



Evaluation of minerals, phenolics and anti-radical activity of three species of Iranian *berberis* fruit

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ABSTRACT

The amounts of substances together with nutritional and medicinal properties of fruits from Berberidaceae family vary between the plant species. Aluminum chloride colorimetric assay was used for flavonoids determination in 250 g of dried fruit. The Folin-Ciocalteu method was used to determine the total phenolic compound contents. The free radical-scavenging activity was examined by stable 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH) assay. The level of total phenol in different *Berberis* Ecotypes was 241.5 mg/g for Black *berberis khorasanica*, 296.8 mg/g for Black *berberis bakhtiarica* and 157.5 mg/g for Red *berberis khorasanica*. Furthermore, anti-radical activities (IC₅₀) of these three Iranian *Berberis* species were 221.1, 58.38 and 123.2 µg/mL respectively. The level of flavonol in Black *berberis khorasanica* was approximately 2.42-fold higher than Black *berberis bakhtiarica* and 5.49-fold higher than Red *berberis khorasanica*. The content of flavonoids in Black *berberis bakhtiarica* was approximately 2 times more than its level in Black *berberis khorasanica* and 3.55 times more than its level in Red *berberis khorasanica*. The highest amount of phenol and total phenols were observed in Black *berberis bakhtiarica* as its anti-radical activity was approximately 3.8 times more than Black *berberis khorasanica* and 2.1 times more than Red *Berberis khorasanica*. We showed high level of antioxidant compounds in Black *berberis bakhtiarica*. Considering the rate of elements and compounds in three Iranian *Berberis* ecotypes and based on their antioxidant and anti-radical effects, the *berberis* can be of benefit to oppose free radicals in the body.

Keywords: *Berberis*, *Berberis khorasanica*, *Berberis Bakhtiarica*, Antioxidants, Anti-radical

INTRODUCTION

Nowadays, due to the potential adverse effects of synthetic drugs and increasing contraindications to their usage, an increasing interest can be seen in the use of medicinal plants [1]. Plants with antioxidant activity have been used to treat various diseases. Recent researches have also shown promising results from these plants in the treatment or prevention of hard curable conditions such as diabetes[2, 3], atherosclerosis[4, 5], hypertension[5-7], and cancer[8, 9]. These herbal medicines have also the capacities to diminish drug induced adverse effects[10, 11] and even heavy metal toxicities[7, 12]. The antioxidant activities of these plants have been attributed to their flavonoids and phenolic components[13, 14].

Berberidaceae family is one of these plants, which are found worldwide, and its family has about 650 species. *Berberis* is native plant of temperate and subtropical regions of Europe, Asia, Africa, North and South America. Various ecotypes of *Berberis* grow well throughout different parts of Iran. Several parts of plant including roots, shoot, leaf and fruit can be used as medicine or food stuff [15-17]. In Iran, *Berberis* seems to have 5 woody species i. e., shrubs and bushes. Two ecotypes of *Berberis* including *Berberis bakhtiarica* and *Berberis khorasanica* are explosively belonged to Iran and *Berberis integerrima* and *Berberis crataegina* are found in Iran as well as other regions including East Anatolia, East Iraq, Afghanistan, Transcaucasia, Turkmenistan, West Pakistan, Kashmir and Central Asia. Several species of this family were recently imported to Iran to use in different, areas as ornamental [18, 19].

Medicinal herbs and their derivatives have long been used as potential remedies for a wide range of illnesses, however, valid researches have not yet fully proved their definite effects[20].

There is a considerable interest for Nutraceuticals and functional foods have as potential alternative therapies in order to for treat various insulin resistance and cardiovascular disorders.

Previous studies on glucose and lipid profile demonstrated the safety and effectiveness of a combination of Barberry extract in patients samples [21, 22] and it has been approved by FDA[23]. This fruit is used as a condiment and flavoring supplmernt in Iran and parts of Asia. *Berberis* fruit has a variety of nutritional constituents such as dextrose, fructose, malic acid, tartaric acid, citric acid, pectin, resin and also is rich in vitamin C, vitamin A, calcium, iron and potassium[24, 25]. The medicinal properties of this plant is attributed to Berberine. Berbamine alkaloids, Oxyaconthine, bervulcine, columbamine and ascorbic acid are found in the chemical composition of different parts of the plant. *Berberis* is used in the treatment of various diseases such as scurvy, hypertension, Alzheimer, depression, diarrhea, diabetes, jaundice, kidney stones, gout, rheumatism and skin diseases[26, 27].

Berberis fruit, as industrial application, is also used as the natural colors in the food industry to its anthocyanin [28]. In Iran, different organs of the plant especially its fruit, has been used in the treatment of diabetes, fatty liver, hypertension, anemia, cardiovascular system, gastrointestinal, genitourinary and breathing complications [29]. According to the geographical conditions, weather, and its different traditionally reported medicinal effects, it sounds to be one of the most important crops in both agricultural and pasture sectors [30, 31]. In the other hand, Iranian local therapists (herbalists) prescribe and recommend it for the treatment of various diseases. Therefore, this study aimed to determine the amount of minerals, antioxidants and anti-radical activity of three Iranian *Berberis* ecotypes including *Black berberis bakhtiarica*, *Black berberis khorasanica* and *Red berberis integerrima* from which the latest is not stone fruits.

MATERIALS AND METHODS

1. Plant material

A total amount of 250 g of dried fruit from each ecotype were acquired from the medicinal herbs stores in Lordegan and Mashhad cities, Iran. The Dried fruit were cleaned from impurities and prepared for laboratory studies.

2. Chemicals

Aluminum chloride, methanol, potassium acetate, sodium acetate, Folin-Ciocalteu reagent, Gallic acid, Na₂CO₃, DPPH and BHA were purchased from Merck, USA. The highest purity available commercially of all other chemicals and reagents were used to ensure the best results.

3. Total flavonoids determination

Aluminum chloride colorimetric assay was used for flavonoids determination as described previously [32]. Each plant extracts (0.5 ml of 1:10 g ml⁻¹) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The solution was incubated at room temperature for 30 min then the absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA).

4. Total phenols determination

The Folin-Ciocalteu method was used to determine the total phenolic compound contents [33, 34]. A diluted sample of each plant extract (0.5ml of 1:10 g ml⁻¹) or gallic acid (standard phenolic compound) was mixed with Folin-Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na₂CO₃ (4 ml, 1 M). The mixtures

were left at room temperature for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg L⁻¹ solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg g⁻¹ of dry mass), which is a common reference compound [35].

5. DPPH radical-scavenging activity

Determination of free radical-scavenging activity of the extracts was performed using the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay [33]. Different concentrations of each extract were added, at an equal volume, to a methanolic solution of DPPH (100 μM). After incubation for 15 min at room temperature, the absorbance was recorded at 517 nm. The IC₅₀ values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals [35].

6. Digestion procedures

In order to compare the minerals and elements in dried fruit samples three sample digestion procedures were tested to prepare dry and wet digestion samples. While the dry digestion was mainly used to measure the amount of Fe, Zn, Cu, Mg and Cd, the wet digestion procedure was chosen for measuring N, P, Ca, Mn and K [36].

6.1. Dry digestion

A total of 1.0 g of dried fruit samples were placed into a high-form porcelain crucible. The furnace temperature was slowly increased to 450 °C. The samples were ashed for about 16 h until a white ash residue was obtained. The ash samples were treated with 5 ml HNO₃ (25% v/v) and the mixtures were heated slowly to dissolve the residue if needed. The resulting solutions were filtered through Whatman filter papers and then transferred to a 10 ml volumetric flask and the volume was adjusted for each sample. Blanks were prepared in the same way but omitting the sample. The determination of metal contents in this clear solution was carried out by ICP-OES. Three replicates (acid digests) were performed for each sample [36].

6.2. Wet digestion

For the digestion of samples, the temperature was maintained at 130 °C for 4 h during digestion of 1.0 g of sample with 6 ml HNO₃ (65%) and 2 ml H₂O₂ (30%) mixtures on the hot plate until complete solubilization of samples, then diluted to 10 ml with distilled water. The residue was filtered through Whatman filter paper and then the samples were diluted to 10 mL with distilled water. Element contents of final solution were determined by ICP-OES. Blanks were prepared in the same way omitting the sample. Three replicates (acid digests) were performed for each sample [36].

7. Statistical tests

All the experiments were performed in three biological replicates. The average and standard deviations were calculated using SPSS software version 22.

RESULTS AND DISCUSSION

The obtained results about flavonol, flavonoids and total phenolic contents in the fruit of three Iranian *Berberis* species are exhibited in Table 1. As shown, flavonol content of *Black berberis khorasanica* was 2 times more than *Black berberis bakhtiarica* and 5 times higher than *Red berberis khorasanica*. Flavonoids content in *Black berberis bakhtiarica* was over 2-fold higher than *Black berberis khorasanica* and over 3-fold higher than *Red berberis khorasanica*. The highest amount of phenolic content was related to *Black berberis bakhtiarica*.

Table 1. Amounts of flavonols, flavonoids and phenolic compounds found in the fruits of three Iranian *Berberis* species

Berberis species	Flavonol Mg/g	Flavonoid Mg/g	Phenol Mg/g	Total phenol Mg/g
<i>Black berberis khorasanica</i>	138.9 ± 2.8	21.73 ± 1.2	0.76 ± 0.021	241.23 ± 4.29
<i>Black berberis bakhtiarica</i>	57.43 ± 1.85	43.36 ± 1.55	0.89 ± 0.025	296.43 ± 7.46
<i>Red berberis khorasanica</i>	25.26 ± 1.65	12.23 ± 0.95	0.53 ± 0.02	157.3 ± 8.15

The anti-radical activities of three Iranian *Berberis* species are listed in Table 2. As seen, the anti-radical activity of *Black berberis bakhtiarica* is around 4 times higher than *Black berberis khorasanica* and 2 times higher than *Red berberis khorasanica*.

Table 2. Anti-radical activities (DPPH) of three Iranian Berberis species

Berberis species	IC ₅₀ µg/mL
<i>Black berberis khorasanica</i>	221.10 ± 5.60
<i>Black berberis bakhtiarica</i>	58.38 ± 7.53
<i>Red berberis khorasanica</i>	123.20 ± 6.48

The amounts of minerals and constituents in three Iranian Berberis species are presented in Table 3. In two species of *Black berberis bakhtiarica* and *Black berberis khorasanica*, the concentration of magnesium in *Black berberis bakhtiarica* was 2 fold higher than *Black berberis khorasanica* and Zn concentration in *Black berberis khorasanica* was 1.5 times more than *Black berberis bakhtiarica* while in other cases both of them were similar. Moreover, iron concentration in *Red berberis khorasanica* was about 5 times greater than *Black berberis*. Also, Zn content in *Black berberis* was lower than half in *Red berberis*. Nitrogen and Cu concentration in both *Black berberis* was lower than threshold.

Table 3. The concentration of elements in fruits of three Iranian Berberis species

Specimen	N %	P %	K %	Ca %	Mg %	Cd mg.kg ⁻¹	Fe mg.kg ⁻¹	Zn mg.kg ⁻¹	Cu mg.kg ⁻¹	Mn mg.kg ⁻¹
<i>Black berberis khorasanica</i>	2.06 ± 0.18	0.24 ± 0.01	1.53 ± 0.28	0.073 ± 0.015	0.197 ± 0.016	1.51 ± 0.22	468.83 ± 42.52	78.43 ± 9.87	47.37 ± 3.46	48.82 ± 1.92
<i>Black berberis bakhtiarica</i>	2.46 ± 0.14	0.26 ± 0.04	1.08 ± 0.08	0.173 ± 0.016	0.479 ± 0.023	1.18 ± 0.12	415.53 ± 26.82	55.37 ± 5.27	49.84 ± 3.03	46.37 ± 1.71
<i>Red berberis khorasanica</i>	1.28 ± 0.16	0.28 ± 0.04	1.06 ± 0.06	0.157 ± 0.021	0.541 ± 0.034	1.63 ± 0.28	2650.34 ± 290.57	27.53 ± 3.52	33.67 ± 2.69	58.61 ± 5.81

This study aimed to determine the amounts of minerals, antioxidants and anti-radical activity involved in the fruits of three Iranian Berberis species. Free radicals play an important and deteriorative effect in human health. Based on the results of present study Berberis fruit with marked antioxidants and anti-radical compounds can lead to prevent different diseases in human beings. The role of copper and zinc elements particularly in Cu/Zn-superoxide dismutase activity is very important[37]. and three Berberis fruits showed significant amount of both elements. Also in accordance with the work of Rezatalab who concluded that anti-oxidant properties of seed oil of *Berberis integerrima* was at high level [38]. The results of present study indicated that the amount of phosphorous, copper, zinc, iron, sodium and potassium were different among the fruits of studied Berberis species. This outcome is not in line with the published results of Berenji work concerning the concentration level of some elements which sounds to be justifiable with regard to measurement method, and different in crop harvesting [17]. Berberis has been recommended in various Iranian dishes. Traditional physicians also use it in order to treat many diseases. It seems that patients with more iron accumulation in their body, including thalassemia and patients treated with blood products should be cautious about using *Red berberis khorasanica* because of high iron level. Otherwise, this maintenance treatment approach will lead to iron accumulation in different tissues, damaging them. However, the antioxidant effect of this fruit might be useful in liver damage resulted from iron accumulation in liver tissue in thalassemic patients due to a crucial role in reduction of fat-soluble antioxidants[39]. So, to come up with this problem use of *Black berberis* should to be useful for the reason of higher amount of anti-oxidant and anti-radical activity in one hand and lower iron contents in the other hands. Furthermore, *Black berberis khorasanica* compared with other Berberis species like *Red Berberis* and some anthocyanin-rich fruits such as berries, blackberries, raspberries, and cranberries contain high level of anthocyanin. The results demonstrated that Berberis as Iranian native and wild fruit is rich in antioxidant compositions which could improve public health level [40,41]. According to the prevalence of iron deficiency anemia throughout the world especially in Iran, the prevalence of this illness in developing countries (estimated to be 28.5%, in southern Iran and 5-8% in industrialized countries), the prevalence of these diseases can be prevented in the community through incorporating more *Red berberis khorasanica* in their diets[42].

CONCLUSION

This study shows that the amount of antioxidant compounds in *Black berberis* of Chaharmahal Va Bakhtiari province was high. With regard to the role of zinc in physiological actions, both *Black berberis* of Khorasan and Chaharmahal Va Bakhtiari provinces contain significant amounts of these two elements. *Red berberis khorasanica*, used mostly in Iranian foods, had significant amounts of iron and seems to be beneficial for iron deficient patients; however, in patients with high iron storage it should be used with caution. Many medicinal plants native to Iran

contain pharmaceutical active ingredients and flavonoid antioxidants and flavonoids, and many medicinal and therapeutic effects due to the presence of these materials [42-74]. These plants may have the same effects and worth examining.

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