

## Antioxidant activity of wild edible plants in the Black Sea Region of Turkey

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### RESUMEN

#### Actividad antioxidante de plantas comestibles salvajes en la región del mar Negro de Turquía

La actividad antioxidante de extractos etanólicos al 80%, obtenidos de once plantas salvajes comúnmente consumidas, fue determinada por el método del fosfomolibdeno, poder reductor, actividad quelatante de metales, actividad captadora de aniones superóxidos y actividad captadora de radicales libres, y comparada con compuestos patrones tales como el butil hidroxianisol (BHA), butil hidroxitolueno (BHT) y Trolox. El contenido de fenoles totales, flavonoides y antocianinas en el extracto fue también determinado. *Trachystemon orientalis*, *Vaccinium myrtillus*, *Rumex acetosella* *Polygonum amphibium*, *Beta vulgaris*, and *Similax Excelsa* tienen las capacidades antioxidantes más altas. En general, los resultados muestran que estas plantas pueden servir como una buena fuente de polifenoles bioactivos en la dieta humana, y pueden ser considerados como buenos candidatos para su uso como suplemento nutricional en formulaciones debido a su alta concentración en fenoles, flavonoides y antocianinas y a su fuerte actividad antioxidante.

**PALABRAS CLAVE:** Actividad antioxidante – Antocianina – Compuestos fenólicos totales – Flavonoides – Plantas salvajes comestibles – Región del mar Muerto.

### SUMMARY

#### Antioxidant activity of wild edible plants in the Black Sea Region of Turkey

The antioxidative activity of the 80% ethanol extract obtained from eleven commonly consumed wild edible plants was determined according to the phosphomolybdenum method, reducing power, metal chelating, superoxide anion and free radical scavenging activity and compared to standard compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and trolox. Total phenolic compounds, flavonoids, and anthocyanins in the extracts were also measured. *Trachystemon orientalis*, *Vaccinium myrtillus*, *Rumex acetosella* *Polygonum amphibium*, *Beta vulgaris*, and *Similax Excelsa* had the highest antioxidant capacities. Overall results showed that these plants can serve as good sources of bioactive polyphenols in the human diet and can be regarded as good candidates for nutritional supplement formulations due to their high concentrations of total phenolic compounds, flavonoids and anthocyanins as well as their strong antioxidant activity.

**KEY-WORDS:** Antioxidant activity – Anthocyanins – Black Sea Region – Flavonoids – Total phenolic compounds – Wild edible plants.

### 1. INTRODUCTION

Reactive oxygen molecules (ROS) such as superoxide ( $O_2^{\cdot-}$ ), hydroxyl ( $OH^{\cdot}$ ) and peroxy ( $ROO^{\cdot}$ ) radicals are generated in a situation of oxidative stress. ROS play an important role in the pathogenesis of various serious diseases, such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, cataracts, and inflammation (Aruoma, 1998). The oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems (Halliwell and Gutteridge, 1999). Due to a depletion of immune system natural antioxidants in different maladies, consuming antioxidants as free radical scavengers may be necessary (Kumpulainen and Salonen, 1999). Several studies have shown that increased dietary intake of natural phenolic antioxidants correlates with reduced coronary heart disease (Deshpande *et al.*, 1996).

There are two basic categories of antioxidants, namely, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids as well as ascorbic acid (Hall and Cuppett, 1997). Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants since the beginning of this century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity (Branen, 1975; Ito *et al.*, 1983). Thus, an interest in natural antioxidants has increased considerably (Löfger, 1991). Recent research investigations have suggested that diets rich in polyphenolic compounds and flavonoids are associated with longer life expectancy (Hu, 2000). Moreover, these compounds have been found effective in many health-related properties, such as anticancer, antiviral, anti-inflammatory activities, effects on capillary fragility and an ability to inhibit human platelet aggregation (Benavente-Garcia *et al.*, 1997).

Turkey has a great deal of plant genetic resources, some of which are wild edible plants. They are used for different purposes as natural food, tea and herbal

medicine. Turkish flora comprises 9300 plant species, 3000 of which are endemic (Güner *et al.*, 2000), rendering it to be one of the most extensive floras in continental Europe (Sezik *et al.*, 2001). The strategic position of the Eastern Black Sea region of Turkey has aided in the development of a rich body of knowledge concerning edible plant species (Sekercioglu *et al.*, 2006). Wild edible plant species appear in the early spring and are consumed as vegetable sources in the Eastern Black Sea region (Ozbucak *et al.*, 2007).

In particular, despite the widespread use of wild edible plants as food in Northern Turkey, the literature contains few reports on the antioxidant activity and chemical composition of these plants. In the present study, the antioxidant activities of *Amaranthus retroflexus*, *Tussilago farfara*, *Trachystemon orientalis*, *Beta Vulgaris*, *Brassica oleracea*, *Vaccinium myrtillus*, *Ornithogalum umbellatum*, *Polygonum amphibium*, *Rumex acetosella*, *Similax excelsa* and *Chaerophyllum byzantinum* were determined using the phosphomolybdenum method, reducing power, metal chelating, superoxide anion and free radical scavenging activity. Another aim was to evaluate whether the total phenolic, flavonoid and anthocyanin contents of wild edible plants were correlated with antioxidant activity. Plant species (or their active constituents) identified as having high levels of antioxidant activity *in vitro* may be of value in the design of further studies to scavenge novel treatment strategies for disorders associated with ROS induced tissue damage.

## 2. MATERIAL AND METHODS

### 2.1. Chemicals

Ferrous chloride and 1,1-diphenyl-2-picryl-hydrazil (DPPH<sup>•</sup>) were purchased from E. Merck. Nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), nicotine adenine dinucleotide (NADH), BHA, BHT,

$\alpha$ -tocopherol, nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH), and potassium ferricyanide were purchased from Sigma-Aldrich Chemical Co. All other chemicals and reagents were of analytical grade or obtained from Across, Sigma-Aldrich, or Merck.

### 2.2. Plant materials

All plant samples were collected from local markets in the center of the Giresun Province, Black Sea Region of Turkey. Prof. Dr. Hamdi G. Kutbay, Department of Biology, Faculty of Arts and Sciences, Ondokuz Mayıs University, confirmed the taxonomic identities of the plants. Designation of the individual plants and their origins are given in Table 1.

Dried samples (20 g) were converted to powder form in a mill and extracted in a Soxhlet apparatus with a hydroalcoholic solution (80% ethanol: 20% dd.H<sub>2</sub>O) until becoming colorless. The extracts were filtered. The filtrates were lyophilized and stored at -20°C until use for assays.

### 2.3. Amount of total phenolic compounds

The total phenolic compound contents were determined using the Folin Ciocalteu reagent (Singleton and Rossi, 1965). 0.1 ml extract (contains 0.1 mg extract) was mixed with water (46 ml). 1 ml of Folin-Ciocalteu reagent was added and mixed thoroughly. 3 ml of Na<sub>2</sub>CO<sub>3</sub> (2 %) were added and incubated in a bath for 2 h. The absorbance was measured at 760 nm. The standard curve was prepared by a 0-100 µg/ml solution of pyrocatechol in ethanol. The concentration of total phenolic compounds in extracts was determined as µg of pyrocatechol equivalent using an equation obtained from the standard pyrocatechol graph and

Table 1  
Names of the wild edible plants traditionally consumed and used in the Black Sea Region of Turk

No	Plant species	Family	Local name	Parts Used	Method of using
1	<i>Amaranthus retroflexus</i> L.	Amaranthaceae	Hoşkiran	Leaf	Roasting with egg
2	<i>Beta Vulgaris</i> var. cicla	Chenopodiaceae	Pazi	Leaf	Roasting with egg
3	<i>Brassica oleracea</i> var. acephala	Cruciferae	Kara lahana	Leaf	Roasting with rice, soap
4	<i>Chaerophyllum byzantinum</i> Boiss	Umbelliferae	Mendek	Leaf	Soap
5	<i>Ornithogalum umbellatum</i> L.	Liliaceae	Sakarca	Stem	Roasting with egg
6	<i>Polygonum amphibium</i> L.	Polygonaceae	Gücündene	Leaf	Soap
7	<i>Rumex acetosella</i> L.	Polygonaceae	Kuzu kulağı	Leaf	Roasting with egg
8	<i>Similax excelsa</i> L.	Smilacaceae	Özdikenimelocan	End of plant	Vegetable and roasting
9	<i>Trachystemon orientalis</i> L.	Boraginaceae	Galdırık	Stem and petiole	Roasting with egg, pickles
10	<i>Tussilago farfara</i> L.	Asteraceae	Gabalak	Stem and petiole	Making pickles
11	<i>Vaccinium myrtillus</i> L.	Ericaceae	Çalı çileği	Leaf	Soap

expressed as mg pyrocatechol / g dry weight (DW) of the plant material. The results were calculated into mg pyrocatechol equivalents / g dried plant materials. The data were presented as the average of three analyses.

## 2.4. Amount of flavonoids

The total flavonoid contents of sample plants were determined according to the colorimetric method (Change *et al.*, 2002). Briefly, each plant extract (0.1 g) was dissolved in 1 ml deionized water. This solution (0.1 ml) was mixed with 10%  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  and 0.1 ml of 1 M potassium acetate ( $\text{CH}_3\text{COOK}$ ). It remained at room temperature for 30 min and the reaction mixture absorbance was measured at 415 nm. Quercetin was chosen as a standard. Using the standard curve (0–100  $\mu\text{g}/\text{ml}$ ), the levels of total flavonoid contents in sample extracts were determined in triplicate, respectively. The results were calculated into mg quercetin equivalents/g dried plant materials.

## 2.5. Amount of anthocyanins

Total anthocyanins were measured according to the methods described earlier (Lee *et al.*, 2005) with slight modifications. The extracts were mixed with acidified methanol (1% HCl/methanol) for 2 h at room temperature in the dark and then centrifuged at  $2000 \times g$  for 10 min. The anthocyanin concentration in the supernatant was measured spectrophotometrically at 530 and 657 nm, respectively. The absorbance values for 530 and 657 nm were indicated as  $A_{530}$  and  $A_{657}$ , respectively. Two different dilutions of the plant samples were prepared, one for pH 1.0 using 0.03 M KCl buffer and the other for pH 4.5 using 0.4 M  $\text{CH}_3\text{CO}_2\text{Na}$  buffer. The absorbance of each sample was measured at 520 nm against distilled water as a blank. The samples had no haze or sediment and thus correction at 700 nm was omitted. The concentration (mg/l) of each anthocyanin was calculated according to the following formula and expressed as Cy-3-glc equivalents:

$$\text{Total anthocyanins (mg/mL, Cy-3-glc):} \\ (A \times \text{MW} \times \text{DF} \times 10^3) / \varepsilon \times L$$

where A is the absorbance  $A = (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH } 1.0} - (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH } 4.5}$ , MW is the Cy-3-glc molecular weight: 449.2 g/mol, DF is the dilution factor (0.2 ml sample is diluted to 2 ml, DF = 10),  $\varepsilon$  is the extinction coefficient ( $L \times \text{cm}^{-1} \times \text{mol}^{-1}$ ) = 26,900 for Cy-3-glc and  $L$  (pathlength in cm) = 1.

## 2.6. Determination of total antioxidant activity

The total antioxidant capacity of the crude hydroalcoholic extracts of plant materials was evaluated by the method of Prieto *et al.* (1999). The antioxidant capacity of the extracts was measured

spectrophotometrically using a phosphomolybdenum method, based on the reduction of Mo (VI) to Mo(V) by the sample analyte and the subsequent formation of green phosphate / Mo(V) compounds. A 0.3 ml aliquot of sample solution (100  $\mu\text{g}/\text{ml}$ ) was combined with 2.7 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). All samples were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm. For the blank, 0.3 ml ethanol was mixed with 2.7 ml of the reagent. A typical blank solution contained 2.7 ml of reagent solution and the appropriate volume of ethanol used for the dissolution of the samples and it was incubated under the same conditions as the rest of the samples. Stock solutions of  $\alpha$ -tocopherol were prepared in methanol just prior to use. The antioxidant activity of extracts was expressed as equivalents of  $\alpha$ -tocopherol using the extinction coefficient of  $4 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$ . Since the samples were of unknown composition, the total antioxidant capacity was expressed as equivalents of  $\alpha$ -tocopherol ( $\mu\text{mol } \alpha\text{-tocopherol/g}$  of extract).

## 2.7. Assay of reducing power

The reducing power of the extracts was determined according to the Method of Oyaizu (Oyaizu, 1986). All species and standard antioxidants (100  $\mu\text{g}/\text{ml}$ ) in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL; 10 g/l). The mixtures were incubated at 50°C for 20 min. Then, a portion of TCA (10%; 2.5 ml) was added to each mixture and centrifuged at  $5000 \times g$  for 20 min. Finally, the supernatants (2.5 ml) were mixed with distilled water (2.5 ml) and  $\text{FeCl}_3$  (0.5 ml; 0.1%). The absorbance of the solutions was measured at 700 nm. A higher absorbance of the reaction mixture indicated that the reducing power had increased.

## 2.8. Metal chelating activity

The chelating of ferrous ions by plant extracts was determined by the method of Dinis *et al.* (1994). Briefly, the samples (extracts or standard antioxidants; 100  $\mu\text{g}/\text{ml}$ ) were added to a solution of 2 mM  $\text{FeCl}_2$  (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the resulting solution was then measured at 562 nm. The metal chelating activities were calculated by the given formula:

$$\text{Metal chelating effect (\%)} = [(A_0 - A_1)/A_0 \times 100]$$

where  $A_0$  was the absorbance of the control and  $A_1$  was the absorbance of extracts or standards. The control contains  $\text{FeCl}_2$  and ferrozine.

### 2.9. Assay of superoxide anion scavenging activity

The determination of the superoxide anion scavenging activity of the plant extracts was measured according to the slightly modified method of Nishimiki *et al.* (1972). Superoxide radicals are generated in phenazine methosulphate (PMS)-nicotinamide adenine dinucleotide (NADH) systems by the oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). One milliliter of all species and standard antioxidants (100 µg/ml), 1.0 ml NBT solution (156 µM NBT in 100 mM phosphate buffer, pH 7.4) and 1.0 ml NADH solution (468 µM in 100 mM phosphate buffer, pH 7.4) were mixed. The reaction was started by adding 100 µl of a PMS solution (60 µM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The mixture was incubated at 25°C for 5 min and its absorbance was measured at 560 nm wavelength against blank samples. A decrease in the absorbance for the mixtures indicates an increasing superoxide anion scavenging activity. The percentage of inhibition of superoxide anion generation was calculated using the following formula:

$$\text{Inhibition of superoxide anion generation (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where  $A_0$  was the absorbance of the control, and  $A_1$  was the absorbance of plant species or standards.

### 2.10. Assay of free radical scavenging activity

The effect of all species on DPPH<sup>·</sup> radical was estimated according to the method of Blois (1958) wherein the bleaching rate of a stable free radical, DPPH<sup>·</sup> is monitored at a characteristic wavelength in the presence of samples. An amount of 0.5 ml of 0.1 mM ethanolic solution of DPPH<sup>·</sup> was added to 3.0 ml of all species extracts or standard antioxidant solutions (100 µg/ml) in water. The mixture was shaken vigorously and held at room temperature for 30 min. Then the absorbance was measured at 517 nm. A decrease in the absorbance of the DPPH<sup>·</sup> solution indicates an increase in DPPH<sup>·</sup> radical-scavenging activity. This activity was calculated by the equation:

$$\text{DPPH}^{\cdot} \text{ Scavenging Effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where  $A_0$  was the absorbance of the control and  $A_1$  was the absorbance of the plant species or standards.

### 2.11. Statistical analysis

The experimental results were given as the mean  $\pm$  S.D. of three parallel measurements. The experimental values were evaluated by using one-way analyses of variance (Tukey test).  $P$  values  $< 0.05$  were regarded as significant and  $P$  values  $< 0.01$  as very significant.

## 3. RESULTS AND DISCUSSION

Several biochemical assays were used to screen the antioxidant properties: total antioxidant capacity (phosphomolybdenum method), reducing power, free radical scavenging, superoxide scavenging and metal chelating activity. The assays were performed on the whole extract, since a bioactive individual component can charge single properties. Crude extract in the plants are responsible for their potent bioactive properties and the benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in whole foods (Liu, 2003).

### 3.1. Extract yield, total phenolics, total flavonoids and total anthocyanin contents in the selected vegetables

The total phenolic compounds, total flavonoids and the total anthocyanins were measured for all the samples. The results are given in Table 2. There was a wide range of phenol concentrations in selected edible plants. The amount of extractable compounds ranged from 5.10 to 22.30 g/g dry plant material for hydroalcoholic extracts. As expected, *Beta vulgaris*, *Polygonum amphibium*, *Rumex acetosella*, *Similax Excelsa*, *Trachystemon orientalis*, *Vaccinium myrtillus* had significantly higher amounts of total phenolics, flavonoids and anthocyanins than the others. *Vaccinium myrtillus* has the highest chemical content.

The relationship between the extraction yield and total phenolic content can be observed in the analysis of the different plant species, where those that had higher yields also had higher levels of total phenolic compounds.

The content of extractable phenolic compounds in extracts, determined from the regression equation of the calibration curve ( $y = 0,4524x + 0,0072$ ;  $r^2 = 0,9975$ ) and expressed as a pyrocatechol equivalent, varied between  $2.11 \pm 0.78$  and  $110.82 \pm 1.15$  mg/g DW (Table 2). These amounts were comparable with results described in the literature for other extracts of plant products (Chun *et al.*, 2005).

The contents of flavonoid (mg/g, DW) in the total extractable compounds of the extracts, determined from the regression equation of the calibration curve ( $y = 2,6018x - 0,0045$ ;  $r^2 = 0,9956$ ) and expressed in catechin equivalents, varied from  $0.23 \pm 0.17$  to  $61.87 \pm 3.11$  mg/g DW (Table 2). Flavonoids are very important plant constituents because of active hydroxyl groups and show antioxidant activity (Kumar *et al.*, 2008).

The contents of total anthocyanosides in the edible plants ranged from  $0.35 \pm 0.23$  to  $24.71 \pm 2.78$  mg/g DW anthocyanins determined as mg cyanidin 3-glucoside/g dry weight (Table 2). It is to be expected that several activities might be related to a possible antioxidant action from anthocyanosides like polyphenol compounds (Einbonda *et al.*, 2004). The extracts of the edible plants were most abundant in phenolics and in anthocyanins (which contribute to

Table 2  
Yield of extractable compounds, total phenolic compounds, flavonoids  
and anthocyanins of 11 wild edible plants

No	Plant species	Yield <sup>a</sup>	Total phenolic compounds <sup>b</sup>	Flavonoids <sup>c</sup>	Anthocyanins <sup>d</sup>
1	<i>Amaranthus retroflexus</i>	5.1	4.3±0.5	1.8±0.1	0.3±0.1
2	<i>Beta vulgaris</i>	21.0	41.0±0.5	7.9±1.6	0.5±0.1
3	<i>Brassica oleracea</i>	11.1	28.8±2.1	4.5±0.7	0.5±0.3
4	<i>Chaerophyllum byzantinum</i>	14.5	23.2±1.1	14.3±0.9	6.0±0.7
5	<i>Ornithogalum umbellatum</i>	5.9	2.1±0.9	0.2±0.2	1.8±0.2
6	<i>Polygonum amphibium</i>	19.6	57.7±1.9	14.7±2.5	6.4±0.4
7	<i>Rumex acetosella</i>	18.8	76.6±1.5	51.6±1.2	10.6±0.7
8	<i>Similax excelsa</i>	20.8	49.9±1.8	12.6±1.1	2.5±0.4
9	<i>Trachystemon orientalis</i>	22.3	82.1±1.5	3.63±1.1	15.2±0.1
10	<i>Tussilago farfara</i>	3.2	7.7±2.8	1.85±0.2	1.5±0.1
11	<i>Vaccinium myrtillus</i>	17.4	110.8±1.2	61.87±3.1	24.7±0.9

<sup>a</sup> Yield, (g crude extract/ g dry plant)

<sup>b</sup> Total phenolic compounds (mg pyrocatechol / g dry weight)

<sup>c</sup> Flavonoids (mg quercetin / g dry weight)

<sup>d</sup> Anthocyanins mg/ml (mg cyanidin 3-glucoside/g dry weight)

the total phenolic levels). Polyphenolic compounds have been found to be one of the most effective antioxidant constituents in plant foods, including fruits, vegetables and grains (Velioglu *et al.*, 1998).

transforming reactive free radical species into more stable non-reactive products. The antioxidants break the free radical chain by donating a hydrogen atom (Gordon, 1990, Dorman *et al.*, 2003).

### 3.2. Total antioxidant activity

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics,  $\alpha$ -tocopherol, and carotenoids (Prieto *et al.*, 1999). Ascorbic acid, glutathione, cysteine, tocopherols, polyphenols, and aromatic amines have the ability to donate hydrogen and electrons and can thus be detected by the five assay models. In general, aqueous alcohol is considered the best solvent for extracting phenolic compounds from plant materials (Negi *et al.*, 2003). The antioxidant activity of edible plant extracts at 100  $\mu$ g/mL concentrations as measured by the phosphomolybdenum method is presented in Figure 1. It can be seen that different vegetables exhibited various degrees of antioxidant activity. All vegetable extracts have antioxidant activity in the phosphomolybdenum method ( $P < 0.05$ ). The results showed that the vegetables exhibited antioxidant activity ranging from  $8260 \pm 59$  to  $18730 \pm 126$   $\mu$ g  $\alpha$ -tocopherol/g and expressed as equivalents of  $\alpha$ -tocopherol acetate; however, the activities were inferior to those of BHA, BHT and trolox. *Trachystemon orientalis*, *Vaccinium myrtillus*, *Rumex acetosella*, *Polygonum amphibium*, *Beta vulgaris*, and *Similax Excelsa* exhibited a higher antioxidant activity than that of the other vegetables, respectively. The results presented above indicate that the antioxidant activity of vegetables seems to be due to the presence of polyphenols, flavonoid and anthocyanoside that may act by donating electrons and free radicals. Flavonoids have been shown to have potent antioxidant activity (Ng *et al.*, 2000). The extracts demonstrated electron-donating capacity and thus they may act as radical chain terminators,

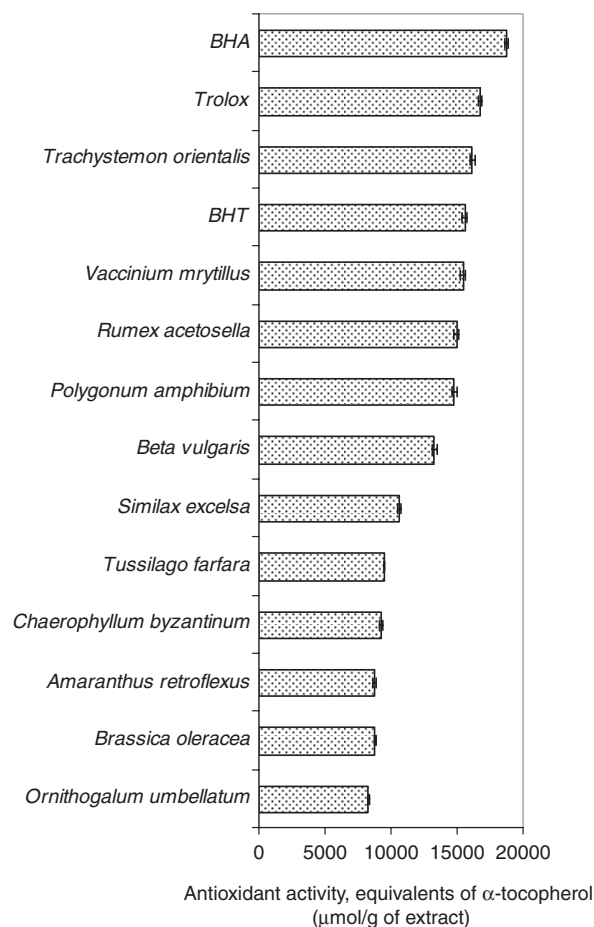


Figure 1  
Antioxidant activity of 11 wild edible plants based on the reduction of Mo (VI) to Mo(V) by the sample.

### 3.3. Reducing power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir *et al.*, 1995). Reducing potential is generally associated with the presence of reductants such as antioxidant substances, which cause a reduction in the  $Fe^{3+}$ /ferricyanide complex to  $Fe^{2+}$ . Accordingly,  $Fe^{2+}$  can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. An electron-donating reducing material deals with antioxidant activity to donate an electron to free radicals from the solution. The reducing power capacities of the samples were compared to BHT, BHT and trolox in Figure 2. Their reducing power values were  $0.247 \pm 0.011$ ,  $0.238 \pm 0.008$ , and  $0.237 \pm 0.006$ , respectively. Plants exhibited reducing power ranging from  $0.185 \pm 0.012$  to  $0.234 \pm 0.001$  at 100  $\mu$ g/ml. All of the plants showed significant reducing power at the same level as standard antioxidants,  $P < 0.05$  because of some degree of electron donation capacity in a concentration dependent manner. Extracts containing the highest amounts of total phenolics had weaker reducing power than compounds although results were close. Similar relations between  $Fe^{3+}$  reducing

activity and total phenol content have been reported in the literature (Gao *et al.*, 2000).

### 3.4. Metal chelating activity

Iron has the most important lipid pro-oxidant. It is known that  $Fe^{+2}$  accelerates lipid peroxidation by breaking down hydrogen and lipid peroxide forms by the Fenton free radicalic reaction;  $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$  (Halliwell and Gutteridge, 1999). The  $Fe^{2+}$  ion can form complexes with ferrozine. In the presence of chelating agents, the complex formation is prevented, resulting in a decrease in the red color of the complex. A measurement of color reduction allows for the determination of metal chelating activity. The measurement of the rate of color reduction allows for the estimation of the chelating activity of the coexisting chelator (Yamaguchi *et al.*, 2000). In this assay, extracts of plant species and standard antioxidants interfered with the formation of a ferrous-ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. As shown in Figure 3, the formation of ferrozine- $Fe^{2+}$  complex is completed in the presence of extracts,

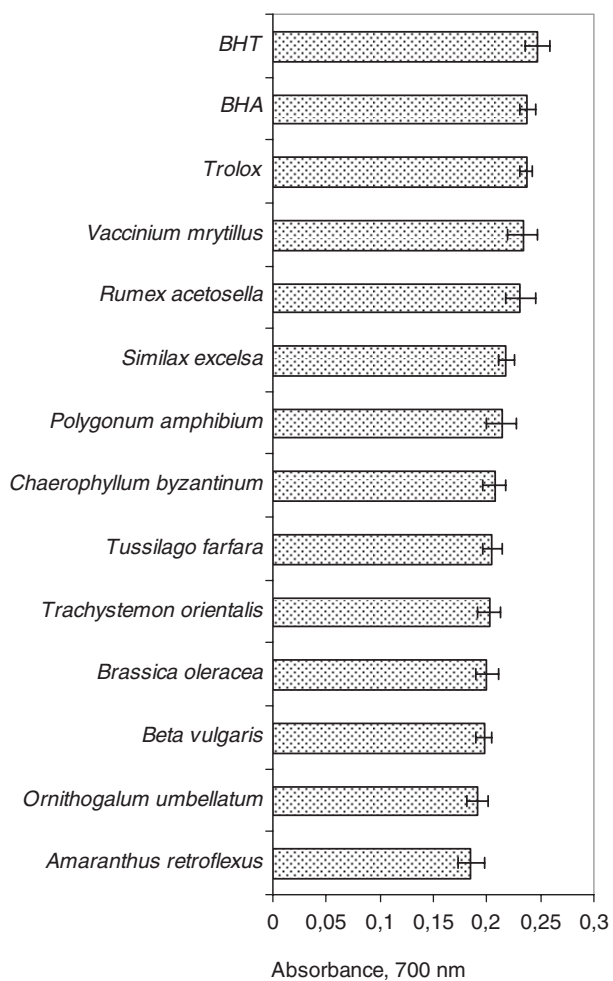


Figure 2

Reducing power of 11 wild edible plants based on the spectrophotometric detection of the  $Fe^{3+}$ - $Fe^{2+}$  transformation.

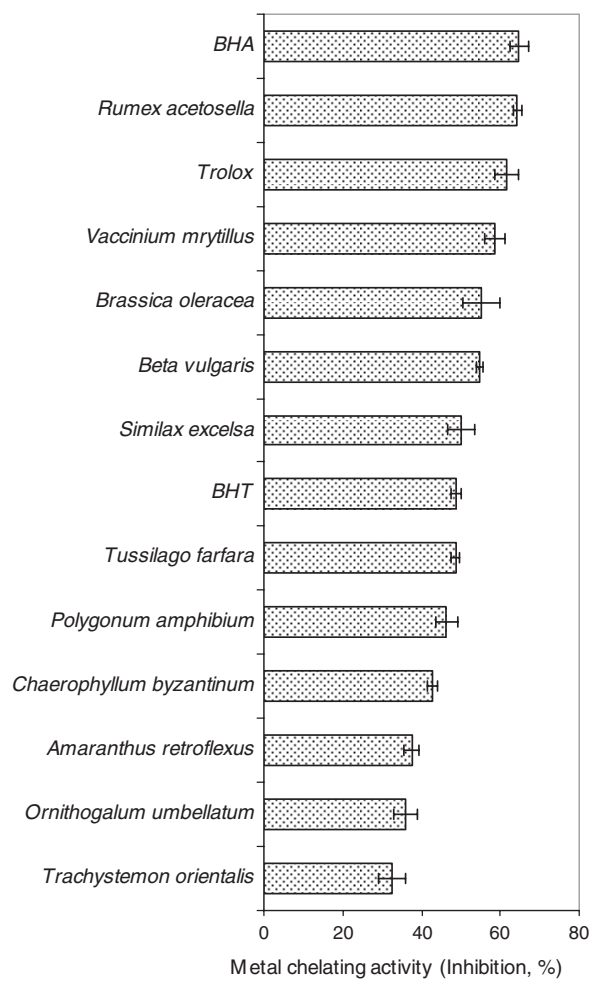


Figure 3

Metal chelating activity of 11 wild edible plants based on the metal chelating effect.

indicating that some plant species change a cheater iron. The results were found statistically significant ( $P < 0.01$ ). At a concentration of 100  $\mu\text{g/ml}$ , the chelating activity of extracts from *Rumex acetosella*, *Vaccinium myrtillus*, *Brassica oleracea*, *Beta Vulgaris*, and *Similax excelsa* showed higher metal chelating activity than that of BHT. It was noted that chelating agents forming  $\sigma$ -bonds with a metal are secondary antioxidants due to the reduction of the redox potential. Iron can stimulate lipid peroxidation by the Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Halliwell and Gutteridge, 1999).

### 3.5. Superoxide anion scavenging activity

The production of highly reactive oxygen species such as superoxide anion radicals, hydrogen peroxide and hydroxyl radicals is also catalyzed by free iron through Haber-Weiss reactions (Haber and Weiss, 1934). The primary free radical in most biological systems is  $\text{O}_2^{\cdot-}$ . Although  $\text{O}_2^{\cdot-}$  itself is quite unreactive compared to the other radicals, the biological systems convert it into more reactive species (Winterbourn and Kettle, 2003).  $\text{O}_2^{\cdot-}$ , which is a reduced form of  $\text{O}_2$ , has been implicated in the initiating oxidation reactions associated with aging. In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. Antioxidants are able to inhibit the blue NBT formation (Cos *et al.*, 1998). The decrease in absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. Figure 4 presents the superoxide radical scavenging activity of 100  $\mu\text{g/ml}$  plant extracts in comparison with the same dose of known antioxidants BHA, BHT, and trolox. *Vaccinium myrtillus* and *Beta Vulgaris* extracts scavenged  $\text{O}_2^{\cdot-}$  significantly ( $P < 0.01$ ) and exhibited higher superoxide radical scavenging activity than BHT trolox at the same concentrations. *Vaccinium myrtillus* and *Beta Vulgaris* could be good  $\text{O}_2^{\cdot-}$  scavengers. Therefore, the spontaneous or catalytic dismutation of  $\text{O}_2^{\cdot-}$  leads to the formation of  $\text{H}_2\text{O}_2$ , which, in the presence of a transition metal ion like  $\text{Fe}^{3+}$ , decomposes into  $\cdot\text{OH}$  radicals, a highly damaging species in free radical pathology (Pardini, 1995).

### 3.6. Free radical scavenging activity

DPPH $\cdot$  is a useful reagent for investigating the free radical-scavenging activities of phenolic compounds and a substrate to evaluate the antioxidative activity of antioxidants (Duh *et al.*, 1999). The free radical DPPH $\cdot$  possesses a characteristic absorption at 517 nm (purple in color), which decreases on exposure to radical-scavengers. In the radical form (DPPH $\cdot$ ), this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to

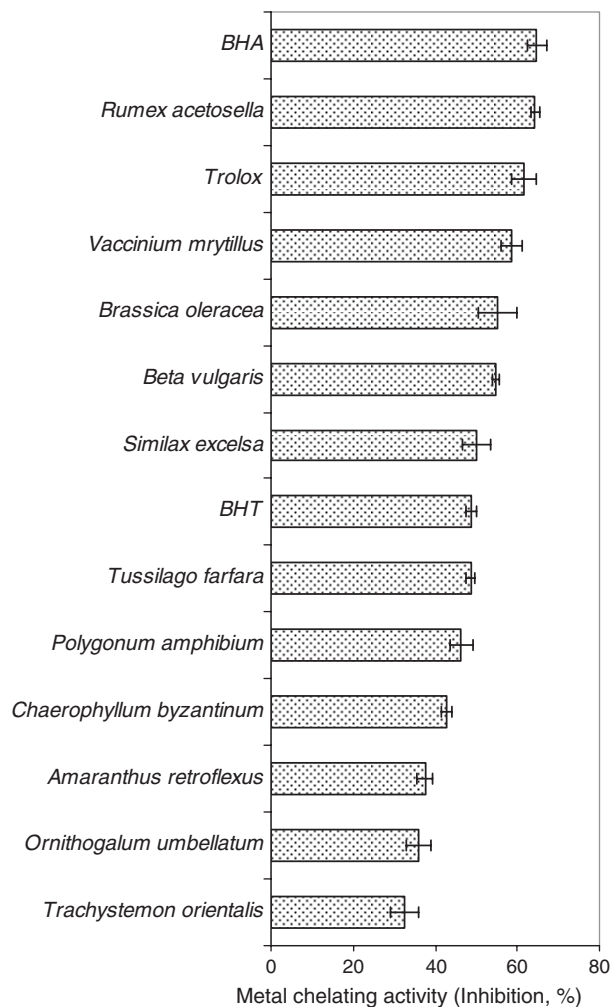


Figure 4  
Superoxide radical scavenging activity of 11 wild edible plants as determined by the PMS/NADH-NBT method.

become a stable diamagnetic molecule (Matthaus, 2002). Figure 5 illustrates a significant decrease ( $P < 0.01$ ) in the concentration of DPPH $\cdot$  radicals due to the scavenging ability of plant extracts and standards. A high correlation between free radical scavenging and the phenolic/polyphenolic contents has been reported for vegetables (Cakir *et al.*, 2006). Hence, the total phenolic compound contents of the extracts of 11 wild edible species were determined. The amounts of total phenolic compounds present in the extracts are shown in Figure 5 as pyrocatechol acid equivalents. As shown in this figure, in general, the extracts of *Vaccinium myrtillus*, *Beta Vulgaris* and *Polygonum amphibium* contained relatively high contents of phenolic compounds and exhibited higher free radical scavenging activity than that of BHA, BHT, and trolox. These results would suggest that there is a correlation between antioxidant potential and the total phenolic contents of the extracts ( $P < 0.01$ ). The results of free radical scavenging activity revealed that the extracts, through hydrogen and electron donation, might prevent ROS species from reaching biomolecules such as amino acids, proteins, sugars, lipoproteins and polyunsaturated

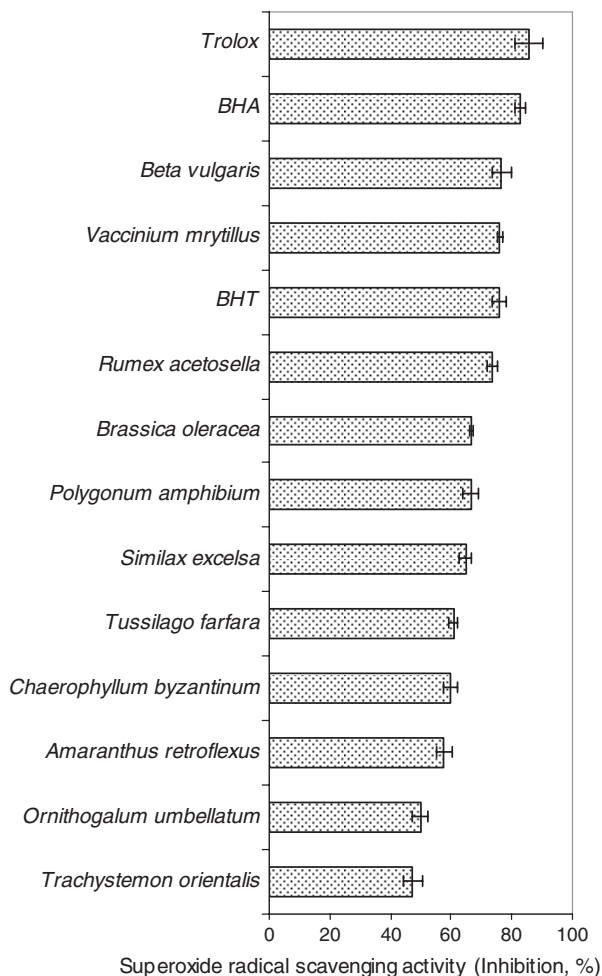


Figure 5  
Free radical scavenging activity of 11 wild edible plants by 1,1-diphenyl-2-picrylhydrazyl radicals.

fatty acids (PUFA) in vulnerable biological and food system (Halliwell *et al.*, 1995).

#### 4. CONCLUSION

Overall, it has been verified that wild edible vegetables contain a wide variety of antioxidant phenolics which contribute a large amount of antioxidant vegetables to be diet in the Giresun Province. Therefore, food modification through the balanced consumption of vegetables is likely to be more important and effective than nutritional supplements for the primary prevention of acute diseases.

#### ACKNOWLEDGEMENTS

The author is thankful to Erdem Çukur and Halil Yılmaz for contribution and would like to thank Prof. Dr. Hamdi Guray Kutbay from Faculty of Arts and Sciences, Department of Biology, Ondokuz Mayıs University for the identification of the plants.

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Recibido: 8/7/09  
Aceptado: 11/9/09