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SOME PHYTOCHEMICAL CONTENTS AND ANTIOXIDANT ACTIVITIES OF TWO *PRANGOS* LINDL. (UMBELLIFERA) SPECIES GROWN IN TURKEY

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Abstract

The genus *Prangos* is a member of the Apiaceae family, and it has been a focus of the attention of researchers due to its high nutritional value and some special medical properties. In this study, the total phenolic and flavonoid contents and antioxidant activities of the leaf and fruit extracts of the *Prangos ferulacea* and *Prangos uloptera* species belonging to this genus were studied, and the properties of these two species were compared to each other. In the study, the Folin-Ciocalteu method was used for the analysis of the total phenolic content, methanolic formaldehyde was used for the total flavonoid content, and both analyses were carried out with a UV-VIS spectrophotometer device. The antioxidant activity of the specimens was measured based on the Trolox equivalent antioxidant capacity (TEAC) method. It was found that the *P. ferulacea* species had higher total phenolic and flavonoid content values than the *P. uloptera* species. The *P. ferulacea* species was also more effective in terms of antioxidant activity, which is known to be associated with these properties. When the fruit and leaf extracts of each species were compared, it was found that the total phenolic and flavonoid content of the fruit extract of the *P. uloptera* species was higher than that of its leaf extract, and the antioxidant activity of the fruit extract was also higher in proportion to these values. In the *P. ferulacea* species, as opposed to the case of *P. uloptera*, all values except for the total flavonoid values were higher in the leaf extract than the fruit extract.

Key words: *Prangos ferulacea*, *Prangos uloptera*, Total flavonoid, Total phenolic.

Introduction

The *Prangos* genus is a genus of the Apiaceae family consisting of perennial plants, which is distributed from Portugal to Tibet and has 45 known species. The diversity of this genus is seen in the region of Iran-Turan. The genus has 17 species and 21 taxa known in the flora of Turkey, among which 10 are endemic. In Turkey, the species of this genus are known among the people as "çakşir otu" (Guner *et al*., 2012). Its species have been used for a long time in several countries for traditional medicine purposes. The fruit and root parts of the plant are turned into extracts, and these are used in the treatment of many conditions including digestion problems, wound-scar healing and stopping bleeding (Kafash-Farkhad *et al*., 2013). Additionally, it is used as a tonic and in the treatment of bloating, hemorrhoids and leukoplakia (Dokoric *et al*., 2004; Yasuhiro *et al*., 2001). Besides these, *Prangos* species are also used as stimulants, aphrodisiacs and natural fertilizers. Some species of this genus are used as spices and food additives (Oke-Altuntas *et al*., 2016; Ozek *et al*., 2018).

Free radicals that are formed at certain stages of metabolism damage cells and the immune system and cause diseases. Antioxidants, on the other hand, minimize the potential damage by binding free radicals to themselves and incapacitating them or reducing their effects. This way, they play an important role in preventing diseases (e.g., cancer, diabetes, dementia) (Abdelhady *et al*., 2011; Shirazi *et al*., 2014).

As it is thought that synthetic antioxidants that are used today have negative health effects, restrictions have started to be issued on the use of these synthetic antioxidants. As a result of this, the trend towards natural antioxidants has increased (Koleva *et al*., 2002). The effects of plants in the field of medicine are associated with their secondary metabolites such as essential oils, phenolics and flavonoids, and it is known that these compounds have antioxidant activities (Riahi *et al*., 2013).

Previous studies showed that *P. ferulacea* is very rich as a source of antioxidants, and it has an even higher antioxidant effect than α-tocopherol (vitamin E) (Coruh *et al*., 2007). Likewise, a potential reason for the analgesic effects of *P ferulacea* was shown as the presence of saponin, anthraquinone, tannin and flavonoids in its extract. Moreover, it was reported that extracts of *P. ferulacea* have antidiabetic properties (Soltani *et al*., 2011).

In previous phytochemical studies conducted on the fruits and roots of *P. uloptera*, the presence of substances known to have antioxidant activity such as various coumarins, monoterpenes and sesquiterpenes has been reported (Abyshev & Denisenko, 1970; Abyshev & Denisenko, 1973; Sefi Dkon & Navaii, 2001; Mazloomifar *et al*., 2004).

In this study, the total phenolic and flavonoid contents and antioxidant activities of aqueous extracts obtained from the fruits and leaves of *P. ferulacea* and *P. uloptera* were compared separately between the two species and between the leaf and fruit extracts of each species for the first time in the literature. The main purpose of our study is to make a comparison between the two species. The results are discussed based on the total phenolic and flavonoid contents of the aqueous extracts of both species and their antioxidant properties in relation to these contents. It is believed that such studies will provide significant contributions both in the field of health and in terms of providing an idea about food supplements by allowing the selection of plant species or their extracts that contain a suitable active compound profile and high antioxidant activity levels.

Material and Method

Plant materials: The *P. ferulacea* and *P. uloptera* species which are highly prevalent in the Nemrut Caldera declared as a Ramsar site in the province of Bitlis in Turkey were collected from their natural habitats at the altitudes of 2000-2250 m. in the July of 2018. The identification of the plant material was performed by Assoc. Prof. Dr. Sukru Hayta (Fig. 1). After fruits and leaves belonging to the *P. ferulacea* and *P. uloptera* plants were dried, they were kept in appropriate conditions until the experiments.

Preparation of the plant extract: Plant extracts were obtained with the method of hydrodistillation from 100 g. dried fruit and leaf specimens belonging to the *P. ferulacea* and *P. uloptera* plants. A Clevenger apparatus was used for this purpose. The plant material was put into the distillation container with water, and as a result of the condensation of the vapor forming following the heating and boiling of the water on the cooler surface, the essential oil and water were collected in the separating tank. The essential oil floating on the water due to the difference in density was taken into vials with the suitable apparatuses. These specimens that were separated were subjected to total flavonoid, total phenolic and antioxidant activity tests.

Total phenolic content analysis: The total phenolic content analysis in this study was carried out based on the Folin-Ciocalteu method as reported by Singleton & Rossi (1965). According to this method, 300 µl of the fruit or leaf extract of *P. uloptera* or *P. ferulacea* was mixed in glass tubes with 1.5 ml 2 N of the Folin-Ciocalteu reactant. After leaving this mixture for 1-2 min., 1.2 ml of the 7.5% sodium carbonate solution was added, mixed with vortex and kept in dark at 25°C for 90 min., after which the absorbance of the mixture was measured by UV-VIS spectrophotometry against the blank (distilled water) at 765 nm. By utilizing the gallic acid calibration curve, the total phenolic content is presented as the gallic acid equivalent (GAE). The gallic acid curve was read with the absorbance values calculated against 5 different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml) of gallic acid.

For calibration, the 0.5 mg/ml stock gallic acid solution that was prepared beforehand for the five different concentrations determined as averages was diluted, and the other concentrations were obtained. The steps described for the phenolic content analysis were followed separately for each solution that was prepared, and the absorbance values of the solution were read. The gallic acid absorbance values read against the different concentration values were coded on the Excel software, and the y=ax+b regression plot and R^2 values were obtained (Fig. 2).

Total flavonoid content analysis: Methanolic formaldehyde was used in the analysis of the total flavonoid content (Lamaison *et al*., 1990). For this analysis, 1 ml of the fruit or leaf extracts of *P. uloptera* or *P. ferulacea* was mixed with the 2% $AlCI_3$ solution. The obtained mixture was kept at room temperature (25°C) for 10 min. The absorbance values of the specimens were read at 394 nm in a UV-VIS spectrophotometer against 2% $AlCI_3$ as the control sample. The flavonoid concentration was calculated by comparison to the calibration curve of quercetin. The calibration curve was read with the absorbance values calculated against 5 different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml) of quercetin. For calibration, the 0.5 mg/ml stock quercetin solution that was prepared beforehand for the five different concentrations determined as averages was diluted, and the other concentrations were obtained. The steps described for the flavonoid content analysis were followed separately for each solution that was prepared, and the absorbance values of the solution were read. The absorbance values read against the different concentration values were coded on the Excel software, and the y=ax+b regression plot and R^2 values were obtained (Fig. 3).

Fig. 1. **a.** *P. ferulacea* **b.** *P. uloptera*

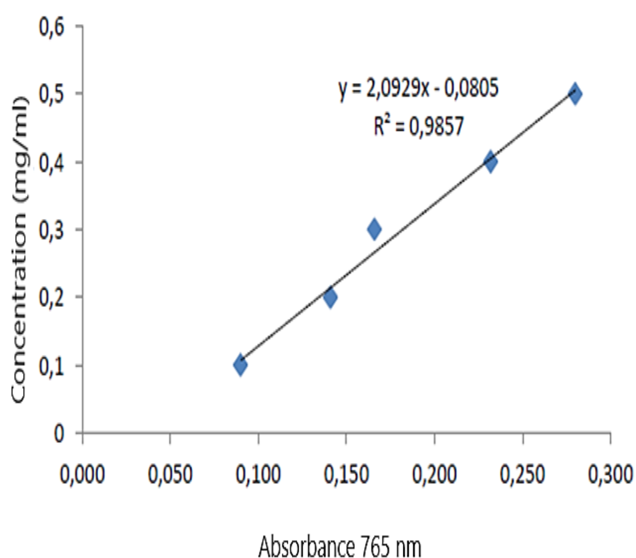

Fig. 2. Gallic acid calibration curve plot.

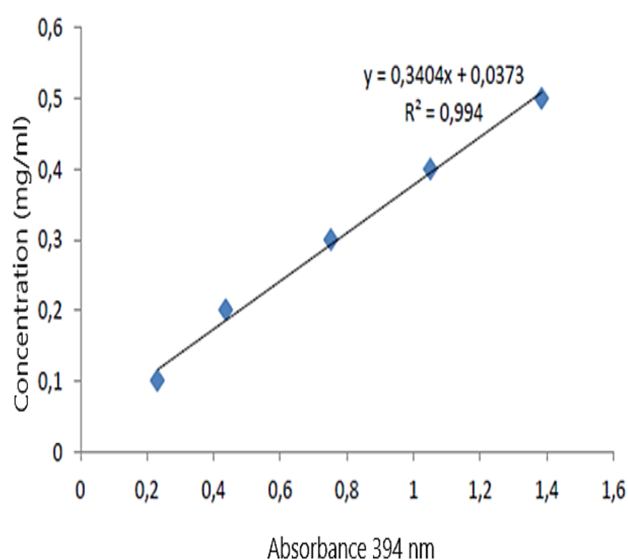

Fig. 3. Quercetin calibration curve plot.

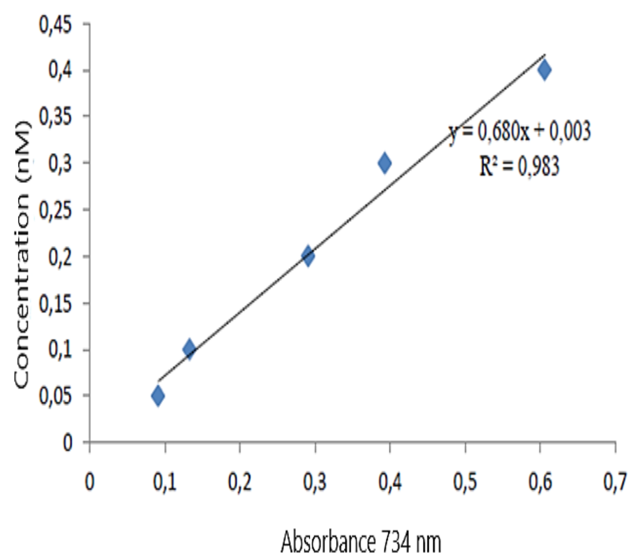

Fig. 4. Trolox calibration curve plot.

Antioxidant capacity determination: The antioxidant capacity values of the *P. uloptera* and *P. ferulacea* extracts were measured based on the radical cation capturing ability of ABTS [2,2''-azino-bis (3- ethylbenzothiazoline-6-sulfonate)]. According to this method, ABTS is oxidized with peroxyl or other oxidants, and the ABTS•+ radical is formed. The ABTS•+ radical cation is formed by the oxidation of the ABTS solution with persulfate. In this solution with a dark blue color that was prepared beforehand, with the effect of the antioxidant, the ABTS•+ cation was degraded, and the color of the dark blue solution became lighter. The lightened color of the specimen solution is an indicator of antioxidant capacity (Miller *et al*., 1995). For the antioxidant capacity analysis, the ABTS stock solution was diluted with a phosphate buffer solution so that the absorbance of the solution would be in the range of 0.7-0.8 at 734 nm. This solution was freshly prepared before each analysis to be conducted, and it was protected from light exposure as much as possible.

Right before the analysis was carried out, 1900 µl of the diluted ABTS solution and 100 µl *P. uloptera* or *P. ferulacea* leaf or fruit extract were separately added to glass tubes and mixed. At the end of the 6th minute, the absorbance values of these mixtures were read against the blind (phosphate buffer) at 734 nm in a UV-VIS spectrophotometer. The method that was used to determine antioxidant capacity is known as the TEAC (Trolox Equivalent Antioxidant Capacity) method, and its results are presented by taking Trolox as the standard. Trolox [6-hydroxy-2-Hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid] is a water-soluble analog of vitamin E. While Trolox is not a compound that is naturally found in living systems, it is used as the standard in several methods of determining antioxidant capacity (Re *et al*., 1999). A calibration plot using Trolox as the antioxidant was prepared, and the capacity of the unknown antioxidant was given as TEAC from this plot (Damar, 2010).

To prepare the calibration curve, the stock Trolox solution that was prepared beforehand was pipetted by respectively 1, 2, 4, 6 and 8 ml into 50-ml flasks. These flasks were completed up to their full capacity line with a phosphate buffer (0.05-0.4 mmol/L). To determine the antioxidant capacity of the Trolox solutions at the 5 different concentrations that were transferred to the flasks and diluted, at the end of the 6th minute, the absorbance values of the mixture solutions in the cuvettes one of which was designated as the witness sample (1900 µl diluted ABTS + 100 µl phosphate buffer) and the other as the analysis sample (1900 µl diluted ABTS + 100 µl Trolox solution) were measured at 734 nm with the UV-VIS spectrophotometer. The absorbance differences between the Trolox samples at the 5 different concentrations and the witness sample were transferred to the Excel software, and the regression equation y=ax+b and R^2 values were determined (Fig. 4).

Statistical data: In each extract that was used in the analyses conducted in determining total phenolic content, total flavonoid content and antioxidant activity values, 3 replicate readings were made for each measurement in the explants that were divided as fruit and leaf, the concentration values were calculated, and these values are presented with their standard deviations.

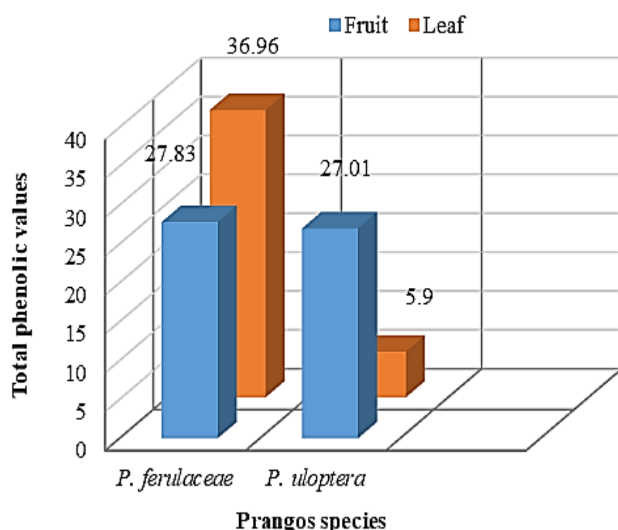

Fig. 5. Graphical comparison of the total phenolic content of the Prangos species (mg GAE/g).

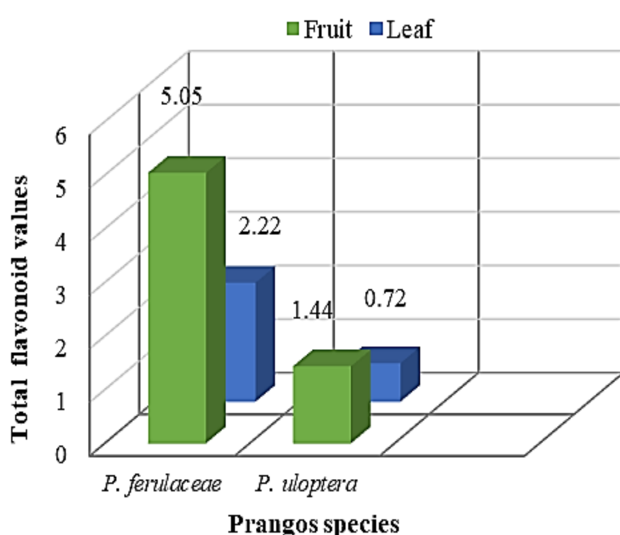

Fig. 6. Graphical comparison of total flavonoid contents of the Prangos species (mg quercetin/g).

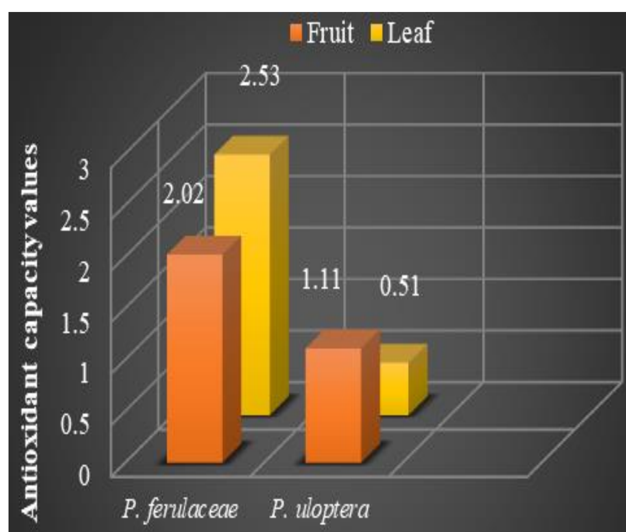

Fig. 7. Graphical comparison of antioxidant capacity values of the Prangos species (mmol/g TEAC).

Table 1. Total phenolic composition of the species.

Species	Fruit extract (mg GAE/g)	Leaf extract (mg GAE/g)
Prangos uloptera	27.01 ± 1.25	5.89 ± 0.69
Prangos ferulaceae	27.83 ± 1.55	36.99 ± 1.97

Table 2. Total flavonoid composition of the species.

Species	Fruit extract (mg quercetin/g)	Leaf extract (mg quercetin/g)
Prangos uloptera	1.45 ± 1.059	0.72 ± 0.79
Prangos ferulacea	5.05 ± 1.66	2.22 ± 0.82

Table 3. Antioxidant capasity (mmol/g TEAC)

Species	Fruit extract (mmol/g TEAC)	Leaf extract (mmol/g TEAC)
Prangos uloptera	1.12 ± 0.40	0.51 ± 1.82
Prangos ferulacea	2.02 ± 1.42	2.53 ± 1.27

Results

Total phenolic content: The total phenolic compositions of the fruit and leaf extracts of the *P. uloptera* and *P. ferulacea* species are presented in Table 1, whereas their comparison is graphically shown in Figure 5. In this study, it was determined that the phenolic compound concentrations of both the fruit and leaf extracts of the *P. ferulacea* species were higher in comparison to those of the *P. uloptera* species. In particular, while this concentration was 39.99 mg/g in the leaf extract of the *P. ferulacea* species, it was 5.89 in the *P. uloptera* species, and the difference was significant. When we analyzed each species to compare the phenolic contents of their fruit and leaf extracts, the total phenolic content of the fruit extract of the *Prangos uloptera* species was found to be noticeably higher in comparison to the leaf extract of the same species. In the *P. ferulacea* species, as opposed to the case above, this value was higher in the leaf extract than the fruit extract (Table 1) (Fig. 5).

Total flavonoid content: The total flavonoid compositions of the fruit and leaf extracts of the *P. uloptera* and *P. ferulacea* species are presented in Table 2, whereas their comparison is graphically shown in Figure 6. In this experiment, as in the case of the experiments to determine total phenolic contents, the total flavonoid content of both the fruit and leaf extracts of the *P. ferulacea* species was noticeably higher than that of the *P. uloptera* species. When we analyzed each species to compare the flavonoid contents of their fruit and leaf extracts, as in the case of their total phenolic contents, the total flavonoid content of the fruit extract of the *P. uloptera* species was higher than that of its leaf extract. However, as opposed to the case of their total phenolic contents, the total flavonoid content of the fruit extract of the *P. ferulacea* species was higher than that of its leaf extract (Table 2) (Fig. 6).

Antioxidant capacity: The results of the Trolox equivalent antioxidant capacity (TEAC) analysis that we conducted to determine the antioxidant capacities of the specimens are shown in Table 3, while their graphical

comparison is presented in (Fig. 7). Accordingly, in the comparison of the two species, in line with their total phenolic and flavonoid content results, the *P. ferulacea* species was found to be more effective in terms of its antioxidant activity in comparison to *P. uloptera.* Furthermore, in the separate comparisons of the fruit and leaf extracts of each species, the fruit extract of the *P. uloptera* species was more effective than its leaf extract, and in contrast, the leaf extract of the *P. ferulacea* species was more effective than its fruit extract in terms of antioxidant activity. This comparison also revealed that there was a direct proportion between the total phenolic compounds of the specimens and their antioxidant activities (Table 3).

Discussion

Aromatic and medicinal plants are rich sources of natural compounds like flavonoids, hydrolyzable tannins and phenolic compounds which have strong antioxidant properties. The phenolic compounds of these plants show antioxidant effects as they serve roles as reducing agents, hydrogen donors and singlet oxygen quenchers (Rong-Zhen & Dao-Wei, 2013; Rice-Evans *et al*., 1995). Accordingly, the antioxidant activities and capacities of several compounds obtained from such plants have been recorded and published in the literature. For example, some commercially accessible natural antioxidants were obtained from rosemary, thyme and sage (Embuscado, 2015.).

Previous studies have reported that various parts of *Prangos* species contain different types of chemical compounds (Tada *et al*., 2002; Tawaha *et al*., 2001; Tsetlin *et al*., 1972; Razavi *et al*., 2008; Baser *et al*., 2000; Başer *et al*., 2000; Ozcan *et al*., 2000). One of such studies reported that alcoholic extracts of this plant showed analgesic effects on formaldehyde-related pain (Emamghoreishi *et al*., 2012), and as the reason for the analgesic effects of *P. ferulacea* extract, the presence of saponin, anthraquinone, tannins and flavonoids was proposed (Bazdar *et al*., 2018). Besides these, another study reported the antidiabetic properties of *P. ferulacea* extracts (Soltani *et al*., 2011).

According to the results of a study where the total phenolic contents and antioxidant activities of 4 *Prangos* species were investigated, the MeOH extracts had generally higher phenolic contents than the aqueous extracts. The highest total phenolic contents were found in the MeOH extracts of fruits, especially in the *P. ferulacea*, *P. uechtritzii* and *P. heyniae* species (respectively, 140.29 µg/mL, 128.23 µg/mL and 127.33 µg/mL). It was reported that the other extracts usually displayed low total phenolic content values. In the DPPH test showing antioxidant activity in the same study, it was determined that especially the MeOH extracts were more active than the aqueous extracts, and the fruit extracts of all species except for that of the *P. meliocarpoides* species showed significant activity. In the TBA test, another indicator of antioxidant activity, the most significant activity was obtained from the MeOH extracts of the fruits. Among the MeOH extracts of the fruits, the highest activity was determined in the extract of the fruits of *P. heyniae* (Ahmed *et al*., 2011). Çoruh et al. found the IC50 value of the lipid peroxidation of a MeOH extract of the *P. ferulacea* species as 152 and its total phenolic content as 65.1 (Coruh *et al*., 2007). Ahmed *et al*., determined these values to be respectively 173 and 119.28 (Ahmed *et al*., 2011). Another study examined the antioxidant activities (DPPH) of ethanolic and methanolic fruit extracts of *P. ferulacea* (L.) Lindl. and determined that all fruit extracts showed weak antioxidant properties (Cesur *et al*., 2017). A similar study observed that extracts of *P. ferulacea* (L.) Lindl. (Apiaceae) showed antioxidant activity (Kafash-Farkhad *et al*., 2013). A different study conducted with two endemic *Prangos* species revealed that aqueous and methanolic extracts of the fruits showed high antioxidant activity (Oke-Altuntas *et al*., 2015). The results of our study were similar to those of other studies in that the antioxidant activity of the *P. ferulacea* species was higher in comparison to the other species.

Other studies showed that the antioxidant activities of *P. ferulacea* extracts may be found at different rates (Kafash-Farkhad *et al*., 2013). While some of these studies reported the antioxidant activity values to be very high (Coruh *et al*., 2007), some others observed low activity (Ahmed *et al*., 2011). Furthermore, Cesur *et al*., (2017) demonstrated that the antioxidant potential of fruits collected in May (6.4-80.3%) was higher than those collected in July (4.4-70.1%). These studies have shown that differences in the total phenolic content may be observed based on differences in the regions from where the plant samples are collected, as well as the collection times. Many secondary metabolites synthesized by plants (essential oils, phenolics and flavonoids) may show substantial differences based on the environmental conditions in which the plant grows. For example, fluctuations in temperature and light exposure promote antioxidant synthesis, whereas bacterial infections promote the synthesis of phenolic and flavonoid compounds (Baydar, 2013; Mammadov, 2014). All these differences are an indicator of the effort of plants to adapt to their environment. Conditions that create differences in the synthesis of these secondary metabolites may be listed as the location and time of collecting the plant sample, the part of the plant that is used, the growth stage of the plant and the analysis methods that are used (Kafash-Farkhad *et al*., 2013; Coruh *et al*., 2007; Ahmed *et al*., 2011; Marotti *et al*., 1994; Akhgara *et al*., 2011). In addition to these, seasonal changes also have a significant effect on the chemical compound contents of plants and the activities of these compounds (Soni *et al*., 2015). These differences stated in previous studies were also observed in the total phenolic and flavonoid contents of the fruit and leaf extracts of the two species examined in our study. While these results may have been caused by the genetic differences of the two species, they may also have been a consequence of other environmental and seasonal conditions.

Some researchers have reported a direct proportion between phenolic content and antioxidant activity (Bendini *et al*., 2006; Dlugosz *et al*., 2006; Wojdyło *et al*., 2007), whereas others argued that there is no direct relationship between these two parameters (Abdelhady *et al*., 2011; Mammadov, 2014; Harish & Shivanandappa, 2006; Hassimotto *et al*., 2005). It may be stated that, in addition to phenolic compounds, other chemical compounds also

have an effect on antioxidant activity (Wojdyło *et al*., 2007). In relation to this issue, in our study, we determined that the phenolic and flavonoid contents of the *Prangos* species could not be solely responsible for their antioxidant activities, and while the antioxidant activities of these plants were found to be proportional to their total phenolic contents, these activities were not much related to their total flavonoid contents. These results showed us that it is not adequate to determine a single type of compounds in such studies, but the effects of other types of compounds should also be investigated.

Conclusion

In this study, we determined that both the phenolic and flavonoid contents and antioxidant properties of the *P. ferulacea* and *P. uloptera* species showed differences based on the part of the plant that was used. According to our findings, the leaf part of the *P. ferulacea* species and the fruit part of the *P. uloptera* species were richer in terms of their total phenolic content, and this total phenolic content was directly proportional to antioxidant activity. In the comparison of the two species, we observed that the *P. ferulacea* species had higher values in terms of both its phenolic and flavonoid contents and its antioxidant activity in comparison to the *P. uloptera* species. However, as it would not be sufficient to completely understand the antioxidant capacities of these two species by looking at these analyses alone, there is a need for more detailed phytochemical analyses.

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