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Original Article

Investigation of enzyme inhibition potentials, and antioxidative properties of the extracts of endemic *Stachys bombycina* Boiss.

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ABSTRACT

Background and Aims: The genus *Stachys* L., is represented by around 300 species worldwide. More than 120 taxa, almost 60 of which are endemic, are widely distributed in Turkey, particularly in the eastern and southern regions. Stachys species have traditionally been used for many diseases such as asthma, rheumatism, cough, genital tumors, ulcers, diabetes, hemorrhoids, kidney stones, and various mental disorders. Among the species, *S. bombycina* Boiss., namely "arıçayçesi" in Turkish, is one of the near-threatened endemic perennial herbs.

Methods: The antioxidant activity of methanol and water extracts of *S. bombycina* was examined utilizing in vitro techniques, including radical scavenging, such as DPPH, and ABTS, an iron-chelating assay, and the total phenol (TPC) and flavonoid contents (TFC). The extracts were also investigated on enzyme inhibition effects using *in vitro* spectrophotometric method. HPLC analysis was also used for the determination of the phytochemical profiles of the extracts.

Results: Based on our results, the methanol extract of *S. bombycina* demonstrated higher DPPH and ABTS radical scavenging activity with the IC_{50} value of 605.7 ± 1.04 and 19.40 ± 0.37 µg/mL, respectively, than the water extract. Otherwise, the water extract was found to have a higher iron chelating activity (IC_{50}= 917.9 ± 3.55 µg/mL) than the methanol extract. The highest TPC of the water extract was determined as 81.07 ± 4.71 µg GAE/mg, although the methanol extract had more TFC at 46.93 ± 1.94 µg QE/mg. In addition, high anti- BChE activity was observed (IC_{50}= 58.09 ± 1.18 µg/mL) in the water extract. In addition, ellagic acid was defined as a major component in the methanol extract, while caffeic acid was detected as the main compound in the water extract.

Conclusion: Consequently, the current study is the first to report the antioxidant and enzyme inhibitory properties of *S. bombycina*. According to our findings on *S. bombycina*, this work can contribute to the development of bioactive agents from natural sources. Moreover, further investigations still need to be conducted on the discovery of the phytoconstituents of *S. bombycina* responsible for the bioactivity, as well as its potential various biological activities.

Keywords: *Stachys bombycina*, Enzyme inhibition, Antioxidant activity

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INTRODUCTION

There are around 290 species of *Stachys* L. (Lamiaceae) throughout the world. These species are widely grown in the Mediterranean, southern Asia, South Africa, and the Americas (Yılmaz, Daşkın, & Kaynak, 2010). Turkey is a country with an endemism rate of 48% in terms of *Stachys* species (Kirkan, 2019). For millennia, the plants from the genus *Stachys* have traditionally been utilized for the treatment of genital organ malignancies, splenic disease, inflammatory disorders, ulcerations, and cough (Tomou, Barda, & Skaltsa, 2020; Tundis, Peruzzi, & Menichini, 2014). Bioactive compounds, phenolic components including phenolic acids, iridoids, and flavonoids, as well as fatty acids are the principal groups of secondary metabolites in these species belonging to the genus (Duru, Çakır, Harmandar, Izumi, & Hirata, 1999). Several studies have found that extracts from *Stachys* spp. exhibit anti-inflammatory, cytotoxic, antibacterial, and antioxidant activities (Háznagy-Radnai et al., 2008; Háznagy-Radnai et al., 2012; Kukić, Petrović, & Niketić, 2006; Saeedi, Morteza-Semnani, Mahdavi, & Rahimi, 2008).

S. bombycina Boiss. is an endemic species to Turkey, and can only be found in abundance in the provinces of Antalya, Muğla, and Mersin (Delazar et al., 2005). This species has been explored for phytochemical and biological activity, but no research on antioxidant and enzyme inhibitory activitieshas been performed on the extracts as far as we know (Kucukbay, Ozgul, Kucukbay, & Akcicek, 2011).

Alzheimer's disease (AD) is a neurodegenerative disorder that causes cognition, memory and behavior problems and is quite common form of dementia., and therefore, gradually, is one of the world's most critical chronic geriatric illnesses. Acetylcholinesterase (AChE) inhibitors are used to treat AD. Cholinesterase inhibitors have been found to be abundant in medicinal plants. Plant materials have been an important source in the search for cholinesterase inhibitors due to the secondary metabolites. These compounds are known for different chemical structures, and there are many scientific studies on them.

Diabetes mellitus (DM) is a metabolic condition characterized by high blood sugar levels caused by the pancreatic failure to make adequate insulin for the organism or the body's failure to properly utilize the insulin effectively. Several anti-diabetic drugs are available on the market, and they are made from natural and/or synthetic sources. However, due to inefficiency, cost, and side effects, the present medications have limitations in their use (Saravanakumar et al., 2021). The extracts and essential oils of various *Stachys* species have previously been tested for antidiabetic activity (Bahadori, Maggi, Zengin, Asghari, & Eskandani, 2020; Bursal, Taslimi, Gören, & Gülçin, 2020; Kang et al., 2017).

Tyrosinase is a copper-containing enzyme that participates in the production of melanin, which protects the dermal layer from the sun. However, excessive accumulation on the skin causes skin problems such as blemishes, skin cancer, and melasma. Therefore, inhibition of the tyrosinase enzyme that produces melanin helps to avoid not only disorders caused by excessive pigmentation, but also neurological diseases (Gou et al., 2017).

As far as we know, there is very little information on the phytochemical components, including phenolics and flavonoids, biological activities, as well as the usages of *S. bombycina*. Therefore, the objective of this research was to examine the antioxidant activity of methanol, and water extracts of *S. bombycina* aerial parts, as well as the inhibitory activity of tyrosinase, AChE, BChE, α-amylase, and α-glucosidase. In addition, we analyzed the extracts for their phytochemical profiles regarding phenolic compounds by HPLC-DAD.

MATERIAL AND METHODS

Plant Material: Before the extraction process, the fresh aerial parts of wild *S. bombycina* were gathered in the west of Antalya, in Yarikpinar canyon, on 12.04.2018. Identification of the plant material was confirmed by Prof. Dr. Hayri Duman, from the Faculty of Science, Gazi University. The plant sample of the specimen was held in Selcuk University, the Herbarium KNYA number 26911.

Extraction: After powdering and drying, 10 g of material from the *S. bombycina* sample was macerated with methanol. After filtering the combined filtrates and extracting them three times, they were concentrated until drieness with a rotary evaporator to give a methanol extract. Then the residue of the plant material was subjected to maceration with distilled water three times. After filtering, the water extract was lyophilized to dryness. The extracts were kept at -20 °C until used for the experiments.

TPC and TFC determination

The TPC and TFC were determined using Folin-Ciocalteu (gallic acid as standard) and aluminum chloride (quercetin as standard). The methods are based on our previously published research (Eruygur & Ayaz, 2021).

Phenolic compounds quantitative analysis by High-Performance Liquid Chromatography (HPLC)

To investigate phytochemical profiles of the methanol and water extracts, chromatographic analysis was conducted by HPLC (Agilent Technologies, Wilmington, DE, USA). The wavelength of the DAD detector was adjusted at 280 nm generally used for the simultaneous determination of different phenolic compounds. For analysis, 25 mg of dry crude extract was diluted in 1 ml of methanol, and a sample volume of 10 µl was injected. The analysis of separations was performed at 30 °C on column C18 (ACE 5,250 x 4.6mm; 5µm; 0.8 ml/min). The mobile phase was composed of water with 0.1% acetic acid (A), methanol with 0.1% acetic acid (B), and acetonitrile with 0.1% acetic acid (C). A gradient elution program for a mixture of A, B, and C was applied at 0-8 min (A: B: C; 80:12: 8). The mobile phase polarity was gradually decreased with 75:15:10, 70:18:12, 65:20:15, 50:35:15, and 25:60:15 at 8-45 min, and then programmed back to the initial elution program (80:12:8) for the reconditioning of the column for 5 min. The samples and mobile phase were filtered utilizing a 0.22 µm filtration apparatus (Millipore Corp., Billerica, MA). Each sample was analyzed in triplicate.

Determination of antioxidant activity

To investigate DPPH radical scavenging activity, experimental procedures were conducted as previously stated (Clarke, Ting,

Wiart, & Fry, 2013). For determining the ABTS scavenging activity, the method applied by Re et al. (Re et al., 1999) was used with minor modifications. The metal chelating test was based on a spectrophotometric measurement of iron-ferrozine absorbance at 562 nm (Chai, Mohan, Ong, & Wong, 2014).

Enzyme inhibitory activity

To evaluate the anticholinesterase activity (AChE, and BChE) of the samples, they were processed as mentioned by Ellman's protocol (Ellman, Courtney, Andres Jr, & Featherstone, 1961) with slight modification. α-Glucosidase inhibition properties of the extracts were assessed by the 96-well plate technique (Lordan, Smyth, Soler-Vila, Stanton, & Ross, 2013). The Caraway-Somogi iodine/potassium iodide design was used to investigate the α-amylase inhibition capabilities as reported previously (Özek, 2018). The tyrosinase enzyme inhibition effect was determined using an original technique as previously mentioned (Jeong et al., 2009).

Statistical analysis

GraphPad Prism 8.0 was used to conduct the data analysis. The report was produced as a mean of three parallel determinations with standard deviation. To evaluate the statistical significance, one-way ANOVA (Tukey test) and Student's t-test were utilized. The results were regarded as significant when the p-value was less than 0.05.

RESULTS

HPLC analysis of phenolics

HPLC–DAD was used to examine the phytochemical profiles of the methanol and water extracts with respect to various phenolic acids and flavonoids discovered (Table 1). The main constituents of the methanol extract were detected as 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) as seen in Figure 1. However, caffeic acid (3.306 µg/mg), catechin (0.411 µg/mg), and quercetin (0.596 µg/mg) were the more predominant phenolic compounds in the water extract (Figure 2).

Antioxidant activity

When phenolics were compared to flavonoids, the level of phenolics was found to be greater in the extracts. The TPC was higher in the water extract (81.07 µg gallic acid (GAE)/mg extract) than in the methanol extract (75.70 ± 3.20 µg GAE/mg extract). On the contrary, the TFC was found to be higher in the methanol extract (46.93 ± 1.94 µg quercetin (QE)/mg extract) than in the water extract (41.22 ± 2.99 µg QE/mg extract). (Table 1). This result is higher than a prior investigation on *Stachys tmolea* and it was stated that methanol was found to be more appropriate for extraction of flavonoid compounds (Elfalleh, Kirkan, & Sarikurkcu, 2019). In this study, the methanol and water extracts exhibited substantial differences in each antioxidant activity assay, such as DPPH, ABTS, and iron chelating, as seen in Table 1. Utilizing the DPPH, and ABTS methods, it was found that the methanol extract possessed the strongest radical scavenging capabilities, with IC_{50} values of 605.7 ± 1.04, and 19.40 ± 0.37 µg/mL, respectively. This could be because of certain flavonoid components in the methanol extract with high radical scavenging abilities. Otherwise, the water extract

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

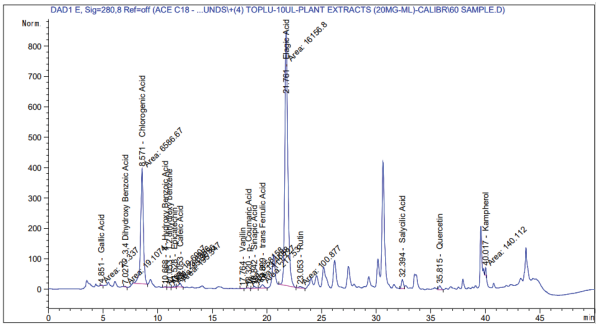

Figure 1. HPLC chromatogram of *S. bombycina* methanol extract.

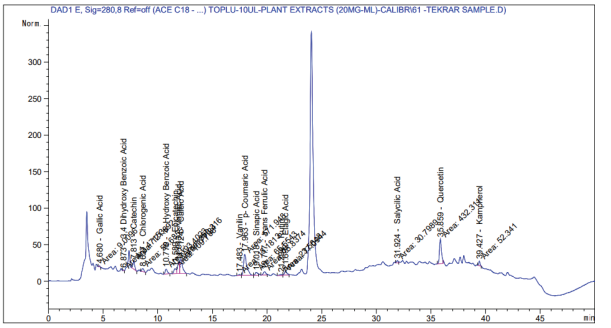

Figure 2. HPLC chromatogram of *S. bombycina* water extract.

exhibited stronger iron chelating activity with IC_{50} value of 917.9 ± 3.55 µg/mL than the methanol extract with IC_{50} value of 3098 ± 1.91 µg/mL. These findings were also consistent with previous research (Elfalleh et al., 2019).

Enzyme inhibitory effects

Table 2 shows the results of testing the enzyme inhibitory effects of the methanol and water extracts prepared from *S. bombycina* aerial parts. The inhibitions of the extracts were also comparable to that of positive control medicines at the same doses. To investigate the ability of cholinesterase enzyme inhibition by *S. bombycina*, the enzymes AChE, and BChE were used. According to our results, the methanol extract showed low inhibitions against AChE and BChE. The water extract demonstrated higher BChE inhibition (IC_{50}: 58.09 ± 1.18µg/mL) than the methanol extract. The potential of tyrosinase inhibition by the extracts progresses in a linear dosage pattern. The methanol and water extracts displayed low tyrosinase inhibitions (Table 2). The antidiabetic activity of *S. bombycina* was investigated by its inhibitory effects on enzymes α-glucosidase and α-amylase. The IC_{50} value of the water extracton α-glycosidase was calculated as 749.3 ± 0.98 µg/mL (with acarbose as the positive control with IC_{50} value of 825.7±1.03µg/mL). Otherwise, the methanol extract showed no activity on α-glycosidase. The strongest α-amylase inhibitory effect was found on the methanol extract with IC_{50} value of 605.7 ± 1.04 µg/mL (with acarbose as the positive control with IC_{50} value of 259.4 ±2.02 µg/mL). The present study showed that the water extract exhibited a higher selectivity against the α-glycosidase enzyme, while the methanol extract showed a stronger sensitivity against the α-amylase enzyme.

DISCUSSION

In our research, TPC and TFC were detected to be more in the methanol extract of *S. bombycina* with 75.70 µg GAEs/mg, and 46.93 µg QEs/mg, respectively, compared to *S. tmolea* from Turkey previously tested by Elfalleh et al. (2019). Therefore, we proposed that variances in polyphenols and antioxidant properties could be caused by different extraction procedures and solvents.

Previous research has found that terpene-rich essential oils and extracts have a substantial inhibitory effect on AChE and BChE. Trans-caryophyllene and β-phellandrene were reported for their prospective cholinesterase inhibition properties (Bonesi et al., 2010). Similar to this, nonacosane, E-9-octadecenoic acid, hexadecanoic acid, *β*-caryophyllene, germacrene D, caryophyllene oxide, and phytol were identified as important components in *S. bombycina* essential oil (Kucukbay et al., 2011). In another investigation on *S. lavandulifolia*, 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 (*µ*g GAEs/mg)[b]	Total flavonoids (*µ*g 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

and dichloromethane extracts had the strongest AChE and BChE inhibition effects with IC_{50} values of 13.7 and 143.9 µg/mL, respectively (Tundis et al., 2015). In another study, the most anticholinesterase activity with IC_{50} values of *S. annua* against different enzymes was as follows: the methanol extract was 119.8 µg/mL on AChE, while the water extract was 186.7 µg/mL on BChE (Bursal et al., 2020).

As for antidiabetic activity, the water extract of *S. annua* was reported as the most active on *α*-glycosidase, and *α*-amylase with IC_{50} values of 18.7 and 11.4 µg/mL, respectively (Bursal et al., 2020). In a previous work, ethyl acetate extract of *S. germanica* subsp. *heldreichii* showed higher α-amylase inhibition activity (IC_{50}: 2.24 mg/mL) than the hexane and methanol extracts. Moreover, it was stated that apigenin in this extract may be contributed to the activity, according to correlation analysis, of chemical composition and activity data (Sarikurkcu, Ceylan, Benabdallah, & Tepe, 2020).

Antityrosinase activity of the methanol extract of *S. germanica* subsp.*heldreichii* was found as important with anIC_{50} value of 2.90 mg/mL (Sarikurkcu et al., 2020). In our findings, the water extract exhibited higher antityrosinase activity (IC_{50}: 1182 ± 0.67 µg/mL), than the result of the above-mentioned study. Otherwise, the methanol extract was found to have lower antityrosinase activity (IC_{50}: 3129 ± 0.21 µg/mL) than the other study.

CONCLUSION

The findings showed that *S. bombycina* has significant antioxidant potentials, such as DPPH, ABTS, and iron chelating, and moderate enzyme inhibitory properties, with the water extract having particularly great data against BChE and α-glucosidase. There is no information on the phenolic content and biological activity of this plant that we are aware of. More chemical screening investigations using various solvents and phytochemical analyses are required to uncover novel antioxidant and key enzyme inhibitors in nature.

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REFERENCES

- Bahadori, M. B., Maggi, F., Zengin, G., Asghari, B., & Eskandani, M. (2020). Essential oils of hedgenettles (*Stachys inflata, S. lavandulifolia,* and *S. byzantina*) have antioxidant, anti-Alzheimer, antidiabetic, and anti-obesity potential: A comparative study. *Industrial crops and products, 145*, 1-8. https://doi.org/10.1016/j.indcrop.2020.112089
- Bonesi, M., Menichini, F., Tundis, R., Loizzo, M. R., Conforti, F., Passalacqua, N. G., Menichini, F. (2010). Acetylcholinesterase and butyrylcholinesterase inhibitory activity of *Pinus* species essential oils and their constituents. *Journal of enzyme inhibition and medicinal chemistry, 25*(5), 622-628. https://doi.org/10.3109/14756360903389856
- Bursal, E., Taslimi, P., Gören, A. C., & Gülçin, İ. (2020). Assessments of anticholinergic, antidiabetic, antioxidant activities and phenolic content of *Stachys annua*. *Biocatalysis and agricultural biotechnology, 28*, 1-22. https://doi.org/10.1016/j.bcab.2020.101711
- Chai, T., Mohan, M., Ong, H., & Wong, F. (2014). Antioxidant, iron-chelating and anti-glucosidase activities of *Typha domingensis* Pers (Typhaceae). *Tropical Journal of Pharmaceutical Research, 13*(1), 67-72. http://dx.doi.org/10.4314/tjpr.v13i1.10.
- Clarke, G., Ting, K. N., Wiart, C., & Fry, J. (2013). High correlation of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. *Antioxidants, 2*(1), 1-10. https://doi.org/10.3390/antiox2010001
- Delazar, A., Celik, S., Göktürk, R., Unal, O., Nahar, L., & Sarker, S. (2005). Two acylated flavonoid glycosides from *Stachys bombycina*, and their free radical scavenging activity. *Die Pharmazie-An International Journal of Pharmaceutical Sciences, 60*(11), 878-880. Retrieved from https://pharmazie.govi.de/
- Duru, M. E., Çakır, A., Harmandar, M., Izumi, S., & Hirata, T. (1999). The volatile constituents of *Stachys athorecalyx* C. Koch. from Turkey. *Flavour and fragrance journal, 14*(1), 12-14. https://doi.org/10.1002/(SICI)1099-1026(199901/02)14:1<12::AID-FFJ763>3.0.CO;2-7
- Elfalleh, W., Kirkan, B., & Sarikurkcu, C. (2019). Antioxidant potential and phenolic composition of extracts from *Stachys tmolea*: An endemic plant from Turkey. *Industrial crops and products, 127*, 212-216. https://doi.org/10.1016/j.indcrop.2018.10.078
- Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology, 7*(2), 88-95. https://doi.org/10.1016/0006-2952(61)90145-9
- Eruygur, N., & Ayaz, F. (2021). Investigation of acetylcholinesterase, butyrylcholinesterase, α-glucosidase, α-amylase, and tyrosinase inhibition and antioxidant activity of methanol and water extracts from aerial parts of *Phlomis lycia* D. DON. *International Journal of Phytocosmetics and Natural Ingredients, 8*(1), 3-3. Retrieved from https://ijpni.org
- Gou, L., Lee, J., Hao, H., Park, Y.-D., Zhan, Y., & Lü, Z.-R. (2017). The effect of oxaloacetic acid on tyrosinase activity and structure: Integration of inhibition kinetics with docking simulation. *International journal of biological macromolecules, 101*, 59-66. https://doi.org/10.1016/j.ijbiomac.2017.03.073
- Háznagy-Radnai, E., Réthy, B., Czigle, S., Zupkó, I., Wéber, E., Martinek, T., . . . Máthé, I. (2008). Cytotoxic activities of *Stachys* species. *Fitoterapia, 79*(7-8), 595-597. https://doi.org/10.1016/j.fitote.2008.06.009
- Háznagy-Radnai, E., Balogh, Á., Czigle, S., Máthé, I., Hohmann, J., & Blazsó, G. (2012). Antiinflammatory activities of Hungarian *Stachys* species and their iridoids. *Phytotherapy Research, 26*(4), 505-509. https://doi.org/10.1002/ptr.3582
- Jeong, S. H., Ryu, Y. B., Curtis-Long, M. J., Ryu, H. W., Baek, Y. S., Kang, J. E., . . . Park, K. H. (2009). Tyrosinase inhibitory polyphenols from roots of *Morus lhou*. *Journal of agricultural and food chemistry, 57*(4), 1195-1203. https://doi.org/10.1021/jf8033286
- Kang, J.-R., Kang, M.-J., Shin, J.-H., Park, J.-H., Kim, D.-i., Chung, S.-Y., & Shin, J.-H. (2017). Antioxidant and antidiabetic activities of various solvent extracts from *Stachys sieboldii* Miq. *Korean Journal of Food Preservation, 24*(5), 615-622. https://doi.org/10.11002/kjfp.2017.24.5.615

- Kirkan, B. (2019). Antioxidant potential, enzyme inhibition activity, and phenolic profile of extracts from *Stachys cretica* subsp. *vacillans*. *Industrial crops and products, 140*, 1-5. https://doi.org/10.1016/j.indcrop.2019.111639
- Kucukbay, F., Ozgul, O., Kucukbay, H., & Akcicek, E. (2011). Composition of the essential oil of *Stachys bombycina* from Turkey. *Chemistry of Natural Compounds, 46*(6), 982-984. https://doi.org/10.1007/s10600-011-9804-9
- Kukić, J., Petrović, S., & Niketić, M. (2006). Antioxidant activity of four endemic *Stachys* taxa. *Biological and Pharmaceutical Bulletin, 29*(4), 725-729. https://doi.org/10.1248/bpb.29.725
- Lordan, S., Smyth, T. J., Soler-Vila, A., Stanton, C., & Ross, R. P. (2013). The α-amylase and α-glucosidase inhibitory effects of Irish seaweed extracts. *Food chemistry, 141*(3), 2170-2176. https://doi.org/10.1016/j.foodchem.2013.04.123
- Özek, G. (2018). Chemical diversity and biological potential of *Tanacetum praeteritum* subsp. *praeteritum* essential oils. *Journal of the Turkish Chemical Society Section A: Chemistry, 5*(2), 493-510. https://doi.org/10.18596/jotcsa.389075
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine, 26*(9-10), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3
- Saeedi, M., Morteza-Semnani, K., Mahdavi, M., & Rahimi, F. (2008). Antimicrobial studies on extracts of four species of *Stachys*. *Indian journal of pharmaceutical sciences, 70*(3), 403. Retrieved from https://www.ijpsonline.com/
- Saravanakumar, K., Park, S., Mariadoss, A. V. A., Sathiyaseelan, A., Veeraraghavan, V. P., Kim, S., & Wang, M.-H. (2021). Chemical composition, antioxidant, and anti-diabetic activities of ethyl acetate fraction of *Stachys riederi* var. *japonica* (Miq.) in streptozotocin-induced type 2 diabetic mice. *Food and Chemical Toxicology, 155*, 1-13. https://doi.org/10.1016/j.fct.2021.112374
- Sarikurkcu, C., Ceylan, O., Benabdallah, A., & Tepe, B. (2020). *Stachys germanica* subsp. *heldreichii* (Boiss.) Hayek: Phytochemical analysis, antioxidant and enzyme inhibitory activities. *South African Journal of Botany, 143*, 291-300. https://doi.org/10.1016/j.sajb.2020.11.009
- Tomou, E.-M., Barda, C., & Skaltsa, H. (2020). Genus *Stachys*: A review of traditional uses, phytochemistry and bioactivity. *Medicines, 7*(63), 1-74. https://doi.org/10.3390/medicines7100063
- Tundis, R., Bonesi, M., Pugliese, A., Nadjafi, F., Menichini, F., & Loizzo, M. R. (2015). Tyrosinase, acetyl-and butyryl-cholinesterase inhibitory activity of *Stachys lavandulifolia* Vahl (Lamiaceae) and its major constituents. *Records of Natural Products, 9*(1), 81-93. Retrieved from http://www.acgpubs.org
- Tundis, R., Peruzzi, L., & Menichini, F. (2014). Phytochemical and biological studies of *Stachys* species in relation to chemotaxonomy: a review. *Phytochemistry, 102*, 7-39. https://doi.org/10.1016/j.phytochem.2014.01.023
- Yılmaz, Ö., Daşkın, R., & Kaynak, G. (2010). *Stachys pseudobombycina* sp. nov. (Lamiaceae) from south Anatolia, Turkey. *Nordic Journal of Botany, 28*(3), 341-343. https://doi.org/10.1111/j.1756-1051.2009.00620.x