

## Biological activities and phytochemical investigation of some *Sideritis* species

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One of the most generally used plants for herbal tea is *Sideritis* L. species (Lamiaceae) which is commonly grown in the Mediterranean region and represented by 46 species and 53 taxa in Türkiye. *Sideritis* species are widely used in Türkiye due to their antispasmodic, antirheumatic, anti-inflammatory, and diuretic activities. In the present research, the effects against oxidative stress, and enzyme activity, as well as chemical compositions of the water and methanol extracts of seven *Sideritis* species (*S. bilgeriana* P.H. Davis), *S. brevidens* P.H. Davis, *S. cilicica* Boiss. & Balansa, *S. erythrantha* Boiss. & Heldr., subsp. *cedretorum* P. H. Davis, *S. hololeuca* Boiss. & Heldr., *S. libanotica* Labill. subsp. *linearis* (Benth.) Bornm., and *S. libanotica* Labill. subsp. *violascens* P. H. Davis.). Its main objective was to assess the suppression of five enzymes, tyrosinase,  $\alpha$ -glucosidase,  $\alpha$ -amylase, acetylcholinesterase (AChE), and butyrylcholinesterase, for the water and methanol extracts of seven different *Sideritis* species. The extracts were also assessed for antioxidant effects using various spectrophotometric methods involving DPPH, ABTS<sup>+</sup>, and iron chelating, as well as the determination of total phenol and flavonoid amounts. As for the phytochemical investigation on *Sideritis* species, the phenolic compounds of the extracts were determined by LC-MS/MS analysis. Because of the LC-MS/MS analysis, Hierarchical Cluster Analysis was performed on four major components: quinic acid, chlorogenic acid, fumaric acid, and acacetin in the present work. As a result, our research indicates that *Sideritis* species, particularly *S. cilicica*, *S. erythrantha*, and *S. libanotica* subsp. *linearis*, deserve to be examined afterwards for their active secondary metabolites, which are thought to be responsible for their potential biological activities. Furthermore, the composition of extracts of *Sideritis* species was statistically analysed using the principal component.

**Keywords:** Antioxidant activity, Enzyme inhibitory effect, LC-MS/MS, Phenolic compounds, *Sideritis*

**IPC Code:** Int Cl.<sup>24</sup>: A61K 36/00, A61K 36/53

The genus *Sideritis* L. (Lamiaceae family) is an annual or perennial shrub, mainly distributed in the Western Palearctic and Mediterranean Regions. It is represented by 150 species in the world. 39 taxa of them are endemic in Türkiye<sup>1,2</sup>. It is referred to as "dağçayı" (mountain tea) and "adaçayı". Infusions and decoctions of its aerial parts have been used for therapeutic purposes since ancient times in addition to serving as a relaxing tea or flavoring ingredient<sup>3</sup>. They are traditionally used to treat stomach disorders, and cough as well as wound healing in Türkiye<sup>4</sup>. The literature report that various *Sideritis* species contain a range of compounds, including essential oils, terpenes, flavonoids, iridoids, coumarins, sterols, and lignans<sup>5</sup>. Previous biological activity studies on extracts or secondary metabolites from a variety of *Sideritis* species indicated antioxidant, antifeedant,

antinociceptive, antiulcer, antiviral, antimicrobial anti-inflammatory, and anticholinesterase properties<sup>1,5-9</sup>. *Sideritis* species have been used in folk medicine for their antimicrobial, antiulcerogenic, and anti-inflammatory properties, as well as in gastritis, gastric ulcer, common cold and flu in the world. Numerous studies have suggested a potential link between this plant's beneficial effects on a number of prevalent illnesses<sup>1,6,7,9</sup>.

Many disorders in brain and heart, Parkinson's disease, arthritis, and diabetes are caused by antioxidant deficiency or failure of physiological antioxidant defense mechanisms<sup>10</sup>. Besides the plant spices are high in bioactive chemicals that fight against oxidative stress and inhibit digestive enzymes linked to the development of diabetes and cardiovascular disease; Therefore, they were

commonly used as food supplements<sup>11</sup>. Phenolic compounds play a crucial role in promoting health by acting as potent antioxidants that help combat oxidative stress and protect cells from damage<sup>12</sup>. Antioxidants and glucosidase inhibition play important roles in diabetes management, particularly in terms of managing blood sugar levels and reducing the risk of complications associated with diabetes<sup>13,14</sup>.

Many investigations previously concentrated on antibacterial, antioxidant, and cytotoxic activities on diverse extracts, and chemical profiles of essential oils for various *Sideritis* species<sup>1,15-21</sup>. The aim of this study was to define the phytochemical compositions of seven *Sideritis* species and assess their biological activities. Statistical analysis were conducted using the four major compounds identified in *Sideritis* extracts.

## Materials and Methods

### Botanical specimens

The flowering aerial parts of *Sideritis* species were harvested from natural habitats and characterized by a specialist in botany, Prof. Dr. Yavuz Bağcı. The specimens were stored at the Herbarium of Necmettin Erbakan University.

### Preparation of extracts

The air-dried aerial parts of *Sideritis* species were ground into powder and subjected to methanol three times through extraction at room temperature. The resulting macerates were then combined, filtered, and dried in a rotary evaporator at 40°C with low pressure. Afterwards distilled water was added to the remaining plant pulp and similar processes were performed for three times to obtain water extracts. Until the studies were completed, each of the extracted materials were kept in a deep freezer at -80°C.

### Total phenolic content (TPC)

The total phenolic content of the extracts was assessed using the Folin-Ciocalteu method<sup>22</sup>. The TPC was calculated as mg gallic acid equivalents/g of dry extract.

### Total flavonoid content (TFC)

The total flavonoid content was measured using the aluminum chloride colorimetric assay<sup>23</sup>. The flavonoid levels were quantified in milligrams of quercetin equivalents per gram of the sample's dry weight, employing an equation derived from a standard quercetin calibration curve.

### *In vitro* antioxidant activity

The capacity of different fractions to scavenge the stable DPPH· free radicals was measured using the method described by Clarke *et al.*,<sup>22</sup>. In 96-well plates, 20 µL of the extract was mixed with 180 µL of freshly prepared 0.04 mg/mL DPPH· methanol solution. After incubating in the dark for 15 min at 25°C, the absorbance was measured at 540 nm using an ELISA reader. The ABTS<sup>•+</sup> cation radical decolorization method was used to determine the capacity for radical scavenging with just a few changes<sup>24</sup>. To prepare the ABTS<sup>•+</sup> stock solution, a 7 mM ABTS<sup>•+</sup> solution was mixed with a 2.4 mM potassium persulfate solution in equal volumes and kept in the dark for 16 h. The working solution was obtained by diluting the stock to an absorbance of 0.70±0.02. Then, 50 µL of the extract was combined with 100 µL of the ABTS<sup>•+</sup> solution and left for 10 min. The absorbance was measured at 734 nm. All tests were performed in triplicate, with results reported as mean±standard deviation.

The previously published analyze was used to evaluate the extracts' capability to chelate ferrous ions<sup>25</sup>. 50 µL of extract were combined with 50 µL of FeSO<sub>4</sub> (0.1 mM) and 100 µL of ferrozine. The absorbance was measured at 562 nm after a 10 min incubation period. EDTA was used as the positive control.

### Enzyme inhibitory activity

The tyrosinase inhibitory activity was measured using the previously described spectrophotometric method with minor modifications<sup>26</sup>. To initiate the reaction, 100 µL of the extracts were mixed with 100 µL of phosphate buffer (0.1 M, pH = 6.8), followed by the addition of 20 µL of tyrosinase (250 U/mL) from Sigma-Aldrich. The plate was then allowed to incubate for approximately 10 min at 25°C. Subsequently, 20 µL of 3 mM L-tyrosine was introduced as the substrate and the mixture was further incubated for 30 min at the same temperature. The absorbance was measured at 492 nm to assess the enzymatic activity. Kojic acid (Acros) served as the positive control in this experiment. The extracts' α-glucosidase inhibitory activities have been measured with a little alteration utilizing the recently published approach<sup>27</sup>. Briefly, in a 96-well plate, 120 µL of 0.1 M phosphate buffer at pH 6.9, along with 10 µL of the test material and 20 µL of α-glucosidase (0.5 U/mL) from Sigma-Aldrich at various concentrations were combined. This mixture was then incubated for

15 min at 37°C. The enzymatic reaction commenced by adding 20 µL of a 5 mM solution of p-nitrophenyl-D-glucopyranoside (pNPG) from Sigma-Aldrich after the preincubation period, followed by another 15 min of incubation at 37°C. To halt the reaction, 80 µL of 0.2 M sodium carbonate solution was added, and the absorbance was measured with an instrument for reading microplate. The Caraway-Somogi iodine/potassium iodide technique reported by Özek was used to assess the extracts for their  $\alpha$ -amylase inhibitory properties with some light adjustments<sup>28</sup>. A fresh starch solution was prepared by heating 100 mg of potato starch in 100 mL of 20 mM sodium phosphate buffer (pH 6.9) with 6.7 mM sodium chloride for 15 min. To initiate the experiment, 20 µL of plant extracts were combined with 1 mL of the prepared starch solution and incubated at 20°C for 20 min. Subsequently,  $\alpha$ -amylase (1 mL) was added, and the mixture was further incubated for 3 min. Following this, 1 mL of 3,5-dinitrosalicylic acid (96 mM) was added, and the solution was heated in a water bath at 75-80°C for 15 min. The resulting color was then measured at 540 nm.

The extracts were tested against the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) using Ellman's method<sup>29</sup> with slight modifications. A mixture comprising 20 µL of either extract sample or positive control at varying concentrations, 20 µL of enzyme (0.1 U/mL for BChE, and 0.22 U/mL for AChE) from Sigma-Aldrich, and 140 µL of 0.1 mM phosphate buffer (pH 6.8) were combined and then incubated with an additional 140 µL of 0.1 mM phosphate buffer (pH 6.8). Afterward, 10 µL of 0.5 mM DTNB (Sigma-Aldrich) was added, followed by 10 µL of substrate (0.2 mM butyrylthiocholine iodide/0.71 mM acetylthiocholine iodide) from Sigma-Aldrich, and the mixture was further incubated for 5 min. Galantamine served as the positive control. Each sample underwent three replicates at five different concentrations in all enzymes inhibitory activity assays.

#### Qualitative and quantitative LC-MS/MS assay

The quantitative assessment of 56 phytochemicals in the examined *Sideritis* species was conducted using a Shimadzu-Nexera ultrahigh performance liquid chromatograph (UHPLC) coupled with a tandem mass spectrometer. For this purpose, a previously established and validated LC-MS/MS method was employed<sup>30</sup>.

A Shimadzu LCMS-8040 tandem mass spectrometer with an electrospray ionization (ESI) source that

might be running in both positive and negative ionization modes was employed for the mass spectrometry detection.

Shimadzu's Lab Solutions software was the tool for their coding and interpretation of LC-ESI-MS/MS data. Quantification of the phytochemicals was performed in MRM (multiple reaction monitoring) mode.

#### Statistical analysis

GraphPad Prism Software Version 8.0 was used for data analysis in order to evaluate differences in results between the experimental and standard groups. The findings are displayed as the average  $\pm$  standard deviation (S.D.). Statistical comparisons were performed utilizing one-way ANOVA followed by Tukey's test in GraphPad Prism 8.0. Significance levels were denoted as \* $p < 0.05$  and \*\*\*\* $p < 0.001$  for different degrees of comparison. Original variables were defined as four major components of *Sideritis* species extracts. Following normalization, the data underwent Hierarchical Cluster Analysis (CA) and principal component analysis (PCA). Statistical analysis were performed using Minitab 19<sup>31</sup>.

## Results and Discussion

#### Plant extract yields

The samples of the extract were made using the maceration method from aerial parts of seven different *Sideritis* species in this study. The extracts were obtained with water and methanol. Table 1 shows the yields of the extracts, herbarium codes, collecting date, and localities of the *Sideritis* species. Compared to methanol extracts, water extracts have less yields.

#### Phytochemical composition of *Sideritis* species extracts with LC/MS-MS

To analyze the methanol and water extracts of *Sideritis* species, a confirmed LC-MS/MS technique was utilized. Among the 53 plant-based compounds used in the established technique, various polyphenols and non-phenolic substances were discovered in the methanol and water extracts of the examined species. The abbreviations of the names of the fourteen extracts and the LC-MS/MS quantitative analysis were listed in Supplementary Table S1. Figure 1 and Figure 2 show the LC-MS/MS-TIC (Total Ion Chromatogram) chromatograms of *S. hololeuca* water extract and methanol extract of *S. cilicia*, respectively. In a supplementary file, the other extracts were provided (Supplementary Fig. S1 to Fig. S14).

Quinic acid is included in green coffee beans and it has obtained as a result of the hydroxylation of chlorogenic acid in the biosynthesis pathway<sup>32</sup>. Also, the quinic acid concentration in the methanol extracts

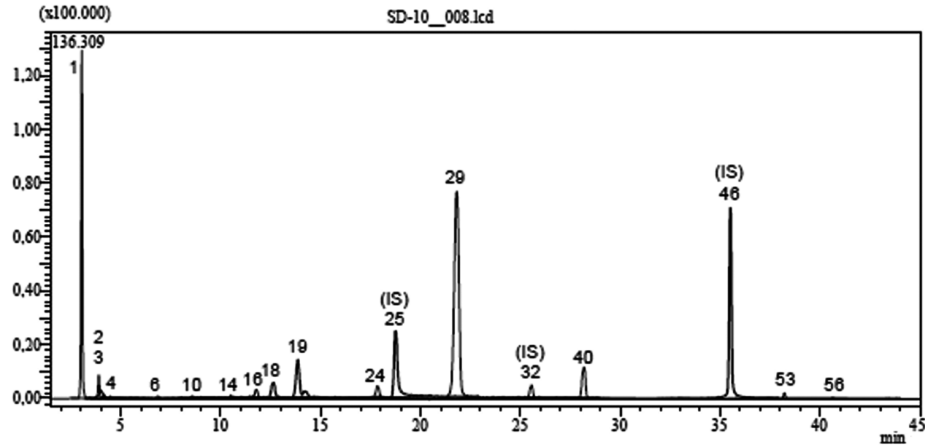


Fig. 1 — TIC (Total Ion Chromatogram) chromatogram of water extract of *S. hololeuca* analyzed by the developed LC–MS/MS method

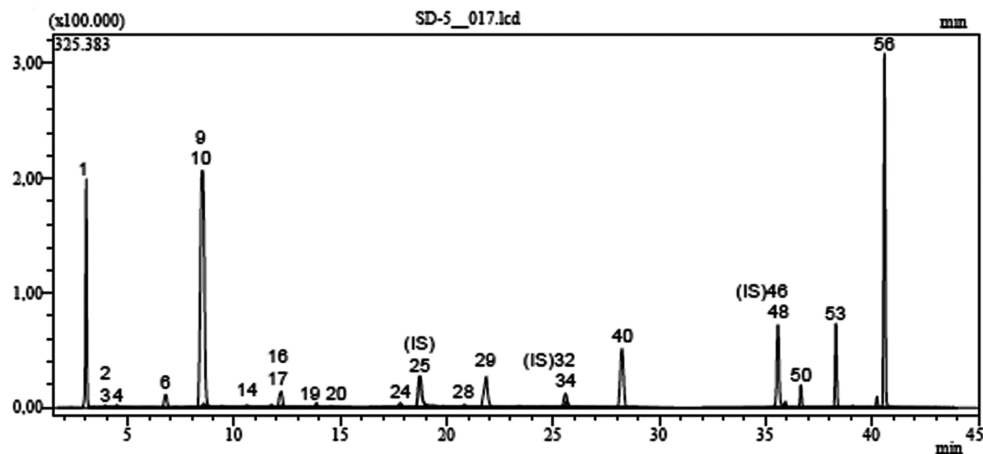


Fig. 2 — TIC (Total Ion Chromatogram) chromatogram of methanol extract of *S. cilicica* analyzed by the developed LC–MS/MS method

Table 1 — List of plant species collected from various parts of Türkiye as well as methanol and extract yields

Plant	Extract	Yield %	Location	Altitude	Date	Herbarium code
<i>Sideritis erythrantha</i> Boiss. & Heldr. subsp. <i>cedretorum</i> P. H. Davis	Water (SEW) Methanol (SEM)	3.84 11.15	C4: Antalya, Mahmutlar, Büyükkarapınar village, roadside	1650 m	27.06.2014	S. DOĞU 2878
<i>Sideritis libanotica</i> Labill. subsp. <i>linearis</i> (Benth.) Bornm.	Water (SLW) Methanol (SLM)	5.70 12.17	C4: Karaman: Karaman-Ayrancı road, 15. km roadside	1050 m	26.06.2014	S. DOĞU 2876
<i>Sideritis cilicica</i> Boiss. & Balansa	Water (SCW) Methanol (SCM)	5.08 13.96	C4: Mersin, Çamlıyayla, Sarıkavak village, Makilıklar	550 m	21.06.2014.	S. DOĞU 2857
<i>Sideritis bilgeriana</i> P. H. Davis	Water (SBiW) Methanol (SBiM)	7.82 1.58	C4: Karaman, Kazancı-Gülнар road section	1255 m	17.07.2015,	S. DOĞU 3188
<i>Sideritis brevidens</i> P. H. Davis	Water (SBW) Methanol (SBM)	6.86 16.24	C4: Mersin, Gülнар, Gülнар-Mut Road 2. km. roadside	850 m	21.06.2014	S. DOĞU 2859
<i>Sideritis libanotica</i> Labill. subsp. <i>violascens</i> P. H. Davis	Water (SLVW) Methanol (SLVM)	9.94 11.95	C4: Karama. Sarıveliler, Sarıpınar plateau, stony slopes	1750 m	23.07.2015	S. DOĞU 3231
<i>Sideritis hololeuca</i> Boiss. & Heldr.	Water (SHW) Methanol (SHM)	4.97 6.80	C4 Karaman, Ermenek, Kazancıkızıllalan, roadside	1450 m	21.06.2014	S. DOĞU 2858

of these species was considerably more than that in the water extracts, with the exception of *S. bilgeriana* and *S. brevidens*.

Chlorogenic acid (CGA), a prominent phenolic acid found in tea and green coffee, has been shown to possess a variety of beneficial pharmacological properties. As a dietary polyphenol, it demonstrates antioxidant, neuroprotective, anti-obesity, free radical scavenging, and central nervous system stimulant effects. Given these desirable features, CGA has the potential to be effectively utilized as a natural food additive to provide protective health benefits<sup>33</sup>. The concentration of CGA in the methanol extracts of these *Sideritis* species was much greater than that in the water extracts. Except the methanol extract of *S. bilgeriana* (0.55 mg analyte/g extract) and *S. brevidens* (14.73 mg analyte/g extract), the other methanol extracts were included in the trace amounts (<0.1). Indeed, the significant CGA content of *S. libanotica* subsp. *violens* methanol extract (40.94 mg analyte/g extract) shows that the *Sideritis* species could be utilized as a natural source of CGA.

Previously, the analysis of the phenolic compounds of the methanol extract of *S. brevidens* aerial parts was performed using LC-MS/MS. Siderol has been obtained from all species, while linearol has only been isolated from *S. brevidens* (690 mg). Also the amounts of major compounds of methanol extract were found as *p*-hydroxybenzoic acid (614±0.6 mg/100 g extract) and kaempferol 3-*O*-glucoside (122.1±2.8 mg/100 g extract)<sup>17</sup>.

According to a study in the literature, the results indicated that major phenolic compounds of *S. bilgeriana* methanol extract were found as apigenin and luteolin (830.6, and 254.4 µg/g extract, respectively)<sup>34</sup>. In another study, the major component in the *S. bilgeriana* methanol extract was detected as CGA. The fumaric acid was determined to be the main compound of both water extracts obtained using infusion and decoction methods, and their quantities were determined to be 39752.76 and 5546538.46 mg/kg, respectively<sup>19</sup>. In another study on *S. libanotica* subsp. *linearis*, *p*-coumaric, caffeic, and ferulic acids were the most common phenolic acids, whereas quercetin, morin, and apigenin were the most abundant flavonoids<sup>35</sup>. HPLC was used to examine the methanol extract of *S. erythrantha* var. *cedretorum*. CGA, one of the primary ingredients, was measured using a previously approved HPLC method. The extract has a quantity of 0.282±0.01 (g/100 g dw)<sup>20</sup>.

#### PCA and HCA analyses of the phenolic constituents of *Sideritis* extracts

The phenolic constituents have been assessed using the PCA and HCA techniques. The four major constituents are chlorogenic acid, quinic acid, acacetin, and fumaric acid (Table 1).

The phenolic component results of methanol and water extracts from *Sideritis* species were analyzed using PCA to illustrate the interrelationships and similarities among the different *Sideritis* species based on their phenolic profiles. Additionally, HCA was employed to evaluate the accuracy of this classification by analyzing the polyphenolic compounds present in the *Sideritis* species extracts. The PCA and HCA analyses are presented in Figure 3 and Figure 4, respectively. For the *Sideritis* species

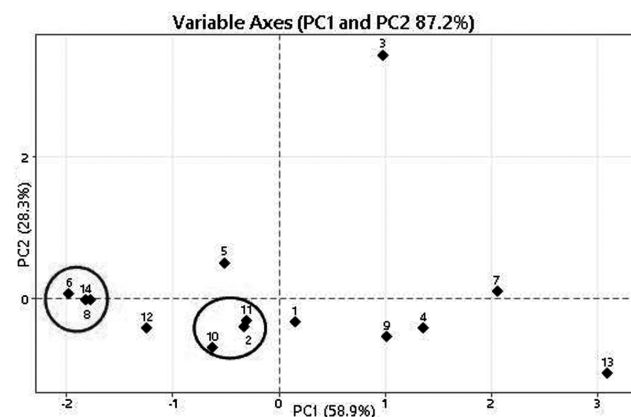


Fig. 3 — PCA analysis of the major components of *Sideritis* genus methanol and water extracts (1: SBiM; 2: SBiW; 3: SBM; 4: SBW; 5: SCM; 6: SCW; 7: SEM; 8: SEW; 9: SHM; 10: SHW; 11: SLM; 12: SLW; 13: SLVM; 14: SLVW)

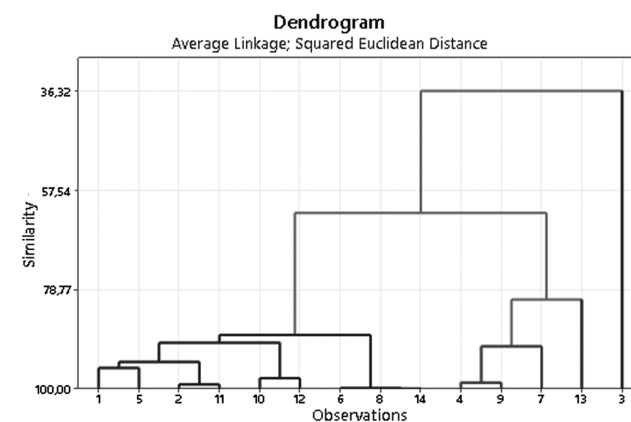


Fig. 4 — Dendrogram obtained by Hierarchical Cluster Analysis (HCA) based on the Euclidean distances between groups of the major components of *Sideritis* genus methanol and water extracts (1: SBiM; 2: SBiW; 3: SBM; 4: SBW; 5: SCM; 6: SCW; 7: SEM; 8: SEW; 9: SHM; 10: SHW; 11: SLM; 12: SLW; 13: SLVM; 14: SLVW)

extracts, the loading plot from the PCA shows that all variables affecting the first two principal components explain 87.2% of the accumulated variation in the analyzed data, indicating that these two principal components capture a significant portion of the overall variance in the phenolic profiles of the *Sideritis* species.

The HCA of the eight primary phytochemical constituents identified three distinct major groupings, with similarity levels varying between 36% and 100% (Fig. 4). The cluster analysis of the water extracts of *S. cilicica*, *S. erythrantha* and *S. libanotica* subsp. *violascens* observations revealed similarities ranging 99.80%-99.92%. Also, the HCA similarity of methanol extract of *S. libanotica* subsp. *violascens* is 36.32%. This suggests that the PCA and HCA analyses provide a comprehensive and reliable assessment of the relationships and similarities between the different *Sideritis* species based on their polyphenolic composition.

#### Analysis of the antioxidant capacities of *Sideritis* species

ABTS<sup>++</sup> and DPPH<sup>•</sup> radical scavenging, iron-chelating, TFC, and TPC assays utilized to assess the antioxidant activity of the methanol and water extracts obtained from the aerial parts of *Sideritis* species (Fig. 5). In this study, the TPCs of the extracts were calculated as gallic acid equivalent while the TFCs of the extracts were expressed as quercetin equivalent.

TPC in the water extracts of all the *Sideritis* species examined was greater than the TFC. This suggests that the antioxidant capacity of these extracts may be primarily attributed to their phenolic compounds rather than their flavonoid content. According to the results of the analysis, it was found that the methanol extract of *S. bilgeriana* contained the highest amount

for TPC (291.25 mgGAE/g extract) and TFC (170.56 mgQE/g extract) among all extracts ( $p < 0.001$ ).

Radical scavenging, iron chelation, and peroxidation delay are only a few of the antioxidant mechanisms. DPPH<sup>•</sup>, ABTS<sup>++</sup>, and iron-chelating assays were used to investigate the antioxidant mechanisms of methanol and water extracts of *Sideritis* species (Fig. 6). The highest antioxidant activities for two methods were observed in the methanol extract of *S. cilicica* with ABTS<sup>++</sup> (IC<sub>50</sub>: 28.01±0.63 µg/mL) and iron chelating (IC<sub>50</sub>: 43.19±1.14 µg/mL). As for the DPPH<sup>•</sup> of *S. erythrantha* methanol extract was the highest one with IC<sub>50</sub>: 1.03±0.1 µg/mL.

In the previous research, the antioxidant capabilities and TPC of 27 *Sideritis* species were investigated. Using a Soxhlet system, plant materials were extracted with petroleum ether<sup>36</sup>. After that, 70% methanol was used to extract the defatted *Sideritis* species. *S. cilicica* (4.5±0.9 mg/mL) had IC<sub>50</sub> values that were close to BHT (4.3±0.3 mg/mL). Furthermore, except for *S. erythrantha* var. *cedretorum* and *S. hololeuca*, the other species' free radical scavenging capabilities were similar but weaker than BHT. The total phenol content of 27 *Sideritis* species was higher than 190 mgGAE/g extracts<sup>33</sup>. By using two complimentary tests: DPPH<sup>•</sup> (IC<sub>50</sub>: 109±0.43 µg/mL) and β-carotene/linoleic acid assays (38.5±2.33%), the aerial parts of *S. libanotica* subsp. *linearis* extract prepared by Soxhlet extraction were separately examined for their probable antioxidant activity<sup>37</sup>.

The natural compounds, sideridiol and flavone were isolated from the *S. libanotica* subsp. *linearis* in another investigation<sup>18</sup>. Total antioxidant activities

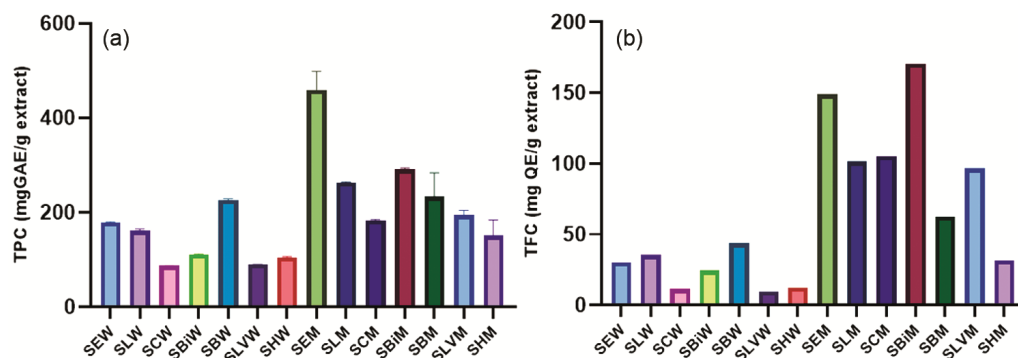


Fig. 5 — TPC and TFC of *Sideritis* species a: TPC, b: TFC, SEW: *S. erythrantha* water. SEM: *S. erythrantha* MEOH. SLW: *S. libanotica* subsp. *Linearis* water. SLM: *S. libanotica* subsp. *Linearis* MeOH. SCW: *S. cilicica* water. SCM: *S. cilicica* MeOH. SBiW: *S. bilgeriana* water. SBiM: *S. bilgeriana* MeOH. SBW: *S. brevidens* water. SBM: *S. brevidens* MEOH. SLVW: *S. libanotica* subsp. *violascens* water. SLVM: *S. libanotica* subsp. *violascens* MEOH. SHW: *S. hololeuca* water. SHM: *S. hololeuca* MEOH

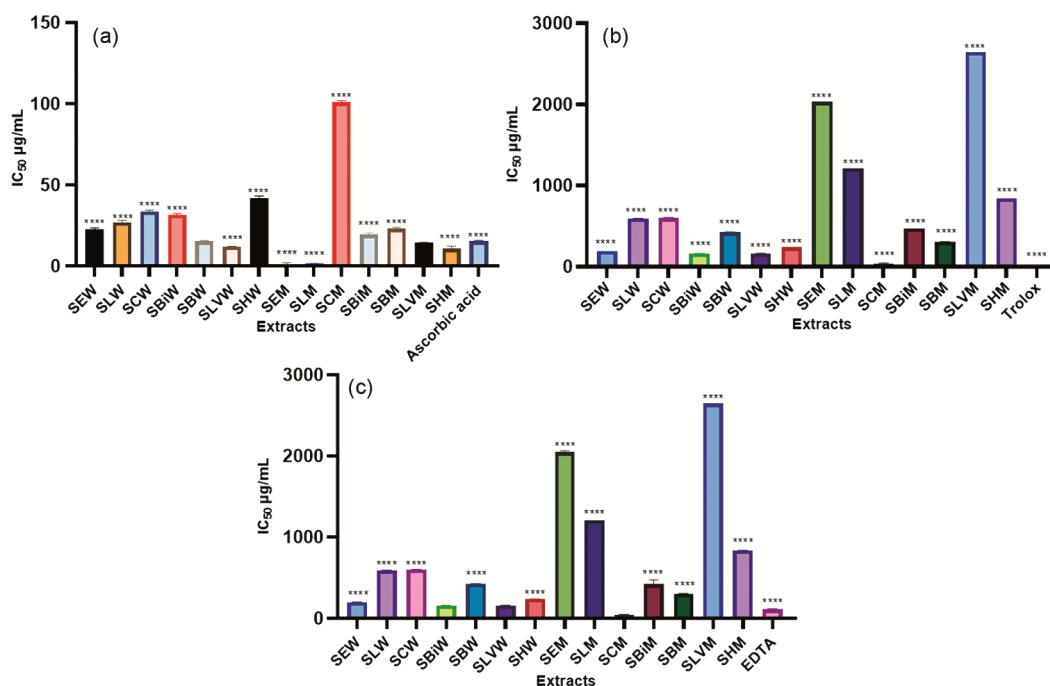


Fig. 6 — Antioxidant activities of *Sideritis* species (IC<sub>50</sub> µg/mL) a: DPPH, b: ABTS, c: Iron chelating, SEW: *S. erythrantha* water. SEM: *S. erythrantha* MEOH. SLW: *S. libanotica* subsp. *Linearis* water. SLM: *S. libanotica* subsp. *Linearis* MeOH. SCW: *S. cilicica* water. SCM: *S. cilicica* MeOH. SBiW: *S. bilgeriana* water. SBiM: *S. bilgeriana* MeOH. SBW: *S. brevidens* water. SBM: *S. brevidens* MEOH. SLVW: *S. libanotica* subsp. *violascens* water. SLVM: *S. libanotica* subsp. *violascens* MEOH. SHW: *S. hololeuca* water. SHM: *S. hololeuca* MEOH, Statistical analysis: one-way ANOVA followed by *t* test. \*\*\*\*Statistically significant ( $p < 0.001$ ) between free radicals treatment with positive control and extracts

were used to assess the antioxidant potentials of flavones and total methanol extract. According to results, flavones have the same antioxidant activity as tocopherol<sup>18</sup>. In another study, the TPC, TFC and antioxidant effect of *S. libanotica* subsp. *linearis* was found in the range of 9.16-10.49 g GAE/kg dw and 13.88-19.04 g dw/g DPPH<sup>•</sup>, respectively. TFC and antioxidant activity were found to be higher in wild grown *Sideritis* species than in cultured samples<sup>32</sup>. Moreover, the total phenolic content of *S. erythrantha* var. *cedretorum* was reported to be 190.78±2.46 mg GAE/g, while the antioxidant effect of the extract was measured using both DPPH<sup>•</sup> (IC<sub>50</sub>: 0.36±0.15 mg/mL) and ABTS<sup>•+</sup> (IC<sub>50</sub>: 0.20±0.01 mg/mL) assays<sup>20</sup>. The different polarities of the extracts of *S. erythrantha* var. *cedretorum* were investigated by the methods. The TPC and yields were found as the highest in the aqueous-MeOH and MeOH extracts<sup>38</sup>.

In the literature, free radical scavenging and  $\beta$ -carotene bleaching activities were utilized to assess the antioxidant capability of the *S. brevidens* methanol and acetone extracts. Both species had similar results and showed modest activities<sup>17</sup>. In addition, TPC content was determined as

1.62±0.04 µg GAE/mL for *S. bilgeriana*. The antioxidant activity results of *S. bilgeriana* extract were found using DPPH<sup>•</sup> (IC<sub>50</sub>: 0.123±0.001 µg/mL) and ferric reducing antioxidant power (7.61±0.01 µg/mL) methods<sup>34</sup>. Conversely, the hexane, acetone, and methanol of *S. bilgeriana* extracts were analyzed using DPPH<sup>•</sup> method. The methanol extract was found to be the most active<sup>19</sup>. Furthermore, the phenolic compounds composition of *S. hololeuca* acetone extract and the decoction sample were reported to be abundant. As expected, the phenol-rich extracts of the species had the highest antioxidant activity in all assays<sup>16</sup>.

#### Enzyme inhibition activities

As for the enzyme activities on this work, the inhibitory effects of the extracts of seven *Sideritis* species were listed in Table 2. In the tyrosinase enzyme inhibition study, the high active findings were found in water extracts of *S. erythrantha* (IC<sub>50</sub>: 1032.40±5.93 µg/mL) and *S. libanotica* subsp. *violascens* (IC<sub>50</sub>: 1663.00±5.52 µg/mL). The inhibitory effects of  $\alpha$ -amylase and  $\alpha$ -glucosidase, which are key players in carbohydrate digestion and glucose regulation, were

Table 2 — Enzyme inhibition activities of *Sideritis* species water and methanol extracts

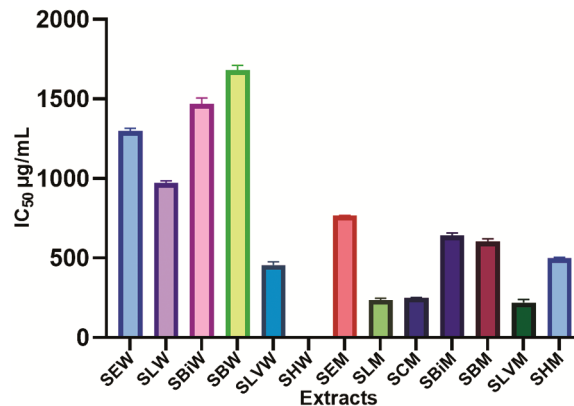
Samples	Inhibitory activity against TYR (percentage±S.D.) 2000 µg/mL	Inhibitory activity against AChE (percentage±S.D.) 2000 µg/mL	Inhibitory activity against BuChE (percentage±S.D.) 1000 µg/mL	Inhibitory activity against $\alpha$ -glucosidase (percentage±S.D.) 100 µg/mL	Inhibitory activity against $\alpha$ -amylase (percentage ±S.D.) 100 µg/mL
SEW	83.97±0.94 <sup>a</sup>	62.31±2.25 <sup>ab</sup>	38.14±0.10 <sup>c</sup>	10.67±3.62 <sup>b</sup>	3.25±4.31
SLW	44.12±1.69 <sup>ab</sup>	68.04±2.64 <sup>b</sup>	38.14±0.10 <sup>c</sup>	7.64±4.45 <sup>bc</sup>	79.99±0.65 <sup>a</sup>
SCW	47.87±3.71 <sup>ab</sup>	43.52±1.31 <sup>c</sup>	38.14±0.13 <sup>c</sup>	12.80±3.29 <sup>b</sup>	55.30±2.57 <sup>b</sup>
SBiW	43.87±1.81 <sup>ab</sup>	58.77±1.06 <sup>ab</sup>	19.54±0.20 <sup>d</sup>	13.92±2.69 <sup>b</sup>	70.58±1.25 <sup>a</sup>
SBW	48.39±2.74 <sup>ab</sup>	61.01±2.76 <sup>ab</sup>	63.90±3.68 <sup>b</sup>	4.26±1.00	14.50±4.73 <sup>c</sup>
SLVW	55.05±1.13 <sup>b</sup>	88.46±2.33 <sup>a</sup>	29.82±0.30 <sup>cd</sup>	9.18±0.67 <sup>b</sup>	59.69±4.18 <sup>ab</sup>
SHW	44.55±1.59 <sup>ab</sup>	94.07±1.40 <sup>a</sup>	20.63±0.27 <sup>d</sup>	n.d.	16.93±5.79 <sup>c</sup>
SEM	34.58±2.11 <sup>bc</sup>	88.41±3.28 <sup>a</sup>	63.55±2.06 <sup>b</sup>	7.63±1.36 <sup>bc</sup>	13.88±1.00 <sup>c</sup>
SLM	34.51±3.88 <sup>bc</sup>	75.98±3.47 <sup>b</sup>	15.91±3.21 <sup>e</sup>	10.54±0.81 <sup>b</sup>	3.03±2.38
SCM	33.55±2.78 <sup>bc</sup>	70.89±1.88 <sup>b</sup>	75.61±5.49 <sup>ab</sup>	4.95±0.58	n.d.
SBiM	24.99±1.13 <sup>c</sup>	69.40±5.19 <sup>b</sup>	45.58±0.10 <sup>c</sup>	6.04±2.40	4.23±2.18
SBM	15.55±1.86 <sup>d</sup>	74.79±1.70 <sup>b</sup>	43.11±5.64 <sup>c</sup>	5.22±1.60	5.91±0.64
SLVM	17.50±4.71 <sup>d</sup>	94.18±0.91 <sup>a</sup>	40.17±0.90 <sup>c</sup>	4.13±4.36	5.53±1.02
SHM	17.19±3.60 <sup>d</sup>	83.93±1.38 <sup>a</sup>	26.92±5.44 <sup>cd</sup>	0.69±2.55	12.09±3.47
Reference	80.96 ±0.51 <sup>a*</sup>	99.10±1.18 <sup>a**</sup>	84.34±4.85 <sup>a**</sup>	53.89±3.24 <sup>a***</sup>	50.65±0.86 <sup>b***</sup>

TYR: Tyrosinase. AChE: Acetylcholinesterase. BuChE: Butyrylcholinesterase. SD: standard deviation. \*: Kojic acid (250 µg/mL). \*\*: Galantamine 10.67 (200 µg/mL). \*\*\*: Acarbose (160 µg/mL for  $\alpha$ -glucosidase and 250 µg/mL for  $\alpha$ -amylase). SEW: *S. erythantha* water. SEM: *S. erythantha* MEOH. SLW: *S. libanotica* subsp. *linearis* water. SLM: *S. libanotica* subsp. *linearis* MeOH. SCW: *S. cilicica* water. SCM: *S. cilicica* MeOH. SBiW: *S. bilgeriana* water. SBiM: *S. bilgeriana* MeOH. SBW: *S. brevidens* water. SBM: *S. brevidens* MEOH. SLVW: *S. libanotica* subsp. *violascens* water. SLVM: *S. libanotica* subsp. *violascens* MEOH. SHW: *S. hololeuca* water. SHM: *S. hololeuca* MEOH. Different letters show statistically significant difference at  $p < 0.05$ . n.d.: not detected.

evaluated to assess the potential antidiabetic properties of *Sideritis* species. This assessment provided insights into the ability of these plant extracts to modulate carbohydrate metabolism and glucose homeostasis, which are important considerations for the management of diabetes. For antidiabetic efficacy, acarbose was utilized as a standard inhibitor. The water extract of *S. libanotica* subsp. *linearis* had a significant inhibitory activity on  $\alpha$ -amylase with 79.99±0.65% inhibition; it was followed by *S. bilgeriana* water extract with 70.58±1.25% inhibition at the concentration of 100 µg/mL which were higher than the reference drug acarbose (50.65±0.86%).

The neuroprotective effect of *Sideritis* species was also evaluated with the inhibition of BuChE and AChE. The enzyme inhibitory activities were measured with spectrophotometric methods (Fig. 7). In our study, all the species had strong inhibitory activities against AChE.

The water extract of *S. cilicica* (43.52±1.31%) exhibited the weakest inhibitory effect on AChE. In contrast, the water extract of *S. hololeuca* demonstrated the strongest AChE inhibitory activity, with an IC<sub>50</sub> value of 4.38±0.51 µg/mL. This indicates that the *S. hololeuca* water extract was the most effective at inhibiting the AChE enzyme, which is involved in the breakdown of the neurotransmitter acetylcholine,

Fig. 7 — AChE Inhibitory activity of *Sideritis* species (IC<sub>50</sub> µg/mL)

suggesting the potential neuroprotective properties of this extract. The highest BuChE inhibition was observed in the water extract of *S. brevidens* (IC<sub>50</sub>: 206.45±1.49 µg/mL).

Previously, the enzyme activity of *S. bilgeriana* preparations was evaluated in hexane, acetone, and methanol. The results showed that none of the extracts inhibited the AChE enzyme, whereas the acetone extracts obtained using both methods moderately inhibited the BuChE<sup>19</sup>. In another investigation on enzymes, human plasma and erythrocytes were utilized as enzyme sources, and they were treated with methanol and hexane extracts of the aerial parts and



root of *S. libanotica* subsp. *linearis*. The hexane extracts had no inhibition effect on the plasma AChE enzyme while the methanol extracts were found  $0.1678 \times 10^{-3}$  and  $0.2136 \times 10^{-3}$ , respectively<sup>39</sup>. In another study, AChE inhibition was examined on *n*-hexane, dichloromethane, acetone, and methanol extracts of *S. hololeuca*. The methanol extract had a moderate inhibition activity on BuChE ( $45.78 \pm 0.72\%$ ), while galantamine showed the highest inhibition activity with  $80.02 \pm 0.38\%$ <sup>16</sup>.

### Conclusion

The antioxidant potentials and enzyme inhibitory activities of *S. erythantha* and *S. cilicica* extracts as well as the TPC and TFC were investigated for the first time. In addition to this, the antidiabetic and tyrosinase enzyme inhibition activities of the seven *Sideritis* species was examined for the first time in our study. The highest antioxidant activities were observed in the methanol extract of *S. cilicica* with ABTS<sup>+</sup> radical scavenging and iron chelating methods. Against AChE, *S. hololeuca* water extract showed strong inhibitory activity. The methanol extract of *S. cilicica* showed strong BuChE inhibitory activity, it was followed by *S. erythantha* methanol and *S. brevidens* water extracts. Moreover, moderate tyrosinase inhibitory activity was exhibited by all extracts. Otherwise, *S. erythantha* water extract exhibited significantly higher tyrosinase inhibitory activity than kojic acid. This plant may be useful for the developing drugs in the treatment of tyrosinase enzyme-related disorders. Our work suggests that *Sideritis* species, especially *S. cilicica*, *S. erythantha*, and *S. libanotica* subsp. *linearis* may be investigated for potent secondary metabolites which are responsible for the promising biological activities of the plants in future.

### Supplementary Data

Supplementary data associated with this article is available in the electronic form at [https://nopr.niscpr.res.in/jinfo/ijtk/IJTK\\_23\(10\)\(2024\)988-998\\_SupplData.pdf](https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_23(10)(2024)988-998_SupplData.pdf)

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### Conflicts of Interest

The authors declare no conflicts of interest for this publication.

### Author Contributions

Conceptualization NE, DK, FA; Data curation NE, DK, FA, MAY; Formal analysis NE, DK, FA, MAY; Funding acquisition NE; Investigation NE, DKFA; Methodology NE; Project administration NE; Resources NE; Supervision NE; Validation NE, DK, MAY; Visualization NE, DK; Roles/Writing - original draft NE, DK, FA, YB, SD, MAY; Writing - review & editing NE, DK, FA, YB, SD, MAY.

### Data Availability

All the data are presented in tables and figures in the manuscript, and supplementary files, as well as further inquiries can be directed to the corresponding author.

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