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Research Article

Biological evaluation of *Stachys iberica* subsp. *stenostachya* (Boiss.) Rech.f. and *Scutellaria orientalis* subsp. *sosnowskyi* (Takht.) Fed. growing in eastern Anatolia

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Abstract: Lamiaceae is one of the largest families in the plant kingdom, including the genus *Stachys* and *Scutellaria,* which are used in many folk medicines throughout the world for the prevention and also the treatment of several disorders. *In vitro* biological potential of *Stachys iberica subsp. stenostachya* and *Scutellaria orientalis* subsp. *sosnowskyi* were investigated in the current study. The aerial parts of the plants were extracted using different solvents such as *n*-hexane, chloroform, and methanol. In addition, infusions of each plant were prepared. The antioxidant potential of the samples was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), ABTS assays, ferrous ion-chelating, and ferric reducing antioxidant power (FRAP) assays. Anticholinesterase activity of the extracts was also determined. Spectrophotometric analysis was used to assess the total phenolic content of the samples. The antimicrobial activities of samples were determined by minimal inhibitory concentration (MIC) against seven bacteria and three *Candida spp*. yeast. According to the findings, the infusion demonstrated significant antioxidant properties, whilst the extracts demonstrated high-to-moderate antioxidant effects. The *n*-hexane extracts showed higher antifungal activity against *C.parapsilosis* and *C.tropicalis*. These outcomes suggest that these two species from Turkey could be employed in the manufacture of phytopharmaceuticals.

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1. INTRODUCTION

For centuries, herbal medicine has been practiced all around the world and remains an important part of treatment not only as a support in therapy but also as the prevention of several disorders (Saad *et al.,* 2005). Nowadays, the value of herbal medicines is increasing day by day in the global pharmaceutical market, which encourages studies on both their chemical composition and pharmacological activities on plants (Pieters *et al.,* 2005). In particular, the members of

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Lamiaceae family have gained attention because these plants have been shown to exhibit a wide variety of biological activities by providing a diverse chemical composition (Frezza *et al.,* 2019).

Stachys L. is considered to be one of the most extensive genera in the Lamiaceae family with 370 species (435 taxa) found around the world (Akçiçek *et al.,* 2016; Açar & Satıl*,* 2019). In Turkey, the genus is represented by 92 species (118 taxa), 53.3% of which are endemic (Güner *et al.,* 2021). These species commonly known as 'mountain tea' are used in many traditional medicines as anti-spasmodic, antimicrobial agent, and herbal tea in order to alleviate asthma problems, to decrease the symptoms of earaches, and to stop the growth of genital tumors and a malignant ulcer (Baytop, 1999; Ebrahimabadi *et al.,* 2010; Sarikurkçu *et al.,* 2016; Satıl & Açar*,* 2020; Tomou *et al.,* 2020). Many studies conducted on these species have confirmed remarkable anti-inflammatory, antioxidant, antimicrobial, anxiolytic, hypotensive, hyaluronidase, anti-Helicobacter pylori, and anti-nephritic activities (Shang *et al.,* 2010; Tundis *et al.,* 2014; Kocak *et al;* 2017; Satıl & Açar, 2020; Tomou *et al.,* 2020). From the chemical point of view, *Stachys* species were discovered rich in different types of secondary metabolites such as iridoids, di- and triterpenes, alkaloids, phenylethanoid glycosides, flavonoids, phenolic acids, and essential oil (Ahmad *et al.,* 2008; Bahadori *et al.,* 2020; Demirci *et al.,* 2016; Kartsev *et al.,* 1994; Kaya *et al.,* 2001).

One of the largest genera of the family Lamiaceae is *Scutellaria* L., which consists of approximately 350 species across the world (Georgieva *et al.,* 2021). In Turkey, it is represented by 39 taxa, almost half of which are endemic (Çiçek, 2012). In several folk medicines, the aerial parts of the species have been used for the treatment of several medical problems, including constipation, genital herpes, kidney stones, stomach ulcers and also used for their neuroprotective, antihelminthic, and diuretic functions (Tao *et al.,* 2016). In Turkish traditional medicine, the infusion and decoctions of the *Scutellaria* species are utilized as a tonic, hemostatic, and wound healing agent (Baytop, 1999; İçen *et al.,* 2016). In regard to the phytochemical analysis of the species, over 300 compounds were identified, which showed the presence of different kinds of substances such as alkaloids, flavonoids, polysaccharides, phytosterols, iridoid glycosides, phenylethanoid glycosides, terpenes, and essential oil (Formisano *et al.,* 2011; Mamadalieva *et al.,* 2017; Doğan *et al.,* 2019; Yılmaz *et al.,* 2019; Zhao *et al.,* 2019). A wide range of biological activities were observed in *Scutellaria* extracts, which was attributed to their diverse chemical composition (Zengin *et al.,* 2019). The pharmacological activities of several *Scutellaria* species have been investigated and some traditional usage has been approved such as antioxidant, antimicrobial, anticancer, hepatoprotective, anti-angiogenesis, anticonvulsant, and neuroprotective activities (Nie *et al.,* 2010; Shang *et al.,* 2010; Vergun *et al.,* 2019; Zhao *et al.,* 2019).

Intensive oxidative stress in the body may result in a number of degenerative illnesses including dermatological problems, cancer, and coronary disease and neurological disorders such as Alzheimer's disease (AD) and Parkinson's disease (Bibi Sadeer *et al.,* 2020). Antioxidants are considered to retard or prevent oxidation or the development of oxidizing chain reactions during the oxidation phase (Shahidi, 2000). Synthetic antioxidants have gained prominence in recent years, however, several studies have revealed that they have severe side effects, limiting their use in the body (Koşar *et al.,* 2008). As a result, researchers have focused their efforts on discovering natural antioxidants. Several studies conducted on natural medicinal plants in order to determine their antioxidant activity demonstrated that the raw extracts or extracted pure secondary metabolites from them were found strong to moderate activity compared to that of synthetic antioxidants (Li *et al.,* 2007; Zhang *et al.,* 2015; Toplan *et al.,* 2017). Numerous investigations have established that plant phenolic compounds have significant free radical scavenging capabilities due to their reactivity as hydrogen-or electron-

donating agents and their metal ion-chelating properties. Hence, determining the total phenol content of food plant extracts would be beneficial (Zengin *et al.,* 2019).

Alzheimer's disease (AD), a progressive neurological condition marked by changes in thinking and behavior and, especially in the developed world, has become one of the major public health issues (Orhan *et al.,* 2011). The primary therapeutic method for AD is to restore diminished acetylcholine levels in the brain by inhibiting acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes (Akkol *et al.,* 2012). For the treatment of AD, researchers have concentrated on determining the phytochemical composition and inhibitory effects of chemical compositions of various plant fractions on enzyme activities (Şenol *et al.,* 2010).

Infectious illnesses are a significant public health problem in hospitals and communities (Gibbons, 2005). Indeed, there is a growing need for plant-derived bioactive as an alternative to antimicrobial synthetic medicines since their extensive and continuous usage has resulted in the mutation of resistant bacteria, decreasing the therapeutic efficacy of these treatments (Orhan, et al., 2010). Plants, especially belonging to the Lamiaceae family, are a fascinating study subject due to their reported inhibitory impact on cholinesterases, showing excellent antioxidant, antibacterial, and antifungal activities (Frezza *et al.,* 2019).

In Turkish traditional medicines, *Stachys* and *Scutellaria* species have been widely used for cough, digestive disorders, and wound healing, remain important, and are suggested by local healers (Minareci *et al.,* 2017; Bardakci *et al.,* 2019; Satıl & Açar, 2020). The goal of this study is therefore to investigate the antibacterial, antifungal, antioxidant, and anticholinesterase properties of various extracts obtained from *S. orientalis* subsp. *sosnowskyi* and *S. iberica* subsp. *stenostachya* growing naturally in eastern Anatolia.

2. MATERIAL and METHODS

2.1. Plant Material

The aerial parts of *S. orientalis* subsp. *sosnowskyi* and *S. iberica* subsp. *stenostachya* were collected in Elle Hamlet, Gölyüzü Village, Doğubeyazıt, Ağrı, Turkey during the flowering stage (2017). After their collection, both of the species were air-dried in a dark place at room temperature, separately, and after that, the laboratory mill was used to powder the materials. The plant was identified by one of us (Gülay Ecevit-Genç). Voucher specimens of *S. orientalis* subsp. *sosnowskyi* and *S. iberica* subsp. *stenostachya* were stored at the Herbarium of the Pharmacy Faculty of Istanbul University (ISTE Number: 116566 and 116567, respectively).

2.2. Preparation of Extracts

Air-dried and powdered aerial parts of *S. orientalis* subsp. *sosnowskyi* and *S. iberica* subsp. *stenostachya* were extracted respectively with *n*-hexane, chloroform, and methanol using the Soxhlet apparatus. An infusion was also prepared via the maceration procedure. The infusion was first filtered and then the filtrates were frozen at −80 °C in an ultra-low temperature freezer, lyophilized, and stored at -20 °C until analysis. Solvents were evaporated to dryness under reduced pressure by a rotary evaporator at a maximum temperature of 50 °C. After the evaporation of the solvents, the crude extracts obtained were stored at +4 ºC until the analysis and used for all experiments.

2.3. Determination of Total Phenolic Contents in Extracts

To each 0.1 mL tube was poured 4.5 mL of water, and varied amounts of extracts (1-5 mg/mL) were mixed. Then, 0.3 mL of 2% sodium carbonate solution and 0.1 mL of the Folin-Ciocalteu reagent (diluted 1/3 with distilled water) were added to the mixture.

After allowing the combination to remain at room temperature for two hours, the absorbance was measured at 760 mm. (4). The total phenolic compounds contained in the extracts were given as mg gallic acid equivalents/mg extract (Ozsoy *et al.,* 2008).

2.4. Antioxidant Activity

2.4.1. *Determination of DPPH• Radical Scavenging Activity*

The free radical scavenging activity of the extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (1). Briefly, DPPH solution (0.1 mM, 240 µL) was added to the extracts (10 µL) prepared at different concentrations (1-5 mg/mL). Then, the mixture was let to rest for 30 minutes at room temperature. A microplate reader (AMR-100, Allsheng) was used to compare the mixture's absorbance to the reference at 517 nm.

The experiment was done three times and using the Graphpad Prism 5 Demo application, the averages of the values and standard deviation were calculated. The concentration of extracts and standard substance, which causes a 50% reduction in initial DPPH concentration, was defined as IC_{50}. The results obtained in the experiment were given as IC_{50} = mg / mL (Wei *et al.,* 2010).

2.4.2. *Determination of the reducing power of (CUPRAC) assay*

The reducing power capacity of the samples was measured using the CUPRAC method. A plate was combined with 1 mL of Cu (II) (1.10-2 M), neocuproine ethanolic mixture (7.3.10-3 M), and 1 M NH_4Ac buffer solution. Extracts 1 mL and 0.1 mL pure EtOH were added to the initial mixture to make the final volume: 4.1 mL. Then, after ten seconds of vortexing, the absorbance of the solution was measured at 450 nm against a reagent blank. Samples of CUPRAC measurements have been demonstrated as equivalents for Trolox (mM Trolox/mg extract). (Apak *et al.,* 2004)

2.4.3. *Ferric reducing antioxidant power (FRAP) assay*

The FRAP reagent was prepared by dissolving 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of the TPTZ solution (10 mM TPTZ in 40 mM HCI) and 2.5 mL of 20 mM $FeCl_3.6H_2O$. Then the FRAP reagent was kept at 37 °C for 30 minutes in an incubator device (Nuve). After 4 minutes, the absorbance of the mixture was measured against a reference at 593 nm using 3.8 mL of the FRAP reagent with 0.2 mL of extract. (3). FRAP values of the samples were reported as mM Fe^{+2}/mg extract (Benzie *et al.,* 1996).

2.5. Determination of Antimicrobial Activity of the Samples

In this study for *in vitro* antimicrobial activities of various extracts obtained from the aerial parts of *S. orientalis* subsp. *sosnowskyi* and *S. iberica* subsp. *stenostachya* were evaluated against *Staphylococcus aureus* ATCC 29213, *S. epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153, *Candida albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, and *C. tropicalis* ATCC 750. The antimicrobial effectiveness of the samples was determined using the broth microdilution technique as described by the Clinical and Laboratory Standards (CLSI, 1997; CLSI, 2020). The MIC values of the samples were defined as the lowest antibiotic concentration that completely inhibited an observable growth. For the positive control, antifungal and antibacterial agents were used such as cefuroxime, cefuroxime-sodium, ceftazidime, amikacin, amphotericin B, and clotrimazole.

2.6. Anticholinesterase Activity of the Samples

The inhibition of cholinesterases was determined using a 96-well microplate reader based on the method developed by Ellman et al. (1961), with some changes. Tris-HCl buffer (50 mM, pH 8.0) was used to prepare all reagent solutions (daily). Shortly, AChE solution (20 μl) was

mixed with 20 μl of the sample and 40 μl of Tris-HCl buffer, and the mixture was kept at room temperature (25 °C) for 10 minutes. Then, 20 μl of ATChI (50 mM) was mixed into the combination and the total mixture was incubated for 5 min. at 25 °C. Then, 100 μl of 20 mM DTNB (containing 1M NaCl and 0.2 M $MgCl_2.6H_2O$) was added to the mixture and its absorbance was read at 412 nm against the reference. Each experiment was conducted in triplicate. Galanthamine was used as a positive control (Ellman *et al.,* 1961).

2.7. Statistical Analysis

Results were expressed as the means ± standard deviation (SD) of three parallel and independent measurements. One-way analysis of variance (ANOVA) was performed, and important differences between means were determined using Tukey's multiple comparisons test. Statistical significance was set at $p < 0.05$.

3. FINDINGS

The antioxidant, anticholinesterase, and antimicrobial activities, as well as the phenolic content, of different solvent extracts obtained from two commonly used Lamiaceae plants are investigated in the present study.

The extract yield and total phenolic content of the samples are presented in Table 1. Of all the extracts, the methanol extracts of both plants had the highest extract yield percentage. When the phenolic components in the extracts were revealed, it was observed that the methanol extract of *S. iberica* subsp. *stenostachya* contained the highest phenolic amounts compared with the other extracts, while the infusion and methanol extracts of *S. orientalis* subsp. *sosnowskyi* contained the high phenolic amounts nearly with similar percentages. Interestingly, the amount of phenolic compound in chloroform extract was found higher than infusion in *S. iberica* subsp. *stenostachya* studied. Additionally, the lowest total phenolic percentages were determined in the *n*-hexane extract of *S. iberica* subsp. *stenostachya.* The *n*-hexane and chloroform extracts of *S. orientalis* subsp. *sosnowskyi* contained a moderate amount of phenolic compounds.

Table 1. The yield and total phenolic content of the samples from *S. iberica* subsp. *stenostachya* and *S. orientalis* subsp. *sosnowskyi.*

Samples	Extracts	Yield[1]	Total phenolics[2]
Stachys iberica subsp. *stenostachya* (1)	*n*-hexane	279.6	6±1.4
	chloroform	497.9	46±2.7
	methanol	5977.2	**74±1.6**
	infusion	504.4	21±1.9
Scutellaria orientalis subsp. *sosnowskyi*. (2)	*n*-hexane	272.6	20±0.5
	chloroform	447	28±0.8
	methanol	3175.3	**52±1.9**
	infusion	510.1	**59±1.2**

[1]Extract yields expressed as milligrams of extract per gram (dry weight) of aerial parts of the plant.

[2]The total phenolic compounds contained in the extracts were given as mg gallic acid equivalents/mg extract.

The evaluation of the antioxidant capacity of the plants is recommended with more than one test in the literature (Zengin *et al.,* 2019). For this purpose, three complementary assays have been conducted to determine the antioxidant potential of different extracts of both plants including DPPH• free radical scavenging, FRAP (ferric reducing antioxidant power), and CUPRAC activity methods. The results are given in Table 2.

Table 2. The antioxidant capacity of the samples from *S. iberica* subsp. *stenostachya* and *S. orientalis* subsp. *sosnowskyi.*

Samples	DPPH (mg AaE/g extract)	CUPRAC (mMtrolox/mg extract)	FRAP assay (mM Fe^{2+}/mg extract)
1-H	-	0.057±0.006	0.085±0.008
1-C	12.7±0.7	**0.103±0.002**	**0.325±0.020**
1-M	**62.3±0.3**	**0.100±0.001**	0.062±0.014
1-I	**53.8±2.3**	0.081±0.005	0.020±0.013
2-H	-	0.059±0.005	0.094±0.018
2-K	-	0.069±0.004	**0.308±0.038**
2-M	**62±0.3**	0.099±0.002	0.082±0.004
2-I	**57.2±0.6**	0.098±0.004	0.105±0.007
BHT		1.1±0.12	
BHA			1.622±0.12

Values are mean of triplicate determination (n =3) ±standard deviation; $^{*}p$ <0.05 compared with the positive control

1: *Stachys iberica* subsp. *stenostachya*, 2- *S. orientalis* subsp. *sosnowskyi*, H: *n*-hexane extracts, C: Chloroform extracts; M: Methanol extracts; I: Infusion; BHA: butylated hydroxyanisole; BHT: butylated hydroxytoluene; AaE: ascorbic acid equivalent

DPPH• is frequently employed to assess the antioxidant capacity of samples, which is the most straightforward, least expensive, and easiest method. The DPPH test results indicated that infusion and methanol extracts of both species exhibited the strongest radical scavenging activity. On the other hand, no activity was observed among other extracts except chloroform extract of *S. iberica* subsp. *stenostachya.* In ferric reducing power assay, FRAP values of the chloroform extracts from both plants were found to be approximately the same and also the greatest compared with those of the other extracts. The cupric reducing antioxidant capacity of chloroform and methanol extracts of *S. iberica* subsp. *stenostachya* was demonstrated to be quite similar and to possess strong effects, while the infusion and *n*-hexane showed moderate effects. Besides, the infusion and methanol extracts of *S. orientalis* subsp. *sosnowskyi* exhibited higher cupric reducing power than that of *n*-hexane and chloroform extracts.

There is vast research on the antioxidant properties of *Stachys* and *Scutellaria* species and remarkable effects have been observed in most of these studies (Koçak *et al.,* 2017; Mamadalieva *et al.,* 2017; Elfalleh *et al.,* 2019; Georgieva *et al.,* 2021). It is generally known that these species are rich in essential oil and phenolic compounds, which may be linked to potent antioxidant action (Sarikurkcu *et al.,* 2016; Zengin *et al.,* 2019).

Generally many previous studies established that there is a positive correlation between the phenolic content and antioxidant capacity (Kartsev *et al.,* 2019; Zengin *et al.,* 2019; Zhao *et al.,* 1994). Following the literature, our study also shows that methanol extracts possessed strong antioxidant activity with high total phenolic content. However, other investigated extracts showed strong to moderate antioxidant potential independent of their total phenolic content. Consequently, the antioxidant capability was present in different degrees in the samples studied. The synthetic antioxidants, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) were used as a positive control and the antioxidant capacity of extracts and their infusions were compared with the capacity of samples. Nevertheless, all samples showed lower activity when compared with the standards.

The Ellman technique was subjected to the comparison of the inhibitory potential of the acetylcholinesterase enzyme in various extracts with galantamine which was used as a reference compound. The results are given in Table 3. As a result, only the infusion and methanol extracts of both species demonstrated inhibition against the acetylcholinesterase enzyme. The other investigated samples were found to be inactive. These findings indicated that the enzyme inhibition properties of the infusion of *S. iberica* subsp. *stenostachya* (92.63%) were almost equaled by galantamine (94.52 %). An early study examined the antioxidant and enzyme inhibitory activity of 33 *Scutellaria* species from Turkey, including *Scutellaria orientalis* subsp. *sosnowskyi*. Its methanol extract showed poor anticholinesterase activity, while strong DDPH radical scavenging activity was determined in both ethyl acetate and methanol extracts (Şenol *et al.,* 2010).

Table 3. The anticholinesterase activity of different extracts from *S. iberica* subsp. *stenostachya* and *S. orientalis* subsp. *sosnowskyi.*

Samples	Enzyme inhibition (%) (500 µg/mL)
1-H	-
1-C	-
1-M	82.032±1.116
1-I	**92.635±1.597**
2-H	-
2-C	-
2-M	75.492±0.721
2-I	**85.206±0.220**
Galanthamine	94.52±0.14

1: *Stachys iberica* subsp. *stenostachya*, 2: *S. orientalis* subsp. *sosnowskyi*, H: *n*-hexane extracts, C: Chloroform extracts; M: Methanol extracts; I: Infusion.

The antimicrobial activities of extracts obtained from the aerial parts of *S. iberica* subsp. *stenostachya* and *S. orientalis* subsp. *sosnowskyi* were studied against 7 bacteria and 3 *Candida spp.* yeast using the broth micro dilutions method. Since the infusions were not found active, only the results of extracts are reported in Table 4. According to our results, the *n*-hexane extract of *S. iberica* subsp. *stenostachya* showed *a* moderate inhibitory effect with MIC values of 625 ug/mL and 156.2 ug/mL against *E. faecalis* and *C. parapsilosis*, respectively. Furthermore, the strong antimicrobial activity was identified in methanol extract of *S.iberica* subsp. *stenostachya* against *S. epidermis* with a MIC value of 625 ug/mL. As to the antimicrobial results of *S. orientalis* subsp. *sosnowskyi,* the *n*-hexane extract showed notably antifungal effects against *C. tropicalis* with a MIC value of 312.5 ug/mL, while methanol extract showed strong to moderate inhibitory effects against *E. faecalis* and *C. tropicalis* with a MIC value of 312.5 ug/mL and 625 ug/mL, respectively. None of the extracts and infusion of both species were active against *E.coli*, *K. pneumoniae*, *P. mirabilis, S. aureus,* and *P. aeruginosa*. The existence of antimicrobial activity in *Scutellaria* and *Stachys* species has been approved by previous investigations despite the fact that their effectiveness rates show changes depending on using different solvents and also the composition of the extracts (Sato *et al.,* 2000).

Table 4. The antimicrobial activity of several extracts from *S. iberica* subsp. *stenostachya* and *S. orientalis* subsp. *sosnowskyi.*

Strains	Extracts					
	1-H	1-C	1-M	2-H	2-C	2-M
P. aeruginosa	>2500	>2500	>2500	>2500	>2500	>2500
E. coli	>2500	>2500	>2500	>2500	>2500	>2500
K. pneumoniae	>2500	>2500	>2500	>2500	>2500	>2500
P. mirabilis	>2500	>2500	>2500	>2500	>2500	>2500
E. faecalis	**625**	>2500	>2500	>2500	>2500	**625**
S. epidermidis	>2500	>2500	**625**	>2500	>2500	>2500
S. aureus	>2500	>2500	>2500	>2500	>2500	>2500
C. albicans	>2500	>2500	>2500	>2500	>2500	>2500
C. parapsilosis	**156.2**	>2500	>2500	>2500	>2500	>2500
C. tropicalis	>2500	>2500	>2500	**312.5**	>2500	**312.5**

1: *Stachys iberica* subsp. *stenostachya*, 2- *S. orientalis* subsp. *sosnowskyi*, H: *n*-hexane extracts, C: Chloform extracts; M: Methanol extracts; I: Infusion; Reference compounds: Ceftazidime: 2.4 mg/L For *P. aeruginosa,* Cefuroxime-Na: 4.9 mg/L for *E. coli* and *K. pneumoniae,* Cefuroxime-Na: 2.4 mg/L for *P. Mirabilis,* Cefuroxime-Na: 1.2 mg/L for *S. aureus*, Cefuroxime: 9.8 mg/L for *S. epidermidis*, Amikacin: 128 mg/L for *E. faecalis*, Clotrimazole: 4.9 mg/L *C. albicans*, Amphotericin B: 0.5 mg/L for *C. parapsilosis*, Amphotericin B: 1 mg/L for *C. tropicalis.*

4. DISCUSSION and CONCLUSION

Not only have plants often offered significant opportunities for pharmaceutical development but also they have produced a wide range of secondary metabolites for their defense systems, allowing us to discover new bioactive chemicals. Lamiaceae family has also been extensively investigated due to the presence of considerable therapeutic potential in its members. *Stachys* and *Scutellaria* are one of the most investigated genus whose extracts possess considerable potential in many biological activities. Hence, investigations into them appear to be extremely beneficial in terms of identifying the potential sources of herbal medicines.

Previous studies on the essential oil of the aerial parts of *Stachys iberica* subsp. *stenostachya* showed linalyl acetate (42.2%), linalool (18.9%), geranyl acetate (8.2%), and α-terpineol (5.3 %) (Kaya *et al.,* 2001). In another study, antimicrobial potential of the methanol extract of *S. orientalis* subsp. s*osnowskyi* was investigated and moderate antibacterial and anticandidal effects were observed (Yılmaz *et al.,* 2020). In this study, the biological potential of different solvent extracts of these two species has been evaluated since they are still used for treatment purposes by the local residents. The activities of several extracts prepared from aerial parts were studied using a variety of methodologies including antioxidant, antibacterial, antifungal, and anticholinesterase activity tests. The antioxidant potential of the extracts ranged from moderate to high while considerable activity was observed in infusion and methanol extracts of both species. The *n*-hexane extract of *S. iberica* subsp. *stenostachya* exhibited notable antimicrobial activity. Further studies are needed to investigate the phytochemical composition of the active extracts in order to find out responsible substances.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship contribution statement

Authors are expected to present author contributions statement to their manuscript such as; **Gizem Gulsoy Toplan**: Investigation, Resources, Analysis, and Writing-original draft, Supervision. **Ayse Civas**: Analysis **Emel Mataraci Kara:** Analysis **Turgut Taskin**: Analysis **Gulay Ecevit Genc:** Identification of plants.

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