

Antioxidant Activity of Fruit Extracts of *Prangos ferulacea* (L.) Lindl. from Turkey

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ABSTRACT: It was aimed to determine antioxidant activity, and amount of total phenolic and flavonoid compounds of methanolic and ethanolic extracts of *P. ferulaceae* (L.) Lindl. fruits from Turkey. The fruits were collected at two different times, in May and July 2014. The total phenolic content of the extracts determined according to the Folin-Ciocalteu Reactifi (FCR) method was calculated as mg Gallic acid equivalent (GAE) g⁻¹ extract. The total flavonoid content of the extracts was expressed as mg Quercetin (QE) g⁻¹ extract. The percentage of antioxidant activity of each samples was assessed by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical assay. Total phenolic and flavonoid contents of the extracts were determined to range from 11.750 to 79.111 mg GAE g⁻¹ extract and from 2.266 to 9.089 mg QE g⁻¹ extract, respectively. According to the results of DPPH test, all fruit extracts exhibited a slightly antioxidant activity.

Keywords: DPPH, *Prangos ferulaceae*, total flavonoid, total phenolic.

Türkiye’deki *Prangos ferulacea* (L.) Lindl.’nin Meyve Özütlerinin Antioksidan Aktivitesi

ÖZET: Türkiye’de doğal olarak yetişen *P. ferulacea* (L.) Lindl.’in metanol ve etanol ile hazırlanan meyve özütlerinin toplam fenolik ve flavonoid miktarları ile antioksidan aktiviteleri belirlendi. Meyveler Mayıs ve Haziran 2014’de toplandı. Ekstraktların toplam fenolik içerikleri Folin-Ciocalteu Reactifi (FCR) yöntemine göre mg Gallik asit eşdeğeri (GAE) g⁻¹ ekstrakt olarak hesaplandı. Ekstraktların toplam flavonoid içeriği mg Kuarsetin (QE) g⁻¹ ekstrakt olarak ifade edildi. Her bir örneğin antioksidan aktivite yüzdesi DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) ile değerlendirildi. Özütlerin toplam fenolik ve flavonoid içerikleri sırasıyla 11.750-79.111 mg GAE g⁻¹ ile 2.266-9.089 mg QE g⁻¹ olarak belirlendi. DPPH testi sonuçlarına göre, tüm meyve özütlerinin zayıf antioksidan aktiviteye sahip olduğu tespit edildi.

Anahtar Kelimeler: DPPH, *Prangos ferulaceae*, toplam fenolik, toplam flavonoid.

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INTRODUCTION

Prangos belonging to the Apiaceae family is a perennial genus. Turkey is an important center for this genus. The genus *Prangos* is represented by 21 taxa including 17 species (*P. acaulis*, *P. corymbosa*, *P. denticulata*, *P. ferulacea*, *P. heynia*, *P. hulusii*, *P. ilanae*, *P. meliocarpoides*, *P. pabularia*, *P. papillaris*, *P. peucedanifolia*, *P. platychlaena*, *P. acaba*, *P. scabrifolia*, *P. turcica*, *P. uechtrizii*, and *P. uloptera*) 2 varieties (*P. meliocarpoides* var. *arcis-romanae* and *P. meliocarpoides* var. *meliocarpoides*) and 2 subspecies (*P. platychlaena* subsp. *engizekensis* and *P. platychlaena* subsp. *platychlaena*) in the flora of Turkey, and 10 taxa of them are endemic. *Prangos* species are known as “çakşir otu” in Turkey (Guner et al., 2012). *Prangos ferulaceae* (L.) Lindl. is spread naturally in Northern and Southern Anatolia Regions of Turkey (Tubives, 2011)

This plant has been used as a medicinal plant in folk medicine of many countries in the world (Kafash-Farkhad et al., 2013). Especially, the extracts from the fruits and roots of the plant have been used for treatment several disorders such as digestive disorders, healing scars, and stop the bleeding. Also, essential oil is obtained from various parts of *P. ferulaceae* (L.) Lindl. such as leaves, flowers, fruits and stems. The previous studies on the essential oils of *Prangos* species showed variations from 0.2 to 1.1% in yield depending on the organs (Baser et al, 1996; Baser et al., 2000; Razavi et al., 2010). Thanks to their chemical compositions, essential oils have various biological activities (antifungal, antioxidant, antibacterial, etc.) (Mirzaei et al., 2007; Mneimne et al., 2016).

The free radicals that occur during normal body functions accelerate aging by damaging the cells and the immune system. Antioxidants bind the free radicals to themselves and inactivating them, they reduce the most possible damage and thus delaying aging. They play an important role in the prevention of human diseases (cancer, diabetes, dementia etc.) (Abdelhady et al., 2011; Shirazi et al., 2014). Nowadays, the organic and inorganic substances such as beta-carotene, C, E vitamins, lycopene, coenzyme, Q-10, selenium, zinc and manganese are the most widely used antioxidants. The vast majority of medicinal and aromatic plants that are rich in secondary metabolites have a strong antioxidant

effect. In such plants, the antioxidant effect is usually related to the presence of phenols and flavonoids and their free radical scavenging activity (Baydar, 2013).

Phenolic compounds are generally colorless or colored, crystalline and amorphous substances which have a special odor and are well soluble in water, organic solvents (alcohol, ester, chloroform, ethyl acetate etc.), and bases liquid solutions. In recent years, these compounds have been studied extensively due to their potential to be protective against deadly diseases such as cancer and coronary heart diseases (Mammadov, 2014). Antioxidants are defined as compounds that prevent or delay oxidative degradation in food. It is known that oxygen is the most important factor in the degradation of many foodstuffs. It is only possible to delay or prevent the peroxidation process by addition of antioxidant compounds in foodstuffs. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary-butylhydroquinone (TBHQ) have been used as synthetic antioxidants to prevent degradation of foodstuffs for years. These synthetic antioxidants are phenolic compounds, which make them effective antioxidants (Abdelhady et al., 2011; Ogut, 2014). The use of BHA and BHT has been limited due to the fact that it causes liver damage and cancer in laboratory animals. For example, TBHQ is prohibited in the European Union countries, although it is free in the United States. For this reason, the demand for natural antioxidant resources is increasing day by day (Abdelhady et al. 2011; Mammadov 2014).

In this study, it is aimed to determine 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and amount of total phenolic and flavonoid compounds of the fruit extracts from *P. ferulaceae* (L.) Lindl. growing naturally in Turkey.

MATERIAL AND METHODS

Plant material

The fruits of *P. ferulaceae* were collected at two different times at an altitude of 1420 m from the area between Tunceli and Pülümür located to the east of Turkey (39° 28' 20" N, 039° 54' 25" E). The first and second collection was held in May and July 2014,

respectively. The plant samples numerated as M. Koç. 1334 were deposited at the Herbarium of the Bozok University Department of Biology (Yozgat/ Turkey).

Solvent extraction

Collected fruits were dried in the shade and ground with a blender. The samples were prepared with three replications for each of the two collection times. 40 mL of methanol was added to 4 g of sample, and these samples were kept in the oven at 40 °C for 24 h. The solutions obtained were centrifuged at 4500 rpm for 10 min. Supernatant fractions of the solutions were removed and the organic solvent was separated by using a rotary evaporator. The extracts were stored in the dark at 4 °C until used.

Determination of total phenolic compounds

The total phenolic content of the extracts was determined according to the Folin-Ciocalteu Reactif (FCR) method (Singleton et al., 1999). 0.2 ml was taken from the sample solutions prepared with ethanol and methanol (2 mg ml⁻¹) and distilled water was added. Then 0.2 ml of Folin-Ciocalteu was added to this solution, and allowed to stand for 3 min. 0.6 ml of Na₂CO₃ (20%) was added on this solution, and the total volume was adjusted to be 10 ml. The obtained solution was incubated in the dark for 2 hours, and then absorbance measurements were made at 760 nm. Gallic acid was used to form the standard calibration curve. 0.2 ml of ethanol was added to each test tube. 0.1 mg/ml was prepared as the main stock from Gallic acid. Ethanol was added to the sample solution (0.2 ml) for the control. According to the Gallic acid standard graph, the total phenolic substance in all plant extracts was calculated as mg Gallic acid equivalent (GAE) g⁻¹ extract.

Determination of total flavonoid compounds

The total flavonoid contents of the extracts were determined by adapting the Arvouet-Grand et al. (1994) method. 8 mg ml⁻¹ from extract solution dissolved in ethanol and methanol was prepared as the main stock. 100 µL of 10% aluminum nitrate and 100 µL of 1 M potassium acetate were added to 250 µL of the sample. The final volume of the solution was 5 ml with 99% ethanol. Absorbance measurements were made at 417

nm after incubation at room temperature for 40 minutes in the dark. 0.5 mg ml⁻¹ was prepared as the main stock for the Quercetin standard. The total flavonoid content of the extracts was expressed as mg Quercetin (QE) g⁻¹ extract. To determine the total phenolic and flavonoid contents of the extracts, spectrophotometric measurements were performed using a Perkin Elmer Lambda 25 UV/VIS spectrometer.

Free radical scavenging activity by DPPH method

The percentage of antioxidant activity (AA %) of each samples was assessed by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Gezer et al. (2006). 4 mg DPPH was added to 100 mL of the solvent (methanol/ethanol) to prepare the concentration. The extracts dissolved in methanol and ethanol was used in this study. 8 mg mL⁻¹ extract solutions were prepared as the main stock, and the dilutions were obtained at different concentrations (100, 200, 400, 600, 800, and 1000 µg mL⁻¹) from this stock. 200 µL of extract solutions in different concentrations were added into 3.2 mL of DPPH solution. The solvent amounts in each test tube were set to be 200 µL of ethanol and 3.2 mL methanol. The absorbance at 517 nm was recorded after 30 min of incubation at room temperature in dark using a Perkin Elmer Lambda 25 UV/VIS spectrophotometer. Ascorbic acid was used as standard antioxidant. The scavenging activity percentage of each sample was determined according to the following equation: $AA (\%) = 100 - [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100]$

where A_{control} is the absorbance of the control with methanol and A_{sample} is the absorbance of essential oils and plant extracts.

Statistical analysis

Experiments were performed in three replicates and two parallel for each replicate. The results were expressed as ±SD. Data obtained were subjected to analyses of variance (ANOVA) and correlation analysis using TARIST statistical program. Means were separated at α=0.05 probability level by the LSD (the least significant difference) test (Acikgoz et al., 2004).

RESULTS AND DISCUSSION

The yield (g) of fruits extracts were 0.4784 ± 0.182 in the first collection in May and

0.1896 ± 0.064 in the second collection in July. Total phenolic and flavonoid contents in the fruit extracts of *P. ferulaceae* given in Table 1.

Table 1. Total phenolic and flavonoid contents in the fruit extracts of *P. ferulaceae*

Extract Types	Total phenolic content (mg GAE g ⁻¹ extract)	Total flavonoid content (mg QE g ⁻¹ extract)
M1	$11.750 \pm 0.3472c^*$	$2.266 \pm 1.646b$
M2	$12.965 \pm 1.7010c$	$3.359 \pm 1.220b$
E1	$79.111 \pm 2.6820a$	$9.089 \pm 2.482a$
E2	$67.479 \pm 1.4456b$	$3.203 \pm 0.004b$

*Each value is the average of three analyses \pm standard deviation. The same letter do not differ significantly at $p < 0.05$.

M1: Methanolic extract, first collection; **M2:** Methanolic extract, second collection; **E1:** Ethanolic extract, first collection; **E2:** Ethanolic extract, second collection

Methanol and ethanol extracts of fruits obtained from the first and second collection were standardized for their contents of phenolic compounds. The calibration curve showed linearity for Gallic acid in the range of $20\text{-}100 \mu\text{g ml}^{-1}$, with a correlation coefficient (R^2) of 0.999 (Figure 1). The results of the total phenolic content determination of the fruit extracts obtained

from the first and second collection, used two different solvents (methanol and ethanol) are given in Table 1. E1 contained the highest content of phenolic ($79.111 \text{ mg GAE g}^{-1}$), followed by E2 ($67.479 \text{ mg GAE g}^{-1}$), M2 ($12.965 \text{ mg GAE g}^{-1}$) and M1 ($11.750 \text{ mg GAE g}^{-1}$). Ethanolic extracts contained higher levels of phenolic than methanolic extracts in our study.

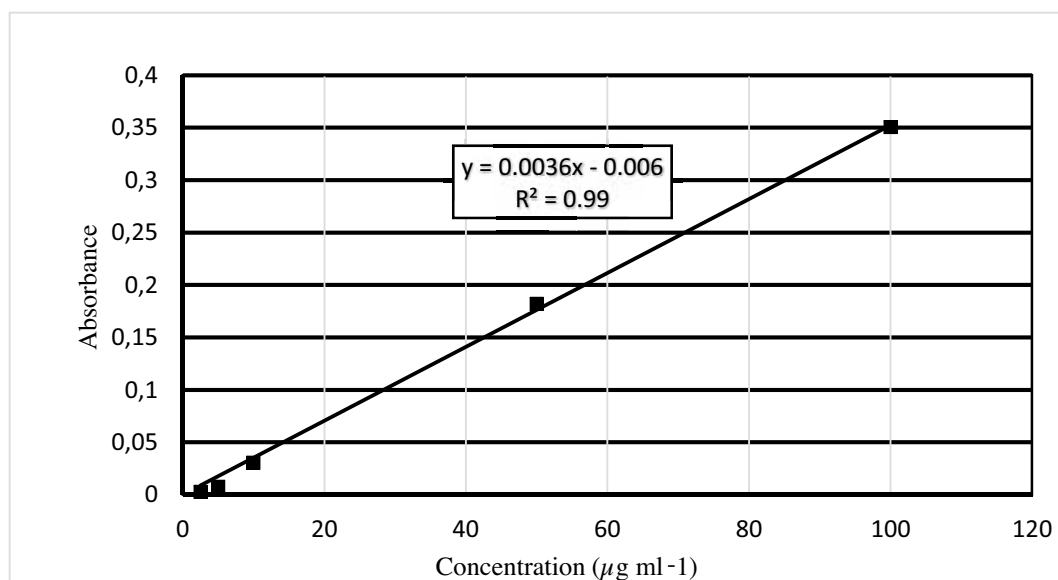


Figure 1. Standard calibration curve of gallic acid at concentration of 20, 40, 60, 80 and $100 \mu\text{g ml}^{-1}$. Spectrophotometric detection was at 760 nm

The calibration curve showed linearity for Quercetin in the range of $100\text{-}600 \mu\text{g ml}^{-1}$, with a correlation coefficient (R^2) of 0.9974 (Figure 2).

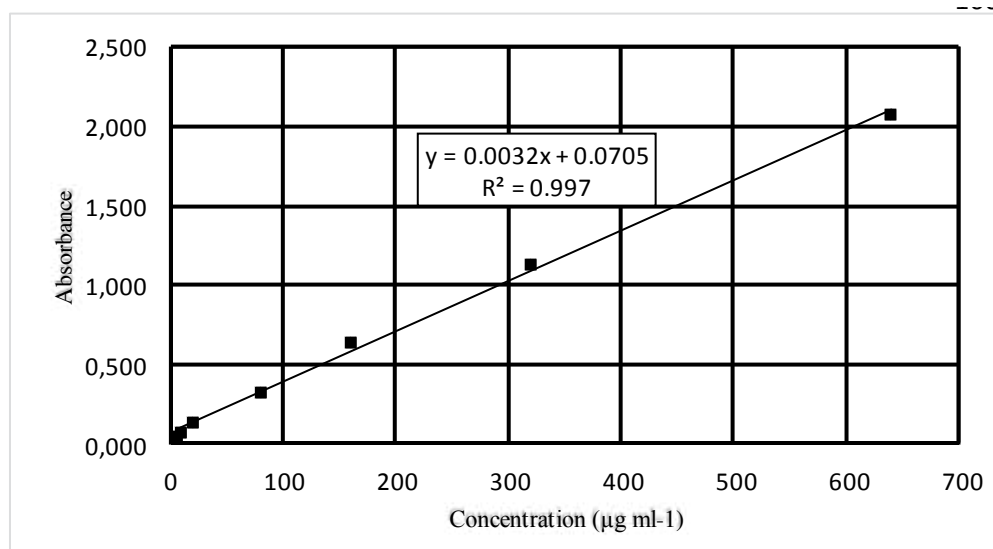


Figure 2. Standard calibration curve of Quercetin at concentration of 100, 200, 300, 400, 500 and 600 $\mu\text{g ml}^{-1}$. Spectrophotometric detection was at 417 nm

Total flavonoid content in fruit extracts ranged from 2.266 (M1) to 9.089 (E1) mg QE/g extract. The ethanol extract of fruits obtained from first collection showed the highest amounts of flavonoid content than the other examined extracts (Table 1). DPPH, a stable free radical, radical scavenging activity is a very popular method used in screening the antioxidant activity of plant extract (Tirzitis and Bartosz, 2010). The Table 2 is given the antioxidant activities of ethanol and methanol extracts of fruit examined using the DPPH radical scavenging recorded in terms of % inhibition. Higher % inhibition value means better scavenging

activity/antioxidant potential. The results obtained were compared with the standard AA (Figure 3). The extracts prepared with methanol exhibit higher DPPH scavenging activity those prepared with ethanol. The antioxidant potential of the fruits collected in May (6.4-80.3%) was found to be higher than those collected in July (4.4-70.1%). According to the values in Table 2, E2 in 100 $\mu\text{g/ml}$ has minimum DPPH scavenging activity (4.4 %) and M1 in 1000 $\mu\text{g/ml}$ has maximum DPPH scavenging activity (80.3 %) among fruits extracts and M1 has shown better DPPH scavenging activity as compared to the other extracts.

Table 2. Free radical scavenging activity of *P. ferulacea* fruit extracts by DPPH methods (%)

C ¹ ($\mu\text{g mL}^{-1}$)	M1	M2	E1	E2	C ($\mu\text{g mL}^{-1}$)	AA
100	6.4±0.058a ²	5.4±1.950ab	6.5±2.252a	4.4±0.400b	10	4.00±0.672
200	15.0±1.000a	13.1±1.100b	10.9±0.351c	7.8±0.600d	20	11.23±1.040
400	33.0±1.650a	29.8±2.000b	19.0±0.058c	15.0±1.050d	40	24.25±0.608
600	48.2±2.600a	45.8±0.400b	26.1±0.451c	22.6±0.800d	60	40.19±0.157
800	63.4±0.551a	58.5±1.050b	32.0±0.400c	29.6±0.751d	80	58.46±0.001
1000	80.3±0.300a	70.1±0.600b	38.3±0.058c	36.5±0.100d	100	73.71±1.048

¹ Concentrations

² Each value is the average of three analyses ± standard deviation. The same letter do not differ significantly at $p < 0.05$.

M1: Methanolic extract, first collection; **M2:** Methanolic extract, second collection; **E1:** Ethanolic extract, first collection; **E2:** Ethanolic extract, second collection; **AA:** Ascorbic acid

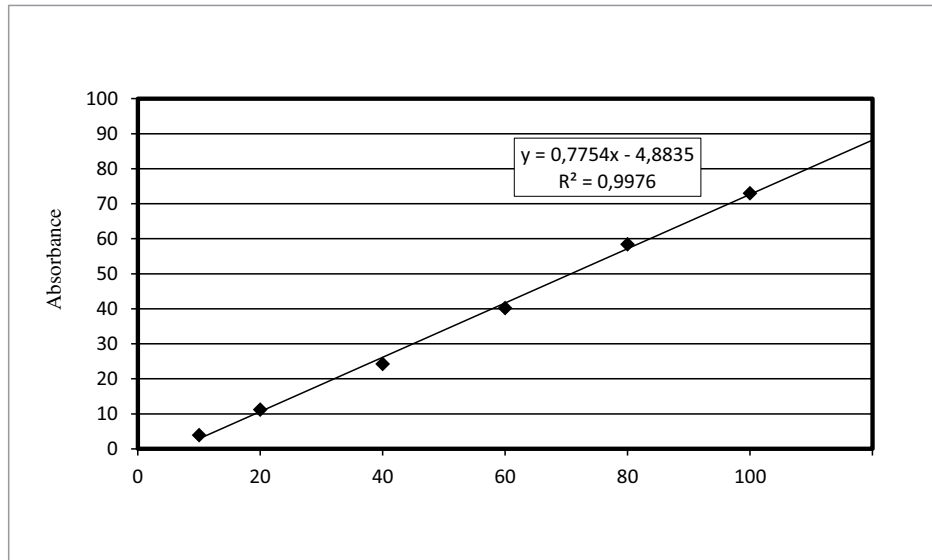


Figure 3. Standard calibration curve of ascorbic acid at concentration of 20, 40, 60, 80 and 100 $\mu\text{g ml}^{-1}$. Spectrophotometric detection was at 517 nm

Studies conducted in previous years have shown that *P. feruleceae* extracts have antioxidant activity at varying ratios (Kafash-Farkhad et al., 2013). Very high antioxidant activity was observed in some studies (Coruh et al., 2007), but very low activity was detected in some (Ahmed et al., 2011). Also, it has been reported that the various chemicals (flavonoids, alkaloids, terpenoids, phenolic compounds etc.) in the plant have high antioxidant activity (Sajjadi and Mehregan, 2003; Coruh et al., 2007). Contrary to this, the flavonoid, quercetin-3-O-glucoside, in the extract from aerial parts of the plant did not exhibit antioxidant activity in the study conducted by Razavi et al. 2009. According to the results of DPPH test in our study, all fruit extracts exhibited a slightly antioxidant activity. Many of the secondary metabolites such as alkaloids, essential oils, phenol and flavonoids synthesized by plants show large variations depending on the environmental conditions under which the plant grows. Temperature and light fluctuations promote the synthesis of antioxidants, bacterial infections promote the synthesis of phenolic and flavonoids (Baydar, 2013; Mammadov, 2014). All of this is the effort of plants to adapt to the environment they grow. Differences in synthesis of secondary metabolites in plants are possible, in view of the differences in collection

sites and timing of collections, the part of the plant used, the growth stage of plant when collected and the methods of analysis etc. (Marotti et al., 1994; Coruh, 2007; Ahmed et al., 2011; Akhgara et al., 2011; Kafash-Farkhad et al., 2013). Similarly, the antioxidant properties of these extract were found to be used solvent, concentration and collection time dependent in our study. The variability in the total phenolic contents of extracts prepared using different solvents may be due to the different solubility of the phenolic compounds (Damyeh and Niakousari, 2016). Bioactive substances in plants show significant changes according to the collection time, organ and life cycle of the plant (Baydar, 2013). The effectiveness and quality of medicinal and aromatic plants depends on their chemical composition. On the other hand, there is a considerable effect of seasonal variation on the chemical composition and biological activities of plants (Soni et al., 2015). Flavonoids which are substances that give color to flowering plants are the most common group of phenolic compounds. Flavonoids are used to prevent tumor formation and diabetes, as antioxidants, anti-inflammatory, anti-viral, anti-allergic, antimicrobial and enzyme inhibitory in medicine (Mammadov, 2014). Flavonoids are powerful antioxidants that protect the cells against antiradicals. Also, they

prevent the growth of bacteria and viruses, and provide resistance against cancer formation and heart attack. Quercetin which is a chemical pigment commonly found in the various organs of many plants is a flavonoid and have a powerful antioxidant activity (Baydar, 2013). It was observed that there was positive correlation ($r= 0.718$) between total

phenolic content and total flavonoid content. This correlation was not significant statistically. Also, correlation coefficients between DPPH, total phenolic content and total flavonoid content and total phenolic content were negatively associated with DPPH ($r=-0.971$, $P<0.05$ and $r=-0.608$, respectively) (Table 3).

Table 3. Correlation coefficients between DPPH, total phenolic content and total flavonoid content

	1	2	3
1. Total Phenolic Content	1.000		
2. Total Flavonoid Content	0.718 ^{ns}	1.000	
3. DPPH	-0.971 [*]	-0.608 ^{ns}	1.000

* $P<0.05$, ns: statistically insignificant

Some researchers support the association between phenolic compounds and antioxidant activity (Bendini et al., 2006; Dlugosz et al., 2006; Wojdylo et al., 2007), while the others argue that there is no direct relationship between these two parameters (Harish and Shivanandappa, 2006; Hassimotto et al., 2005; Abdelhady et al., 2011; Mammadov, 2014). In addition to phenolic compounds, other chemical compounds may be said to have an effect on antioxidant activity (Harish and Shivanandappa, 2006). Also, it has been stated that the flavonoid content of *P. ferulaceae* is not solely responsible for antioxidant activity and that there is no sufficient study on the detection of compounds responsible for antioxidant activity (Ahmed et al., 2011).

CONCLUSION

In this study, the DPPH radical scavenging activity, and amount of total phenolic and flavonoid compounds of the extracts from fruit of *P. ferulaceae* were analyzed.

As known, collection time is an important factor affecting the quality of medicinal and aromatic plants. Therefore, the plants were collected at two different times (May and July) to see the variation between collection times, and effect of seasonal variation on the DPPH radical scavenging activity, and amount of total phenolic and flavonoid compounds of the extracts was evaluated in this study.

The findings from the analysis results demonstrated that the extracts of fruits collected at two different times exhibited a slightly antioxidant activity. However, the antioxidant properties of these extract were influenced by the solvent and concentration used, and the collection time of the fruits. According to the findings obtained from our study, we recommend collecting the fruit of *P. ferulaceae* in May. Detailed phytochemical analyzes are needed to fully understand the antioxidant capacity of this species.

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