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The Anti-Lipid Peroxidative, Metal Chelating, and Radical Scavenging Properties of the Fruit Extracts From Endemic *Prangos meliocarpoides* Boiss var. *meliocarpoides*

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ABSTRACT

In this study, the anti-lipid peroxidative, metal chelating and radical scavenging properties of the various extracts from fruit parts of *Prangos meliocarpoides* Boiss var. *meliocarpoides* were analyzed. Moreover, chlorogenic acid/rutin profiles and total phenolic/carotenoid contents of the extracts were determined. The water extract exhibited the highest plasma lipid peroxidation inhibitory (45.3 ± 1.1 % at 2 mg/mL) and *β*-carotene bleaching (83.4 ± 0.2 % at 2 mg/mL) effects. According to HPLC analysis, the highest rutin (3.17 ± 0.06 µg/mg) and chlorogenic acid (2.97 ± 0.22 µg/mg) contents were determined in the methanol extract. The results suggest that the active extracts of *Prangos meliocarpoides* var. *meliocarpoides* may serve as a potential source of natural antioxidants for food industry.

Keywords: *Prangos meliocarpoides Boiss var. meliocarpoides, chlorogenic acid, rutin, antioxidant activity, carotenoid*

1. INTRODUCTION

Plants synthesize many compounds with biological activity, as secondary products, which are mainly phenolics serving in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to avoid oxidative damage [1]. The antioxidant activities of phenolics are related to the different mechanisms, such as free-radical scavenging, hydrogen donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxyl [2].

Extracts of plant materials rich in polyphenolics are increasingly of interest to the food industry because they have the capacity to retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [3]. The importance of the antioxidant

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constituents of plants in the maintenance of health and protection from many diseases is also raising interest among scientists, food manufacturers and consumers since the future trend is toward functional foods with specific health effects [4, 5].

The genus *Prangos* Lindl. (Apiaceae) is represented by 17 taxa in Turkey, including 9 endemic species [6-11]. *Prangos* species are known as "Çakşır otu" in Turkey and used to make herbed cheese to give the desired aroma and taste in the eastern part of Turkey [12]. *Prangos* has been used in folk medicine for treatment of leukoplakia, digestive disorders, healing scars and stopping bleeding [13].

The aim of the study was i) to investigate the anti-lipid peroxidative, metal chelating, and radical scavenging properties of *P. meliocarpoides* var. *meliocarpoides* ii) to determine the total phenolic and carotenoid contents of the extracts, and iii) to quantify the chlorogenic acid and rutin contents of the extracts by HPLC.

2. EXPERIMENTAL

2.1. Plant materials

P. meliocarpoides var. *meliocarpoides* fruit materials (voucher specimen numbered as HD 10025) were collected from Konya (Turkey) in July 2009. The identification of plant materials were confirmed by taxonomist in the Department of Biology, Gazi University, Ankara, Turkey.

2.2. Preparation of the extracts

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 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, acetone, ethyl acetate, and water (ultra pure) were used as solvents.

2.3. Anti-lipid peroxidation inhibitory assays

The plasma lipid peroxidation inhibitory effect was analyzed by the method developed by Rodriquez et al. [14], with some modifications [15].

β-Carotene bleaching method test was carried out following the spectrophotometric method of Miller [16] based on the ability to decrease the oxidative bleaching on β-carotene in a β-carotene/linoleic acid emulsion.

2.4. DPPH radical scavenging assay

Free radical scavenging activity of the samples was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to method of Blois [17]. Scavenging of DPPH radical was calculated according to the formula: Scavenging % = $[(A_{control} - A_{sample}) / A_{control}]$ x 100

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

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

Metal chelating activity was determined according to the method of Decker and Welch, [18] with some modifications [19]. 2 mM $FeCl_2$ solution (0.05 mL) was added to the extract solutions (0.5 mL). Then, the reaction was initiated by adding 5 mM ferrozine solution (0.1 mL). The mixture was diluted with 2 ml of methanol and left to stand at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. EDTA was used for comparison.

2.6. Determination of total phenolic contents

Total phenolic contents of the extracts were determined using the modified Folin-Ciocalteu method as described by Singleton and Rossi [20]. Total phenol contents were expressed as µg gallic acid equivalents per mg of the extracts.

2.7. Determination of the carotenoid contents

β-Carotene and lycopene were determined according to the method of Nagata and Yamashita [21]. 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.

2.8. 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. The peak area was calculated with a Winchrom integrator. 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/ 40mM formic acid; flow rate, 1 $mL \cdot min^{-1}$; 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 [22]. The amount of each phenolic compound is expressed as µg per mg of the extracts.

2.9. Statistical analysis

All experiments were done in triplicate, and mean values are presented. 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 phenolic contents and the

antioxidant activity of the extracts. The level of statistical significance was taken at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Anti-lipid peroxidative effect of the extracts

The results of the effects of the extracts on plasma lipid peroxidation are presented in Table 1. Among the extracts, the water extract exhibited the highest plasma lipid peroxidative effect (45.3 ± 1.1 % at 2 mg/mL). The process of lipid peroxidation results in a range of intermediates and end products, including lipid hydroperoxides, aldyehydes, and malondialdehyde (MDA). These aldehydes and lipid hydroperoxides form DNA adducts and may result in extensive single-strand and double-strand breaks [23]. Treatment with the extract for 12 h was observed to significantly reduce plasma MDA level indicating a protective role of the extract against H_2O_2 induced plasma lipid peroxidation.

β-Carotene bleaching assay is based on the loss of the yellow color 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 [24]. Inhibition of linoleic acid oxidation is an important issue in food processing and preservation. The relative antioxidative activities (RAAs) of the extracts were calculated from the equation, RAA = Asample/Aα-tocopherol, where Aα-tocopherol is the absorbance of the control (α-tocopherol) and Asample is the absorbance of the extract. The calculated RAAs of the extracts are given in Table 1. The extracts exhibited in the range of 32.3 ± 0.2% – 83.4 ± 0.2% inhibition against linoleic acid oxidation. The high anti-lipid peroxidation activity of the water extract may be attributed to the presence of high phenolics and flavonoids contents.

3.2. DPPH radical scavenging activity of the extracts

DPPH scavenging activity of the extracts decreased in the order: BHA > α-tocopherol > BHT > PMME > PMWE > PMAE > PMEE (Table 1). The highest DPPH radical scavenging effect was detected in the methanol extract (IC_{50} = 0.088 ± 0.001 mg/mL). Acetone and ethyl acetate extracts of these species exhibited weak radical scavenging activity (Fig. 1.). Antioxidant activity studies had been carried out on different *Prangos* species [12, 25, 26]. Ahmed et al. [26] reported DPPH radical scavenging activities of 4 *Prangos* species collected from Konya province using qualitative DPPH test, and their results indicated that the lowest inhibition zones were shown in *P. meliocarpoides* var. *meliocarpoides* among the whole methanol extracts of studied *Prangos* species.

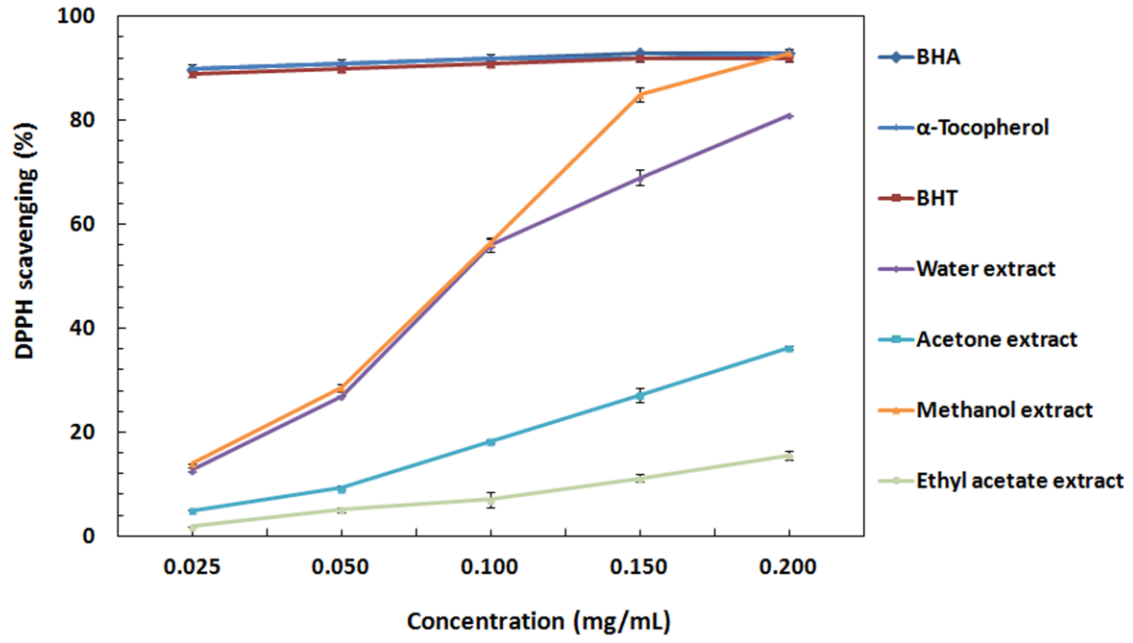

Fig. 1. DPPH radical scavenging activity of the extracts

3.3. Metal chelating activity of the extracts

Iron is an essential mineral for normal physiology, but an excess of it may result in cellular injury. If they undergo Fenton reaction, these reduced metals may form reactive hydroxyl radicals and thereby contribute to oxidative stress [27]. An important mechanism of antioxidant activity is the ability to chelate transition metals. Therefore, it is considered important to evaluate the iron chelating ability of extracts [28]. The methanol

extract had the highest chelating ability (IC_{50} = 0.87 ± 0.04 mg/mL). On the other hand, the acetone and ethyl acetate extracts exhibited low chelating activity (> 5 mg/mL) (Table 2). Synthetic chelating agent EDTA had potent excellent chelating ability with a 93.7 at 2 mg/mL and the methanol extract exhibited similar chelating activity at the same concentration. The radical scavenging and metal chelating abilities of the antioxidants are dependent upon their unique phenolic structures and the number and position of hydroxyl groups [29].

Table 1. Antioxidant activities of the fruit extracts from *Prangos meliocarpoides* var. *meliocarpoides*[1]

Material	DPPH IC_{50} (mg/mL)	Metal chelating IC_{50} (mg/mL)	Plasma lipid peroxidation (%)	β-carotene bleaching (%)
Water extract	0.090 ± 0.002[d]	0.98 ± 0.04[a]	45.3 ± 1.1[a]	83.4 ± 0.2[d]
Methanol extract	0.088 ± 0.001[d]	0.87 ± 0.04[b]	44.2 ± 0.7[a]	80.5 ± 0.3[e]
Acetone extract	0.277 ± 0.003[e]	>5	23.4 ± 0.2[b]	56.3 ± 1.4[f]
Ethyl acetate extract	> 0.5	>5	10.3 ± 0.2[c]	32.3 ± 0.2[g]
α-Tocopherol	0.011 ± 0.001[b]	NS[2]	NS[2]	100.0 ± 0.0[a]
BHA	0.003 ± 0.000[a]	NS[2]	NS[2]	98.8 ± 0.1[b]
BHT	0.023 ± 0.000[c]	NS[2]	NS[2]	95.6 ± 0.1[c]

[1] Values represent averages ± standard deviations for triplicate experiments. Values in the same column with different superscript lower case letters are significantly ($p < 0.05$) different. [2] Not studied.

3.4. Determination of bioactive compounds contents

The contents of total phenolic compounds in the extracts were found in a range of 13.29 ± 0.64 to 48.01 ± 0.40 µg/mg (Table 2). The methanol extract had the highest total phenolic content (48.01 ± 0.40 µg/mg) and exhibited the highest chelating (IC_{50} = 0.87 ± 0.04 mg/mL) and DPPH scavenging activity (IC_{50} = 0.088 ± 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 that phenolics are primarily responsible compounds for these activities. Total phenolic contents of *P. meliocarpoides* var. *meliocarpoides* fruit methanol and water extracts were found as 101.48 ± 4.35 mg/g and 63.24 ± 2.64 mg/g, respectively, by Ahmed et al. [26]. In our study, these values were determined as 48.01 ± 0.40 µg/mg and 43.29 ± 0.66 µg/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 (0.106 ± 0.002 µg/mg) and lycopene (0.025 ± 0.002 µg/mg) contents were determined in the acetone extract. Our results indicated that the water and methanol extracts had higher total phenolic contents than the acetone extract, but the acetone extract had higher carotenoid content than the other extracts. On the other hand, the ethyl acetate extract had low total phenolic and carotenoid contents.

3.5. Quantification of chlorogenic acid and rutin in the extracts by HPLC

Phytochemical tests have shown that *Prangos* species are rich source of coumarin derivatives and terpenoids [30]. The quantitative data of chlorogenic acid and rutin in *Prangos* species were calculated using their respective concentration vs. peak area calibration curves. According to HPLC analysis, the highest rutin (3.17 ± 0.06 µg/mg) and chlorogenic acid (2.97 ± 0.22 µg/mg) contents were determined in the methanol extract. The extracts showed the presence of chlorogenic acid. The methanol extract had higher chlorogenic acid content than the water extract. Rutin was not detected in the water extract (Table 2).

Table 2. The yields and the antioxidant compounds contents of the fruit extracts[1]

Material	Yield (w/w%)	Total phenolic (µg/mg)	β-Carotene (µg/mg)	Lycopene (µg/mg)	Chlorogenic acid (µg/mg)	Rutin (µg/mg)
Water extract	17.5	43.29 ± 0.66[b]	ND[2]	ND[2]	1.16 ± 0.12[b]	ND[2]
Methanol extract	14.1	48.01 ± 0.40[a]	0.031 ± 0.002[b]	0.016 ± 0.002[b]	2.97 ± 0.22[a]	3.17 ± 0.06
Acetone extract	3.4	26.74 ± 0.09[c]	0.106 ± 0.002[a]	0.025 ± 0.002[a]	NS[3]	NS[3]
Ethyl acetate extract	6.6	13.29 ± 0.64[d]	0.021 ± 0.001[c]	0.010 ± 0.001[c]	NS[3]	NS[3]

[1] Values represent averages ± standard deviations for triplicate experiments. Values in the same column with different superscript lower case letters are significantly ($p < 0.05$) different. [2] Not determined. [3] Not studied.

In conclusion, the results of all the assays are in agreement that the methanol and water extracts of *P. meliocarpoides* var. *meliocarpoides* displayed remarkable antioxidant activity. A positive and significant correlation was observed between antioxidant activity and total phenolic content, revealing that phenolic compounds were the effective antioxidant components in these extracts. The results suggest that the active extracts of *Prangos meliocarpoides* var. *meliocarpoides* may serve as a potential source of natural antioxidants for food industry.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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