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Phenolic Combination and Comparison of Antioxidant Activity in Three Different Alcoholic Extracts of *Dracocephalum moldavica* L.

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Research Article

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A B S T R A C T

Dracocephalum moldavica L. has been used as both treatment of coronary heart disorders and food supplements. In the present study, determination of total phenolic and flavonoid content in methanol, ethanol and methanol/ethanol extracts of leaf was studied in Iranian *Moldavian balm* for the first time. Antioxidant activity of the studied plant was also measured. The methanol extract had the highest phenolic and flavonoid content, anthocyanin, DPPH and H_2O_2 radical scavenging activity. The ethanol extract showed the least amount of all. The methanol/ethanol extract showed the highest amount in two oxides including nitric and superoxide radical scavenging activities; it also showed the highest Ferric Reducing Ability Power. The obtained chromatograms of the plant using High Performance Liquid Chromatography showed that the highest and the lowest found phenolic compounds were caffeic acid and vanilic acid, respectively. The results show that this plant is a suitable natural antioxidant to reduce the oxidative stress in human being.

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Introduction

Plants have been used for treating different kinds of disease in our ancient traditional method of medicine. Plants make many chemical compounds that are active for many various biological functions. Recently the focus of pharmacologs has been on the development of new drugs obtained from plants (Ivanova et al., 2005). Herbal compounds are also used as preservatives in foods and cosmetics (Makari et al., 2008).

Phenols and flavonoids are essential compounds of medical plants containing antibacterial, antioxidant and antitumor properties; actually these compounds have been proved as potential preservatives (Nychas et al., 2003). Antioxidants have been shown to counteract oxidative stress in organisms and they have the ability of increasing the risk of chronic and degenerative diseases such as atherosclerosis, cancer and diabetes. Natural antioxidant compounds obtained from plants are much healthier and safer than those produced synthetically (Hentschel et al., 2002; Dastmalchi et al., 2007). Phenolic compounds known as hydrophilic antioxidants and together with Pneumatic cell metabolisms are produced to defend injured plants against pathogens (Bors et al., 1999). They can scavenge free radicals, break radical chain reactions, and chelate metals. Reactive oxygen species (ROS) having lots of pathological effects like DNA damage, carcinogenesis and various degenerative disorders such as cardiovascular diseases, aging and neurodegenerative diseases are generated in living organisms during metabolism (Ahmad et al., 2011; Noda et al., 1997; Gyamfi et al; 2002).

The genus *Moldavian balm (Dracocephalum moldavica L.)* from Lamiaceae (Labiatae) family has been used for many purposes. This family is well known as a rich source of essences and phenolic compounds (Hentschel et al., 2002). It is an annual herbaceous aromatic plant native to some areas of central Asia and naturalized in central and Eastern Europe. It is mostly found in northern and northwestern parts of Iran, especially in mountains of West Azerbaijan province. Aqueous extracts from *Dracocephalum moldavica* is distilled and used as a beverage and a cardiotonic agent in the folk medicine of Iran (Rechinger, 1986). *Moldavian balm* containing essential compounds is commonly consumed for medical and nutritional purposes such as stomach treatment, liver disorders, congestion, headaches and cardio protection in different parts of the world. It has antitumor properties (Chachoyan and Oganesyan, 1996), liver disorder treating properties (Chinese Pharmacopoeia

Commission, 1999), antimicrobial and assedative (El-Gengaihi and Wahba 1995), peripheral antinociceptive and anti-inflammatory activity (Le Bars D et al., 2001). *Moldavian balm* can also be used as a food ingredient, herbal drug and a demulcent in the treatment of fevers headache and toothache (Hentschel et al., 2002; Proestos et al., 2004).

Geographic location, climate and cultivar are some of the most effective external factors in composition content of the plants; So that, the plants cultivated from different parts of the world may be containing different effective materials with totally different bioactivities. (Lee and Talcott, 2004).

Very little research has been done regarding the antioxidant activity and nonvolatile chemistry of *Moldavian balm*; therefore less information has been published regarding antioxidative properties of *Moldavian balm* (Kakasy et al., 2002; Povilaityte et al., 2001; Povilaityte and Venskutonis, 2000). Solvents show various extraction capabilities depending not only on structure of their own but also on the structure of the solute; So that the extractive effect of phenolic compounds highly depends on the organic solvent used for extraction. The present study focuses on evaluation of the antioxidant activities of different leaf extractions of Peppermint gathered from Iran (Sardasht in West Azerbaijan province) and introduction of the best extraction method among three different alcoholic extracts. Furthermore, this study tries to determine the total phenolic compounds and antioxidant activities of leaf part of *Moldavian balm* and to analyze the main phenolic compounds in the extract of leaf by high performance liquid chromatography (HPLC) method.

Materials and Methods

Chemicals

1-1-diphenyl-2-picrylhdrazyl (DPPH) was bought in from Sigma-Aldrich Company Ltd, Gillingham, UK. The solvents, all other materials and reagents were HPLC and analytical grade purchased from Merck in Germany.

Plant Material

The plant *Moldavian balm (European Dracocephalum moldavica L.)* was collected from the mountains of Sardasht city of West Azerbaijan Province in May 2012. The material was identified and confirmed by Dr. Abbas Siami and was entrusted to the herbarium of Science Faculty, Urmia University, Urmia, Iran.

Preparation of Extracts

The leaf parts of the *Moldavian balm* plant were cut and dried in a dark place in the room temperature at 25°C, then these dried parts of *Moldavian balm* were powdered in a mixture grinder. Separately 2 g of powdered sample was extracted for 3h with 25 mL of different solvents including methanol, ethanol, and methanol/ethanol (1:1) at room temperature on an orbital shaker set at 200 rpm due to extraction in three various dishes at low speed. After filtration with Whatman filter paper No.1 (Whatman Ltd., England using vacuum pump), the residue was re-extracted in the similar way to get the maximum compounds by adding 75 mL solvent to make up the final extract (100 mL). The concentrated extracts were kept in dark bottles at 4°C for being utilized. Light exposure was avoided during the extraction process.

Determination of Total Phenolic Content **(TPC**)

Total phenolic content (TPC) of the studied plant was measured refer to the Folin-Ciocalteu colorimetric method with a little modification (Kahkonen et al., 1999). To start with, Folin Ciocalteu's phenol reagent (1 mL) (and 7.5% w/v Na2CO3 (0.8 mL) were added to sample M, E and methanol/ethanol ME extracts (20µL), afterwards the solvent placed in the room temperature for 30 min to be incubated at 25°C in a dark place. Finally, the absorbance of the final reaction was determined in 765nm. The results were expressed in terms of gallic acid equivalents/100 g *Moldavian balm* powder. (The calibration equation for Gallic acid: $y = 0.042 x - 0.023$, $R^2 = 0.998$).

Determination of Total Flavonoid Content **(TFC)**

Total flavonoid content (TFC) of the *Moldavian balm* was measured by using the method of aluminum chloride colorimetric (Chanda and Dave, 2009) with a little modification. During this study, deionized water (1 mL) was added to 20µL of plant extract; then 0.075 mL $NaNO_2$ (5%) was added to the mixture and after 5 min staying in room temperature, 0.15 mL of 10% AlCl3_6H_2O was added into the solvent and was stayed for 6 min in room temperature again; adding 0.5 mL NaOH (mol/L) made the solvent complete; finally, the outcome was reached to the final volume of 3mL using deionized water. The absorbance of the final mixture was measured in 510 nm. The results were expressed in terms of g quercetin equivalents /100 g *Moldavian balm* powder (The calibration equation for quercetin: $y = 0.077 x - 0.013$, $R^2 = 0.997$).

Determination of Ferric Reducing Ability Power (FRAP)

The FRAP was determined using FRAP assay (Benzieet al., 2002) with some modifications. The FRAP solvent included 5 mL of 10 mMol/L TPTZ (2, 4, 6-tripyridyl- s- triazine) solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3$, and 25 mL of 0.3 M acetate buffer (pH 3.6) (0.3 mol/L, pH = 3.6). 3 mL FRAP was added to 100µL of mixed sample and the final solution was measured at the absorbance of 593 nm after incubation at 37˚C for 10 min. Methanolic solutions of known Fe (II) concentration, in the range of 0.6–10 µmol/L ($FeSO_4$), were used for obtaining the calibration curve. The FRAP value shows the ratio between the slope of the linear scheme for reducing Fe^{3+} -TPTZ reagent by *Moldavian balm* extract in comparison with the slope of the scheme for $FeSO_4$.

Determination of superoxide radical scavenging activity

The system of pyrogallol autoxidation was used for evaluation of superoxide scavenging (Jing and Zhao, 1995). A volume of 9 mL Tris-HCl buffer solution (50mmol/L, pH = 8.2) was added into a test tube, and the

tube was incubated in a water bath at 25°C for 20 min. A volume of 40µL pyrogalol solution (45 mmol/L in 10 mmol/L HCl) which was pre-incubated for 20 min at 25°C was injected to the mentioned tube by a microlitre syringe and mixed together. The absorbance of this solvent showing the speed of pyrogallol autoxidation by being measured at 420 nm after 5 min. Ascorbic acid was announced as the reference compound. Certain concentration of extract was added to the previous solution and standard into Tris-HCl buffer solution and indicated the autoxidation speed. The less absorbance of the reaction mixture there was, the more superoxide anion scavenging activity there was. The superoxide radical scavenging activity using the following equation:

$$\text{Superoxide scavenging activity\%} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 was the absorbance of control (blank, without extract) and A_1 was the absorbance of the solution in the presence of ascorbic acid and the extract.

Determination of Hydrogen Peroxide Radical Scavenging Activity (H_2O_2)

A method to determine the H_2O_2 scavenging ability previously mentioned was used with a little modification (Ruch et al., 1989). *Moldavian balm* extracts (2 mg/mL) were dissolved in 3 mL of a 0.1 M phosphate buffer (pH=7.4) solution and mixed with 600µL of a 43 mM solution of hydrogen peroxide previously prepared in the same buffer. A blank solution was prepared the same way without presence of H_2O_2. The absorbance of the solutions was measured for recognizing the concentration of hydrogen peroxide at 230 nm. Gallic acid was used as the reference compound. The hydrogen scavenging activity was calculated using the following equation:

$$H_2O_2 \text{ scavenging activity\%} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 was the absorbance of control (blank, without extract) and A_1 was the absorbance of the solution in the presence of the extract and gallic acid.

Determination of DPPH Radical Scavenging Activity

DPPH radical scavenging activity in *Moldavian balm* extracts were determined using the method described by Hatano (Hatano et al., 1988). A volume of 40 µL of each extract in various concentrations (3-20 mg/mL) was added to 1 mL of DPPH (1-1-diphenyl 2-picryl hydrazyl) radical solution dissolved in ethanol with a 0.2 mM final concentration. Two different standards called ascorbic acid and gallic acid (0.125–20 mg/mL) were reference standards. The different tubes containing the solutions were placed in a dark room for 30 min to be incubated. The absorbance of mixtures was measured at 520 nm immediately. The DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging activity\%} = \frac{A_0 - A_1}{A_0} \times 100$$

A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance of the whole materials including extract, ascorbic acid, gallic acid, DPPH.

Determination of Nitric Oxide Radical Inhibition Assay

Nitric oxide radical inhibition can be measured using Griess Ilosvay reaction reaction (Garrat 1964). naphthyl ethylene diamine dihydrochloride (0.1% w/v) was used instead of napthylamine (5%). The final combination of mixture (3 mL) containing phosphate buffer saline (0.5 mL), sodium nitroprusside (10 mM, 2 mL) and the alcoholic extract of *Moldavian balm* (0.5 mL) was incubated at room temperature for 150 min. After incubation, 1mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was added to 0.5 mL of the prepared mixture and allowed to stand for 5 min for complementation of diazotization. Then, 1 mL of naphthyl ethylene diamine dihydrochloride was added and allowed to stay at room temperature for 30 min. The final solution had a pinkish color in diffuse light. Ascorbic acid and gallic acid were utilized as the reference compounds. The absorbance of the solutions was measured at 540 nm in the comparison of blank. The nitric oxide radical scavenging activity was calculated using the following equation.

$$\text{Nitric oxide scavenging activity\%} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance of the whole materials including extract, ascorbic acid and gallic acid.

Determination of Total Anthocyanin Content (TAC)

Total anthocyanin content (TCA) in the studied plant extract was determined utilizing pH-differential method previously described with a little modification (Lee et al., 2005). The whole anthocyanins show the maximum absorbance at 520 nm. The colorless hemiketal form at pH 4.5, and the colored oxonium form predominate at pH 1.0. The method of PH differentiation is the reaction producing oxonium forms and permits accurate and rapid measurement of the total anthocyanins. Absorbance of the results was calculated using the following equation:

$$A = (A_{520} - A_{700})\ pH_{1.0} - (A_{520} - A_{700})\ pH_{4.5}$$

The concentration (mg/L) of each anthocyanin was calculated according to the following equation and expressed as Cyanidin-3-glucoside (Cy-3-glc) equivalents:

$$\text{Concentration (mg/L) of each anthocyanin} = \frac{A \times MV \times DF \times 10^2}{\Sigma \times L}$$

A is the absorbance = (A of pH 1.0- A of pH 4.5), MW is the molecular weight (g/moL) = 449.2 g/mol for Cy-3-glc, DF is the dilution factor, Σ is the extinction coefficient (L× cm-1× mol-1) = 26,900 for Cy3-glc, and L (path length in cm) = 1, so result reported is expressed as Cy-3-glc equivalents.

HPLC Analysis

For the analysis of HPLC, 1 g of the powdered and dried plant material was extracted with 50% methanol/water (1×10 mL) for 2 h at room temperature. After that, 1.2 N HCl was added to the resulted extract and it was hydrolyzed by reflux method in a water bath for 1 h (Lin-Chin et al., 2000). Samples were filtered through a 0.45-µm pore size syringe-driven filter before injection. A 20 µL aliquot of sample solution was separated utilizing a HPLC Knauer system equipped with UV-V detector and a Eurospher 100-5 C-18 column (25 cm × 4.6 mm; 5 µm) and processed the hydrolysate to qualitative and quantitative analysis (Bajpai et al., 2005). The system includes a mobile phase full of purified water with 2% acetic acid (A) and acetonitrile (B) at a flow rate of 0.8 mL/min. Phenolic compounds were eluted under the following conditions: 0.8 mL/min flow rate and the temperature was set at 25 °C, isocratic conditions from 0 to 10 minute with 0% B, gradient conditions from 0% to 20% B in 5 min, from 20% to 25% B in 10 min, from 25% to 100% B in 15 min, followed by washing and reconditioning the column. Phenolic compounds were detected in a wavelength of 280 nm. Results were gained by using the comparison of peak areas of the samples with those of standards.

Statistical Analysis

All the investigations were carried out in triplicate. Experimental information was revealed as mean ± standard deviation (SD). SPSS software, Version 19.0, was used to analyze the data. One-way analysis of variance was provided by ANOVA procedures. Values of $P<0.05$ were organized as significant utilizing Duncan's multiple range tests to show the differences between means.

Result and Discussion

Total Phenolic Content (TPC)

Moldavian balm species are proved to have secondary metabolites like flavonoids and terpenoids. They not only have glycosides, aglycones but also have ohydroxybenzoic, hydroxycinnamic acids (Dastmalchi et al., 2007). There are numerous investigations proving the effect of antioxidant supplementation for reducing and preventing the oxidative stresses (Jahanban Sfahlan et al., 2009). Plant phenolics, strong free radical scavengers, are highly used for prevention of increasing the number of free radicals. In this study, phenolic acids, flavonoids and anthocyanins were the major phenolics analyzed. The TPC of leaf part of the M extract 3.0450±0.11 g gallic acid/100 g *Moldavian balm* dry shown in Table 1 had the highest total phenolic content as compared to the other extracts. The ME extract (2.308473±0.97) also showed the good amount of total phenolic compound compared to M extract (1.5673±0.077).

Total Flavonoid Content (TFC)

Flavonoids are a ubiquitous member of plant polyphenolic compounds which act as common components with dietary, biological actions. Nowadays, flavonoids have attracted lots of interests of antioxidant studies. There are numerous biomolecules and proteins in living organisms with a significant free radical scavenging actions such as, flavonoids, phenols and other dietary supplements (Patricia et al., 2005). In present study, we have worked on finding and comparing the total flavonoids in different extracts of the leaf part of *Dracocephalum moldavica L* and the possible mechanisms. The values of TFC demonstrated in Table 2 conclude that M extract (1.8813±0.04 g quercetin /100 g *Moldavian balm* powder) and E (1.25552±0.70 g quercetin /100 g *Moldavian balm* powder) extracts were the highest and the least amount of total flavonoid content respectively. Also, there was an important point that comparing the TPC and TFC in all extracts shows that M extract of leaf part of *Moldavian balm* contains the highest amount of total phenol and flavonoid compounds.

Ferric Reducing Antioxidant Power (FRAP)

Wide differences of *in vitro* antioxidant power were resulted in three different extracts of *Moldavian balm*. The results showed that various extracts of *Moldavian balm* plant had wide differences. The outcome antioxidant capacity was correlated with the total phenolic contents. The DE (185.4379±1.5819%) and ME (101.4027±6.0876%) extracts had the highest and the least FRAP, respectively shown in Table 3. The E extract was the highest of all but this fact never confirmed that *Moldavian balm* extracts contain the highest levels of antioxidant compounds because the FRAP activities of extracts were lower than standards.

Superoxide Radical Scavenging Activity

The superoxide anion is produced by mitochondrial respiration, NADPH oxidase, xanthine oxidase, cyclooxygenase, lipoxygenase, nitric oxidase synthetase (NOS) and cytochrome P450 (30-31-32-33-34-35). Not only phenols but also flavonoids belonging to the class of phenols can gather harmful superoxide radicals effectively. The combination of superoxide and nitric acid peroxynitrite can be a reason of producing toxic compounds such as hydroxyl radical and nitric dioxide (Sainani et al., 1997). Furthermore anions of superoxide can accelerate the autoxidation, thereupon autoxidation don't have the opportunity taking place in the presence of antioxidants collectors. Although anion of superoxide is a weak oxidator, but oxygen and hydroxyl radical can be produced by superoxide which both involve in oxidative stresses (Halliwell, 1995). The capacity of superoxide scavenging activity using the different alcoholic extract of *Moldavian balm* has been obtained by method of pyrogallol autoxidationin presence of Tris-HCl buffer. The percentage of scavenging activity was more than 60% in all the obtained extracts. Respectively, the ME (56.7226±1.8564%) and E (44.4327±6.472%) extracts had the highest and the least amount of Superoxide radical scavenging activity shown in Table 2.

Table 1 Total phenolic, flavonoid and anthocyanin contents of 20 mg/mL alcoholic extracts of *Dracocephalum moldavica* L.*

Extract	Total phenols content (g Gallic acid/100 g)	Total flavonoid content (g Quercetin/100 g)	Anthocyanin contents (g /100 g)
Methanol	3.04 ± 0.1^{c}	1.88 ± 0.04^{b}	0.24 ± 0.01^{a}
Ethanol	1.56 ± 0.07^{a}	1.25 ± 0.70^{a}	0.11 ± 0.01^{b}
Methanol/Ethanol (1:1)	2.308 ± 0.9^{b}	1.70 ± 0.60^{c}	0.16 ± 0.03^{c}

*Values are presented as mean ± SD (n = 3). Values of P<0.05 are regarded as significant.

Table 2 DPPH, nitric oxide and superoxide radical scavenging activities values and IC50 values of 20 mg/mL alcoholic extracts of *Dracocephalum moldavica* L.*

Extract	IC50 (mg/mL)	DPPH	NORSA	SRSA
Methanol	3.624 ± 1.26^{d}	87.77 ± 0.31^{a}	37.60 ± 1.95^{a}	44.43 ± 6.47^{c}
Ethanol	8.040 ± 0.25^{c}	60.88 ± 5.03^{b}	73.54 ± 1.63^{c}	56.72 ± 1.85^{b}
Methanol/Ethanol(1:1)	2.860 ± 69^{b}	25.96 ± 1.07^{b}	87.90 ± 5.28^{a}	57.14 ± 1.21^{a}
Gallic acid	0.16 ± 0.03^{a}	92.00 ± 0.35^{c}	68.28 ± 2.63^{b}	-
Ascorbic acid	0.18 ± 0.02^{a}	91.20 ± 0.29^{c}	65.26 ± 0.99^{b}	65.61 ± 1.88^{d}

*Values are presented as mean± SD (n = 3). Values of P<0.05 are regarded as significant. DPPH: DPPH radical scavenging activity%, NORSA: Nitric oxide radical scavenging activity (%); SRSA: Superoxide radical *S*cavenging activity (%)

Nitric Oxide Radical Scavenging Percentage

A highly participated reactive molecule in the systems of immune and cardiovascular is called Nitric oxide (NO) which is involved in many physiologic reactions. This nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite anion and proxynitrite which are well known as free radicals (Sainani et al., 1997). Under high concentration of NO, a number of extremely reactive nitrogen oxide species, such as N_2O_3 and ONOO- can be produced and they cause toxic reactions including lipid peroxidation and DNA modification (Bartasch and Montesano, 1984). The extract inhibits nitrite formation by competing with oxygen. The ME (87.9095±5.2856%) and M (37.6074±1.9518%) had the highest and the least nitric oxide radical scavenging percentage shown in Table 2. The ME and E extracts had more nitric oxide radical scavenging percentage than gallic acid (61.16±2.89%) and ascorbic acid (68.16±0.90%) but M extract had lower nitric oxide radical scavenging percentage than standards.

Hydrogen Peroxide Radical Inhibition Assay

The toxicity of hydrogen peroxide can be derived from its conversion to other species of ROS such as hydroxyl radical which initiate lipid peroxidation and cause DNA damage (Nakayama et al., 1993). Hydrogen radicals ($OH^{\cdot}$) may be produced by decomposition of H_2O_2 into oxygen and water. Thus, removing hydrogen peroxide as well as other ROS is very important to protect lots of cellular compounds such as protein and DNA (Zhao et al., 2008). The hydrogen peroxide scavenging activities of M (7.93±0.658) and E (4.48±2.030) extracts had the highest and the least scavenging capacity against H_2O_2 respectively. The M extract had more hydrogen peroxide scavenging activity than gallic acid (16.91±2.86%) at a concentration of 2 mg/mL, strongly suggesting that this extract contains the necessary compounds for hydrogen peroxide deletion shown in Table 3.

DPPH Radical Scavenging Assay

DPPH is a radical with purple color which can get a hydrogen or electron to be a yellow colored compound called diphenyl-picrylhdrazine at 25°C. Antioxidants can cause a reduction in the DPPH radical and in the absorbance of the different extracts of *Moldavian balm* (Brand-Williams et al., 1995). The IC_{50} values were calculated and they are negatively associated with antioxidant activity. The DPPH radical scavenging activity values for gallic acid and ascorbic acid were 93.00±0.45% with IC_{50}=0.15±0.01 mg/mL and 92.30±0.26% with IC_{50}=0.16±0.01 mg/mL, respectively. The results showed that the DPPH radical scavenging activities of the M extract (87.77±0.31%, IC_5=3.624±1.26) and E extract (60.88±5.03%, IC_{50}=8.040±0.25) were significant. The amount of DPPH radical scavenging activity in all extracts was lower than both gallic acid and ascorbic acid shown in Table 2.

Total Anthocyanin Contents (TAC)

Anthocyanins are some compounds classified in flavonoids and found in almost all fruit and vegetables. They are not only protectors against oxidative stresses but also reducer of their effects. Table 1 shows the amount of anthocyanin content of the different 3 extracts. Respectively, the M (0.24±0.013%) and E (0.11±0.010%) extracts were the highest and the least TCA shown in Table 1.

HPLC Analysis of the Moldavian Balm Extracts

The content of phenolic substances was determined by quantitative HPLC analysis after extraction and acid hydrolysis. Analyzing *Moldavian balm* using HPLC analysis method revealed that some compounds such ferulic acid, caffeic acid, vanilic acid, 1-naphtol, 4-hydroxy-benzoic acid, cholorogenic acid were identified. Refer to HPLC analysis caffeic acid (9.49±4.97 mg/100 g *Moldavian balm* powder and vanillic acid (0.43±0.008 mg/100 g *Moldavian balm* powder) were the highest and lowest phenolic compound in *Moldavian balm* respectively. Injection of 9 standards of phenols are represented in figure 1 and figure 2, and comparison of the obtained chromatogram of the results are shown in Table 5. HPLC analysis proved that *Moldavian balm* extract contains caffeic acid and 1-naphtol as other phenols which are shown in table 4, but some phenols like

ascorbic acid, gallic acid and rutin shown in table 5 were not detected.

Table 3 H_2O_2 radical scavenging activities and FRAP values of 2mg/mL alcoholic extracts of *Dracocephalum moldavica* L*

Extract	H_2O_2 radical Scavenging activity (%)	FRAP value (µmol/100g)
Methanol	7.93±0.65[d]	180.98±1.02[a]
Ethanol	4.48±2.030[c]	101.40±6.08[a]
Methanol/Ethanol (1:1)	6.17±0.813[b]	185.437±1.58[a]
Gallic acid	21.81±2.34[a]	811.65±5.21[c]
Ascorbic acid	-	521.16±10.94[b]

Values are presented as mean ± SD (n = 3). Values of P<0.05 are regarded as significant

Table 4 Linear calibration curves for the HPLC-UV analysis of the phenolic compounds of extract from *Moldavian balm* (*Dracocephalum moldavica* L.).

No	Ascorbic acid	Retention Time (min)	Equation of linear Regression (peak area concentration)	R^2	Linear range (mg/L)
1	Ascorbic acid	3.6	Y=2e+06X-1e+06	0.998	0.005-5
2	Rutin	5.4	Y=E+06x-2E+06	0.998	0.5-10
3	Gallic acid	7.2	Y=68759x+3509.5	0.997	0.5-10
4	Chlorogenic acid	10.3	Y=165138x-136553	0.989	0.5-10
5	Ferulic acid	11.0	Y=83185x-1E+5	0.988	0.5-10
6	4-Hydroxy benzoic acid	11.4	Y=3E+06X-29609	0.998	1-10
7	Vanilic acid	12	Y=1E+06X-2E+06	0.999	0.05-10
8	Caffeic acid	14.7	Y=1E+06X-76169	0.988	1-10
9	Naphtol	22.3	Y=2e+06X-1e+06	0.998	1-10

Table 5 Content of phenolic compounds in extract of *Moldavian balm*

No	Phenolic compound	(mg/100 g dragonhead powder)
1	Ascorbic acid	Nd
2	Rutin	Nd
3	Gallic acid	Nd
4	Chlorogenic acid	1.57±0.06
5	Ferulicacid	4.53±0.03
6	4-Hydroxybenzoicacid	7.23±0.14
7	Vanilicacid	0.43±0.008
8	Caffeicacid	9.49±4.97
9	1-Naphtol	4.18±0.14

Values are presented as mean± SD (n = 3). Values of p<0.05 are regarded as significant. Nd: Not detected.

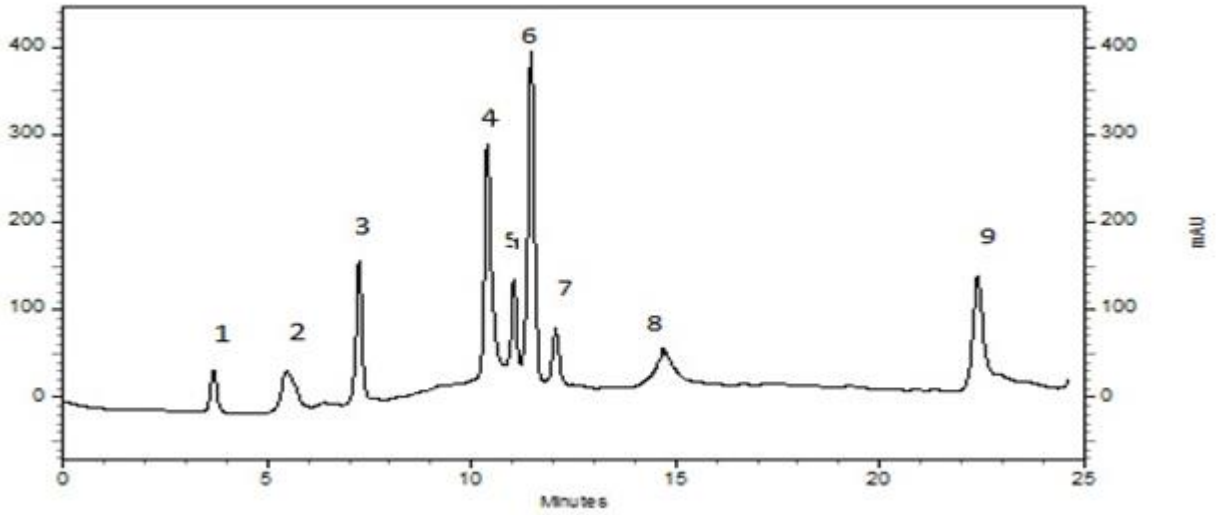

Figure 1 HPLC chromatograms of Moldavian balm extracts at 280 nm. Peak: (1) Ascorbic acid, (2) Rutin, (3) Gallic acid, (4) Chlorogenic acid, (5) Ferulic acid, (6) 4-Hydroxy benzoic acid, (7) Vanilic acid, (8) Caffeic aicd, and (9) 1-Naphtol P<0.05

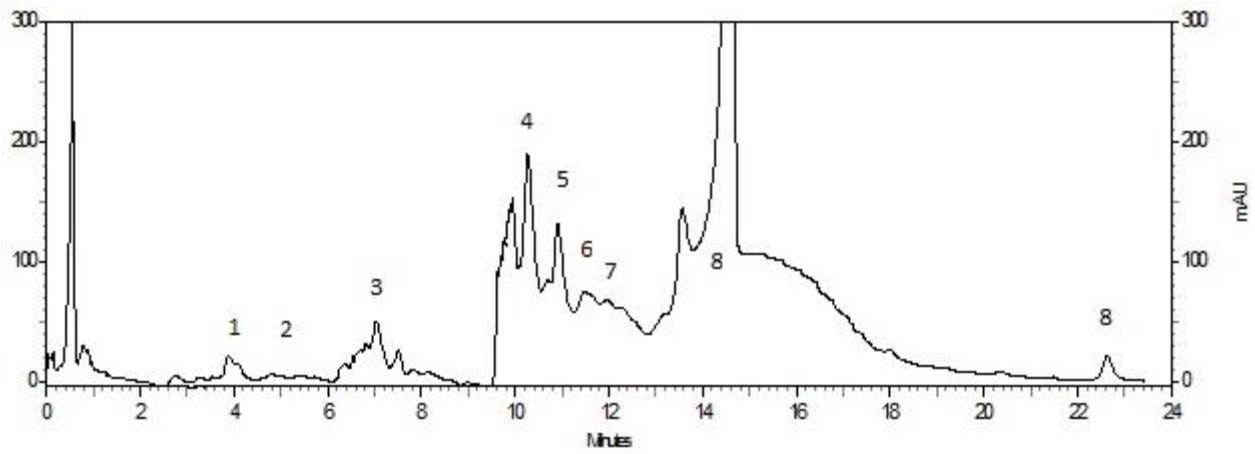

Figure 2 Mixture of standard phenolic compounds at 280 nm. Peak: (1) Ascorbic acid, (2) Rutin, (3) Gallic acid, (4) Chlorogenic acid, (5) Ferulic acid, (6) 4-Hydroxy benzoic acid, (7) Vanilic acid, (8) Caffeic aicd, and (9) 1-Naphtol, $p < 0.05$

Conclusion

The present study showed that *Moldavian balm (Dracocephalum moldavica L.)* growing in Iran, which is often present in Iranian food and folk medicine, is a strong radical scavenger. The extracts of alcoholic solvents were found to have different levels of antioxidant activity in the systems tested. This could be due to the presence of an enormous amount of flavonoid and phenolic compounds. The study also revealed the possible antioxidant mechanism of the extracts such as electron-donating ability and direct free radical scavenging properties. The high scavenging property of *Moldavian balm (Dracocephalum moldavica L.)* may be due to hydroxyl groups existing in the phenolic compounds that can scavenge the free radicals. These extracts can be used as easily accessible source of natural antioxidants and a possible food supplement or in pharmaceutical applications. It can also be used in stabilizing food against oxidative deterioration.

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