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Original Article

COMPARATIVE EVALUATION OF TOTAL PHENOLIC/CAROTENOID CONTENTS, CHLOROGENIC ACID/RUTIN PROFILES, AND ANTIOXIDANT PROPERTIES OF TWO *PRANGOS* SPECIES (*P. UECHTRITZII* AND *P. PABULARIA*)

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ABSTRACT

Objective: The aim of the study was to investigate the antioxidant activities, chlorogenic acid/rutin profiles, and bioactive compounds' contents of the various extracts from *Prangos uechtritzii* Boiss. & Hausskn. and *Prangos pabularia* Lindl.

Methods: The antioxidant capacities of the extracts were evaluated by various methods, including the plasma lipid peroxidation inhibitory, *β*-carotene/linoleic acid bleaching, free radical scavenging activity, and metal chelating activity assays. Chlorogenic acid and rutin contents of the extracts were determined qualitatively and quantitatively by high-performance liquid chromatography (HPLC). Total phenolic, *β*-carotene, and lycopene contents of the extracts were also determined.

Results: In the assays, the methanol and the water extracts showed higher antioxidant activities than the acetone and ethyl acetate extracts. According to HPLC analysis, the richest extracts in terms of rutin and chlorogenic acid were determined as *P. pabularia* methanol extract (12.61±0.11 µg/mg) and *P. uechtritzii* methanol extract (4.76±0.12 µg/mg), respectively.

Conclusion: It could be suggested that these *Prangos* species, especially the water extract of *P. uechtritzii* may be used a potential source of natural antioxidants for food and pharmacy industries.

Keywords: *Prangos pabularia*, *Prangos uechtritzii*, Chlorogenic acid, Rutin, Antioxidant activity.

INTRODUCTION

Prangos Lindl. is a perennial genus of the Apiaceae represented by 30 species in the world. Turkey is an important center for the genus *Prangos.* It is represented by 17 taxa in Turkey, including 9 endemic species [1-6]. *Prangos uechtritzii* Boiss. & Hausskn. is an endemic species of this genus in Turkey. *Prangos pabularia Lindl.* is the widespread species of the genus that is distributed from East Europe to central and Eastern Asia [1].

The aerial parts of some *Prangos* species have been used as an aromatic in cheese and milk products in the eastern part of Turkey [7]. Roots and fruits of *Prangos* have medicinal value. This genus has been considered as an aphrodisiac [8] and has also been used in the treatment of leukoplakia, digestive disorders, scars, and bleeding [9].

Natural antioxidants from plant materials have recently drawn the substantial interest of researchers. Flavonoids and other polyphenols that classified major antioxidant phytochemicals from plants have been reported to inhibit the propagation of free radical reactions and to protect the human body from diseases [10].

Previous phytochemicals tests have shown that *Prangos* genus is a rich source of coumarin derivatives [11, 12, 9, 13], but few studies have been reported on their phenolic acids and flavonoids [14, 15, 16]. In this study, antioxidant effects, total phenolic and carotenoid contents, and two phenolic compounds (rutin/chlorogenic acid) of *P. uechtritzii* and *P. pabularia* were investigated.

MATERIALS AND METHODS

Chemicals

Anhydrous sodium carbonate, Folin-Ciocalteu's phenol reagent, iron (II) sulfate heptahydrate ($FeSO_4{}^*7H_2O$), acetone (analytical reagent and HPLC gradient grade), ethyl acetate (analytical reagent and HPLC gradient grade), 1-butanol, chloroform, hydrogen peroxide (H_2O_2), glacial acetic acid, *n*-hexane, acetonitrile (HPLC gradient grade), methanol (analytical reagent and HPLC gradient grade), and *o*-phosphoric acid (analytical grade) were purchased from Merck (Darmstadt, Germany). Ethylenediaminetetraacetic acid (EDTA), *β*-carotene, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (ferrozine), iron (II) chloride ($FeCl_2$), gallic acid, butylated hydroxyanisole (BHA), 2,6-di-*tert*-butyl-4-methylphenol (BHT), trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), α-tocopherol, linoleic acid, Tween 40, rutin, and chlorogenic acid were purchased from Sigma-Aldrich GmbH. (Steinheim, Germany). All other chemicals were analytical grade and obtained from either Sigma or Merck.

Plant materials

Prangos uechtritzii (voucher specimen numbered HD 9885) fruit materials were collected from Kayseri (Turkey) and *P. pabularia* (voucher specimen numbered HD 9882) fruit materials were collected from Adıyaman (Turkey) in July 2008. The identifications of plant materials were confirmed by a taxonomist in the Department of Biology, Gazi University, Ankara, Turkey.

Preparation of the extracts

Collected plant materials were dried in the shade and ground in a grinder with a 2 mm in diameter mesh. Thirty grams of the dried and powdered plant materials were separately extracted with solvents by using Soxhlet apparatus for 6 h. The extracts were filtered and concentrated under vacuum at 50 °C (for water at 95 °C) by using a rotary evaporator (Heidolph, Laborota 4000, Schwabach, Germany) and stored in the dark at 4 °C until used within a maximum period of one week. Methanol (HPLC grade), acetone, ethyl acetate, and water (ultra pure) were used as solvents.

Plasma lipid peroxidation inhibitory assay

The plasma lipid peroxidation was analyzed by the method developed by Rodriquez *et al.* [17], with some modifications [18].

400 µl of plasma (Blood Center, Gazi University, Turkey), 100 µl of $FeSO_4$ solution (0.5 mM), 100 µl of H_2O_2 (0.5 mM), and 200 µl of the extracts (2 mg/ml) were mixed and incubated at 37 °C. After 12 h of incubation, the reaction solution was mixed with 375 µl of TCA (4 %) and 75 µl of BHT (0.5 mM) and held in an ice bath for 5 min. The upper phase was obtained by centrifugation at 5000 *g* for 15 min. TBA (200 µl; 0.6 %) was then added. This mixture was incubated at 95 °C for 30 min and allowed to cool. The mixture was mixed with the same volume of 1-butanol. The absorbance was then measured at 532 nm.

Inhibition of linoleic acid oxidation (*β*-Carotene bleaching method)

The test was carried out following the spectrophotometric method of Miller [19]based on the ability to decrease the oxidative bleaching on *β*-carotene in a *β*-carotene/linoleic acid emulsion. A stock solution of *β*-carotene/linoleic acid mixture was prepared as follows: 0.5 mg *β*-carotene was dissolved in 1 ml of chloroform (HPLC grade); 25 µl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated by using a vacuum evaporator. Then, 100 ml of distilled water was added with oxygen (30 min at a flow rate of 100 ml/min) vigorous shaking. Aliquots (2.5 ml) of this reaction mixture were dispensed to test tubes, and 350 µl of the extract prepared at 2 mg/ml concentration was added, and the emulsion system was incubated for up to 48 h at room temperature. After this incubation period, absorbances of the mixtures were measured at 490 nm.

DPPH radical scavenging assay

Free radical scavenging activity of the samples was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to a method of Blois [20]. The samples were added to 0.004 % methanol solution of DPPH. The mixture was left to stand at room temperature for 30 min in the dark. The absorbance of the samples was determined using a spectrophotometer (Hitachi, U-1800, Tokyo, Japan) at 517 nm. Scavenging of DPPH radical was calculated according to the formula:

$$\text{Scavenging \%} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

BHA, BHT and α-tocopherol were used as positive controls.

Metal chelating activity on ferrous ions (Fe^{2+})

Metal chelating activity was determined according to the method of Decker and Welch, [21] with some modifications [22]. Briefly, 0.5 ml of the extracts was mixed with 0.05 ml of 2 mM $FeCl_2$ and 0.1 ml of 5 mM ferrozine. Total volume was diluted with the solvent. Then, the mixture was left standing at room temperature for ten minutes. The absorbance of the solution was measured spectrophotometrically at 562 nm. EDTA was used for comparison.

Determinations of bioactive component contents

Total phenolic contents of the extracts were determined using the modified Folin-Ciocalteu method as described by Singleton and Rossi [23]. The extract solutions were mixed with 0.2 ml of 50 % Folin-Ciocalteu reagent and allowed to react for 3 min and 1 ml of aqueous solution of 2 % Na_2CO_3 was added. At the end of incubation for 45 min at room temperature, the absorbance of each mixture was measured at 760 nm. Total phenol contents were expressed as µg gallic acid equivalents per mg of the extracts.

β-Carotene and lycopene were determined according to the method of Nagata and Yamashita [24]. The dried extract (100 mg) was vigorously shaken with 10 ml of acetone–hexane mixture (4:6) and filtered through disposable filters (0.45 µm, Millipore). The absorbance of the filtrate was measured at 453, 505, 645, and 663 nm. Contents of *β*-carotene and lycopene were calculated according to the following equations: lycopene (mg/100 ml) =-0.0458 A_{663}+0.204A_{645}+0.372 A_{505}-0.0806 A_{453}; *β*-carotene (mg/100 ml) = 0.216 A_{663}− 1.22A_{645}-0.304 A_{505}+0.452 A_{453}. The results were expressed as µg of carotenoid/mg of the extract.

Quantification of rutin and chlorogenic acid in the extracts by HPLC

The HPLC system (Agilent Technologies 1200 series) was equipped with a binary pump, DAD detector, and an injector with a loop size of 20 µl. The peak area was calculated with a Winchrom integrator. The reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (150 mm × 4.6 mm i.d., particle size 5 µm, Agilent Zorbax Eclipse XDB-C18) at 25 °C. Running conditions included: injection volume, 10 µl; mobile phase, acetonitrile/40 mM formic acid; flow rate, 1 ml/· min; and UV detection at 254 nm and 330 nm. Samples were filtered through an ultra membrane filter (pore size 0.45 µm; Millipore) prior to injection in the sample loop. Rutin and chlorogenic acid in the samples were identified by comparing chromatographic peaks with the retention time (Rt) of individual standards and further confirmed by co-injection with isolated standards. Standard solutions containing rutin or chlorogenic acid were prepared in ethanol (70 %). Five concentrations of rutin (4.99-998 g/ml) and chlorogenic acid (5.2-1040 g/ml) were subjected to regression analysis to calculate calibration equation and correlation. The amount of each phenolic compound is expressed as µg per mg of the extracts.

Statistical analysis

All experiments were carried out in triplicate. The results were expressed as means±standard deviations (SD). Statistical analyses were performed using the SPSS 11.5 (SPSS, Chicago, IL). Differences among means were done by analysis of variance (ANOVA) and averages were compared using Tukey test. Pearson's correlation analysis was used for comparisons of total phenol contents and the antioxidant activity of the extracts. The level of statistical significance was taken at $p<0.05$.

RESULTS AND DISCUSSION

Plasma lipid peroxidation inhibitory effects of the extracts

Plasma concentration of thiobarbituric acid reactive substances (TBARS) is an index of lipid peroxidation and oxidative stress. The polyunsaturated fatty acids located in cells and in blood are highly prone to attack, which results in the generation of lipid peroxides[25]. *P. uechtritzii* water extract exhibited the highest inhibition against TBARS formation (69.4±1.1 %). The TBARS formation inhibitory effects of the water and the methanol extracts were higher than those of the other extracts (table 1).

Inhibition of linoleic acid oxidation by the extracts

β-Carotene bleaching method is based on the loss of the yellow colour of *β*-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. The rate of *β*-carotene bleaching can be slowed down in the presence of antioxidants [26]. Inhibition of linoleic acid oxidation is an important issue in food processing and preservation. The relative antioxidative activities (RAA) of the extracts were calculated from the equation, RAA = Absorbance of the sample (extract)/Absorbance of the control (α-tocopherol). The calculated RAA of the extracts are given in table 1. The extracts exhibited in the range of 55.9 to 96.7 % inhibition against linoleic acid oxidation. *P. uechtritzii* fruit water extract showed the highest inhibition against linoleic acid oxidation (96.7±0.3 % at 2 mg/ml).

DPPH radical scavenging activity of the extracts

The IC_{50} values for DPPH scavenging activities of the extracts and positive controls BHA, *α*-tocopherol and BHT are compared and shown in table 1. Lower IC_{50} values indicate higher free radical scavenging activity. DPPH scavenging activity of the extracts decreased in the order: BHA>α-tocopherol>BHT>*P. uechtritzii* water extract (PUWE)>*P. uechtritzii* methanol extract (PUME)>*P. pabularia* water extract (PPWE)>*P. pabularia* methanol extract (PPME)>*P. uechtritzii* acetone extract (PUAE)>*P. pabularia* acetone extract (PPAE)>*P. pabularia* ethyl acetate extract (PPEE)>*P. uechtritzii* ethyl acetate extract (PUEE). The highest DPPH radical scavenging effect was detected in PUWE (IC_{50} = 0.066±0.001 mg/ml) followed by PUME (IC_{50} = 0.071±0.001 mg/ml). Acetone and ethyl acetate extracts of these species exhibited weak antioxidant activity (fig. 1).

Antioxidant activity studies were carried out on different *Prangos* species [7, 27, 28]. Ahmed *et al.* [28] reported DPPH radical scavenging activities of *P. uechtritzii* fruit methanol and water extracts using qualitative DPPH test, and their results demonstrated that the extracts of *P. uechtritzii* display low antioxidant activities. In

our study, we used quantitative DPPH test, and the results showed *P. uechtritzii* fruit water extract exhibited moderate scavenging activity on DPPH radicals with an IC_{50} value of 0.066 mg/ml.

Metal chelating activity of the extracts

Significant differences in chelating activities were observed in the extracts (table 1). The chelating activities of the methanol extracts were higher than those of the water extracts. *P. uechtritzii* methanol extract showed the highest ferrous iron chelating ability (IC_{50} = 0.71±0.01 mg/ml). On the other hand, the acetone and ethyl acetate extracts exhibited weak chelating activity. Synthetic chelating agent EDTA had the potent chelating ability with a 93.7 % at 2 mg/ml and *P. uechtritzii* methanol extract (92.3 %) exhibited similar chelating activity at the same concentration.

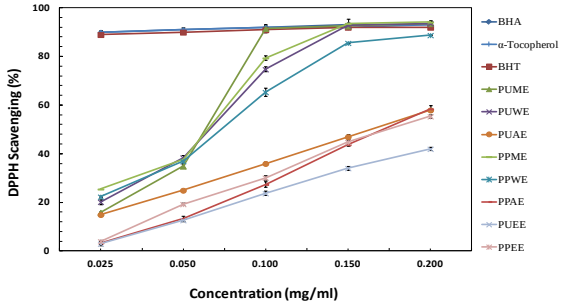

Fig. 1: DPPH radical scavenging activity of the extracts and the positive controls

Table 1: Antioxidant activities of the fruit extracts from *Prangos* species*

Material	DPPH IC_{50} (mg/ml)	Metal chelating IC_{50} (mg/ml)	Plasma lipid peroxidation (%)	β-carotene bleaching (%)
PUWE	0.066±0.001^{a}	3.37±0.03^{d}	69.4±1.1^{a}	96.7±0.3^{a}
PUME	0.071±0.001^{a,b}	0.71±0.01^{a}	67.9±0.2^{b}	93.1±0.2^{b}
PUAE	0.164±0.003^{c}	>5	40.1±0.2^{d}	77.0±0.3^{e}
PUEE	0.224±0.005^{f}	>5	29.1±0.4^{g}	55.9±0.3^{g}
PPWE	0.073±0.002^{a,b}	2.98±0.03^{c}	67.6±0.2^{b}	90.6±0.1^{c}
PPME	0.076±0.002^{b}	1.70±0.02^{b}	66.0±0.2^{c}	88.2±0.2^{d}
PPAE	0.172±0.001^{d}	>5	35.5±0.1^{e}	70.0±0.1^{f}
PPEE	0.177±0.001^{e}	>5	31.5±0.1^{f}	66.5±0.2^{h}
α-Tocopherol	0.011±0.002^{g}	Ns	Ns	100.0±0.0^{i}
BHA	0.003±0.000^{h}	Ns	Ns	98.8±0.1^{j}
BHT	0.023±0.004^{i}	Ns	Ns	95.6±0.1^{a}

* Values represent averages±standard deviations for triplicate experiments. Values in the same column with different superscript lowercase letters are significantly (p<0.05) different. Ns: Not studied.

Determination of bioactive compounds' contents

The contents of total phenolic compounds in the extracts were found in a range of 23.90 to 57.71 µg/mg (table 2). *P. uechtritzii* methanol extract had the highest total phenolic content (57.71±0.11µg/mg). This extract exhibited the highest chelating activity (IC_{50} = 0.71±0.01 mg/ml) and remarkable DPPH scavenging activity (IC_{50} = 0.071±0.001 mg/ml). A significant (p<0.01) correlation was observed between total phenolic content and the antioxidant activity of the extracts. It is indicating those phenolics are primarily responsible compounds for these activities. Total phenolic contents of *P. uechtritzii* fruit methanol and water extracts were found as 128.23±2.17 mg/g and 79.92±5.11 mg/g, respectively, by Ahmed *et al.* [28]. In our study, these values were determined as 57.71±0.11µg/mg and 54.14±0.16µg/mg, respectively. These differences in the results of the studies could be due to differences in the plant collection time and site, and the used method (e. g., incubation time).

β-carotene and lycopene were only found in vestigial amounts in the extracts (table 2). *β*-carotene and lycopene were not found in the water extracts due to their fat-soluble nature. The highest *β*-carotene content was determined in the acetone extracts of *P. uechtritzii* and *P. pabularia* (0.105 µg/mg). On the other hand, the highest lycopene content was determined in *P. pabularia* acetone extract (0.026 µg/mg). Our results indicated that the water and methanol extracts had higher total phenol contents than the acetone extracts, but the acetone extracts had higher carotenoid contents than the other extracts.

Table 2: The yields and the antioxidant compounds contents of the fruit extracts*

Material	Yield (w/w %)	Total phenolic (µg/mg)	β-Carotene (µg/mg)	Lycopene (µg/mg)
PUWE	23.2	54.14±0.16^{b}	ND	ND
PUME	12.9	57.71±0.11^{a}	0.038±0.002^{c}	0.014±0.001^{c}
PUAE	4.3	34.73±0.24^{d}	0.105±0.004^{a}	0.020±0.002^{b}
PUEE	4.9	23.90±0.34^{g}	0.104±0.002^{a}	0.014±0.001^{c}
PPWE	29.3	53.67±0.24^{b}	ND	ND
PPME	21.7	55.41±0.79^{b}	0.076±0.001^{b}	0.020±0.002^{b}
PPAE	9.9	31.53±0.11^{c}	0.105±0.000^{a}	0.026±0.003^{a}
PPEE	11.5	28.98±0.33^{f}	0.025±0.001^{d}	0.012±0.000^{c,d}

*Values represent averages±standard deviations for triplicate experiments. Values in the same column with different superscript lowercase letters are significantly (p<0.05) different. ND: Not determined.

Quantification of chlorogenic acid and rutin in the extracts by HPLC

HPLC chromatographic fingerprint profile of PPME is shown in fig. 2. Phytochemicals tests have shown that *Prangos* species are a rich source of coumarin derivatives and terpenoids [29]. In this study, chlorogenic acid and rutin contents of these two *Prangos* species were characterized. The quantitative data of chlorogenic acid and rutin in *Prangos* species were calculated using their respective concentration vs. peak area calibration curves.

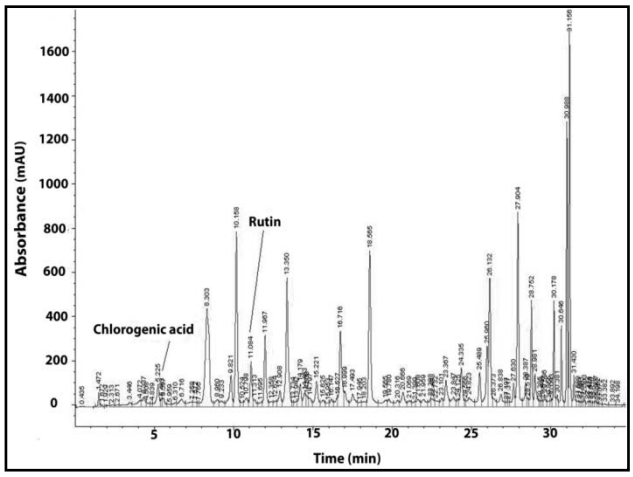

Fig. 2: High-performance liquid chromatogram of *Prangos pabularia* fruit methanol extract

According to the calibration curve, the richest extracts in terms of chlorogenic acid and rutin were determined as *P. uechtritzii* methanol extract (4.76±0.12 µg/mg extract) and *P. pabularia* methanol extract (12.61±0.11 µg/mg extract), respectively. All the extracts showed the presence of chlorogenic acid. Rutin was not detected in *P. uechtritzii* methanol extract. The methanol extracts had higher chlorogenic acid content than the water extracts (table 3).

Dietary polyphenols are thought to be beneficial for human health by exerting various biological effects such as free-radical scavenging, metal chelation, modulation of enzymatic activity, and alteration of signal transduction pathways [30]. It was widely reported that chlorogenic acid and related compounds are well known to be antioxidants [31, 32]. Chlorogenic acid may be an essential ingredient in the antioxidant activity of these *Prangos* species. In our previous study, we reported the contribution of the chlorogenic acid to the antioxidant capacities of two endemic *Prangos* species (*P. heyniae* and *P. denticulata*) [33]. Mullen *et al.* [31] reported that the results showed a statistically significant correlation between the total chlorogenic acid content of the coffee fruit samples (extracts and powders) and their ability to scavenge peroxyl radicals, hydroxyl radicals, peroxynitrite, and singlet oxygen. On the other hand, the rutin content seems to be irrelevant to the antioxidant activity of the species. *P. pabularia* have higher rutin content than *P. uechtritzii*, but exhibited lower DPPH scavenging and plasma inhibitory activity than the other water and methanol extracts. Zielinska *et al.* [34] showed a low contribution of rutin to the antioxidant activity of buckwheat samples.

Table 3: Chlorogenic acid and rutin contents of the fruit extracts*

Material	Chlorogenic acid (µg/mg)	Rutin (µg/mg)
PUWE	3.45±0.12^{c}	0.57±0.09^{c}
PUME	4.76±0.12^{a}	ND
PPWE	2.84±0.08^{d}	8.22±0.07^{b}
PPME	3.93±0.13^{b}	12.61±0.11^{a}

*Values represent averages±standard deviations for triplicate experiments. Values in the same column with different superscript lowercase letters are significantly ($p<0.05$) different. ND: Not determined.

CONCLUSION

In this study, antioxidant activity, total carotenoid and phenolic contents, and chlorogenic acid/rutin profiles of *P. uechtritzii* and *P. pabularia* were analyzed. The results indicate that the extracts rich in polyphenol have significant antioxidant activity. These results showed that the fruit extracts from two Prangos species, especially the water extract of *P. uechtritzii* could become a useful supplement for food products as a new antioxidant agent.

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CONFLICTS OF INTERESTS

All authors have none to declare.

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