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Evaluation of Antioxidant Activity of *Crataegus* Species Collected from Different Regions of Turkey

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Abstract: *Crataegus* species (Rosaceae), known as "Howthorn" have found special medicinal use for the treatment of mild heart diseases. This work aims to measure the antioxidant capacities of various *Crataegus* species growing in Turkey. In this study, the flowers and leaves from 52 samples belonging to 17 taxa of 14 *Crataegus* species naturally growing in Turkey have been investigated for their antioxidant activity/capacity. Four different methods (CUPRAC, FRAP, ABTS/Persulfate and Folin: FCR assays) were used for determination of the antioxidant capacities of the samples. The leaves and the flowers of the plants were studied separately. Samples representing the same species collected from different locations were studied separately. The results have indicated that the samples differing by some minor morphological characteristics exhibit considerably different antioxidant capacities. Among the flower samples, the most effective species was *C.* × *sinaica* Boiss. nothosubsp. *sinaica* and among the leaf samples *C. pentagyna* Waldst and Kit. ex. Willd. were the most active. Generally, *C. monogyna* Jacq. samples have exhibited markedly high antioxidant activity. Moreover, the species collected from Bolu district (surrounded by several forests and lakes) have shown significantly high activity regardless of the species differences among the samples.

Keywords: *Crataegus;* antioxidant capacity assays; CUPRAC; ABTS; FRAP; Folin.

1. Introduction

Crataegus species (Rosaceae), known as "Howthorn" have found special medicinal use for the treatment of mild heart diseases. Flavonoids and procyanidins are the main constituents responsible for the observed biological activities. Generally the leaves and flowers of the plant are used. The most important feature of *Crataegus* extracts is their positive inotropic effect. They increase the activation of the heart muscle cells, provide them a well feeding, regulate the blood flow, and are coronary dilatators [1,2].

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Today, the plant is mainly used for treating cardiovascular diseases in addition to its use as therapeutic agent for cancer, diabetes and sexual weakness in Arab traditional medicine, and is considered to be generally safe and well-tolerated [3]. The different parts (leaf, flower, sprout, root) of *Crataegus* species have been used in traditional medicine for various diseases such as cough, flu, asthma, stomach ache, rheumatic pain, nephritis, hemorrhoids, cardiac diseases in Turkey [4-7].

The genus *Crataegus* is represented with many species in Turkey and botanical studies are still continuing on this genus [2, 8-10]. Investigation of *Crataegus* species growing in Turkey in terms of identification of chemical composition and biological activities has been started by certain members of our research group [4, 11-14]. Some experimental work was conducted to determine the antioxidant activities of *Crataegus* species [15, 16]. These studies have exhibited that the antioxidant activity is essentially correlated to phenolic procyanidine and flavonoid content [3], and to a lesser extent to total phenols [15]. The flowers and leaves of *Crataegus* species are the parts being used as herbal drug with medical importance. In this study, the flowers and the leaves from 52 samples belonging to 14 *Crataegus* species taxa growing in Turkey (Table 1) have been investigated for their antioxidant capacity.

In general, the total antioxidant capacities (TAC) reported by ET-based assays show acceptable correlations [17, 18] among each other. In this regard, Folin (FCR), ABTS/TEAC (trolox equivalent antioxidant capacity), FRAP (ferric reducing antioxidant power), and CUPRAC (cupric reducing antioxidant capacity) assays are all classified as electron-transfer (ET) based assays, and it is emphasized that the reaction rate differences between antioxidants and oxidants are not reflected in the ABTS/TEAC values because the TEAC assay is an end-point assay [17]. The diverse antioxidant activity/capacity assay methods existing in literature depending on the consumption of chromogenic radicals, i.e., ABTS [19] and of ferric ions in the FRAP [20] test have been extensively criticized for their inadequacies [21] Ou *et al.* [22], concluded that there is no "total antioxidant" as a nutritional index available for food labeling because of the lack of standard quantification methods.

Therefore the selected chromogenic redox reagent for the assay of plant material should be easily accessible, stable, selective, respond to all types of known antioxidants regardless of chemical type or hydrophilicity; the concerned redox reaction should be rapid, and the resulting colour should be stable for a reasonable period of time. These requirements have been primarily met by the CUPRAC method introduced to world literature in 2004 [21].

This work aims to measure the antioxidant capacities of various *Crataegus* species growing in Turkey, using the electron transfer−based antioxidant assays of CUPRAC [21], ABTS/persulfate [23], Folin [24] and FRAP [20]. The results expressed as trolox equivalent antioxidant capacities were compared among themselves to produce meaningful results. It is known that the environmental conditions can be effective on chemical composition of the plants, especially with respect to antioxidant contents. In this context, we found it appropriate to evaluate the samples for a given species collected from different locations. Furthermore, minor differences detected for some samples belonging to the same species from the same location were evaluated separately. The antioxidant capacity results were compared among themselves, and correlation between the antioxidant capacity and morphological character was evaluated.

2. Material and methods

2.1. Plant material

All the *Crataegus* samples were collected by one of the authors (A.A. Dönmez) and voucher specimens have been kept at the herbarium of Hacettepe University (HUB). The samples were separated, left to dry at room temparature, and were powdered. Collected samples and their locations are depicted in Table 1.

Table 1. Voucher specimens of the studied taxa.

Sample No	Plant Material	Locations and collection code
1	*C. ambigua* Becker subsp. *ambigua*	Bitlis: Bölükyazı, below Arıdağ village, field edge, 38^0 20’ 296’’ N, 42^0 09’ 246’’ E,1540 m, 29. 5. 2002, *A.A.Dönmez* 10738.
2	*C. ambigua* Becker subsp. *ambigua*	Bitlis: Doğruyol, Yolcular village, hedge, 38^0 17’ 811’’ N, 42^0 16’ 167’’ E, 1751 m, 29. 5. 2002, *A.A.Dönmez* 10729.
3	*C. ambigua* Becker subsp. *ambigua*	Bitlis: Doğruyol, Yolcular village, hedge, 38° 17' 811" N, 42° 16' 167" E, 1751 m, 29. 5. 2002, *A.A.Dönmez* 10739.
4	*C. azarolus* L. var. *aronia* L.	Sivas: 14 km from Divriği to Sincan, rocky slopes 39° 25' 960" N, 38° 03' 663" E, 1113 m, 1. 6. 2002, *A.A.Dönmez* 10879.
5	*C. azarolus* L. var. *aronia* L.	Hakkari: 1.5 km from Şemdinli to Yüksekova, *Quercus* scrub, 37° 21' 733" N, 44° 32' 404" E, 1660 m, 30. 5. 2002, *A.A.Dönmez* 10775.
6	*C. azarolus* L. var. *aronia* L.	Gaziantep: 33 km from Nurdağı to Gaziantep, hedge, 37° 10' 272" N, 037° 07' 008" E, 1140 m, 27. 5. 2002, *A.A.Dönmez* 10694.
7	*C. caucasica C.Koch*	Iğdır: Tuzluca, Eğrekdere village, hedge, 40° 00' 356" N, 43° 38' 805" E, 1275 m, 31. 10. 2002, *A.A.Dönmez* 10855.
8	*C. caucasica C.Koch*	Artvin: Ardanuç, the road of Kutul pasture, open deciduous forest, 41° 04' 902" N, 42° 12' 113" E, 1960 m, 3.11. 2002, *A.A.Dönmez* 11079.
9	*C. caucasica* C.Koch	Iğdır: Tuzluca, Eğrekdere village, hedge, 40° 00' 356" N, 43° 38' 805" E, 1275 m, 31. 10. 2002, *A.A.Dönmez* 10860.
10	*C. davisii* Browicz	Hakkari: 11.5 km from Şemdinli to Yüksekova, in *Quercus* scrub, 370 21' 733" N, 44° 32' 404" E, 1660 m, 30. 5. 2002, *A.A.Dönmez* 10797.
11	*C. davisii* Browicz	Bitlis: Doğruyol, Yolcular village, hedge, 38° 17' 811" N, 42° 16' 167" E, 1751 m, 29. 5. 2002, *A.A.Dönmez* 10735.
12	*C. davisii* Browicz	Hakkari: 6 km from Şemdinli to Yüksekova, in *Quercus* scrub, 37° 20' 224" N, 44° 32' 862" E, 1765 m, 30. 5. 2002, *A.A.Dönmez* 10793.
13	*C. heterophylloides* Pojark. ex K.I.Christ.	Bitlis: Doğruyol, Yolcular village, hedge, 38° 17' 811" N, 42° 16' 167" E, 1751 m, 29. 5. 2002, *A.A.Dönmez* 10726.
14	*C. meyeri* Pojark.	Bingöl: 1.5 km from Yolçatı village to Bingöl, roadside, 38° 56' 032" N, 40° 18' 844" E, 1556 m, 28. 5. 2002, *A.A.Dönmez* 10722.

Sample No	Plant Material	Locations and collection code
15	*C. meyeri* Pojark.	Tunceli: Pülümür, 1 km from Kırmızı Bridge to Tunceli, open *Quercus* scrub, 39° 23' 109" N, 39° 49' 075" E, 1236 m, 1. 6. 2002, *A.A.Dönmez* 10867.
16	*C. meyeri* Pojark.	Sivas: Divriği, Kayaburun village, in steppe, 39° 16' 896" N, 38° 00' 517" E, 1315 m, 1. 6. 2002, *A.A.Dönmez* 10884.
17	*C. meyeri* Pojark.	Tunceli: Pülümür, 1 km from Kırmızı Bridge to Tunceli, open *Quercus* scrub, 39° 23' 109" N, 39° 49' 075" E, 1236 m, 1. 6. 2002, *A.A.Dönmez* 10868.
18	*C. meyeri* Pojark.	Sivas: Divriği, in steppe, 39° 16’ 896’’ N, 38° 00’ 517’’ E, 1315 m, 1. 6. 2002, *A.A.Dönmez* 10886.
19	*C. meyeri* Pojark.	Van: from Edremit to Van, the end of the city, hedge, 1670 m, 16. 6. 2002, *A.A.Dönmez* 10904.
20	*C. monogyna* var. *lasiocarpa* (Lange) K.I.Christ.	Tunceli: Pülümür, Gökçekonak village, open *Quercus* scrub, 39° 23' 995" N, 39° 50' 087" E, 1252 m, 1. 6. 2002, *A.A.Dönmez* 10871.
21	*C. monogyna* Jacq. var. *monogyna*	Tokat: Reşadiye, below Yuvacık village, field edge 40° 20' 357" N, 037° 33' 124" E, 602 m, 25. 5. 2002, *A.A.Dönmez* 10567.
22	*C. monogyna* Jacq. var. *monogyna*	Karabük: from Karabük to Safranbolu, open deciduous forest, 41° 21’ 921’’ N, 32° 42’ 477’’ E, 1055 m, 17. 5. 2002, *A.A.Dönmez* 10593.
23	*C. monogyna* Jacq. var. *monogyna*	Bolu: 7 km from Mengen to Bolu, meadow, 40° 52' 626" N, 032° 05' 536" E, 702 m, 17. 5. 2002, *A.A.Dönmez* 10606.
24	*C. monogyna* Jacq. var. *monogyna*	Bolu: 6 km from Mengen to Bolu, nearby the Bridge of Çapak River, open *Pinus nigra* area, c. 550 m, 17. 5. 2002, *A.A.Dönmez* 10605.
25	*C. monogyna* Jacq.var. *monogyna*	Tokat: 2 km from Koyulhisar to Reşadiye, open *Quercus-Pinus* area, 40° 22' 759" K, 37° 33' 124" D, 505 m, 25. 5. 2002, *A.A.Dönmez* 10569.
26	*C. monogyna* Jacq. var. *monogyna*	Tokat: Reşadiye, below Yuvacık village, field edge 40° 20' 357" N, 37° 33' 124" E, 602 m, 25. 5. 2002, *A.A.Dönmez* 10568.
27	*C. monogyna* Jacq. var. *monogyna*	Malatya: the road of Elazığ, Kapıkaya village, hedge, 38° 20' 993" N, 38° 33' 181" E, 930 m, 21. 4. 2002, *A.A.Dönmez* 10515.
28	*C. monogyna* Jacq. var. *monogyna*	Bolu: 2.5 km from Karacasu to Seben, Kuşköyü, in view of the fountain, 40° 40' 434" N, 31° 38' 493" E, 931 m, 18. 5. 2002, *A.A.Dönmez* 10610.

Sample No	Plant Material	Locations and collection code
29	*C. orientalis* M.Bieb. var. *szovitsii* Pojark.	Afyon: The road of Dinar, nearby the National Park, in *Quercus* scrub, 30° 41' 160" N, 030° 19' 520" E, 930 m, 19. 6. 2002, *A.A.Dönmez* 10907.
30	*C. orientalis* M.Bieb. var. *szovitsii* Pojark.	Gaziantep: 27 km from Nurdağı to Gaziantep, in vineyard, 37° 10' 182" N, 037° 07' 008" E, 1088 m 27. 5. 2002, *A.A.Dönmez* 10693.
31	*C. pentagyna* Waldst. and Kit.ex Willd.	Karabük: Karakuş village, under mixed forest 41° 09' 532" N, 32° 31' 031" E, 267 m, 17. 5. 2002, *A.A.Dönmez* 10598.
32	*C. pentagyna* Waldst and Kit. ex Willd.	İstanbul: From Şile to Kaynarca, below Yeniceli village, near the graveyard, 41° 05' 368" N, 29° 40' 387" E, 184 m, 18. 5. 2002, *A.A.Dönmez* 10621.
33	*C. pontica* C.Koch	Bitlis: Doğruyol, Yolcular village, hedge, 38° 17' 811" N, 42° 16' 167" E, 1751 m, 29. 5. 2002, *A.A.Dönmez* 10707.
34	*C. pontica* C.Koch	Artvin: Ardanuç, the road of Kutul pasture, open deciduous forest, 41° 04' 902" N, 42° 12' 113" E, 1960 m, 3. 7. 2002, *A.A.Dönmez* 11080.
35	*C. pseudoheterophylla* Pojark. subsp. *pseudoheterophylla*	Bartın: Arıt, Kayadibi village, *Buxus-Phillyrea* scrub 41° 39' 342" N, 32° 29' 628" E, 158 m, 7. 4. 2002, *A.A.Dönmez* 10493.
36	*C. pseudoheterophylla* Pojark. subsp. *pseudoheterophylla*	Sivas: Divriği, Kayaburun village, in steppe, 39° 16' 896" N, 38° 00' 517" E, 1315 m, 1. 6. 2002, *A.A.Dönmez* 10885.
37	*C. pseudoheterophylla* Pojark. subsp. *pseudoheterophylla*	Bitlis: Bölükyazı, below Arıdağ village, field edge 38° 20' 296" N, 42° 09' 246" E, 1540 m, 29. 5. 2002, *A.A.Dönmez* 10737.
38	*C. pseudoheterophylla* Pojark. subsp. *pseudoheterophylla*	Ankara: 17 km from Seben to Nallıhan, Danışman 2 bridge, 40° 20' 154" N, 31° 25' 325" E, 899 m, 18. 5. 2002, *A.A.Dönmez* 10617.
39	*C. pseudoheterophylla* Pojark. subsp. *turcomanica* (Pojark.) K.I.Christ	Bolu: 10 km from Seben to Bolu, in steppe, 40° 28' 392" N, 31° 35' 929" E, 1183 m, 18. 5. 2002, *A.A.Dönmez* 10615.
40	*C. rhipidophylla* Gand. var. *kutahyaensis* Dönmez	Kütahya: the road of Eskişehir, hedge, 900 m, 20. 5. 2002, *A.A.Dönmez* 10652.
41	*C. rhipidophylla* Gand. var. *kutahyaensis* Dönmez	Kütahya: the road of Eskişehir, Ahmetoluk village, hedge, 39° 33' 151" N, 030° 03' 883" E, 893 m, 20. 5. 2002, *A.A.Dönmez* 10647.
42	*C. rhipidophylla* Gand. var. *rhipidophylla*	Sivas: the road of Tokat, Koyulhisar, Yukarıkale village, hedge, 40° 16' 230" N, 037° 52' 073" E, 602 m, 25. 5. 2002, *A.A.Dönmez* 10565.
43	*C. rhipidophylla* Gand. var. *rhipidophylla*	Kütahya: the road of Eskişehir, Ahmetoluk village, hedge, 39° 3' 151" N, 030° 03' 883" E, 893 m, 20. 5. 2002, *A.A.Dönmez* 10651.

Sample No	Plant Material	Locations and collection code
44	*C. rhipidophylla* Gand. var. *rhipidophylla*	Bolu: 2.5 km from Karacasu to Seben, Kuşköyü, around the fountain, 40° 40' 434" N, 031° 38' 493" E, 931 m, 18. 5. 2002, *A.A.Dönmez* 10612.
45	*C. rhipidophylla* Gand. var. *rhipidophylla*	Kütahya: the road of Eskişehir, Ahmetoluk village, hedge, 39° 33' 151" N, 030° 03' 883" E, 893 m, 20. 5. 2002, *A.A.Dönmez* 10649.
46	*C. rhipidophylla* Gand. var. *rhipidophylla*	Kütahya: Domaniç, Eskiyayla, open *Quercus* scrub,1200 m, 19 .5. 2002, *A.A.Dönmez* 10640.
47	*C.* × *sinaica* Boiss. nothosubsp. s*inaica*	Elazığ: 38 km from Sivrice to Maden, 38° 28' 613" N, 39° 35' 363" E, 1232 m, 28. 5. 2002, *A.A.Dönmez* 10701.
48	*C.* × *sinaica* Boiss. nothosubsp. *sinaica*	Bingöl: 1.5 km from Yolçatı village to Bingöl, roadside, 38° 56' 032" N, 40° 18' 844" E, 1556 m, 28. 5. 2002, *A.A.Dönmez* 10709.
49	*C.* × *sinaica* Boiss. nothosubsp. *sinaica*	Bolu: 7 km from Mengen to Bolu, meadow 40° 52' 626" K, 32° 05' 536" D, 702 m, 17. 5. 2002, *A.A.Dönmez* 10616.
50	*C.* × *sinaica* Boiss. nothosubsp. s*inaica*	Karabük: from Safranbolu to Ulus, Karaveli village, Pyrus-Rosa scrub, 41° 29' 647" N, 032° 42' 340" E, 973 m, 17. 5. 2002, *A.A.Dönmez* 10591.
51	*C.* × *sinaica* Boiss. nothosubsp. s*inaica*	Elazığ: the road of Bingöl, Çağlar village, hedge, 38° 35' 062" N, 39° 21' 966" E, 850 m, 21. 4. 2002, *A.A.Dönmez* 10519.
52	*C. tanacetifolia* (Poir.) Pers.	Sivas: 27 km from Yıldızeli to Akdağmadeni, in *Quercus* scrub, 39° 48' 604" N, 36° 04' 136" E, 1330 m, 27. 6. 2002, *A.A.Dönmez* 10931.

2.2. Chemicals and solutions

Neocuproine and Folin Ciocalteou reagent (FCR) were purchased from Sigma Chemical Co. (Steinheim, Germany). Trolox was obtained from Aldrich Chemicals Co. (Steinheim, Germany). Ammonium acetate, copper (II) chloride, potassium persulfate, hydrochloric acid, sodium hydroxide, copper (II) sulfate, sodium carbonate, sodium potassium tartarate, glacial acetic acid, sodium acetate trihydrate, ferric chloride hexahydrate, ethanol (96%) and methanol were purchased from Merck (Darmstadt, Germany), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) from Fluka Chemical Co. (Buchs, Switzerland). All other chemicals and solvents were of analytical reagent grade.

$CuCl_2$ solution, $1.0x10^{-2}$ M, was prepared by dissolving 0.4262 g $CuCl_2.2H_2O$ in water, and diluting to 250 mL. Ammonium acetate (NH_4Ac) buffer at pH=7.0, 1.0 M, was prepared by dissolving 19.27 g NH_4Ac in water and diluting to 250 mL. Neocuproine (Nc) solution, $7.5x10^{-3}$ M, was prepared daily by dissolving 0.039 g Nc in 96% ethanol, and diluting to 25 mL with ethanol. Trolox, $1.0x10^{-3}$ M, was prepared in 96% ethanol. The chromogenic radical reagent ABTS, at 7.0 mM concentration, was prepared by dissolving 0.1920 g of the compound in water, and diluting to 50 mL. To this solution was added 0.0331 g $K_2S_2O_8$ such that the final persulfate concentration in the mixture be 2.45 mM. The resulting ABTS radical cation solution was left to mature at room temperature in the dark for 12-16 h, and then used for TEAC assays. The solutions used in the Folin assay of

polyphenolics were prepared as follows: Lowry A: 2% aqueous Na_2CO_3 in 0.1 M NaOH; Lowry B: 0.5% $CuSO_4$ aqueous solution in 1% $NaKC_4H_4O_6$ solution; Lowry C: prepared freshly as mixture (50 mL Lowry A + 1 mL Lowry B); Folin-Ciocalteau reagent was diluted with H_2O at a volume ratio of 1:3 prior to use. All percentages are given as (w/v), and distilled and deaerated (N_2-bubbled) water was used throughout. The FRAP solutions were prepared as folllows: A suitable mass of $FeCl_3.6H_2O$ was weighed so that the final concn. of Fe(III) in solution would be $2.0x10^{-2}$ M; 1 mL of 1 M HCl solution was added, dissolved in some water and diluted to 50 mL with H_2O. A suitable mass of TPTZ was weighed such that its final concentration would be $1.0x10^{-2}$ M, dissolved in 96% EtOH, and diluted to 50 mL. In order to prepare 0.3 M CH_3COOH/CH_3COONa buffer solution at pH 3.6, 3.1 g of $CH_3COONa.3H_2O$ was weighed and 16 mL glacial acetic acid was added, diluted with water to 1 L. The FRAP reagent was prepared as follows: The pH 3.6 acetic acid buffer, $1.0x10^{-2}$ M TPTZ solution, and $2.0x10^{-2}$ M $FeCl_3.6H_2O$ solution were mixed in this order at a volume ratio of 10:1:1. The FRAP reagent was prepared and used freshly.

2.3. Instruments

All spectrophotometric measurements were made with a pair of matched Hellma quartz cuvettes using a Varian CARY 1E UV-Vis spectrophotometer. The pH measurements were made with the aid of a E512 Metrohm Herisau pH-meter using a glass electrode; the centrifugations were performed with an Adams Dynac Centrifuge apparatus. Ultra-Turrax CAT X 620 apparatus was used for the extraction of plant material. An Adams Dynac centrifuge apparatus was used for separation of the clear fractions of plant extracts.

2.4. Solvent extraction of plant materials

The antioxidant capacities of plant samples were reported based on dry matter content (5 g) for each sample that was exhausted in a Soxhlet apparatus with extra pure methanol for six hours on a water bath at 80ºC. The extracts were evaporated by a rotary evaporator to 10 mL-volume.

2.5. Assessment of total antioxidant capacity

2.5.1. CUPRAC assay

To a test tube were added 1 mL of $CuCl_2$ solution ($1.0x10^{-2}$ M), 1 mL of neocuproine alcoholic solution ($7.5x10^{-3}$ M), and 1 mL NH_4Ac buffer solution, and mixed; 0.5 mL of dilute plant extract (previously diluted with H_2O at a volume ratio of 1:20) followed by 0.6 mL of water were added (total volume = 4.1 mL), and mixed well in stoppered tubes. Absorbance against a reagent blank was measured at 450 nm after 30 min. Since the calibration curve for pure trolox is a line passing through the origin, the trolox equivalent molar concentration of the plant extract sample in final solution may be found by dividing the observed absorbance to the molar absorptivity (ε) for trolox (optical cuvette thickness = 1 cm). The trolox equivalent antioxidant capacity may be traced back to the original extract considering all dilutions, and proportionated to the initial mass of plant sample taken to find the capacity in the units of mmol TR/g dry matter. The recommended technique was applied thrice to three different 0.5 mL-aliquots of each plant extract. If the above practice is followed, then

TEAC of plant (mmol TR g^{-1}) = (Absorbance/ε_{TR})(4.1/0.5)(20/1)(100/g-plant weight) (1/dry matter %)

where the molar absorptivity of trolox in the CUPRAC method is $\varepsilon_{TR} = 1.67x10^4$ $Lmol^{-1}cm^{-1}$.

2.5.2. ABTS/Persulfate assay

Unlike all other antioxidant capacity assays involving the measurement of coloured products, the ABTS/persulfate method is basically a decolorization assay. The matured ABTS radical solution of blue-green colour was diluted with ethanol at a ratio of 1:10. The absorbance of the 1:10 diluted

$ABTS^{\cdot+}$ radical cation solution was 1.28 ± 0.04 at 734 nm. To 1 mL of the radical cation solution, 4 mL of ethanol were added, and the absorbance at 734 nm was read at the end of the sixth minute. The procedure was repeated for the unknown plant extract by adding 1 mL of the radical cation solution to x mL (x= 0.1 or 0.5 mL) of dilute plant extract (previously diluted with H_2O at a volume ratio of 1:20) and (4-x) mL of ethanol, and recording the absorbance. The absorbance difference (ΔA) was found by subtracting the extract absorbance from that of the reagent blank (pure radical solution), and this was correlated to trolox equivalent antioxidant concentration with the aid of a linear calibration curve. The recommended technique was applied thrice to three different 1.0 mL-aliquots of each plant extract.

TEAC of plant (mmol TR g^{-1}) = (Absorbance/ε_{TR})(5.0/1.0)(20/1)(100/g-plant weight) (1/dry matter %)

where the molar absorptivity of trolox in the ABTS method is $\varepsilon_{TR} = 2.6x10^4$ $Lmol^{-1}cm^{-1}$.

2.5.3. FRAP assay

To 3 mL of the FRAP reagent was added 0.3 mL H_2O. Then 50 or 100 μL aliquots of the plant extracts were taken, and 96 % EtOH was added to make the final volume 3.4 mL. The absorbance at 595 nm (A_{595}) was read against a reagent blank after 6 min.

TEAC of plant (mmol TR g^{-1}) = (Absorbance/ε_{TR})(3.4/mL-plant extract) (20/1)(100/g-plant weight) (1/dry matter %)

where the molar absorptivity of trolox in the FRAP method is $\varepsilon_{TR} = 4.63x10^4$ $Lmol^{-1}cm^{-1}$.

2.5.4. Folin total phenolic content

To 0.5 mL of the dilute plant extract (previously diluted with H_2O at a volume ratio of 1:20) was added 1.5 mL H_2O. An aliquot of 2.5 mL of Lowry C solution was added, and the mixture was let to stand for 10 min. At the end of this period, 0.25 mL of Folin reagent was added, and 30 more min was allowed for stabilization of the blue colour formed. The absorbance against a reagent blank was measured at 750 nm. The recommended technique was applied thrice to three different 0.5 mL-aliquots of each plant extract.

TEAC of plant (mmol TR/g) = (Absorbance/ε_{TR}) (4.75/0.5) (20/1)(100/g-plant weight) (1/dry matter %)

where the molar absorptivity of trolox in the Folin method is $\varepsilon_{TR} = 4.65x10^3$ $Lmol^{-1}cm^{-1}$.

3. Results and Discussion

Due to their ability to scavenge free radicals and reactive species, thereby reducing oxidative stress and associated tissue damage, various health claims have been made regarding the use of exogenous, dietary antioxidants. As a result, numerous studies have been performed to examine the possible beneficial health effects of antioxidant supplementation. Additionally, more direct beneficial health effects of antioxidants can be expected in patients suffering from a disease that is actually associated with increased levels of oxidative stress, such as diabetes, chronic lung diseases and coronary heart diseases. The use of exogenous antioxidants to support the treatment of these diseases has recently gained a lot of interest [17].

The antioxidant activity studies on *Crataegus* species have exhibited that these species possess considerable antioxidant potential due to their polyphenolic compounds such as flavonoids and procyanidines. In this study, the indigenous *Crataegus* species of Turkey were compared in terms of their antioxidant capacities using four methods. The leaves and the flowers of the plants were studied separately. All of the leaf samples were collected during the flowering period. Samples obtained from the same species carefully collected (with a botanical vision) from different locations were studied separately. Different samples belonging to the same species which only differed in some morphological characteristics (less deeply-deeply divided sinuses; loosely-densely flowered inflorescence; slightly-deeply divided leaves; dark green colour leaves) were also evaluated separately

even if they were collected from the same locations. The samples differing in some minor morphological characteristics exhibited considerably different antioxidant capacities. This result hints to the fact that some minor morphological differences can refer to a variety of chemical content for *Crataegus* species.

As for the differences of results obtained with the four antioxidant capacity assays employed, the hierarchial order of total antioxidant capacities (TAC values) was: Folin>CUPRAC>ABTS/TEAC>FRAP. The Folin test gave the highest results due to the indefinitely high standard redox potential of the Folin reagent of not exactly known composition [25] while FRAP yielded the lowest results due to incomplete reaction of tripyridyltriazine-iron(III) reagent with some flavonoids and phenolic acids [26]. In fact, the ABTS/TEAC test was the most competent assay compared to CUPRAC, because both assays gave acceptable results with hydrophilic and lipophilic antioxidants [27].

In general, CUPRAC assay highly correlated with other electron transfer-based antioxidant assays for a number of plant materials [28-31]. In this study, the linear correlation coefficients of CUPRAC results with those of ABTS/TEAC (Fig. 1), FRAP (Fig. 2), and Folin (Fig. 3) were r: 0.812, 0.870, and 0.883, respectively, pointing out to curvi-linear correlations. The ABTS/TEAC test is considered by some researchers to lie at the interface of electron transfer (ET)- and hydrogen atom transfer (HAT)-based assays [17-18] and therefore it yielded the lowest r coefficient with the CUPRAC assay, whereas other similar ET-based assays (i.e., FRAP and Folin) correlated with CUPRAC with better linearity due to the similarity of reaction mechanisms and responsive substrates. As for correlations reported in the literature between ET-based test results and individual antioxidant constituents, Bahorun et al. [15] have reported r values ranging between 0.7 and 0.9 for correlations of ABTS/TEAC and FRAP results with total phenols, total proanthocyanidins, and total flavonoids content of *Crataegus monogyna* Jacq. callus extracts.

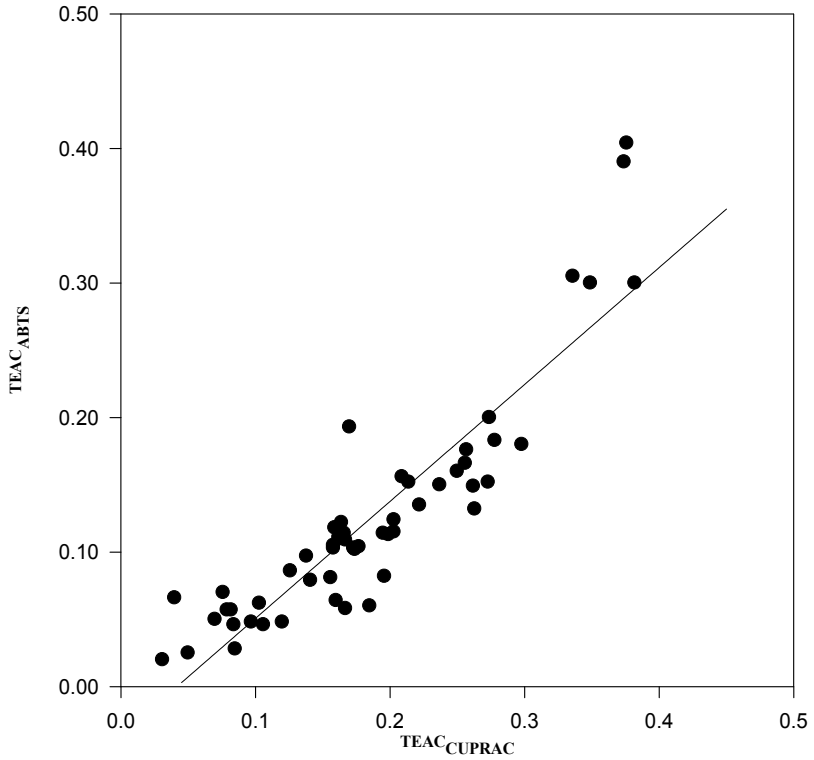

Figure 1. The correlation of CUPRAC assay results with those of ABTS (r= 0.812).

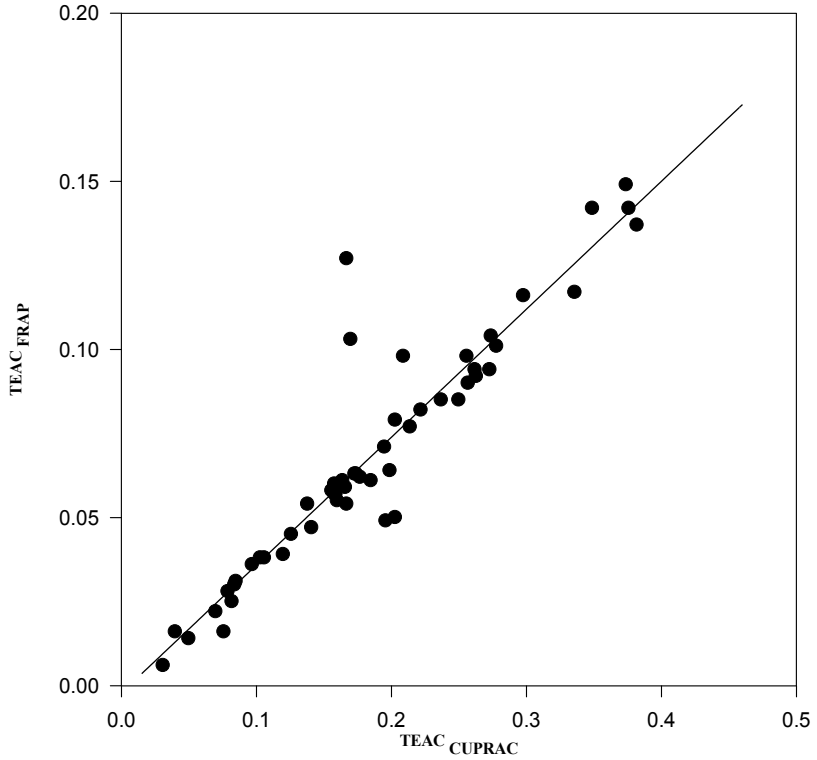

Figure 2. The correlation of CUPRAC assay results with those of FRAP (r= 0.870).

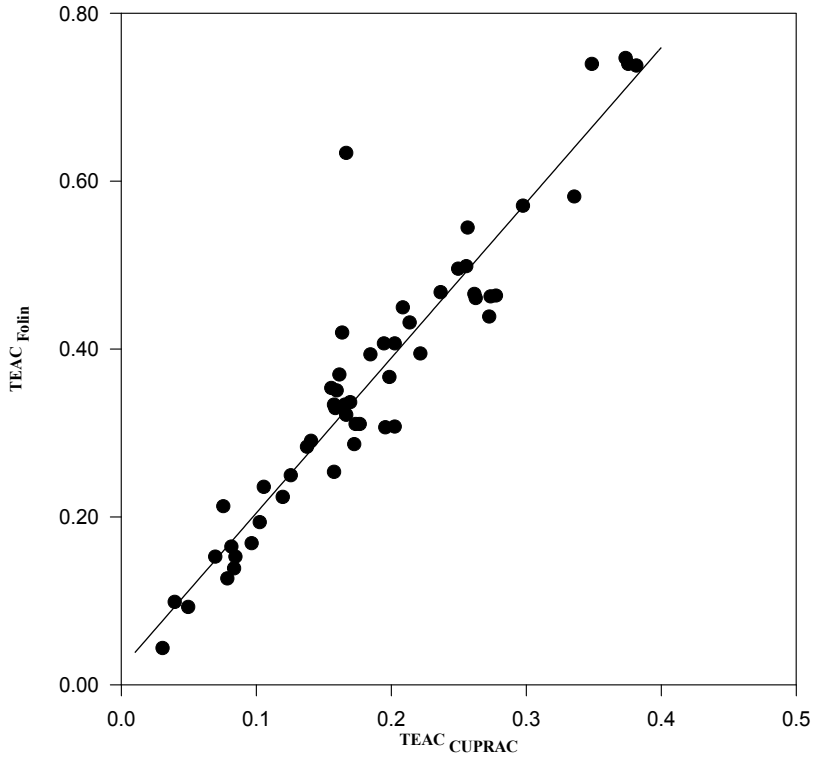

Figure 3. The correlation of CUPRAC assay results with those of Folin (r= 0.883).

Table 2. The antioxidant capacity results obtained from four different methods.

Plant material	Codes	$TEAC_{CUPRAC}$ (mmol TR g^{-1})	$TEAC_{FRAP}$ (mmol TR g^{-1})	$TEAC_{ABTS}$ (mmol TR g^{-1})	$TEAC_{Folin}$ (mmol TR g^{-1})
C.ambigua Becker subsp. *ambigua*	**F-1**	0.159±0.002	0.057±0.002	0.118±0.039	0.329±0.052
	L-1	0.255±0.020	0.070±0.005	0.138±0.002	0.549±0.018
	F-2	0.084±0.012	0.030±0.002	0.046±0.005	0.138±0.035
	L-2	0.109±0.005	0.034±0.001	0.062±0.017	0.240±0.035
	F-3	0.195±0.010	0.071±0.003	0.114±0.015	0.406±0.010
	L-3	0.259±0.002	0.090±0.007	0.175±0.016	0.524±0.142
C. azarolus L. var. *aronia* L.	**F-4**	0.106±0.001	0.038±0.002	0.046±0.009	0.235±0.064
	L-4	0.117±0.036	0.066±0.003	0.075±0.003	0.336±0.013
	F-5	0.177±0.007	0.062±0.006	0.104±0.004	0.310±0.038
	L-5	0.146±0.002	0.065±0.003	0.090±0.023	0.381±0.071
	F-6	0.196±0.011	0.049±0.002	0.082±0.016	0.306±0.030
	L-6	0.357±0.009	0.132±0.005	0.379±0.025	0.728±0.239
C.caucasica C.Koch	**F-7**	0.158±0.004	0.060±0.002	0.103±0.007	0.253±0.082
	L-7	0.177±0.003	0.063±0.005	0.097±0.012	0.317±0.063
	F-8	0.120±0.010	0.039±0.001	0.048±0.006	0.223±0.057
	L-8	0.230±0.007	0.103±0.006	0.151±0.007	0.475±0.013
	F-9	0.085±0.001	0.031±0.001	0.028±0.010	0.152±0.020
	L-9	0.169±0.014	0.095±0.002	0.118±0.014	0.318±0.091
C. davisii Browicz	**F-10**	0.185±0.004	0.061±0.002	0.060±0.002	0.393±0.049
	L-10	0.118±0.005	0.096±0.004	0.169±0.004	0.473±0.016
	F-11	0.031±0.003	0.006±0.001	0.005±0.007	0.043±0.023
	L-11	0.075±0.001	0.024±0.002	0.021±0.035	0.153±0.018
	F-12	0.082±0.005	0.025±0.001	0.057±0.003	0.164±0.008
	L-12	0.038±0.001	0.016±0.001	0.032±0.006	0.111±0.013
C. heterophylloides Pojark.	**F-13**	0.170±0.015	0.103±0.004	0.193±0.012	0.336±0.054
	L-13	0.173±0.004	0.081±0.000	0.110±0.003	0.470±0.043
C. meyeri Pojark.	**F-14**	0.160±0.009	0.055±0.004	0.064±0.008	0.350±0.025
	L-14	0.098±0.005	0.042±0.001	0.053±0.007	0.240±0.048
	F-15	0.250±0.010	0.085±0.002	0.160±0.019	0.495±0.100
	L-15	0.101±0.005	0.050±0.005	0.059±0.024	0.256±0.062
	F-16	0.256±0.007	0.098±0.002	0.166±0.004	0.498±0.025
	L-16	0.155±0.007	0.070±0.001	0.133±0.018	0.371±0.060
	F-17	0.141±0.011	0.047±0.001	0.079±0.005	0.290±0.058

	L-17	0.084±0.008	0.043±0.002	0.080±0.021	0.240±0.070
Plant material	**Codes**	**$TEAC_{CUPRAC}$** (mmol TR g^{-1})	**$TEAC_{FRAP}$** (mmol TR g^{-1})	**$TEAC_{ABTS}$** (mmol TR g^{-1})	**$TEAC_{Folin}$** (mmol TR g^{-1})
C. meyeri Pojark.	**F-18**	0.336±0.014	0.117±0.006	0.065±0.016	0.581±0.158
	L-18	0.170±0.007	0.059±0.004	0.104±0.007	0.375±0.058
	F-19	0.076±0.000	0.016±0.000	0.070±0.0012	0.212±0.017
	L-19	0.034±0.002	0.020±0.001	0.046±0.006	0.101±0.022
C. monogyna var. *lasiocarpa*	**F-20**	0.174±0.010	0.063±0.001	0.102±0.005	0.310±0.027
	L-20	0.288±0.005	0.117±0.003	0.232±0.008	0.645±0.101
C. monogyna Jacq. var. *monogyna*	**F-21**	0.262±0.008	0.094±0.002	0.149±0.012	0.465±0.053
	L-21	0.367±0.004	0.141±0.011	0.330±0.052	0.688±0.138
	F-22	0.278±0.026	0.101±0.007	0.183±0.003	0.463±0.015
	L-22	0.320±0.004	0.126±0.001	0.226±0.107	0.614±0.159
	F-23	0.214±0.009	0.077±0.002	0.152±0.004	0.431±0.022
	L-23	0.127±0.011	0.064±0.000	0.098±0.011	0.282±0.078
	F-24	0.273±0.006	0.094±0.002	0.152±0.013	0.438±0.048
	L-24	0.249±0.012	0.111±0.004	0.200±0.006	0.508±0.020
	F-25	0.040±0.005	0.016±0.001	0.066±0.010	0.098±0.005
	L-25	0.298±0.010	0.137±0.007	0.230±0.008	0.652±0.085
	F-26	0.257±0.005	0.090±0.004	0.176±0.016	0.544±0.002
	L-26	0.141±0.004	0.066±0.001	0.077±0.018	0.309±0.043
	F-27	0.162±0.009	0.060±0.003	0.111±0.002	0.369±0.050
	L-27	0.159±0.006	0.080±0.004	0.130±0.012	0.443±0.099
	F-28	0.374±0.004	0.149±0.003	0.390±0.018	0.746±0.123
	L-28	0.338±0.014	0.123±0.004	0.035±0.004	0.666±0.053
C. orientalis M.Bieb. var. *szovitsii* Pojark.	**F-29**	0.203±0.065	0.079±0.012	0.124±0.016	0.406±0.017
	L-29	0.061±0.002	0.042±0.001	0.092±0.026	0.476±0.079
	F-30	0.079±0.009	0.028±0.001	0.057±0.016	0.126±0.013
	L-30	0.073±0.007	0.038±0.001	0.069±0.004	0.200±0.010
C. pentagyna Waldst. and Kit.ex Willd.	**F-31**	0.103±0.002	0.038±0.002	0.062±0.007	0.193±0.032
	L-31	0.191±0.007	0.093±0.003	0.166±0.024	0.443±0.021
	F-32	0.222±0.007	0.082±0.001	0.135±0.013	0.394±0.058
	L-32	0.378±0.004	0.132±0.001	0.423±0.112	0.752±0.201
C. pontica C.Koch	**F-33**	0.050±0.006	0.014±0.001	0.025±0.012	0.092±0.013
	L-33	0.215±0.009	0.082±0.004	0.140±0.007	0.386±0.069
	F-34	0.070±0.005	0.022±0.002	0.050±0.019	0.152±0.014
	L-34	0.136±0.007	0.044±0.002	0.076±0.025	0.251±0.038

Plant material	Codes	$TEAC_{CUPRAC}$ (mmol TR g^{-1})	$TEAC_{FRAP}$ (mmol TR g^{-1})	$TEAC_{ABTS}$ (mmol TR g^{-1})	$TEAC_{Folin}$ (mmol TR g^{-1})
C. pseudoheterophylla Pojark. subsp. *pseudoheterophylla*	**F-35**	0.156±0.022	0.058±0.002	0.081±0.007	0.353±0.040
	L-35	0.325±0.013	0.152±0.000	0.204±0.012	0.760±0.130
	F-36	0.167±0.005	0.127±0.001	0.058±0.007	0.633±0.033
	L-36	0.066±0.004	0.063±0.002	0.107±0.008	0.381±0.107
	F-37	0.203±0.005	0.050±0.007	0.115±0.019	0.307±0.044
	L-37	0.264±0.013	0.073±0.001	0.136±0.011	0.455±0.036
	F-38	0.097±0.003	0.036±0.002	0.048±0.003	0.168±0.040
	L-38	0.138±0.006	0.050±0.002	0.082±0.005	0.194±0.083
C. pseudoheterophylla Pojark. subsp. *turcomanica* (Pojark.) K.I.Christ	**F-39**	0.164±0.004	0.061±0.004	0.122±0.009	0.419±0.077
	L-39	0.253±0.006	0.068±0.007	0.090±0.025	0.374±0.067
C. rhipidophylla Gand. var. *kutahyaensis* Dönmez	**F-40**	0.173±0.003	0.063±0.000	0.103±0.013	0.286±0.015
	L-40	0.217±0.005	0.066±0.001	0.130±0.012	0.376±0.095
	F-41	0.274±0.014	0.104±0.004	0.200±0.018	0.462±0.040
	L-41	0.205±0.007	0.074±0.002	0.126±0.016	0.384±0.125
C. rhipidophylla Gand. var. *rhipidophylla*	**F-42**	0.158±0.002	0.057±0.002	0.105±0.020	0.333±0.021
	L-42	0.100±0.029	0.100±0.029	0.174±0.020	0.522±0.150
	F-43	0.209±0.021	0.098±0.002	0.156±0.003	0.449±0.016
	L-43	0.253±0.006	0.119±0.006	0.202±0.005	0.558±0.001
	F-44	0.349±0.005	0.142±0.002	0.080±0.004	0.739±0.103
	L-44	0.259±0.011	0.123±0.004	0.186±0.011	0.509±0.070
	F-45	0.199±0.011	0.064±0.007	0.113±0.019	0.366±0.048
	L-45	0.105±0.004	0.048±0.001	0.088±0.006	0.286±0.020
	F-46	0.263±0.002	0.092±0.007	0.132±0.011	0.460±0.114
	L-46	0.217±0.007	0.064±0.000	0.131±0.007	0.375±0.064
C. × *sinaica* Boiss. nothosubsp. s*inaica*	**F-47**	0.167±0.010	0.054±0.004	0.109±0.002	0.321±0.013
	L-47	0.218±0.003	0.092±0.003	0.172±0.010	0.462±0.042
	F-48	0.237±0.037	0.085±0.006	0.150±0.016	0.467±0.066
	L-48	0.178±0.002	0.077±0.007	0.118±0.007	0.486±0.038
	F-49	0.382±0.005	0.137±0.003	0.068±0.008	0.737±0.067
	L-49	0.267±0.027	0.086±0.004	0.156±0.004	0.470±0.069
	F-50	0.298±0.010	0.116±0.004	0.180±0.018	0.570±0.091
	L-50	0.222±0.007	0.082±0.003	0.130±0.012	0.391±0.065
	F-51	0.126±0.005	0.045±0.002	0.086±0.006	0.249±0.057
	L-51	0.157±0.011	0.046±0.001	0.081±0.002	0.305±0.015
C. tanacetifolia (Poir.) Pers.	**F-52**	0.166±0.017	0.059±0.001	0.114±0.034	0.333±0.068
	L-52	0.162±0.004	0.049±0.002	0.088±0.012	0.372±0.010

According to the results of the CUPRAC assay, among the flower samples, the most effective species are *C.* × *sinaica* Boiss. nothosubsp. *sinaica* (Table 1 No:49), *C. monogyna* Jacq. var. *monogyna* (Table 1 No:28), *C. rhipidophylla* Gand. var. *rhipidophylla* (Table 1 No:44) and among the leaf samples, *C. pentagyna* Waldst et Kit. ex Willdenow (Table 1 No:32) is the most active (Table 2). Different antioxidant capacities were determined in *C. ambigua* Becker subsp. *ambigua*, *C. caucasica* C.Koch, *C. meyeri* Pojark., *C. monogyna* Jacq. var. *monogyna* species with some minor morphological characteristics like divided sinuses, flowered inflorescence, hairs on its leaves and the colour of the leaves. As a general observation, *Crataegus monogyna* samples have exhibited markedly high antioxidant activity. Bahorun and co-workers have shown that in the leaf extracts of *Crataegus monogyna* Jacq., flavonoids account for most of the antioxidant activity observed, whereas proanthocyanidins and catechins do the same activity in flowers [32]. Moreover, the species collected from Bolu district have shown significantly high activity regardless of the species differences among the samples. Bolu is surrounded by several forests and lakes which provide oxygen-rich and clean air to this district. Additionally, Bolu is known for its cold weather conditions -enriched with drought- during winter months, which is also a demonstrated factor in raising the TAC values of *Crataegus* leaves [33].

In Europe, the preparations of *Crataegus* species which are used for medicinal purposes are *C. monogyna* Jacq. and *C. laevigata* (Poir.) DC, the latter not being grown in Turkey. It is demonstrated that among the *Crataegus* species in Turkey there are *Crataegus* samples which have a higher antioxidant capacity than the medicinal species *C.monogyna* Jacq.

Though there are differences in antioxidant capacity between the same species collected from different regions, these differences can be due to the factors as land characteristics, unpolluted air, oxygen concentration and height. This study indicates that *Crataegus* species yielded from nature can not be standardized for medicinal use. We think that *Crataegus* species in Turkey should be cultured and it is necessary to make use of species with known characteristics such as origin, height, etc.

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