Industrial Crops & Products 107 (2017) 305–311

Contents lists available at ScienceDirect

Industrial Crops & Products

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

# Antioxidant and antibacterial activities of the essential oils obtained from seven Iranian populations of *Rosmarinus officinalis*

Iman Bajalan[a,*], Razieh Rouzbahani[a], Abdollah Ghasemi Pirbalouti[b,c], Filippo Maggi[d]

[a] Young Researchers and Elite Club, Borujerd Branch, Islamic Azad University, Borujerd, Iran
[b] Medicinal Plants Department, Shahrekord Branch, Islamic Azad University, PO Box 166, Shahrekord, Iran
[c] Medicinal Plants Program, College of Natural Sciences, Massachusetts University, Amherst 01003, MA, USA
[d] School of Pharmacy, University of Camerino, Camerino, Italy

ARTICLE INFO

*Keywords:*
Rosemary
Iranian accessions
Essential oil
Antibacterial
Antioxidant

ABSTRACT

Rosemary (*Rosmarinus officinalis*) is an aromatic shrub belonging to the Lamiaceae family and widely used as herbal remedy and spices. In this work, the leaf essential oil from seven populations of rosemary growing in Western Iran was obtained by hydrodistillation and analyzed by GC-FID and GC–MS. The major chemical groups occurring in the essential oils were monoterpene ketones (14.55–37.31%), hydrocarbons (28.36–36.81%) and oxygen-containing compounds (9.05–27.60%). The major constituents of the essential oils were 1,8-cineole (5.63–26.89%), camphor (1.66–24.82%) and α-pinene (14.69–20.81%). The essential oils were screened for antibacterial and antioxidant activities. The antibacterial activity of the essential oils was evaluated on two gram-positive and two gram-negative strains, namely *Streptococcus agalactiae*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* by the agar disc diffusion method. Results of antibacterial test indicated that the largest inhibition (inhibition zone of 18.51 mm) was achieved with essential oil from Ma population against *E. coli*. This sample showed high level of 1,8-cineole. Antioxidant activity of the essential oils was evaluated using the DPPH radical inhibition assay (at 0.4, 0.8, 1.6 and 3.2 mg/ml). Overall, the essential oils from the seven populations, at 3.2 mg/ml, exhibited high antioxidant activity (more than 50% radical inhibition). Statistical analysis revealed a significant correlation between some essential oil components and biological activity displayed.

## 1. Introduction

Secondary metabolites occurring in aromatic and medicinal plants have been recognized for their antifungal, antibacterial and antioxidant properties (Giordani et al., 2008; Adrar et al., 2016; Bajalan et al., 2017). They are widely used in medicine and in food preservation (Bendif et al., 2017).

With the increase of bacterial resistance to antibiotics, there is remarkable interest in evaluating the antimicrobial effects of plant secondary metabolites against a range of pathogens, in order to discover new classes of safe and effective antimicrobials useful for infection control and/or food preservation. Therefore, the identification and evaluation of natural products for the control of food pathogens is of pivotal importance to assure consumers a safe, wholesome, and nutritious food supply (Bajpai et al., 2009).

Antioxidants have also great importance because they can reduce oxidative stress causing damage to biological molecules. Antioxidants play a key role in the treatment of many illnesses related to degenerative disorders, cardiovascular and brain diseases, diabetes, cancer, arthritis, and immune system decline, by acting as free radical scavengers, and reducing the extent of oxidative damage (Kivrak et al., 2009; Valko et al., 2007).

Rosemary (*Rosmarinus officinalis* L., Lamiaceae) is an aromatic plant native to the Mediterranean area and used worldwide for its antimicrobial and antioxidant activities. This plant has also many other beneficial effects given by its anti-inflammatory activity (Rahbardar et al., 2017). *R. officinalis* is considered to be one of the most important sources of both volatile and non-volatile bioactive compounds (Ojeda-Sana et al., 2013). To our knowledge, no reports on the variation of essential oil composition and biological activity of *R. officinalis* from western provinces of Iran are available. Therefore, the present study aimed to screen the antibacterial properties of seven Iranian rosemary populations against four worldwide food-borne spoilage and pathogenic bacteria, and to find correlations with their chemical compositions. In addition, the antioxidant activity of essential oils obtained from the same populations, in terms of inhibition on the DPPH radical,

* Corresponding author.
*E-mail addresses:* bajalan_iman@yahoo.com, ibajalan@yahoo.com (I. Bajalan).

http://dx.doi.org/10.1016/j.indcrop.2017.05.063
Received 22 February 2017; Received in revised form 28 May 2017; Accepted 31 May 2017
Available online 08 June 2017

**Table 1**
Collection sites of different *Rosmarinus officinalis* accessions.

| No. | A. n. [a] | Collection site | Longitude | Latitude | Altitude (m a.s.l) |
|---|---|---|---|---|---|
| 1 | Do | Dorud, Lorestan Province, Iran | 48° 5 ' 23.59879 | −56° 44 ' 33.59709 | 1455 |
| 2 | Kh | Khoramabad, Lorestan Province, Iran | 47° 5 ' 49.77636 | −56° 42 ' 59.77208 | 1368 |
| 3 | Bo | Borujerd, Lorestan Province, Iran | 47° 36 ' 18.0734 | −56° 18 ' 49.25095 | 1581 |
| 4 | Ma | Malayer, Hamedan Province, Iran | 47° 49 ' 44.7595 | −55° 57 ' 47.71415 | 1840 |
| 5 | Jo | Jowkar, Hamedan Province, Iran | 47° 36 ' 13.4772 | −55° 47 ' 45.34017 | 1705 |
| 6 | Bi | Bisotun, Kermanshah Province, Iran | 54° 34 ' 59.1054 | −55° 49 ' 2.46673 | 1312 |
| 7 | Ke | Kermanshah, Kermanshah Province, Iran | 54° 5 ' 32.54122 | −55° 50 ' 27.50578 | 1343 |

[a] Association name.

was investigated as well.

## 2. Materials and methods

### 2.1. Plant material and site description

The leaves of different rosemary accessions were collected in the Zagros regions, western Iran, in April 2015 (Table 1). Three leaf samples were collected from ten shrubs representative of each accession and then they were mixed each other. For each population, a voucher specimen number has been deposited in the Herbarium of Borujerd University, Iran. Once collected, the leaves were air dried in the laboratory (25 °C) for ten days protected from light (Bajalan and Pirbalouti, 2015).

### 2.2. Essential oil isolation

The dried leaves of rosemary (100 g) were subjected to hydrodistillation for 3 h, using a modified Clevenger-type apparatus. The oil was dried over anhydrous $Na_2SO_4$ and preserved at 4 °C prior to further analysis (up to one month). Essential oil yields, expressed as ml/100 g (n = 3), were calculated on dry basis.

### 2.3. GC-FID and GC–MS analysis

Composition of the essential oils was determined by GC-FID and GC–MS analyses. They were achieved on an Agilent Technologies 7890 GC equipped with FID and mass spectrometer detectors using a HP-5MS (5% phenylmethylpolysiloxane) capillary column (30.00 m × 0.25 mm, 0.25 μm film thicknesses; J & W Scientific, Folsom, CA). The carrier gas was helium at a flow rate of 0.8 ml/min. Initial column temperature was 60 °C and programmed to increase up to 280 °C at 4 °C/min. The split ratio was 40:1. The injector temperature was set at 300 °C. The acquisition range was 50–550 *m/z* in electron-impact (EI) mode using an ionization voltage of 70 eV. The essential oils were diluted 1:100 in *n*-hexane, then 0.1 μl were injected into the GC systems. The constituents were identified by comparison of their retention indices and mass spectra with those recorded in the NIST 08 (NIST 08, 2008), ADAMS (Adams, 2007) and Willey MS libraries. The percentage composition (average of three independent analyses) was computed from the GC peak areas without using any correction factors.

### 2.4. Antioxidant activity

The evaluation of the free radical-scavenging activity of oils was based on the measurement of the reducing ability of antioxidants toward the DPPH radical. The method described by Ali et al. (2015) with some corrections was used. From each sample, different concentrations of essential oils were prepared in methanol: 0.4, 0.8, 1.6, and 3.2 mg/ml. Twenty μl of different oils (at different concentrations) were added to 980 μl of the methanolic DPPH solution (90 μM). After a 60 min-incubation at room temperature and under darkness, the absorbance was measured at 517 nm against a blank sample as the negative control (methanol solution) using a UV–vis spectrophotometer. The scavenging of DPPH radical was calculated according to the following equation: $I\% = [(AB–AA)/AB] \times 100$, where I is the DPPH· inhibition, %; AB and AA are the absorbance values of the control and of the test sample, respectively. Thee food preservative butylhydroxyanisole (BHA), from Sigma Inc. (St. Louis, MO, USA), was used as positive control.

### 2.5. Antibacterial assay

Bacteria strains including *Streptococcus agalactiae* (ATCC 12386), *Staphylococcus aureus* (ATCC 33591), *Escherichia coli* (ATCC 8739) and *Klebsiella pneumoniae* (ATCC 700603) were obtained from Food Microbiology Laboratory, Veterinary Medicine Faculty, Borujerd University (Iran). Bacteria inhibition growth was determined by the agar disc diffusion method. Briefly, bacterial suspensions were adjusted to $1 \times 10^7$ CFU/ml and spread in tryptic soy agar (TSA) or plate count agar (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 (20 μl) and negative controls were prepared with DMSO. After keeping at 4 °C for 2 h, all Petri dishes were incubated at 37 °C for 24 h. Antibacterial activity was evaluated according to the zone of inhibition by measuring the radius of the inhibition zones to the nearest millimeter (Bajalan and Pirbalouti, 2014). Experiments were repeated three times.

### 2.6. Statistical analysis

Data were statistically analyzed using SPSS ver. 21 statistical software. Each treatment was carried out with three replicates. To compare means of each treatment, Duncan's multiple range test was used. Differences were considered to be significant at $p \leq 0.05$ level.

## 3. Results and discussion

### 3.1. Essential oil composition

The chemical compositions and yields of essential oils from leaves of different populations of rosemary are reported in Table 2. Essential oil yields of rosemary accessions in this study varied from 0.6 in Jo population to 2.35 ml/100 g in Kh population. Regarding the essential oil compositions, the monoterpene ketones and hydrocarbons were dominant in all Iranian rosemary samples. However, their distribution varied between populations. Ketones ranged from 14.55% in Ma to 37.31% in Bo population. As illustrated in Table 2, Ma population showed the lowest levels of ketones (14.55%) whereas Bo population the highest amount (37.31%). Camphor was the main component in this group and ranged from 1.66 to 24.82%. The Kh population was rich in monoterpene hydrocarbons (36.81%). The latter ranged from 28.36% in Ma to 36.81% in Kh population, with α-pinene as the most abundant component (14.69% in Do to 20.81% in Kh). The amounts of monoterpene oxides ranged from 7.28% in Bo to 27.60% in Ma population, with 1,8-cineole as the most representative compound, ranging from 5.63% in Bo to 26.89% in Ma population.

As shown in previous studies, the rosemary essential oils are rich in α-pinene, 1,8-cineole and camphor, associated with variable amounts of other compounds such as borneol and verbenone (Ojeda-Sana et al., 2013). Our results were in agreement with these studies (Porte et al.,