, limonene, linalyl acetate, linalool oxide, and geranyl acetate were present in oils of myrtle. The highest negative correlation ($p \leq 0.05$) was between relative humidity and linalyl acetate (−0.683), followed by precipitation and geranyl acetate (−0.658) (Table 3).

Hierarchical cluster analysis of all identified components grouped essential oil of the 12 populations into four distinct clusters (Fig. 3). The first cluster formed by oils of five populations (Gachsaran, Dehdasht, Cheshmeh-Ali, Madan and Seyedan) and contained α-pinene (17.5–25.7 %), 1,8-cineole (19.9–26.9 %), linalool (12.1–23.1 %), and limonene (0–0.6 %). This cluster had a higher linalool concentration as compared with other clusters (Chemotype I: α-pinene/1,8-cineole/linalool). The plant populations in the first cluster were collected from nearby geographical areas. For intra-population tests, genetic distances were estimated as described in the second cluster formed by oil of one population (Seyedan), which contained α-pinene (24.6 %), linalool (15.3 %), 1,8-cineole (10.59 %), and limonene (1.8 %) (Chemotype II: α-pinene/linalool). Isolation of this population was probably due to the low elevation in which the plant grows in comparison with the other geographic regions.

The third cluster formed by oils of three populations (Dezful, Mongere, and Chavoni) contained the highest α-pinene (29.7–37.1 %) and 1,8-cineole (21.9–29.8 %) concentrations in comparison with the other clusters

Table 3 Pearson's simple correlation between the main constituents in myrtle oil and environmental conditions

Characteristics	P	T	RH	H	α-Pinene	Limonene	Terpinolene	1,8-Cineol	Linalool oxide	Linalool	Linalyl acetate	Geranyl acetate
Precipitation (P)	1											
Temperature (T)	−0.935**	1										
Relative humidity (RH)	−0.461	0.584*	1									
Altitude (H)	0.469	−0.652*	−0.512	1								
α-Pinene	0.151	0.006	0.660*	−0.401	1							
Limonene	0.221	−0.117	0.311	0.139	0.211	1						
Terpinolene	−0.187	0.068	0.123	−0.033	−0.079	−0.337	1					
1,8-Cineole	−0.384	0.424	0.204	−0.266	0.047	−0.578*	0.529	1				
Linalool oxide	−0.118	0.005	−0.419	0.238	−0.724**	−0.24	−0.125	−0.308	1			
Linalool	−0.5	0.248	−0.192	0.144	−0.627*	−0.209	0.132	−0.229	0.727**	1		
Linalyl acetate	−0.003	−0.081	−0.683*	0.241	−0.717**	−0.144	−0.324	−0.321	0.627*	0.577*	1	
Geranyl acetate	−0.658*	0.48	0.297	−0.357	−0.121	−0.403	0.659*	0.286	0.117	0.559	−0.128	1

* Correlation is significant at the 0.05 level; ** correlation is significant at the 0.01 level

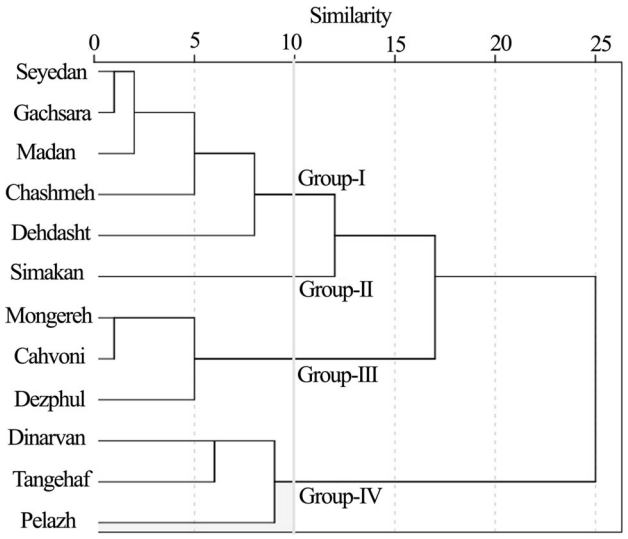

Fig. 3 Dendrogram obtained by hierarchical cluster analysis (HCA), based on the main constituents of the essential oil from various populations of myrtle

(Chemotype III: α-pinene/1,8-cineole). The populations of these plants were collected from nearby geographical areas and classified in a cluster. The fourth cluster was formed by essential oils of three populations (Dinarvand, Tangehaft, and Pelazh) and contained α-pinene (26.4–28.6 %), and 1,8-cineole (9.9–23.0 %) and limonene (12.9–22.7 %). This cluster had higher limonene in comparison with the other clusters (Chemotype IV: α-pinene/1,8-cineole/limonene).

Pearson's correlation indicated a negative correlation ($p \leq 0.05$) between annual precipitation and oxygenated monoterpenes (−0.596) (Table 4). To examine the relationships between the different populations of myrtle, PCA was applied to the volatile oil constituent data, and the first four eigenvalues corresponded to 33, 29, 15, and 9 % of the total variance (Table 5). The first principal component (PC_1) explained 33 % of the variation and had an eigenvalue of 3.96, which consisted of α-pinene and 1,8-cineole as major constituents (Table 5). PC_2 accounted for 29 % of the variation, with an eigenvalue of 3.54. PC_2 was bipolar with linalool, linalool oxide, linalyl acetate, and geranyl acetate. PC_3 accounted for 15 % of the variation, and had an eigenvalue of 1.78. Limonene, a major volatile oil constituent, was responsible for this bipolar vector. PC_4 explained 9 % of variation, mainly due to limonene, terpinolene, linalool, geranyl acetate.

Discussion

The chemical differentiation observed in the myrtle samples sourced in this study is in agreement with the inter-population chemical polymorphism observed by other

Table 4 Pearson's simple correlation between monoterpenes in myrtle oil and environmental conditions

Characteristics	P	T	RH	H	HM	OM
Precipitation (P)	1					
Temperature (T)	−0.935**	1				
Relative humidity (RH)	−0.461	0.584*	1			
Altitude (H)	0.469	−0.652*	−0.512	1		
Hydrocarbons monoterpenes (HM)	0.375	−0.206	0.509	−0.068	1	
Oxygenated monoterpene (OM)	−0.596*	0.458	−0.235	−0.101	−0.906**	1

** Correlation is significant at the 0.01 level; * correlation is significant at the 0.05 level

Table 5 Eigenvalues and loadings on principal component analysis of variation in volatile oil constituents in myrtle populations

	Principal axes			
	I	II	III	IV
Eigenvalue	3.96	3.54	1.79	1.1
Proportion (%)	0.33	0.29	0.15	0.09
Cumulative (%)	0.33	0.62	0.77	0.87
Loadings				
P	−0.29	−0.36	−0.24	0.06
T	0.34	0.26	0.31	−0.23
RH	0.42	−0.1	0.28	0.23
H	−0.35	−0.05	−0.21	0.33
α-Pinene	0.29	−0.37	0.07	0.03
Limonene	−0.06	−0.28	0.45	0.39
Terpinolene	0.2	0.17	−0.49	0.44
1,8-Cineole	0.3	0.11	−0.43	−0.35
Linalool oxide	−0.28	0.35	0.13	0.03
Linalool	−0.12	0.45	0.17	0.29
Linalyl acetate	−0.35	0.27	0.15	−0.27
Geranyl acetate	0.26	0.36	−0.1	0.38

researchers (Boelens and Jimenez 1991; Chalchat et al. 1998; Jerkovic et al. 2002; Messaoud et al. 2005; Gardeli et al. 2008; Mulas and Melis 2011). The plant populations having significantly higher percentages of the major components in the essential oil obtained from different populations of myrtle contained monoterpenes, oxygenated monoterpenes, hydrocarbons, phenylpropanoids, and sesquiterpenes. The essential oil of the Pelazh population had the highest concentration of phenylpropanoids [methyl eugenol (2.1 %) and methyl chaviol (2.1 %)]. In contrast, samples from Fars province, including the Dehdasth (5.2 %) and Simakan (5.1 %) populations, had the highest concentrations of sesquiterpenes.

Earlier reports (see Table 6) on the chemical constituents in various ecotypes, cultivars, and varieties from Iran, plus other countries were comparable with the chemical compounds in myrtle oil obtained from our populations. The recurring polymorphism observed within the genus produces a large number of subspecies, varieties, and plant forms in varying degrees of abundance that produce essential oils with different chemical composition. In most cases hydrocarbons monoterpenes (α-pinene) and oxygenated monoterpenes (linalool, 1,8-cineole, limonene, myrtenyl acetate, and linalyl acetate) and phenylpropanoid (methyl chavicol, methyl cinnamate, eugenol, and methyl eugenol) are the major components of the oils.

The secondary metabolite production is believed to be stimulated by stressful environmental conditions such as water deficiency (Sangwan et al. 2001). The strongest positive correlation ($p \leq 0.01$) was between the relative linalool ($C_{10}H_{18}O$) and linalool oxide ($C_{10}H_{18}O_2$) levels, compounds that have similar structure with only a difference is in the position of the oxygen group (O). In addition, the strongest positive correlations were between the relative linalool, linalool oxide, and linalyl acetate ($C_{12}H_{20}O_2$). Linalyl acetate is the acetate ester of linalool, and the two often occur in conjunction (Ghasemi Pirbalouti 2010). The highest negative correlations were between α-pinene and linalool, α-pinene and linalool oxide and α-pinene and linalyl acetate. Linalool can be synthesized by *a*-pinene through kinetic peculiarities of catalytic steps (Semikolenov et al. 2001). We found negative correlation between 1,8-cineol and limonene. 1,8-cineole and limonene are both correlated strongly with various formylated phloroglucinol compounds in eucalyptus (Moore et al. 2004).

The clusters of plants with similar essential oil profiles may be due to enzymatic reactions induced by shortage of water or other stress that leads to the conversion of oxygenated monoterpenes to monoterpene hydrocarbons. While little experimental data is available to support this notion, Hendawy and Khalid (2005) have reported variations in essential oil yield and composition due to environmental effects on enzyme activity and metabolic pathways. The highest negative correlation was between oxygenated monoterpenes and monoterpenes hydrocarbons, a relationship that could be explained by a change in the biosynthetic pathway for monoterpene hydrocarbons and oxygenated monoterpenes (Selmar and Kleinwächter 2013).

Table 6 The essential oil yield and chemical composition of myrtle ecotypes

Ecotype/cultivar	Essential oil yield	Chemical composition	References
Croatian myrtle	0.19–0.37 % (v/w)	Myrtenyl acetate (13.5–30.7 %), 1,8-cineole + limonene (12.6–29.8 %), linalool (10.8–18.3 %), and α-pinene (6.6–16.4 %)	Jerkovic et al. (2002)
Portuguese myrtle	0.33–0.74 % (v/w)	Limonene + 1,8-cineole (39.5 %), myrtenyl acetate (24.8 %), α-pinene (21.5 %), and linalool (6.2 %)	Pereira et al. (2009)
Cultivated at Botanical Garden, (Tehran) Iran	0.45 % (w/w)	α-Pinene (29.4 %), limonene (21.2 %), 1,8-cineole (18 %), linalool (10.6 %), linalyl acetate (4.6 %), and α-terpineole (3.1 %)	Rasooli et al. (2002)
Cultivated at Kashan, Central Iran	–	1,8-cineole (36.1 %), α-pinene (22.5 %), linalool (8.4 %), bornyl acetate (5.2 %), α-terpineol (4.4 %), linalyl acetate (4.2 %) and limonene (3.8 %	Mahboubi and Ghazian Bidgoli (2010)
Natural habitat in Southwest Iran (Dezful, Khuzestan province)	–	α-Pinene (29.1 %), limonene (21.5 %), 1,8-cineole (17.9 %), and linalool (10.4 %)	Yadegarnia et al. (2006)
Moroccan myrtle	0.3–0.4 % (v/w)	1,8-Cineole (43 %), myrtenyl acetate (25 %), and α-pinene (10 %),	Farah et al. (2006)
Tunisian myrtle	0.5 (v/w) based on fresh the plant	α-Pinene (19.20 %), 1,8-cineole (15.96 %), linalool (7.66 %), α-terpineol (7.51 %), and limonene (5.75 %).	Messaoud et al. (2005)
Sardinia, Italy	0.22–0.90 % (v/w)	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	Tuberoso et al. (2006)
Greek island, Greece	1.25–1.45 % (v/w)	Myrtenyl acetate (23.7–39.0 %), 1,8-cineole (12.7–19.6 %), α-pinene (10.1–11.6 %) and linalool (7.0–15.8 %)	Gardeli et al. (2008)
9 Cultivars, Sardinia, Italy	0.6–10.7 (g/kg)	α-Pinene (up −81.68 %), limonene (up −46.88 %), 1,8-cineole (up −22.18 %), and linalool (up −11.03 %)	Mulas and Melis (2011)
var. *Italica*	0.14–0.61 % (v/w)	α-Pinene (28.3–58.0 %), 1,8-cineole (12.7–30.7 %), linalool (2.4–21.5 %), and limonene (0.1–13.3 %)	Aidi Wannes et al. (2010)
Albanian myrtle	–	α-Pinene (11.4–22.5 %), 1,8-cineole (13.8–21.8 %), linalool (8.8–16.7 %) and myrtenyl acetate (11.3–17.7 %)	Asllani (2000)
Turkish myrtle	–	1,8-Cineole (18.3 % and 10.5 %), linalool (16.3 % and 18.6 %) and myrtenyl acetate (14.5 % and 10.8 %) in leaves, and branches + leaves, respectively.	Ozek et al. (2000)
Corsica (France)	–	α-Pinene (47.9–59.5 %) and 1,8-cineole (19.8–28.1 %)	Bradesi et al. (1997)
Caprione Promontory, Italy	–	α- Pinene (41.6 %), 1,8-cineole (25.5 %), limonene (9.5 %), and *trans*-myrtanol acetate (4.2 %)	Flamini et al. (2004)
Spanish myrtle	0.4–0.5 % (v/w)	α-Pinene (7–20 %), 1, 8-cineole (16.5–61.5 %), and myrtenyl acetate (0.1 to 36 %)	Boelens and Jimenez (1991)

Conclusion

Essential oil components of wild populations of myrtle varied by chemotype, environmental conditions, and geographic origin. Essential oils of myrtle leaves were characterized by high levels of oxygenated monoterpenes, hydrocarbon monoterpenes, phenylpropanoids, and sesquiterpenes. Monoterpenes were the main constituents of the essential oil of the leaves of the collected plants. The level of oxygenated monoterpenes, including 1,8-cineole, linalool, and α-terpineol, were higher than the monoterpene hydrocarbons, such as α-pinene and limonene. These monoterpenes are widespread components of the essential oils and used as fragrances and flavors in the cosmetic, perfume, drug and food industries.

Our results suggest that samples of myrtle used in this study can be assigned to chemotypes, α-pinene/linalool α-pinene/1,8-cineole/linalool, α-pinene/1,8-cineole, and α-pinene/1,8-cineole/limonene due to the relatively high grouping of these chemical constituents in the essential oil. The variation in oil composition and oil yield of myrtle can result from genetic diversity, differences in environmental conditions, and diversity/environment interactions. The main source of variability in chemical composition and oil yield of the studied populations seemed to be due to differences in environmental conditions and chemotypes. Plant populations collected from close geographical areas could be classified in a cluster.

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