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Examining the antioxidant capabilities and enzyme-inhibiting potentials of the Stachys bombycina Boiss extracts.

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ABSTRACT

Scope and Context: Roughly 300 species of Stachys L. are known to exist throughout the globe. Across Turkey, especially in the south and east, you may find more than 120 different taxa, with about 60 of them being endemic. Stachys species have a long history of medicinal usage, with applications ranging from the treatment of asthma and rheumatism to coughs, ulcers, genital tumors, diabetes, hemorrhoids, kidney stones, and a host of mental illnesses. S. bombycina Boiss., also known as "arıçayçesi" in Turkish, is a species of indigenous perennial plant that is almost endangered.

We used in vitro methods comprising radical scavenging (DPPH and ABTS), an iron-chelating assay, total phenol content (TPC) and flavonoid content (TFC) analysis, and water and methanol extracts of S. bombycina were tested for antioxidant activity. Using an in vitro spectrophotometric technique, the extracts were also examined for their effects on enzyme inhibition.

To further understand the extracts' phytochemical profiles, HPLC analysis was also used.

Conclusion: Our findings indicate that compared to the water extract, the methanol extract of S. bombycina exhibited superior radical scavenging activity against DPPH and ABTS, with IC50 values of 605.7 ± 1.04 and 19.40 ± 0.37 µg/mL, respectively. In contrast to the methanol extract, the water extract was shown to possess a greater iron chelating activity (IC50= 917.9 ± 3.55 µg/mL). The water extract had the greatest total phenolic content (TPC) at 81.07 ± 4.71 µg GAE/mg, whereas the methanol extract had a higher total flavonoid content (TFC) at 46.93 ± 1.94 µg QE/mg. Further, the water extract exhibited a significant level of anti-BChE activity (IC50= 58.09 ± 1.18 µg/mL). Furthermore, the water extract had caffeic acid as its primary component, and the methanol extract contained ellagic acid as its significant component.

In sum, As a result, no previous research has documented S. bombycina's antioxidant and enzyme inhibitory capabilities till now.

Our results on S. bombycina suggest that this study has the potential to aid in the search for naturally occurring bioactive compounds. Additionally, additional research is required to determine the specific phytoconstituents of S. bombycina that are responsible for its bioactivity and other possible biological effects.

Stachys bombycina, antioxidant properties, and enzyme inhibition

INTRODUCTION

Globally, the Lamiaceae family counts over 290 species of Stachys L. The Americas, southern Asia, the Mediterranean, and South Africa are major cultivators of these species (Yılmaz, Daşkın, & Kaynak, 2010). The percentage of Stachys species that are considered extinct in Turkey is 48% (Kirkan, 2019).

Tomou, Barda, & Skaltsa (2020) and Tundis, Peruzzi, & Menichini (2014) state that for millennia, members of the genus Stachys have been used to cure cough, genital organ cancer, splenic illness, inflammatory diseases, ulcerations, and other conditions. The main categories of secondary metabolites found in these species of the genus include bioactive chemicals, phenolic components (including phenolic acids), iridoids, flavonoids, and fatty acids (Duru, Çakır, Harmandar, Izumi, & Hirata, 1999). According to many research (Háznagy-Radnai et al., 2008; Háznagy-Radnai et al., 2012; Kukić, Petrović, & Niketić, 2006; Saeedi, Morteza-Semnani, Mahdavi, & Rahimi, 2008), extracts from Stachys spp. have antioxidant, anti-inflammatory, cytotoxic, and antibacterial properties.

The provinces of Antalya, Muğla, and Mersin are the only places where the endemic species S. bombycina Boiss. is abundant (Delazar et al., 2005). To the best of our knowledge, no studies have investigated the antioxidant and enzyme inhibitory effects of extracts from this species, despite extensive investigation into its phytochemical and biological activities (Kucukbay, Ozgul, Kucukbay, & Akcicek, 2011).

Dementia comes in many forms, but one of the most prevalent and devastating is Alzheimer's disease (AD), a neurodegenerative disease that slowly but surely becomes one of the most important chronic disorders affecting the elderly throughout the globe. One medicine that may help with Alzheimer's disease is an inhibitor of acetylcholinesterase (AChE). Research has shown that medicinal plants contain high concentrations of cholinesterase inhibitors. Because of their abundance of secondary metabolites, plant materials have played a significant role in the hunt for cholinesterase inhibitors.

Numerous scientific investigations have focused on these molecules, which are well-known for their diverse chemical structures. High blood sugar levels, produced by either insufficient insulin production by the pancreas or ineffective insulin use by the body, define the metabolic disorder known as diabetes mellitus (DM). Various anti-diabetic medications derived from both natural and synthetic sources are commercially accessible. Unfortunately, there are restrictions on the usage of current drugs because of their inefficiency, high cost, and adverse effects (Saravanakumar et al., 2021). Previous research has investigated the potential anti-diabetic effects of extracts and essential oils from several species of Stachys (Bahadori, Maggi, Zengin, Asghari, & Eskandani, 2020; Bursal, Taslimi, Gören, & Gülçin, 2020; Kang et al., 2017).

The copper-containing enzyme tyrosinase is involved in the synthesis of the sun-protective pigment melanin. Skin issues including melasma, acne, and skin cancer result from an overabundance of the substance on the skin. So, to prevent neurological illnesses and problems induced by excessive pigmentation, it is helpful to block the tyrosinase enzyme (Gou et al., 2017). To the best of our knowledge, there is little data about the biological activities, uses, and phytochemical components (such as flavonoids and phenolics) of S. bombycina. Hence, this study set out to investigate the antioxidant capabilities of methanol and water extracts of aerial portions of S. bombycina, together with the inhibitory activity of tyrosinase, AChE, BChE, α-amylase, and α-glucosidase. Furthermore, we used HPLC-DAD to examine the phytochemical profiles of the extracts with respect to phenolic components.

MATERIAL AND METHODS

As a prerequisite to extraction, on April 12, 2018, we collected the fresh aerial parts of wild S. bombycina in Yarikpinar canyon, west of Antalya. Professor Dr. Hayri Duman of Gazi University's Faculty of Science verified the plant material's identification. Selcuk University's Herbarium KNYA number 26911 houses the specimen plant.

Recovering information: Ten grams of the S. bombycina sample was dried and powdered before being macerated with methanol. The mixed filtrates were extracted three times and then concentrated to dryness using a rotary evaporator to get a methanol extract. Maceration with distilled water was then performed three times on the plant material residue. The aqueous extract was lyophilized until it was dry after filtration. The samples were stored at -20 °C until they were needed for the tests determining TPC and TFC

Folin-Ciocalteu was used to determine the TPC and TFC, using gallic acid as the standard, and aluminum chloride as the quercetin standard. Our earlier published study (Eruygur & Ayaz, 2021) served as the basis for the methodology.

Analyzing phenolic chemicals quantitatively using the HPLC method. The chromatographic study was carried out using HPLC (Agilent Technologies, Wilmington, DE, USA) to evaluate the phytochemical profiles of the water and methanol extracts. Typically, many phenolic compounds may be measured simultaneously using a DAD detector with a wavelength set at 280 nm. In order to conduct the analysis, a mix of 25 mg of dry crude extract and 1 ml of methanol was used, and then 10 µl of the diluted sample was injected.

In order to analyze the separations, column C18 (ACE 5,250 x 4.6mm; 5µm; 0.8 ml/min) was run at 30 °C. Three different solutions were used to make up the mobile phase: water (A), methanol (B), and acetonitrile (C). Each solution included 0.1% acetic acid. Between 0 and 8 minutes, a gradient elution program was implemented using a combination of A, B, and C in the following order: 80:12: 8. Using the following steps: 8–45 minutes of 75:15:10, 70:18:12, 65:20:15, 50:35:15, and 25:60:15 for the mobile phase polarity, and then 5 minutes of reconditioning the column with the original elution program (80:12:8). Filtration was performed on the samples and mobile phase using a 0.22 µm filtration system from Millipore Corporation in Billerica, MA. We measured each sample three times.

Finding the antioxidant capacity

The experimental methods were carried out in the same way as previously mentioned (Clarke, Ting,

Fry and Wiart (2013). We adjusted the procedure described by Re et al. (Re et al., 1999) somewhat to find the ABTS scavenging activity. Spectrophotometry of iron-ferrozine absorbance at 562 nm constituted the basis of the metal chelating test (Chai, Mohan, Ong, & Wong, 2014).

Activity of enzyme inhibitors

The samples were prepared in a slightly modified version of Ellman's procedure (Ellman, Courtney, Andres Jr., & Featherstone, 1961) in order to assess their anticholinesterase activity (AChE and BChE). According to Lordan, Smyth, Soler-Vila, Stanton, and Ross (2013), the 96-well plate method was used to evaluate the extracts' α-Glucosidase inhibitory capabilities. According to Özek (2018), the α-amylase inhibition capabilities were examined using the Caraway-Somogi iodine/potassium iodide design. The impact of inhibiting the tyrosinase enzyme was measured using an innovative method, as stated before (Jeong et al., 2009).

Data analysis using statistical methods

In order to analyze the data, GraphPad Prism 8.0 was used. Three independent determinations were averaged and their standard deviations were included in the report. We used Student's t-test and one-way ANOVA (Tukey test) to check for statistical significance. A p-value of less than 0.05 was considered a statistically significant finding.

RESULTS

Perfetic acid HPLC analysis

The phytochemical profiles of the water and methanol extracts were examined using HPLC-DAD in relation to the different flavonoids and phenolic acids that were found (Table 1). As shown in Figure 1, the primary components of the methanol extract were found to be ellagic acid (92.807 µg/mg), chlorogenic acid (11.817 µg/mg), salicylic acid (3.182 µg/mg), and caffeic acid (1.875 µg/mg). Figure 2 shows that the water extract included mostly caffeic acid (3.306 µg/mg), catechin (0.411 µg/mg), and quercetin (0.596 µg/mg) among its phenolic components.

Activity of antioxidants

There was a higher concentration of phenolics in the extracts as compared to flavonoids. In comparison to the methanol extract, which had a TPC of 75.70 ± 3.20 µg GAE/mg, the water extract had a higher concentration of gallic acid (GAE) at 81.07 µg/mg. However, compared to the water extract, which had a TFC of 41.22 ± 2.99 µg QE/mg, the methanol extract had a higher concentration of 46.93 ± 1.94 µg QE/mg.

As seen in Table 1. Elfalleh, Kirkan, and Sarikurkcu (2019) reported that methanol was deemed more suitable for the extraction of flavonoid chemicals, and this result is greater than that of a previous study on Stachys tmolea. Table 1 shows that among the antioxidant activity tests conducted in this investigation, there were significant differences

between the water and methanol extracts. These tests included DPPH, ABTS, and iron chelating. The methanol extract was discovered to have the most potent radical scavenging capabilities when the DPPH and ABTS techniques were used. The IC50 values for the methanol extract were 605.7 ± 1.04 and 19.40 ± 0.37 µg/mL, respectively. This may be due to the fact that the methanol extract contains flavonoids that are very effective in scavenging free radicals. Under no circumstances

Table 1. The phenolic contents of the methanol and water extracts of *S. bombycina* (µg/mg, n=3).

Analyte	Retention time (min)	Methanol extract	Water extract
Gallic acid	4.69	0.008	-
3,4-dihydroxy benzoic acid	6.98	0.065	0.022
Catechin	7.97	-	**0.411**
Chlorogenic acid	8.79	**11.817**	0.113
4-hydroxy benzoic acid	10.65	0.047	0.227
1,2-dihydroxy benzene	11.09	0.066	-
Epicatechin	11.40	0.294	0.378
Vanillic acid	11.80	-	0.169
Caffeic acid	12.18	**1.875**	**3.306**
Vallinin	17.63	0.029	0.007
p-Coumaric acid	18.27	-	0.272
Sinapic acid	19.17	0.510	0.293
Trans-Ferulic acid	20.07	0.262	0.091
Ellagic acid	21.17	**92.807**	0.294
Rutin	22.40	0.207	0.091
Salicylic acid	32.88	**3.182**	0.201
Quercetin	36.26	0.241	**0.596**
Kaempferol	39.97	0.327	0.129

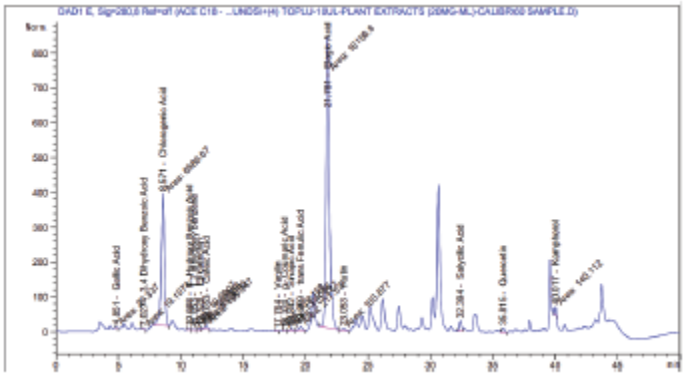

Figure 1. HPLC chromatogram of *S. bombycina* methanol extract.

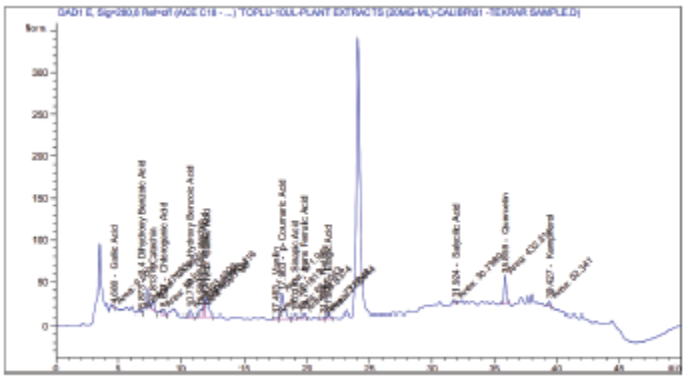

Figure 2. HPLC chromatogram of *S. bombycina* water extract.

The IC50 value of 917.9 ± 3.55 µg/mL for the iron chelating activity was higher than that of 3098 ± 1.91 µg/mL for the methanol extract. In line with other studies, these results were likewise obtained (Elfalleh et al., 2019).

Interactions between enzymes

The results of the enzyme inhibitory effect tests on the methanol and water extracts made from the aerial portions of S. bombycina are shown in Table 2. There was no significant difference between the extracts' inhibitory effects and those of positive control medications when tested at the same dosages. We used AChE and BChE enzymes to study S. bombycina's capacity to suppress cholinesterase activity. Our findings indicate that the methanol extract had weak inhibitory effects on AChE and BChE. The inhibition of BChE was greater in the water extract (IC50: 58.09 ± 1.18µg/mL) compared to the methanol extract. There is a linear dose pattern to the extracts' putative tyrosinase inhibition. Tyrosinase inhibitions were minimal in the water and methanol extracts (Table 2). The inhibitory effects on the enzymes α-glucosidase and α-amylase were used to study the antidiabetic activity of S. bombycina. With acarbose as the positive control and an IC50 value of 825.7±1.03 µg/mL, the water extracton α-glycosidase was found to have an IC50 value of 749.3 ± 0.98 µg/mL. On the other hand, the α-glycosidase enzyme was not activated by the methanol extract. With an IC50 value of 605.7 ± 1.04 µg/mL, the methanol extract exhibited the most potent inhibitory action on α-amylase, in comparison to the positive control, acarbose, which had an IC50 value of 259.4 ± 2.02 µg/mL. The current research shown that the α-glycosidase enzyme was more selectively targeted by the water extract, but the α-amylase enzyme was more strongly affected by the methanol extract.

DISCUSSION

The methanol extract of S. bombycina had higher levels of TPC and TFC in our study (75.70 µg GAEs/mg and 46.93 µg QEs/mg, respectively), as compared to S. tmolea from Turkey, which had been examined earlier by Elfalleh et al. (2019). Hence, we hypothesised that variations in solvents and extraction methods may account for observed polyphenol and antioxidant property differences.

Evidence suggests that essential oils and extracts high in terpenes significantly inhibit AChE and BChE. The potential cholinesterase inhibitory effects of trans-caryophyllene and β-phellandrene were shown by Bonesi et al. (2010). Important components of S. bombycina essential oil include nonacosane, E-9-octadecenoic acid, hexadecanoic acid, β-caryophyllene, germacrene D, caryophyllene oxide, and phytol (Kucukbay et al., 2011). A separate study on Salvia lavandulifolia found that the hexane

Table 2. Extract yield, total phenol and flavonoid content, and antioxidant activities of *S. bombycina* methanol and water extracts.

Extract/ Reference	Extract yield (% g/g)	Total phenolic (μg GAEs/mg)[b]	Total flavonoids (μg QEs/mg)[c]	Antioxidant activity (μg/mL)		
				DPPH (IC_{50})	ABTS (IC_{50})	Iron chelating (IC_{50})
Methanol	17.93	75.70 ± 3.20	46.93 ± 1.94	605.7 ± 1.04	19.40 ± 0.37	3098 ± 1.91
Water	8.51	81.07 ± 4.71	41.22 ± 2.99	1960 ± 0.69	109.2 ± 1.03	917.9 ± 3.55
Quercetin		-	-	9.62 ± 0.09	-	-
BHT		-	-	-	0.7 ± 0.22	-
EDTA		-	-	-	-	437.3 ± 2.31

a: Values expressed are means ± S.D. of three parallel measurements and values were calculated according to negative control. Values with different letters in the same column were significantly different ($p < 0.05$)
b: GAEs. Gallic acid equivalents ($y = 0.003x + 0.0578$ gallic acid (μg) ($r^2 = 0.999$))
c:QEs. Quercetin equivalents ($y = 0.0068x + 0.0928$ quercetin (μg) ($r^2 = 0.9982$)).

Table 3. Enzyme inhibitory activity of methanol and water extracts of *S. bombycina* (IC_{50} μg/mL)[a]

Samples	Extract	AChE	BChE	Tyrosinase	α-glucosidase	α-amylase
S. bombycina	methanol	5668 ± 0.83	3028 ± 0.54	3129 ± 0.21	N.E.	605.7 ± 1.04
	water	1418 ± 1.05	58.09 ± 1.18	1182 ± 0.67	749.3 ± 0.98	3686 ± 0.97
Galanthamine		28.16 ± 2.01[b]	27.34 ± 1.86[b]	-	-	-
Kojic acid		-	-	107.3 ± 0.66[b]	-	-
Acarbose		-	-	-	825.7±1.03[b]	259.4 ±2.02[b]

a: IC_{50} values are given as the mean and standard deviation (Mean ± SD) of three parallel measurements
b: Reference compound
N.E.: not active

According to Tundis et al. (2015), the AChE and BChE inhibition effects were most pronounced in dichloromethane extracts, with IC50 values of 13.7 and 143.9 μg/mL, respectively. According to Bursal et al. (2020), S. annua exhibited the highest anti-cholinesterase activity with IC50 values against several enzymes in the following study: AChE was inhibited by the methanol extract at 119.8 μg/mL, and BChE by the water extract at 186.7 μg/mL. According to Burstal et al. (2020), the antidiabetic action was best shown by the aqueous extract of S. annua against α-glycosidase and α-amylase, with IC50 values of 18.7 and 11.4 μg/mL, respectively. Prior research shown that compared to the hexane and methanol extracts, the α-amylase inhibitory activity of the ethyl acetate extract of S. germanica subsp. heldreichii was stronger (IC50: 2.24 mg/mL). According to a correlation study of chemical composition and activity data, it was also suggested that the extract's apigenin might have contributed to the activity (Sarikurkcu, Ceylan, Benabdallah, & Tepe, 2020).

The IC50 value of 2.90 mg/mL for the antityrosinase activity of the methanol extract of S. germanica subsp. heldreichii was determined to be rather relevant (Sarikurkcu et al., 2020). We found that the antityrosinase activity of the water extract was stronger (IC50: 1182 ± 0.67 μg/mL) than that of the research reported before. Negligible antityrosinase activity was observed in the methanol extract (IC50: 3129 ± 0.21 μg/mL) compared to the other research.

CONCLUSION

The research demonstrated that S. bombycina had formidable antioxidant capabilities, including DPPH, ABTS, and iron chelating, as well as moderate enzyme inhibitory capabilities. Notably, the water extract shown very promising results against BChE and α-glucosidase. Our knowledge of this plant's phenolic content and biological activity is limited. To find new antioxidants and important enzyme inhibitors in nature, further chemical screening studies utilizing different solvents and phytochemical analyses are needed.

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