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Impact of Extraction Solvents on Total Antioxidant Capacity of Barberry (*Berberis Crataegina*) Plant

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ABSTRACT

Defense mechanisms involving antioxidant molecules play a key role in neutralizing free radicals formed in the human body. It is important to determine the content of these molecules, which have become important in human nutrition in recent years, in consumed foods. This study aims to determine the total antioxidant capacity of the barberry (*Berberis Crataegina*) plant, which is consumed in many regions of Turkiye. For this purpose, firstly, barberry plant samples were collected from Mersin in summer season, the extracts were prepared with different solvents and CERAC method was used to determine the total antioxidant capacities of the samples. The highest value of total antioxidant capacity was obtained by 80 % (v/v) methanol solution for flesh part of the fruit. Total phenolic compound and total antioxidant capacities of the flesh part of barberry samples were calculated as 1.954 mmol gallic acid (GA) g^{-1} and 1.288 mmol Tr (TR) g^{-1}, by Folin-Ciocalteu method and Cerium Reducing Antioxidant Capacity Assay (CERAC), respectively.

Keywords: *Berberis crataegina*, Total antioxidant capacity, Cerium Reducing Antioxidant Capacity, Solvent effect.

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Introduction

Oxidative stress is a widely used term in medical sciences that explains the physiology of very common diseases, like diabetes, high blood pressure, preeclampsia, atherosclerosis, acute renal failure, Alzheimer's and Parkinson's. Antioxidants are molecules that prevent the formation of free radicals or destroy existing radicals, disabling cell damage by sweeping and generally have a phenolic function in their structure [1]. These compounds function as shields in the body, and neutralize free radicals by donating their own electrons without producing free radicals in the process [2,3]). Furthermore, it is more significant for an antioxidant compound to prevent cell damage by scavenging free radicals, inhibiting the formation of more stable ones [4]. These natural compounds may be found in many fruits, herbs, vegetables, and plants [5]. One of the most important parameters for the evaluation of the nutritional value of foods is to measure their antioxidant activity/capacity [6]. Various assays, involving different mechanisms may be suggested to monitor antioxidant activity/capacity such as: (i) electron transfer-based assays, (ii) hydrogen atom transfer-based assays, (iii) mixed-mode assays, (iv) lipid peroxidation assays, (v) ROS/RNS scavenging assays, (vi) oxidative stress biomarkers and cellular-based assays, and (vii) chromatographic, chemometric, and electrochemical assays [7]. Although these methods are generally divided into two categories as hydrogen atom transfer (HAT) reaction and electron transfer (ET) reaction-based methods, ET and HAT mechanisms often operate together in various examples. The most widely used ET-based spectrophotometric total antioxidant capacity (TAC) assays may be listed as Folin−Ciocalteu, ABTS/ TEAC (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid/Trolox equivalent antioxidant capacity), Cupric Reducing Antioxidant Capacity (CUPRAC), and Ferric Reducing Antioxidant Power (FRAP). Besides, HAT- based antioxidant activity assays are; oxygen radical absorbance capacity (ORAC), total peroxyl radical trapping antioxidant parameter (TRAP) and total oxyradical scavenging capacity (TOSC) [8–10].

Another user-friendly and applicable spectrophotometric method, Cerium IV Reducing Antioxidant Capacity (CERAC) based on the oxidation of antioxidant compounds with Ce(IV) sulfate at room temperature under controlled conditions [11] was chosen to observe the solvent effect for the determination of total antioxidant capacity of *Berberis crataegina* which is well-known as "karamuk" in Turkiye, belongs to the family Berberidaceae. CERAC method is based on the measurement of the absorbance value of unreacted CeIV at 320 nm under adjusted conditions as pH and oxidant concentration. The method was previously applied to determine the total antioxidant capacities of various samples such as herbs, herbal teas and common fruits [11]. The fruits of the plant vary from dark purple to black in colour, ripening in late summer or autumn [12]. Various diseases may be treated according to traditional methods, using all parts of the plant [13]. Sun-dried fruits of *Berberis*

crataegina are a popular snack-food consumed by Anatolian people due to their health effects. Bioactive characteristics, potential antioxidant and antimicrobial features of Berberis crataegina was previously discussed in conducted literature [14,15]. However, this study is important as it indicates the impact of extraction solvent on total phenolic content and total antioxidant capacity of barberry fruit, installing CERAC method for the first time.

Extraction solvent is one of the major factors to evaluate the efficiency of extraction in terms of bioactive compounds and potential health effects [16]. In this study, total antioxidant capacity of of *Berberis crataegina* extracts was assessed by CERAC method. The compositions of extraction solutions were varied as 50.0 mL water, 50.0 mL 100 % (v/v), 80 %(v/v) and 50 %(v/v) for methanol and ethanol. The total antioxidant capacity and reducing phenolics content of the samples were recorded as Trolox equivalents. The obtained results of antioxidant assay were correlated to those obtained by reference methods such as CUPRAC [17] and Folin-Ciocalteu (FC) [18].

Materials and Methods

Chemicals and Instrumentation

All the reagents used during experiments were of analytical grade. Cerium(IV) sulfate tetrahydrate [$Ce(SO_4)_2.4H_2O$], Folin– Ciocalteu phenol reagent, sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), sodium potassium tartarate, copper(II) sulfate ($CuSO_4$), absolute ethyl alcohol (EtOH), pure acetone, methanol, lanthanum(III)chloridehеptahydrate, ethylacetate(EtAc) and CH_3COONH_4, sodium sulfate, trolox were from Merck (Germany).

Quartz cuvettes were used for the measurements of the visible spectra and absorption using a Varian Cary 100 UV–visible spectrophotometer (Mulgrave,Victoria,Australia). The solutions were homogenised by using a Heidolph vortex stirrer (Nuremberg, Germany). Samples of *Berberis crataegina* were ground by blender (Magic Bullet Blender) and prepared solutions of them were centrifuged using MSE Mistral 2000 centrifuge (Sanyo Gallenkamp,Middlesex,UK) prior to analysis.

Sample Preparation

The plant was collected from Mersin, Turkiye during summer season. The flesh part of the fruit was separated from the seeds and investigated separately as flesh and seeds. The samples were first pestled into small pieces in a porcelain mortar and then ground with a blender and stored in polyethylene bags in 22^0C.

For the observation of the impact of extraction solvents, seven types of solvents were defined. For the extraction of the samples, 5.0 g of flesh and seed samples were weighed accurately and mixed with 50.0 mL water, 50.0 mL 100 % (v/v), 80 %(v/v) and 50 %(v/v) methanol and 50.0 mL 100 %(v/v), 80 %(v/v) and 50 %(v/v) ethanol in a shaker for 60 minutes at 350 rpm. Afterwards, the extracts were filtered using filter paper (Whatman, 11µm pore size). For further evaluation, total phenolic content (TPC), and total antioxidant capacities (TAC) were determined installing Folin-Ciocalteu (FC), CERAC and CUPRAC assays, respectively.

Determination of Antioxidant Properties

CeIV based reducing antioxidant capacity (CERAC) method:

For the determination of the TAC of the extract solutions CERAC method (Ozyurt et. al., 2007) was installed. 1 mL of 2×10^{-3} molL^{-1} $Ce(SO_4)_2$ + 1 mL sample + 8 mL - H_2O (final volume of 10 mL) solutions were prepared and allowed to stand at room temperature for 30 min. The absorbance values were recorded at 320 nm using blank reagent as distilled water. TAC values were calculated as Trolox-Equivalent Antioxidant Capacity (TEAC) and displayed as mg Trolox equivalent/100 g sample. The experiments were held as three replicates.

Trolox equivalent antioxidant capacity values of the sample extracts were calculated using the equation given below;

$$TEAC\ \left(mmol\frac{Trolox}{g}\right) = [(Ao - A_1)/\varepsilon_{Trolox}] \times V_f/V_i \times DF \times V_e/m \quad (1)$$

Where;

A_o: Initial absorbance value of CeIV solution (λ_{max} = 320 nm); A_1: Final absorbance value after barberry extract addition (λ_{max} = 320 nm); $\varepsilon_{Troloks}$: Molar absorption coefficient for trolox (mol^{-1} L cm^{-1}); V_f: Final volume (mL); V_s: Sample volume (mL); DF: Dilution Factor; V_e: Extract volume (mL); m: mass of the sample (g)

Cupric ion reducing antioxidant capacity (CUPRAC) method:

CUPRAC assay was installed by addition of 1 mL of 1×10^{-2} molL^{-1} copper (II) chloride solution, 1 mL of 7.5×10^{-3} molL^{-1} neocuproine solution and 1 mL of 1 molL^{-1} ammonium acetate buffer (pH 7.0) to a test tube, respectively. 1.0 mL of extract solution was added to the mixture and allowed to stand at room temperature for 30 min. The absorbance values of the final solutions were recorded at 450 nm against the blank solution. TEAC values of the samples was calculated in mg Trolox equivalent/ 100 g sample [17]. All the experiments were performed in triplicates.

Folin-Ciocalteu (FC) method:

The determination of total polyphenol content of the samples was performed referring FC method as described by Singleton et al. (1999) [18]. The total phenolic content of the samples was estimated as gallic acid equivalent (GAE) in micromole per gram of sample extract with the aid of constructed gallic acid calibration graph. The analyses were conducted in triplicates.

Results And Discussion

Impact of Extraction Solvent on Determination of TAC of Barberry Samples by Spectrophotometric CERAC Method

Defining the appropriate type of extraction solvent is crucial for the effective determination of total antioxidant capacity and the total phenolic content of antioxidant-rich fruits or plants. Initially, the TAC values of phenolic standards that have potential to be found in the barberry samples were calculated as trolox equivalents, and for this purpose the calibration graph of freshly prepared Trolox (TR) solution was constructed as molar concentration of TR versus absorbance values measured at 320 nm which is the maximum absorption wavelength of Ce (IV). The linear calibration equation was computed as y= -1.43c +1.094 (R^2= 0.9989). Prior to the evaluation of impact of extraction solvents, barberry samples were classified as flesh and seeds for the determination of total antioxidant capacities installing spectrophotometric CERAC assay. Each part of the fruit was extracted using different solvents in accordance with the procedure mentioned in Section "Sample Preparation", to observe the influence of the extraction solvents on TAC values. The findings were displayed as TAC values versus solvent type by the column graph, for a better understanding.

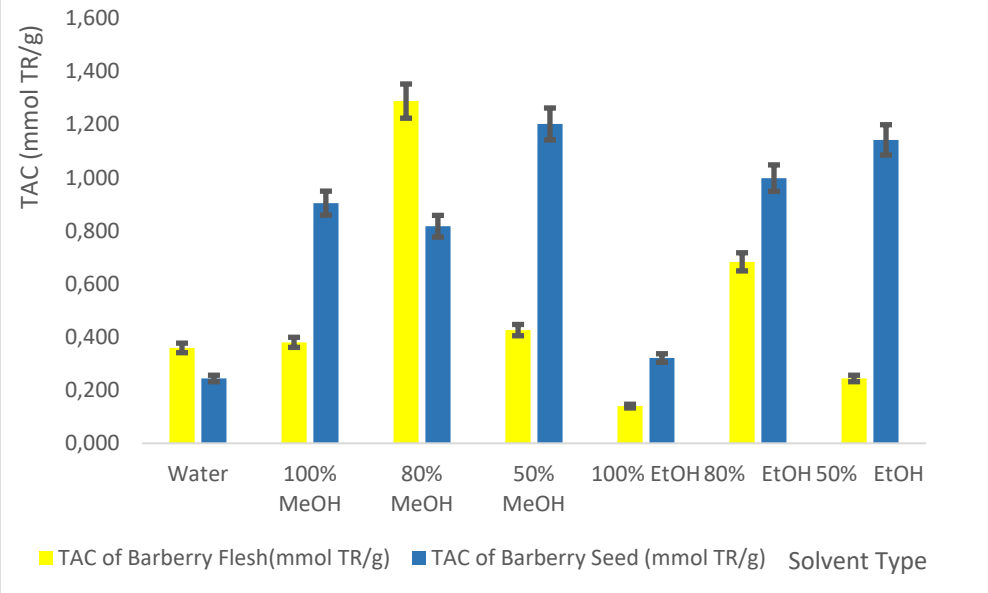

Figure 1. Effect of solvent type on total antioxidant capacities of flesh and seed parts of barberry.

As displayed in Figure 1, the highest antioxidant capacity belongs to the 80 % methanol extract of the flesh of barberry fruit, followed by 50 % methanol extract of seed of barberry fruit. The variation in the findings may be explained by the variation of bioactive compounds found in the samples as well as the variation at the polarity of solvents. Similar results were obtained in the study reported by Chavan et. al [19], however several studies were previously conducted proposing a different order of extraction solvents [20]. Additionally, the study investigating the effect of extraction solvent on the antioxidant capacity of berries [21], it was revealed that the organic solvent-water mixtures showed higher efficacy when compared to pure solvents, in compatible with the findings of the current study.

Determination of TAC and TPC of Barberry Samples

80 % methanol (v/v) extracts and water infusions of seed and flesh parts of the collected samples were prepared and TAC and TPC of the extracts were calculated as trolox and gallic acid equivalents, respectively. Infusions were prepared so as to evaluate the total antioxidant intake in form of fruit tea. For this purpose, the calibration graphs were constructed as molar concentration values of freshly prepared trolox / gallic acid solution versus absorbance values and linear calibration equations were computed as follows y= -1.43 $\times$ 10^4 c +1.094 (R^2= 0.9989) for CERAC; y= 1.82c $\times$ 10^4 + 0.0024 (R^2= 0.9984) for CUPRAC and y= 0.62c $\times$ 10^4 -0.092 (R^2= 0.9923) for FC assay. Table 1 summarizes the findings for the comparison of TAC and TPC of seed and fruit parts of barberry.

Table 1. The Total Antioxidant Capacities and Total Phenolic Content of Flesh and Seed Parts of Barberry (expressed in 1g of fresh weight)

Sample	Total Phenolic Content by FC Method (mmol GA g^{-1})		Total Antioxidant Capacity			
			CERAC (mmol TR g^{-1})		CUPRAC (mmol TR g^{-1})	
	Infusion	80 % (v/v; MeOH:Water,)	Infusion	80 % (v/v;MeOH:Water,)	Infusion	80 % (v/v;MeOH:Water,)
Flesh samples	1.689±0.033	1.954±0.038	0.359±0.090	1.288±0.024	0.411±0.039	0.765±0.045
Kernel Samples	0.600±0.013	1.103±0.028	0.244±0.069	0.817±0.011	0.230±0.018	0.602±0.032

There are various methods to evaluate the antioxidant potential with specific mechanisms of action such as hydrogen atom transfer, single electron transfer, and targeted scavenging activities. The selection of an appropriate method or combination of assays is essential for valid assessment of antioxidant activity and eventually the potential of antioxidants as health-promoting agents or preservative food additives [22].

The study involves the comparison of spectrophotometric assays that are based on electron transfer. Different parameters including the antioxidant mechanism, type of substrate, oxidation initiator and type of extraction solvent have influence on evaluation explaining the achievement of various antioxidant capacities [23].

Regardless from the assay installed for analysis, highest total antioxidant capacity was achieved after extraction with 80 % MeOH solution and 50 % MeOH solution, respectively for flesh and seed parts of the barberry samples. As it is displayed in Table 1, the values obtained by three methods vary from each other. This difference is due to the fact that the Folin-Ciocalteu method measures the total phenolic content of the samples. Besides, the reason of values obtained with the aid of Folin-Ciocalteu method operating in an alkaline environment (pH = 10) is that phenolic antioxidants lose their protons at pH 10, are more prone to oxidation, and the Folin reagent has an indefinitely high redox potential [24]. When the total antioxidant capacity values of medicinal plant infusions and herbal extracts are examined, the capacity values obtained are not the same as each other since the different methods have different redox potentials and different mechanisms [6]. TAC content of flesh samples achieved by CUPRAC assay were higher than the total phenolic content, in agreement with the study of Dimitrijevic et. al. [14]. The difference at the mechanisms of the assays allows the detection of different compounds. Folin-Ciocalteu method is capable for the detection of polyphenols whereas the CUPRAC assay allows the detection of not only polyphenols but also thiols, ascorbic acid, and some of the lipophilic antioxidants . Consequently, it may be said that the barberry plant has a significant amount of total antioxidant capacity.

Berries are the most important group among the fruits to prevent inflammation, cardiovascular diseases and eliminate the effects of carcinogenic diseases with their enhancing vitamin, mineral and polyphenol content [25]. When total phenolic content and total antioxidant capacities of different types of berries were evaluated, the results were observed to vary within a range due to the differences at the mechanism of the installed assays and extraction procedures [24]. In addition, it may be concluded that barberry fruit possess considerable values of total antioxidant capacity and total phenolic content.

Conclusion

This study focuses on the impact of extraction solvents on the total antioxidant capacity of barberry fruit (grown in Mersin, Turkiye) determined by assays with different mechanisms. Among these methods, CERAC method was applied for the first time in the literature for the evaluation of total antioxidant capacity of barberry fruit. As the fruit matrix is significantly complex involving both hydrophilic and hydrophobic biocompounds, the extraction solvent has a direct influence on the total antioxidant capacities of foods and must be considered. Our findings indicate that barberry fruit represents a valuable dietary source, combining a high polyphenol content with a strong total antioxidant capacity.

Conflicts of interest

There are no conflicts of interest in this work.

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