Journal of Medicinal Plants and By-products (2023) 2: 181-188
https://doi.org/10.22092/jmpb.2022.356303.1413

Original Article

Determination of Phenolic Compounds and Antioxidant Activities of 55 Iranian *Berberis* Genotypes

Zahra Behrad[1,2], Fatemeh Sefidkon[1*], Hossein Ghasemzadeh[2], Hassan Rezadoost[3] and Ahmad Balandari[4]

[1]*Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran*
[2]*Department of Chemistry, Imam Khomeini International University, Qazvin, Iran*
[3]*Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran*
[4]*Department of Food Safety and Quality Control, Research Institute of Food Science and Technology, Mashhad, Iran*

Article History

Received: 14 October 2021
Accepted in revised form: 05 December 2021

ABSTRACT

The genus *Berberis* belongs to the Berberidaceae family, with 15 genera and 650 species around the world. It has valuable potential in the medicinal and food industries. In this study, the phytochemical diversity of 55 fruits of the Iranian Berberis genotype were investigated. The results of this study could be used in the breeding and determination of superior genotypes in the future. Plant materials were collected from the barberry Collection Garden of Mashhad and also different natural habitats of barberry in various provinces of Iran. The samples were air-dried, finely grounded, and extracted by methanol at room temperature. Then, total phenol and total flavonoid were measured by Folin-Ciocalteu and AlCl3 assays, respectively. Radicals neutralizing effects of extracts were examined through the 2,2-Diphenyl-1- picrylhydrazyl (DPPH) method. The results showed a significant difference between phenolic content, flavonoid content, and antioxidant properties of various extracts, at a probability level of 1%. A methanol extract of *B. integerrima* × *crataegina* (genotype code 4-1) resulted in the highest phenolic content with an average of 4.2 mg gallic acid equivalents (GAE) ml^{-1} extract, while the lowest content was recorded for *B. integerrima* (genotype code 23-4) with an average of 1.7 mg gallic acid equivalents (GAE)/ ml extract. The highest flavonoid content was observed with extracts of *B. integerrima* (genotype code 4-4) and *B. orthobotrys* × *crataegina* (genotype code 15-4) with an average of 6.3 mg quercetin equivalents (Q)/ ml extract. The least was recorded for *B. integerrima* (genotype code 23-4) with an average of 0.4 mg quercetin equivalents (Q)/ ml extract. The highest and lowest rates of free radical scavenging DPPH were 59.06% and 12.3%, respectively. The results showed that barberry has a great diversity in terms of phytochemical characteristics in different genotypes and is a valuable genetic source for breeding research.

Keywords
Phenolic compound
Folin-Ciocalteu
Flavonoid
Antioxidant
Berberis

INTRODUCTION

The genus *Berberis* belongs to the Berberidaceae family, containing 15 genera and 650 species found in the northern hemisphere. In the southern hemisphere, the genus *Berberis* is found in the temperate zone of South America. It is the largest genus in this family, including more than 500 species [1,2]. They can be found as shrubs and small trees with evergreen leave and thorny shoots. The flowers are single or aggregate and arranged as spikes [3]. In Iran, five wild species were reported, including *Berberis vulgaris*, *B. orthobotrys*, *B. khorasanica*, *B. crataegina*, and *B. integerrima*. The above-mentioned species contain seeds. Seedless barberry is cultivated in South Khorasan region (East of Iran) and different names are reported for this cultivar in literature including: *B. vulgaris*, *B. orientalis* Ck Schh.var. asperma and *B. vulgaris* L var. asperma [4].

*Correspondence author: Department of Chemistry, Imam Khomeini International University, Qazvin, Iran
Email Address: sefidkon@gmail.com

Based on research from the last decade, it has been identified as *B. integerrima* [5, 6, 7].

Extracts and herbal teas have shown antibacterial, antiviral, and antifungal properties [8]. Researchers reported polyphenolic and flavonoid compounds (Rutin, Quercetin, kaempferol) in barberry fruit.These compounds are antioxidants in lowering blood pressure, heart rate regulation, anticholinergic, anti-inflammatory, antibacterial, and antifungal healing effects on liver disorder [9], cardiovascular system, nervous system, hypertension treatment, epilepsy, and cramping [10]. Fruits are a source of phenols and antioxidants and contain nutrients and minerals [11]. The occurrence of phenolic compounds, tannin, and alkaloid was demonstrated by phytochemical analysis of *B. vulgaris* extract [12]. The barberry phenol compounds contain anthocyanins and carotenoid pigments [13].

Phenolic compounds and polyphenols are diverse chemicals containing simple phenol and complex polymers such as flavonoid and anthocyanin pigments. Flavonoids and anthocyanins act as an antioxidant in a biological system. This plant has a complex chemical composition that, in some cases, its synthesis in laboratory conditions is difficult or even impossible. Also, the quality and quantity of chemical compounds are affected by environmental factors and plant genotype. Therefore, studying the chemical compound variation of different plant species such as barberry, grown and cultivated under different climate and field conditions, is a priority in the valuable medicinal plants research field. In this study, the phytochemicals were screened to evaluate the total flavonoid and phenolic contents and antioxidant activity of methanol extract of 55 Iranian *Berberis* genotypes.

MATERIALS AND METHODS

Chemicals and Reagents

Sodium carbonate, Folin–Ciocalteu reagent, sodium acetate, aluminum chloride, hydrochloric acid from Merk Co. (Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and Phenolic standards (gallic acid, quercetin) were procured from Sigma–Aldrich Chemical Co. (USA). Other chemicals and solvents had an analytical grade.

Plant material and site description

The 32 barberries (*Berberis* spp.) accessions originated from the Barberry Collection Garden at the Research Institute of Food Science and Technology, Mashhad, Iran. The Barberry Collection Garden is located at 36° 44′N latitude, 59° 4′E Longitude, and 1116 m above the sea level with an annual average temperature of 14.3 °C, and 251 mm precipitation. The names of the genotypes are given in Table 1. The 23 barberry accessions were collected from different natural habitats of barberry (Table 1). The samples were collected during Sep–Oct 2019. The plant identification was determined by Mr. Mohammadreza Joharchi in the Research Institute of Plant Sciences, Ferdowsi University of Mashhad. The fresh ripe fruits were dried in the shade, powdered, and then used for extraction.

Phenolic Extraction Procedure

Dried fruit samples (1 g) were grounded and soaked in 30 ml of methanol: water (80:20) for 1 h by shaking. The extracts were filtered and dried by vacuum. Finally, it was dissolved in 10 ml of methanol-water (80:20) and stored in a refrigerator at 4 °C for further use.

Flavonoids Extraction Procedure

For this purpose, 2 grams of dried barberry of each genotype code was ground and then poured in vials. Then, 20 ml of 50% methanol was set in the fridge for 24 hours and then extracted. After 24 hours, the upper layer of the liquid was moved to another container and the leftover was again extracted with 20 ml of methanol 50%. Finally, both liquids were mixed and filtered with specific paper. 9 ml of the extract was dried with rotavapor at 35 °C and was solved in 3.5 ml of methanol 50% -Dimethyl Sulfoxide (ratio 50-50) and stored in a refrigerator at 4 °C for further use.

Total Phenolics (TP) Analysis

The amount of total phenolics in extracts was determined according to the spectrophotometric method based on Folin-Ciocalteu's procedure [15]. Briefly, 0. 5 ml of diluted extract and 0.45 ml water were mixed with 2.5 ml of 1:10 diluted Folin–Ciocalteu's phenol reagent, followed by 2 ml of 7.5% (w/v) sodium carbonate.

After 5 min at 50 °C, absorbance was measured at 760 nm. Phenol content was estimated from a standard curve of gallic acid, and results were expressed as mg gallic acid equivalents (GAE)/ ml extract.

Table 1 Main characteristics of selected sites of *Berberis*

No	Genotype code	Species name *	Region of Iran	Latitude	Longitude	Altitude (m asl **)
1	1-4	*B. integerrima*	Tehran Province	354851	505933	1236
2	2-4	*B. vulgaris*	Tehran Province	354851	505933	1236
3	3-4	*B. integerrima*	Golestan Province	361433	540120	1565
4	4-4	*B. integerrima*	North Khorasan Province	412081	496885	1735
5	5-4	*B. integerrima*	Kohgiluyeh and Boyer-Ahmad Province	302526	514549	2500
6	6-4	*B. integerrima*	Chaharmahal and Bakhtiari Province	347653	524158	2620
7	7-4	*B. vulgaris*	Isfahan Province	326212	514641	1612
8	8-4	*B. integerrima*	Isfahan Province	326212	514641	1612
9	9-4	*B. vulgaris*	South Khorasan Province	334359	591433	1453
10	10-4	*B. integerrima*	Razavi Khorasan Province	364879	580944	1770
11	11-4	*B. integerrima*	Semnan Province	363501	544309	2657
12	12-4	*B. integerrima*	Semnan Province	363501	454309	2661
13	13-4	*B. integerrima*	Semnan Province	363501	454309	2661
14	14-4	*B. orthobotrys*	Golestan Province	364125	543413	1862
15	15-4	*B. ortho* × *crat.*	Golestan Province	364044	54344	2167
16	16-4	*B. ortho* × *integ.*	Alborz Province	360113	510901	1783
17	17-4	*B. vulg* × *integ.*	Alborz Province	360112	510900	1798
18	18-4	*B. ortho* × *crat.*	Mazandaran Province	361451	511809	1895
19	19-4	*B. ortho* × *crat.*	Mazandaran Province	361451	511809	1896
20	20-4	*B. ortho* × *integ.*	Mazandaran Province	360404	530405	732
21	21-4	*B. ortho* × *integ.*	Mazandaran Province	360404	530405	711
22	22-4	*B. integerrima*	Ghazvin Province	362541	500510	1833
23	23-4	*B. integerrima*	Zanjan Province	362141	481224	1613

* The name of species and hybrids are still under investigation and are not defined. The naming was done by studying 40 morphologic investigations [14]

** meters above sea level (m asl)

Calibration graphs (Fig. 1) were plotted subsequently for linear regression analysis of the peak area with concentration 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 µg/ml.

Total Flavonoids (TF) Analysis

The flavonoid content was determined according to the spectrophotometric method based on the formation of the aluminum-flavonoid complex [16]. All analyses were carried out in triplicate. An aliquant of AlCl3 solution (0.5 mL, 2 %, w/v) was added to 1 ml of the test solution (standard or sample), and subsequently, 0.5 mL of water, HCl, CH3COONa, or CH3COONH4 (each at a concentration of 1 M) was added. The concentrations of standard solutions of flavonoids were 100 µM. The mixture was vigorously shaken and then subjected to spectral analysis at 425 nm after 10 min of incubation at room temperature. The amount of AlCl3 solution was substituted by the same amount of water in the blank. Flavonoid content was estimated from a standard curve of quercetin, and results were expressed as mg quercetin equivalents (Q)/ml extract. Calibration graphs (Figure 2) were plotted subsequently for linear regression analysis of the peak area with concentrations 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 µg/ml.

Antioxidant Capacity by DPPH Assay

The extract DPPH radical scavenging activity was obtained against the stable free radical DPPH described previously [17]. Briefly, one ml of each sample (1 mg/ml) was incubated with a methanolic solution of DPPH lM. After 30 min of incubation at room temperature, the absorbance at 517 nm was measured by a spectrophotometer. The percentage of inhibition (%I) of the radical was calculated according to the following formula:

$$\%I = [(ADPPH - AP)/ADPPH] \times 100$$

where ADPPH and AP presented the absorbance of the DPPH solutions containing ethanol and plant extracts, respectively.

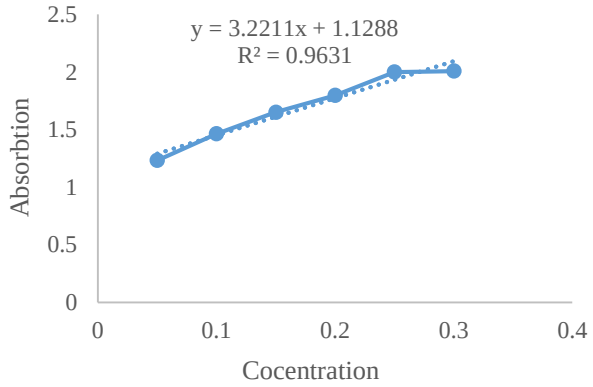

Fig. 1 Calibration curve of gallic acid

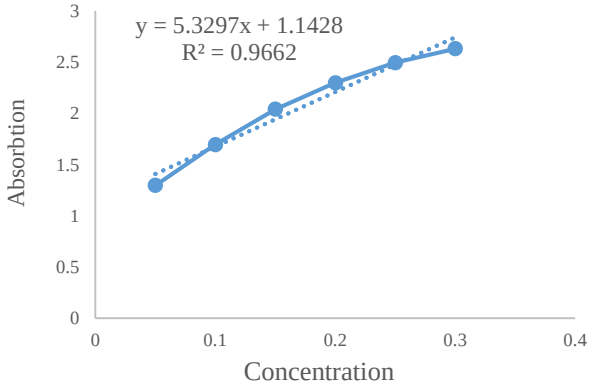

Fig. 2 Calibration curve of quercetin

Statistical analysis

This project was implemented by using a completely randomized design with three replications and 55 accessions. Analysis of variance (ANOVA) was accomplished by using the SAS software (version 9.3). The mean comparisons were performed by Duncan's multiple range test (DMRT) at a 5% probability level.

RESULTS AND DISCUSSION

Total phenol (TP) and total flavonol (TF) Contents

Analysis of variance of the data showed a significant difference in the amount of phenol and flavonoid of the extracts from different genotypes at the level of 1% probability (Table 2).

The results of the mean comparison showed that the highest and lowest flavonoids measured in different genotypes were in separate groups A and T. The rest of the genotypes were grouped as an intermediate between these two statistical groups. The observed variation in phenol was divided into three separate statistical groups, A, B, and S, and the other genotypes showed an intermediate statistical grouping. *B. integerrima* × *crataegina* (genotype code 4-1) with an average of 4.2 had the highest phenol content, and *B. integerrima* (genotype code 23-4) with an average of 1.7 had the lowest. The flavonoid in *B. integerrima* (genotype code 4-4) and *B. orthobotrys* × *crataegina* (genotype code 15-4) with an average of 6.3 had the highest content, and in *B. integerrima* (genotype code 23-4) with an average of 0.4 had the lowest (Table 3).

In the study of Akbulut *et al*. (2009), the total phenolic content of fresh fruits of *B. vulgaris* in Turkey has been reported to be 789.32 ±88.50 mg/100 g [18]. Total phenol content in barberry genotypes was reported as 689.82, 675.68, and 702.94 [19]. Sasikumar *et al.* (2012) found the value of total phenolic as 410 ± 0.02 mg/100 g for the fresh barberry fruits [20]. Total phenol content as 8530 and 3450 mg/100 g fresh fruit was reported for *Berberis integerrima* and *B. vulgaris*, respectively [21]. In a study, Yıldız *et al*., (2014) found total phenolic values of barberry fruits ranged from 2500 mg to 3720 mg GAE/L of fruit juice [22]. The total phenolic content of fruits of barberry genotypes in the Central Anatolia region of Turkey has been reported between 2560- 3630 mg GAE per L. [23]. Some studies have shown, the amount of measured phenol in fresh samples is higher than in dried samples [24,25]. On the other hand, Vinson *et al.* (2005) showed that the level of the phenolic compounds of dried fruits is higher than fresh fruits [26]. The total amount of phenol and flavonoid obtained in this study has great diversity in all genotypes. Genotype and environmental conditions affect the biosynthesis of phenol and flavonoid accumulation in different parts of the plant [27, 28]. Phenolic compounds are highly effective free radical scavengers and antioxidants [29]. In the study of Sasikumar *et al*. (2012), the total flavonoid content of fresh fruit barberry has been reported to be 320 mg equal to Eq quercetin/100 g [20]. Awan *et al*. (2014) stated total flavonoids as 385.52, 376.93, and 395.09 [19]. Pyrkosz-Biardzka *et al*. (2014) found that the methanolic extract of *B. vulgaris* contains significant amounts of phenolic compounds and flavonoids [30]. In a study conducted by Balandari *et al*. (2017), a different method of analysis and extraction was done on 12 genotypes of the understudy genotypes. A significant difference was seen on genotypes, according to the total phenol and flavonoid. Genotype code 5-1 with the average mean 1482/61 mg GAE/100g fruit dry weight (DW), and after that code 4-1 had the highest total phenol content. Genotype code 12-1 had the lowest amount

with a mean of 756/30 mg GAE/100g fruit dry weight (DW). The total amount of phenol in genotype codes 4-1 ،2-2 ،5-2 ،5-3 ،11-1 ،10-1 and seedless were not significantly different from 5-1 code. There are various reports about the amount of phenol in seedless barberry, referred to different extractions and measurements. Among the studied genotypes, genotype code 14-2 with 837/53 mg QUE/100g fruit DW had the highest amount of flavonoid due to the high anthocyanin of this genotype. After this genotype code, 5-1,11-1, seedless,12-1 and 10-1 were placed respectively which all had dark color except seedless genotype. It shows the high amount of flavonoid (492/56 mg QUE/100g Fruit DW) and anthocyanin in seedless barberry (significant difference with genotype code 14-2). The lowest amount was genotype code 13-1 (219/72 mg QUE/100g Fruit DW) [31].

DPPH radical-scavenging activity

The analysis of variance showed that the percentage of antioxidant activity was significantly different between the genotypes at the level of 1% probability (Table 1).

Comparing the means showed that according to the obtained statistical grouping, due to a large number of genotypes and the differences between them, the genotypes were divided into two separate statistical groups A and U, and more than ten intermediate groups. The results of the mean comparison table showed that *B. integerrima* (genotype code seedless), with an average of 59.06%, had the highest antioxidant activity. After that, *B. integerrima* (genotype code 4-23) with an average of 54.9% was in the next group. The level of antioxidant activity decreased from 59.06 to 12.3% in *B. orthobotrys* × *crataegina* with genotype code 18-4 (Table 5).

This method is based on the reduction of free radical DPPH methanolic solution by antioxidant compounds such as phenols. These compounds have hydrogen donor groups and lead to the formation of non-radical DPPH forms. In this state, the color of DPPH containing solution changes from purple to dark yellow, and the absorbance drops at 517nm [32, 28]. The antioxidant property of 15 barberry genotypes was assessed by four methods, DPPH, ferric reducing antioxidant power (FRAP), beta-carotene linoleic acid, and total antioxidant activity based on the TFPL method. The results showed that the total antioxidant activity in different barberry genotypes was directly related to phenolic compounds. Moreover, genotypes with the higher phenolic compounds, especially total phenols, had higher antioxidant properties. Comparing antioxidant activity in different methods for berry genotypes showed that total antioxidant activity based on the TFPL method is a better technique for measuring antioxidant activity in barberry genotypes. It is due to its higher association with antioxidant compounds such as phenols [33]. Hassanpour and Alizadeh (2016) found a significant correlation among all three antioxidant assays (DPPH, Fe2 + chelating and ferric reducing antioxidant power (FRAP) [34]. Hosseini *et al*. (2014) studied several existing laboratory methods to evaluate the inhibitory capacity based on its work, method, and strengths and weaknesses. Their studies showed that despite the different methods for assessing antioxidant capacity, there is a gap in the standardization of antioxidant capacity measurement. There is a theoretical consensus among researchers that the use of several methods combination can be useful to assess antioxidant capacity in laboratory conditions [35]. Some studies have shown a positive correlation between phenolic contents and antioxidant activity in plants [20, 28,36, 37]. On the other hand, some authors could not find such a relationship [28,38,39].

In the present study, all accessions contained significant phenolic contenets and antioxidant activity. Motalleb *et al*. (2005) did not find any relationship between antioxidant activity and total phenolic contents in *B. vulgaris* fruit extract; however, they found that *B. vulgaris* fruit extract had higher antioxidant activity in ethanol than water. Based on that, Motalleb *et al*. (2005) concluded that *B. vulgaris* fruit in each type of solvent had a different antioxidant activity. It could be attributed to different antioxidant components such as β-carotene, vitamin C, butylhydroxytoluene, and phenolic compounds [40].

Phenotype is a function of the genotype, the environment, and the differential phenotypic responses of genotypes to different environments, also known as genotype by environment interactions [41]. Phytochemical content is affected not only by genetic variation, but also by environmental conditions and seasonal and year-to-year differences [42].

Table 2 Analysis of variance of difference in the phenol and flavonoid content of extracts between different accessions

S.O.V	df	Average of squares	
		phenol	Flavonoid
bloc	2	0.03 ^{ns}	7.3 **
accessions	54	0.9 **	7.7 **
error	108	0.08	0.7
Cv (%)	-	11.4	24

ns and ** mean non-significance and significance at the level of 1% probability, respectively.

Table 3 Mean comparison of the accessions as the phenol and flavonoid contents

Genotype code	Flavonoid content	Phenol content	Genotype code	Flavonoid content	Phenol content
1-1	2.3 m-s	2.8 c-i	10-3	2.6 k-s	2.4 g-q
2-1	3.3 h-q	3.7 b	11-3	5.6 a-d	2.1 ls
4-1	2.8 j-s	4.2 a	12-3	1.3 rst	2.3 k-s
5-1	3.3 h-q	2.4 g-p	seedless	4.2 c-l	2.4 g-q
7-1	1.4 rst	3.4 bc	1-4	4.2 c-l	2.4 e-p
8-1	2.04 o-t	2.5 e-n	2-4	6.05 ab	3.4 bcd
9-1	3.7 f-n	1.9 p-s	3-4	4.2 c-l	1.8 rs
10-1	1.9 p-t	1.9 o-s	4-4	6.3 a	1.8 rs
11-1	5.9 abc	3.3 bcd	5-4	5.4 a-f	2.6 e-n
12-1	2.3 m-s	2.3 h-s	6-4	4.9 a-h	2.5 e-o
13-1	2.1 n-t	2.3 i-s	7-4	5.1 a-g	2.2 k-s
14-1	2.2 m-s	2.1 k-s	8-4	4.6 a-i	3.7 B
1-2	3.7 g-o	2.3 j-s	9-4	6.1 Ab	2.2 k-s
2-2	5.7 a-d	2.8 d-j	10-4	4.6 b-j	2.3 h-r
3-2	3.3 h-q	2 n-s	11-4	5.1 a-g	1.9 o-s
4-2	2.6 l-s	3.4 bcd	12-4	1.6 q-t	1.8 Rs
5-2	4.8 a-h	2.3 i-s	13-4	2.9 i-r	2.3 j-s
8-2	1.7 q-t	1.8 qrs	14-4	4.3 b-k	2.5 f-p
10-2	4.7 a-h	2.4 g-p	15-4	6.3 A	1.9 o-s
11-2	1.6 q-t	1.9 o-s	16-4	1.8 q-t	2.6 e-m
12-2	3.5 g-p	1.8 rs	17-4	1.2 st	1.9 o-s
13-2	2.7 k-s	3.05 c-f	18-4	3.9 e-m	2.9 c-g
14-2	4.5 b-j	2.4 g-p	19-4	5.5 a-d	2.7 e-k
2-3	4.9 a-h	2.4 g-p	20-4	6.02 ab	3.07 cde
3-3	3.3 h-q	2.3 i-s	21-4	1.8 q-t	1.8 qrs
4-3	1.5 rst	2.3 h-s	22-4	3.7 g-o	2.09 m-s
5-3	1.4 rst	2.7 e-l	23-4	0.4 t	1.7 s
8-3	4.1 d-l	2.9 c-h	-	-	-

similar letters indicate no significant difference between the means

Table 4 Analysis of variance of difference in the percentage of antioxidant activity between different accessions

S.O.V	df	Antioxidant activity
accessions	54	381.03 **
error	110	25.14
Cv (%)	-	14.2

** stand for significant at 1%

Table 5 Comparison of the mean of different accessions as a percentage of antioxidant activity

Genotype code	Species name *	Antioxidant activity (%)	Genotype code	Species name *	Antioxidant activity (%)
1-1	*B. integerrima* × *crataegina*	51.2 abc	10-3	*Berberis spp.*	38.6 g-k
2-1	*B. integerrima* × *crataegina*	36.8 h-l	11-3	*B. integerrima* × *crataegina*	36.4 h-l
4-1	*B.integerrima* × *crataegina*	39.2 e-j	12-3	*B. integerrima* × *crataegina*	38.9 f-i
5-1	*B. integerrima* × *crataegina*	20.1 q-u	seedless	*B. integerrima*	59.06 a
7-1	*B.integerrima* × *crataegina*	48.9 b-e	1-4	*B. integerrima*	48.7 b-f
8-1	*B. integerrima* × *orthobotrys*	34.4 h-m	2-4	*B. vulgaris*	24.7 m-s
9-1	*B. orthobotrys*	50.3 a-d	3-4	*B. integerrima*	33.7 i-n
10-1	*B. integerrima*	29 k-q	4-4	*B. integerrima*	48.1 b-g
11-1	*B. integerrima*	16.7 r-u	5-4	*B. integerrima*	27.8 l-q
12-1	*B. integerrima*	15.4 r-u	6-4	*B. integerrima*	42.4 c-i
13-1	*B. vulgaris*	28.2 l-q	7-4	*B. vulgaris*	30.1 j-p
14-1	*B. integerrima* × *crataegina*	24.03 n-s	8-4	*B. integerrima*	38.5 g-k
1-2	*B. integerrima* × *crataegina*	19.4 q-u	9-4	*B. vulgaris*	30.3 j-p
2-2	*B. vulgaris*	41.9 c-i	10-4	*B. integerrima*	36.5 h-l
3-2	*B. integerrima* × *crataegina*	48.9 b-e	11-4	*B. integerrima*	13.9 tu
4-2	*B. vulgaris*	28.3 l-q	12-4	*B. integerrima*	42.1 c-i
5-2	*B. vulgaris*	25.9 m-r	13-4	*B. integerrima*	49.2 b-e
8-2	*B.integerrima* × *crataegina*	47.2 b-g	14-4	*B. orthobotrys*	30.5 j-p
10-2	*Berberis spp.*	42.3 c-i	15-4	*B. orthobotrys* × *crataegina.*	17.2 r-u
11-2	*B. integerrima*	28.5 l-q	16-4	*B. orthobotrys* × *integerrima*	32.4 i-p
12-2	*B. orthobotrys*	47.9 b-g	17-4	*B. vulgaris* × *integerrima*	36.4 h-l
13-2	*B. vulgaris*	32.6 i-o	18-4	*B. orthobotrys* × *crataegina*	12.3 u
14-2	*B. integerrima*	39.4 c-h	19-4	*B. orthobotrys* × *crataegina*	22.7 o-t
2-3	*B. crataegina* × *vulgaris*	30.3 j-p	20-4	*B. orthobotrys* × *integerrima*	41.8 d-i
3-3	*B. integerrima* × *crataegina*	41.6 c-i	21-4	*B. orthobotrys* × *integerrima*	39.5 e-j
4-3	*B.integerrima* × *crataegina*	50.5 a-d	22-4	*B. integerrima*	22.6 p-t
5-3	*B. integerrima* × *orthobotrys*	43.9 c-h	23-4	*B. integerrima*	54.9 ab
8-3	*B. vulgaris*	30.05 j-p	-	-	-

similar letters indicate no significant difference between the means

* The name of species and hybrids are still under investigation and are not defined. The naming was done by studying 40 morphologic investigations [14]

For instance, the phytochemical and antioxidant activity of 172 soybeans (*Glycine max* L.) landraces differed between two cultivation years [43]. The phytochemical composition and biological activity of Parkia speciosa seeds varied significantly depending on where the plants had been cultivated [44]. The obtained results of the study also showed the efficient role of the growth location due to their different ecological and climatic characteristics on the accumulation of secondary metabolites. Different phenol and flavonoid contents were observed in all accessions due to the similarity of the samples in species, solvents, and extraction methods. Existing differences in the growing area, changes in ambient temperature, changes in the intensity of solar radiation, and annual rainfall can be the reasons for the observed differences in the synthesis and accumulation of the collected plant compounds. The main purpose of studying intraspecific diversity is to preserve genetic resources and ultimately preserve and propagate them [45]. Research has proven that plants belonging to a species that grow in different ecological and geographical conditions show distinct characteristics that lead to different populations [46]. Gohari *et al*. (2011) examined the antioxidant activity of some medicinal plants and several species of the mint family. They reported that the chemical composition of the extracts varied depending on the geographical area, type of tissue, and harvest time [47]. Research on medicinal species has shown a direct relationship between increasing altitude and ecological stress with the amount of phenolic and flavonoid substances and antioxidant activities of plant extracts [48].

CONCLUSION

The present study focused on phenolic compounds and antioxidant activities of 55 Iranian Berberis genotypes. The results showed that Barberry has high diversity in phytochemical properties in different genotypes and is a valuable genetic source for breeding research. The highest phenolic content was observed with extract of *B. integerrima* × *crataegina* (genotype code 4-1), and the highest flavonoid content was observed with extracts of *B. integerrima* (genotype code 4-4) and *B. orthobotrys* × *crataegina* (genotype code 15-4). Also, the highest rates of free radical scavenging DPPH were observed with extracts of *B. integerrima* (genotype code seedless). These genotypes need further investigation to determine the superior genotype in the future. The present information is published for the first time.

REFERENCES

1. Bhardwaj D., Kaushik N. Phytochemical and pharmacological studies in genus Berberis. Phytochem Rev. 2012; 11:523–542.
2. Ahrendt L., Berberis and Mahonia: a taxonomic revision. Bot J Linn Soc. 1961; 57:1–410.
3. Bottini M.C.J., De Bustos A., Jouve N., Poggio L. AFLP characterization of natural populations of Berberis (Berberidaceae) in Patagonia Argentina. Plant Sys & Evolution. 2002; 231: 133-142.
4. Kafi M., Balandary A. Berberis: Production and Processing. Zaban va Adab Press. 2002; 210 p. [In Persian]
5. Heydary S., Marashi H., Farsi M., Mirshamsi Kakhki, A. Assessment of genetic structure and variation of native Berberis populations of Khorasan provinces (Iran) using AFLP markers versus morphological markers. Iranian J Biotech. 2009; 7:101–107.
6. Sodagar N., Bahrami A.R., Memariani F., Ejtehadi H., Vaezi J., Khosravi A.R. Biosystematic study of the genus Berberis L. (Berberidaceae) in Khorassan, NE Iran. Plant Syst Evol. 2012; 298:193-203.
7. Rezaei M., Ebadi A., Reim S., Fatahi R., Balandary A., Farrokhi N., Hanke M.V. Molecular analysis of Iranian seedless barberries via SSR. Scientia Horticulturae. 2011; 129:702–709.
8. Gosh A.K., Bhattacharyya F.k., Ghosh D.K. Leishmania donovani: Amastigote inhibition and mode of action of berberin. Exp Parasite. 1985; 60:404-413.
9. Jiménez C.D.C., Flores C.S., He J., Tian Q., Schwartz S.J., Giusti M.M. Characterisation and preliminary bioactivity determination of Berberis boliviana Lechler fruit anthocyanins. Food Chem. 2011; 128: 717-724.
10. Rajaian H., Jalaee J., Aghajani A. Berberis vulgaris as growth promoter in broiler chickens. Int J Poultry Sci. 2006; 5: 395-397.
11. Belwal T., Dhyani P., Bhatt I.D., Singh Rawal R., Pande V. Optimization extraction conditions for improving phenolic content and antioxidant activity in Berberis asiatica fruits using response surface methodology (RSM). Food Chem. 2016; 207:115-124.
12. Rounsaville T.J., Ranney T.G. Ploidy levels and genome Sizes of Berberis L. and Mahonia Nutt. Species, hybrids, and cultivars. HortScience. 2010; 45: 1029–1033.
13. Minaiyan M., Ghannadi A., Mahzouni P., Jaffari-Shirazi E. Comparative Study of Berberis vulgaris Fruit Extract and Berberine Chloride Effects on Acetic Acid-Induced Colitis in Rats. Iranian J Pharmaceutical Res. 2011; 10: 97-104.
14. Balandari A., Joharchi M.R., Kaveh M. Morphological studies phase report of the evaluation plan for registration and reproduction of the top populations of the barberry collection garden of Iran. Food industrial and Technology Research Institute and the headquarters for the development of science and technology of medicinal plants and traditional medicine. 2019. [In Persian]
15. Pantelidis G.E., Vasilakakis M., Manganaris G.A., Diamantidis G.R. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. Food Chem. 2007; 102: 777–783
16. Pękal A., Pyrzynska K. Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay. Food & Methods. 2014; 7:1776–1782
17. Brand-Williams W., Cuvelier M.E., Berset CLWT. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci and Tech. 1995; 28: 25–3.
18. Akbulut M., Ercisli S., Tosun M. Physico-chemical characteristics of some wild grown European elderberry (Sambucus nigra L.) genotypes. Pharmacog Magazine. 2009; 5: 320-323.
19. Awan M.S., Ali S., Ali A., Hussain A, Ali M. A comparative study of barberry fruits in terms of its nutritive and medicinal contents from CKNP Region, Gilgit-Baltistan, Pakistan. J Bio & Env Sci. 2014; 5: 9-17.
20. Sasikumar J.M., Maheshu V., Smilin A.G., Gincy M.M., Joji C. Antioxidant and antihemolytic activities of common Nilgiri barberry (*Berberis tinctoria* Lesch.) from south India. Int Food Res J. 2012; 19: 1601- 1607.
21. Ardestani S.B., Sahari M.A., Barzegar M., Abbasi S. Some physicochemical properties of Iranian native barberry fruits (abi and poloei): Berberis integerrima and Berberis vulgaris. J Food & Pharmaceutical Sci. 2013; 1: 67-74.
22. Yıldız H., Ercisli S., Sengul M, Topdas EF, Beyhan O, Cakir O, Narmanlioglu HK, Orhan E. Some physicochemical characteristics, bioactive content and antioxidant characteristics of non-sprayed Barberry (Berberis vulgaris L.) fruits from Turkey. Erwerbs-Obstbau. 2014;56: 123-129.

23. Ozgen M., Saracoglu O., Gecer E.N. Antioxidant capacity and chemical properties of selected barberry (Berberis vulgaris L.) fruits. Hort Environ Biotech, 2012; 53: 447-451.
24. Siow L.F., Hui Y.W. Comparison on the antioxidant properties of fresh and convection oven-dried guava (Psidium guajava L.). Inter Food Res J. 2013; 20: 639-644.
25. Beyhan Ö., Elmastaş M., Gedikli F. Total phenolic compounds and antioxidant capacity of leaf, dry fruit and fresh fruit of feijoa (Acca sellowiana, Myrtaceae). J Medicinal Plants Res. 2010; 4:1065-1072.
26. Vinson J.A., Zubik L., Bose P., Samman N., Proch J. Dried Fruits: Excellent in vitro and in vivo Antioxidant. J the American college of Nutrition. 2005; 24: 44-50.
27. Kawashty S.A., Mosharrafa S.A.M., EI-Gibali M., Saleh N.A.M. The flavonoids of four Pistacia species in Egypt. Biochemical Systematic and Ecology. 2000; 28: 915-917.
28. Sharifi F., Poorakbar L. The survey of antioxidant properties of phenolic compounds in fresh and dry hybrid Barberry fruits (Berberis integerrima× vulgaris). Sci J (CSJ). 2015; 36:1609-1617.
29. Van Acker S.A.B.E., Van Den Berg D.J., Tromp M.N.J.L., Griffioen D.H., Van Bennekom W.P., Van der Vijgh W.J.F., Bast A. Structural aspects of antioxidant activity of flavanoids. Free Radical Biology and Medicine. 1996; 20: 331-342.
30. Pyrkosz-Biardzka k., Kucharska A.Z., Sokół-Łętowska A., Strugała P., Gabrielska J. A Comprehensive Study on Antioxidant Properties of Crude Extracts from Fruits of Berberis vulgaris L., Cornus mas L. and Mahonia aquifolium Nutt. Pol J Food Nutr Sci. 2014; 64: 91-99.
31. balandari A., Azizi M., Khodabandeh M. Investigation and comparison of some bioactive and nutritious compounds of twelve native barberry masses with emphasis on their antioxidant properties. Cooperative Research Project of Food Industrial and Technology Research Institute and Fund for Researchers and Technologists support of the country. 2017. [In Persian]
32. Paixao N., Perestrelo R., Marques J.C., Camara J.S. Relationship between antioxidant capacity and total phenolic content of red, rose´and white wines. Food Chem. 2007; 105: 204 –214.
33. Talebi S., Alizadeh M., Ramezanpour S.S., Ghasemnezhad A. The antioxidant properties of some endemic barberry genotypes of Iran. Iranian J Hortic Sci. 2020; 51:91-107. [In Persian]
34. Hassanpour H., Alizadeh S. Evaluation of phenolic compound, antioxidant activities and antioxidant enzymes of barberry genotypes in Iran. Sci Hortic. 2016; 200: 125-130.
35. Hosseini S., Gharachorlo M., Ghiasi Tarzi B., ghavami M. An overview of antioxidant capacity determination methods (basis of the reaction, method and strength & weekness point of view). Food Technol & Nutrition. 2014; 11: 89-111. [In Persian]
36. Brighente I.M.C., Dias M., Verdi L.G., Pizzolatti M.G. Antioxidant activity and total phenolic content of some Brazilian species. Pharmaceutical Biology, 2007; 45: 156-161.
37. Okatan V., Colak A.M. Chemical and phytochemical content of barberry (Berberis vulgaris L.) fruit genotypes from sivasli district of usak province of western Turkey. Pakistan J Botany. 2019; 51: 165-170.
38. Agbor G.A., Oben J.E., Ngogang J.Y., Xinxing C., Vinson J.A. Antioxidant capacity of some herbs/spices from Cameroon: A comparative study of two methods. J Agric & Food Chem. 2005; 53: 6819-6824.
39. Zovko Koncˇic M., Kremer D., Karlovic K., Kosalec I. Evaluation of antioxidant activities and phenolic content of Berberis vulgaris L. and Berberis croatica Horvat. Food and Chemical Toxicology. 2010; 48: 2176–2180
40. Motalleb G., Hanachi P., Kua SH., Fauziah O., Asmah R. Evaluation of phenolic content and total antioxidant activity in Berberis vulgaris fruit extract. J Biol Sci. 2005; 5: 648–653.
41. Falconer D.S. Selection for large and small size in mice. J Genet. 1953; 51: 470–501
42. Lee K.J., Baek D.Y., Lee G.A., Cho G.T., So Y.S., Lee J.R., Ma KH, Chung J.W., Hyun D.Y. Phytochemicals and Antioxidant Activity of Korean Black Soybean (*Glycine max* L.) Landraces. Antioxidants. 2020; 9: 213.
43. Kumar B.S.A., Lakshman K., Jayaveera K.N., Shekar D.S., Kumar A.A., Manoj B. Antioxidant and antipyretic properties of methanolic extract of Amaranthus spinosus leaves. Asian Pac J Trop Med. 2010; 3:702–706.
44. Ghasemzadeh A., Jaafar H.Z., Bukhori M.FM., Rahmat M.H., Rahmat A. Assessment and comparison of phytochemical constituents and biological activities of bitter bean (Parkia speciosa Hassk.) collected from different locations in Malaysia. Chem Cent J. 2018; 12: 1-9.
45. Rezapour M., Fattahi M. Evaluation of diversity in some morphological characteristics and essential oil antioxidant activity among of wild-growing populations of Golpar Heracleum persicum Desf. Fischer ،from Northwest of Iran. Ecophytochemical J Med Plants. 2018; 6: 40-55.
46. Ottai M.E.S., Abdel-Moniem A.SH. Genetic parameter variations among milk thistle, Silybum marianum varieties and varietal sensitivity to infestation with seed-head weevil, Larinus latus Herbst. International J Agric & Biology. 2006; 6: 862-866.
47. Gohari A.R., Hajimehdipoor H., Saeidnia S., Ajani Y., Hadjiakhoondi A. Antioxidant activity of some medicinal species using FRAP assay. Med Plants. 2011; 10: 54-60.
48. Moghaddam P.Z., Mazandarani M., Zolfaghari M.R. Antibacterial and antioxidant activities of root extract of Onosma dichroanthum Boiss. In North of Iran. African J Microbiol Res. 2012; 6: 1776-1781.