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Essential oil variation among 21 wild myrtle (*Myrtus communis* L.) populations collected from different geographical regions in Iran

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ABSTRACT

Myrtle (*Myrtus communis* L.) is an industrial medicinal plant with various pharmaceutical and nutritional applications. The leaf essential oils in 21 wild populations of myrtle collected from natural habitats were investigated for their chemical components and oil yield diversity. The leaf essential oil yield ranged from 0.6 to 1.4 ml/100 g based on dry matter. GC–MS analyses revealed 38 compounds, constituting 94.1–98.3% of the essential oils. The main constituents were α-pinene, 1,8-cineole, limonene, linalool, α-terpineol, and linalyl acetate. According to the results, two chemotypes were determined for Iranian myrtle populations, including high α-pinene/1,8-cineole, and high limonene/low α-pinene groups. Among populations the Fars possessed the highest amount of α-pinene and 1,8-cineole. The highest correlation coefficient was between α-pinene and 1,8-cineole (+0.90), while the highest negative correlation was between 1,8-cineole and limonene (−0.90). The analyses indicated that populations collected from higher altitudes with loamy and clay soils, had higher amounts of α-pinene and 1,8-cineole, while the populations collected from sandy soils rich in organic matter possessed higher contents of limonene.

1. Introduction

Myrtle (*Myrtus communis* L.) belongs to the family of Myrtaceae, and is a diploid shrub typical representative of the Mediterranean flora. Myrtle is an evergreen shrub, which grows as wild in several regions all over the world (Aydın and Ozcan, 2007). It is traditionally used as an antiseptic, disinfectant drug and hypoglycaemic agent (Messaoud et al., 2011). Different parts of the plant have found various uses in the food industry, such as for flavoring meat and sauces, in the cosmetic and pharmaceutical industries (Aidi Wannes et al., 2010; Messaoud et al., 2011). Myrtle has been also used in folk medicine because of its astringent and balsamic properties (Flamini et al., 2004). The decocted leaves are traditionally used for sore washing, vaginal lavage and enemas and against respiratory diseases (Flamini et al., 2004). At the folk medicine, leaf and fruits decoction or infusion of this plant is used as stomachic, hypoglycemic, cough and oral diseases, antimicrobic, for constipation, appetizing, antihemorrhagic and externally for wound healing (Aydın and Ozcan, 2007). The oils extracted by steam distillation of fruits are used both in flavor and fragrance industries. Fruits have high amount of tannin. The fruits are very astringent and are used as a condiment as a substitute for pepper and considered a rich source of tanin (Aydın and Ozcan, 2007).

In Iran, the species commonly known as "Mord or Mort" is abundant in the Zagros Mountainous Range (Ghasemi Pirbalouti, 2009). Myrtle has also been widely employed in Iranian folk medicine where a decocted leaves are used for various purposes such as skin and digestive discords, astringent activity, hair conditioning and bronchodilator activity (Zargari, 1982–1992; Ghasemi Pirbalouti, 2009). Furthermore, essential oils obtained from the leaves are also mainly used for treatment of lung disorders and wound healing (Ghasemi et al., 2011). Leaves and berries are known as sources of essential oils with medicinal properties, including antimicrobial (Ghasemi Pirbalouti et al., 2010; Hayder et al., 2004; Yadegarinia et al., 2006), antioxidant activity (Chryssavgi et al., 2008; Montoro et al., 2006), and antimutagenic (Mimica-Dukić et al., 2010). Furthermore, the myrtle fruits also have high oil contents and are rich in polyunsaturated acid (Aidi Wannes et al., 2010; Messaoud and Boussaid, 2011).

Essential oil content and composition of plants may be highly affected by genetic and environmental factors (Rahimmalek et al., 2009). There are several reports regarding composition of myrtle essential oils in different countries, including Algeria (Bouzabata et al., 2010; Brada et al., 2012), Tunisia (Wannes et al., 2011; Snoussi et al., 2011), Albania (Asllani, 2000), Iran (Pezhmanmehr et al., 2010), Italy (Flamini et al., 2004); Turkey (Cakir, 2004), and

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Table 1
Collection site, geographical and soil characteristics of different myrtle accessions.

No.	Accession name	Collection site	Latitude	Longitude	Altitude (m)	Clay	Silt	Sand	Organic matter%	Acidity
1	Ch1	Anjoo, Charmahal and Bakhtiari, Iran	3474315 N	470942 E	1121	38	42	20	0.243	7.82
2	Ch2	Anjoo, Charmahal and Bakhtiari, Iran	3474315 N	470942 E	1121	38	42	20	0.243	7.82
3	Ch3	Anjoo, Charmahal and Bakhtiari, Iran	3474315 N	470942 E	1121	38	42	20	0.243	7.82
4	Ch4	Armand olia, Charmahal and Bakhtiari, Iran	3503544 N	478314 E	1091	28	30	42	2.379	7.45
5	Ch5	Armand olia, Charmahal and Bakhtiari, Iran	3503544 N	478314 E	1091	28	30	42	2.379	7.45
6	Ch6	Armand olia, Charmahal and Bakhtiari, Iran	3503544 N	478314 E	1091	28	30	42	2.379	7.45
7	Fa1	Noorabad mamasani, Fars, Iran	3323722 N	553816 E	1637	34	44	22	1.541	7.54
8	Fa2	Noorabad mamasani, Fars, Iran	3323722 N	553816 E	1637	34	44	22	1.541	7.54
9	Fa3	Noorabad mamasani, Fars, Iran	3323722 N	553816 E	1637	34	44	22	1.541	7.54
10	Fa4	Marvdasht, Fars, Iran	3325723 N	655652 E	1040	26	38	36	1.736	7.38
11	Fa5	Marvdasht, Fars, Iran	3325723 N	655652 E	1040	26	38	36	1.736	7.38
12	Fa6	Marvdasht, Fars, Iran	3325723 N	655652 E	1040	26	38	36	1.736	7.38
13	Lo1	Cham-murd, Lorestan	3700903 N	775975 E	924	24	16	60	0.117	7.78
14	Lo2	Cham-murd, Lorestan	3700903 N	775975 E	924	24	16	60	0.117	7.78
15	Lo3	Cham-murd, Lorestan	3700903 N	775975 E	924	24	16	60	0.117	7.78
16	Lo4	Gar-murd, Lorestan	373719 N	760925 E	1161	28	30	42	3.471	7.26
17	Lo5	Gar-murd, Lorestan	373719 N	760925 E	1161	28	30	42	3.471	7.26
18	Lo6	Gar-murd, Lorestan	373719 N	760925 E	1161	28	30	42	3.471	7.26
19	Ko1	Basht, Kohgiluyeh and Boyer-Ahmad	3361181 N	511174 E	865	24	46	30	1.775	7.41
20	Ko2	Basht, Kohgiluyeh and Boyer-Ahmad	3361181 N	511174 E	865	24	46	30	1.775	7.41
21	Ko3	Basht, Kohgiluyeh and Boyer-Ahmad	3361181 N	511174 E	865	24	46	30	1.775	7.41

Greece (Chryssavgi et al., 2008). High chemical variations have been observed in these researches.

The main constituents of the myrtle essential oil are 1,8-cineole and α-pinene (Flamini et al., 2004). In previous studies, the reported ratio of α-pinene to 1,8-cineole was significantly correlated to the geographical origins of collected plants (Bradesi et al., 1997). The composition of these compounds is highly affected by the site of collection. Therefore, studying the variability of natural populations in each country may have a great role in identification and introduction of new germplasm with high contents of essential oils and major compounds in order to be used in food and pharmaceutical industries. There are limited studies about variability of the essential oil composition in natural populations. For example, Messaoud et al. (2011) assessed the variability of essential oil constituents in Tunisian natural populations of *M. communis* originated from different climatic conditions. The chemical composition of myrtle oils in some populations from Italy was studied by Flamini et al. (2004). Bazzali (2012) compared the essential oil composition of some Mediterranean *M. communis*, populations including Corsica, Sardinia, Portugal, Morocco, Algeria, and Tunisia. Natural populations of myrtle have an extensive geographical distribution in Iran. To our knowledge, no documented reports on essential oil variation of Iranian *M. communis* are available.

The aims of this study were to (1) categorize the populations based on their essential oil yield and composition, (2) regroup the chemotypes of Iranian myrtle based on their leaf major compounds, and (3) assess the relationship between variations of essential oil yield and composition with some climatic factors and morphological characters in Iranian populations.

2. Materials and methods

2.1. Plant material and site description

The leaves of 21 myrtle accessions belonging to six myrtle populations were harvested at 50% flowering stage in June 2012 from natural growing plants in South and Southwest Iran. The collection sites characteristics are shown in Table 1. Plant identification was confirmed by H. Shirmardi, and voucher specimen (No. 231) has been placed in the Herbarium of I.A.U., Shahrekord Branch, Iran. Each sample was labeled and its location was recorded using a Global Positioning System (GPS, Vista Garmin) receiver. Environmental properties, soil physical and chemical characteristics of different locations, including pH, electrical conductivity (EC), and organic carbon (OC%) were determined and presented in Table 1. Some morphological characteristics were also measured in collection site in three replicates.

2.2. Essential oil isolation

Fresh leaves were dried for five days at room temperature. Dried leaves were ground, and 100 g of the powdered tissue was distillated with 1 l of water for 4 h using a Clevenger-type apparatus. Then, the essential oil was collected in a container. The essential oil content was determined on the basis of dry matter and measurements were done in triplicates.

2.3. Gas chromatography–mass spectrometry (GC–MS)

The essential oils were analyzed using an Agilent 7890, a gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), with a HP-5MS 5% phenylmethylsiloxane capillary column (30.00 m × 0.25 mm, 0.25 μm film thickness). Oven temperature was kept at 60 °C for 4 min initially, and then raised at the rate of 4 °C/min to 260 °C. Injector and detector temperatures were set at 290 °C 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. Peaks area percents were used for obtaining quantitative data. The gas chromatograph was coupled to an Agilent 5975C (Agilent Technologies, Palo Alto, CA, USA) mass selective detector. The EI-MS operating parameters were as follows: ionization voltage, 70 eV; ion source temperature, 200 °C.

2.4. Identification of component

Compounds were identified by comparing of their RI (retention indices) relative to C5–C24 *n*-alkanes obtained on a nonpolar DB-5MS column, with those provided in the literature (Adams, 1995). The individual components were identified by retention indices and compared with compounds known from the literature. The yield of each component was calculated per kg of the plant material, while its percentage of composition was calculated summation of the peak areas of the total oil composition.