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ANTIOXIDANT PROPERTIES AND PHENOLIC COMPOSITION OF *Sideritis* SPECIES

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The antioxidant properties and phenolic composition of 27 Sideritis *species were studied. Plant samples were extracted with petroleum ether using a Soxhlet apparatus. The defatted plant materials were extracted with 70% methanol. Antioxidant activities of the extracts were measured using Fe^{+2} induced linoleic acid peroxidation, as indicated by thiobarbituric acid reactive substance (TBARS) production. Free radical scavenging activities were determined based on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). Results were compared with standard BHT. Total phenol concentration of the extracts was estimated with Folin-Ciocalteu reagent using gallic acid as standard, and phenolic components were quantified by HPLC-DAD.*

Key words: *Sideritis*, antioxidant, HPLC, free radical scavenging activity, lipid peroxidation, composition.

The genus *Sideritis* consists of 46 species and 53 taxa in Turkey. This genus is interesting because the ratio of endemism is very high (almost 80%) [1–3]. These species named "Dag cayi or Ada cayi" in Turkey, are widely used as herbal tea and folk medicine for treatment of cough and curing gastrointestinal disorders [4–6].

These species are attributed to have antispasmodic, anti-feedant, carminative, analgesic, nervous system stimulant, sedative, antitussive, stomachic, anticonvulsant, antibacterial, antiinflammatory, antimicrobial and antioxidant activities [7–16]. Most of the studies on *Sideritis* species, subject to the present study, comprise their essential oils, fatty oils, diterpenoids and flavonoids [17–22].

The objective of this work was to evaluate the antioxidant activity of the methanol extracts of 27 *Sideritis* species in different *in vitro* antioxidant test systems.

The extract yields and total phenol data for the methanol-soluble *Sideritis* extracts are shown in Table 1.

The amount of extractable components from different *Sideritis* plants ranged from 158 mg/g (*S. hololeuca*) to 279 mg/g (*S. germanicopolitana* subsp. *germanicopolitana*). The phenolic content, expressed as milligrams of gallic acid per gram of extract, in decreasing order was *S. scardica* subsp. *scardica* > *S. amasiaca* > *S. germanicapolitana* subsp. *viridis* > *S. cilicica* > *S. phlomoides* > *S. gulendamiae* > *S.huber-morathii* (from 402.5±2.5 $mg_{GAE}/g_{extract}$ to 321.1±0.5 $mg_{GAE}/g_{extract}$) (Table 1). Amounts of total phenols of the other species were found to be lower than 300 $mg_{GAE}/g_{extract}$.

For the identification of compounds in *Sideritis* extracts, high pressure liquid chromatography-diode array detection was used. The identification of phenolic compounds within the extracts based on their UV spectral data. Relative percentage of phenolic groups were calculated as benzoates, hydroxycinnamates, and flavonoids. In all the extracts except for *S. amasiaca*, *S. argyrea*, *S. brevidens*, and *S. niveotomentosa*, flavonoid derivatives were found as main the compounds (Table 1). The most active samples in both test systems, *S. amasiaca* and *S. germanicopolitana* subsp. *viridis*, contained the highest amount of flavonoids (36.2% and 48.5%, respectively) and hydroxycinnamic acid (41.9% and 32.5%, respectively) derivatives. Both groups of compounds are known strong free radical scavengers [23]. In previous investigations diterpenoids and flavonoids by HPLC methods were reported in *Sideritis* species [24–26]. However, no data are avaliable on the *Sideritis* species investigated here.

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TABLE 1. Collection Sites, Extract Yields, and Total Phenol Contents and HPLC Qualitative Data of *Sideritis* Species

Species	Collection sites	Yields, %	Total phenol [$mg_{GAE}/g_{extract}$]	HPLC qualitative data, %		
				benzoates	hydroxy-cinnamates	flavonoids
S. amasiaca Bornm.* (**A**)	A5: Corum	21.5	389.9±0.6[a]	-	41.9	36.2
S. argyrea P. H. Davis* (**B**)	C4: Antalya	31.2	262.8±7.4	-	46.0	14.8
S. armeniaca Bornm.* (**C**)	A7: Giresun	24.2	310.3±0.9	1.7	21.7	58.0
S. bilgerana P. H. Davis* (**D**)	C4: Icel	21.0	306.2±2.5	-	12.9	55.8
S. brevidens P. H. Davis* (**E**)	C4: Icel	18.0	272.4±7.9	-	37.6	10.9
S. caesarea H. Duman, Aytac & Baser* (**F**)	B6: Kayseri	22.4	264.4±5.5	-	19.2	58.4
S. cilicica Boiss. et Bal.* (**G**)	C5: Adana	23.8	382.1±4.5	0.6	12.8	50.8
S. dichotoma Huter* (**H**)	A2: Kutahya	18.2	193.5±7.1	0.5	4.4	69.9
S. erytrantha Boiss. & Heldr. var. *cedretorum** (**I**)	C4: Antalya	23.2	206.7±7.2	-	26.8	41.7
S. erythrantha Boiss. & Heldr. var. *erythrantha** (**J**)	C3: Antalya	20.6	252.8±4.2	-	21.1	26.6
S. galatica Bornm.* (**K**)	A4: Ankara	25.3	232.1±4.3	0.3	8.9	60.5
S. germanicopolitana Bornm. ssp. *germanicopolitana** (**L**)	A4: Cankiri	27.9	304.7±5.8	2.9	22.9	55.0
S. germanicapolitana ssp. *viridis* Hausskn. ex Bornm.* (**M**)	A4: Kastamonu	23.8	389.1±5.3	-	32.5	48.5
S. gulendamiae H. Duman & Karaveliogullari* (**N**)	B3: Eskisehir	23.4	321.5±4.9	-	16.3	63.7
S. hololeuca Boiss. & Gueter.* (**O**)	C4: Icel	15.8	234.5±9.3	-	17.7	46.9
S. huber-morathii gueter & Burdet* (**P**)	C6: Hatay	18.4	321.1±0.5	-	5.9	71.6
S. libanotica Labill. ssp. *linearis* (Bentham) Bornm.* (**Q**)	C4: Karaman	23.4	265.2±7.0	-	17.2	37.8
S. libanotica Labill. ssp. *kurdica* (Bornm.) Hub.-Mor.* (**R**)	C7: Mardin	26.0	288.3±1.0	-	14.9	43.3
S. niveotomentosa Hub.-Mor.* (**S**)	C4: Icel	17.8	252.8±4.2	-	41.9	32.6
S. ozturkii Aytac & Aksoy* (**T**)	C3: Konya	27.0	265.5±1.1	-	18.2	55.2
S. phlomoides Boiss. et Bal.* (**U**)	B5: Kayseri	25.3	325.2±3.2	0.6	29.9	53.8
S. phrygia Bornm.* (**V**)	B4: Afyon	26.2	328.9±4.9	-	26.6	51.3
S. scardica Griseb. ssp. *scardica* (**W**)	A1: Kirklareli	27.0	402.5±2.5	0.2	11.9	53.4
S. serratifolia Hub.-Mor.* (**X**)	B4: Konya	25.2	353.3±3.4	-	24.7	57.4
S. taurica Stephan ex Willd. (**Y**)	A4: Karabuk	27.5	328.3±8.8	0.4	29.4	38.1
S. vulcanica Hub.-Mor.* (**Z**)	B7: Elazig	27.7	191.6±0.9	0.6	17.5	49.2
S. vuralii H. Duman & Baser* (**ZT**)	C4: Icel	16.8	198.6±6.3	-	16.4	54.9

*Endemic, [a]Values are expressed as mean±standard deviation.

As can be seen in Fig. 1, a linear relationship was found between the total phenol content and free radical scavenging activities. The lowest IC_{50} indicated the highest free radical scavenging activities. Free radicals are involved in the process of lipid peroxidation and are considered to play a major role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others, and are implicated in the aging process. Therefore, the extracts were assessed against DPPH˙ radicals to determine their free radical scavenging properties. In this assay, the DPPH˙ radical serves as the oxidizing substrate, which can be reduced by an antioxidant compound to its hydrazine derivative *via* hydrogen donation, and as the reaction indicator molecule. IC_{50} values of *S. amasiaca*, *S. cilicica*, and *S. taurica* (4.6±0.6, 4.5±0.9, 4.4±0.4 mg/ml) were found to be similar to BHT (4.3±0.3 mg/ml). In addition, the free radical scavenging activities of the other species, except for *S. vulcanica*, *S. vuralii*, *S. erytrantha* var. *cedretorum*, and *S. hololeuca*, were similar but lower than that of BHT. The correlation between free radical scavenging activities and total phenolic contents of the extracts is shown in Fig. 1.

The antioxidant activities of methanol extracts from *Sideritis* species at concentration of 0.02% and 1% on linoleic acid peroxidation with Fe^{+2} induced linoleic acid peroxidation-TBA reactive substances were investigated, and the results are shown in Fig. 2. A higher inhibitory concentration indicates higher antioxidant activity. In all extracts, antioxidant activity increased with increasing concentration. According to Fig. 2, inhibition percentage of all extracts except *S. armeniaca* (21.1%), *S. hololeuca* (19.2%), and *S. libanotica* subsp. *linearis* (24%) were lower than BHT (24.6%) at a concentration of 0.02%. On the other hand, antioxidant activity of BHT increased with increasing concentration (74.8%). None of the extracts showed activity stronger than BHT at a concentration of 1% whereas *S. amasiaca* (61.2%) and *S. germanicopolitana* subsp. *viridis* (60.6%) have shown similar activities.

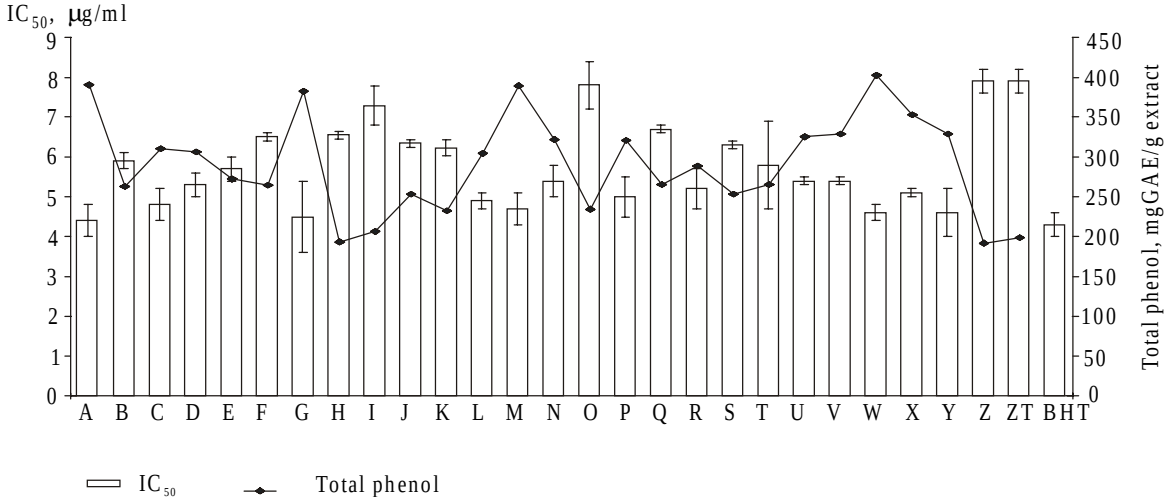

Fig. 1. Free radical scavenging activities and total phenol contens of the extracts.

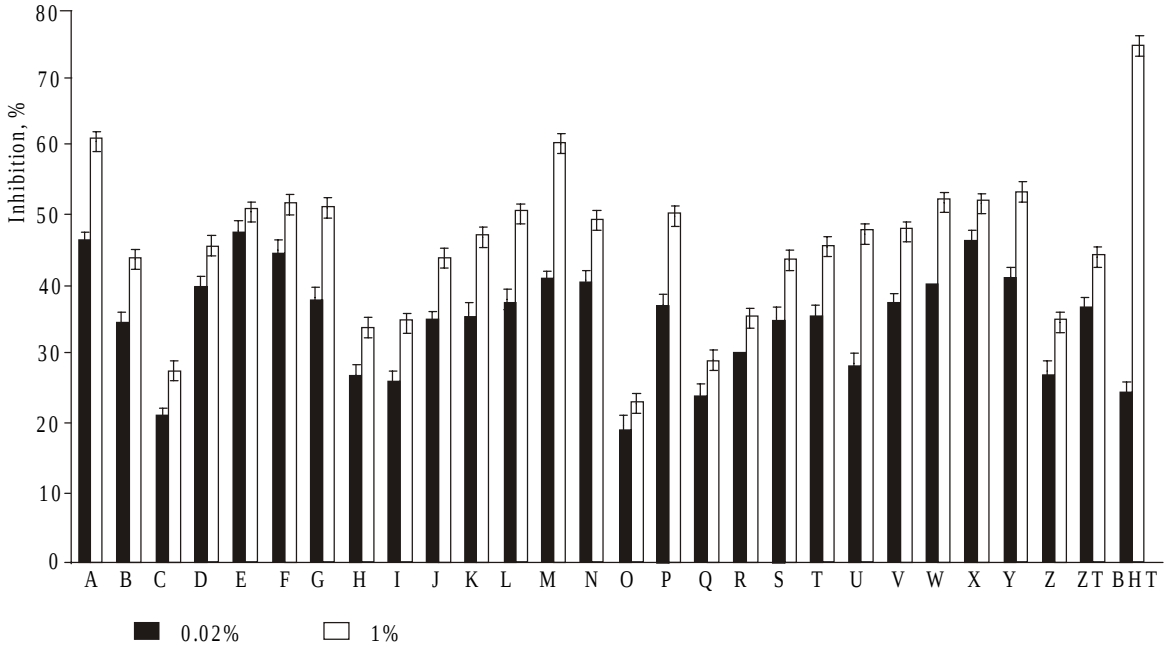

Fig. 2. Effect of Sideritis extract on lipid peroxidation at concentrations of 0.02% and 1%.

Here, we report for the first time on the antioxidant activity of 27 species. These species except for *S. scardica* ssp. *scardica* and *S. taurica* are endemic species.

The following activities were reported previously for some *Sideritis* species investigated here. *S. libanotica* ssp. *linearis*, antibacterial and anti-inflammatory activity [15]; *S. libanotica* ssp. *kurdica*, antistress activity [8]; *S. niveatomentosa*, *S. brevidens*, and *S. gulendamiae*, antifeedant activity [10]; and *S. argyrea*, antimicrobial, antibacterial, and antiinflammatory activity [16]. Antioxidant activity of the different polarites of extracts of *S. scardica* seeds was investigated by three method: (DPPH˙) free radical scavenging method, static headspace gas chromatograpty (HS-GC), and β-carotene bleaching test (BCBT). The sample polarity was important for the exhibited activity in the HS-GC and BCBT methods but not for the DPPH method [14].

The results in this study clearly indicate that *S. scardica* subsp. *scardica*, *amasiaca*, *germanicopolitana* subsp. *viridis*, *germanicapolitana* subsp. *germanicapolitana*, *cilicica*, *serratifolia*, *taurica*, *huber-morathii*, and *armeniaca* showed remarkable antiradical and lipid peroxidation activity. In addition, the highest amounts of total phenol were also obtained from these species. A linear correlation was found between total phenol contents and antioxidant activities in the extracts.

EXPERIMENTAL

Plant Materials and Reagents. Aerial parts of the plant materials were collected from different regions of Turkey. Voucher specimens are kept at the Herbarium of the Faculty of Pharmacy, Anadolu University, Eskisehir. Information on collection sites is given in Table 1. Butylated hydroxytoluene (BHT), linoleic acid, and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^{\cdot}$) were purchased from Aldrich (Steinheim, Germany). All the solvents used in experimental work were analytical grade.

Extraction Procedure. Powdered samples were extracted with petroleum ether to remove fats. Fat-free materials were air dried and macerated in a water bath with aqueous methanol. Methanol (%70, v/v) was evaporated under vacuum at 40°C and the remaining aqueous fraction was freeze dried and the residue used for determine the extract yields, total phenolic contents, and their antioxidant activities.

Determination of Total Phenolics. The total phenolics were determined colorimetrically according to the Folin-Ciocalteaeu method [27]. Gallic acid was used as a standard. Samples (0.5 ml, 0.5 mg/ml) were introduced into a test tube; 2.5 ml Folin-Ciocalteaeu reagent solution (10% h/h in water) and 7.5 ml sodium carbonate solution (%20'lik, a/h, water) were added. The tubes were shaken and allowed to stand at room temperature in the dark for 2 hours. Absorption was measured at 750 nm. Total phenol content was expressed as gallic acid equivalents (GAE) in ($mg_{GAE}/g_{extract}$) dry material. The results are expressed as the average of five measurements.

High Performance Liquid Chromatography (HPLC) Set-up. The liquid chromatographic apparatus (Shimadzu LC 10A*vp*) consisted of an in-line degasser, pump and controller coupled to a photodiode array detector (Shimadzu SPD 10A*vp*) equipped with an automatic injector (20 mL injection volume) interfaced to a PC running Class VP chromatography manager software (Shimadzu, Japan). Separations were performed on a reverse-phase C18 Ultrasphere analytical column (250× 4.6 mm *i.d.*, particle size 5 mm , Beckman) operating at room temperature with a flow rate of 1 mL/min. Detection was carried out with a sensitivity of 0.1 a.u.f.s. between the wavelengths of 200 to 550 nm. Elution was effected using a nonlinear gradient of the solvent mixture MeOH:H_2O:CH_3COOH (10:88:2, v/v/v) (solvent A) and MeOH:H_2O:CH_3COOH (90:8:2, v/v/v) (solvent B). The composition of B was increased from 0% to 15% in 15 min, increased to 50% in 5 min, increased to %70 in 9 min, then increased to %100 in 6 min, held for 10 min, and finally decreased to 0% in 1 min and held for 4 min. Components were identified by comparison of their retention times to those of authentic standards under analysis conditions and UV spectra with our in-house PDA library. A 10 min equilibrium time was allowed between injections.

1,1-Diphenyl-2-picrylhydrazyl (DPPH^{+}) Scavenging Activity. Free radical scavenging effects of the extracts on DPPH$^{\cdot}$ (1,1-diphenyl-2-picrylhidrazyl) were estimated according to the method of Sanchez-Moreno and co-workers as follows [28]: 0.5 ml of sample solution (approx. 40 mg/ml) was introduced into a test tube, and 3ml of DPPH$^{\cdot}$ solution (20 mg/mL) was added. The mixture was shaken and left standing at room temperature for 30 min. Absorbance of the resulting solutions was measured spectrophotometrically at 517 nm. Concentration range of DPPH$^{\cdot}$ used in calibration was 4.0 – 20.0 mg/mL. The calibration equation was A_{517nm}(mg/mL) = 28.717′(DPPH) + 1.9×10^{-3} (r = 0.9999). IC_{50} values of extracts were calculated by using the calibration equation and expressed in mg/ml.

Antioxidant Activity in Linoleic Acid System. Antioxidant activity of the extract was determined using the Fe^{+2} induced-linoleic acid-TBA peroxidation method [29]. The sample was mixed with linoleic acid emulsion (0.25 mL, 0.1 M, in a mixture of phosphate buffer and ethanol 1:1) at the level of 1% and 0.02% (w/w) of linoleic acid. Following the addition of 0.1 ml $FeCl_2$ (1 mM) solution the mixture was incubated at 40°C for 24 h. Then, HCl (0.5 mL, 0.1 M), sodium dodecyl sulfate (0.2 mL, 9.8%), water (0.9 mL), and TBA (2 mL, 0.6%) were added to the mixture. The mixture was heated at 80°C in a water bath for 30 min. After cooling for 10 min in an ice-bath the solution was measured spectrophotometrically at 532 nm. The values are presented as the mean of 5 measurements.

A low absorbance value indicated a high antioxidant activity. Percent inhibition of linoleic acid peroxidation was calculated from the following equation:

$$\text{Percentage inhibition} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100.$$

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