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Evaluation of phenolic compound, antioxidant activities and antioxidant enzymes of barberry genotypes in Iran

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ABSTRACT

Antioxidant capacity, total phenol (TP), total anthocyanin (TA), total flavnoid (TF) and antioxidant enzymes of a number of selected barberry genotypes from *Berberis vulgaris* and *Berberis integerrima* species were investigated. To evaluate the antioxidant capacity; 2,2-diphenyl-1-picrylhydrazyl (DPPH), Fe^{2+} chelating and ferric reducing antioxidant power (FRAP) assays were performed. The results showed genotype-specific differences in most of the physicochemical characteristics, antioxidant capacity and antioxidant enzymes. The range of TP content of genotypes was 261.68–623.07 mg gallic acid (GAE) per 100 g fresh weight (FW) basis. Genotype N1 had the highest antioxidant capacity based on DPPH and FRAP assays. The highest guaiacol peroxidase (G-POD) and catalase (CAT) activity was observed in berries of SH1 genotype. Significant correlation among all three antioxidant assays (DPPH, Fe^{2+} chelating and FRAP) were found. The present study shows the potential of barberry genotypes studied for improvement of nutritional value through germplasm enhancement programs.

1. Introduction

The genus *Berberis* belongs to family Berberidaceae are commonly used in nutrition and medicine. This genus has been widely distributed in Europe, North Africa, Asia, especially in Iran (Gundogdu, 2013). They are usually consumed in various forms as fresh fruit, juice, jam, beverage and other processed products. Furthermore, the fruits of barberry are used in Persian and Georgian cuisines (Siow et al., 2011). Recently, much attention to Nutraceutical foods has led breeders to initiate selection of plants with higher than the normal antioxidant capacity, such as sea buckthorns (Ercisli et al., 2007), cornelian cherry (Hassanpour et al., 2011), apples and strawberries (Scalzo et al., 2005).

Barberry has long been used as an herbal remedy since ancient times. Barberry is one of the medicinal plants that have various biological properties such as anti-proliferative, anti-migratory, anti-bacterial and antioxidant activities (Mahata et al., 2011; Villinski et al., 2003). Previous studies mentioned that the phytochemical constituents of barberry such as antioxidants, phenolic and anthocyanins compound increased its bioactivity and was affected by different extraction methods (Abd El-Wahab et al., 2013; Annegowda et al., 2011). This crop is very important for human nutrition and health due to many bioactive compounds, antibiotic alkaloids and antioxidants (Koncic et al., 2010; Stermitza et al., 2001). The consumption of barberry fruits plays an important role in disease prevention, such as kidney discomforts, gastrointestinal tract, liver diseases and bronchial discomforts (Blumenthal et al., 1998). Also, barberry can be applied for sore healing and for soothing minor mouth and throat irritations (Villinski et al., 2003).

Furthermore, extracts of barberry fruit appear to have natural antihistamine and anti-allergy potential (Ozgen et al., 2012). The health benefits of natural antioxidants are believed to be achieved via possible mechanisms by directly reacting with and scavenging free radicals, chelating transition metals, reducing peroxides, and stimulating the antioxidative defense enzyme systems (Li et al., 2009). These results have stimulated research to determine different types of small fruits with regards to bioactive compounds and antioxidant capacity (Korekar et al., 2011). The concentration of enzymatic and non-enzymatic antioxidant compounds in plant depends on the genotype and is influenced by processing and environmental factors (Hulya Orak et al., 2012).

Most of the studies on barberry antioxidant properties focused on the bark (Tomosaka et al., 2008), twigs and leaves of crop (Koncic et al., 2010). Previously only few studies have been reported on the phenolic compound and antioxidant activities of barberry fruits in different countries (Motalleb et al., 2005; Koncic et al., 2010; Ozgen et al., 2012; Gundogdu, 2013; Yildiz et al., 2014).

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However, very little information is available regarding the phenolic content, antioxidant capacity, and antioxidant enzymes of barberry fruits from different genotypes grown in Iran. Therefore, more data are required about the biodiversity of bioactive compounds of this berry to identify the most nutritious varieties. Thus, the purpose of the present study was to determine antioxidant capacity, total anthocyanin, total phenol, total flavonoid, and antioxidant enzymes of a number of selected barberry genotypes in Iran. This study is a first attempt at investigating of antioxidant capacity and antioxidant enzymes of barberry fruits grown in Iran.

2. Materials and methods

2.1. Collection and preparation of barberry samples

All of the selected samples of wild growing plants of barberry were obtained from west Azerbaijan, in Iran (Table 1). Twenty genotypes of barberry, according to high yield and free of pest and disease characteristics, were selected. Approximately 500 g of fully matured, fresh barberry fruits per genotype were harvested manually in September 2014. The berries were sorted based on uniformity of shape and color and then immediately transported to the laboratory and frozen with liquid nitrogen and kept at −80 °C, until needed for analysis. All used chemicals were analytical degree (Sigma–Aldrich Company, St. Louis, MO, USA).

2.2. Total phenol content (TP)

Total phenolic content in extracts was determined by Folin–Ciocalteu colorimetric method according to Slinkard and Singleton (1977). The gallic acid (GAE) was used as a standard and results were expressed as mg GAE equivalents per 100 g FW basis.

2.3. Total anthocyanin content (TA)

Some of frozen tissue was ground to a fine powder under liquid nitrogen by cold mortar and pestle and 1 g of the resultant powder was added to 10 mL of methanol containing HCl (1%, v/v) and held at the 0 °C for 10 min (Cordenunsi et al., 2003). The slurry was centrifuged at 17,000 × *g* for 15 min at 4 °C and then the supernatant was used. TA content was estimated by the pH differential method (Cheng and Breen, 1991). Absorbance was measured at 520 and 700 nm in buffers at pH 1.0 (hydrochloric acid–potassium chloride, 0.2 M) and 4.5 (acetate acid–sodium acetate, 1 M). TA content was calculated using a molar extinction coefficient of 29600 (cyanidin 3-glucoside).

$$\text{Absorbance}\,(A) = (A_{520\,\text{pH}\,1} - A_{700\,\text{pH}\,1}) - (A_{520\,\text{pH}\,4.5} - A_{700\,\text{pH}\,4.5})$$

The results were expressed as mg cyanidin 3-glucose equivalents per 100 g FW basis.

2.4. Total flavonoid content (TF)

Some of frozen tissue was ground to a fine powder under liquid nitrogen by cold mortar. One gram of the powder was mixed with 10 mL of methanol containing HCl (1%, v/v) and held at room temperature for 24 h (Cordenunsi et al., 2003). The slurry was centrifuged at 4000 × *g* for 15 min at 4 °C, and the supernatant was used. Content of TF was determined by a colorimetric assay based on aluminium chloride complex formation (Youngjae et al., 2007). One milliliter of diluted sample was mixed with 4 mL of deionized water. Then 0.3 mL of 5% $NaNO_2$ solution was added to this mixture, which was allowed to stand for 5 min at room temperature, and 0.6 mL of 10% $AlCl_3{\cdot}6H_2O$ solution was added. The mixture was allowed to stand for 6 min at room temperature, and 2 mL of 1 mol L^{-1} NaOH was added, and the total was made up to 10 mL with deionized water. The absorbance of the solution was measured immediately at 510 nm. TF content was calculated from calibration curve of catechin and results are expressed as catechin (CAT) equivalents per 100 g FW basis.

2.5. Antioxidant capacity

2.5.1. DPPH free radical scavenging activity

For the determination of DPPH free radical scavenging activity, barberry samples were extracted with methanol. Then, they were centrifuged (Sigma 3K30, Germany) at 15,000 × *g* for 10 min. The supernatants were concentrated under reduced pressure at 40 °C. The dried extracts were dissolved in methanol. The scavenging effect for DPPH was measured according to the method of Nakajima et al. (2004) with minor modifications. Briefly, 1 mL of 6×10^{-5} mol L^{-1} methanolic DPPH solution were added to 50 μL of the diluted extracts (concentrations 2–20 mg mL^{-1}) of either methanolic solution of extract (sample) or methanol (control). The mixture was shaken and left at room temperature in the dark. After 30 min, the absorbance was measured spectrophotometrically at 515 nm. The percent of reduction of DPPH was calculated using the following equation:

$$\%\text{inhibition of DPPH} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Abs control is the absorbance of DPPH solution without the extract.

2.5.2. Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was measured according to the method of Benzie and Strain (1996). Briefly, the FRAP reagent was freshly prepared by mixing 100 mM acetate buffer (pH 3.6), 10 mM 4,6-tripryridylstriazine (TPTZ) in 40 mM HCl and 20 mM ferric chloride in a ratio 10:1:1 (by volume) before measurement. One hundred microliters of extracts and 4.9 mL of FRAP reagent were transferred into vials and incubated at 37 °C. After 10 min, the absorbance was determined at 593 nm relative to a reagent blank also incubated at 37 °C. The FRAP-value was expressed as mmol trolox equivalent per liter of fruit juice.

2.5.3. Ferrous ions (Fe^{2+}) chelating activity

Chelating ability was measured according to the method of Dinis et al. (1994). Each sample (0.1– 10 mg/mL, 1 mL) in 0.2% acetic acid solution was mixed with 3.7 mL of methanol and 0.1 mL of 2 mM ferrous chloride. The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine. After 10 min, the absorbance was measured at 562 nm against a blank. A lower absorbance indicates a higher chelating ability. Citric acid and EDTA were used for comparison. Iron chelating activity (*R*) was calculated using the following equation:

$$R(\%) = \left(1 - \frac{\text{As}}{\text{Ac}}\right) \times 100$$

where Ac is the absorbance of control reaction (without extract) and As is the absorbance in the presence of an extract.

2.6. Antioxidant enzymes

2.6.1. Guaiacol peroxidase (G-POD)

Beery tissue (4 g) was ground in a cold mortar and pestle with 4 mL potassium-phosphate buffer (0.1 mol L^{-1}, pH 7.3) containing 1 mmol L^{-1} EDTA and 2 mmol L^{-1} DTT. The homogenate was centrifuged at 12,000 × *g* for 10 min at 4 °C. The supernatant was used for G-POD assay. The G-POD enzyme assay mixture containing 0.1 mol L^{-1} phosphate buffer (pH 6.1), 4 mmol L^{-1} guaiacol as donor, 3 mmol L^{-1} H_2O_2 as substrate and 1.0 mL crude enzyme