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Antioxidant and Anticholinesterase activities of *Stachys pumila*

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ABSTRACT: Numerous plant species have been utilized in traditional medicine since antiquity. Plants are essential natural resources for those possessing these attributes. The total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index of *Stachys pumila* Banks & Sol. were assessed in our study. The inhibitory activities of acetylcholinesterase and butyrylcholinesterase in the plant were assessed. The aerial components of the plant were extracted using ethanol in a Soxhlet system. The TAS, TOS, and OSI values were ascertained with Rel Assay kits. Anticholinesterase activity were assessed using the Elman technique. The experiments determined the TAS value of the plant's ethanol extract to be 5.447±0.024 mmol/L, the TOS value to be 6.974±0.043 µmol/L, and the OSI value to be 0.128±0.001. The anti-AChE value was measured at 38.28±1.95 µg/mL, whereas the anti-BChE value was measured at 63.12±1.54 µg/mL. It was established that the plant's biological activities were within normal ranges and that it possesses potential for pharmaceutical applications.

Keywords: Antioxidant, Anticholinesterase, *Stachys*, Medicinal Plants.

INTRODUCTION

Plants are significant natural entities distinguished by their diverse characteristics. They have emerged as significant natural resources for individuals in various capacities (Turkmen et al., 2023). They have been extensively utilized for various functions, including sustenance, habitation, thermal regulation, equipment manufacturing, flavoring, and illness management. Their nutritional qualities render plants indispensable for humans (Karcioglu et al., 2011). Besides their nutritional attributes, they are also significant natural products in the realm of medicine (Güler et al., 2022). Numerous studies have demonstrated that plants exhibit various biological activities, including antiproliferative, anticancer, anti-inflammatory, antioxidant, DNA protective, antiallergic, and hepatoprotective effects (Karaman et al., 2007; Daştan et al., 2019; Saraç et al., 2019; Dursun et al., 2021; Manzione et al., 2022; Mohammed et al., 2023; Sevindik et al., 2023; Uysal et al., 2023; El-Chaghaby et al., 2024; Sabik et al., 2024). In this setting, assessing the biological activity of plants is crucial. The antioxidant and anticholinesterase properties of *S. pumila* were assessed in our study.

Stachys is among the largest genera within the Lamiaceae family. Approximately 300 species are dispersed globally (Tundis et al. 2014; Kepekçi et al., 2017). The aerial components of various *Stachys* species have been utilized in traditional medicine for addressing genital malignancies, splenic sclerosis, inflammatory conditions, cough, and ulcers (Tundis et al. 2014; Kepekçi et al., 2017; Tomou et al., 2020). *S. pumila* is a medicinal plant utilized in traditional medicine for the treatment of bronchitis, asthma, abdominal pain, and problems of the bile and liver (Tepe et al. 2011; Kepekçi et al., 2017).

MATERIALS AND METHODS

Plant samples were collected from Hatay (Turkey). The aerial parts of the plant were used. In this context, herbarium samples of the plant were created after the field studies. These samples are kept in Osmaniye Korkut Ata University, Pharmacy Services Laboratory. The identification of the plant samples was made using Flora of Turkey and the East Aegean Islands, Vol. 7 (Davis, 1982). The plant samples were dried after the field studies. After the drying process, they were powdered. Then, 30 g of the samples were weighed. After the weighing process, they were extracted with 250 mL of ethanol in a Soxhlet apparatus at 50 °C for approximately 6 hours. Then, the solvents were evaporated at 40 °C using a Buchi R100 Rotary Evaporator. Crude extracts were stored at +4 °C for the experiments.

Antioxidant tests

The antioxidant capacities of ethanol extracts from the aerial portions of the plant were assessed using Rel Assay TAS and TOS kits. At this juncture, the protocol provided in the manufacturer's kit was adhered to. Trolox was utilized to standardize

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TAS assessments. Hydrogen peroxide was employed to calibrate TOS assessments. TAS levels were denoted in mmol/L. TOS values were denoted in μmol/L. In calculating the OSI value, the units of TOS and TAS were standardized; TOS values were derived by dividing TOS by TAS and expressing the result as a percentage (Erel, 2004; Erel, 2005; Sevindik, 2019).

Anticholinesterase activity tests

The anticholinesterase activity of ethanol extracts from the aerial portions of the plant was assessed using the Ellman method (Ellman et al., 1961). Galantamine used as the standard. Stock solutions were produced from the plant extracts at doses ranging from 200 to 3.125 μg/mL. Subsequently, 130 μL of 0.1 M phosphate buffer at pH 8 was introduced to the microplate. 10 μL of stock solution and 20 μL of enzyme solution (AChE or BChE) were included. Subsequent to this procedure, incubation was conducted at 25 °C for 10 minutes. Subsequently, 20 μL of DTNB (5.5”-dithiobis-(2-nitrobenzoic acid)) solution and 20 μL of substrate (acetylcholine iodide or butyrylcholine iodide) were introduced, and the measurement was conducted at a wavelength of 412 nm. IC_{50} values of % inhibition for samples were given in μg/mL.

RESULTS AND DISCUSSION

Antioxidant And Oxidant Potential

Free radicals are a primary contributor to oxidative stress in biological organisms. Although low concentrations of these substances can be tolerated, cellular harm becomes evident as their concentrations rise (Saridogan et al., 2021). The antioxidant defense mechanism is stimulated, inhibiting free radicals. Nonetheless, the antioxidant defense mechanism may be inadequate in the presence of elevated free radical concentrations (Sevindik, 2021). Oxidative stress transpires in this instance. Oxidative stress is associated with numerous severe diseases, including cancer, diabetes, multiple sclerosis, Parkinson's disease, Alzheimer's disease, and cardiovascular problems (Akgül et al., 2016; Islek et al., 2021). Supplemental antioxidants may mitigate or diminish the potential impacts of oxidative stress (Sevindik and Akata, 2019). In this context, plants constitute significant natural resources. We assessed the total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) of *S. pumila* in our study. The results are presented in Table 1.

Table 1. *TAS, TOS and OSI values of Stachys pumila*

Sample	TAS mmol/L	TOS μmol/L	OSI
Stachys pumila	5.447±0.024	6.974±0.043	0.128±0.001

* Values are presented as mean±SD

The antioxidant capacity of *Stachys pumila* has been documented in the literature employing various methodologies (Kepekçi et al., 2017). The study provided the TAS, TOS, and OSI values of *Stachys pumila*. The literature contains TAS, TOS, and OSI investigations on many plant species. The TAS value of *Salvia absconditiflora* was documented as 7.350 mmol/L, the TOS value as 8.501 μmol/L, and the OSI value as 0.116 (Akgul et al., 2020). The TAS of *Alcea kurdica* was recorded at 3.298 mmol/L, the TOS at 8.312 μmol/L, and the OSI at 0.252 (Mohammed et al., 2022). The TAS of *Glycyrrhiza glabra* was documented as 8.770 mmol/L, the TOS as 14.590 μmol/L, and the OSI as 0.167 (Mohammed et al., 2021). The TAS of *Glaucium alakirensis* was recorded at 3.496 mmol/L, the TOS at 2.204 μmol/L, and the OSI at 0.063 (Özçandır et al., 2024). The TAS of *Dittrichia graveolens* was documented at 6.93 mmol/L, the TOS at 12.53 μmol/L, and the OSI at 0.18 (Korkmaz et al., 2023). The TAS of *Hypericum spectabile* has been documented 9.306 mmol/L, a (TOS of 13.065 μmol/L, and an OSI of 0.140 (Gürgen et al., 2024). In comparison to these studies, the TAS value of *S. pumila* in our research was found to be lower than that of *S. absconditiflora, G. glabra, D. graveolens*, and *H. spectabile*, while being higher than *A. kurdica* and *G. alakirensis*. The TAS value signifies the aggregate of antioxidant chemicals generated in natural goods (Bal et al., 2023). In this context, it was established that *S. pumila* utilized in our study possesses antioxidant capability.

The TOS value signifies the aggregate of oxidant chemicals generated in natural goods (Bal et al., 2023). The TOS value of *S. pumila* in our study was lower than that of *S. absconditiflora, A. kurdica, G. glabra, D. graveolens,* and *H. spectabile*, but greater than *G. alakirensis*. In this setting, the quantities of oxidant chemicals generated by *S. pumila* were found to be within normal ranges.

The OSI number indicates the extent to which antioxidant chemicals inhibit the oxidant compounds generated by natural products. In this situation, it is advisable to utilize natural items with elevated OSI values judiciously (Bal et al., 2023). The OSI value of *S. pumila* in our study was higher than that of S. absconditiflora and G. alakirensis, but lower than *A. kurdica, G. glabra, D. graveolens*, and *H. spectabile*. *S. pumila* demonstrates considerable promise in inhibiting oxidant chemicals.

Anticholinesterase activity

Alzheimer's is among the most prevalent diseases observed in individuals over the age of 65 today. It is projected that there will be approximately 80 million individuals with Alzheimer's disease in the next two decades. Currently, there is no universal treatment for this condition. Nonetheless, research exists in this domain. Numerous investigations focus on the suppression of cholinesterase enzymes (Çömlekçioğlu et al., 2024; Seğmenoğlu et al., 2024). This study assessed the anticholinesterase activity of *S. pumila*. The results (IC_{50} values) are presented in Table 2.

Table 2. *Anti-AChE and anti-BChE values of Stachys pumila*

Sample	AChE µg/mL	BChE µg/mL
Stachys pumila	38.28±1.95	63.12±1.54
Galantamine	7.42±0.11	12.55±0.26

The literature contains no findings regarding the anticholinesterase activity of *S. pumila*. Nonetheless, research exists on various *Stachys* species. In this context, the acetylcholinesterase value of *Stachys guyoniana* was reported as 5.78 µg/mL, whereas the butyrylcholinesterase value was 39.10 µg/mL (Ferhat et al., 2017). In comparison to this study, it was noted that the activities of both acetyl and butyrylcholinesterase in *S. pumila* utilized in our research were diminished. Furthermore, it was established that it demonstrated reduced anticholinesterase activity relative to galantamine, which served as the reference in our investigation. Identifying enzymes responsible for the genesis of human diseases is crucial in combating illnesses. The inhibition of these enzymes can substantially aid in the management of illnesses (Świątek et al., 2021). In this regard, *S. pumila*, utilized in our study, is identified as one of the natural products applicable to Alzheimer's disease.

CONCLUSION

Plants are extensively utilized in traditional medicine to treat ailments. The antioxidant and anticholinesterase properties of *S. pumila* were assessed in our study. Research in this regard has established that the aerial components of the plant possess antioxidant properties. Furthermore, the inhibitory potentials of acetylcholinesterase and butyrylcholinesterase were shown to be minimal. In this context, it was established that the biological activities of *S. pumila* were within normal parameters. Consequently, it is believed to possess potential as a natural resource in pharmaceutical formulations.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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