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Antioxidant Activity Studies on Selected *Sideritis* Species Native to Turkey

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Abstract

Sideritis L. species are widely used as medicinal plants and as herbal teas in Turkey, in which 45 species of the genus are naturally found. The aim of this study was to determine the antioxidant activity of *Sideritis* species found in the Mediterranean region. In this study, the antioxidant activities of the lyophilized extracts obtained from aerial parts of 17 species (18 taxa) of *Sideritis* were compared, of which 15 taxa were endemic. The antioxidant activities of aqueous extracts were studied by two different techniques: qualitative DPPH (1,1-diphenyl-2-picrylhydrazyl radical) assay to detect the free radical scavenging activity and the TBA assay to detect liposome lipid peroxidation. All the extracts (except *S. erithrantha* subsp. *erithrantha*, *S. dichotoma*, *S. syriaca* subsp. *nusariensis*, *S. tmolea*) showed a strong antioxidant activity with the DPPH test. High activity was observed in the *S. brevibracteata* (IC_{50} mg/ml = 0.16), *S. condensata* (IC_{50} mg/ml = 0.33), *S. serratifolia* (IC_{50} mg/ml = 0.31) extracts with the lipid peroxidation assay method.

Keywords: Antioxidant, Lamiaceae, *Sideritis*, Turkey.

Introduction

The genus *Sideritis* L. (Lamiaceae) is represented by more than 150 species, distributed from Bahamas to Western China and from Germany to Morocco, and mainly found in the Mediterranean basin. The genus *Sideritis* was represented by 38 species in the 7th volume of *Flora of Turkey* (Davis, 1982), but this number has increased to 45 (53 taxa) by the year 2000 (Duman et al., 1995, 1998; Aytaç & Aksoy, 2000) of which 39 taxa are endemic in Turkey. Infusion of some *Sideritis* species are widely used in the treatment of gastrointestinal disorders, for the common cold, and as diuretics (Sezik & Ezer, 1983, 1988), as well as an herbal tea in folk medicine in Turkey (Baytop, 1999). Dried leaves and spikes or heads of *Sideritis* species are served as tea, instantly prepared by dipping one or two spikes and leaving them in a cup of hot water for half a minute or so, to extract the pleasant aroma (Baser, 1995).

Sideritis species contain flavonoids (Ezer et al., 1992; Gil et al. 1993), essential oils (Baser et al., 1997), diterpenes (Garcia-Granados et al., 1985), phenylpropanoid glycosides (Ezer et al., 1992), and iridoid glucosides (Ezer et al., 1995). Anti-inflammatory (Barberan et al., 1987; Alcaraz et al., 1989; Zarzuelo et al., 1993) and antibacterial (Ezer et al., 1994, 1995) activities of *Sideritis* species have been reported, but not many studies on their antioxidant activity have been reported (Alcaraz et al., 1990; Rios et al., 1992). This study was undertaken to investigate the antioxidant properties of freeze-dried extracts of the aerial parts of 17 species of *Sideritis*, as these may account for the beneficial properties traditionally ascribed to infusions.

Materials and Methods

Plant material

The aerial parts of *Sideritis* species used in this study were collected in the flowering season from the Mediterranean

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Table 1. Locations of *Sideritis* species.

Sideritis species	Location and voucher number
S. albiflora Hub.-Mor. (E)	C1 Muğla: 5 km from Muğla to Yatağan, 720–750 m, in macchie clearings, calcareous rocks, 23. 6. 2000, N 37° 13′, E 28° 19′: *Hayri Duman* 7270
S. arguta Boiss. & Heldr. (E)	C4 Antalya: Alanya, 24 km from Alanya to Hadim, 950–1000 m, in *Pinus brutia* clearings, calcareous bedrock, 26. 6. 2000, N 36° 30′, E 32° 11′: *Hayri Duman* 7294
S. brevibracteata P. H. Davis (E)	C3 Antalya: Alanya, Ulaş, 10 m, in macchie clearings, calcareous rocks, 22. 5. 2000: *Hayri Duman* 7147
S. condensata Boiss. & Heldr. (E)	C3 Burdur: Ağlasun, Ağlasun to Sagalassos, 1300–1400 m, in *Quercus coccifera* clearings, in calcareous habitat, 25. 6. 2000, N 37° 40′, E 30° 31′: *Hayri Duman* 7284
S. congesta P. H. Davis & Hub.-Mor.	C3 Antalya: Manavgat-Akseki road, around Hacıobası village, 50 m, in phrygana clearings, calcareous rocks, 26. 6. 2000, N 36° 44′, E 31° 36′: *Hayri Duman* 7290
S. dichotoma Huter (E)	A2 Kütahya: Domaniç, Domaniç-Darı Tepe, at firewatch tower, 1400–1450 m, in *Pinus nigra* clearings, calcareous rocks, 27. 7. 2000, N 39° 59′, E 29° 35′: *Hayri Duman* 8399
S. erythrantha Boiss. & Heldr. var. *Cedretorum* P. H. Davis (E)	C4 Antalya: Alanya, between Elmalısu and Gökbel Y., 1320 m, in *Pinus nigra* and *Cedrus libani* clearings, 16. 7. 2000, N 36° 37′, E 32° 20′: *Hayri Duman* 8391
S. erythrantha Boiss. & Heldr. var. *Erythrantha* (E)	C3 Antalya: Serik to Gebiz, Bozburun Da., Boğaz-Tozlu Çukur Y., 1450–1500 m, scree calcareous slopes, 30. 7. 2000, N 37° 21′, E 31° 02′: *Hayri Duman* 8409
S. huber-morathii Greuter & Burdet (E)	C6 Hatay: Antakya, Yayla Da. Road, Şenköy to Ziyare D., 900–1200 m, in machie clearings, calcareous rock, 11. 7. 2000, N 36° 02′, E 36° 06′: *Hayri Duman* 8353
S. leptoclada O. Schwarz & P. H. Davis (E)	C2 Muğla: Fethiye-Kaş road, 10th km, 300 m, serpentine rocks, in phrygana, 24. 6. 2000, N 36° 38′, E 29° 16′: *Hayri Duman* 7273
S. libanotica Labill. subsp. *libanotica*	C6 Hatay: Arsus, between Haymaseki and Avcılar suyu, 150 m, *Pinus brutia* forest, serpentine, 12. 7. 2000: *Hayri Duman* 8361
S. phrygia Bornm. (E)	B3 Afyon: Çay, Sultandağı, Çay to Yalvaç, 1300–1350 m, volcanic rocky slopes, 31. 7. 2000, N 38° 32′, E 31° 01′: *Hayri Duman* 8418
S. pisidica Boiss. & Heldr. var. *termessi* P. H. Davis (E)	C3 Antalya: Termessus National Park, 950–1000 m, in macchie clearings, calcareous rocks, 25. 6. 2000, N 36° 59′, E 30° 28′: *Hayri Duman* 7283
S. rubriflora Hub.-Mor. (E)	C4 İçel: Taşucu, 10 m, in machie clearings, calcareous rocks, 23. 5. 2000: *Hayri Duman* 7154
S. serratifolia Hub.-Mor. (E)	B4 Konya: 50 km from Konya to Beyşehir, 1350 m, *Pinus nigra* clearings, calcareous rocks, 31. 7. 2000, N 37° 52′, E 32° 01′: *Hayri Duman* 8417
S. sipylea Boiss. (E)	B1 Manisa: Sipil Da., 930–950 m, calcareous slopes, 22. 6. 2000, N 38° 34′, E 27° 24′: *Hayri Duman* 7261
S. syriaca L. subsp. *nusairiensis* (Post) Hub.-Mor.	C6 Hatay: Belen–Atik valley, 930–980 m, in *Pinus brutia* clearings, calcareous rocks, 12. 7. 2000, N 36° 30′, E 36° 13′: *Hayri Duman* 8357
S. tmolea P. H. Davis (E)	B2 İzmir: Ödemiş, Boz Da., (Tmolus), 1550– 1650 m, on rocky schistose slopes, 22. 6. 2000, N 38° 21′, E 28° 05′: *Hayri Duman* 7264

(E): Endemic.

region of Turkey, and details are given in Table 1. Voucher specimens are deposited in the Botany Department Herbarium at Gazi University (GAZI), Turkey.

Extraction and preparation of test solutions

Plant materials were dried at room temperature. For each species, 50 g of plant was subjected to extraction by refluxing with distilled water (600 ml) for 30 min, followed by filtration. The resultant extracts were then freeze-dried, and for each lyophylized product tested, seven different test solutions of the following concentrations were prepared: 1, 0.5, 0.25, 0.125, 0.0625, 0.031 mg/ml.

DPPH test for antioxidant activity

The DPPH assay was used as a rapid TLC screening method to evaluate the antioxidant activity of the freeze-dried extracts of *Sideritis* species due to free-radical scavenging. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), a purple-colored stable free radical, on reduction gives yellow-colored diphenylpicryl hydrazine. When it is sprayed onto a thin-layer chromatography (TLC) plate, any antioxidant compound is seen as a yellow zone on a purple background (Conforti et al., 2002). Using Wiretrol II micropipettes, 2 µl of a 1 mg/ml aqueous solution prepared from the lyophylized extracts was applied to the silica gel TLC plates (Merck, Darmstadt, Germany), which were then developed with ethyl acetate:formic acid:glacial acetic acid:distilled water (100:11:11:26) and sprayed with 0.2% DPPH solution in MeOH, left at 20°C, and examined at 30 min after spraying.

As parallel TLC studies, replicate plates were sprayed either with 1% vanillin in H_2SO_4 or with 0.5% diphenylboryloxyethylamine followed by 5% polyethylene glycol before observing under UV light at 365 nm.

Thiobarbituric acid (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 compounds to protect liposomes from lipid peroxidation. The TBA reaction is based on the fact that peroxidation of most membrane systems leads to the formation of small amounts of free malonaldehyde (MDA). One molecule of MDA reacts with two molecules of TBA to yield a colored product, which in an acidic environment absorbs light at 532 nm, and it is readily extractable by organic solvents. It can thus be measured and quantified spectrophotometrically, and the intensity of color is a measure of MDA concentration. Absorbance at 532 nm was measured by a Perkin Elmer Lambda 40 (Boston, MA, USA) UV-Vis spectrophotometer. The incorporation of any antioxidant compound in the mixture will lead to a reduction of the extent of peroxidation (Conforti et al., 2002) and hence a reduction in color formation and absorbance.

The lyophilized extracts of *Sideritis* species were tested in phosphate-buffered saline (5 mg/ml). Peroxidation of the samples was done by adding 0.1 ml $FeCl_3$ (1 mM) and 0.1 ml ascorbic acid (1 mM) followed by incubation at 37°C for 20 min. Ascorbic acid is a well-known antioxidant but also has pro-oxidant properties in the presence of certain transition metal ions, such as iron or copper. 2,6-Di-tert-butyl-4-methylphenol (BHT) in ethanol was added to prevent lipid peroxidation during the TBA test itself (Fernandez et al., 1997). Propyl gallate was used as a positive control in seven different concentrations ranging from 1 mg/ml to 6.4×10^{-5} mg/ml. Four replicate experiments were performed for each extract.

Percentage inhibition of lipid peroxidation was assessed by comparing the absorbance of the reaction mixture containing no inhibitor with that of the test extract reaction mixtures where the substance to be assessed was included. The absorbance readings of the extract alone and the liposomes alone were also taken into account as follows:

$$\%\ \text{inhinition} = 100 \times \frac{(\text{FRM} - \text{B}) - (\text{ET} - \text{B} - \text{EA})}{(\text{FRM} - \text{B})}$$

where FRM is the absorbance of the full reaction mixture (liposomes and iron source plus solvent water without the test substance), B is the absorbance of the blank mixture (liposomes only), ET is absorbance of the extract test mixture (full reaction mixture plus test substance), and EA is the absorbance due to the extract alone (Halliwell & Chirico, 1993). The half-maximal inhibitory concentrations (IC_{50}) of the *Sideritis* species were calculated by linear regression analysis using PRISM software.

Results

In the DPPH test, yellow zones were very prominent for extracts of *S. arguta*, *S. brevibracteata*, *S. condensata*, *S. leptoclada*, *S. libanotica* subsp. *libanotica*, *S. phyrigia*, *S. serratifolia*, and *S. spilea*, whereas extracts of *S. erythrantha* var. *cedretorum*, *S. dichotoma*, *S. syriaca* subsp. *nusariensis*, and *S. tmolea* gave only faint yellow zones.

After development on TLC, prominent yellow zones were seen only with extracts of *S. brevibracteata*, *S. phyrigia*, and *S. serratifoli*.

The antioxidant activities of the *Sideritis* species on liposomes obtained from the TBA test are given in Table 2.

Discussion

The results of the DPPH test demonstrate that lyophylized extracts of most of the *Sideritis* species have free-radical quenching activity. The extracts of *S. erythrantha*

Table 2. Antioxidant activities of the lyophilized extracts of *Sideritis* species in the TBA test.

Species	IC_{50} value (mg/ml) ± SD
Sideritis albiflora	10.2 ± 0.66
S. arguta	0.44 ± 0.23
S. brevibracteata	0.16 ± 0.02
S. condensata	0.33 ± 0.04
S. congesta	1.27 ± 0.05
S. dichotoma	0.61 ± 0.04
S. erythrantha var. *cedretorum*	14.62 ± 0.02
S. erythrantha var. *erythrantha*	2.81 ± 0.01
S. huber-morathii	30.8 ± 0.24
S. leptoclada	0.48 ± 0.05
S. libanotica subsp. *libanotica*	0.65 ± 0.01
S. phyrigia	0.75 ± 0.19
S. psidica var. *termesii*	12.37 ± 0.93
S. rubriflora	7.14 ± 1.9
S. serratifolia	0.31 ± 0.02
S. spilea	0.71 ± 0.05
S. syriaca subsp. *nusariensis*	2.28 ± 1.2
S. tmolea	15.33 ± 2.2
Propyl gallate	0.05 ± 0.01

var. *cedretorum*, *S. dichotoma*, *S. syriaca* subsp. *nusariensis*, and *S. tmolea* display little antoxidant activity.

In the TBA test, the antioxidant activity of water-soluble compounds was determined. Previous studies have shown that essential oils, diterpenes, flavonoids, phenylpropanoid glycosides, and iridoid glucosides are present in *Sideritis* species (Ezer & Akcos, 1995). When the plates were sprayed with 1% vanillin-H_2SO_4 and 0.5% diphenylboryloxyethylamine followed by 5% polyethylene glycol reagents; it was obvious that antioxidant compounds were mainly flavonoids together with phenylethanoid glycosides because of their fluorescent characteristics, the phenylethanoids giving a bright blue fluorescence in UV light (365 nm) before spraying and the flavonoids giving yellow and orange fluorescence in UV light (365 nm) after spraying (Wagner & Blatt, 1995). Previous investigations (Rios et al., 1992) have shown that hypolaetin-8-glucoside isolated from *S. javalambrensis* has antioxidant activity. The antioxidant activity of natural flavonoids is governed by number and location of their aromatic hydroxy groups, probably due to the loss of a proton and formation of a stable free intermediate because of resonance delocalization (Conforti et al., 2002).

In the TBA method, highest activity was observed with the *S. brevibracteata* extract (IC_{50} mg/ml = 0.16). This species is endemic in Alanya (Antalya) province and is widely used as an herbal tea in this province. *S. condensata* (IC_{50} mg/ml = 0.33) and *S. serratifolia* (IC_{50} mg/ml = 0.31) are species also widely used as herbal teas and have high antioxidant activity. However, results from the TBA experiment showed that the aqueous extracts of *Sideritis albiflora*, *S. erythrantha* var. *cedretorum*, *S. huber-morathii*, *S. psidica* var *termesii*, *S. rubriflora*, and *S. tmolea* have low antioxidant activity as compared with the other species. It is evident that herbal tea prepared from these species does not have much antioxidant activity.

Future studies to determine the identity of the constituents responsible for the antioxidant activity of *S. brevibracteata*, *S. condensata*, and *S. serratifolia* are in progress.

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