REPORT

Regional variation in the chemical composition and antioxidant activity of *Rosmarinus officinalis* L. from China and the Mediterranean region

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Abstract: Rosemary is an aromatic evergreen shrubby herb that is native to the Mediterranean region. This herb is now widely cultivated in many regions of the world. Rosemary is widely used in traditional Chinese medicines, foods, nutraceuticals and cosmetics. Hydro distilled essential oils, obtained from rosemary in China and the Mediterranean region, were analysed by gas chromatography-mass spectrometry (GC–MS). Thirty-seven compounds accounting for 94.97%-99.72% of the oils were identified. The majority of the compounds in the essential oils exhibited no significant differences (table 1 and fig. 1). The extracts were prepared with three solvents of different polarity (dichloromethane, ethyl acetate and aqueous). The ethyl acetate fractions exhibited the highest phenol content and were found to be significantly more active than the dichloromethane and aqueous fractions (fig. 2). Antioxidant activity (by DPPH radical scavenging, ferric ion reduction (FRAP) and thiobarbituric acid reactive substance (TBARS)) was also assessed. The ethyl acetate extracts of Yunnan had the highest amount of antioxidant capacity from China by DPPH and TBARS, with the lowest IC_{50} values being 0.0011 mg/ml, and 1.6611 mg/ml, respectively. In conclusion, the antioxidant activities of the essential oils and ethyl acetate extracts from rosemary obtained by three different testing methods revealed higher antioxidant activity from rosemary grown in China than in the Mediterranean region. These results suggested that Chinese rosemary should be widely used in food, traditional medicine, cosmetics and perfume products, as well as other chemical industries.

Keywords: *Rosmarinus officinal* L., essential oil, antioxidant activity, DPPH, TBARS.

INTRODUCTION

Rosemary *(Rosmarinus officinalis* L.) is an aromatic evergreen shrubby herb that is native to the Mediterranean region. It is a well-known and greatly valued medicinal herb that is widely cultivated in many regions of the world (Sui *et al*., 2012), such as China, India Uttaranchal, Algerian Sahara, North America, Northern Europe and England (Liu *et al*., 2011). According to the Compendium of Materia Medica, rosemary was widely used in a sachet carried on clothes and as an aromatherapy incensed in the room, and with further important roles in refreshing oneself, the removal of niff and killing insects. Although rosemary is broadly planted in Yunnan, Guizhou, Zhejiang, Sichuan province and Shanghai in China at present, many Chinese still buy rosemary from the Mediterranean region as a flavouring agent, preservative, medicine and cosmetic (Miroddia *et al*., 2014). This finding indicates a perception among the Chinese that rosemary from the Mediterranean region possesses good qualities. Recent research has not demonstrated a quality variation to date in rosemary grown in China and the Mediterranean region. Therefore, it is highly significant to evaluate the quality of rosemary originating from different regions.

Currently, over 80% of the developing world population still relies on rosemary for primary healthcare. For example, rosemary can be utilized as a treatment for skin infections (Miroddia *et al*., 2014), inflammation disorders, cardiovascular diseases, diabetes, cancer (Chun *et al*., 2014, Johnson, 2011), gynecopathy (Ulbricht *et al*., 2010), anti-aging diseases (Suggs *et al*., 2014) and as a remedy for mental fatigue and depression (Fernández *et al*., 2014). As is well-known, antioxidants have a degree of preventive and therapeutic effects on these disorders. These compounds can be used to help the human body in reducing oxidative damage caused by free radicals (Ouariachi *et al*., 2014). The essential oil and phenolic constituents of rosemary may contribute to its biological activity (Aruoma *et al*., 1992, Sui *et al*., 2012). The essential oil is extensively used in cosmetic and perfume products, as well as in food because of its unique aroma and antioxidant activity (Liu *et al*., 2011). Rosemary

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leaves have a high content of essential oil containing α-pinene, camphene, 1,8-cineole and camphor (Celiktas *et al*., 2007, Da *et al*., 2015, Liu *et al*., 2011). In addition, the polar extracts of compounds referenced in the literature exhibited a high phenol content and strong antioxidant capacity (Formisano *et al*., 2014).

The objective of this research was to study the variety of rosemary collected from China and the Mediterranean and to compare the differences in chemical composition and antioxidant activities from three regions.

MATERIALS AND METHODS

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Xiya Reagent Company (Chengdu, China). Butylated hydroxytoluene (BHT) and ascorbic acid (VC) were purchased from Kaixin Chemical Company (Tianjin, China). Ferrous sulphate heptahydrate and potassium ferricyanide were purchased from Guangfu Technology Development Co., Ltd (Tianjin, China). Trichloroacetic acid (TCA) was purchased from Kaitong Chemical Company (Tianjin, China). 2-Thiobarbituric acid (TBA) was purchased from Guangnuo Technology Development Co., Ltd (Shanghai, China). Dibasic potassium phosphate, sodium hydroxide, Folin-Ciocalteu reagent, sodium nitrite, aluminium chloride, gallic acid, anhydrous sodium sulphate, anhydrous sodium carbonate and ferric chloride were purchased from Damao Chemical Company (Tianjin, China). The solvent used for preparing standard solutions was methanol supplied by Tianhuang Chemical Company (Xuzhou, China).

Plant materials

The plant material rosemary was collected from the Zhejiang Hisun Pharmaceutical Co., Ltd (Hangzhou, China) plantation of Yunnan, Guizhou in China, and other plant material was commercially available from the Islamic Republic of Iran (Iran) at the flowering period. Professor Changcai Bai at the Pharmacognosy Department, College of Pharmacy, Ningxia Medical University identified this plant material.

Essential oils isolation

The essential oil (Eo) was obtained from 50 g of rosemary leaves and 500 mL water placed in a Clevenger type apparatus for hydrodistillation over 4 hours. The obtained essential oil was separated and dried over anhydrous sodium sulphate. All essential oils were kept in a sealed dark vial at 4°C until further analysis.

Extraction procedure

The residual liquid of the essential oils isolation system was successively partitioned with dichloromethane (CH_2Cl_2) and ethyl acetate (EtOAc) fractions, as well as an aqueous (H_2O) residue. Each fraction was collected, joined and concentrated under vacuum at 55°C using a vacuum rotary evaporator and stored at 4°C until used for further analyses.

Essential oils analysis

The main constituents were analysed by GC/MS. The analytical GC was carried out on a Shimadzu QP 2010 plus gas chromatograph with a Rxi-5Sil MS (length =30 m, i.d.=0.25mm, thickness =0.25μm) fused-silica capillary column with a helium flow of 1.0mL/min. The injector and detector temperatures were maintained at 250°C. The oven temperature was programmed to increase from 60°C to 240°C at a rate of 5°C/min and finally held for 5 min. The mass spectra were taken at 70 eV from 35 to 500 Da. The compounds of essential oil were identified by comparison of their retention indices relative to a homologous series of n-alkanes and by comparison of their mass spectra with those recorded in the database from the National Institute of Standards and Technology (NIST); additional RI values for comparison were obtained from the literature (Da *et al*., 2015, Mukazayire *et al*., 2011, Napoli *et al*., 2010, Sadgrove *et al*., 2014, Tian *et al*., 2014). Further comparisons were made via the co-injection of available reference compounds. The samples were analysed in triplicate.

Determination of total phenol content

The concentration of total phenolics was measured through the method described by (Dashipour *et al*., 2015), with several modification. The mixture was stirred thoroughly and kept at ambient temperature for 2 h prior to an abso rbance reading at 765 nm against water on a UV-Visible spectrophotometer (Shimadzu UV-VIS 2550, Japan). Total phenolic content (TPC) was expressed as mg gallic acid equivalents (GAE)/L of sample solution.

Antioxidant activity determination

1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Assay

The free-radical scavenging activity of the various solvent each fractions of rosemary and Eos were determined using DPPH (Sun *et al*., 2015, Brand, 1995). Next, 1mL of each sample was added to 2mL of a 6×10^{-5} M solution of DPPH in methanol. The reaction mixtures were well-shaken and later kept at room temperature in a dark room for 30 min. The decrease in absorbance at 517 nm was determined using a UV-Visible spectrophotometer. The methanol with respective samples served as blank. Absorbance of the radical without antioxidant (control) was measured daily. The IC_{50} value was calculated as the concentration of sample required to scavenge 50% of DPPH free radicals. Inhibition of the DPPH free radical was calculated as percent inhibition using the equation below:

% Inhibition of DPPH radical = $100\ (A_0 - A_1)/A_0$

where A_1 refers to the absorbance of the control and A_0 is the absorbance of the sample. Ascorbic acid (VC; in the

same concentration) and butylated hydroxytoluene (BHT; in the same concentration) were used as references. The assay was carried out in triplicate.

Ferric reducing antioxidant power assay

The ferric reducing power (FRAP) of the samples was determined using the potassium ferricyanide-ferric chloride method (Benzie and Strain, 1996, Tian *et al*., 2014) with slight modifications. Different methanol concentrations of the samples (1 mL) were each added to 2mL 1% potassium ferricyanide ($K_3Fe(CN)_6$) and 2mL 0.2M phosphate buffer (pH 6.6). The mixture was heated in a water bath at 50 °C for 20 min. Afterward, 2mL 10% trichloroacetic acid (TCA) was added to the reaction mixture, which was later centrifuged at 3000 rpm for 10 min. Finally, the upper layer of the solution (2 mL) was mixed with 0.5mL 1% ferric chloride ($FeCl_3$) and 2 mL distilled water. The absorbance was measured at 700 nm using a UV-Visible spectrophotometer after 30 min for all samples. The sample blank was prepared under the same conditions. Increased absorbance indicated increased reducing power. BHT and VC were used as reference compounds, and all assays were carried out in triplicate.

Thiobarbituric acid reactive species assay

The methods of Daker *et al*. and Viuda-Martors *et al*. (Daker *et al*., 2007, Viuda *et al*., 2010) were modified to determine the thiobarbituric acid reactive substance (TBARS), a secondary product of lipid per oxidation. Specifically, different dilutions of samples (1mL) were added to a mixture that contained 1mL fowl egg yolk, 1 mL 0.1M phosphate buffer (pH 7.4) and 1mL 1 mM Fe^{2+} ($FeSO_4$). The mixture was heated in water bath at 37°C for 1h, after which it was added to 1mL of 1% thiobarbituric acid (TBA) and 0.5mL freshly prepared 15% trichloroacetic acid (TCA). The mixture was kept in a boiling water bath for 10 min followed by centrifugation at 3000 rpm for 10 min in order to remove precipitated protein. Finally, the supernatant absorbance was measured at 532 nm. The blank consisted of buffered egg yolk with Fe^{2+} only. BHT and VC were used as reference. The percentage inhibition ratio was calculated using the following equation:

% Inhibition of TBARS ability =100 $(A_0 - A_1)/A_0$

where A_0 was the absorbance of the blank sample and A_1 was the absorbance of the sample. The EC_{50} value is the inhibitory concentration at which inhibition was 50%, and the value was obtained by plotting against sample concentration. Tests were carried out in triplicate.

STATISTICAL ANALYSIS

Data were analysed by using SPSS (version 19.0) statistical software. Statistical analysis was carried out using one-way ANOVA. Tukey's multiple range test was used to compare the significance of differences between the test samples at P values of <0.05. All of the experiments were performed in triplicate and were repeated at least twice. The data were expressed as the mean ± SD (standard deviation) for three replicates.

RESULTS

Essential oils composition

In this study, the oil from rosemary aerial parts was obtained from Yunnan, Guizhou and Iran. The essential oil was obtained in the same yield of 1.80% from the rosemary of different origins. GC–MS analysis of the rosemary essential oil identified 37 compounds that accounted for 94.97%-99.72% of the oils (table 1). fig. 1 shows a comparison between the percentages of some ample components in the oils obtained from the three areas. The major compounds were 1,8-cineole (42.86-46.76%), camphor (16.26-23.42%), α-pinene (6.37-9.19%), camphene (2.27-4.37%), borneol (4.00-4.33%), linalol (2.62-3.56%), α-terpine (2.78-3.41%), decane (2.24-3.12%), limonene (2.23-2.44%) and ρ-cymene (1.69-1.72%). These results are in accord with earlier studies on rosemary found to be rich in 1,8-cineol and camphor (Liu *et al*., 2011, Ma *et al*., 2013, Sui *et al*., 2012, Wang *et al*., 2008). The main compound content of 1,8-cineol was not significantly different from three areas.

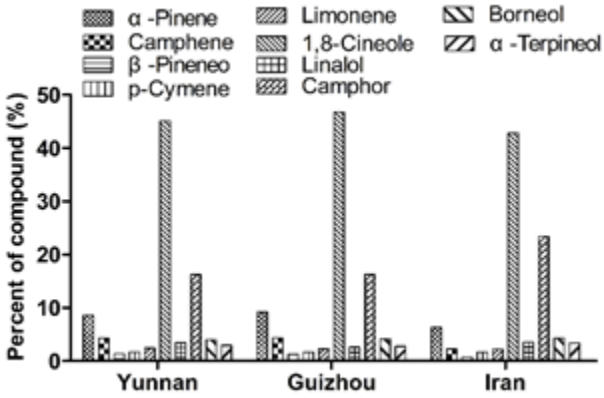


Fig. 1: Comparison of major components of rosemary oils from three sites in China and Iran.

Total phenol content

The yields of each fraction of rosemary are presented in Table 2. On the one hand, fig. 2 details the each fraction of rosemary total phenols content (TPC). The aqueous fraction contains the highest yields (13.67–16.49%) but contains the lowest TPC values of Yunnan (19.53%), Guizhou (25.12%) and Iran (16.28%). On the other hand, the ethyl acetate fraction had the lowest yields (0.81–1.17%). As we can clearly see, however, the ethyl acetate fraction from Yunnan shows the highest TPC (68.80%) followed by the Guizhou (34.70%) and Iran (20.13%). Finally, the dichloromethane fraction yields (0.24–0.46%) show that the Yunnan, Guizhou and Iran include the lowest TPC (14.70%, 12.90% and 2.13%, respectively). These results showed that the ethyl acetate fraction of rosemary shows the lower yields, but this fraction is rich in TPC.

Table 1: Chemical compounds of essential oils obtained from aerial parts of rosemary (%) from Yunnan, Guizhou and Iran.

No	Component	RI_{lit}	RI_{cal}	RT	Yunnan Area (%)	Guizhou Area (%)	Iran Area (%)
1	Nonane	900	899	4.953	0.35	0.37	0.47
2	α-Pinene	934	934	5.960	8.62	9.19	6.37
3	Camphene	951	952	6.472	4.32	4.37	2.27
4	β-Pinene	980	980	7.264	1.40	1.28	0.72
5	Myrcene	990	990	7.537	0.93	0.97	0.80
6	Decane	1000	1000	7.831	3.12	2.24	2.45
7	3-Carene	1008	1008	8.062	--	0.11	--
8	α-Terpinene	1016	1018	8.369	0.47	0.46	0.29
9	p-Cymene	1027	1026	8.591	1.72	1.69	1.69
10	Limonene	1031	1031	8.735	2.44	2.31	2.23
11	1,8-Cineole	1034	1034	8.849	45.09	46.76	42.86
12	cis-Ocimene	1053	1056	9.485	0.08	--	--
13	γ-Terpinene	1059	1059	9.588	0.42	0.38	0.20
14	cis-Sabinene hydrate	1072	1070	9.916	--	0.08	--
15	Linalool	1084	1087	10.394	0.26	0.21	0.14
16	Linalol	1100	1100	10.793	3.49	2.62	3.56
17	Camphor	1148	1149	12.228	16.28	16.26	23.42
18	Pinocarvone	1157	1157	12.475	--	--	0.15
19	cis-Verbenol	1164	1164	12.671	0.27	0.33	0.27
20	3-Pinanone	1169	1171	12.880	0.47	0.46	0.54
21	Borneol	1173	1174	12.952	4.00	4.13	4.33
22	Terpinen-4-ol	1180	1182	13.198	0.77	0.75	0.66
23	α-Terpineol	1191	1196	13.599	2.94	2.78	3.41
24	γ-Terpineol	1200	1200	13.725	0.17	0.12	0.15
25	Verbenone	1209	1209	13.993	0.28	0.31	0.31
26	Cyclohexane	1281	1282	16.122	--	0.49	--
27	Bornyl acetate	1285	1285	16.121	0.52	0.55	0.56
28	Copaene	1377	1379	18.635	0.09	--	--
29	E-Caryophyllene	1425	1422	19.801	0.86	--	1.02
30	α-Humulene	1459	1460	20.715	--	--	0.15
31	Myristicin	1519	1520	22.220	0.11	--	--
32	Caryophyllene oxide	1587	1587	23.809	0.12	0.12	0.20
33	Guaiol	1600	1600	25.475	--	--	0.45
34	Tetradecanal	1624	1624	25.066	0.12	--	--
35	α-Muurolol	1646	1642	25.074	--	0.11	--
36	Bulnesol	1660	1660	25.473	0.29	0.27	--
37	14-Hydroxy-9-epi-E-Caryophyllene	1674	1674	25.800	--	--	0.16
	Total identified (%)				94.97	99.72	96.92
	Essential oil yields (%)				1.80	1.80	1.80

Compounds are listed in order of their elution from Rtx-1 column.
RI_{cal}: Retention indices on the Rtx-1 column were calculated using homologous series of n-alkanes (C_7–C_{20}).
RI_{lit}: Retention indices on Rtx-1 reported in literature.

Table 2: The yields of various each fraction and essential oils of rosemary from regional variation

Various fractions	Regional variation		
	Yunnan	Guizhou	Iran
Dichloromethane fraction	0.46%	0.28%	0.24%
Ethyl acetate fraction	1.17%	1.04%	0.81%
Aqueous fraction	13.67%	14.27%	16.49%

Table 3: Antioxidant activity of essential oil and ethyl acetate fraction of rosemary from regional variation at different concentrations using DPPH assay

Sample		DPPH inhibition（%）				IC_{50}*mg/ml)
		2 mg/ml	5 mg/ml	10 mg/ml	15 mg/ml	
Yunnan	Ethyl acetate fraction	94.85 ± 0.14^{aA}	95.69 ± 0.31^{bA}	96.66 ± 0.80^{cA}	97.22 ± 0.74^{dA}	0.0011
	Essential oil	12.48 ± 1.51^{aB}	22.21 ± 0.98^{bB}	35.65 ± 1.80^{cB}	42.02 ± 1.17^{dB}	21.5600
Guizhou	Ethyl acetate fraction	87.61 ± 0.27^{aC}	89.33 ± 0.77^{bC}	91.05 ± 0.21^{cC}	93.29 ± 0.75^{dC}	0.0025
	Essential oil	13.71 ± 1.23^{aD}	26.54 ± 1.33^{bD}	34.84 ± 1.18^{cD}	41.64 ± 0.99^{dD}	23.8000
Iran	Ethyl acetate fraction	86.55 ± 0.45^{aE}	89.76 ± 0.36^{bE}	91.07 ± 0.56^{cE}	93.21 ± 0.54^{dE}	0.0083
	Essential oil	10.92 ± 1.21^{aF}	24.25 ± 1.63^{bF}	39.02 ± 1.45^{cF}	47.06 ± 0.78^{dF}	16.4500
BHT*		92.40 ± 0.24^{aG}	92.52 ± 0.51^{bG}	93.82 ± 0.49^{cG}	93.14 ± 0.35^{dG}	0.0030
VC*		96.78 ± 0.13^{aH}	96.79 ± 0.16^{bH}	96.67 ± 0.23^{cH}	97.02 ± 0.11^{dH}	0.0013

* IC_{50}, concentration (mg/ml) for a 50% inhibition.
*BHT, Butylated hydroxytoluene (BHT).
*VC, ascorbic acid (VC)
Date are presented as mean ± S.D. (n = 3)
Values followed by the same small letter within the same line are not significantly different ($p>0.05$) according to Tukey's multiple range test.
Values followed by the same capital letter within the same column are not significantly different ($p>0.05$) according to Tukey's multiple range test.

Table 4: Antioxidant activity of essential oil and ethyl acetate fractions of rosemary from regional variation at different concentrations using TBARS assay

Sample		TBARS inhibition (%)				IC_{50}*mg/ml)
		2 mg/ml	5 mg/ml	10 mg/ml	15 mg/ml	
Yunnan	Ethyl acetate fraction	53.37 ± 0.61^{aA}	62.60 ± 0.50^{bA}	73.87 ± 0.30^{cA}	77.11 ± 0.54^{dA}	1.6611
	Essential oil	38.37 ± 0.30^{aB}	47.84 ± 0.18^{bB}	48.92 ± 0.14^{cB}	55.16 ± 0.07^{dB}	8.5922
Guizhou	Ethyl acetate fraction	51.92 ± 0.18^{aC}	59.99 ± 0.21^{bC}	68.70 ± 0.28^{cC}	72.46 ± 0.25^{dC}	1.7780
	Essential oil	39.93 ± 0.60^{aD}	42.09 ± 0.36^{bD}	54.08 ± 0.18^{cD}	59.35 ± 0.25^{dD}	7.0241
Iran	Ethyl acetate fraction	48.80 ± 0.84^{aE}	63.67 ± 0.80^{bE}	67.27 ± 0.30^{cE}	69.54 ± 0.54^{dE}	1.9135
	Essential oil	28.90 ± 1.08^{aF}	33.57 ± 0.95^{bF}	46.76 ± 0.78^{cF}	52.64 ± 0.76^{dF}	13.3400
BHT*		59.95 ± 1.70^{aG}	63.31 ± 0.39^{bG}	66.19 ± 0.87^{cG}	73.50 ± 1.70^{dG}	1.2871
VC*		57.91 ± 0.45^{aH}	63.55 ± 0.66^{bH}	68.94 1.22^{cH}	78.30 ± 1.21^{dH}	1.0636

* IC_{50}, concentration (mg/ml) for a 50% inhibition.
*BHT, Butylated hydroxytoluene (BHT).
*VC, ascorbic acid (VC)
Date are presented as mean ± S.D. (n = 3)
Values followed by the same small letter within the same line are not significantly different ($p>0.05$) according to Tukey's multiple range test.
Values followed by the same capital letter within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.

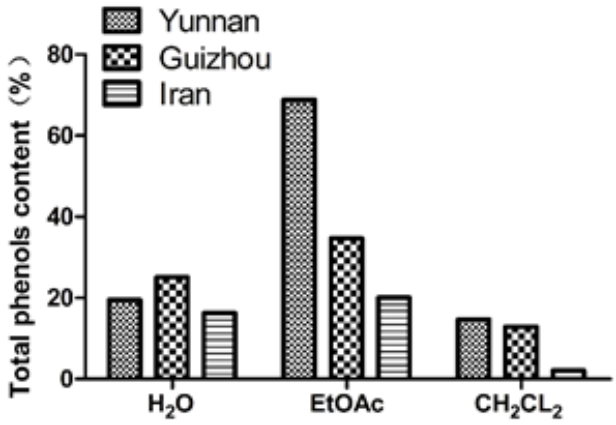
Antioxidant activity

The antioxidant activities of the essential oil have been widely demonstrated (Viuda *et al*., 2010), although the mechanism of such activity is not completely understood. There are several possible explanations, among them the following: acceptor of free radicals, donation of a hydrogen atom (Dobravalskytė *et al*., 2012), interrupting chain oxidation reactions (Viuda *et al*., 2010), chelating metals (Ye *et al*., 2013) or inducing lipid per oxidation (Cabral *et al*., 2015). The activities of essential oils such as antioxidants rely not only on their construction features but also on many other factors, such as temperature, concentration, light, physical state of the system and type of substrate (Yanishlieva, 2001). The use of various *in vitro* assays can be helpful in clarifying the action of latent antioxidants.

DPPH radical scavenging assay

The antioxidant properties of plants could be correlated with radical scavenging capacity and oxidative stress defense (Ulukanli *et al*., 2014). The DPPH scavenging assay is a feasible method for the evaluation of the antioxidant activity of a compound, they convert it into a yellow coloured compound Di-phenylhydrazine. The decrease in solution absorbance gives a measure of scavenging potential of the samples (Arora and Pandey,

2014). In antioxidant activity assay, the results were presented in Table 3. The oils and ethyl acetate extracts exhibited varying degrees of scavenging ability. The ethyl acetate from Yunnan, Guizhou and Iran an exhibited striking radical scavenging effect at 15 mg/mL with percentage inhibition values of 97.22±0.74%, 93.29± 0.75% and 93.21±0.54%, respectively, which are close to those of the positive controls BHT and VC (93.14± 0.35% and 97.02±0.11%, respectively). Under the same experimental conditions, oils obtained from Iran, Yunnan and Guizhou exhibited a radical scavenging effect of 15 mg/mL with percentage inhibition values of 47.06± 0.78%, 42.01±1.17% and 41.64±0.99%, respectively, which are lower than those observed for the positive controls BHT and VC.



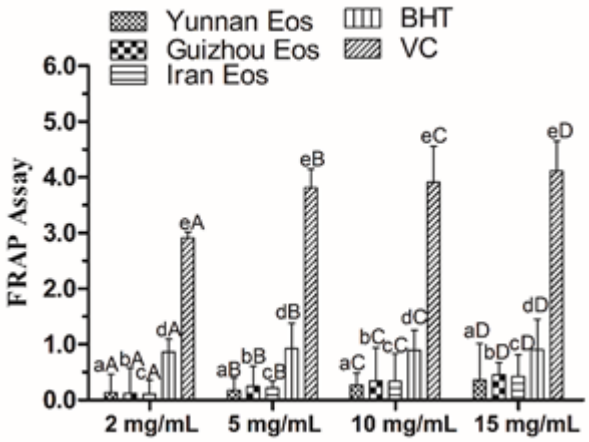
H_2O: aqueous fraction, EtOAc: ethyl acetate fraction and CH_2Cl_2: dichloromethane fraction.
*Means followed by the same letter(s) are not significantly different at $p \leq 0.05$ (one-way ANOVA was used).

Fig. 2: Total phenolic content in various each fraction from regional variation rosemary.

The DPPH assay detects the abilities of the samples to donate hydrogen to the DPPH radical, resulting in bleaching of the DPPH solution. IC_{50} is reflected in the level of bleaching action and antioxidant activity (Tian *et al*., 2014). The greater the bleaching action, the higher the antioxidant activity, which is reflected in a lower IC_{50} (Viuda *et al*., 2010). The rosemary from the ethyl acetate fraction of Yunnan (IC_{50}=0.0011mg/mL) had the strongest antioxidant activity, slightly lower than those of VC and BHT (IC_{50}=0.0013mg/mL and IC_{50}=0.0030 mg/mL, respectively), followed by the ethyl acetate fraction from Guizhou and the ethyl acetate fraction from Iran. However, the oils from Guizhou, Yunnan and Iran showed little antioxidant activity with lower IC_{50} values of 23.8000, 21.5600 and 16.4500mg/mL, respectively. The ethyl acetate fractions from rosemary from China were found to possess better antioxidative properties than those from Mediterranean rosemary tested during the study. The essential oil shows that the Mediterranean sample's antioxidant capacity is higher than that from China; however, it exhibited lower radical scavenging.

Ferric reducing antioxidant power assay

Reductive ability has been investigated by the Fe^{3+} to Fe^{2+} transformation in the presence (Djouahri *et al*., 2015) of the rosemary. As shown in fig. 3 and fig. 4, all rosemary samples exhibited good, dose-dependent reducing power, and there is concentration dependence. fig. 3 (Eos) shows that the reduction capacity of Guizhou at a concentration of 15g/mL was 0.455±0.22, while the reduction capacities of VC and BHT at a concentration of 15g/mL were 4.114±0.54 and 0.905±0.55, respectively. However, in fig. 4 (ethyl acetate fraction), the highest reduction capacity corresponded to Yunnan 4.310±0.14 at a concentration of 15g/mL (positive control: VC and BHT, at the same concentration). In brief, the reducing power of the ethyl acetate fraction of rosemary exhibited superior characteristics to those of VC and BHT.



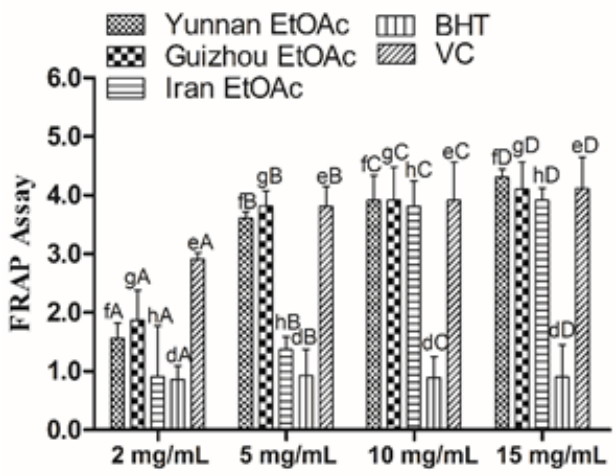
Date are presented as mean ± S.D. (n=3)
Values followed by the same small letter within the same concentration are not significantly different (>0.05) according to Tukey's multiple range test.

Fig. 3: Rosemary Eos (Essential oils) FRAP assay.

Thiobarbituric acid reactive species assay

The TBARS assay is sensitive, requires small sample amounts and provides reproducible results (Viuda *et al*., 2010). The capacity for preventing lipid per oxidation could only be determined in lipophilic samples (Boulanouar *et al*., 2013). The effect of the rosemary on lipid per oxidation was screened and monitored using the TBARS method. table 4 shows the essential oils of Yunnan, Guizhou and Iran and various Each fraction was examined for their ability to act as lipid per oxidation and compared with the ability of VC and BHT. The essential oils of rosemary from Yunnan, Guizhou and Iran each possessed lower inhibition values (55.16±0.07%, 59.35± 0.25% and 52.64±0.76%, respectively). Nevertheless, the ethyl acetate fraction of Yunnan shows the highest inhibition (77.11±0.54%) of all the rosemary samples analysed (table 4). This value was followed by that of the ethyl acetate fraction of the Guizhou sample (72.46± 0.25%) and the ethyl acetate fraction of the Iran sample (69.54±0.54%). The ethyl acetate fractions were better at

lipid per oxidation than the essential oils of the same concentration, while BHT showed the highest radical scavenging activity of all.



Date are presented as mean ± S.D. (n = 3)
Values followed by the same small letter within the same concentration are not significantly different ($p>0.05$) according to Tukey's multiple range test.

Fig. 4: Ethyl acetate fraction FRAP assay.

The EC_{50} values ranged from 1.0636 to 13.3400 mg/mL, and the lipid per oxidation inhibitory potency decreased in the following order: Essential oil (Iran) > essential oil (Yunnan) > essential oil (Guizhou) > ethyl acetate fraction (Iran) > ethyl acetate fraction (Guizhou) > ethyl acetate fraction (Yunnan) > VC > BHT. These findings suggest that all of the ethyl acetate fractions and essential oils prevent oxidative stress by inhibiting lipid per oxidation. However, all of the ethyl acetate fractions from rosemary showed higher lipid per oxidation than the essential oils of rosemary. This result showed that as an effective inhibitor of lipid per oxidation, Chinese rosemary is better than Iranian rosemary.

DISCUSSION

Preservation of food, drug materials and cosmetic products from instability during production, storage and marketing is an important issue in the food and cosmetic industries. Adding antioxidants can attenuate oxidation of these products (Stich, 1991). Therefore, there has been increasing interest in finding natural, easily obtainable, effective, cheaper, and safe antioxidants because they can protect the human body from free radicals and oxidative stress and retard the progress of many chronic diseases (Nandita and Rajini, 2004). Among these antioxidants, the essential oils may be used as an antioxidant additive (Olmedo *et al*., 2014). In the present study, the results of an *in vitro* antioxidant test showed that the rosemary had high antioxidant activity. The result is considered together with the essential oil and phenolic compounds. The antioxidant activity could be mainly due to the 1,8-cineole and α-pinene of the essential oils. At the same time, the phenolic compounds content could be used as an important indicator of the antioxidant properties, which may be used as a preliminary screen for rosemary from different regions when intended as natural sources of antioxidants in foods and chemical industries (Liu *et al*., 2008, Viuda *et al*., 2010). Many authors have described the potential antioxidant activity of polyphenols (Aruoma *et al*., 1992, Hernández *et al*., 2009).

The reactive facets of phytochemicals are sufficiently complex that the antioxidant properties of plant extracts cannot be evaluated by only a single method. However, there are at least two test systems recommended for the determination of antioxidant properties to establish authenticity (Schlesier *et al*., 2002, Ye *et al*., 2013). For this reason, the antioxidant capacity of rosemary samples was determined by three spectrophotometric methods: DPPH, FRAP and TBARS assays. The results of this study showed that the antioxidant properties of the rosemary plant have been evaluated by three methods: in all cases, the essential oil shows the lowest antioxidant activity. These results reveal that the essential oils were of no significant differences in antioxidant properties when comparing the three regions. This finding is probably attributable to the chemical composition bearing no significant differences in the samples from China and the Mediterranean region. However, the ethyl acetate fractions from rosemary exhibited considerable activity toward scavenging DPPH, reducing power, lipid peroxidation and total antioxidant properties. The antioxidant abilities of the ethyl acetate fraction from rosemary is probably due the high content of phenolic compounds determined in these samples. The ethyl acetate fraction from rosemary from Yunnan exhibited strong antioxidant capacity because of the highest TPC of 68.80%. The ethyl acetate fraction of Iran shows only a moderate TPC of 20.13%, and it shows low antioxidant properties. In general, the antioxidant activity of rosemary determined by three different testing methods revealed that rosemary grown in China outperformed that of the Mediterranean region. However, further studies are warranted to confirm these findings.

Overall, the total phenols content and the antioxidant activity of rosemary showed that samples grown in China perform better than those of the Mediterranean region. In conclusion, based on the results of this study we concluded that Chinese rosemary could serve as a good source for perfume, cosmetic products, food, traditional medicine, pharmaceutical industries, agribusiness and chemical industries.

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