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Phytochemical characterization of several hawthorn (*Crataegus* spp.) species sampled from the Eastern Mediterranean region of Turkey

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ABSTRACT

Background: We evaluated the total phenolic content, antioxidant capacity as well as antioxidant activity of five *Crataegus* species (A1, A2, Y1, Y2, Y4 accessions of *Crataegus aronia* var. *aronia*; B2, B3, B5, B6, B7, B9, Y5 accessions of *C. aronia* var. *dentata*; B10 accession of *C. aronia* var. *minuta*; Y3 accession of *Crataegus orientalis* var. *orientalis* and A3 accession of *Crataegus monogyna* subsp. azarella). **Materials and Methods:** Antioxidant activity and total phenolic content of fruits were determined by β-carotene bleaching and Folin–Ciocalteu assays. Antioxidant capacity was determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. **Results:** *C. monogyna* subsp. *azarella* had the highest total phenol, antioxidant activity and antioxidant capacity of 55.2 mg gallic acid equivalents (GAE)/g dry weight (DW), 81.9% and 31.2%, respectively. *C. aronia* var. *aronia* was found to have the lowest total phenolic content (35.7 mg GAE/g DW). The antioxidant activities of fruit extracts increased in the order of *C. orientalis* var. *orientalis* < *C. aronia* var. *minuta* < *C. aronia* var. *dentata* < *C. aronia* var. *aronia* < *C. monogyna* subsp. *azarella* according to β-carotene/linoleic acid assay. In recent years, *C. aronia* var. *dentata* has gained importance as a commercial species in this region. B3 and B7 accessions had fruit weight more than 14 g, and considerable total phenol content, antioxidant activity and antioxidant capacity. **Conclusion:** This investigation shows the potential value of hawthorn fruit species as a good source of natural antioxidants and that consumption of hawthorn fruit or its products may contribute substantial amounts of antioxidants to the diet.

Key words: Antioxidant, *Crataegus* sp., hawthorn, total phenolic

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INTRODUCTION

Turkey is one of the genetic centers for *Crataegus*,[1] but there are few studies that have attempted to describe the *Crataegus* genus of Turkey. Browicz[2] described some of the *Crataegus* species from flora of Turkey, and Christensen[3] made many additions to this list. From the studies of Donmez, new species and information are being added on the *Crataegus* species.[4-6] Currently, there are more than 20 species of *Crataegus* in Turkey, including *Crataegus monogyna* Jacq., *Crataegus pentagyna* Willd., *Crataegus azarolus* L., *Crataegus orientalis* M. Bieb., *Crataegus rhipidophylla* Gaud. and *Crataegus laevigata* (Poir) DC. Although Turkey has numerous *Crataegus* species and many geographical areas with diverse wild growing *Crataegus* accessions, hawthorn is still an underutilized crop in Turkey.[7] There have been few reports describing the characteristics of some *Crataegus* species accessions.[7-10]

Although there are cultivated species of some hawthorn in the world,[11,12] usually they are selected from nature. Hawthorn is generally used for health treatment in Turkey.[8,13] Due to its positive effects on the cardiovascular system, *Crataegus* genus has recently become quite a popular herbal medicine in phytotherapy.[14] Recent studies have focused on the health benefits of aromatic and medicinal plants, which have antioxidant, antimicrobial, and mutagen properties.[15] Dietary intake of antioxidant compounds is important for health.[16] Also, increasing interest in

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nutraceuticals and functional foods has led plant breeders to initiate selection of crops with higher than normal phenolic and antioxidant properties, such as blueberries,[17] plums and peaches[18] and sea buckthorns.[19]

Similar to most parts of Turkey, growing of hawthorn is not yet made commercial in the Hatay province. In general, hawthorn plants grow in village gardens and as border plants in the arid areas of this region. In recent years, hawthorn fruits have been sold at good prices in the local markets. Therefore, the identification of Hatay hawthorn species and determination of the economic value of this crop become important.[7] The objectives of the present study were to determine antioxidant activities and total phenolic content of several *Crataegus* spp. accessions sampled from the Eastern Mediterranean region of Turkey, and investigate the variability among the hawthorn species.

MATERIALS AND METHODS

Chemicals

All the chemicals used were obtained from Sigma and Aldrich Company (St. Louis, MO, USA).

Plant materials

A total of 15 accessions of five hawthorn species (*Crataegus aronia* var. *aronia*, *C. monogyna* subsp. *azarella*, *C. aronia* var. *minuta*, *C. aronia* var. *dentata*, and *C. orientalis* var. *orientalis*) selected by Serçe *et al.*[7] from Eastern Mediterranean region of Turkey were used.

Approximately 3 kg of hawthorn fruits were harvested from each accession when 30–70% of fruit had reached color development. The fruits were selected according to the uniformity in shape and color and then transported to the laboratory for analysis. Samples were dried, ground to fine powder with a mortar and pestle, and kept at room temperature prior to extraction. The dried samples were packed into new plastic bags and stored in desiccators for a maximum of 3 days until antioxidant activity determination and total phenol analysis. The sample weighing about 100 g was extracted in a soxhlet with methanol (MeOH) at 60°C for 6 h. The extract was then filtered and concentrated in vacuum at 45°C. Finally, the extracts were lyophilized and kept in the dark at 4°C until analyzed.

Total phenolics in the methanol extracts were determined colorimetrically using Folin–Ciocalteu reagent as described by Slinkard and Singleton.[20] Gallic acid was used as a standard and results were expressed on the basis of milligrams of gallic acid equivalents (GAE) per gram dry weight (DW). Total antioxidant activity of samples was determined by hydrogen atom transfer reactions (β-carotene bleaching) assay. In β-carotene bleaching assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation.[21]

For determining the antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) method of Burits and Bucar[22] was used. The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of the purple-colored methanol solution of DPPH. This spectrophotometric assay uses stable radical DPPH as a reagent (Sigma-Aldrich). One hundred microlitres of various concentrations of the extracts in methanol were added to 5 ml of 0.004% methanol solution of DPPH After 30 min of incubation at room temperature, the absorbance was read against a blank at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH radical scavenging effect was calculated according to the following equation:

DPPH scavenging effect (%) = $[(AC_{(0)} - AC_{(\tau)})/AC_{(0)}] \times 100$,

where $AC_{(0)}$ is the absorbance of the control DPPH solution at 0 min, and $AC_{(\tau)}$ is the absorbance in the presence of test samples at 30 min.

The antioxidant activity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation.[23] A stock solution of β-carotene/linoleic acid (Sigma-Aldrich) was prepared as follows. First, 0.5 mg of β-carotene was dissolved in 1 ml of chloroform (HPLC grade), and then 25 µl of linoleic acid and 200 mg of Tween 40 (Merck) were added. The chloroform was subsequently evaporated using a vacuum evaporator. Next, 100 ml of distilled water saturated with oxygen (30 min at 100 ml/min) was added with vigorous shaking. Aliquots (2.5 ml) of this reaction mixture were transferred to test tubes, and 350 µl portions of the extracts (2 g/l in methanol) were added before incubating for 48 hours at room temperature. The same procedure was repeated with α-tocopherol at the same concentration and a blank containing only 350 µl of ethanol. After the incubation period, the absorbance of the mixtures was measured at 490 nm. The antioxidant capacities of the samples were compared with those of α-tocopherol and the blank.

For each quantitative trait measured, the means and the standard deviations were calculated using the TABULATE procedure of SAS.[24] Correlation analyses were conducted using CORR procedure.

RESULTS AND DISCUSSION

The results are the first evaluation of phytochemical characteristics of hawthorn species grown in Turkey. Considerable differences in all phytochemical properties were evident among the 15 hawthorn accessions within the five species, *C. aronia* var. *aronia*, *C. monogyna* subsp. *azarella*, *C. aronia* var. *minuta*, *C. aronia* var. *dentata*, and *C. orientalis* var. *orientalis*. Pomological analysis and genetic characterization of accessions of these species were made by Serçe *et al*.[7] However, phytochemical characteristics of these species and their associations with fruit traits were not investigated. The evaluation of correlations between the phytochemical and pomological characteristics indicated significant correlations.

Total phenolic contents and antioxidant activities of 15 accessions of hawthorn fruits are shown in Table 1. The accession B6 had the highest total phenolic content (57.1 mg GAE/g DW), followed by Y3 (51.2 mg GAE/g DW) and B7 (50.8 mg GAE/g DW). The overall average total phenolic content was 40.8 mg GAE/g DW [Table 2]. Our findings on hawthorn fruit phenolic content provide opportunities to improve hawthorn fruit quality and nutritive value. The total antioxidant activity among the hawthorn accessions ranges from 42.7% (Y4) to 82.9% (B6), as was determined by β-carotene/linoleic acid assay. The results of DPPH assay were similar to those of β-carotene assay and showed that B6 (33.2%) had the highest antioxidant capacity and Y3 (21.4%) had the lowest capacity.

Results from this study suggest that the antioxidant activities were significantly different among hawthorn species [Table 2]. The antioxidant activities of fruit extracts increased in the order *C. orientalis* var. *orientalis* < *C. aronia* var. *minuta* < *C. aronia* var. *dentata* < *C. aronia* var. *aronia* < *C. monogyna* subsp. *azarella*, according to β-carotene bleaching assay. *C. monogyna* subsp. a*zarella* had the highest total phenolic, antioxidant activity and capacity of 55.2 mg GAE/g DW, 81.9% and 31.2%, respectively. *C. aronia* var. *aronia* was found to have the lowest total phenolic content (35.7 mg GAE/g DW). However, the lowest antioxidant capacity determined by β–carotene/linoleic acid and DPPH assays was observed for *C. orientalis* var. *orientalis*. Similar results were obtained in the total phenolic content in previous studies, i.e. 12.8 mg GAE/g DW for *C. monogyna*,[25] 2.9 mg GAE/g DW for *C. pinnatifida*,[26] and 26.4 mg GAE/g DW for *C. monogyna*.[27] Also, Froehlicer *et al*.[25] reported a total antioxidant capacity of 54.0 mmol Trolox Equivalent /kg Fruit Weight for *C. monogyna*. According to our results, total phenolic and antioxidant capacity obtained were higher than these values. The average antioxidant activity of accessions (65.9%) was higher than that of some other fruits such as apple (25.7%), quince (60.3%), pear (14.0%),[28] and cactus pear (64.3%).[29]

C. aronia var. *dentata* was found to be commercially very

Table 1: Total phenolic content, antioxidant activity and antioxidant capacity of hawthorn accessions sampled from Hatay, Turkey

Accession	Species	Total phenolic content (mg GAE/g DW)	Antioxidant activity (β-carotene/linoleic acid assay, %)	Antioxidant capacity (DPPH, %)
A1	*C. aronia* var. *aronia*	26.6^{h*}	73.6^{de}	32.0^{ab}
A2	*C. aronia* var. *aronia*	40.6^{cd}	75.8^{cd}	29.9^{bcd}
A3	*C. monogyna* subsp. *azarella*	55.2^{a}	81.9^{ab}	31.2^{ab}
B10	*C. aronia* var. *minuta*	40.1^{d}	60.9^{gh}	24.8^{gfh}
B2	*C. aronia* var. *dentata*	32.6^{f}	56.2^{i}	22.4^{hi}
B3	*C. aronia* var. *dentata*	36.0^{e}	57.9^{hi}	23.6^{ghi}
B5	*C. aronia* var. *dentata*	42.2^{c}	74.6^{cde}	28.2^{cde}
B6	*C. aronia* var. *dentata*	57.1^{a}	82.9^{a}	33.2^{a}
B7	*C. aronia* var. *dentata*	50.8^{b}	78.4^{bc}	30.9^{abc}
B9	*C. aronia* var. *dentata*	39.3^{d}	62.3^{g}	25.6^{efg}
Y1	*C. aronia* var. *aronia*	30.2^{g}	71.0^{ef}	29.4^{bcd}
Y2	*C. aronia* var. *aronia*	41.2^{cd}	68.5^{f}	27.6^{def}
Y3	*C. orientalis* var. *orientalis*	51.2^{b}	57.3^{hi}	21.4^{i}
Y4	*C. aronia* var. *aronia*	39.8^{d}	42.7^{j}	24.4^{gh}
Y5	*C. aronia* var. *dentata*	28.2^{hg}	44.3^{j}	22.3^{hi}
Mean		40.8	65.9	27.1
$LSD_{0.05}$		2.1	4.1	3.0

*means with different letters are significantly different, at 0.05, by LSD method

promising because of its size, total soluble solids (TSS), total phenolic, antioxidant activity and antioxidant capacity. Accessions of this species are widely distributed in Hatay, Eastern Mediterranean region of Turkey, and fruits of this species have been sold in local markets at higher prices in recent years. Therefore, some farmers, especially in Belen County, are trying for the orchard establishment with plants of this species. Due to the smaller fruit size and TSS of *C. monogyna* subsp. *azarella*, commercial production has not become significant. Red colored fruits of this species with high phytochemical content may be used with the other hawthorn species in breeding studies. Also, Orhan *et al*.[30] reported that *C. monogyna* spp. could be a good alternative source of crude drug material. Therefore, it should be rational to cultivate the plant in the optimized agricultural conditions instead of collecting it from the wild. *C. orientalis* var. *orientalis* is found in the Kışlak village, Yayladağı county. Although this species has medium fruit size, TSS is higher than the other species; fruits with the red color as well as high total phenol content make it promising for cultivation.

Table 2: Total phenolic content, antioxidant activity and antioxidant capacity of hawthorn species sampled from Hatay, Turkey

Species	Total phenolic content (mg GAE/g DW)	Antioxidant activity (β-carotene/ linoleic acid assay, %)	Antioxidant capacity (DPPH, %)
C. aronia var. *aronia*	35.7 c*	66.3 ab	28.7 ab
C. monogyna subsp. *azarella*	55.2 a	81.9 a	31.2 a
C. aronia var. *minuta*	40.1 c	60.9 b	24.8 bc
C. aronia var. *dentata*	40.9 bc	65.2 ab	26.6 ab
C. orientalis var. *orientalis*	51.2 ab	57.3 b	21.4 c
$LSD_{0.05}$	4.49	4.45	4.48

*Means with different letters are significantly different, at 0.05, by LSD method

Correlation coefficients between antioxidant capacity of DPPH and total phenolic contents, and among these traits and other pomological characteristics are presented in Table 3. Among the pomological characteristics, flesh/seed ratio, TSS, fruit skin color b and C significantly correlated with antioxidant capacity, DPPH and total phenolic contents. It is also known that within the same fruit species and the accessions, the smaller fruits tend to have higher TP content and total antioxidant capacity since the compounds having higher activities are usually found in large amounts in fruit skin and smaller fruits have relatively larger skin area when compared to their larger counterparts.[31-33] In this study including five hawthorn species, a correlation was not found between fruit size and phytochemical characteristics. However, we found important correlation between antioxidant capacity and fruit color C (–0.59*) and between DPPH and flesh/seed ratio (–0.59*). Also, total phenolic content was affected by TSS (0.61*), fruit color b (–0.75**) and C (–0.71**). Similar to our results, Hegedú´s *et al*.[34] observed that an important correlation existed between antioxidant capacity and fruit color C in apricot. The antioxidant capacity highly correlated with DPPH in hawthorn fruits (0.89**). But antioxidant capacity and DPPH poorly correlated with

Table 3: Correlation coefficients of several fruit characteristics of hawthorn accessions grown in Hatay, Turkey

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14
V2	0.95**													
V3	0.92**	0.97**												
V4	0.44	0.56*	0.49											
V5	−0.27	−0.43	−0.37	−0.42										
V6	0.03	0.12	0.04	0.47	–0.67**									
V7	0.79**	0.76**	0.77**	0.10	0.14	−0.36								
V8	0.41	0.60*	0.56*	0.22	−0.65**	0.24	0.32							
V9	−0.30	−0.43	−0.40	0.04	0.46	−0.19	−0.29	−0.91**						
V10	0.35	0.49	0.33	0.50	−0.69**	0.51	0.04	0.69**	−0.58*					
V11	0.27	0.36	0.20	0.59*	−0.59*	0.51	−0.09	0.38	−0.22	0.92**				
V12	0.30	0.45	0.42	−0.01	−0.49	0.17	0.30	0.94**	−0.99**	0.59**	0.24			
V13	−0.04	−0.11	−0.02	−0.48	0.33	−0.48	0.22	−0.16	0.05	−0.50	−0.59*	−0.06		
V14	−0.14	−0.18	−0.16	−0.59*	0.26	−0.50	0.17	−0.04	−0.11	−0.33	−0.47	0.11	0.89**	
V15	−0.02	−0.15	0.01	−0.40	0.61*	−0.48	0.32	−0.41	0.27	−0.75**	−0.71**	−0.30	0.48	0.32

Significant coefficients at 0.05 and 0.01 are indicated by * and **, respectively, V1: fruit weight, V2: fruit width, V3: fruit length, V4: flesh/seed ratio, V5: TSS, V6: pH; V7: acidity, V8: L, V9: a, V10: b, V11: C, V12: *h°*, V13: antioxidant activity, V14: antioxidant capacity, V15: total phenolics

total phenolics in hawthorn fruits, thus suggesting that other components contribute more significantly to the total antioxidant capacity. Previously, Toplu *et al*.[29] Usenik *et al*.[35] and Egea *et al*.[36] had reported that antioxidant activity of some fruits is not related to phenolic contents.

CONCLUSION

The potential value of wild hawthorn species from the Eastern Mediterranean region of Turkey has been reported for the first time here. In our study, the fruit of hawtorn species was found to contain very high amount of total phenolics and high antioxidant capacity and activity. Furthermore, there was no correlation between these substances. The fruits of five hawthorn species have higher phytochemical properties in comparison with other fruit species. They could become a valuable source of nutritionally important substances for humans. This investigation shows the potential value of fruits of hawthorn species as a good source of natural antioxidants and that consumption of hawthorn fruit or its products may contribute substantial amounts of antioxidants to the diet. In addition, the hawthorn species presented significant differences in all the parameters. Individual accessions which combine higher antioxidant capacity, total phenolic and antioxidant activity may be important to consider them for commercial production. *C. aronia* var. *dentata* was found to be commercially very promising because of larger fruit size, TSS, total phenolic, antioxidant activity and antioxidant capacity. Accessions of this species are widely distributed in Hatay, and fruits of this species have been sold at higher prices in the local markets in recent years. Based on the results of this study, we anticipate very promising results in the future researches on both *C. aronia* var. *dentata* and *C. orientalis* var. *orientalis*.

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