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Mineral Elements, Phenolics and Organic Acids of Leaves and Fruits from *Berberis crataegina* DC.

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The objective of the study is to determine the elemental composition, phenolic compounds and organic acid concentrations of fruits and leaves from *Berberis crataegina* DC. Calcium and potassium were the most abundant element in leaves and fruits, respectively. While the calcium concentration of leaves (11130.00 μg/g) was higher than in fruit (2389.00 μg/g), the potassium concentration of fruits (11210.00 μg/g) was the higher than in leaves (7857.00 μg/g). The predominant major phenolic compounds of leaves and fruits were rutin 170.87 mg/kg and chlogenic acid 70.24 mg/kg, respectively. Rutin and apigenin 7-O-glucoside were the highest concentration in fruits. Malic acid and citric acid of leaves were the highest and found as 4338.00 and 155.00 μg/g, respectively. The results showed that the elemental composition, phenolic compounds and organic acid concentrations were obvious difference in fruits and leaves from *B. crataegina*.

Key Words: *B. crataegina*, Elemental composition, Organic acids, Phenolic compounds.

INTRODUCTION

In the family Berberidaceae in Turkey, there are naturally four *Berberis* species (*B. vulgaris* L., *B. integerrima* Bunge, *B. cretica* L. and *B. crataegina* DC). One of them *B. crataegina* DC. is a shrub up to 2 m tall. It is the typical Central Anatolian *Berberis* and especially has a distribution on the rocky slopes and gullies between 800-1500 m[1]. Its main characteristics are spines usually shorter than the leaves and fruits black when ripe and red while immature[1]. It is one of the most important shrub species and widespread vegetation in the lakes district of Turkey[2,3].

The fruits and leaves of the plant has been used for healing purposes as a medicinal plant in traditional medicine and used as a food additive in Turkey. Fresh or dried roots and bark from this plant has been also repeatedly mentioned as a dye plant.

Medicinal properties for all parts of the *Berberis* species have been reported and its leaves, fruits and roots have been used for liver and gastrointestinal disorders along with enteritis and diarrhea[4], antimicrobial[5-7], antihistaminic and anticholinergic[8], antiinflammatory[9,10] and vasodilator[11]. Fruit extract from *B. vulgaris* L. has beneficial effects on both cardiovascular and neural system suggesting a potential use for treatment of hypertension, tachycardia and some neuronal disorders, such as epilepsy and convulsion[12,13].

In addition, barberry fruits (*B. vulgaris* L.) have been used for human consumption in the worldwide and their consumption is safe that is approved by FDA[14]. Tiny fruits from the *B. crataegina* DC. size of rice grains have been also commonly consumed as food and food additive in Turkey due to their delicious astringent and sourish flavour when they ripen in autumn. The limited literature was reported that the barberry fruits (*B. vulgaris* L.) include carbohydrates, organic acids, some vitamin, polyphenolic compounds, pectin, tannin, mineral elements[15]. The phenolic compounds in fruit contribute to the quality properties such as colour, flavour, astringency and bitterness[16] and they are directly related to some characteristics of fruit and vegetables such as taste, palatability and nutritional value and have particular importance for the characteristics and quality. Barberry also has plant alkaloids including berbamine, jatrorrhizine, berberine and palmatine[17]. The other compound which is responsible for organoleptic properties of fruit and fruit products is also organic acids. The contents of organic acids in fruit influence the balance of the flavour, the chemical stability and pH and thus the quality of the fruit[18,19]. Especially α-hydroxy acids including tartaric acid, malic acid, lactic acid and citric acid are responsible for these characteristics. According to our best of knowledge, there is no detailed data on chemical composition of fruits and leaves from *B. crataegina* DC.

In this study, it is aimed to determine the elemental composition, phenolic compounds and organic acid concentrations of fruits and leaves from *B. crataegina* DC.

EXPERIMENTAL

The study was conducted in Yukari Gökdere National protected area (37°35'-37°50'N; 30°50'-30°25'E) in the Lake Egirdir basin in the Lakes District of Turkey. It has a transition climate between the Mediterranean climate and the Central Anatolian continental climate with dry and hot summers and harsh and rainy winters. The mean annual precipitation for 62 years[20] is 581 mm and the mean annual temperature is 12 ºC, the average humidity is 61 % and the average annual evaporation is 1221.9 mm.

The ripe fruits and leaves of *B. crataegina* DC., growing wild in the area, were collected from between altitude 1000 and 1600 m in September 2008. The samples were transported in carton bags from the area to the laboratory and dried to constant weight at 105 ºC before analysis. The authentic specimen was identified by Dr. R. Süleyman Göktürk (R.S. Göktürk), from the department of Biology, Faculty of Science, Akdeniz University. A representative specimen was deposited in the herbarium of the Akdeniz University, Faculty of Science (7427-AKDU).

Elemental composition analysis by ICP-OES: The nitric acid used was of supra pure grade (Merck, Germany). The single element standard solutions used throughout the analysis were purchased from inorganic ventures/iv Labs, USA. The deionized water used was prepared using the Millipore, Milli-Q system (France).

Sample digestion was performed using a microwave digestion system ETHOS 900 Plus, Milestone, Italy. The instrument used for the determination of major elements in fruits and leaves from *B. crataegina* DC. were an inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin-Elmer, Optima 5300 DV, USA). A cross-flow type nebulizer was used for pneumatic nebulization in the ICP-OES instrument.

Samples of fruits and leaves from *B. crataegina* DC. were dried to constant mass at 105 ºC and powdered with a Teflon rod in a Teflon vessel. About 0.500 g of powdered fruits and leaves samples were weighed and 1 mL of hydrogen peroxide and 5 mL of nitric acid (65 %) were added into the digestion vessel. After completing the digestion, the solution was made up to 25 mL with nanopure water and analyzed by ICP-OES.

Phenolic compounds by HPLC analysis: About 5 g of accurately weighed fruits and leaves samples (dried and powdered) were homogenized with 100 mL solvent mixure of aceton:methanol:acetic acid (50:49.5:0.5) and then ultrasonicated using an ultrasonic bath (Super RK 255 H, Bandelin electronic, Berlin Germany) for 2 h. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated at reduced pressure (Rotavator, T < 40 ºC) under vacuum, to constant weight and then dissolved in methanol.

Phenolic compounds of fruits and leaves from *B. crataegina* DC. were evaluated by reversed phase-high performance liquid chromatography (RP-HPLC). Detection and quantification was carried out with a SCL-10Avp System controller, a SIL-10AD vp Autosampler, a LC-10AD vp pump, a DGU-14a degasser, a CTO-10 A vp column heater and a diode array dedector with wavelengths set at 278 nm. The 250 mm × 4.6 mm i.d. column used was filled with Agilent EclipseXDB-C_{18}, 5 µ. The flow rate was 0.8 mL/min, injection volume was 20 µL and the column temperature was set at 30 ºC. Gradient elution of two solvents was used: solvent A consisted of: acetic-water (3:97 v/v), solvent B: methanol and the gradient program used is given Table-1.

TABLE-1
SOLVENT GRADIENT CONDITIONS WITH LINEAR GRADIENT

Final time	A (%)	B (%)	Final time	A (%)	B (%)
(Initial)	100	0	60	67	33
3	95	5	62	58	42
20	72	28	70	50	50
28	75	25	75	20	80
35	70	30	80	0	100
45	67	33	–	–	–

The data were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software system. The fruit and leave samples, standard solutions and mobile phases were filtered by a 0.45 µm poure size membrane filter. The amount of phenolic compounds in the samples was calculated using external calibration curves, which were obtained for each phenolic standard. Phenolic compositions of samples were determined by the modified method of Capanio *et al.*[21]. All determinations were done three times by using three different samples.

Organic acid analyses by HPLC: Dried and powdered fruit and leaves samples (5 g) were homogenized with 25 mL of phosphoric acid (2 %) and filtered. The filtrates of samples were mixed with 0.01 M potassium dihydrogen phosphate (pH 8). 1 mL of mixure was passed through a C_{18} Sep-Pac cartridge. The eluates of samples were filtered through 0.45 µm membrane filter before 20 µL injections. High pressure liquid chromatography (HPLC) analysis of organic acids was performed by HPLC on a Shimadzu class LC VP HPLC system with class LC-VP software equipped with a photo-diode array detector (DAD) and a pump (LC-6AD). Distilled water adjusted to pH 2.3 with phosphoric acid (H_3PO_4) was used as mobile phase. The flow rate was 0.5 mL/min and the column temperature was set at 30 ºC. ACE C_{18} (250 mm × 4.6 mm i.d., 5 µm) column was used. The DAD detector was set at 210 nm. Initial identity assignment of organic acids was based on comparison retention data obtained with DAD detector for standard compounds and sample components. Quantification was achieved by using peak areas from external calibration with standard (Galactronic, tartaric, malic, lactic, acetic, citric, succinic, fumaric acids) solutions. All determinations were done three times by using three different samples.

RESULTS AND DISCUSSION

The elemental composition of leaves and fruits from *B. crataegina* DC. was given in Table-2. Among the 13 elements for fruits and 12 elements for leaves were determined. Calcium

TABLE-2
ELEMENTAL COMPOSITIONS OF LEAVES AND FRUITS FROM *B. crataegina* DC.

No.	Element and wavelength (nm)	Composition (μg/g dried sample)	
		Leaves	Fruits
1	Zn 206.200	14.00 ± 1.00	25.00 ± 1.00
2	Cu 327.393	0.00 ± 0.00	3.00 ± 1.00
3	Fe 238.204	95.00 ± 1.00	44.00 ± 1.00
4	Mg 285.213	1777.00 ± 10.00	711.00 ± 10.00
5	Mn 257.610	41.00 ± 10.00	14.00 ± 2.00
6	Cd 228.802	2.00 ± 1.00	3.0 ± 1.00
7	B 249.677	38.00 ± 10.00	11.00 ± 0.00
8	P 213.617	4.00 ± 1.00	10.00 ± 0.00
9	As 188.979	3.00 ± 1.00	2.00 ± 1.00
10	Ba 233.527	17.00 ± 1.00	3.00 ± 0.00
11	Na 589.592	116.00 ± 10.00	86.00 ± 10.00
12	K 766.490	7857.00 ± 10.00	11210.00 ± 10.00
13	Ca 317.933	11130.00 ± 10.00	2389.00 ± 10.00

and potassium were the most abundant element in leaves and fruits, respectively. While the calcium concentration of leaves (11130.00 μg/g dried sample) was higher than in fruit (2389.00 μg/g dried sample), the potassium concentration of fruits (11210.00 μg/g dried sample) was the higher than in leaves (7857.00 μg/g dried sample). The magnesium content of leaves and fruits were also found as 1777.00 and 711.00 μg/g dried sample, respectively. Copper was not found in leaves. In addition, the fruits and leaves from *B. crataegina* DC. contained the highest concentration of sodium, iron, manganese, boron, barium zinc and were the minor elemental constituents. In the fruit from *B. aristata* DC., the percentage of different mineral elements such as phosphorus, potassium, calcium, magnesium and iron were found as 0.079, 0.439, 0.065, 0.061 and 0.011, respectively[22]. Gulfraz *et al.*[23] found that calcium, sodium, iron and zinc in fruits of *B. lyceum* were 1.8, 0.6, 0.2 and 0.8 g/100 g, respectively. Shah *et al.*[24] also determined Mn (136.12 μg/g), P (1315.00 μg/g) and Ca (2389.00 μg/g) in leaves of *B. lyceum*. Results were similar to the values found in the literature. Differences of elemental composition are probably originated from species, collect time, soil characteristics, fertilization and climate.

Reversed phase high-performance liquid chromatography (RP-HPLC) was used for seperation of phenolic compounds. As can be seen separation was achivied for 9 components including phenolic acids and flavonoids such as flavanons, flavons, flavanols and flavonols. Phenolic compounds of leaves and fruits from *B. crataegina* DC. (mg/kg dried sample) were shown in Table-3. The major differences were observed in fruits and leaves samples. While the rutin, vitexin, luteolin and eriodictiol were detected in leaves, chlorogenic acid, rutin, apigenin 7-O-glukozid, (+)-catechin, (+)-epicatechin and *p*-coumaric acid were found in fruits. The most abundant phenolic compounds detected were rutin as a flavonol (170.87) in leaves and chlogenic acid as a phenolic acid (70.24) in fruits.

The seperation and determination of organic acids in leaves and fruits from *B. crataegina* DC were carried out by HPLC and the results were given in Table-4. As it was shown in the table, malic and citric acids were seperated. The malic and citric acid values were 4338.00 and 155.00 μg/g dried sample in leaves and 96.00 μg/g dried sample in fruits, respectively. While malic acid was the most abundant organic

TABLE-3
PHENOLIC COMPOUNDS OF LEAVES AND FRUITS FROM *B. crataegina* DC.

Phenolic compounds	Composition (mg/kg dried sample)	
	Leaves	Fruits
Phenolic acids		
Chlogenic acid	0.00 ± 0.00	70.24 ± 1.54
p-Coumaric acid	0.00 ± 0.00	1.10 ± 0.01
Flavanones		
Eriodictiol	0.15 ± 0.00	0.00 ± 0.00
Flavons		
Apigenin 7-O-glukozid	0.00 ± 0.00	20.08 ± 3.71
Luteolin	0.24 ± 0.02	0.00 ± 0.00
Vitexin	0.29 ± 0.02	0.00 ± 0.00
Flavanols		
(-)-Epicatechin	0.00 ± 0.00	1.60 ± 0.00
(+)-Catechin	0.00 ± 0.00	8.41 ± 0.08
Flavonols		
Rutin	170.87 ± 2.99	27.09 ± 0.97

TABLE-4
ORGANIC ACID CONCENTRATIONS OF LEAVES AND FRUITS FROM *B. crataegina* DC.

Organic acids	Composition (μg/g dried sample)	
	Leaves	Fruits
Malic acid	4338.00 ± 8.00	96.00 ± 6.00
Citric acid	155.00 ± 5.00	15.00 ± 2.00

acid in leaves and fruits, both malic acid and citric acid were the highest in leaves. The other organic acids were not detected in leaves and fruits from *B. crataegina* DC. This result agrees with the findings of Pozniakovskii *et al.*[15]. They reported that citric acid and malic acid were the main organic acids in fruits of *B. vulgaris* L. Hanachi and Golkho[25] found malic acid of *Berberis* vulgaris fruit as 116.03 μg/100 g. It was also determined ascorbic acid as 256.48 in fruit of *B. vulgaris* L by Akbulut *et al.*[26]. According to our best of knowledge, there was no study conducted on the determination of the organic acid in fruits of *B. crataegina* DC and no study organic acids in leaves of all *Berberis* sp.

Conclusion

Fruits and leaves of *B. crataegina* DC were identified with the highest concentrations of phenolics such as chlogenic acid and rutin and organic acids such as malic acid and citric acid, respectively. The mineral elements present in both leaves and fruits. Calcium and potassium were the most abundant element found in leaves and fruits, respectively. Calcium in leaves and potassium in fruits were significantly higher.

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