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Orange, red and purple barberries: Effect of *in-vitro* digestion on antioxidants and ACE inhibitors

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ABSTRACT

Bioactive compounds such as phenolics, anthocyanins and carotenoids were investigated in *Berberis* plant with purple (PB), red (RB) and orange (OB) fruits (barberries). The study was aimed to evaluate the bioaccessibility of the antioxidants and ACE (angiotensin converting enzyme) inhibitory compounds of barberry plants upon their transit through *in-vitro* digestion. Among barberries, (PB) exhibited the strongest antioxidant activity due to its higher phenolic and flavonoid content. The order of total phenolics and anthocyanins was PB > RB > OB. Chlorogenic acid was the main phenolic compound in all barberries even after *in-vitro* digestion. All barberries had similar amounts of total carotenoids (4.45 mgβ-carotene/100gDW). After *in-vitro* digestion, decreasing phenolics, anthocyanins and carotenoids led to a reduced antioxidant activity. Anthocyanins were found the most sensitive compounds *in-vitro* digestion. In terms of ACE inhibition activity, only RB exhibited ACE inhibition before (73.84%) and after (65.51%) *in-vitro* digestion. No ACE inhibition activity was detected at all in RB and OB samples. Leaves and branches of *Berberis* had also over 50% ACE inhibitory activity. To our knowledge, this is the first study on the carotenoids of barberry and the effect of *in-vitro* digestion on its bioactive compounds and ACE inhibitors.

1. Introduction

The genus *Berberis* wild plant belongs to the Berberidaceae family and is widely grown in Europe, Asia, Iran and in the Central Anatolia of Turkey. Roots, leaves, branches, and fruits of *Berberis*, which have been used as herbal treatment since ancient times, are currently employed in pharmaceutical and food industries. The fruit of *Berberis* (barberry) known as''Karamuk'' in Turkish, is usually consumed in various forms as fresh fruit, juice, jam, beverage, food colorant, and other processed products (Siow, Sarna, & Karmin, 2011).

Barberries are rich in bioactive compounds such as phenolics, anthocyanins, carotenoids, alkaloids and vitamin C (Ardestani, Sahari, Barzegar, & Abbasi, 2013; Končić, Kremer, Karlović, & Kosalec, 2010). Polyphenols are secondary plant metabolites which show a wide range of biological and physiological functions, such as anti-allergenic, anti--inflammatory, antimicrobial and antioxidant activities (Balasundram, Sundram, & Samman, 2006; Middleton, Kandaswami, & Theoharides, 2000). Anthocyanins, a sub-class of polyphenols, have potential as natural water-soluble colorants and powerful antioxidants in food, pharmaceutical and cosmetic industries. In addition, polyphenols including flavonoids and anthocyanins have been proved to be responsible for regulating blood pressure (anti-hypertensive effect) which is closely related to ACE inhibition activity (Kwon et al., 2010; Ojeda et al., 2010; Sharifi, Souri, Ziai, Amin, & Amanlou, 2013). Carotenoids are other bioactive compounds of barberries that provide therapeutic effects on a variety of chronic diseases such as cancer, cardiovascular, diabetes (Stahl & Sies, 2005). They are liposoluble and more stable food colorants than anthocyanins. However, stability of polyphenols and carotenoids is affected by temperature, pH, oxygen, enzymes, food matrix (Fang & Bhandari, 2010), food processing and post-harvest treatment (Saini & Keum, 2018) while insufficient gastric residence time, low permeability and/or low solubility impacts their bioavailability (McDougall, Dobson,

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Smith, Blake, & Stewart, 2005; Rein et al., 2013).

Barberries exhibit ACE inhibition activity as well as antioxidant activity (Fatehi-Hassanabad, Jafarzadeh, Tarhini, & Fatehi, 2005). Previous studies only focused on antioxidant properties, phenolics, alkaloids and anthocyanins of *Berberis* plant (Gundogdu, 2013; Končić et al., 2010; Özgen, Saraçoğlu, & Geçer, 2012). On the other hand, several authors have reported that *in-vitro* digestion induces significant changes in polyphenols of different food sources leading to changes in antioxidant and ACE inhibitory activities (Fatehi-Hassanabad, Jafarzadeh, Tarhini, & Fatehi, 2005; Fernández & Labra, 2013; Pérez-Vicente, Gil-Izquierdo, & García-Viguera, 2002). To the best of our knowledge, there is no report available regarding barberry carotenoids and the effect of *in-vitro* digestion on its antioxidants and ACE inhibitors. Therefore, the aim of this work was to evaluate the effects of *in-vitro* simulated gastrointestinal digestion on ACE inhibitory and antioxidant activities of the *Berberis* plant including barberry, leaf and branch parts taking the polyphenolic compounds into account.

2. Materials and metods

2.1. Material

Three *Berberis* genus with PB, RB and OB were obtained from Bayburt Province in Blacksea region of Turkey. After washing, barberries, leaves and branches were separated and milled using liquid nitrogen. Then, the powdered samples were lyophilized and stored at −20 °C until the analysis.

2.2. Chemical and reagents

All chemicals and reagents used in this study were of analytical or high performance liquid chromatography grade. Pepsin from porcine (0.7 FIP-U/mg), pancreatin from porcine pancreas, bile salts, dialysis tubing cellulose membrane, angiotensin converting enzyme (from rabbit lung, 2.0 U/mg protein) and hippuryl-histidyl-leucine (HHL) were purchased from Sigma-Aldrich (Steinheim, Germany).

2.3. Extraction of phenolics and carotenoids

Polyphenol extraction was employed using a modified version of the method of Končić et al. (2010). Lyophilized samples (0.5 g) were extracted using 15 mL mixture of methanol/MQ-water/formic acid (80:19:1, v/v/v). After ultrasonication at 4 °C for 30 min, the samples were centrifuged at 1968 *g* at 4°C for 30 min. Extraction was repeated 3 times for each sample. Finally, the pooled extracts were filtered through a 0.45 μm membrane.

Carotenoid extraction was performed based on the method described by Chuyen, Roach, Golding, Parks, and Nguyen (2017). Lyophilized samples (0.2 g) were extracted with 20 mL of a mixture of hexane/acetone/ethanol (50:25:25, v/v/v) for 30 min using a magnetically stirrer. The liquid phase was transferred to a beaker and stored in a dark room. The extraction was then repeated until the residual solid became colourless. To remove chlorophyll pigment, 5 mL of methanolic KOH (10 g/100 mL) was added to the collected liquid phase and incubated for 2 h in a dark room for saponification. After hexane layer was separated by a separatory funnel, extract dehydrated with anhydrous sodium-sulphate was filtered through a 0.45 μm membrane.

2.4. In-vitro digestion

Only barberries were exposed to *in-vitro* digestion procedure under simulated gastrointestinal tract conditions adapted from McDougall et al. (2005) since they are only edible parts. At the end of incubation, the solutions inside and outside of the dialysis tubing were taken as the "IN" and "OUT" samples representing the material that entered the serum and the material that remained in the gastrointestinal tract, respectively. Prior to HPLC analysis, samples were filtered through a 0.45 μm membrane filter. Bioaccessibility of bioactive compounds were calculated according to equation (1).

$$\%\ \text{Bioaccessibility} = \text{IN/INITIAL}*100 \quad (1)$$

2.5. Total phenolic and flavonoid content

Total phenolic content (TPC) was determined using Folin-Ciocalteu assay (Tezcan, Gültekin-Özgüven, Diken, Özçelik, & Erim, 2009). The results were expressed as mg gallic acid equivalents (GAE) per g on dry weight (DW) basis (Lucas - Gonzales et al., 2016) (Chuyen et al., 2017).

Total flavonoid content (TFC) was detected using the method of Valcarcel, Reilly, Gaffney, and O'Brien (2015). The results were expressed as mg rutin equivalents (RE) per g on DW basis.

2.6. Total anthocyanin content (TA)

The pH-differential method was conducted according to the method described by Lee, Durst, and Wrolstad (2005). TA was calculated as follows:

$$\text{mg cyn} - 3 - \text{gly/L} = \frac{A*MW*DF*1000}{\varepsilon*0.75} \quad (2)$$

where A=(A_{520}nm–A_{700}nm)pH 1.0–(A_{520}nm–A_{700}nm)pH 4.5; ε = 26900 M extinction coefficient, in $\text{Lxmole}^{-1}\text{x cm}^{-1}$ for cyanidin-3-glycoside; DF = dilution factors; MW = 449.2 g/mol for cyanidin-3-glycoside; 0.75 = pathlength (cm).

2.7. Determination of total carotenoid content (TCC)

The absorbance of carotenoid extract was directly measured at 450 nm. TCC (mgβ-carotene/100gDW) was calculated as follows (Nowacka & Wedzik, 2016); (Fatehi - Hassanabad et al., 2005) (

$$\text{TCC} = \frac{A450*V*10^6}{E^{1\%}*1000*m} \quad (3)$$

where A_{450} is the absorbance value of the hexane extract at 450 nm; V is the volume of extract (ml); $E^{1\%}$ 2505 cm is the extinction coefficient for β-carotene in hexane solution, m is the weight of lyophilized sample.

2.8. Determination of individual phenolics, anthocyanins and carotenoids using UFLC-PDA

Individual phenolics, anthocyanins and carotenoids were detected using an ultra-fast liquid chromatography with a PDA detector (SPD M20A, Schimadzu). The chromatographic separation for phenolics was performed on an ACE C18 column (250 mm × 4.6 mm, 3 μm) with a guard column (4.0 × 10 mm, 2 μm) (Advanced Chromatography Technologies Ltd., UK). A gradient of mobile phase A (MQ-water/formic acid, 99.9/0.1 v/v) and mobile phase B (acetonitrile) was used. The flow rate was 0.5 mL/min and the injection volume was 10 μL for each standard mixture and the column temperature was set to 40 °C. A 55 min gradient program was used with the gradient profile as follows: 0–5 min: 10% B, 5–45 min: 55% B, 45–48 min: 90% B, 48–55 min: 10%, 50–55min:10% B.

The chromatographic separation for anthocyanins was performed on a Luna-5μ-Phenyl-Hexyl column (250 mm × 4.6 mm, 5 μm) (Phenomenex, USA). A gradient of mobile phase A (MQ-water/formic acid, 95:5 v/v) and mobile phase B (acetonitrile) was used. The flow rate was 0.5 mL/min and the injection volume was 10 μL for each standard mixture and the column temperature was set to 40 °C. A 55 min gradient program was used with the gradient profile as follows: 0–40 min: 5% B, 40 min: 30% B, 55 min: 50% B, 57–59 min: 100% B, 60–65 min:5%.

The chromatographic separation for carotenoids was performed on a YMC-C30 column (250 mm × 4.6 mm, 5 μm) (YMC Europe Gmbh,

Germany). A gradient of mobile phase A (methanol/MTBE/MQ-water; 82:16:2 v/v/v) and mobile phase B (methanol/MTBE/MQ-water; 10:88:2 v/v/v) was used (Mokhtar et al., 2016). The flow rate was 1 mL/min and the injection volume was 10 μL for each standard mixture and the column temperature was set to 40 °C. A 17 min gradient program was used with the gradient profile as follows: 0–3 min: 0% B, 3–13 min: 100% B, 13–14 min: 0% B, 14–17 min: 0%B.

2.9. Antioxidant activity (AA)

AA was determined by DPPH radical scavenging (Tezcan et al., 2009) and CUPRAC (cupric ion reducing antioxidant capacity) (Apak, Güçlü, Özyürek, Karademir, & Altun, 2005) assays. All results were expressed as mg Trolox equivalents (TE) per gram DW.

2.10. ACE inhibitory activity

The ACE inhibition assay developed by Rayaprolu et al. (2015) was employed with a small modification. Lyophilized samples were dissolved in borat buffer (100 mmol/L). 50 μL of diluted sample and HHL (5 mmol/L) was mixed and pre-incubated at 37 °C for 15 min. The reaction was initiated by addition of 20 μL of ACE solution (100mU/mL), and the mixture was incubated at 37 °C for 60min. The reaction was terminated with addition of 250 μL of HCl (1 mol/L). Then, ethyl acetate was added for extraction of hippuric acid and the samples were centrifuged at 4000 g for 5 min. After centrifugation, 1 mL of supernatant was heated at 95 °C for 30 min to evaporate ethyl acetate. The remained residue was dissolved in MQ-water and the absorbance was measured at 228 nm. Results were expressed as %inhibition of ACE and calculated using the following formula:

$$\text{Inhibition activity } (\%) = \frac{Acontrol - Asample}{Acontrol - Ablank} \text{ x } 100 \quad (4)$$

where A_{blank} is the absorbance of the blank (HCl was added prior to the addition of ACE) $A_{control}$ is the absorbance without sample solution (buffer solution added instead of sample) and A_{sample} is the absorbance in the presence of the ACE and sample solution.

2.11. Statistical analysis

The data obtained from three independent experiments was reported as mean ± standard deviation. Statistical analysis was carried out using IBM SPSS Statistics 22(Chicago, USA) software. The significance ($p < 0.05$) of the differences between the means was determined using one-way analysis of variance (ANOVA) with Tukey's post-hoc test. The correlation coefficients (R^2) were calculated using Microsoft Office Excel 2017 software (Microsoft Corporation, USA).

3. Results and discussion

3.1. Effect of in-vitro digestion on phenolic acids and flavonoids

The results of TPC and TFC of barberries before and after *in-vitro* digestion are shown in Table 1. Among barberries, PB contained the highest amount of phenolics (81.42 mgGAE/gDW) followed by RB (74.74 mgGAE/gDW) and OB (67.70 mgGAE/gDW) ($p < 0.05$). Similarly, Ardestani et al. (2013) reported a higher phenolic content in PB (*B. integerrima*) (47.80 g/100 g) than in RB (*B.vulgaris*) (27.99 g/100 g). In contrast, Hassanpour and Alizadeh (2016) reported higher phenolics content for RB (*B.vulgaris*) (10.46–18.43 mgGAE/gDW) than PB (*B. integerrima*) (5.98–14.16 mgGAE/g DW). This variation in phenolic content depends on genotype, agronomic practices, maturity level at harvest, postharvest storage and environmental factors (Benvenuti, Pellati, Melegari, & Bertelli, 2004; Yilmaz, Ercisli, Zengin, Sengul, & Kafkas, 2009). Meanwhile, TFC and TPC results followed a similar order between barberries as PB(180.83 mg RE/g DW) >RB (170.04 mgRE/gDW) >OB(160.82 mg RE/gDW) ($p < 0.05$). In accordance with the literature, barberry extracts were shown to hold higher TFC than TPC (Atanassova, Georgieva, & Ivancheva, 2011; El Atki et al., 2019). After *in-vitro* digestion, the amount of recovered phenolics (IN) was found to be approximately 16 fold lower than the initial phenolics for all barberries. The highest concentration of bioaccessible phenolics was observed in OB due to the absence of unstable anthocyanins and the presence of more stable phenolic acids, predominantly chlorogenic acid. Approximately 90% of TPC in RB and PB degraded during *in-vitro* digestion due to alkaline pH of intestines (Bouayed, Hoffmann, & Bohn, 2011). Earlier, Lucas-Gonzalez et al. (2016) reported the phenolic recovery in maqui berry was to be 17.14%.

11 individual phenolic compounds were identified in barberries (Table 2). Main phenolic was chlorogenic acid in all barberries with the highest amount found in OB (32.9 mg/gDW). Gundogdu (2013) reported that chlorogenic acid (0.752 g/kg Fresh weight (FW)) was the dominant of twelve different phenolic compounds in RB (*B.vulgaris*). After *in-vitro* digestion, recovered phenolic acids were higher than recovered flavonoids in all barberries, especially for chlorogenic acid found in PB (24%), RB (16%) and OB (11%). Recovery of chlorogenic acid in acai and soursop fruits was also reported to be 18.28% and 11.08%, respectively (Dantas et al., 2019). Trace amount of bioaccessible flavonoids was recovered after *in-vitro* digestion while post digestion recovery of phenolic acids was higher than flavonoids including anthocyanins. An explanation to this change may be the interference of flavonoids with sugar proteins and dietary fiber

Table 1
Changes in bioactive compounds and bioactivities of barberries before and after *in-vitro* digestion.

	Red Barberry			Purple Barberry			Orange Barberry		
	Initial	IN	OUT	Initial	IN	OUT	Initial	IN	OUT
TPC (mgGAE/g)	$74.74 \pm 1.17^{b,A}$	$5.16 \pm 0.21^{b,C}$	$18.59 \pm 0.82^{c,B}$	$81.42 \pm 1.99^{a,A}$	$5.46 \pm 0.03^{b,C}$	$20.9 \pm 0.22^{b,B}$	$67.70 \pm 4.63^{c,A}$	$7.48 \pm 0.23^{a,C}$	$34.90 \pm 0.79^{a,B}$
TFC (mgRE/g)	$170.04 \pm 4.45^{b,A}$	$0.08 \pm 0.00^{c,B}$	T.A	$180.83 \pm 1.90^{a,A}$	$0.10 \pm 0.00^{b,B}$	T.A	$160.82 \pm 1.59^{c,A}$	$0.12 \pm 0.00^{a,B}$	T.A
TA (mgCYN-3-GLY/100 g)	$366.52 \pm 0.08^{b,A}$	$7.76 \pm 0.35^{a,C}$	$44.16 \pm 4.45^{a,B}$	$455.91 \pm 0.05^{a,A}$	$1.63 \pm 0.09^{b,B}$	$5.15 \pm 1.52^{b,B}$	$42.01 \pm 0.01^{c,A}$	$1.16 \pm 0.14^{c,C}$	$5.97 \pm 0.52^{b,B}$
TCC (mgβ-carotene/100 g)	$4.45 \pm 0.17^{a,A}$	$1.02 \pm 0.11^{a,C}$	$1.48 \pm 0.18^{a,B}$	$4.42 \pm 0.22^{a,A}$	$0.91 \pm 0.26^{a,B}$	$1.38 \pm 0.12^{a,B}$	$4.49 \pm 0.02^{a,A}$	$0.85 \pm 0.09^{b,C}$	$0.96 \pm 0.02^{b,B}$
DPPH (mgTE/g)	$96.63 \pm 9.89^{a,A}$	$0.02 \pm 0.00^{c,B}$	$0.01 \pm 0.00^{c,C}$	$112.70 \pm 1.10^{a,A}$	$0.05 \pm 0.00^{b,B}$	$0.03 \pm 0.00^{b,C}$	$74.59 \pm 0.53^{b,A}$	$0.07 \pm 0.00^{a,B}$	$0.03 \pm 0.00^{b,C}$
CUPRAC (mgTE/g)	$286.00 \pm 3.03^{a,A}$	$0.22 \pm 0.00^{b,C}$	$0.13 \pm 0.00^{a,B}$	$290.10 \pm 4.52^{a,A}$	$0.27 \pm 0.00^{b,B}$	$0.15 \pm 0.00^{a,C}$	$182.92 \pm 2.11^{b,A}$	$0.53 \pm 0.08^{a,B}$	$0.14 \pm 0.04^{a,C}$
ACE inhibition (%)	$73.84^{a,A}$	65.51^{B}	29.59^{C}	N.D	–	–	N.D	–	–

Data represent average values ± standard deviation of three independent samples. Different letters in the rows represent statistically significant differences ($p < 0.05$). ND: Not detected; TA: Trace Amount, TPC: Total phenolic content, TFC: Total flavonoid content, TA: Total anthocyanins, TCC: Total carotenoid content, GAE: Gallic acid equivalents, RE: Rutin equivalents, TE: Trolox equivalents, CYN-3-GLY: cyanidin-3-glycoside.

Table 2
Changes in individual phenolics, anthocyanins and carotenoids of barberries before and after *in-vitro* digestion.

mg/g	Red Barberry			Purple Barberry			Orange Barberry		
	initial	in	out	initial	İn	out	initial	in	out
Phenolic acids									
Gallic Acid	0.08 ± 0.01^{f}	0.03 ± 0.00^{d}	0.11 ± 0.00^{d,e}	0.07 ± 0.00^{d}	0.01 ± 0.00^{d}	0.1 ± 0.00^{c}	0.09 ± 0.00^{e}	T.A	0.08 ± 0.00^{c}
Chlorogenic acid	22.42 ± 0.24^{a}	2.60 ± 0.00^{a}	6.53 ± 0.01^{a}	22.53 ± 1.05^{a}	2.75 ± 0.00^{a}	9.73 ± 0.03^{a}	33.00 ± 0.00^{a}	4.00 ± 0.00^{a}	4.4 ± 0.00^{a}
Caffeic acid	0.23 ± 0.00^{e,f}	0.13 ± 0.00^{c}	0.36 ± 0.00^{c}	0.27 ± 0.00^{d}	0.14 ± 0.00^{b}	–	0.03 ± 0.00^{e}	0.27 ± 0.00^{b}	0.5 ± 0.00^{b}
Vanilic Acid	0.25 ± 0.14^{e,f}	–	–	0.51 ± 0.00^{d}	–	–	0.20 ± 0.00^{d}	–	–
Flavonoids									
EGCG	1.97 ± 0.00^{c}	–	–	0.36 ± 0.00^{d}	–	0.50 ± 0.00^{b}	–	–	–
Rutin	0.47 ± 0.02^{e}	0.02 ± 0.00^{e}	0.07 ± 0.00^{f}	0.70 ± 0.00^{d}	0.03 ± 0.00^{c,d}	0.14 ± 0.00^{c}	0.30 ± 0.00^{b}	T.A	0.08 ± 0.00^{c}
Luteolin	–	–	–	0.03 ± 0.00^{d}	–	–	0.05 ± 0.00^{e}	–	–
Quarcetin	–	–	–	–	–	–	0.08 ± 0.00^{e}	–	–
Epicatechin	1.21 ± 0.00^{d}	–	–	–	–	–	–	–	–
Phenolic aldehydes									
Vanilin	–	–	–	0.30 ± 0.00^{d}	–	–	–	–	–
Protocatechuic ethyl esters									
Ethyl-3,4dihydroxybenzoate	0.30 ± 0.00^{e,f}	0.05 ± 0.00^{d}	0.09 ± 0.00^{e,f}	0.37 ± 0.00^{d}	0.04 ± 0.00^{c}	0.10 ± 0.00^{c}	0.27 ± 0.00^{c}	0.04 ± 0.00^{c}	0.15 ± 0.00^{c}
Anthocyanins									
Delphinidin-3-glycoside	–	–	–	3.45 ± 0.00^{b}	–	T.A	T.A	–	–
Cyanidin-3-glycoside	T.A	–	–	1.81 ± 0.00^{c}	T.A	T.A	–	–	–
Petunidin-3-glycoside	–	–	–	0.30 ± 0.00^{d}	–	–	–	–	–
Delphinidin-chloride	0.51 ± 0.00^{e}	0.02 ± 0.00^{e}	0.13 ± 0.00^{d}	0.10 ± 0.00^{d}	T.A	T.A	0.12 ± 0.00^{e}	T.A	T.A
Peonidin-3-glycoside	0.02 ± 0.00^{f}	0.01 ± 0.00^{f}	0.04 ± 0.08^{g}	0.12 ± 0.00^{d}	–	T.A	–	–	–
Malvidin-chloride	0.17 ± 0.00^{f}	–	–	0.14 ± 0.00^{d}	–	–	–	–	–
Peonidin-chloride	0.03 ± 0.00^{f}	–	0.03 ± 0.00^{g}	0.03 ± 0.00^{d}	–	–	–	–	–
Petunidin-3-galactoside	3.70 ± 0.00^{b}	0.15 ± 0.00^{b}	0.96 ± 0.00^{b}	–	–	–	0.15 ± 0.00^{e}	–	–
Petunidin-chloride	0.01 ± 0.00^{f}	–	–	–	–	–	–	–	–
Pelargonidin-3-glycoside	–	–	–	T.A	–	–	–	–	–
Carotenoids									
Lutein and α-carotene	–	–	–	T.A	–	–	T.A	–	–

Data represent average values ± standard deviation of three independent samples. Different letters in the rows represent statistically significant differences ($p < 0.05$). Trace amount expressed as T.A.

compounds during *in-vitro* digestion (Gil-Izquierdo, Zafrilla, & Tomás-Barberán, 2002). After pancreatic digestion liberates these compounds, they may combine with flavonoids and could not cross the dialysis membrane and hence remained in the OUT fraction (Vallejo, Gil-Izquierdo, Pérez-Vicente, & García-Viguera, 2004).

Moreover, the order of recovered phenolics was barberries > leaves > branches (Fig. 1). TPC ranged from 24.81 to 42.62 mgGAE/gDW among leaf samples while its concentration varied between 21.36 and 15.02 mgGAE/gDW among branch samples of the plant ($p > 0.05$).

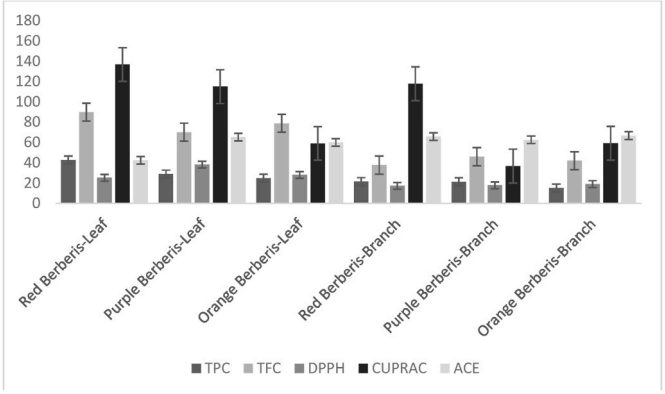

Fig. 1. Changes in bioactive compounds and bioactivities of *Berberis* leaf and branch samples.
TPC: total phenolic content, TFC: total flavonoid content.

Comparable TPC values reported to be 10.98–12.53 mgGAE/gDW for branch and 20.38–52.54 mgGAE/gDW for leaf in RB *(B.vulgaris)* by Končić et al. (2010). TFC of the leaves changed between 78.74 and 89.75 mgRE/gDW while that of branches ranged between 37.59 and 45.91 mgRE/gDW. Similar to barberries, all leaf samples contained chlorogenic acid as the major phenolic compound with the highest content present in PB (17.55 mg/gDW) (Table 3). EGCG was the major one in branch samples of PB and RB while cholorogenic acid was found dominantly in OB. Branch samples also contained chlorogenic and vanilic acids. In addition, trace amounts of luteolin and quercetin were present in all *Berberis* leaves and branches.

3.2. *Effect of in-vitro digestion on anthocyanins*

TA of barberries before and after *in-vitro* digestion is shown in Table 1. PB had the highest TA (455.91 mgCYN-3-GLY/100gDW) followed by RB (366.52 mgCYN-3-GLY/100gDW), OB (42.01 mgCYN-3-GLY/100gDW) ($p < 0.05$). Earlier, TA was reported to be 35.1–100.4 mgCYN-3-GLY/100gFW in Turkish RB genotypes (Yildiz et al., 2014). Several authors determined TA for RB (*B.boliviana*) as 7 g/100gDW (Jiménez et al., 2011), for RB (*B.vulgaris)* as 22.96–65.28 mgCYN-3-GLY/100gDW, and for PB (*B.integerrima*) as 39.15–417.07 mgCYN-3-GLY/100gDW (Ardestani et al., 2013; Hassanpour & Alizadeh, 2016). Although OB contains considerable amounts of anthocyanins, there is no available study. After *in-vitro* digestion, the most of anthocyanins in the IN fraction were degraded in all barberries. Although a significant reduction in TA was observed after *in-vitro* digestion, this didn't indicate a complete loss of these compounds. The

Table 3
Changes in individual phenolics, anthocyanins and carotenoids of *Berberis* leaf and branch samples.

mg/g	Leaf			Branch		
	Red	Purple	Orange	Red	Purple	Orange
Phenolic Acids						
Gallic Acid	–	–	0.04 ± 0.00^{c}	0.12 ± 0.00^{d}	0.10 ± 0.00^{d}	–
Chlorogenic acid	7.12 ± 0.14^{a}	17.56 ± 0.67^{a}	9.70 ± 0.64^{a}	2.23 ± 0.03^{c}	4.64 ± 0.05^{b}	6.00 ± 0.02^{a}
Caffeic Acid	0.17 ± 0.01^{d,e}	0.54 ± 0.01^{d}	0.35 ± 0.03^{c}	0.16 ± 0.00	0.26 ± 0.00^{d}	–
Vanilic Acid	0.32 ± 0.01^{d}	1.75 ± 0.00^{c}	0.35 ± 0.03^{c}	6.10 ± 0.19^{b}	3.60 ± 0.09^{c}	2.16 ± 0.02^{c}
Flavonoids						
EGCG	0.87 ± 0.00^{c}	–	–	12.60 ± 0.48^{a}	6.54 ± 0.14^{a}	4.25 ± 0.00^{b}
Rutin	5.00 ± 0.04^{b}	7.03 ± 0.38^{b}	4.10 ± 0.00^{b}	0.30 ± 0.00^{d}	0.86 ± 0.00^{d,e}	0.52 ± 0.00^{d}
Luteolin	0.03 ± 0.00^{e}	0.01 ± 0.00^{d}	0.001 ± 0.00^{c}	–	–	–
Quercetin	0.05 ± 0.00^{e}	0.07 ± 0.00^{d}	0.03 ± 0.00^{c}	–	0.04 ± 0.00^{e}	–

Data represent average values ± standard deviation of three independent samples. Different letters in the rows represent statistically significant differences ($p < 0.05$). Trace amount expressed as T.A.

highest recovery of bioaccessible anthocyanins was determined in OB (2.84%). Anthocyanins were metabolized, oxidized or degraded to different chemical compounds in intestinal phase due to alkaline pH (Pérez-Vicente et al., 2002). Consequently, bioaccessibility and metabolism of anthocyanins are negatively affected. Several authors demonstrated a low absorption of anthocyanins (1–20%) in other fruits (Fernández & Labra, 2013; Manach, Mazur, & Scalbert, 2005; Mosele, Macià, Romero, & Motilva, 2016).

Given in Table 2, PB had mainly delphinidin-3-glycoside (3.45 mg/g) and cyanidin-3-glycoside (1.81 mg/g) while RB contained petunidine-3-galactoside (3.7 mg/g). OB had only trace amounts of delphinidine-3-glycoside, petunidine-3-galactoside and delphinidin-chloride. After *in-vitro* digestion, only trace amounts of cyanidine-3-glycoside and delphinidin-chloride were remained in PB while peonidin-3-glycoside (50%), petunidin-3-galactoside (4%) and delphinidin-chloride (3.9%) were protected in RB. In RB, the amount of recovered petunidin-3-galactoside was higher than other anthocyanins while bioaccessibility of peonidin-3-glycoside (50%) was the highest in all anthocyanins. It is probably due to the fact that stability of anthocyanins depends on anthocyanin structure. Meanwhile, only delphinidin-chloride was remained in OB. Bioaccessible anthocyanins were not detected in acai, caja, and jabuticaba while anthocyanins were recovered in blueberry (malvinidin-3-glycoside, 2%; cyanidin-3-glycoside, 2.56%; pelargonidin-3-glycoside 1.81%), in raspberry (cyanidin-3-glycoside, 6.56%; pelargonidin-3-glycoside, 28.33%; delphinidin-3-glycoside, 15.26%) in the report of Dantas et al. (2019). These results are consistent with our data.

3.3. Effect of in-vitro digestion on carotenoids

Carotenoids of barberries before and after *in-vitro* digestion are given in Table 1. Barberries composed of similar amounts of carotenoids (4.5 mgβ-carotene/100 g) ($p > 0.05$). Although carotenoid content of PB and RB was similar to OB, the presence of anthocyanidins suppressed its yellow-orange colour. After *in-vitro* digestion, TCC was found to be the highest in RB (22.90%), followed by PB(20.44%) and OB(18.90%). Bioaccessible carotenoids of orange, plums, amaranth were reported between 3% and 11% (Veda, Kamath, Platel, Begum, & Srinivasan, 2006). This difference may be related to the acidification treatment in IN and OUT samples after *in-vitro* digestion. In terms of individual carotenoids, trace amounts of lutein and α-carotene was found in PB and OB. However, they could not be detected in any barberries after *in-vitro* digestion (Table 2). The bioaccessibility of carotenoids from plant sources is low owing to the inadequate degradation or digestion of carotene–protein, -plant cell walls and -fibers complexes to release carotenoids (Rein et al., 2013). To our knowledge, carotenoid content of barberries have not been investigated previously.

3.4. Effect of in-vitro digestion on antioxidant activity of Berberis plants

AA of *Berberis* was measured by DPPH and CUPRAC assays before and after *in-vitro* digestion is shown in Table 1. PB had the highest AA (112.70–290.10 mgTE/gDW) followed by RB (96.63–286.00 mgTE/gDW), OB (74.59–182.92 mgTE/gDW) regarding both DPPH and CUPRAC assays, respectively. Antioxidant assays utilized are in agreement, but values obtained by CUPRAC assay were higher than DPPH assay. Hassanpour and Alizadeh (2016) stated that AA of RB *(B.vulgaris)* and PB *(B.integerrima)* was in the range of 20.47–74.72% of DPPH inhibition and 20.2–70.39 mmolTE/L of ferric reducing antioxidant power (FRAP). In Turkish RB genotypes, AA was reported to be between 44.2 mmolTE/L and 52.0 mmoleTE/L in terms of TEAC and FRAP assays, respectively (Özgen et al. (2012). There is no available data on the antioxidant activity of barberries regarding CUPRAC assay. After *in-vitro* digestion, AA was not detected in considerable amounts in and OUT fractions by DPPH assay. AA of barberries in the IN and OUT fractions by CUPRAC assay was ordered as OB > PB > RB. PB which contains the highest TPC, TFC and TA exhibited the highest antioxidant activity, but these bioactive compounds were not remained after *in-vitro* digestion. Therefore, decreased antioxidant activity was indicated. This reduction of antioxidant activity in the intestinal phase was found in agreement with the reports of Correa-Betanzo et al. (2014) for blueberry (over 50%), Schulz et al. (2017) for juçara fruits (64%) and Pinto et al. (2017) for elderberry (%50). On the other hand, *Berberis* leaves and branches had lower AA than all barberries. As seen in Fig. 1, the leaves and branches of all varieties showed similar AA ($p > 0.05$). The descending order of antioxidant activity of *Berberis* is PB ~ RB > OB > leaves > branches for both DPPH and CUPRAC assays. Previously, Končić et al. (2010) reported that leaf exhibited a higher antioxidant activity than branch for RB (*B.vulgaris)*.

3.5. Effect of in-vitro digestion on ACE inhibition of Berberis plants

ACE inhibition activity of barberries before and after *in-vitro* digestion is shown in Table 1. The highest ACE inhibition was detected in RB (73.84%). In contrast, OB and PB showed no ACE inhibition. It was suggested that ACE inhibition exhibited no correlation with AA and TFC. A similar situation was declared for raspberry, pepper fruit and strawberry extracts in preceding works of Cheplick, Kwon, Bhowmik, and Shetty (2010); Ranilla, Kwon, Apostolidis, and Shetty (2010); Cheplick, Kwon, Bhowmik, and Shetty (2007). ACE inhibition may be related to some specific phenolic compounds. While Suzuki et al. (2002) found that chlorogenic acid and its derivatives decreased blood pressure in hypertensive rats, Cheplick et al. (2010) reported that there was no correlation between chlorogenic acid of strawberry and ACE inhibition activity. We also did not find any correlation between ACE inhibition activity and either total phenolics or presence of chlorogenic acid. On the other hand, several pharmacological studies indicated that ACE inhibition of *B. vulgaris* was related to its alkoloid constituent called berberine (Fatehi-Hassanabad, Jafarzadeh, Tarhini, & Fatehi, 2005).

After *in-vitro* digestion, ACE inhibition of RB was found to be 65.51% whereas it was not detected in PB and OB at all. Fernández and Labra (2013) reported that ACE inhibition of raw grape extracts was over 90% while it was over 80% after *in-vitro* digestion. This reduction might stemmed from berberine, which could inhibit ACE, could not cross the membrane to serum fraction.

On the other hand, *Berberis* leaves and branches had shown ACE inhibition over 50%. The order of ACE inhibition in *Berberis* plant was

RB > orange *Berberis* branch > purple *Berberis* leaf ~ red *Berberis* branch > purple *Berberis* branch > orange *Berberis* leaf ~ red *Berberis* leaf ($p < 0.05$) (Fig. 1). There is no research paper related to ACE inhibition activity of *Berberis* plant parts.

4. Conclusion

To our knowledge, this is the first report on bioaccessibility and stability of barberry bioactive compounds. Barberries are significant carriers of phenolics and anthocyanins, and hence are considered as a good source of natural antioxidants. *Berberis* holds high radical scavenging activity and ACE inhibition properties while these biological properties significantly varied depending on the plant part and genetic diversity. Our results suggest PB as a significant source of total phenolics and antioxidants while RB was ranked the highest for ACE inhibition activity. However, stability of bioactive compounds, especially anthocyanins, is affected in the intestinal digestion.

After *in-vitro* digestion antioxidant activity declined due to altered chemical composition. Morever, leaf and branches parts of *Berberis* indicated over 50% ACE inhibition activity. The current results support the therapeutic properties of *Berberis* plants, which are locally used and cheap, and its potential as source of natural bioactive compounds for further utilization in the functional food industry. Further studies are warranted for chemical screening of other compounds such as alkaloids using other solvents in order to identify new natural bioactive molecules from different plant parts and *in vivo* bioassays should be conducted.

CRediT authorship contribution statement

Eda Şensu: Investigation, experimental study, Methodology, Writing - original draft. **Kadriye Nur Kasapoğlu:** Investigation, Methodology, Writing - review & editing. **Mine Gültekin-Özgüven:** Investigation, Methodology, Writing - review & editing. **Evren Demircan:** Investigation, Methodology, Writing - review & editing. **Ayla Arslaner:** Investigation, Methodology. **Beraat Özçelik:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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