Industrial Crops and Products 61 (2014) 303–307

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Variation in antibacterial activity and chemical compositions of essential oil from different populations of myrtle

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ARTICLE INFO

Article history:
Received 12 April 2014
Received in revised form 6 July 2014
Accepted 9 July 2014
Available online 2 August 2014

Keywords:
Myrtus communis L.
Diversity
Biological activity
α-Pinene

ABSTRACT

Myrtle (*Myrtus communis* L.) belonging to the family Myrtaceae, is an evergreen shrub that grows mainly in Mediterranean climates. In Iran, the plant grows in the Zagros Mountainous Range of the country. The essential oils from the leaves and fruits of the plant are widely used to enhance the flavor of foods, and cosmetic and pharmaceutical industries. In this study, essential oil was extracted from the leaves of *M. communis* collected from six natural habitats in three provinces (Ilam, Lorestan, and Kermanshah), Western Iran. The hydrodistillated essential oil was analyzed by GC/MS. Results indicated that there were significant differences ($p \leq 0.05$) among the various populations for the main constituents in the essential oils. The major components of the essential oils from different populations of *M. communis* were α-pinene (24.42–31.57%), limonene (trace to 23.55%), 1,8-cineole (5.92–21.21%), and linalool (8.72–11.56%). Results of the antibacterial activity *in vitro* indicated that the essential oils from *M. communis* have good inhibitory activities against bacteria, especially *Staphylococcus aureus* (PTCC 1112). Results obtained in this study revealed that there is a high potential of the essential oil composition variability among the populations of myrtle. The results can be used in selection programs for production of aromatic myrtle with suppressing effects on pathogens.

1. Introduction

The development of drug resistance as well as the appearance of side effects of certain antibiotics has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures that overcome the above disadvantages (Lewis and Ausubel, 2006). Thus, the food industry at present uses chemical preservatives to prevent the growth of food-borne and spoiling microbes and to extend the life of foods. Mainly due to undesirable effects such as toxicity and carcinogenicity of synthetic additives, interest has considerably increased for finding naturally occurring antimicrobial compounds suitable for use in food (Feng and Zheng, 2007; Haddouchi et al., 2013). Herbs, spices, and essential oils are also well known for their various beneficial effects on human health. The use of herbs in phytotherapy is mostly due to the essential oils and their various biological activities, such as spasmolytic, carminative, hepatoprotective, antiviral, and anti-carcinogenic properties (Bakkali et al., 2008; Mimica-Dukíc et al., 2010; Ghasemi Pirbalouti et al., 2014).

Myrtus communis L. ("Myrtle" in English and "Mord" in Persian), belongs to the family Myrtaceae, is an evergreen shrub that grows mainly in Mediterranean climates. (Gardeli et al., 2008; Ghasemi Pirbalouti et al., 2010). The steam or hydro-distillation are good and commonly used methods for extraction of essential oil of myrtle from the leaves, branches, fruits, and flowers (Ghasemi Pirbalouti et al., 2010). Earlier studies have been identified the chemical compositions of the essential oil from different ecotypes (Bradesi et al., 1997; Koukos et al., 2001; Flamini et al., 2004; Rahimmalek et al., 2013) that the main components of myrtle essential oils, included 1,8-cineole, α-pinene, limonene, and linalool. The leaves of *M. communis* are traditionally used as an antiseptic, disinfectant drug and hypoglycaemic agent (Elfellah et al., 1984). Different parts of the plant find various uses in the food industry, such as for flavoring meat and sauces, and in the cosmetic industry (Gardeli et al., 2008). Leaves and berries are sources of essential oil that have medicinal properties, including antimicrobial and antioxidant activities (Ghasemi Pirbalouti et al., 2010; Hayder et al., 2004; Yadegarinia et al., 2006). In Iran, myrtle grows wild in different bioclimatic zones extending from the upper semi-arid to the lower humid. Populations of *M. communis* grow at altitudes ranging from 900 to 1700 m, under a rainfall ranging from 400 to 600 mm/year (Ghasemi Pirbalouti, 2010).

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http://dx.doi.org/10.1016/j.indcrop.2014.07.023
0926-6690/

Table 1
Geographical properties of natural habitats of *M. communis* L. in Western Iran.

No.	Accession name	Collection site	Latitude (UTM)	Longitude (UTM)	Altitude (m a.s.l)
1	Ha	Hamzeh, Lorestan Province, Iran	3,716,721	250,198	1269
2	Di	Dinarvand, Lorestan Province, Iran	3,698,096	253,851	761
3	MK	Mareh khell, Kermanshah Province, Iran	3,879,030	601,413	1216
4	Gh	Ghelane Gharb, Kermanshah Province, Iran	3,776,583	585,867	833
5	Ch	Chavar, Ilam Province, Iran	3,729,724	618,499	976
6	Ma	Maymeh, Ilam Province, Iran	3678008	677917	1154

To our knowledge, no documented reports on variation of chemical composition and antibacterial activity of the essential oils from different populations of *M. communis* in western provinces of Iran are available. The aims of this study were (i) to determine the variation of chemical constitutes of essential oils from *M. communis* leaves collected from natural habitats in Western Iran, and (ii) to evaluate diversity in the antibacterial activity of the essential oils of wild populations of myrtle against two Gram-positive and two Gram-negative bacteria.

2. Materials and methods

2.1. Plant material and site description

The leaves of myrtle belonging to six myrtle populations were harvested from natural habitats in the Zagros regions, western Iran in September 2013. Each sample was labeled and the location was recorded using a Global Positioning System (GPS, Vista Garmin) receiver (Table 1).

2.2. Essential oil extraction

Fresh leaves were dried for 10 days at room temperature (25 °C). Dried leaves were ground using food processor, and 40 g of the powdered tissue was distillated with 1 L of water for 3 h using a Clevenger-type apparatus. The collected essential oil was dried over anhydrous sodium sulphate and stored at 4 °C until analyzed. The yield based on dry weight of the sample was calculated.

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

GC analysis was done on an Agilent Technologies 6890 gas chromatograph equipped with a HP-5MS 5% capillary column (30.00 m × 0.25 mm, 0.25 μm film thicknesses). The oven temperature was maintained at 50 °C for 3 min then programmed to 290 °C at 15 °C/min then remind for 6 min. Helium (99.99%) was used as carrier gas at a flow rate of 1.5 ml/min, and 0.1 μL samples were injected manually in the split mode (50:1). Operating parameters for the EI–MS were: ionization voltage, 70 eV; interface temperature, 280 °C; detector voltage, 1.66 kV; mass range, 30–450 u; scan speed, 2.86 scans/s; interval, 0.01 min (20 Hz).

2.4. Identification of components

Constituents were identified by comparison of their KI (Kovats index) relative to *n*-alkanes (C_5–C_{24}) obtained on a nonpolar HP-5 MS column by comparison of the KI, provided in the literature, by comparison of the mass spectra with those recorded by the NIST 08 (National Institute of Standards and Technology) and Willey (ChemStation Data System). 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 (Adams, 2007).

2.5. Antibacterial test

Isolates of bacteria strains obtained from Veterinary Medicine Faculty, Tabriz University, Iran. The antibacterial activity of the essential oils against *Staphylococcus aureus* (PTCC 1112), *Bacillus subtilis* (PTCC 1254), *Klebsiella pneumonia* (PTCC 1053), and *Escherichia coli* (PTCC 1270) were determined with the disk diffusion method (NCCLS, 2002). Briefly, bacterial suspensions were adjusted to 1.0×10^7 CFU/ml, and spread in TSA or PCA using sterile cotton swabs. Subsequently, filter paper discs (6 mm Ø; Whatman #1) were placed on the surface of Petri dishes and impregnated with 20 μl of essential oil. Positive controls were prepared with oxytetracycline and solphamecine, but negative control was prepared only with DMSO. After incubation at 37 °C for 24 h, antibacterial activity was evaluated by measuring the radius of the inhibition zones to the nearest millimeter (Teixeira et al., 2013). Experiments were performed in triplicate at three different times.

2.6. Statistical analysis

Data were analyzed with three replications using the SAS ver. 9.1 statistical software. The significance of differences among means was tested using Duncan's multiple range test at $p \leq 0.05$ level. Calculation of correlation among the compounds and antibacterial activity was performed using Minitab ver. 16.

3. Results and discussion

3.1. Chemical composition of essential oil

Essential oils extracted from the populations of *M. communis* were analyzed by GC/MS. Generally, 27 constituents in total oil were identified from the essential oil of *M. communis*, which represented 90.59–96.91% of the oil (Table 2). According to the result of this study, monoterpene hydrocarbons, alcohols, and oxides were the most important of chemical groups in *M. communis* essential oil.

The main constituents of myrtle essential oils were α-pinene (24.42–31.57%), limonene (trace to 23.41%), 1,8-cineole (5.92–21.21%), linalool (8.72–11.56%), α-terpineol (7.04–8.12%), linalyl acetate (trace to 7.12%), and geranyl acetate (2.33–5.12%). Furthermore, α-pinene was the most abundant constituent in all samples. Limonene and 1,8-cineole were the second and third major compounds, respectively (Table 2). Similarly, the essential oils of nine populations from *M. communis* leaves collected from Alghero region (Northwestern Sardinia, Italy) were analyzed to evaluate variation in oil yield and the chemical composition (Mulas and Melis, 2011). They reported that the main components of the essential oils were α-pinene, limonene, 1,8-cineole, linalool, and geranyl-acetate. In other study by Flamini et al. (2004), the main constituents of the essential oil from myrtle collected from Italy were 1,8-cineole and α-pinene. In addition, Messaoud et al. (2011) reported that the main constituents of the essential oil from myrtle leaves collected from natural populations in Tunisia were α-pinene, camphene, and 1,8-cineole. Yadegarinia et al. (2006) reported that α-pinene (29.1%), limonene (21.5%),