



In Vitro Investigation of the Natural Antioxidant and Potential Neuroprotective Properties of *Sideritis perfoliata*

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ABSTRACT: In this study, the antioxidant and anticholinesterase biological activities of ethanol extracts of *Sideritis perfoliata* were evaluated using a comprehensive approach. The total antioxidant capacity (TAS), total oxidant level (TOS), and oxidative stress index (OSI) of the extracts obtained from the above-ground parts of the plant using the Soxhlet method were determined using commercial kits. Furthermore, free radical scavenging activity was assessed using the DPPH method, and reducing power using the FRAP method. Anticholinesterase potential was investigated based on the Ellman method using acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes, with galantamine used as a positive control. According to the results obtained, the TAS value of *S. perfoliata* extract was determined as 8.736 ± 0.180 mmol/L, the TOS value as 15.466 ± 0.180 μ mol/L, and the OSI value as 0.177 ± 0.002 . DPPH and FRAP analyses yielded values of 179.083 ± 3.075 mg TE/g and 227.557 ± 2.604 mg TE/g, respectively. In anticholinesterase tests, the inhibitory effects of the extract on AChE and BChE enzymes were determined as 60.363 ± 1.050 μ g/mL and 80.537 ± 1.519 μ g/mL, respectively. The results indicate that *S. perfoliata* possesses a significant antioxidant capacity and exhibits a moderate but consistent inhibitory effect on cholinesterase enzymes. These findings suggest that the species can be evaluated in pharmacological and nutraceutical applications as a natural antioxidant and potential neuroprotective resource.

Keywords: Plant bioactives, redox balance, free radical scavenging, enzyme inhibition, neurodegenerative processes, ethanol extract.

INTRODUCTION

Medicinal and aromatic plants stand out as natural bioreservoirs exhibiting multifaceted biological activities thanks to their unique chemical composition (Turkmen et al., 2025). The phenolic acids, flavonoids, terpenes, and essential oil components synthesized in these plants play a role in the plant's defense against environmental stresses, while also exhibiting antioxidant, antimicrobial, antiproliferative, and anti-inflammatory effects on human health (Mohammed et al., 2023; Sevindik et al., 2023; Colak et al., 2025). They are reported to have a protective effect on biological systems, particularly through the neutralization of free radicals, the inhibition of lipid peroxidation, and the modulation of cellular signaling pathways. Current research reveals the potential of extracts and purified compounds obtained from medicinal and aromatic plants for use in pharmaceutical, nutraceutical, and cosmeceutical applications; This places these plants in a strategic position in the development of naturally derived bioactive products (Sağlıker and Darici, 2005; Sağlıker and Darici, 2007; Yaşar et al., 2009; Uysal et al., 2023). In this study, the antioxidant and anticholinesterase activity of *Sideritis perfoliata* was determined.

Sideritis perfoliata is a medicinal-aromatic plant native to the Eastern Mediterranean, known colloquially as "mountain tea" or "ironwort," and widely used in traditional medicine for the treatment of colds, digestive disorders, inflammation, and rheumatic diseases. In recent years, this species has attracted attention due to its high protein and mineral content (especially K, P, Mg, Fe, Zn, and Cu); The chemical profile, rich in phenolic acids, phenylethanoid glycosides (especially verbascoside/actoside), flavonoid glycosides, iridoids, diterpenes, and predominantly monoterpene–sesquiterpene volatile oil components, has been detailed (Charami et al., 2008; Chrysargyris et al., 2019; Lall et al., 2019; Sarikurkcu et al., 2020; Tomou et al., 2021; Çarıkcı et al., 2023). It is reported that terpenes such as α -pinene, β -pinene, β -phellandrene, and valeranone are dominant in its volatile oil (Lall et al., 2019). In biological activity studies performed on extracts and isolated compounds, strong antioxidant and anti-inflammatory effects were confirmed by DPPH, lipid peroxidation, and lipoxygenase tests; These findings were compatible with the traditional uses of the plant (Charami et al., 2008; Chrysargyris et al., 2019; Sarikurkcu et al., 2020; Żyżelewicz et al., 2020; Çarıkcı et al., 2023). In addition, moderate antimicrobial activity, significant inhibition of acetyl/butyrylcholinesterase, α -glucosidase, α -amylase and tyrosinase enzymes, selective cytotoxic effect in HepG2 cell line, antiviral activity against herpes simplex virus and significant antiparasitic effect against *Echinococcus granulosus* protoscolexes have been reported (Deveci et al., 2019; Lall et al., 2019; Sarikurkcu et al., 2020; Żyżelewicz et al., 2020; Çelik et al., 2021; Çarıkcı et al., 2023; Tomou et al., 2025). Antihyperglycemic and blood pressure-lowering effects on the metabolic and

cardiovascular systems have also been reported, supporting the pharmaceutical and nutraceutical potential of *S. perfoliata* (Çelik et al., 2021). However, the vast majority of current studies are limited to in vitro and animal models, and human clinical trials and comprehensive toxicological data are still insufficient (Aneva et al., 2019; Çelik et al., 2021).

MATERIALS AND METHODS

The *S. perfoliata* samples evaluated in this study were collected from within the borders of Osmaniye province in Turkey. The above-ground parts of the collected plant material were dried under controlled conditions in a laboratory environment to prepare them for experimental analysis. In the extraction stage, 10 g of the dried material was taken and processed with 250 mL of ethanol at 50 °C for approximately 6 hours using a Soxhlet extraction system. After the completion of the extraction process, the obtained extract was concentrated using a Buchi R100 rotary evaporator operating at 40 °C to remove the solvent. The final extracts obtained were stored at +4 °C until the analyses were performed.

Antioxidant and Oxidant Tests

In this study, the total antioxidant capacity (TAS) and total oxidant level (TOS) of extracts obtained from the above-ground parts of *S. perfoliata* using ethanol were determined. Analyses were performed using commercial test kits from Rel Assay Diagnostics, following the application steps reported by the manufacturer. Trolox was used as the standard reference substance for TAS measurements, while TOS analyses were calibrated using a hydrogen peroxide standard. The obtained TAS values were expressed in millimol/L (mmol/L), and TOS values in micromol/L ($\mu\text{mol/L}$) (Erel, 2004; Erel, 2005). In calculating the oxidative stress index (OSI), TAS and TOS data were converted to a common unit; then the TOS/TAS ratio was calculated, and the results were presented as percentages (%) (Sevindik, 2025).

The free radical scavenging potential of the plant extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. In this context, the extracts were prepared as stock solutions in DMSO at a concentration of 1 mg/mL; 1 mL of the prepared solutions was mixed with 160 μL of DPPH solution prepared at a concentration of 0.267 mM. After incubating the reaction mixture at room temperature for 30 minutes under light-proof conditions, absorbance values were measured at a wavelength of 517 nm. Antioxidant activity results were expressed in mg Trolox equivalent/g extract (mg TE/g) units (Ünal et al., 2025).

The antioxidant capacities of the extracts based on their reducing power were evaluated using the FRAP (Ferric Reducing Antioxidant Power) method. For the analysis, 100 μL of extract solution was mixed with 2 mL of FRAP reagent. The FRAP reagent; The reaction mixture was prepared by combining 300 mM acetate buffer (pH 3.6), 40 mM HCl, 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and 10 mM 2,4,6-tris(2-pyridyl)-S-triazine solutions in a 10:1:1 ratio. The reaction mixture was incubated at 37 °C for 4 minutes, and absorbance measurements were performed at a wavelength of 593 nm. FRAP results were reported in mg Trolox equivalent/g extract (mg TE/g) (Ünal et al., 2025).

Anticholinesterase Activity Tests

In this study, the anticholinesterase potential of plant extracts was evaluated based on the colorimetric method described by Ellman et al. (1961). Galantamine was used as a positive control to determine the inhibitory effect on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. Stock solutions prepared from plant extracts were prepared as serial dilutions in the concentration range of 200-3125 $\mu\text{g/mL}$. In the experimental application, 130 μL of phosphate buffer (0.1 M, pH 8.0), 10 μL of test sample, and 20 μL of enzyme solution (AChE or BChE) were added to each well of a microtiter plate, respectively; the resulting mixture was pre-incubated for 10 minutes at 25 °C under light-free conditions. Following this time, the reaction was initiated by adding 20 μL of DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] and 20 μL of the appropriate substrate (acetylcholine iodide or butyrylcholine iodide). Changes in enzymatic activity were recorded spectrophotometrically at a wavelength of 412 nm. All experiments were performed in triplicate, and IC_{50} ($\mu\text{g/mL}$) values were calculated using the obtained inhibition percentages.

RESULTS AND DISCUSSION

Antioxidant Activity

Plants can exhibit strong antioxidant properties thanks to the phenolic compounds, flavonoids, and other secondary metabolites they synthesize during their metabolism. These bioactive components play a significant role in reducing cellular oxidative damage by neutralizing free radicals (Mohammed et al., 2024). Plant-derived antioxidants support the organism's endogenous defense system, contributing to the suppression of oxidative stress associated with aging and various chronic diseases (Sevindik et al., 2025). Therefore, determining the antioxidant capacities of natural plant resources is of great importance both

in developing protective strategies for health and in the design of functional foods and drugs (El-Chaghaby et al., 2024). In this study, the antioxidant potentials of the above-ground parts of *S. perfoliata* were evaluated. The data obtained are presented in Table 1.

Table 1. TAS, TOS, OSI, DPPH and FRAP values of *Sideritis perfoliata*

Plant	TAS mmol/L	TOS μ mol/L	OSI (TOS/(TASx10))	DPPH mg TE/g	FRAP mg TE/g
<i>Sideritis perfoliata</i>	8.736 \pm 0.180	15.466 \pm 0.180	0.177 \pm 0.002	179.083 \pm 3.075	227.557 \pm 2.604

In our study, the antioxidant capacity of *S. perfoliata* extract was comprehensively evaluated using tests reflecting total antioxidant-oxidant balance parameters and free radical scavenging and reducing power. The TAS value determined in our study (8.736 \pm 0.180 mmol/L) reveals that the plant has a high total antioxidant capacity, while the relatively low TOS value (15.466 \pm 0.180 μ mol/L) indicates that the oxidative load can be kept at a limited level. The low OSI value of 0.177 \pm 0.002, calculated by dividing these two parameters, suggests that *S. perfoliata* extract can effectively maintain antioxidant defense against oxidative stress and preserve the oxidant-antioxidant balance in favor of antioxidants. When the free radical scavenging capacity of the extract was evaluated by the DPPH test, the obtained value of 179.083 \pm 3.075 mg TE/g indicates that *S. perfoliata* has a strong hydrogen-donating capacity that can directly interact with free radicals. Similarly, the value of 227.557 \pm 2.604 mg TE/g determined in the FRAP analysis reveals that the extract has a high capacity to reduce ferric ions to ferrous form and exhibits a significant electron-donating property. The agreement between the DPPH and FRAP results supports the idea that the antioxidant effect of the extract is not due to a single mechanism but occurs through both radical scavenging and redox-based reduction pathways.

In the literature, the antioxidant activity of *S. perfoliata* has been previously reported using different extraction methods and analytical approaches (Sarikurku et al., 2020; Cocelli et al., 2021). However, no direct findings regarding the TAS, TOS, and OSI parameters of *S. perfoliata* have been found in the existing literature. In this respect, our study provides a unique contribution to the literature by presenting data on the total antioxidant-oxidant balance profile of the species. When compared with the TAS (7.934 mmol/L), TOS (10.626 μ mol/L), and OSI (0.134) values reported for *Sideritis libanotica* subsp. *kurdica* in the literature (Uysal et al., 2023), it is observed that *S. perfoliata* used in our study has higher values for all three parameters. This difference is thought to be due to interspecies variability in phytochemical composition, ecological growing conditions, and genetic factors. However, the higher levels of both antioxidant capacity and oxidative load in *S. perfoliata* suggest that the species may have a more dynamic redox profile. Furthermore, studies conducted on different plant species showed that TAS values for *Salvia absconditiflora*, *Glycyrrhiza glabra*, *Thymus vulgaris*, *Barbarea vulgaris*, and *Dryopteris raddeana* were 7,350, 8,770, 3,549, 7,317, and 5,099 mmol/L, respectively; and TOS values were 8,501, 14,590, 2,780, 13,524, and 7,354 μ mol/L, respectively. OSI values have been reported as 0.116, 0.167, 0.078, 0.185, and 0.144 (Akgül et al., 2020; Mohammed et al., 2021; Erdoğan et al., 2025; Sağlıker et al., 2025; Uygun et al., 2025). Compared with these data, the TAS value of *S. perfoliata* used in our study was found to be higher than that of *S. absconditiflora*, *T. vulgaris*, *B. vulgaris*, and *D. raddeana*, and close to that of *G. glabra*. Considering that the TAS value is a holistic indicator of antioxidant compounds synthesized in natural products (Gürgen and Sevindik, 2022), it can be said that *S. perfoliata* has a rich antioxidant metabolite profile. On the other hand, the fact that the TOS value of *S. perfoliata* was determined to be higher than all the compared plants suggests that the extract also contains some compounds that can exhibit oxidative properties. Since the TOS parameter reflects the total number of compounds present in natural products that can create an oxidative effect (Gürgen and Sevindik, 2022), this can be considered a natural consequence of the multi-component and bidirectional redox properties of herbal extracts. When viewed in terms of the OSI value obtained by evaluating these two parameters together, it is seen that the OSI value of *S. perfoliata* is higher than *S. absconditiflora*, *G. glabra*, *T. vulgaris* and *D. raddeana*, but lower than *B. vulgaris*. Considering that the OSI value is an index showing the percentage extent to which oxidant compounds are suppressed by antioxidant compounds (Gürgen and Sevindik, 2022), it is understood that although *S. perfoliata* contains an oxidative load, its antioxidant capacity can largely balance this load. In conclusion, the findings reveal that *S. perfoliata* has a significant antioxidant potential, can be evaluated as a natural and functional antioxidant source in biological processes associated with oxidative stress, and thus holds promise for pharmacological and nutraceutical applications.

Anticholinesterase Activity

It is known that some plant-derived secondary metabolites, primarily phenolic compounds, flavonoids, and terpenoids, can suppress cholinesterase enzyme activity. This inhibitory effect contributes to the longer retention of acetylcholine in the synaptic cleft, thus supporting the continuity of neuronal transmission (Owokotomo et al., 2015). In recent years, plant-derived cholinesterase inhibitors have been addressed with increasing scientific interest as natural and complementary treatment options in the control of neurodegenerative diseases (Gürgen et al., 2025). Accordingly, the identification of naturally derived

cholinesterase inhibitors and the elucidation of their biological properties both pave the way for the development of therapeutic agents with safer side effect profiles and contribute to the evaluation of plant-based functional products (Adewusi et al., 2010; Kabaktepe et al., 2025). In this study, the anticholinesterase potential of extracts from *S. perfoliata* was investigated, and the findings are summarized in Table 2.

Table 2. Anti-AChE and anti-BChE values of *Sideritis perfoliata*

Sample	AChE µg/mL	BChE µg/mL
<i>Sideritis perfoliata</i>	60.363 ± 1.050	80.537 ± 1.519
Galantamine	7.650 ± 0.422	15.593 ± 0.345

In our study, the potential effect of *S. perfoliata* extract on enzymes associated with neurodegenerative diseases was evaluated through the inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The AChE inhibition value determined for *S. perfoliata* extract in our study was 60.363 ± 1.050 µg/mL, and the BChE inhibition value was 80.537 ± 1.519 µg/mL. These findings indicate that the extract exhibits a measurable and significant inhibitory effect on both cholinesterase enzymes, but has a lower inhibitory potential when compared to galantamine (AChE: 7.650 ± 0.422 µg/mL; BChE: 15.593 ± 0.345 µg/mL), which was used as a reference inhibitor. However, considering that galantamine is a pure and potent pharmaceutical inhibitor, these results obtained for a multi-component herbal extract can be considered biologically significant. The literature reports that the acetylcholinesterase inhibitory activity of *S. perfoliata* ranges from 0.14-0.26 mg galantamine equivalent/g extract, and its butyrylcholinesterase inhibitory activity ranges from 0.09-0.53 mg galantamine equivalent/g extract (Sarikurkcu et al., 2020). These data support the observation of AChE and BChE inhibitory effects in our study, which are consistent with the literature, and indicate that *S. perfoliata* exhibits moderate but consistent inhibitory activity on cholinesterase enzymes. Quantitative differences between studies may be due to methodological differences in the inhibitory expression units used, extract concentrations, solvent systems, and enzyme analysis protocols. Inhibition of AChE and BChE enzymes is considered a fundamental therapeutic approach, particularly in preserving cholinergic synaptic transmission in neurodegenerative disorders such as Alzheimer's disease (Korkmaz et al., 2025). In this context, it is thought that the inhibitory effect of *S. perfoliata* extract against both enzymes may be related to bioactive metabolites such as phenolic compounds and flavonoids found in the plant. It is widely reported in the literature that phenolic structures can interact with enzyme active sites and suppress cholinesterase activity through reversible inhibition mechanisms (Yazar et al., 2024). In conclusion, these findings make a significant contribution to the literature regarding the evaluation of *S. perfoliata* as a functional plant and natural neuroprotective agent.

CONCLUSION

This study investigated the antioxidant and anticholinesterase biological activities of ethanol extracts of *S. perfoliata* using multifaceted analytical methods. The combined assessment of total antioxidant and oxidant status demonstrated that the plant maintains its redox balance in favor of antioxidants and possesses an effective defense potential against oxidative stress. High values obtained from DPPH and FRAP tests support the conclusion that the extract exhibits an effective antioxidant profile through both free radical scavenging and electron-donating mechanisms. However, while the inhibitory effects of *S. perfoliata* extract on acetylcholinesterase and butyrylcholinesterase enzymes are lower compared to galantamine, they are biologically significant for a multi-component extract of plant origin. This suggests that phenolic compounds and flavonoids present in the plant may interact with cholinesterase enzymes. Overall, the findings indicate that *S. perfoliata*, in addition to being a natural antioxidant source, has the potential to be considered as a complementary or supportive agent in processes associated with neurodegenerative diseases. However, further phytochemical studies aimed at isolating the active components, as well as in vivo and clinical-based research, are important for a clearer understanding of the therapeutic potential of the species.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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