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Total phenolic contents and antioxidant activities of *Prangos* Lindl. (Umbelliferae) species growing in Konya province (Turkey)

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Abstract: *Prangos* species are widely used as medicinal plants in Turkey, and 14 species of this genus grow naturally in Anatolia. In the present study, the phenolic contents and antioxidant activities of the water and methanol (MeOH) extracts obtained from the root, herb, and fruits of 4 species of *Prangos* (*Prangos ferulacea, P. heyniae*, *P. meliocarpoides* var. *meliocarpoides*, and *P. uechtritzii*) collected in Konya province were compared. The phenolic contents of the samples were determined using Folin-Ciocalteu's phenol reagent. Antioxidant activities of the extracts were studied by qualitative DPPH• (1,1-diphenyl-2-picrylhydrazyl radical) assay to detect the free radical scavenging activity and by thiobarbituric acid (TBA) assay to detect their liposome lipid peroxidation. Total phenolic contents of the MeOH extracts were found to range from 77.99 to 140.29 mg/g and the water extracts ranged from 37.53 to 97.29 mg/g in dry weight expressed as gallic acid equivalents (GAE). All extracts showed a slightly antioxidant activity with the DPPH• test. High activity was observed in the MeOH extracts when compared to the water extracts in the TBA test.

Key words: *Prangos ferulacea* Lindl., *Prangos heyniae* H. Duman & M. F. Watson., *Prangos meliocarpoides* Boiss. var. *meliocarpoides*, *Prangos uechtritzii* Boiss. & Haussk., Umbelliferae, total phenolic contents, antioxidant activity

Konya (Türkiye) çevresinde yetişen *Prangos* Lindl. (Umbellifrae) türlerinin total fenolik madde miktarları ve antioksidan aktiviteleri

Özet: *Prangos* türleri Türkiye'de yaygın olarak kullanılan tıbbi bitkilerdir ve Anadolu'da 14 türü doğal olarak yetişmektedir. Bu çalışmada, Konya çevresinden toplanan 4 *Prangos* türünün, kök, herba ve meyvelerinden su ve metanol (MeOH) ile hazırlanan ekstrelerinin total fenolik madde miktarları ve antioksidan aktiviteleri karşılaştırılmıştır. Örneklerin total fenolik madde içerikleri Folin-Ciocalteu'nun fenol reaktifi kullanılarak tayin edilmiştir. Ekstrelerin antioksidan aktiviteleri; serbest radikal süpürücü aktivite kalitatif DPPH• (1,1-difenil-2-pikrilhidrazil radikal) yöntemi ile, lipozom lipit peroksidasyonu ise tiuobarbitürik asit (TBA) yöntemi ile çalışılmıştır. Total fenolik madde miktarları kuru ağırlık üzerinden gallik asit eşdeğeri olarak (GAE) MeOH ekstrelerinde 77,99-140,29 mg/g arasında; sulu ekstrelerde ise 37,53-97,29 mg/g arasında hesaplanmıştır. Bütün ekstreler DPPH• testinde zayıf bir antioksidan aktivite göstermiştir. TBA testinde MeOH ekstrelerinde sulu ekstrelere oranla daha yüksek aktivite gözlemlenmiştir.

Anahtar sözcükler: *Prangos ferulacea* Lindl., *Prangos heyniae* H. Duman & M. F. Watson., *Prangos meliocarpoides* Boiss. var. *meliocarpoides*, *Prangos uechtritzii* Boiss. & Haussk., Umbelliferae, total fenolik içerik, antioksidan aktivite

Introduction

Different endogenous and exogenous sources can produce various forms of activated oxygen and nitrogen. The major constituents of biological membranes are lipids and proteins. The number of functions of the membranes increases as protein amount increases (1). Reactive oxygen species can easily initiate the lipids causing damage to the cell membrane constituents, such as phospholipids and lipoproteins, by propagating a reaction cycle (1). Free radicals play a major role in the pathogenesis of many diseases, and free radical scavenging is facilitated by utilization of both exogenous and endogenous antioxidants (2). The currently used synthetic antioxidants have been suspected to cause or promote negative health effects; hence stronger restrictions have been placed on their application, and there is a trend to substitute them with naturally occurring antioxidants (3). Many medicinal plants (1,3,4), vegetables (2,5,6), and spices (7) have been found to be excellent sources of phenolic compounds, which have been reported to show good antioxidant activity.

Prangos Lindl. (Umbelliferae) genus has 43 species worldwide; these species grow in different centers of the Irano-Turanian phytogeographic region. This genus is widely distributed in Turkey and represented by 14 species, of which 8 are endemic (8-12).

Extracts of *Prangos* species were used to stop bleeding and heal scars in Central Asia (13). Furthermore, *Prangos* species have been used in traditional medicine in Turkey as tonic, antiflatulent, anthelmintic, in the treatment of wounds, and to stop external bleeding. Roots of the *Prangos* species, like those of *Ferula* and *Ferulago,* are used as aphrodisiacs (14). The aerial parts of *Prangos ferulacea* Lindl. collected during flowering have been used as an aromatic in cheese and milk products in the eastern part of Turkey (2). In addition, dried herbs of *P. ferulacea* were used as cattle feed (15). According to the chemical investigations, the presence of volatile oils (14,16,17), coumarins (13,18-20) and flavonoids (21) in *Prangos* species was reported. Antimicrobial (13), antioxidant (2,15,22,23), and cytotoxic activity (19) studies were carried out on different *Prangos* species.

In the present study, we investigated the antioxidant activities of water and methanol (MeOH) extracts of the root, herb, and fruits of 4 *Prangos* species collected in Konya province, 3 of which are endemic [*P. ferulacea* Lindl., *P. heyniae* H. Duman & M. F. Watson. (endemic = E), *P. meliocarpoides* Boiss. var. *meliocarpoides* (E), and *P. uechtritzii* Boiss. & Hausskn. (E)] (7-9). Total phenolic contents of the extracts were determined as using Folin-Ciocalteu's phenol reagent (23-26). Antioxidant activity of the extracts was evaluated by two methods. Free radical scavenging activity was analyzed by qualitative DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl radical). The DPPH assay was used as TLC screening method (4). This method is easy and rapid for the determination of total antioxidant activity. The thiobarbituric acid (TBA) method was used to determine the inhibition of lipid peroxidation (4) and propyl gallate was used as positive control in this method. TBA reaction has been extensively used for the detection of oxidative deterioration in lipids in recent_years (4,27).

Materials and methods

Plant material

Plant material: *P. ferulacea*, *P. uechtritzii, P. heyniae*, and *P. meliocarpoides* var. *meliocarpoides* were collected from Konya province during the flowering and fruiting periods. The voucher specimens were deposited at the Herbarium of Ankara University, Faculty of Pharmacy (AEF). Locations of the investigated plant samples are given in Table 1.

Extraction and preparation of test solutions

1- *Preparation of MeOH extracts*: 20 g of root, herb, and fruits of each species were powdered and macerated with 200 mL of MeOH for 8 h at room temperature with magnetic stirrer and the extracts were filtered. Under the same conditions, this procedure was repeated twice with 150 mL of MeOH. The collected extracts were dried under vacuum using rotavapor at 40 °C. The dried extracts were dispersed in water and lyophilized.

2- *Preparation of water extract*: 5 g of root, herb and fruits of each species were powdered and boiled with 100 mL distilled water for 30 min. The water extracts were filtered when hot and then the resultant extracts were lyophilized.

Table 1. Locations of the studied *Prangos* species.

Species	Part	Location	Herbarium no.
P. ferulacea	Herb, Radix	C4 Konya: Hadim- Bozkır road, 1 km to Korulan, Roadside, Rocky places, 1450 m, 11-06-2005.	AEF 23643
	Fructus	C4 Konya: Bozkır- Hadim road, 20 km to Hadim, near Didemli, Roadside, 1340 m, 24-06-2006.	AEF 23803
P. uechtritzii	Herb, Radix	C4 Konya: Taşkent, Over Mihrap park, Near the stairs, Against Pirler-Kontu hotel, 1350 m, 11-06-2005.	AEF 23644
	Fructus	C4 Konya: Hadim- Taşkent road, Exit of Hadim, roadsides, Over the rocks, 1300 m, 25-06-2005.	AEF 23646
P. heyniea	Herb, Radix	C4 Konya: Hadim-Korulan road, 14 km to Hadim, roadside, Rocky slopes, 1580 m, 25-06-2005.	AEF 23647
	Fructus	C4 Konya: Korulan-Hadim road, 12 km to Hadim, roadsides, Rocky slopes, 1570 m, 24-06-2006.	AEF 23804
P. meliocarpoides var. *meliocarpoides*	Herb, Radix	C4 Karaman: Karaman to Mut, 6-11 km, roadsides, 1250 m 24-06-2006.	AEF 23805
	Fructus	C4 Karaman: Karaman to Mut, 9-11 km, near the *Pinus* trees, 1320 m 02-07-2005.	AEF 23645

Determination of total phenolic compounds in the extracts

Generally, measurement of color occurred by reaction between Folin-Ciocalteu's phenol reagent (22,23), and this method is a preferred method for the determination of the phenolic compounds present in plants, because the majority of plant antioxidants are polyphenols (22-25).

Total contents of the phenolic compounds in the extracts were determined by Folin-Ciocalteu's method (23) as gallic acid equivalents (GAE) (25). Then 250 µL of Folin-Ciocalteu's phenol reagent was mixed with 50 µL of the samples, and 500 µL of 20% water solution of Na_2CO_3 was added to the mixture. Mixtures were vortexed and completed with water to 5 mL. As control, reagent without adding extract was used. After incubation of the samples at room temperature for 30 min, their absorbance was measured at 765 nm. The calibration curve created by using fresh prepared gallic acid solutions was used as a base in calculations of total phenolic compound contents in the extracts. Experiments were repeated 3 times for every extract and the total phenolics were given in average values as GAE (mg gallic acid/g extract) (5).

For the calibration curve, 10 mg of gallic acid was dissolved in 10 mL of MeOH using an ultrasonic bath (stock solution). Different dilutions of stock solution were prepared and were determined by Folin-Ciocalteu's method (23,24). Experiments were repeated 3 times for every dilution and a calibration curve was created.

DPPH test for antioxidant activity

DPPH assay was used as a rapid thin layer chromatography (TLC) screening method to evaluate the antioxidant activity of the freeze-dried extracts of *Prangos* species due to free radical scavenging. $DPPH^{\bullet}$ is a purple-colored stable free radical, which on reduction gives yellow-colored diphenyl picryl hydrazine. When it is sprayed onto a TLC plate, any antioxidant compound is seen as a yellow zone on a purple background. Using Wiretrol II micropipettes, 2 µL of 1 mg/mL aqueous and methanolic solutions prepared from the lyophilized extracts was applied

to the silica gel TLC plates (Merck, Darmstadt, Germany), which were sprayed with 0.2% DPPH solution in MeOH, left at 20 °C, and examined at 30 min after spraying (4).

TBA test for antioxidant activity using liposomes

The in vitro antioxidant activity tests were carried out by lipid peroxidation of liposomes, where TBA was used to assess the efficacy of the extracts to protect liposomes from lipid peroxidation. It can be measured and quantified spectrophotometrically, and the intensity of color is a measurement of MDA (malonyldialdehyde) concentration. The absorbances of the upper layers, which contain the chromogen, were determined by a Shimadzu UV-1601 UV/VIS spectrophotometer at 532 nm. The incorporation of any antioxidant compound in the mixture will lead to a reduction in the extent of peroxidation. The calculation of percentage inhibitions of lipid peroxidation was assessed by comparing the absorbance of the full reaction mixture with that of the extract test reaction mixtures where the substance to be assessed was included. The absorbance readings of the extract alone and of the liposomes alone were also taken into account as follows:

$$\% \text{ Inhibition} = 100 \times (FRM - B) - (ET - B - EA) / (FRM - B),$$

where FRM is the absorbance of the full reaction mixture (liposomes and iron source plus solvent without the test substance), B is the absorbance of the blank mixture (liposomes only), ET is the absorbance of the extract test mixture (full reaction mixture plus test substance), and EA is the absorbance due to the extract alone. The half-maximal inhibitory concentrations (IC_{50}) of the extracts were calculated by linear regression analysis (4,26). Concentrations of 1, 0.5, 0.25, 0.125, 0.0625, and 0.031 mg/mL of water and MeOH extracts were prepared to use in TBA. Propyl gallate was used as a reference compound in the 7 different concentrations (1, 0.20, 0.04, 0.008, 0.0016, 0.00032, and 0.000064 mg/mL).

Results and discussion

The results of total phenolic contents obtained for *Prangos* species extracts are given in Table 2.

The results of the qualitative DPPH test demonstrated that the water and MeOH extracts of the *Prangos* species display low antioxidant activities. However, in the DPPH• test, yellow zones on a purple background were prominent for the MeOH

Table 2. Total phenolic contents of the MeOH and water extracts of *Prangos* species.

Species	Total phenolic contents mg/g ± SS	
	MeOH extracts	Water extracts
P. ferulacea root	96.67 ± 1.84	49.93 ± 1.04
P. ferulacea herb	119.28 ± 5.61	89.86 ± 2.49
P. ferulacea fruit	140.29 ± 1.73	97.29 ± 2.67
P. uechtritzii root	120.73 ± 2.64	75.71 ± 1.94
P. uechtritzii herb	101.79 ± 5.44	56.39 ± 0.65
P. uechtritzii fruit	128.23 ± 2.17	79.92 ± 5.11
P. heyniae root	95.99 ± 2.78	46.67 ± 2.94
P. heyniae herb	79.80 ± 2.13	65.61 ± 3.70
P. heyniae fruit	127.33 ± 5.10	96.11 ± 2.69
P. meliocarpoides var. *meliocarpoides* root	77.94 ± 0.23	37.53 ± 4.11
P. meliocarpoides var. *meliocarpoides* herb	100.33 ± 3.03	69.38 ± 3.25
P. meliocarpoides var. *meliocarpoides* fruit	101.48 ± 4.35	63.24 ± 2.64

MeOH: Methanol

extracts of the fruits of *P. uechtritzii*, *P. heyniae*, and *P. ferulacea*. The lowest inhibition zones were shown on the whole MeOH extracts of *P. meliocarpoides* var. *meliocarpoides*. The water extracts of fruits of the *P. ferulacea* and *P. uechtritzii* gave faint yellow zones when compared with their roots and herbs. The highest DPPH$^{\bullet}$ scavenging effects, expressed as yellow zones of the root water extracts, in decreasing order were determined as *P. heyniae* > *P. uechtritzii* > *P. ferulacea* > *P. meliocarpoides* var. *meliocarpoides* (Figure).

The antioxidant activities of the *Prangos* species on liposomes obtained from the TBA test are given in Table 3. When the obtained data were evaluated according to the antioxidant activity of propyl gallate (IC_{50}: 0.18 µg/mL), which was used as positive control in this study, the MeOH extracts of the fruits of *P. heyniae*, *P. ferulacea*, and *P. uechtritzii* were observed to have medium activity (IC_{50}: 20.96 µg/mL, IC_{50}: 47.85 µg/mL, and IC_{50}: 49.89 µg/mL respectively), while the MeOH and water extracts of *P. ferulacea* herb, the water extracts of *P. meliocarpoides* var. *meliocarpoides* herb and *P. uechtritzii* fruit, and the MeOH extracts of *P. meliocarpoides* var. *meliocarpoides* fruit and of *P. heyniae* root showed weak activity. Using the TBA method, it was observed that the other extracts showed no activity.

In the present study, we evaluated the total phenolic contents of the water and MeOH extracts of 4 *Prangos* species collected from Konya province. We also demonstrated the free radical scavenger activities and detected the liposome lipid peroxidation of these extracts using the TBA assay.

According to the results obtained from the determination of total phenolic contents, it was generally found that the MeOH extracts contained more phenolic contents than the water extracts. The highest total phenolic content was determined in the MeOH extracts of the fruits, especially in *P. ferulacea, P. uechtritzii*, and *P. heyniae* (140.29 µg/

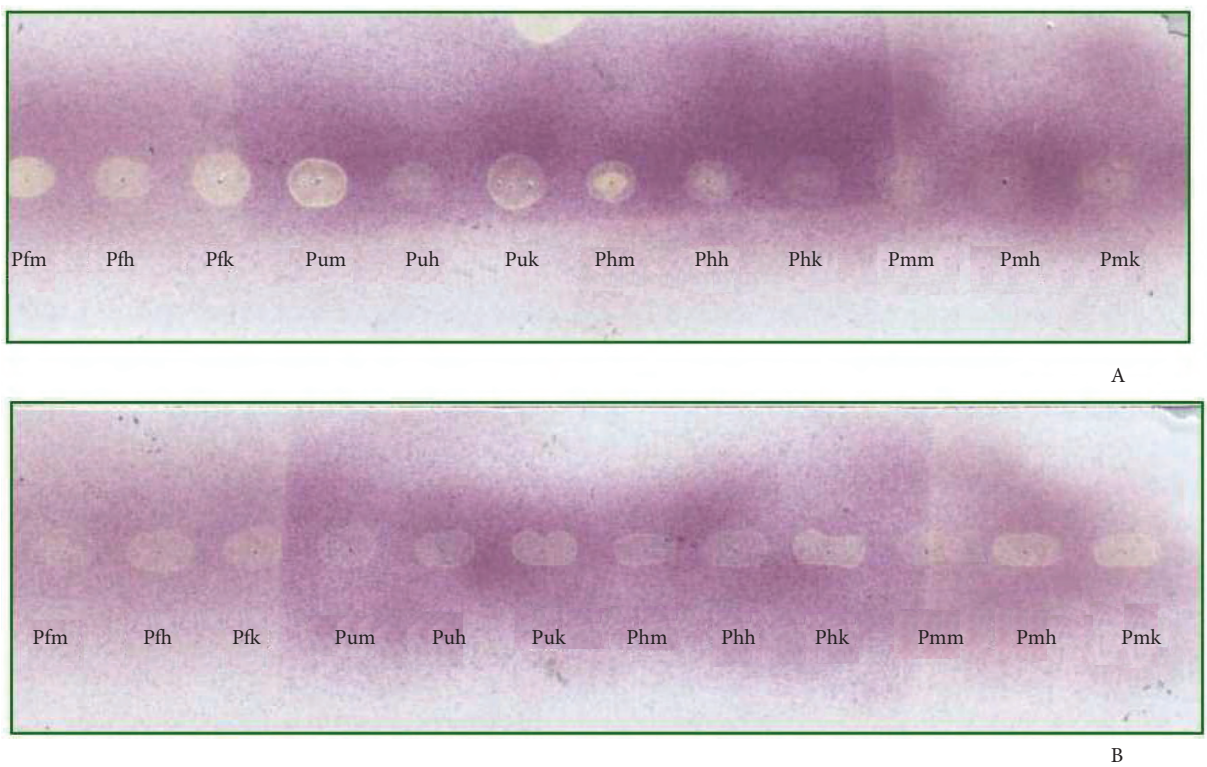

Figure. Antioxidant activity by qualitative DPPH test on TLC of *Prangos* species. A- Methanolic extracts B- Water extracts.
Pfm: *P. ferulacea* fruit; Pfh: *P. ferulacea* herb; Pfk: *P. uechtritzii* root; Pum: *P. ferulacea* fruit; Puh: *P. uechtritzii* herb; Puk: *P. uechtritzii* root; Phm: *P. heyniae* fruit; Puh: *P. heyniae* herb; Phk: *P. heyniae* root; Pmm: *P. meliocarpoides* var. *meliocarpoides* fruit; Pmh: *P. meliocarpoides* var. *meliocarpoides* herb; Pmk: *P. meliocarpoides* var. *meliocarpoides* root.

Table 3. Antioxidant activities of the MeOH and water extracts of *Prangos* species in the TBA test.

Species	IC_{50} value (μg/mL) ± SS	
	MeOH extracts	Water extracts
P. ferulacea root	≥250 ± 1.99	≥250 ± 9.18
P. ferulacea herb	173.69 ± 1.55	137.61 ± 3.12
P. ferulacea fruit	47.85 ± 2.81	≥250 ± 2.72
P. uechtritzii root	≥250 ± 1.29	≥250 ± 5.87
P. uechtritzii herb	101.25 ± 6.43	≥250 ± 4.65
P. uechtritzii fruit	49.89 ± 0.72	125.17 ± 2.81
P. heyniae root	193.86 ± 1.46	≥250 ± 6.23
P. heyniae herb	≥250 ± 1.46	≥250 ± 2.81
P. heyniae fruit	20.96 ± 1.99	≥250 ± 4.14
P. meliocarpoides var. *meliocarpoides* root	≥250 ± 2.89	≥250 ± 5.25
P. meliocarpoides var. *meliocarpoides* herb	≥250 ± 1.42	234.32 ± 5.05
P. meliocarpoides var. *meliocarpoides* fruit	233.32 ± 1.65	≥250 ± 3.01
Propyl gallate	0.18 ± 0.03	

MeOH: Methanol. TBA: Thiobarbituric acid. IC: Inhibitory concentration

mL, 128.23 μg/mL, and 127.33 μg/mL, respectively). Other extracts generally possessed low total phenolic contents.

Results of the DPPH• test showed that the MeOH extracts of *Prangos* species are more active than the water extracts. Among the MeOH extracts, the fruit extracts (except for the extract of the fruit of *P. meliocarpoides* var. *meliocarpoides*) demonstrated prominent activity. While the water extracts of 4 *Prangos* species have shown moderate activity in this test, the root MeOH extracts (except the root extract of *P. ferulacea*) demonstrated the lowest activity.

The most significant activity in the TBA test was obtained from the MeOH extracts of the fruits. The highest activity of the MeOH extracts of the fruits was observed in the extract of the fruit of *P. heyniae*. Furthermore, it was observed that the water extract of the fruit of *P. uechtritzii* had weak activity. Except for the MeOH extract of *P. heyniae,* the water and MeOH extracts of the roots did not show any activity. These results are in accordance with the data obtained from the total phenolic contents, the free radical scavenging activities, and peroxidation inhibition of the root of 4 *Prangos* species. According to the results obtained from previous studies, determination of antioxidant activity of the MeOH extract of *P. ferulacea* was recorded (2,23). These results are in accordance with the results obtained from the tests carried out on the MeOH extracts of *P. ferulacea* herb in our study. In a previous study by Mavi et al., use of a positive control was not recorded and the investigated plant extracts were compared with each other (23). In the present study, the amount of phenolic compound and lipid peroxidation inhibition (IC_{50}: 201) have been demonstrated in the 5% MeOH extract of the stem of *P. ferulacea*. The phenolic compound and peroxidation inhibition of this plant has been also found to be low. In the screening study by Çoruh et al., α-tocopherol was used as positive control (2). While IC_{50} value of lipid peroxidation of the MeOH extract of *P. ferulacea* herb was found to be 152 and the total phenolic content 65.1 in the study carried out by Çoruh et al. (2), these values were determined

as 119.28 and IC_{50}:173, respectively, in the present study. Differences in total phenolic content in plants are possible, in view of the differences in collection sites and timing of collections.

This investigation is the first report on the comparative analysis of total phenol and antioxidant activity of the MeOH and water extracts of *P. heyniae*, *P. meliocarpoides* var. *meliocarpoides*, and *P. uechtritzii*. Quantitative differences in the total phenol profiles between MeOH and water extracts of 4 *Prangos* species have been shown. There is correlation between total phenol and antioxidant activity. Phenolic compounds inhibited MDA concentration during lipid peroxidation; thus, they exhibited antioxidant activity. MeOH extracts contain both the nonpolar and polar compounds (aglycones and glycosides) in the plant. Therefore, results of the present showed that MeOH extracts of *P. heyniae*, *P. ferulacea*, and *P. uechtritzii* have higher activity than the water extracts in both the DPPH test and TBA method.

Previous studies have shown that different types of chemical constituents were found in the various parts of *Prangos* species. These were mainly coumarins (13,18-20) and essential oils (14,16,17); but not many studies have been reported on their flavonoids (21). However, the active antioxidant components of *Prangos* species have not been investigated so far. Therefore, the flavonoid content cannot be only responsible for the antioxidant activity of this genus. The components responsible for the antioxidant are currently unclear; therefore, the bioassay-guided fractionation procedure to isolate and characterize the active constituents is still needed to be determined.

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