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## Evaluation of Phenolic Content and Total Antioxidant Activity in *Berberis vulgaris* Fruit Extract

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**Abstract:** This study was carried out to determine the total antioxidant activity and phenolic content of barberry (*Berberis vulgaris*) fruit extracts. The extract was prepared with distilled water and ethanol and methanol (80%), respectively. The  $\beta$ -carotene bleaching and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay were used to determine antioxidant properties of barberry fruit by measuring the decrease in absorbance at 470 and 517 nm. In distilled water, *Berberis vulgaris* showed 82.52 $\pm$ 0.64% free radical scavenging activity. In ethanol, *Berberis vulgaris* fruit extract showed 73.62 $\pm$ 1.87% free radical scavenging activity, BHT 67.50 $\pm$ 0.53% and vitamin C 88.56 $\pm$ 0.43%, respectively. *Berberis vulgaris* fruit extract exhibited the higher free radical scavenging activity in distilled water with 82.52% and EC50 = 0.64 mg mL<sup>-1</sup>. The methanol 80% extract of *Berberis vulgaris* showed 60.15%  $\beta$ -carotene bleaching assay and vitamin C 91.17%. *Berberis vulgaris* fruit extract in 80% methanol had the highest phenolic content followed by extract in water. The results of showed significant differences ( $p < 0.05$ ) in the means of free radical scavenging activities of barberry fruit in water and ethanol.

**Key words:** Total antioxidant activity, total phenolic content, *Berberis vulgaris* fruit

### INTRODUCTION

Fruits and vegetables account for a small part of our daily caloric intake; however their benefits to health surpass their caloric contribution. The contributory factors are due to the presence of vitamins and provitamins, such as ascorbic acid, tocopherols and carotenoids and in addition to that, they are also rich in a wide variety of phenolic substances<sup>[1]</sup>. Phenolic substances are category of phytonutrients that exert strong antioxidant properties. They can be classified into simple phenols, phenolic acids, hydroxycinnamic acid derivatives and flavonoids. The ability of some of the phenolic substances to act as potent antioxidant components has been reported<sup>[2]</sup>. The principle function of antioxidants is in delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and they may reduce oxidative damage to the human body<sup>[3]</sup>. The occurrence of such oxidative damage may be a significant causative factor in the development of many chronic diseases, such as cancer and cardiovascular diseases<sup>[4,5]</sup>. Several epidemiological studies have shown a negative association between intake of fruits and vegetables and

certain diseases<sup>[5]</sup>. Barberry (*Berberis vulgaris* L., Var. *Asperma* Don., family Berberidaceae) grows in Asia and Europe. The plant is well known in Iran and has been used extensively as a medicinal plant in traditional medicine. The fruits of the plant have been used as food. Medicinal properties for all parts of the plant have been reported, including tonic, antimicrobial, antiemetic, antipyretic, antipruritic and cholagogue actions and it has been used in some cases like cholecystitis, cholelithiasis, jaundice, dysentery, leishmaniasis, malaria and gall stones<sup>[6-8]</sup>. Over the last decade, bisbenzylisoquinoline (BBI) and especially protoberberine alkaloids (e.g., berberine and jatrorrhizine) have attracted considerable attention. Protoberberine represents a structural class of organic cations and have been found to be predominantly distributed in several genera of the families Ranunculaceae and Berberidaceae (e.g., *Berberis*, *Mahonia*, *Coptis*)<sup>[9-12]</sup>. BBI alkaloids are excellent scavengers of Reactive Oxygen Species (ROS) such as single oxygen and/or superoxide anion radical<sup>[13]</sup>.

Barberry fruit is not commonly used in Iran but not consumed as vegetable in Malaysia. Thus, it was the purpose of this study to determine the total antioxidant activity and phenolic content of barberry fruit.

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## MATERIALS AND METHODS

***Berberis vulgaris*:** Barberry (*Berberis vulgaris*) fruit was purchased from Iranian markets at KL in Malaysia.

**Chemicals:** Linoleic acid,  $\beta$ -carotene, Tween 20, gallic acid, Butylated Hydroxyl Toluene (BHT) was purchased from Sigma Chemical Co. (St Louis, MO, USA). Other chemicals used were analytical grade.

**Preparation of plant extract:** Barberry fruit extract was prepared according to method modified Shamsa *et al.*<sup>[14]</sup>. Three hundred and ten gram *B. vulgaris* fruit that was free from fungus, bacteria and any other plant diseases were selected randomly. The fruits were dried in an oven (Memmert SLM 400, GmbH, Germany) for 3 days at a constant temperature of 65°C. The fruits were cut into small pieces and were grounded into fine powder using a dry grinder. The grounded samples were sieved to get uniform particle size, then were kept in air-tight container and stored for further extraction. The sample was extracted by boiling in water for 20-30 min at a temperature of 75°C. Water and ethanol were added in the ratio of 1:10 and stirred at 250 rpm in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH and Co. KG, Germany) for 1 h at room temperature. The extract was then separated from the residue by filtration through Whatman No.1 filter paper. The remaining residue was re-extracted twice and then the two extracts were combined. The residual solvent of ethanolic extract was removed under reduced pressure at 50°C using a rotary evaporator (BÜCHI Rotavapor R-200, Germany) until thick syrup was collected. The thick syrup was evaporated completely using freeze drying system (FreeZone 77520, LABCONCO, USA) for the determination of total antioxidant activity and phenolic content.

**Determination of total antioxidant activity:** Antioxidant activity of *Berberis vulgaris* extract was measured according to the method described by Gazzani *et al.*<sup>[15]</sup>. One milliliter of  $\beta$ -carotene solution (0.2 mg mL<sup>-1</sup>) was pipetted into a round-bottom flask (50 mL) containing 0.02 mL of linoleic acid and 0.2 mL of 100% Tween 20. The mixture was then evaporated at 40°C for 10 min by means of rotary evaporator to remove chloroform. After evaporation, the mixture was immediately diluted with 100 mL of distilled water. The distilled water was added slowly to the mixture and agitated vigorously to form an emulsion. Five milliliters aliquots of the emulsion were transferred into different test tubes containing 0.2 mL of samples in 80% methanol at final concentration of 1 mg mL<sup>-1</sup>. The tubes were then gently shaken and placed

at 45°C in a water bath for 2 h. The absorbance of the samples was measured at 470 nm using a spectrophotometer (SECOMAM ANTHELIE, Advanced-FRANCE) at initial time (t=0) against a blank, consisting of an emulsion without  $\beta$ -carotene. Standards of the same concentration as samples were used for comparison; 0.2 mL of 80% methanol in 5 mL of the above emulsion was used as the control. The measurement was carried out at 15 min intervals for 120 min. All samples were assayed in triplicate. The Antioxidant Activity (AA) was measured in terms of successful bleaching of  $\beta$ -carotene by using the following equation:

$$AA = 1 - (A_t - A_o) / (A_o - A_o^o) \times 100$$

Where,  $A_o$  and  $A_o^o$  are the absorbance values measured at the initial incubation time for samples and control, respectively, while  $A_t$  and  $A_t^o$  are the absorbance values measured in the samples or standards and control at t = 120 min.

**Free radical scavenging assay:** Effect of *Berberis vulgaris* fruit extract on DPPH radical was measured based on Yen and Hsieh<sup>[16]</sup>. The reaction mixture (final volume 1.5 mL, final concentration 0.3 mM) of 1 mL of 0.45 mM 1, 1-diphenyl-2-picrylhydrazyl (DPPH) in absolute ethanol and 0.5 mL of 5 mg mL<sup>-1</sup> extracts, BHT and L-ascorbic acid (vitamin C) was left in the dark at room temperature for 30 min. A control was set up by replacing the samples with 0.5 mL absolute ethanol. The remaining DPPH was measured spectrophotometrically at 517 nm. The antioxidant activities were calculated as below:

$$\text{Antioxidant activity} = (OD_{\text{control}} - OD_{\text{sample}}) / OD_{\text{control}} \times 100$$

Where,  $OD_{\text{sample}}$  is the optical density of sample and  $OD_{\text{control}}$  is optical of control.

**Determination of total phenolic content:** The amount of total phenolic was determined according to the method modified of Velioglu *et al.*<sup>[17]</sup> which used Folin-Ciocalteu reagent. Extract was prepared at a concentration of 1 mg mL<sup>-1</sup>. One hundred microliter of extract was transferred into a test tube and 0.75 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with deionised water) were added and mixed. The mixture was allowed to stand at room temperature for 5 min. 0.75 mL of 6% (w/v) sodium carbonate was added to the mixture and then mixed gently. After standing at 725 nm using a UV-Vis spectrophotometer. The standard calibration (0.02-0.1 mg mL<sup>-1</sup>) curve was plotted using gallic acid.

The total phenolic content was expressed as gallic acid equivalents in milligrams 100 g *B. vulgaris* fruit extract.

**Statistical analysis:** The results obtained were analyzed using one-way ANOVA for mean differences. The Statistical Package for Social Science for windows version (12.0) was used to analyze the data.

## RESULTS

**Total antioxidant activity:** The total antioxidant activity, which reflected the ability of the extracts to inhibit the bleaching of  $\beta$ -carotene, was measured and compared with the control which contained no antioxidant component. The  $\beta$ -carotene bleaching rates of the extracts are shown in Fig. 1 and 2. There was a decrease in absorbance values of  $\beta$ -carotene in the absence of extracts due to the oxidation of  $\beta$ -carotene and gallic acid. The high absorbance values indicated that BV fruit extract possessed antioxidant activity. The absorbance value of the control was significantly lower ( $p < 0.05$ ) than *B. vulgaris* fruit extract and standards. *B. vulgaris* fruit extract in 80% methanol showed a significance value until 80 min. This indicated that *B. vulgaris* fruit extract has acted as effective antioxidant in the  $\beta$ -carotene system, which inhibited the oxidation activity of  $\beta$ -carotene (Fig. 1). The comparison of mean total antioxidant activity of *B. vulgaris* fruit extract in water and ethanol is presented in Fig. 3. Mean antioxidant activity of *B. vulgaris* fruit extract in water and ethanol were  $82.52 \pm 0.64\%$ ,  $73.62 \pm 1.876\%$ , respectively (Fig. 3). Results of ANOVA analysis indicated that antioxidant activity of *B. vulgaris* fruit extract in water is significantly higher ( $p < 0.05$ ) than in ethanol.

Table 1 shows the comparison of the mean concentration for 50% free radical scavenging activity ( $EC_{50}$ ) of water and ethanolic extracts of *Berberis vulgaris* against 250  $\mu$ M DPPH radical. The  $EC_{50}$  of vitamin C and BHT were  $0.252 \pm 0.01$  mg mL<sup>-1</sup>,

$0.612 \pm 0.01$  mg mL<sup>-1</sup>, respectively, which is stronger than water and ethanolic extracts. The  $EC_{50}$  values of water extract of *Berberis vulgaris* was  $82.52 \pm 0.64$  mg mL<sup>-1</sup>. For ethanolic extract, the  $EC_{50}$  value of *Berberis vulgaris* was  $0.658 \pm 0.03$  mg mL<sup>-1</sup>. The  $EC_{50}$  value in water and ethanol for *Berberis vulgaris* fruit extract was nearly equal. The results of ANOVA analysis show significant differences ( $p < 0.05$ ) in the means of free radical scavenging activity of barberry fruit extract in water and ethanol.

**Total phenolic content:** The total phenolic content of the *Berberis vulgaris* fruit extracts is shown in Fig. 4. Among all the extracts, *Berberis vulgaris* fruit extract in 80% methanol had the highest phenolic content 28000 mg/100 g fruit extract, followed by *Berberis vulgaris* fruit extract in water 10000 mg/100 g. ANOVA showed significant differences ( $p < 0.05$ ) in the total phenolic content between *Berberis vulgaris* fruit extract in water and 80% methanol (Fig. 4). There was not any relationship between antioxidant activity and total phenolic content of *Berberis vulgaris* fruit extracts (Fig. 5).

## DISCUSSION

Environmental factors, such as climatic growth conditions, growth, ripening stage, temperature, duration of storage and thermal treatment may have influenced the antioxidant activity<sup>[1,5]</sup>. Antioxidant activity of vegetable

Table 1: Free radical scavenging activity ( $EC_{50}$ ) of *Berberis vulgaris* Fruit Extract (BFE) in water and ethanol

Extract and standards	Free radical** scavenging activity (%)	$EC_{50}$ * (mg mL <sup>-1</sup> )
BFE in water	$82.52 \pm 0.6407^a$	$0.650 \pm 0.00231^a$
BFE in ethanol	$73.62 \pm 1.876388^c$	$0.658 \pm 0.0326^b$
BHT	$67.50 \pm 0.5268^e$	$0.612 \pm 0.0085^c$
Vitamin C	$88.56 \pm 0.4332^b$	$0.252 \pm 0.0006^d$

\*\* Values are expressed as mean  $\pm$  standard deviation of three replicate measurements. Different letter(s) indicate significant difference at the level of  $p < 0.05$ . Comparison was made between two extracts of the respective *Berberis vulgaris*. The  $EC_{50}$  value is defined as the amount of (BFE) necessary to decrease the initial DPPH radical concentration by 50%.

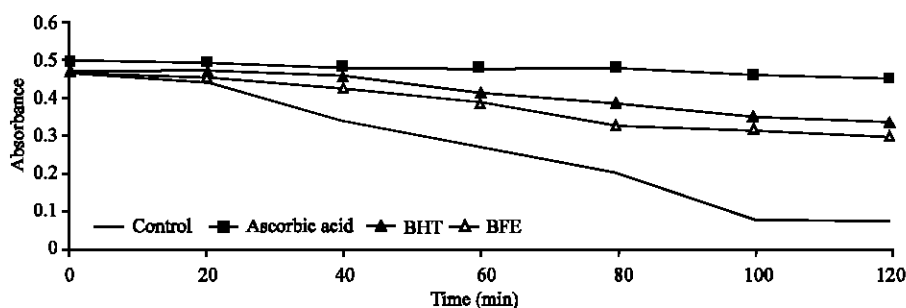


Fig. 1: Antioxidant activity of *Berberis vulgaris* 80% methanol extract (BFE) compared with, Ascorbic acid (Vit. C) and Butylated Hydroxyl Toluene (BHT) at 1 mg mL<sup>-1</sup> using a  $\beta$ -carotene-linoleate system

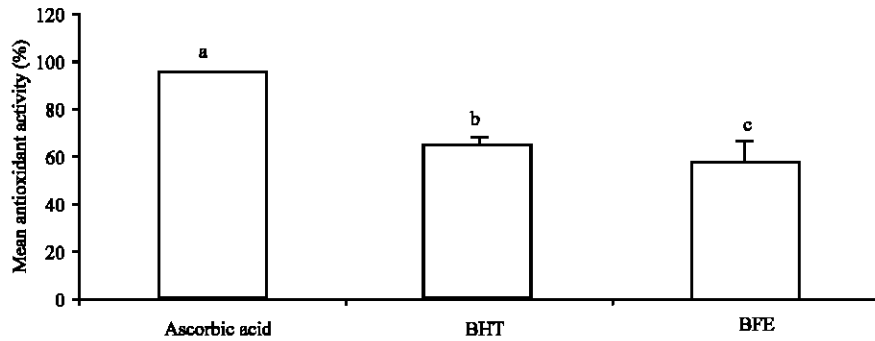


Fig. 2: Mean total antioxidant activity of *Berberis vulgaris* 80% methanol extract, vitamin C and BHT. a, b, c values are significantly different at  $p < 0.05$ . Antioxidant activity was measured using a  $\beta$ -carotene-linoleate system. Results are means of three determinations

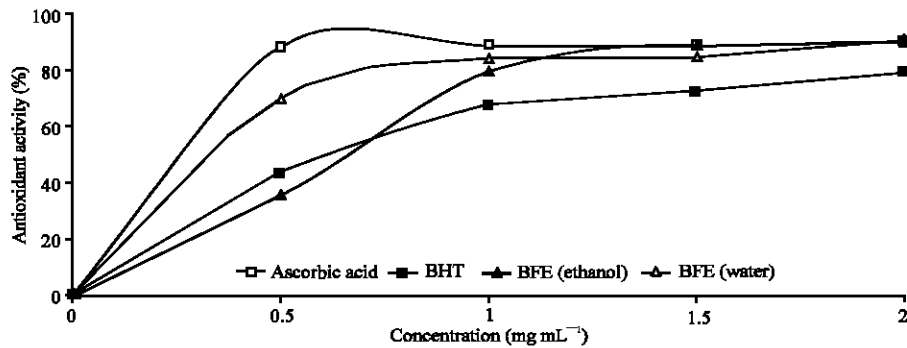


Fig. 3: Antioxidant activity percentage of BFE in water and ethanol compared to ascorbic acid and Butylated Hydroxyl Toluene (BHT) at different concentrations using the DPPH free radical scavenging assay

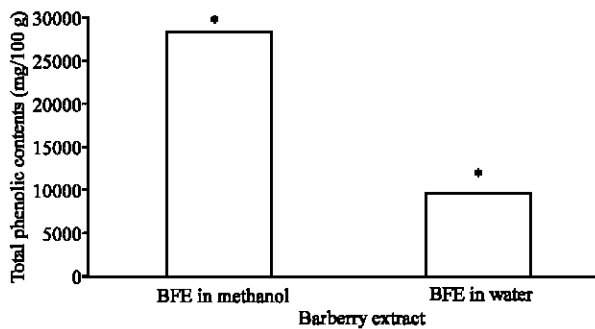


Fig. 4: Mean total phenolic content of *B. vulgaris* Fruit Extract (BFE). Asterisk (\*) indicates significant differences ( $p < 0.05$ ) between water and 80% methanol extract

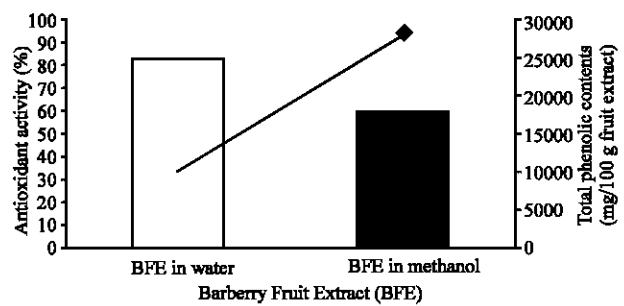


Fig. 5: Comparison of antioxidant activity and total phenolic content in *Berberis vulgaris* Fruit Extract (BFE) in water and 80% methanol

extracts also depends on the type and polarity of the extracting solvent, the isolation procedures and purity of active compounds, as well as the assay techniques and substrate used<sup>[18]</sup>. Tsud *et al.*<sup>[19]</sup> reported that less polar solvents provided slightly more active extracts than mixtures with ethanol or methanol, or methanol alone for tamarind seed coats. The extraction solvents in this

study were 80% methanol (v/v) for  $\beta$ -carotene, water and pure ethanol for DPPH assay. This factor may also have affected the results in the different findings. The dose-response curve for the free radical scavenging of studied samples of water and ethanolic extracts and standards at different concentrations are presented in Fig. 3. The scavenging activity of all samples on the DPPH radical was found to be strongly dependent on concentration. In general, the scavenging effects on the DPPH radical

increased sharply with increasing concentration of all the samples and standards to a certain extent and then slowly increased. The findings indicated that total phenolic content were different in water and 80% methanol. Several studies have reported on the relationships between phenolic content and antioxidant activity. Kähkönen *et al.*<sup>[19]</sup> found that the total phenolic content of vegetables was very low compared to fruits. Vinson *et al.*<sup>[20]</sup> found that beets had the highest total phenolic content, followed by red onion, broccoli and kidney beans. Meanwhile, Velioglu *et al.*<sup>[2]</sup> reported that red onion had a higher total phenolic content than other plant materials. Some authors found a correlation between the phenolic content and the antioxidant activity, while others found no such relationship. Velioglu *et al.*<sup>[2]</sup> reported a strong relationship between total phenolic content and antioxidant activity in selected fruits, vegetables and grain products. Kähkönen *et al.*<sup>[19]</sup> reported there was not any correlation between antioxidant activity and phenolic content in some plant extracts. In this study, the findings do not show any relationship between antioxidant activity and total phenolic contents (Fig. 5). For example, *B. vulgaris* fruit extract had the lowest total phenolic content in water whereas its antioxidant activity was higher in ethanol than water. Robards *et al.*<sup>[21]</sup> reported that there is a wide degree of variation between different phenolic compounds and in their effectiveness as antioxidant. The findings of this study indicate that BV fruit in each type of solvent had a different antioxidant activity, contributed by different antioxidant components, such as  $\beta$ -carotene, vitamin C, Butylated Hydroxy Toluene (BHT), phenolic compounds. The high antioxidant activity of barberry fruit might be due to its flavonoid contents.

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