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Comparison chemical composition and antioxidant properties of *Rosmarinus officinalis* L. essential oil cultivated in the farm (Green space) in four different regions of Iran

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Abstract

Essential oils and extracts of medicinal plants with antioxidant compounds due to the presence of free radicals eliminating factors are very significant as new and natural pharmaceutical compounds both in the area of health, disease treatment and protecting raw and processed foods. The aim of this study was to determine the chemical composition of the leaves of the rosemary *Rosmarinus officinalis* L. and compare the antioxidant properties in the cities of Mashhad, Birjand, Bojnoord and Bardaskan that this Comparison was done for the first time. Rosemary leaves were collected and then extraction was performed by Clevenger apparatus. Composition of the oils using coupled gas chromatography with mass spectrometry (GC/MS) identified and compared that some of the major essential oil samples included: Bornyl acetate, 1, 8 cineole, camphene and Alpha phenyl acetate which 12 components were common among the compounds of essential oils. In this study, three different concentrations (0.1, 0.2 and 0.3 mg per ml) of rosemary were prepared and antioxidant activity each of dilutions using DP PHP (DPPH) was determined. Results showed that inhibition percent of essential oil with increasing the concentration increased.

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Introduction

Rosemary with scientific name of *Rosmarinus officinalis* L. is from Labiatae family. Rosemary is always a green shrub with sturdy and thin leaves which grow blue multi- part flowers from the upper side of it. This plant from great distances because of its perfume can be detected. It grows in the Mediterranean region and if not exposed to wind and other factors in the rest of Europe can be planted in gardens as an ornamental plant. This plant was used for perfume and medicinal properties in the past. Leaves and even its essential oil are used in many combinations of anti rheumatic drugs because there is too much power in irritation of the skin (alcohol, bath soap, camphor). Brewed leaves, especially during menopause calm nerves with booster effect. This herb is also a zero diuretic and laxative and improves digestive activity and its use for pregnant women is toxic (Akhondzade *et al.,* 2000; Zaouali *et al.,* 2010).
In laboratory experiments, the analgesic, antioxidant, antimicrobial and anti-inflammatory effects have been proved (Najid, 1992; Chevallier, 2007) In the essential oils of the plant, there are materials such as borneol, limonene, camphene, Camphor and other plant compounds such as phenolic acids, including rosemaric acid, caffeic acid and chlorogenic acid (Chevallier, 2007; Wagnera and Ulrich- Merzenich, 2009).

Antioxidants are compounds that reduce the harmful effect of free radicals in biological and food systems in effective and different ways and cause detoxification (Shrififar, 2007). Natural antioxidants are usually herbal phenolic compounds obtained from various plant sources. Phenolic compounds are usually multifunctional and can be used as reducing agents (free radicals), chelating of metals and suppressive function of singlet oxygen (Zhang *et al,* 2006). Researches have shown that rich foods composed of phenolic compounds with a series of physiological characteristics such as antioxidant, antimicrobial, (Fatemi, 2003) anti-mutagenic, (Esterbauer *et al.,* 1993) inhibiting oxidation of lipoproteins and accumulation of plaques, (Markesbery and Lovell, 1993) anti-inflammatory activity, (Jadhav, 1995) and so on.

Studies showed rosemary essential oil with the use of flavoring used in food and also due to having antimicrobial and antioxidant properties widely known as a medicinal plant (Wang, 2008).

Also, various studies on the microbial properties of different rosemary species and its constituents have been done, including the kestin and colleagues in the Turkey, which have investigated the antimicrobial properties of Gilhanas well as Rosemary. Another study was related to Pintotr and his colleagues who investigated antimicrobial properties of rosemary grown in the two different regions of Italy (Keskin *et al.,* 2008; Pintore *et al.,* 2007).

Ahmadi *et al* study showed that rosemary essential oil can be used instead of chemical drugs to treat infections. This means that high dilutions of rosemary oil had inhibition and lethality effect on the growth of Enterococcus faecalis, Staphylococcus epidermidis and Escherichia coli, indicating the strong bactericidal effect on these bacteria (Ahmadiasbchin *et al.,* 2016).

Several methods to evaluate the antioxidant activity of natural ingredients that most of these complement each other (Pereira, 2007). Among these methods, the power of radicals 2, 2-diphenyl- 1-pykrylhydrazyl (DPPH)) can be mentioned, (Wang, 1999) which is used in this study.

Materials and methods

Chemical materials

All chemicals and solvents used high purity; BHT, sodium sulfates anhydride, chloroform, sulfuric acid, ethyl acetate, butyl toluene from Merck (Germany) 2, 2-diphenyl 1- pykryl hydrazyl (DPPH) from Sigma-Aldrich was prepared.

Collection, extraction and analysis of oil

Studies have shown that the leaves before flowering and in the warm season had the most essential oil (Hasanzadeh *et al.,* 2014) so the sampling time has been done in June. Since, the time of harvest is effective on the amount and quality of essential oil, all samples at 11 am in June 2015 before flowering have

been collected from Mashhad, Bojnourd, Birjand and Bardaskan and after storing and sending to a cool environment (refrigerator) were extracted within 24 hours.

The essential oils obtained by gas chromatography device connected to mass spectrometry (GC/MS) were analyzed. The chromatography device was 8690Agilent type used a column with the length of 30 mm and diameter of 0.25 mm and layer thickness 0.25 µm of the HP-5MS type. Column temperature program have been adjusted in this way: the initial temperature of the oven is 60 ° C and for 5 minutes at this temperature have been maintained. Temperature gradient is 5 ° C per minute, increasing the temperature to 220 ° C and up to 5 minute stopped at this temperature, and gas helium as the carrier gas with flow rate of 1 ml/min is used.

Mass spectrometer with the model of Agilent 5973 with Voltage 70 eV was ionization energy. Then, all the chemical composition of rosemary leaves essential oil from four different locations (Mashhad, Bojnourd, Birjand, Bardaskan) with relative percentages in the Table (1) is visible.

Determine the strength of essential oil to trap free radicals DPPH

To determine the strength of essential oil to trap free radicals DPPH, sample S to the amount of 0.1, 0.2 and 0.3 mg /ml used and added to 1 ml of 0.2 solution free radical DPPH in chloroform. Solution absorbance after 50 minutes at 517 nm wavelength is read with a spectrophotometer Table (2). A sample of chloroform and 1 ml of DPPH solution is used as a control group. Butyl hydroxy toluene is used as a positive control in this experiment. Eliminating activity of DPPH radical by essential oils which is a measure of radical antioxidant activity is calculated according to the following formula:

100 × control absorption rate (control absorption rate - sample absorption rate) =% of trapping.

Results and discussion

Essential oils composition

With careful study of compounds retention times, Kvansretention indices and mass spectra was found that rosemary essential oils in terms of values and even in some other material were similar (Table1).

Table 1. All the chemical composition of rosemary leaves essential oil from four different locations (Mashhad, Bojnourd, Birjand, Bardaskan) with relative percentages.

Peak number	Combining name	% Area (Mashhad)	% Area (Bojnourd)	%Area (Birjand)	%Area (Bardaskan)
1	Gama-Thujene	%2.12	%4.18	%1.02	—
2	Alpha-Pinene	%5.04	%3.95	%2.65	%5.37
3	Camphene	%11.23	%12.47	%11.12	%11.46
4	Sabinene	%0.85	—	—	—
5	Beta-Pinene	%3.31	%4.97	%3.14	%3.75
6	myrcene	%0.41	—	—	—
7	1,8-Cineole	%20.67	%21.93	%11.42	%8.41
8	Camphor	%5.57	%4.76	%6.15	%7.43
9	Isoborneol	%3.14	%0.73	%2.49	%1.85
10	Terpinen-4-ol	%2.38	%0.57	%2.76	%4/47
11	alpha-Fenchyl acetate	%7.98	%5.44	%4.35	%2.17
12	Bornyl acetate	%23.30	%22.25	%23.75	%18.25
13	Alpha-Copaene	%0.19	—	—	%0.45
14	Caryophyllene	%5.16	%2.48	%1.57	%0.45
15	Alpha-Humulene	%0.12	%0.65	—	%0.18
16	Gama-Muurolene	%0.21	%0.71	%0.09	—
17	Germacrene B	%1.44	%0.95	—	—
18	Gama-Cadinene	%0.45	%0.12	%0.26	%0.12
19	Caryophyllene oxide	%1.18	%2.45%	%5.24	%12.24
20	Globulol	%0.14	%0.38	—	—

21	alpha-Fenchene	—	%0.65	%0.98	—
22	Delta3-Carene	—	%0.27	%1.18	%2.18
23	eucaliptol	—	%0.65	—	—
24	trans-beta-Ocimene	—	%0.26	%0.19	—
25	Benzene	—	%1.05	%1.74	%0.12
26	linalool	—	%1.47	%0.21	%1.46
27	Terpinolene	—	%0.94	%0.45	%1.75
28	verbenone	—	%0.69	—	—
29	Carvacrol	—	%0.85	%1.74	%0.97
30	Alloaromadendrene	—	%0.54	—	—
31	Para-Cymene	—	—	%4.94	%2.17
32	Limonene	—	—	%2.06	%3.46
33	Borneol	—	—	%0.49	%1.02
34	p-Cymen-3-ol	—	—	%3.05	%2.47
35	Thymol	—	—	%2.48	%1.75

The number of compounds in rosemary leave essential oil in the cities of Mashhad, Bojnourd, Birjand and Bardaskan were obtained 20, 27, 27 and 24 respectively.12 composition was common between Rosemary components with the highest percentage related to Bornyl acetate obtained from rosemary essential oil of Birjand (23.75%), Mashhad (23.30%), Bojnoord (22.25%) and Bardaskan (18.25 %), followed by the highest combination of (20.67%) 1,8-Cineole of Mashhad rosemary leave essential oil and the lowest percentage of components related to the Gama-Cadinene of leaves rosemary essential oil and Bardaskan 0.12.

Table 2. Absorbance read at 517 nm.

Concentration	Essential oil of rosemary from Mashhad	Essential oil of rosemary from Bojnourd	Essential oil of rosemary from Birjand	Essential oil of rosemary from Bardaskan	BHT
0.1 mg/ml	25/00	22/63	80/29	33/55	94/68
0.2 mg/ml	37/85	34/64	87/91	48/43	94/74
0.3 mg/ml	45/55	40/85	90/69	54/84	94/80

Evaluation of antioxidant activity

According to the anti-oxidant results, the lowest, percentage of essential oil inhibition at a concentration of 0.1 mg /ml Bojnoord and the highest were Birjand**.** Also the lowest percentage of inhibition at a concentration of 0.2 mg /ml was Bojnoord, while the highest was Birjand. The lowest percentage of inhibition at a concentration of 0.3 mg/ml was Bojnoord, while the highest was Birjand (Fig. 1)**.**

The results of the chemical composition of rosemary essential oil in the current study partially was consistent with other studies conducted in this regard. Malakoutian and colleagues, (Malakotian and Hatami, 2013) in their study identified 20 compounds, which 82.9 percent were 1, 8 Verbenone -limonene, Camphene, Linalool, Camphor, α-pinen, Borneo, Cineole as the most essential components of the study and in the current study, 1, 8, Camphene, Camphor, α-pinen, Cineole were major components of the essential oil. Also, Miladi *et al.* (2013) compared the chemical composition of rosemary and thyme plants as well as their antioxidant activity in France. In this study, the method to identify compounds was MS-GC and most important combination of rosemary was 1, 8 cineole (24.1%), which was much closer to our results Miladi *et al.* 2013.

This study can be an introduction to practical application of green cumin essential oil due to the chemical composition and proper antioxidant capacity.

Thus, it is possible to use a readily available and affordable source. Also it is preventing from product waste and damage caused by it and finally, the steps taken to promote health and food safety.

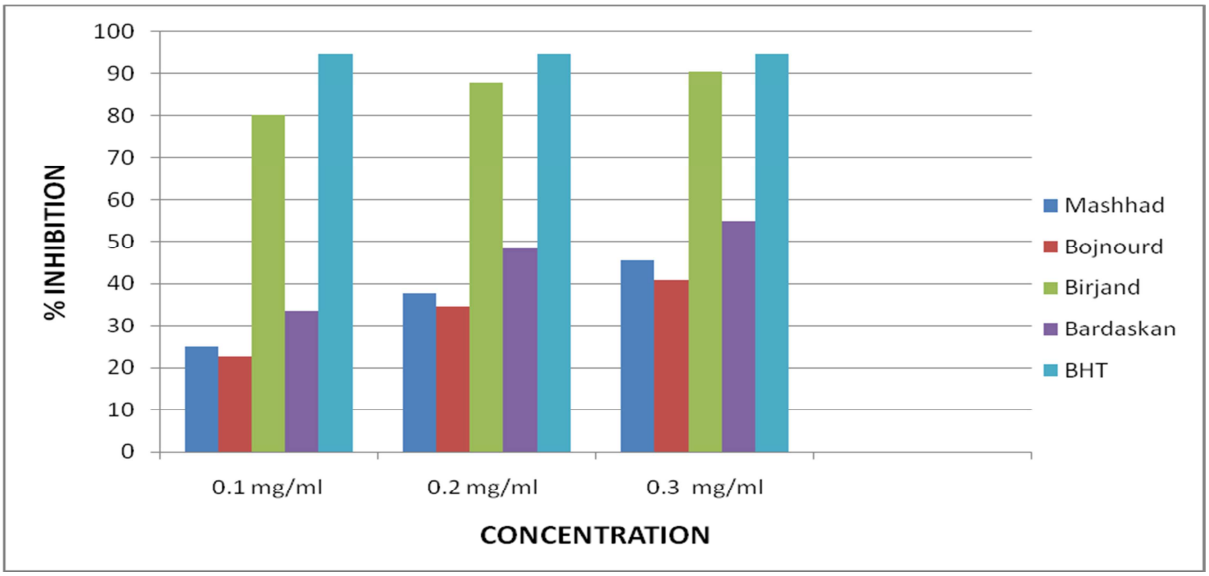

Fig. 1. Comparison of inhibition percentage of rosemary leave essential oils in Mashhad, Bojnourd, Birjand, Bardaskan and BHT in three different concentrations.

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