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# The survey of antioxidant properties of phenolic compounds in fresh and dry hybrid Barberry fruits (*Berberis integerrima× vulgaris*)

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**Abstract.** Berberis (Barberry) belongs to the family Berberidaceae and its use in traditional medicine has a long history. This plant is native to America and Europe and can be found in abundance in Iran. This research aims to assess and compare total phenolic and flavonoid content and measure antioxidant activity with DPPH radical scavenging and to measure Fe reduction capacity in aqueous, ethanolic and methanolic extract of fresh and dried fruits of hybrid barberry *Integerrima ×Vulgaris*. Total phenol and flavonoid content was measured by spectrophotometry and antioxidant activity of extracts using free radical DPPH and Fe reduction with FRAP assay. Maximum amount of phenol (59.57±0.83 mg of gallic acid/10 g barberry) was seen in aqueous extract of dried fruits while highest flavonoid content (1.93±0.03 mg of quercetin/10 g barberry) and DPPH radical scavenging activity (44.62±0.99) was observed in methanolic extract of fresh barberry and maximum Fe reduction level with FRAP (18.15±0.84) in ethanolic extract of dried of barberry. Results showed that barberry is a rich source of antioxidant compounds having significant antioxidant activity.

**Keywords:** Berberis (Barberry), antioxidant activity, phenol, flavonoid, FRAP

## 1. INTRODUCTION

Free radicals are highly reactive and unstable molecules that can damage cells, DNA and ultimately cause mutagenesis. Free radicals that are produced in human body, as a part of metabolic function, may lead to further production or failure in neutralization of oxidative stress and cause damage to the tissues [1]. When the inhibitory mechanisms of the human body that includes antioxidant systems fail, free radicals cause cancer, atherosclerosis and other adverse physiological conditions [2]. Therefore, taking antioxidants through food is necessary. In different countries, fruits and vegetables have been subjected to researches on phenolic content and antioxidant activity evaluation. Replacing synthetics with natural antioxidants have proven to improve human health, Inhibit cardiovascular disease, variety of cancers and aging [3]. Among plant antioxidants, phenolic compounds are widely distributed in plants. Phenolic compounds inhibit lipid oxidation reactions by giving electrons to free radicals [4]. Barberry plant (Berberis Integerrima× Vulgaris) is from the family Berberidaceae. bioactive compounds of Barberry are widely used in food and medical industries [5]. Barberry extract contains various flavonoids including quercetin, krysantemin, hyperosid, delphinidin-3-O-beta-D-glucoside, plargonin and petionidin-3-O-beta- D-glucoside [6]. It also contains compounds such as ascorbic acid, Alpha-Tocopherol and beta-carotene; all considered antioxidants [7]. Motalleb and colleagues (2005) showed that barberry fruit extract is rich in antioxidant compounds and its root bark in phenolic compounds and antioxidant [8]. Phenolic compounds and anthocyanins are the most important secondary metabolites in barberry as antioxidant [9].nowadays, due to the adverse effects of synthetic antioxidants, there is an increasing tendency to use natural antioxidants. For this reason, in this study, we try to consider the best method for preserving barberry by comparing the phenolic compounds and antioxidant capacity of different extracts of

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dried and fresh fruits of Barberry since it has many applications in various fields ranging from expenditures and different products of food to pharmaceutical benefits and applications.

## 2. MATERIALS AND METHODS

**Plant collection:** Hybrid Berberis integerrima × Vulgaris was collected from Qomchqay, Bijar city in November 2012 and stored in the freezer until assay.

**Extraction:** Studied plant extracts were prepared by maceration method. Using a digital scale, 1 g of dried fruits and 2 g of fruit removed from the freezer was weighted. Then, fruits were completely crushed in a mortar and finally the mashed fruits were mixed with 25 ml methanol for 2 h in a shaker. The resulting mixture was filtered through a filter paper. Obtained extracts were placed in special tubes away from light and transferred to the refrigerator to conduct further experiments. To prepare ethanolic and aqueous extract, the same steps were repeated.

### 2.1. Determination of phenol and flavonoid content of the extracts

#### 2.1.1. Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu detector [10]. According to this method, 1 ml of tenfold diluted reagent Ciocalteu-Folin was mixed with 1 mL of the extract. After 3 minutes, 1 ml of sodium carbonate (10%) was added to the solution and then its absorbance was read at 750 nm using APPEL model spectrophotometer (Figure 2-2). The total phenolic content was calculated using the standard curve of gallic acid according to gallic acid Eq (GAE) per 10 g of fresh and dry weight of the sample.

#### 2.1.2. Determination of total flavonoid

The total flavonoid content was determined using Bonvehi et al (2001) method [11] with a slight modification. Thus, 1 ml of fresh barberry extract and a total of 0.2 ml distilled water and 0.8 ml dried barberry was added to 1 ml aluminum chloride ($AlCL_3$) 2% dissolved in methanolic solution of 5% acetic acid. After 10 min, absorbance was measured using a spectrophotometer at a wavelength of 430 nm. Flavonoid content was determined using the standard curve of quercetin.

#### 2.1.3. DPPH radical scavenging capacity

Stable DPPH (2,2- Diphenyl- 1- pykryl hydrazyl)radical scavenging was determined using Cuendent et al (1997) approach [12]. 2.5 mL of the extract was added to 2.5 mL of methanolic solution of DPPH 0.004%. After 30 min of incubation in the dark, the absorption of solution was read using a spectrophotometer at 517 nm. Scavenging percentage was calculated according to the following formula.

$$\text{DPPH radical scavenging percentage} = 1 - (A_{sample} / A_{blank}) \times 100 \quad (1)$$

$A_{sample}$: absorbance with sample, $A_{blank}$: absorbance without sample

#### 2.1.4. Scavenging capacity by FRAP

Scavenging capacity of different extracts were investigated using Benzie et al (1996) method [13]. First, FRAP reagent was freshly prepared by by mixing 2.5 ml TPTZ (2, 4,6 tripridyl-S-triazin) 10 mM in HCl 40 mM and 2.5 ml $FeCl_3$ 20 mM and 25 ml acetate buffer 0.3 M (pH=03.6). 3 microliter of each extract was mixed with 3 mL of FRAP reagent and reaction absorption was measured at the wavelength of 595 nm.

## 3. STATISTICAL ANALYSIS

All experiments were performed in triplicate and results were expressed as mean values and standard deviations (SD). The difference between the samples were analyzed with two-way variance analysis and significance level was considered 5% ($P < 0.05$). Figures and tables were drawn using SPSS and EXCEL soft wares.

## 4. DISCUSSION AND CONCLUSION

### 4.1. Determination of total phenolic content

The total phenolic contents of aqueous, ethanolic and methanolic extracts of fresh and dried barberry fruit are shown in Figure 1. There was significant difference between phenolic content of different extracts of fresh and dried barberry. Generally, the aqueous extract showed the greatest amount of phenol in both types of barberry. In all three types of solvent, dried barberry showed the highest levels of phenolic compounds. Methanolic fresh barberry had the lowest phenol (12.18±1.56) and aqueous dried barberry the highest (59.57±0.83). In this study it was concluded that aqueous extract and methanolic extract had the highest and lowest phenolic content, respectively. Studies have shown that oral intake of antioxidants through food plays an important role in maintaining and promoting health. For example, the risk of coronary heart disease and some cancers is inversely related to the consumption of foods rich with polyphenols. Studies have led to a special focus on natural resources in order to find antioxidant molecules [14]. One of the best sources of natural antioxidants is plant phenolic compounds [15]. Chemical antioxidants which are mostly used in the food industry include BHT, BHA, TBHQ and propyl gallate and the carcinogenicity and negative effects of these compounds on human health have been identified [16]. The total phenolic content of fresh fruit Berberis vulgaris in Turkey has been reported to be 789.32 ±88.50 mg/100 g [17]. In the study of Sasikumar et al (2012), total phenolic content of fresh Barberry fruits was reported 410±0.02 mg/100 g [18]. In a study, Berenji Ardestani and colleagues (2013) reported the total phenol content as 8530 and 3450 mg/100 g fresh fruit for Berberis integerrima and Berberis vulgaris, respectively [19]. The total amount of phenol obtained in this study was less and this may be due to the ripening steps of fruits or some environmental factors [20]. In the study of Siow and Hui (2013) on fresh and dried plant of *Psidium guajava*, the amount of measured phenol in fresh sample is greater than dried samples [21]. Beyhan et al (2010) reported greater total phenol content in fresh fruits of feijoa (*Acca sellowiana, Myrtaceae*) compared to its dried form [22]. Vinson et al (2005) showed that phenolic compounds level of dried fruits is higher than fresh fruits which were consistent with the results of this study [23].

### 4.2. Determination of total flavonoid content

Results of the total flavonoid evaluation based on quercetin/10 g barberry sample are shown in Figure 2. Unlike the results of the phenol, methanolic extract and aqueous extract had the highest and lowest flavonoids in both fresh and dried barberries, respectively. Results varied between 0.65 and 1.93. The highest level of flavonoids was observed in the methanolc extract of fresh barberry (1.93±0.03) and the lowest amount in aqueous extract of dried barberry (0.65±0.03). In the research that has been applied, genotype and environmental condition have great effects in biosynthesis and phenol and flavonoid accumulation in different parts of the plant and plant types [24]. Phenol and polyphenolic compounds such as flavonoids have been widely found in food products and have been shown to have significant antioxidant activity [25]. Important flavonoids in fruits and vegetables are anthocyanins, flavonoids and catechins [26]. Flavonoids such as quercetin and quercetin glycoside have shown a good ability in inhibiting free radicals [27]. Sasikumar et al (2012) reported a flavonoid content of 320 mg equal to Eq quercetin/100 g fresh fruit barberry [18]. Pyrkosz-Biardzka and colleagues (2014)

showed that the methanolic extract of *Berberis vulgaris* contains significant amounts of phenolic compounds and flavonoids [28].

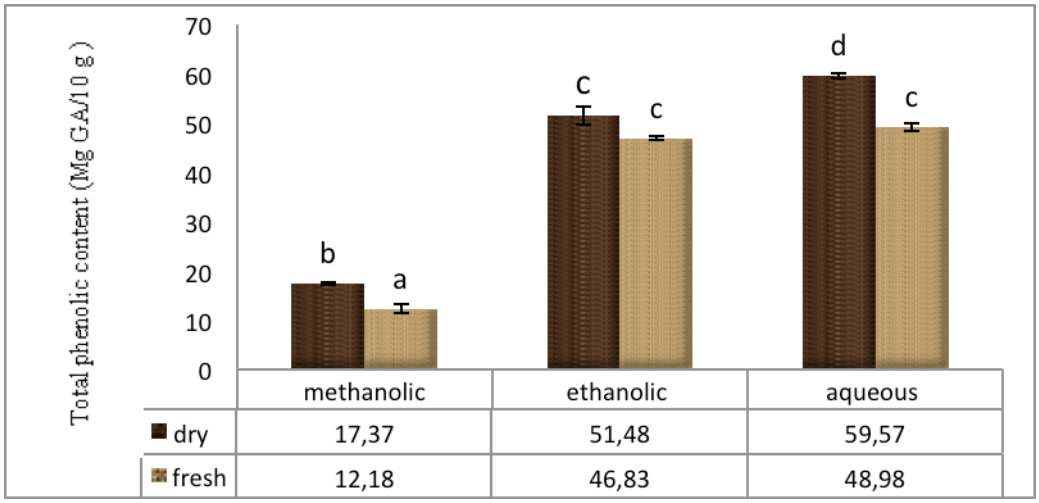


**Figure 1.** The total phenolic content (mg of gallic acid/ 10 g of fresh and dry sample) in B. integerrima×vulgaris fruit in different methanolic, ethanolic and aqueous extraction (three replicates ± SE, $p < 0.05$)

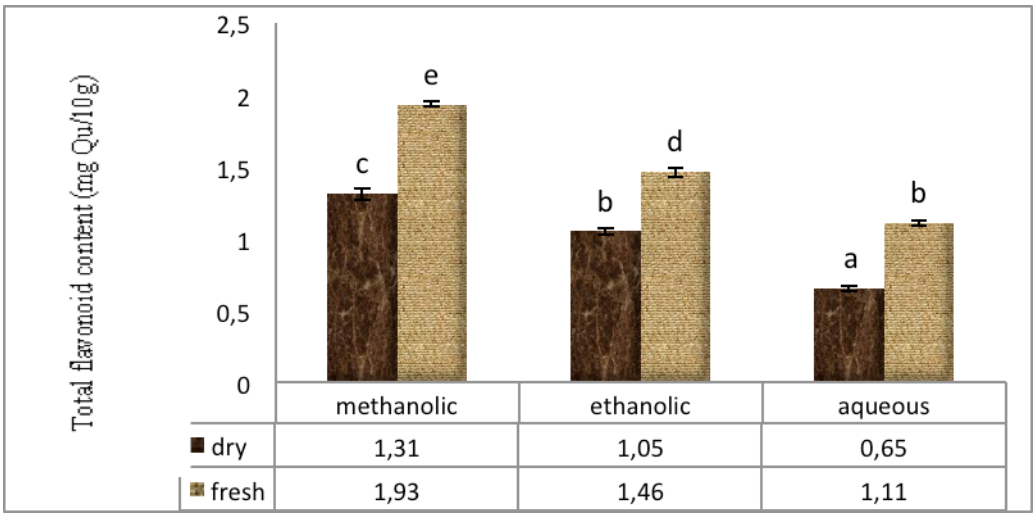


**Figure 2.** Total flavonoid content (mg quercetin/ 10 g fresh and dried sample) in B. integerrima×vulgaris fruit in different methanolic, ethanolic and aqueous extraction (three replicates ± SE, $p < 0.05$)

### 4.3. DPPH radical scavenging activity

The capacity of methanolic, ethanolic and aqueous extracts of fresh and dried barberry in donating hydrogen or oxygen to the DPPH radical which results in DPPH radical becoming neutralized to DPPH-H was investigated. In all tested extracts, color change from purple (radical) to yellow (neutral) wa observed. Antiradical activity of the methanolic extract of fresh barberry (44.62±0.99) and aqueous extract of dried barberries (19.88± 1.39) had the highest and lowest percentage of DPPH radical scavenging activity (Figure 3). The methanolic extract was introduced as the best to trap DPPH radical. Fresh fruit showed the highest level of DPPH free radical scavenging activity compared with dried barberry. Due to its simplicity, sensitivity and speed, DPPH scavenging method is used to evaluate the antioxidant activity of compounds [29]. This method is based on 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, DPPH containing solution changes color from purple to dark yellow and the absorbance at 517nm drops (this radical is lipophilic and its maximum absorption is at 517 nm) [30]. Many studies have found a direct link between the content of phenols, flavonoids and DPPH radical scavenging capacity [31]. However, there are reports that do not show this relationship[32]. In the study of Beyhan et al (2010) on dried and fresh fruits of feijoa (*Acca sellowiana*, *Myrtaceae*), DPPH radical scavenging activity has been reported to be higher in dried fruit than fresh ones [22]. Sasikumar et al (2012) investigated the DPPH radical scavenging activity of different concentrations of methanolic extract of barberry fruit (*Berberis tinctoria*) in comparison with BHT which was lower [18]. In 2011, a study on *Berberis vulgaris* by Pogačnik and Poklarulrih showed that antioxidant activity of methanolic extract of the plant is more than its aqueous extract. It was also reported that compared to the fresh fruit, the antioxidant activity of dried barberry fruit decreases [33]. Similar results were obtained in the present study, so that the antioxidant activity of methanolic extract was higher than the other extracts and among the various extracts, aqueous extract had a poor performance in relation to the free radical scavenging and antioxidant activity of fresh fruit was higher than dried fruits.

### 4.4. Determination of Fe reduction capacity using FRAP assay

Fe reduction capacity of methanolic, ethanolic and aqueous extracts in fresh and dried fruits of barberry using FRAP assay is shown in Figure 4. Based on data analysis, the highest and lowest level of reduction capacity was observed in the ethanolic extract of dried barberries (18.15±0.84) and aqueous extract of fresh barberry (3.93±2.40), respectively. The reduction capacity in dried barberries was higher than fresh ones and among extracts of dried barberries, ethanolic extract and among extracts of fresh barberries, methanolic extract had the highest reduction capacity. Among popular methods of measuring antioxidant properties, 1,1-diphenyl-2- pykryl hydrazyl (DPPH) radical trapping method based on hydrogen donation of the extract [34], study of the linoleic acid oxidation inhibition (BCB) and measurement of antioxidant activity of the extracts based on $Fe^{2+}$ reduction capacity (FRAP)can be named [35].

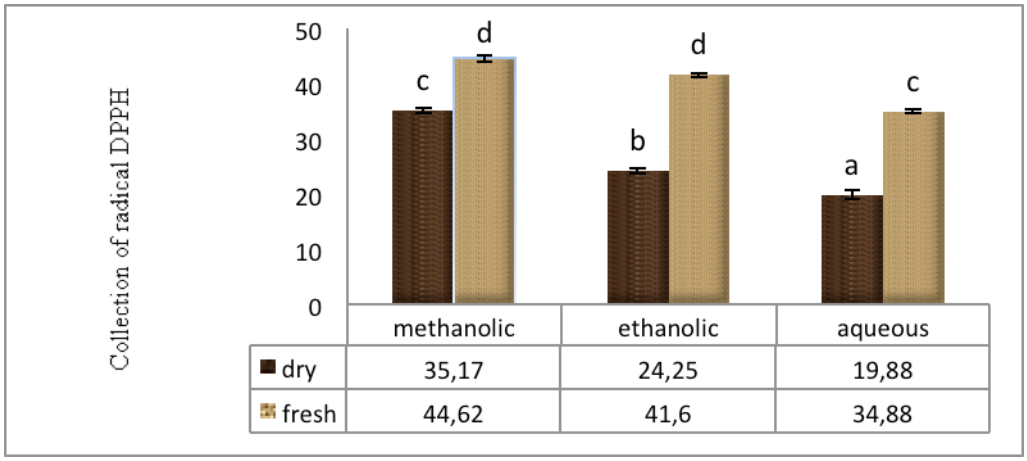


**Figure 3.** Percentage of DPPH radical scavenging in fresh and dried fruits of B. integerrima× vulgaris in different methanolic, ethanolic and aqueous extraction (three replicates ± SE, $p < 0.05$)

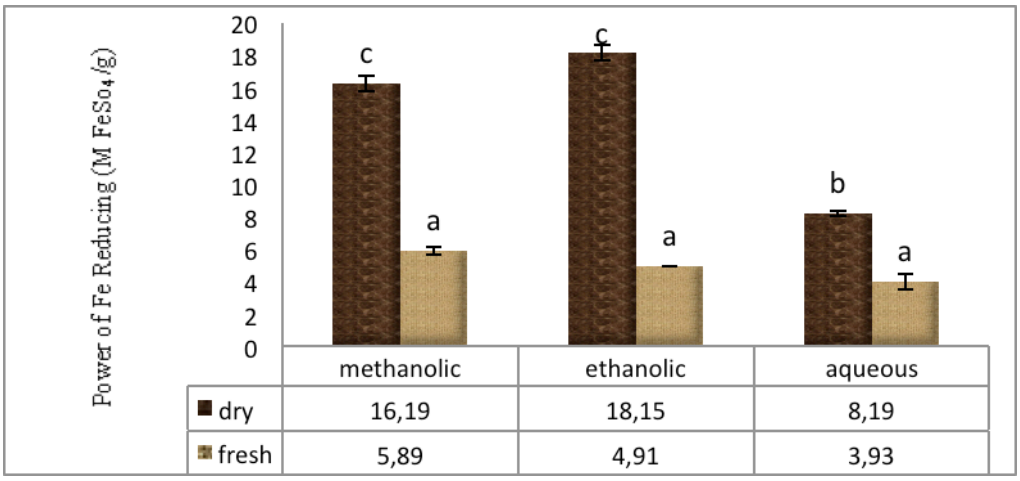


**Figure 4.** Fe reduction capacity by FRAP assay in fresh and dried B. integerrima× vulgaris in different methanolic, ethanolic and aqueous (three replicates ± SE, $p < 0.05$)

These three methods differ from each other based on characteristics such as substrate type, reaction conditions, data quantitation methods and etc. [36]. Depending on the method used for assay, plant extracts show different antioxidant capacities in different periods [37]. FRAP assay measures antioxidant activity of the extracts based on their Fe reduction capacity and in fact, according to Deepa et al (2007) shows estimated antioxidant properties of water-soluble compounds [38]. Using FRAP assay and measuring the amount of ascorbic acid in orange juice, Gardner et al (2000) showed that ascorbic acid content is directly associated with antioxidant properties of extract measured by FRAP [37]. Several studies have shown that the electron donating capacity reflects the bioactive compounds reduction capacity along with antioxidant activity [39]. Phenolic compounds such as catechins may play a role in determining FRAP. As it is known, th barberry has a significant level of antioxidant compounds like catechin [40]. In this study, both DPPH and FRAP methods was used to evaluate the antioxidant activity of the extracts. Depending on the method used, extracts showed different antioxidant capacities. As mentioned earlier, the role of phenolic compounds such as total phenol in antioxidant activity by DPPH assay is not essential, whereas according to present reports [41], antioxidant capacity measured by FRAP can be related to these compounds. Özgen et al (2012) examined the Fe reduction capacity by FRAP method in different varieties of barberry (Berberis vulgaris) [22]. For the different varieties, reduction level has been reported to be between 41.0±0.08 to 65.6±2.5. Reports have shown that in plants such as blackberry, raspberry and strawberry, there is a linear relationship between total phenolic content and antioxidant activity of leaves and fruits measured by FRAP assay [42]. In this research, it also can be noted that this relationship between the phenol and antioxidant capacity by FRAP method exists so that dried samples showed higher antioxidant capacity in comparison with fresh samples.

## 5. CONCLUSION

Phenolic compounds such as flavonoids are natural plant compounds that their antiradical and antioxidant effect has been proven in several studies. This research leads us to this conclusion that barberry fruit is a rich source of phenolic compounds that are used in food and pharmaceutical industry. Dried barberry fruit has more phenol and higher Fe reduction capacity by FRAP compared to fresh fruits but instead, fresh fruits contain more flavonoids and a higher percentage of DPPH radical scavenging. So, both fresh and dried fruit intake is recommended. Fresh and dried types of barberry have higher antioxidant activity and are introduced as rich source of phenolic compounds. According to the obtained results, various solvents have a lot of value but in general, using aqueous extract is recommended due to it being innocuous.

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