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Determination of total phenolic content, total flavonoid content and total antioxidant capacity in some endemic *Sideritis* L. (Lamiaceae) species grown in Turkey

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ABSTRACT: In this study, total phenolic, total flavonoid and antioxidant activities of the some endemic species *Sideritis rubriflora* Hub.-Mor., *Sideritis libanotica* Labill. subsp. *violascens* (P.H.Davis) P.H.Davis, *Sideritis erythrantha* Boıss. Et Heldr. Apus Bentham var. *cedretorum* P.H.Davis, *Sideritis congesta* P. H. Davis Et Hub.-Mor., *Sideritis brevidens* P.H.Davis and *Sideritis vuralii* H. Duman Et Başer, which were collected from Anamur district of Mersin province in Turkey, were analyzed. Total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (DPPH, ABTS, FRAP) of the ground surface parts were evaluated. As a result of the study, the highest TPC value was observed in *S. erythrantha* subsp. *cedretorum* and *S. rubriflora* extracts as being 366.9 and 328.3 mg/g DW, respectively; the highest TFC value was observed in *S. rubriflora* extract as being 155.7 mg/g; the highest DPPH radical scavenging activity was observed in *S. congesta* and *S. brevidens* extracts as being 39.1% and 38.9%, respectively; the highest ABTS radical scavenging activity was observed in *S. erythrantha* subsp. *cedretorum* and *S. rubriflora* extracts as being 54.9% and 51.9%, respectively; the highest FRAP value was observed in *S. libanotica* subsp. *violascens* extract as being 1500.2 µmol/g. In the light of the acquired findings, it is suggested that *Sideritis* species used in the study can be used as a possible natural source in the pharmaceutical and food industries.

Keywords: *Sideritis*; Antioxidant; Total flavonoid; Total phenolic; Turkey.

1. INTRODUCTION

Turkey, located in the mild regions of the world, has quite a rich diversity of habitats due to geomorphological, topographic and climatic characteristics [1]. One of the reasons for the presence of such a rich floristic diversity in Turkey is that Turkey is located at the intersection of three phytogeographic regions such as Mediterranean, Europe-Siberia and Iran Turan [2]. The *Lamiaceae* family encompasses important aromatic plants consisting of approximately 7173 species and 236 genera. These plants are used in the traditional and modern medicine, pesticides industries, food, cosmetic and pharmaceutical industry and they contain a high volatile oil ratio [3-6]. The genus *Sideritis,* a member of the *Lamiaceae* family, has more than

150 species annual and perennial herbs and small shrubs distributed in the temperate and tropical regions of the Northern Hemisphere [7-9]. In the Flora of Turkey *Sideritis* L. genus is represented by 46 species and together 55 taxa, 42 taxa of which being endemic [10]. *Sideritis* species are calcicolous and heliophilous plants that usually grow in dry and semi-arid regions [8]. This genus contains antimicrobial and antioxidant polyphenolics such as flavonoids [11]. *Sideritis* species are widely used in the treatment of gastrointestinal disorders and coughs, colds and diuretic therapy, also in herbal teas and folk medicine in Turkey [12,13]. Plant chemical compounds are classified as primary and secondary metabolites according to their metabolic pathway and functions [14]. Free radicals play an important role in the pathogenesis of various diseases and therefore antioxidants have an important role in preventing diseases [15]. Phenolic substances found in plants are bioactive compounds that are important antioxidant sources [16]. Flavonoids composed a large group of polyphenolic compounds that have a benzo-p-pyron structure and are ubiquitous in plants [17]. In this study, total phenolic and total flavonoid content, and antioxidant activities of some *Sideritis* species spreading in Anamur/Mersin/Turkey were analyzed.

2. MATERIALS AND METHODS

2.1. Plant materials

In this study, *Sideritis rubriflora, S. libanotica* subsp. *violascens, S. erythrantha* var. *cedretorum, S. congesta, S. brevidens* and *S. vuralii* species were collected from Anamur district of Mersin/Turkey province in 2017 (Figure 1) and moved to the herbarium. Specimens were identified and prepared for experimental study.

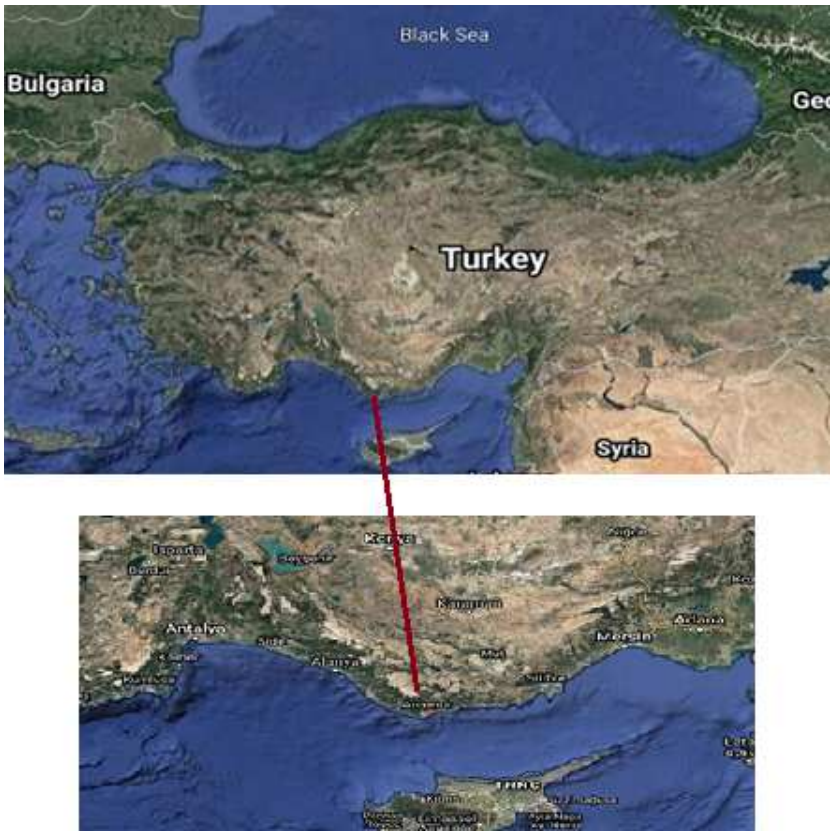

Figure 1. Location of the Mersin/Anamur (https://www.google.com/maps).

2.2. Extraction

Dried aerial parts were ground with an electrical blender. The powdered aerial parts (30 g) were placed in Soxhlet apparatus, and extraction was performed in 300 mL of methanol (polarity index: 5.1) for 6 h. The solution was filtered and concentrated at 40°C by vacuum (SCILOGEX RE100-Pro, USA). Extracts were frozen (–18°C) until used for determining the TPC, TFC and antioxidant activities.

2.3. Total Phenolic Content (TPC)

TPC of *Sideritis* sp. extracts was determined using Folin-Ciocalteu procedure described by Spanos and Wrolstad [18] with slight modifications. The absorbance was measured by UV-Vis spectrophotometer (UNICO S1205, USA) at 765 nm. The results are expressed as milligrams of gallic acid equivalents (GAE) per g of dry weight (DW).

2.4. Total Flavonoid Content (TFC)

TFC of extracts was detected using spectrophotometric method described by Quettier et al. [19]. The absorbance was measured at 415 nm. The results are expressed as milligrams of quercetin equivalents per g of dry weight (DW).

2.5. Antioxidant activity

According to 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3 ethylbenzothiazoline-6-sulfonic acid (ABTS), and Ferric Reducing Antioxidant Power (FRAP) methods antioxidant activity of extracts was determined.

2.6. DPPH method

The diluted extract (50µL) was mixed with DPPH solution (950 µL, 0.1 N). The mixture was placed in a shaker at room temperature in the dark for 30 min. The sample was then measured at 515 nm by UV-Vis spectrophotometer. The inhibition of the DPPH radical by the sample was calculated using the formula: (Absorbance control-absorbance sample/Absorbance control)×100 [20].

2.7. ABTS method

This assay was carried out as described earlier [21]. ABTS solution and 2.45 mM potassium persulfate solution were stirred (1:1 v/v). The mixture was left for 12-16 h at room temperature in the dark. After an absorbance value of 0.70 at 734 nm was reached, the mixture was diluted with methanol. The diluted extract (0.15 mL) was then mixed with 2.85 mL of diluted ABTS solution followed by incubation for 2 h at room temperature in the dark. Absorbance was measured at 734 nm by spectrophotometer. Percentage of ABTS was calculated using the formula: (Absorbance control–absorbance sample/Absorbance control)×100.

2.8. FRAP method

FRAP assay was performed according to the procedure described by Benzie and Strain [22] with slight modifications. To prepare the FRAP reagent, 25 mL of 300 mM sodium acetate buffer (pH 3.6), 2.5 mL of 2,4,6-tripiridil-s-triazin (TPTZ), 10 mM of 40 mM HCl, and 2.5 mL of iron(III) chloride hexahydrate ($FeCl_3$ $6H_2O$) (20 mM) were mixed. The initial absorbance value of 900 µL of reagent was measured at 593 nm. The diluted extract (20 µL) and 2.98 mL of FRAP reagent were mixed followed by incubation for 10 min at room temperature. Absorbance was measured at 593 nm using spectrophotometer. The ferric ion reducing ability of

extracts was determined using the calibration curve and reported as µmol of $FeSO_4$ equivalents per gram of sample.

3. RESULTS AND DISCUSSION

Natural products derived from plants provide many opportunities for new medicines [23,24]. Phenolic compounds, also known as secondary metabolites, are among the most important and functional components produced by plants. These components are involved in activities such as color formation, taste formation, aroma formation, and plant defense systems in plants [25]. The amount of phenolic compounds found in plants varies depending on the variety, soil structure, habitat, climatic and seasonal characteristics [26]. The results of TPC and TFC of *Sideritis* extracts are presented in Table 1.

Table 1. Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities of endemic *Sideriti*s species.

***Sideritis* species**	**TPC (mg/g)**	**TFC (mg/g)**	**DPPH (%)**	**ABTS (%)**	**FRAP (µmol/g)**
S. rubriflora	328.3±14.8^{a}	155.7±38.9^{a}	18.3±4.3^{c}	51.9±5.2^{a}	1160.3±29.2^{d}
S. libanotica subsp. *violascens*	172.3±13.5^{c}	74.8±1.9^{c}	33.3±1.6^{b}	43.6±2.5^{b}	1500.2±38.6^{a}
S. erythrantha var *.cedretorum*	366.9±19.4^{a}	93.5±8.4^{b}	21.8±3.8^{c}	54.9±1^{a}	970.8±5.8^{e}
S. congesta	52.5±2.7^{e}	31.7±4.7^{d}	39.1±8.2^{a}	12.5±1.7^{e}	1390.3±20.1^{b}
S. brevidens	205.5±12.8^{b}	105.9±4.4^{ab}	38.9±9.9^{a}	42.8±1.1^{b}	1170.2±18.4^{d}
S. vuralli	35.5±2.9^{f}	14.2±0.9^{f}	32.8±5.5^{b}	18.9±2.9^{d}	1230.8±5.6^{c}

All values a represented as means ±SD (n = 3). Different letters (a-f) within the columns indicate statistically significant differences by Duncan's multiple range test at $p<0.05$.

Statistically significant differences among samples were observed ($p<0.05$). The highest TPC value was detected in *S. erythrantha* subsp. *cedretorum* and *S. rubriflora* extracts as being 366.9 and 328.3 mg/g DW, while the lowest TPC value was determined in *S. rubiflora* as 328.3 mg/g DW.

It was determined that TFC values of samples vary between 14.2 mg/g DW (*S. vuralli*) and 155.7 mg/g (*S. rubriflora*). Gökbulut et al. [27] reported that the amount of total phenolic matter in the methanol extracts of the ground surface parts of *Sideritis argyrea, S. congesta* and *S. erythrantha var. cedretorum* species obtained from local markets varied between 121.7 and 190.8 mg GA/g DW, and that the highest TPC value was observed in *S. erythrantha var. cedretorum*. Radojevic et al. [28] reported that TPC amount in methanolic extract of *S. montana L.* species was found as 97.85 mg/g, and TFC amount was found as 159.54 mg/g. Also, Tadić et al. [29] reported that TPC amount in ethanolic extracts of *S. scardica* Griseb was found as 188.5 mg/g. Alipieva et al. [30] found out that in the tea of *S. scardica x S. syriaca* hybrid plants, TPC content was 32.2 mg/g, and TFC content was 9.6 mg/g. Nakiboğlu et al. [31] reported that TPC value was 0.089 µg (GAE/µg extract) in the methanolic extract of *S. spylea* species. Tunalıer et al. [32] reported that TPC values varied between 191.6 and 402.5 mg/g among 27 *Sideritis* species. Sağdıç et al. [33] reported TPC values as 39.35 and 93.79 mg/g in two endemic *Sideritis* species. In general, the results we obtained in our study are similar to the results reported in the literature. In the study, by using 3 different methods, antioxidant activity values of plant extracts were determined. Antioxidant capacity results of samples are given in Table 1. According to this, DPPH radical scavenging activity of the samples ranged from 18.3 to 39.8%. The highest DPPH radical scavenging activity was observed in *S. congesta* and *S. brevidens* extracts as being 39.1% and 38.9%, and the lowest activity was observed in *S. brevidens* extracts as being 38.9. ABTS radical scavenging

activity results of plant extracts were in parallel with TPC and TFC results. The highest ABTS radical sweep activity was observed in *S. erythrantha* subsp. *cedretorum* and *S. rubriflora* extracts as being 54.9% and 51.9%, respectively; the highest FRAP value was observed in *S. libanotica* subsp. *violascens* extract as being 1500.2 µmol/g. Gökbulut et al. [27] reported that the highest antioxidant activity in the methanol extracts of *Sideritis argyrea, S. congesta* and *S. erythrantha* var. *cedretorum* species was observed in *S. erythrantha* var. *cedretorum*. Sağdıç et al. [33] reported that DPPH radical sweep activities of *S. ozturkii* and *S. caesarea* plant extracts were 41.68% and 72.47%, respectively. Koleva et al. [34] reported that the radical sweep activity in the extracts of *Sideritis scardica, S. syriaca* and *S. montana* which were obtained by using different solvents was above 90%. The differences between the results we obtained in our study and those reported in the literature may be due to differences in species, methods, growing conditions or solvent.

5. CONCLUSIONS

As a result, the maximum TPC value in this study; in *Sideritis erythrantha* subsp. *cedretorum* and *S. rubriflora* species, the highest TFC value was in *S. rubriflora* species detected. It has been determined that antioxidant activity values vary according to the method and species. It has been explained that *Sideritis* taxa can also be used in the pharmaceutical industry due to their TFC, TPC and antioxidant activities.

Authors' Contributions: İG: Collecting plant samples. ZTM: Determination of total phenolic content, total flavonoid content and total antioxidant capacity. ES and ZTM: wrote the manuscript. All authors interpreted the results. All authors read and approved the final manuscript.

Conflict of Interest: The authors have no conflict of interest to declare.

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