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CHEMICAL COMPOSITIONS AND ANTIOXIDANT ACTIVITIES OF FIVE ENDEMIC *ASPERULA* TAXA

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Abstract - In this study, the proximate chemical composition, total flavonoid and α-tocopherol amounts and antioxidant activities of five endemic *Asperula* taxa (Rubiaceae) (*A. brevifolia, A. pseudochlorantha* var. *antalyensis, A. pseudochlorantha* var. *pseudochlorantha, A. purpurea* subsp. *apiculata and A. serotina*) were determined. Antioxidant properties of methanol extracts were studied by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. Among the all *Asperula* extracts *A. brevifolia* showed the most potent radical scavenging activities.

Key words: Asperula, chemical composition, antioxidant activity, flavonoid, Turkey

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INTRODUCTION

Oxidation is essential to many living organisms for the production of energy for biological activities. However, the uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis and atherosclerosis as well as in degenerative processes associated with aging (Turkoglu et al., 2007). Almost all organisms are well-protected against free radical damage by enzymes such as superoxide dismutase and catalase or compounds such as ascorbic acid, tocopherols and glutathione (Elmastas et al., 2005). When the mechanism of antioxidant protection becomes unbalanced by factors such as ageing, deterioration of physiological functions may occur, resulting in diseases and accelerated ageing. However, antioxidant supplements or antioxidant-containing foods can be used to help the human body to reduce oxidative damage (Cazzi et al., 1997).

Many species of fruits, vegetables, herbs, cereals, sprouts and seeds have been investigated for their antioxidant activities during the past decade (Halliwell and Gutteridge, 2003). Natural antioxidants are being extensively studied for their capacity to protect organisms and cells from damage brought on by oxidative stress, the latter being considered a cause of ageing and degenerative diseases (Niki et al., 1994).

Turkey has an extraordinarily rich flora of nearly 10,000 natural plant species (Ates and Erdogrul, 2003), because of its geographic location and climate. There is also a wide knowledge of their medicinal properties. The genus *Asperula* is represented in the Turkish flora by 39 species, 19 of which are endemic. Most of them grow in the south-western and north-eastern parts of Anatolia (Davis, 1988). Certain species from this genus contain quinonic compounds (anthraquinones, naphthoquinones, naphthohydroquinones and their glycosides), iridoids, coumarins, triterpenes and flavonoids. Some *Asperula* species

are used in folk medicine as a diuretic and tonic and against diarrhoea (Guvenalp and Demirezer, 2005).

Despite the considerable medicinal potential of certain plants in Turkey, knowledge of this area and studies on these plants is scarce (Kalyoncu et al., 2006). To the best of our knowledge, no information is available on the antioxidant nature of these plants. Our objective was to evaluate the proximate chemical content and antioxidant activities of five endemic *Asperula* taxa by the free radical scavenging method.

MATERIALS AND METHODS

Plant material

In this study, five *Asperula* taxa (Rubiaceae) (*A. brevifolia* Vent., *A. pseudochlorantha* var. *antalyensis* (Ehrend.) Minareci et Yildiz, *A. pseudochlorantha* Ehrend. var. *pseudochlorantha*, *A. purpurea* (L.) Ehrend. subsp. *apiculata* (Sibth. et Sm.) Ehrend. and *A. serotina* (Boiss. Et Heldr.) Ehrend) collected from the south-western and north-eastern parts of Anatolia were analyzed for their proximate chemical contents and antioxidant activities. The origin and herbarium numbers of these semi-shrubs are given in Table 1. Voucher specimens were deposited in the Herbarium of Botany, Department of Biology, Celal Bayar University. The aerial parts of these plants used in present study.

Antioxidant activity assay

The aerial parts of the plants were dried at room temperature and then reduced to a coarse powder. A fine dried plant sample (1 g) was continuously extracted with methanol in a Soxhlet apparatus for 24 h. The methanolic extract was evaporated to dryness at 45^0C and re-dissolved in methanol, and stored at 4°C prior to further use (Barros et al., 2007). The capacity to scavenge the "stable" free radical DPPH was monitored according to the method of Barros et al. (2007). Different concentrations of methanolic extracts from plants (2 ml) were mixed with 2 ml of a methanolic solution containing DPPH radicals (6x10-5 mol/L). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation:

$$\% \text{ RSA} = [(A_{DPPH} - A_S) / A_{DPPH}] \times 100$$

where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. The assays were carried out in triplicate and the results expressed as mean values ± standard deviations. BHT was used as standard.

Total flavonoid content

The flavonoid content in *Asperula* extracts was determined according to the method of Ahn et al (Ahn et al., 2007). To 3.0 ml of the ethanol extracts of *Asperula* taxa were added 3 ml of a 2% $AlCl_3$-ethanol solution. After 1 h at room temperature, the absorbance values were measured at 420 nm. The total flavonoid contents were calculated as quercetin equivalents (milligrams per gram) from a calibration curve.

Quantitative determination of α-tocopherol (ATOC) by HPLC-UV

The plant material was collected and dried at room temperature and reduced to a coarse powder with a Retsch mill. One g of the powdered sample was extracted by 50 ml of n-hexane under ultrasonic stirring. The organic phase was filtered using a 0.45 µm pore diameter filter and evaporated in vacuum. The sample solution was prepared by dissolving the n-hexane extract in methanol and a twenty ml aliquot was injected on the HPLC-UV system (Hiroshi, 2000).

Chemical composition assay

The water content and total carbohydrates in plant samples were determined according to the Association of Official Analytical Chemists (AOAC, 1995).

Table 1. Herbarium numbers and origins of *Asperula* taxa

Taxa	Coordinate	Altitude	Herbarium no
A. brevifolia	N 36° 42', E 27°34'	480 m	451-23
A. pseudochlorantha var. *antalyensis*	N 36° 40', E 30° 32'	60 m	453-02
A. pseudochlorantha var. *pseudochlorantha*	N 36° 35', E 30° 30'	100 m	452-49
A. purpurea subsp. *apiculata*	N 40° 50', E 27° 23'	20 m	455-64
A. serotina	N 36° 36', E 33° 03'	885 m	454-89

Table 2. Extraction yields and antioxidant activity values of *Asperula* species

Taxa	Extraction yields (%)	RSA (%)	Concentration (mg/ml)
A. brevifolia	8.40	92.32 ± 0.21	1.68
A. pseudochlorantha var. *antalyensis*	8.29	91.88 ± 0.42	1.66
A. pseudochlorantha var. *pseudochlorantha*	9.41	90.77 ± 0.73	1.88
A. purpurea subsp. a*piculata*	7.91	87.30 ± 0.63	1.58
A. serotina	6.08	85.38 ± 0.21	1.22
BHT		98.24	3.00

Table 3. Total flavonoid and α-tocopherol contents in *Asperula* taxa

Taxa	**Total flavonoid (%)**	**α-tocopherol (%)**
A. brevifolia	2.49	0.22
A. pseudochlorantha var. *antalyensis*	2.72	0.66
A. pseudochlorantha var. *pseudochlorantha*	2.74	0.65
A. purpurea subsp. a*piculata*	2.90	0.32
A. serotina	1.69	0.32

Table 4. Proximate composition (% dry weight) of five *Asperula* taxa

Taxa	**Ash**	**Fat**	**Moisture**	**Protein**	**Carbohydrate**
A. brevifolia	4.66	3.71	7.35	4.20	81.94
A. pseudochlorantha var. *antalyensis*	4.89	3.98	7.44	3.85	79.85
A. pseudochlorantha var. p*seudochlorantha*	3.10	5.15	6.94	4.85	79.98
A. purpurea subsp. a*piculata*	3.74	5.37	6.53	4.31	81.93
A. serotina	5.70	5.37	7.48	6.13	78.01

Total protein was determined by the Kjeldahl method. Protein was calculated using the general factor of 6.25. The weight of fat extracted from 5 g of plant sample was determined to calculate the lipid content (AOAC, 1995). Diethyl ether was used as an extraction solvent and the extraction was performed for 4

h. Two grams of the sample were placed in a porcelain container and were ignited and incinerated in a muffle furnace at about 550^{0}C for 8 h until a grayish white ash was obtained (AOAC, 1995).

Statistical analysis

The data presented are averages of the results of three replicates with a standard error of less than 5%.

RESULTS AND DISCUSSION

Free-radical scavenging activity

The methanol extracts of plant samples were subjected to screening for possible antioxidant activity by the DPPH free radical scavenging method. The model of scavenging the stable DPPH radical is widely used to evaluate antioxidant activities over a relatively short time compared to other methods. DPPH is a stable free radical with a characteristic absorption at 517 nm. As antioxidants donate protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging. Free radical scavenging values of *Asperula* extracts as percentage are shown in Table 2.

Methanol extracts of *A. brevifolia* showed the strongest radical scavenging effect (92.32%) at 1.68 mg/ml. This activity was followed by *A. pseudochlorantha* var. *antalyensis* (91.88%) and *A. pseudochlorantha* var. *pseudochlorantha* (90.77%) respectively (Table 2). The lowest scavenging activity was exhibited by *A. serotina* (85.38%). However, the scavenging effect for BHT was 98.24% at 3.0 mg/ml. The yields of methanol extracts of *Asperula* taxa are given in Table 2.

In previous studies, the antioxidant activities of methanolic extracts of several plants have been reported (Assimopoulou et al., 2004, Al Fatimi et al., 2007). Özgen et al. (2003) found that methanolic extracts of *Onosma argentatum* and *Rubia peregrina* scavenged 98.00 and 94.20% of DPPH radicals, respectively. At 1.0 mg/ml, the methanolic extracts of *Acacia nilotica*, *Aerva javanica*, *Solanum nigrum* and *Tamarindus indica* scavenged 94.62, 91.80, 95.30 and 93.86%, respectively (Al Fatimi et al., 2007).

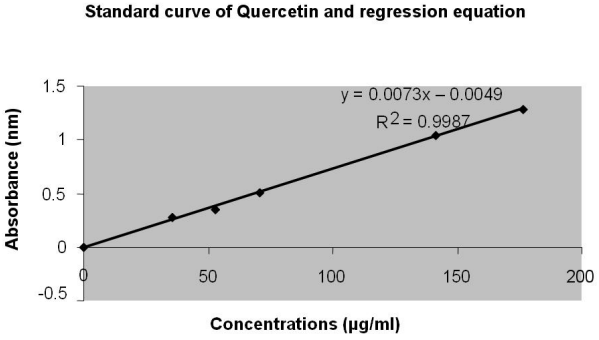

Fig. 1. Standard quercetin curve used for the determination of the total flavonoid content

Total flavonoid contents

Table 3 shows the total flavonoid contents of *Asperula* taxa. Using the standard curve of quercetin (R^2=0.9987) (Fig. 1), the total flavonoid contents in *Asperula* taxa ranged from 1.69 to 2.90%. *A. purpurea* subsp. *apiculata* had the highest value (2.90%), while *A. serotina* had the least (1.69%).

Flavonoids are commonly found in both edible and non-edible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Flavonoids have been suggested to play a preventive role in the development of cancer and heart disease (Ahn et al., 2007). The $AlCl_3$ coloration is currently used to determine the total flavonoid contents (Liu et al., 2007). Prasad et al. (2009) reported that, total flavonoid contents of *Cinnamomum burmanni* and *C. tamala* are 0.274 and 0.056%, respectively.

Quantitative determination of α-tocopherol (ATOC) by HPLC-UV

Tocopherols are considered to be the most effective lipid phase natural antioxidants. They prevent lipid peroxidation by acting as peroxyl radical

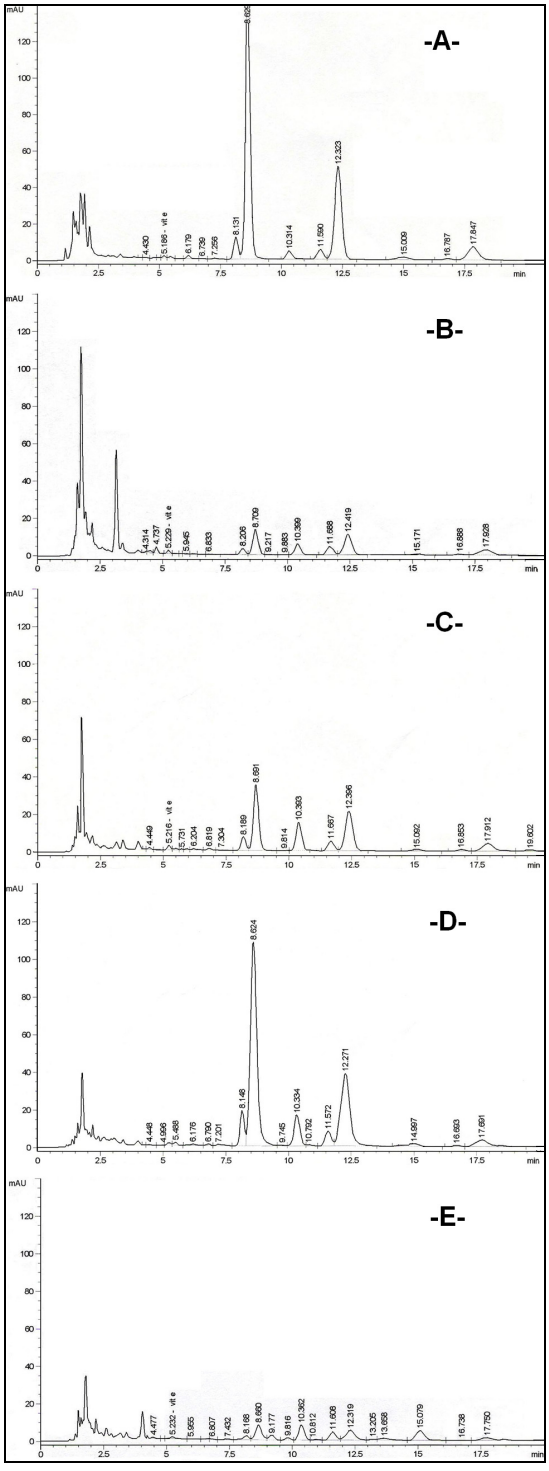

Fig. 2. Quantitative determination of α-tocopherol by HPLC-UV; **A**, *A. brevifolia*; **B**, *A. pseudochlorantha* var. *antalyensis*; **C**, *A. pseudochlorantha* var. *pseudochlorantha*; **D**, *A. purpurea* subsp. *apiculata*; **E**, *A. serotina*.

scavengers that terminate chain reactions in membranes and lipoprotein particles (Traber and Atkinson, 2007). The levels of ATOC (vitamin E) in medicinal plants would make them suitable sources of these antioxidants. Theu could be used commercially to retard rancidity in fatty materials, to reduce the effects of ageing and to help to prevent oxidative stress-related diseases such as cancer and heart disease (Dewick, 2002). Peaks obtained during quantitative determination of ATOC are given as Fig. 2.

The amounts of alpha-tocopherol in five *Asperula* taxa are presented in Table 3. *A. pseudochlorantha* var. *antalyensis* had the highest content of ATOC (0.66%) and *A. brevifolia* had the lowest (0.22%). Barros et al. (2010) reported that the ATOC contents of *Castanea sativa* and *Helichrysum stoechas* were 0.124 and 0.066%, respectively.

Proximate analysis assay

Proximate analysis was carried out on five endemic *Asperula* taxa. The results of proximate composition are presented in Table 4. *A. serotina* had the highest concentration of protein (6.13%), followed by *A. pseudochlorantha* var. *pseudochlorantha* and *A. purpurea* subsp. *apiculata*, while *A. pseudochlorantha* var. *antalyensis* had the least (3.85%). With respect to the moisture content, *A. serotina* had the highest value (7.48%) and *A. purpurea* subsp. *apiculata* the lowest (6.53%). *A. brevifolia* had the highest carbohydrate content (81.94%); ash was highest in *A. serotina* (5.70%). The ether extract (fat) values are between 3.71% (*A. brevifolia*) and 5.37% (*A. serotina* and *A. purpurea subsp. apiculata*) (Table 4).

The protein contents of the plants analyzed in this study were lower than those obtained in previous studies; Madibela et al. (2002) reported that the protein contents of *Tapinanthus lugardii*, *Viscum verrucosum* and *V. rotundifolium* are 11.9, 7.9 and 12.8% respectively. Also, the protein contents of *Centella asiatica*, *Erythrina crista* and *Lasia spinosa*, were as reported by Maisuthisakul

et al. (2008), i.e. 12.7, 24.2 and 17.9% respectively. In the same study, the ash contents were 12.6, 7.7 and 1.3%, the fat contents were 6.2, 5.1 and 3.8%, and the carbohydrate contents were 53.1, 40.3 and 45.5% respectively.

CONCLUSIONS

Antioxidant properties of plants are usually related to low-molecular weight compounds, in particular to the phenolic fractions. A wide range of these potentially beneficial phenolic compounds could be natural substrates of oxidative enzymes such as peroxidases or polyphenol oxidases that are present in high levels in some plants (Barros et al., 1997).

On the basis of the presented results, the extract of *Asperula* taxa could be of use as an easily accessible source of natural antioxidant and used for nourishment. However, at present, the active component in the extract which is responsible for the observed antioxidant activity is unknown. Therefore, further work, involving the isolation and purification of the active components from the crude extracts of *Asperula* taxa is required in order to examine the mode of action. As far as our literature survey could ascertain, there is no information about the antioxidant activities and chemical compositions of the *Asperula* taxa presented here.

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