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Research Article

Antioxidant activity and total phenolic content of some *Crocus* (Iridaceae) species

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Abstract
The antioxidant activity was determined for the corm and the aerial parts of the species of *Crocus olivieri* subsp. *istanbulensis, C. flavus* subsp. *flavus, C. danfordiae* subsp. *danfordiae* and *C. antalyensis* subsp. *antalyensis.* The antioxidant activity was investigated by determining the percentage of 2.2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of ethanolic extracts at a concentration of 1 mg/mL. The highest antioxidant activity was found in the corm part of *C. flavus* subsp. *flavus*, whereas, the lowest value was detected in the aerial part of *C. danfordiae* subsp. *danfordiae*. For the total phenolic content, the highest and the lowest values were observed in the corm of *C. olivieri* subsp. *istanbulensis* at 390 mg GAE/100 g and in the aerial part of *C. danfordiae* subsp. *danfordiae* at 24.4 mg GAE/100 g, respectively.
Keywords: *Crocus*, DPPH activity, Sakarya, total phenolic content, Turkey

Bazı *Crocus* (Iridaceae) türlerinin antioksidan aktivitesi ve toplam fenolik içerikleri

Özet
Bu çalışmada *Crocus olivieri* subsp. *istanbulensis, C. flavus* subsp. *flavus, C. danfordiae* subsp. *danfordiae* ve *C. antalyensis* subsp. *antalyensis* türlerinin korm ve toprak üstü kısımlarının antioksidan aktivitesi belirlenmiştir. Antioksidan aktivite, 1 mg/mL yoğunluğundaki etanolik ekstraktların % 2,2-diphenyl-1-picrylhydrazyl (DPPH) süpürme aktivitesi belirlenerek araştırılmıştır. En yüksek antioksidan aktivite *C. flavus* subsp. *flavus*'un korm kısmında, en düşük ise *C. danfordiae* subsp. *danfordiae*'nın toprak üstü kısmında belirlenmiştir. Toplam fenolik içerik için en yüksek değer *C. olivieri* subsp. *istanbulensis* korm kısmında 390 mg GAE/100 g olarak, en düşük değer ise *C. danfordiae* subsp. *danfordiae*'nın toprak üstü kısmında 24.4 mg GAE/100 g olarak tespit edilmiştir.
Anahtar kelimeler: *Crocus*, DPPH aktivitesi, Sakarya, toplam fenolik içerik, Türkiye

INTRODUCTION

Plants tend to improve an ability to produce secondary phenolic metabolites, which are the vital parts of their interaction mechanisms with their surroundings, reproduction strategies and defensive behaviors. These phenolic compounds also contain natural antioxidants that have beneficial and protective influences on human health. The oxidative stress is thought to be the reason for various disorder-related illnesses in different parts of the human body/organs due to the overproduction of reactive nitrogen and oxygen species. Antioxidants may retard the development of chronic diseases and are also widely used as food additives for protection of foods against oxidative degradation (Zou et al. 2016; Zengin et al. 2019; Tunç et al. 2020).

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Medicinal, aromatic and many other plants contain chemical compounds that exhibit antioxidant properties. However, there is still little scientific knowledge about the antioxidant properties of plants, especially those used less frequently in the kitchen and medicine (Miliauskas et al. 2004).

Turkey has 78 *Crocus* L. species and 54 of them are endemic (Yüzbaşıoğlu 2012). *Crocus* is a perennial herb from the Iridaceae family and widely cultivated in Turkey, Iran, Spain and Greece. It has some traditional uses against some cardiovascular illnesses, diabetes, Parkinson's disease, depression, apoptosis, atherosclerosis, etc. (Esmaeili et al. 2011; Baba et al. 2015). Medicinal and aromatic plants are being used by the people of Sakarya as in many parts of the world (Sağıroğlu et al. 2012; 2017).

The aim of this study was to determine the antioxidant and the total phenolic activity of the corm and the aerial parts of *Crocus flavus* Weston subsp. *flavus* and endemic species of *C. olivieri* J.Gay subsp. *istanbulensis* B.Mathew, *C. danfordiae* Maw subsp. *danfordiae* and *C. antalyensis* B.Mathew subsp. *antalyensis*.

MATERIAL AND METHOD

Crocus species have been collected from their natural populations at flowering. The specimens collected were identified according to the *Flora of Turkey and the East Aegean Islands* (Mathew 1984; 1988; 2000) and deposited at the Sakarya University Department of Biology Herbarium.

Plant materials

The collection information on the *Crocus* specimens is listed below:

Crocus olivieri subsp. ***istanbulensis.*** Turkey. A3 Sakarya: Between Mesruriye and Pamukova road, 5th km, 760 m, 03.02.2019, M.Sağıroğlu 6457.

Crocus flavus subsp. ***flavus.*** Turkey. A3 Sakarya: Sakarya University Campus, Faculty of Political Sciences surroundings, in *Quercus* forest, 200 m, 01.03.2019, M.Sağıroğlu 6471.

Crocus danfordiae subsp. ***danfordiae.*** Turkey. A3 Sakarya: Pamukova, Çilekli village, 750 m, 12.02.2019, M.Sağıroğlu 6465.

Crocus antalyensis subsp. ***antalyensis.*** Turkey. A3 Sakarya: between Mesruriye and Pamukova road, 3rd km, 740 m, 22.02.2019, M.Sağıroğlu 6467.

Ethanolic extracts of the plants

Specimens were dried at room temperature for seven days. Ten grams of the dried specimens were ground into a capped bottle and 150 mL of ethanol was added. The prepared mixtures were kept at room temperature in a dark environment and stirred for 3 days. The solvents in the extracts were evaporated by using a rotary evaporator (Heidolph) under vacuum at 55°C for 15 minutes and the dried extracts were then used for all investigations. The extract concentrations were adjusted by adding the same solvent (ethanol) to each extract at the doses of 1mg/mL for the antioxidant activity and the total phenolic analyses.

Antioxidant activity (DPPH assay)

Antioxidant activity was determined by the modified Blois method (1958). A total of 1 ml of 0.004% DPPH (2.2-Diphenyl-1-picrylhydrazyl) radical solution in ethanol was mixed with 1 mL of the extract solution (in ethanol). These solutions were then kept in a dark environment for 30 mins

and the optical density was measured with a spectrophotometer (Shimadzu UV mini-1240) at 517 nm. Throughout the process, ethanol was used as the blank. The inhibition percentage of the specimens was calculated according to the following formula: % Inhibition = (A control – A specimen) / (A control) × 100.

Determination of total phenolic content

To determine the total phenolic content, the Folin-Ciocalteu method (Singleton & Rossi 1965) was employed. A total of 100 µL of ethanolic extract (1 mg/mL) was mixed with 200 µL of Folin-Ciocalteu (50%) and held for 2 minutes. Then, 1 mL of 2% Na_2CO_3 solution was added and shaken well. The mixture was kept in a dark environment for 1 hour. The absorbance of the mixture was measured with a spectrophotometer at 760 nm. The total phenolic content values were evaluated from a calibration curve obtained with a series of gallic acid standards (50, 100, 200, 300, 400 mg/L). The results were expressed as mg of GAE/100 g.

RESULTS AND DISCUSSION

The antioxidant and the antimicrobial properties of the extracts obtained from many plants are of great interest in the food and health industry. The *Crocus* species are being intensely used by the public for various purposes in Turkey and especially in Sakarya Province. The DPPH is a stable free radical, that has been generally used as a tool for evaluating the free radical-scavenging activity of the antioxidants (Neagu et al. 2018; İnceçayır et al. 2019). The percentage DPPH scavenging activity was given in Figure 1 for the extracts in a concentration of 1 mg/mL. The percentage DPPH radical-scavenging rate for *C. olivieri* subsp. *istanbulensis* at the corm was found to be 87%, whereas it was 81% for the aerial part. These values were found to be 93%, 41%, 92%, 27%, 84% and 74% for the corm and aerial parts of *C. flavus* subsp. *flavus, C. danfordiae* subsp. *danfordiae* and *C. antalyensis* subsp. *antalyensis*, respectively. The corms of all *Crocus* species showed higher antioxidant activity than the aerial parts.

Khalili et al. (2016) studied the polyphenolic fractions for DPPH scavenging activity in the corms and aerial parts of *Crocus caspius* Fisch. & C.A. Mey. ex Hohen. and found that the corm fractions have higher activities, which is like our findings. Acar Doğanlı et al. (2010) have investigated the DPPH scavenging activity of the *C. baytopiorum* B. Mathew, *C. flavus* and *C. biflorus* Mill. species extracts in the concentration of 1.6 mg/mL and concluded that the results were 78%, 90% and 76%, respectively. In another work, the ethanolic extracts of *C. sativus* L. leaves in the concentrations of 0.1 mg/mL showed a DPPH scavenging activity at 84% (Ökmen et al., 2016). It was concluded that the preparation techniques of extracts, the collection areas and species-dependent differences may have a great effect on the antioxidant activity level.

Polyphenolic compounds are frequently encountered in both edible and inedible plants and they are accepted to have various biological outcomes, especially on antimicrobial and antioxidant effectiveness (Ouerghemmi et al. 2017). The amount of total phenolic content was measured by the Folin-Ciocalteu method.

It can be easily seen that the highest phenolic content was in the corm of *Crocus olivieri* subsp. *istanbulensis*, whereas the lowest value was observed in the aerial part of *C. danfordiae* subsp. *danfordiae*. The corms of *C. olivieri* subsp. *istanbulensis, C. antalyensis* subsp. *antalyensis* and *C. danfordiae* subsp. *danfordiae* were found to have higher phenolic contents, which were parallel to the antioxidant activity results (Table 1).

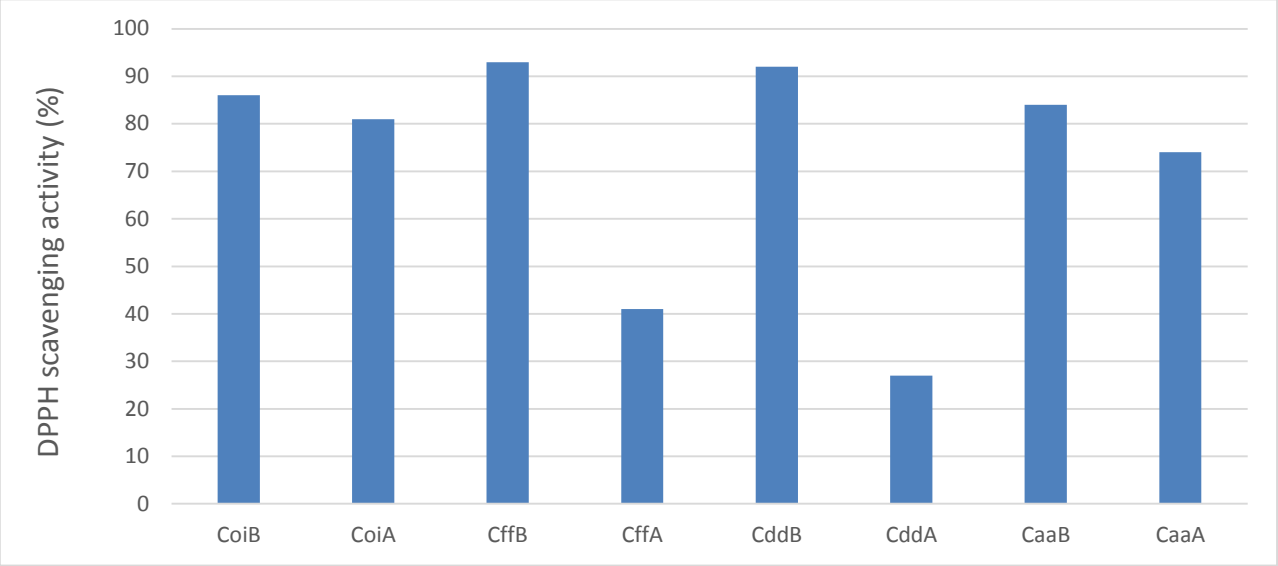

Figure 1. DPPH scavenging-activity (%) of the *Crocus* species used in this study (CoiB: *C. olivieri* subsp. *istanbulensis* corm; CoiA: *C. olivieri* subsp. *istanbulensis* aerial; CffB: *C. flavus* subsp. *flavus* corm; CffA: *C. flavus* subsp. *flavus* aerial; CddB: *C. danfordiae* subsp. *danfordiae* corm; CddA: *C. danfordiae* subsp. *danfordiae* aerial; CaaB: *C. antalyensis* subsp. *antalyensis* corm; and CaaA: *C. antalyensis* subsp. *antalyensis* aerial).

However, the aerial parts of *Crocus flavus* subsp. *flavus* were found to have higher levels of phenolics than its corms. In the literature, there exists various works showing that there are close and positive relationships between the antioxidant activity and the amount of phenolic contents in plants (Cai et al. 2004; Do et al. 2014), though there are other studies showing inverse relationships (Aksoy et al. 2013). The method used to measure the total phenolic content provides a rough evaluation for the total phenolic compounds remaining in the extract. This is not special to polyphenols however, various interfering compounds can interact with the reactants, resulting in a raised phenolic concentration level (Prior et al. 2005). Besides, various phenolic compounds may react in a contrasting manner in this assay, depending on the number of phenolic groups contained (Singleton & Rossi 1965), and total phenolics content does not necessarily integrate all the antioxidants that may be included in an extract.

Table 1. Total phenolic content of the *Crocus* species used.

Species	Total phenolic (mg GAE/100 g) ± STD	
	Corm	Aerial parts
C. olivieri subsp. *istanbulensis*	390 ± 3.2	73 ± 5.1
C. flavus subsp. *flavus*	126.8 ± 2.1	202.4 ± 1.2
C. danfordiae subsp. *danfordiae*	53.2 ± 1.3	24.4 ± 2.8
C. antalyensis subsp. *antalyensis*	220 ± 1.8	140 ± 1.6

Nowadays, there is an increasing trend in the use of natural resources instead of synthetic preservatives and the need for new plant species with antioxidant potential. From that point of view,

this study is valuable in that it states that the *Crocus olivieri subsp. istanbulensis, C. flavus subsp. flavus, C. danfordiae subsp. danfordiae* and *C. antalyensis subsp. antalyensis* species have antioxidant properties. This study shows that similar studies can be performed for other *Crocus* species to find out whether they are valuable or not to be employed as antioxidant contents.

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REFERENCES

Acar Doğanlı, G., Doğan, N.M., Duru, M.E. & Kıvrak, İ. (2010). Phenolic profiles, antimicrobial and antioxidant activity of the various extracts of *Crocus* species in Anatolia. *Afr J Microbiol Res* 4(11): 1154–1161.

Aksoy, L., Kolay, E., Ağılönü, Y., Aslan, Z. & Kargıoğlu, M. (2013). Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic *Thermopsis turcica*. *Saudi J Biol Sci* 20(3): 235–239. DOI: 10.1016/j.sjbs.2013.02.003.

Baba, S.A., Malik, A.H., Wani, Z.A., Mohiuddin, T., Shah, Z., Abbas, N. & Ashraf, N. (2015). Phytochemical analysis and antioxidant activity of different tissue types of *Crocus sativus* and oxidative stress alleviating potential of saffron extract in plants, bacteria, and yeast. *S Afr J Bot* 99: 80–87. DOI: 10.1016/j.sajb.2015.03.194.

Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature* 181: 1199–1200.

Cai, Y., Luo, Q., Sun, M. & Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci* 74(17): 2157–2184. DOI: 10.1016/j.lfs.2003.09.047.

Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S. & Ju, Y.H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila* aromatica. *J Food Drug Anal* 22(3): 296–302. DOI: 10.1016/j.jfda.2013.11.001.

Esmaeili, N., Ebrahimzadeh, H., Abdi, K. & Safarian, S. (2011). Determination of some phenolic compounds in *Crocus sativus* L. corms and its antioxidant activities study. *Pharmacog Mag* 7(25): 74–80. DOI: 10.4103/0973- 1296.75906.

İnceçayır, D., Semerci, A.B., Mustafa, N. & Tunç, K. (2019). *Catalpa bignonioides* metanolik çiçek ekstraktının biyolojik ve kimyasal aktivitesi (The biological and chemical activity of the *Catalpa bignonioides* methanolic flower extract). *Türk Tarım ve Doğa Bilimleri Dergisi* 6(2): 230–234. DOI: 10.30910/turkjans.557101 (in Turkish).

Khalili, M., Fathi, H. & Ebrahimzadeh, M.A. (2016). Antioxidant activity of bulbs and aerial parts of *Crocus caspius*, impact of extraction methods. *Pak J Pharm Sci* 29(3): 773–777.

Mathew, B. (1984). [*Crocus* L.] In: Davis, P.H. (Ed.). *Flora of Turkey and the East Aegean Islands*. Vol. 8. Edinburgh University Press, Edinburgh, pp. 413–438.

Mathew, B. (1988). [*Crocus* L.] In: Davis, P.H., Mill, R.R. & Tan, K. (Eds.). *Flora of Turkey and the East Aegean Islands*. Vol. 10. Edinburgh University Press, Edinburgh, pp. 228.

Mathew, B. (2000). [*Crocus* L.] In: Güner, A., Özhatay, N., Ekim T. & Başer, K.H.C. (Eds.). *Flora of Turkey and the East Aegean Islands*. Vol. 11. Edinburgh University Press, Edinburgh, pp. 271–274.

Miliauskas, G., Venskutonis, P.R. & Beek, T.A. van. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem* 85(2): 231–237. DOI: 10.1016/j.foodchem.2003.05.007.

Neagu, E., Radu, G.L., Albu, C. & Paun, G. (2018). Antioxidant activity, acetylcholinesterase and tyrosinase inhibitory potential of *Pulmonaria officinalis* and *Centarium umbellatum* extracts. *Saudi J Biol Sci* 25(3): 578–585. DOI: 10.1016/j.sjbs.2016.02.016.

Ökmen, G., Kardaş, S., Bayrak, D., Arslan, A. & Çakar, H. (2016). The antibacterial activities of *Crocus sativus* against mastitis pathogens and its antioxidant activities. *WJPPS* 5(3): 146–156.

Ouerghemmi, I., Rebey I.B., Rahali, F.Z., Bourgou, S., Pistelli, L., Ksouri, R., Marzouk, B. & Tounsi, M.S. (2017). Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian *Ruta chalepensis*. *J Food Drug Anal* 25(2): 350–359. DOI: 10.1016/j.jfda.2016.04.001.

Prior, R.L., Wu, X. & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem* 53(10): 4290–4302. DOI: 10.1021/jf0502698.

Sağıroğlu, M., Olgaç, E., Ertürk, B. & Turna, M. (2012). An ethnobotanical survey from Şile (İstanbul) and Karasu (Sakarya). *Ot Sis. Bot. Dergisi* 19(2): 93–104.

Sağıroğlu, M., Turna, M. & Köseoğlu, S.T. (2017). Medicinal plants in flora of İkramiye (Sapanca-Sakarya-Türkiye). *Sakarya University Journal of Science* 21(3): 527–539. DOI: 10.16984/saufenbilder.292196.

Singleton, V.L. & Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *Am J Enol Vitic* 16(3): 144–158.

Tunç, K., Semerci, A.B. & Okur, I. (2020). Antioxidant activity of the fruits of *Pyracantha coccinea* using ethanolic extract method. *Food Health* 6(1): 35–40. DOI: 10.3153/FH20005.

Yüzbaşıoğlu, S.İ. (2012). [*Crocus* L.]. Güner, A., Aslan, S., Ekim, T., Vural, M. & Babaç, M.T. (Eds.). *Türkiye Bitkileri Listesi (Damarlı Bitkiler)* (Plant list of Turkey {Vascular plants}). ANG Vakfı Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul, pp. 530–534 (in Turkish).

Zengin, G., Aumeeruddy, M.Z., Diuzheva, A., Jeko, J., Cziáky, Z., Yıldıztugay, A., Yıldıztugay, E. & Mahomoodally, M.F. (2019). A comprehensive appraisal on *Crocus chrysanthus* (Herb.) Herb. Flower extracts with HPLC–MS/MS profiles, antioxidant and enzyme inhibitory properties. *J Pharm Biomed Anal* 164: 581–580. DOI:10.1016/j.jpba. 2018.11.022.

Zou, Z., Xi, W., Hu, Y. Nie, C. & Zhou, Z. (2016). Antioxidant activity of *Citrus* fruits. *Food Chem* 196: 885–896. DOI: 10.1016/j.foodchem.2015.09.072.