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Original Article

Antioxidant and antimicrobial activities of various extracts from *Stachys cretica* subsp. *bulgarica* Rech.f., *Stachys byzantina* K. Koch and *Stachys thirkei* K. Koch

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ABSTRACT

Background and Aims: *Stachys* L. species, which are members of the Lamiaceae family, have long been applied for their therapeutic benefits especially to cure cough, infection, genital tumors, ulcers, inflammatory illnesses, and wounds in Anatolian traditional medicine. In the current study, various extracts prepared from the aerial parts of *Stachys cretica* subsp. *bulgarica* Rech.f. (SC), *Stachys byzantina* K. Koch (SB), *Stachys thirkei* K. Koch and were tested for their in vitro antioxidant, antibacterial, and anticandidal properties.

Methods: The aerial parts of three *Stachys* species were sequentially extracted using n-hexane, chloroform, and methanol. Aqueous extracts of each sample was also prepared by infusion process. The total phenolic content of each extract was determined and the contribution of the biological activities in the samples was evaluated. To assess the antioxidant capacity, samples were studied using CUPRAC activity, DPPH• free radical scavenging, and FRAP methods. The antimicrobial activity of the extracts was tested against 7 bacteria and 3 yeast.

Results: The infusion and methanol extract exhibited the strongest antioxidant potential and also had the highest percentage of phenolics among the studied extracts. The *n*-hexane extracts of all studied species showed considerable antifungal activity with MIC values ranging from 312.5-78.12 mg/L.

Conclusion: According to our results, three *Stachys* species were found to be beneficial for their antioxidant and antimicrobial properties.

Keywords: *Stachys*, antioxidant activity, total phenolic, antimicrobial activity

INTRODUCTION

Plants have been evaluated for different purposes throughout humankind's history such as food, hunting, prevention and treatment of diseases, and preparation for spiritual ceremonies. Several studies have confirmed the beneficial properties of plants in

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the pharmaceutical, food, and cosmetic industries and that has led to an increase in their economic value (Silva, & Fernandes Júnior, 2010). In particular, Lamiaceae species offer a broad range of activities due to their rich chemical composition (Frezza, Venditti, Serafini, & Bianco, 2019).

Stachys L. is classified as one of the most extensive genera of the Lamiaceae family, containing over 300 species with almost worldwide distribution (BilušićVundać, 2019; Tundis, Peruzzi, & Menichini, 2014). The flora inTurkey is represented by approximately 90 species including 115 taxa, 54 of which are endemic (Akcicek, Dirmenci, & Dündar, 2012). The species of *Stachys* are annual, perennial herbs or tiny shrubs with simple leaf segments that attach directly to the stem or are sessile. They are commonly known as 'Dağ Çayı' in Anatolian folk medicine and utilized as an appetizer, healer for digestive complaints, stimulant, antispasmodic, and also as carminative (Baytop, 1999; Goren, 2014). Ethnopharmacological usage of *Stachys* species is supported by several studies in the worldwide literature, mainly demonstrated by antibacterial, anti-*Helicobacter pylori*, anti-inflammatory, anticancer, and antioxidant properties (Salehi, Sonboli, &Asghari, 2007; Khanavi et al., 2009; Goren et al., 2011; Tomou, Barda, & Skaltsa, 2020; Tundis et al., 2014). The promising findings shown in research conducted on several antioxidant test systems also indicate the great potential for preventing diseases that correlate with the deficiency of antioxidant mechanisms (Erdemoglu, Turan, Cakıcı, Sener, & Aydın, 2006; Kukić, Petrović, & Niketić, 2006; Hajdari, Novak, Mustafa, & Franz, 2012; Tundis, et al., 2014). The diversity in its pharmacological properties can be explained via its containing multiple classes of secondary metabolites in combination (Pieters & Vlitenick, 2005). An extensive range of investigations have been conducted on the phytochemistry of *Stachys* species that have revealed the presence of iridoids, di- and triterpenes, alkaloids, phenylethanoid glycosides, flavonoids, phenolic acids, and essential oil (Kaya, Demirci, & Baser, 2001; Asnaashari et al., 2010; Demirtas, Gecibesler, & Yaglioglu, 2013; Tomou et al., 2020).

Free radicals have become of concern since they appear to play a role in a wide range of diseases and food deterioration (Fang, Yang, & Wu, 2002). Multiple studies have indicated that the production of reactive oxygen species (ROS) and the resulting oxidative stress are important in the initiation and progression of many major disorders, including cancer and degenerative diseases (Valko et al., 2007). According to the literature, phenolic compounds have significant effects on the protection of degenerative disorders due to slowing down free radical reactions and reducing lipid oxidation (Toplan et al., 2017). Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are commonly employed in the foodservice industry as synthetic antioxidants to prevent oxidative degradation in prepared foods (Branen, 1975). Because of the potentially harmful consequences of these agents, there has been a tremendous rise in international importance and utilization of plants for their antioxidant, anti-aging, and antimicrobial benefits, and besides, they have fewer adverse effects (Li et al., 2012). Therefore, this encourages researchers to investigate plants, especially those rich in phenolics, for their natural antioxidants which keep the human body healthy and preserve food from rotting.

Today, antibiotic resistance is a significant public health problem. There is substantial evidence of resistance to nearly all of the antimicrobial drugs now in use, and this evidence is growing (Kwapong, Soares, Teo, Stapleton, & Gibbons, 2020). In recent decades, the amount of new antibacterial medications has decreased and the design and discovery of new antibacterial chemicals have become one of the key fields of antibacterial research (Coqueiro, Regasini, Stapleton, da Silva Bolzani, & Gibbons, 2014). Another key issue in the food, beverage, cosmetic, and pharmaceutical industries is microbial contamination (Tiwari et al., 2009). Researchers worldwide are keen to discover new natural antimicrobial compounds in response to growing cases of microbe resistance to currently used preservatives. Numerous investigations have approved the promising antibacterial and antifungal characteristics of plant extracts as well as secondary metabolites (Pieters & Vlietinck, 2005). Nowadays, a large percentage of prescribed medicines consist of plant-based products (Atanasov, Zotchev, Dirsch, & Supuran, 2021).

Three *Stachys species,* namely *Stachys cretica* subsp. *bulgarica, S. byzantina* and *S. thirkei* whose traditional names are 'Kızıl Deliçay', 'Bozkarabaş', and 'Kestere' respectively, are three of the most used species in Turkish traditional medicine; their infusions or decoctions are consumed as a tea by local people for mainly gastrointestinal problems (Satıl & Acar, 2020). In the current study, the antioxidant and antimicrobial activities of different extracts obtained from the aerial parts of these three *Stachys* species were screened for their total phenolic content.

MATERIALS AND METHODS

Plant material

The aerial parts of *Stachys cretica* subsp. *bulgarica* (SC)*, S. byzantina* and *S. thirkei* were collected from Saray-Güngörmez, Tekirdağ, and Kastamonu during the flowering stage. After that, the collected plants were dried at room temperature in a dark storeroom. Dried plant material was ground usinga laboratory mill before the experiments. Specimens were identified by one of us (Gulay, Ecevit-Genç) and vouchers were deposited in in Herbarium of İstanbul University Faculty of Pharmacy (ISTE). The herbarium number of each plant are given in Table 1.

Table 1. The location of the collected plants with herbarium number.

Species	Location	Date	Herbarium Number	Sample Code
Stachys cretica subsp. *bulgarica* (SC)	Tekirdağ	15.07.2015	ISTE 117262	SC
Stachys byzantina (SB)	Ilgaz Yolu- Kastamonu	02.08.2015	ISTE 117263	SB
Stachys thirkei (ST)	Saray-Güngörmez	15.07.2015	ISTE 117264	ST

Extraction of samples

The aerial parts of each plant were crushed and extracted sequentially with *n*-hexane, chloroform, and methanol using a Soxhlet apparatus. A rotary evaporator was used to evaporate the solvents under reduced pressure, with a maximum temperature of 50°C. Following solvent evaporation, the crude extracts were kept at +4 °C until analysis and used in all studies.

The aqueous extracts of aerial parts of each plant were prepared by infusing 10.0 g of dried material in 200 mL of distilled water for 15 min at 80 °C. The infusion was filtered, and the filtrates were frozen and stored at -80°C in an ultra-low degree freezer. After that, the solution was lyophilized, and the freeze-dried product was stored at -20°C until the screening.

Total phenolic content of the samples

4.5 mL of distilled water was added to 0.1 mL of the extracts produced at 0.5-5 mg/mL concentrations. The absorbance of a blue color after 2 hours at room temperature was measured at 760 nm against reference standards using 0.1 mL of Folin-Ciocalteu reagent (diluted 1/3 with distilled water) and 0.3 mL of 2% carbonate solution. The total phenolic content was measured as milligram gallic acid equivalents per milligram of extract (Taskın, Taskın, & Rayaman, 2018).

Antioxidant capacity of the samples

The cupric reducing antioxidant capacity (CUPRAC)

CUPRAC technique was used to assess the antioxidant capacity of the samples. 60 µL Cu (II) (1.10-2 M), neocuproine ethanolic solution (7.3.10-3 M), and 1 M ammonium acetate buffer solution were added to a microplate well. The samples were diluted with solvent mixture of methanol:DMSO (2:1 v/v). 60 µL of the diluted extracts and 10 µL pure ethanol were added to the original mixture. The absorbance of the solution was measured at 450 nm against a reagent blank after ten seconds of vortexing. The Trolox equivalents (mM Trolox/mg extract) were used to calculate the CUPRAC values of the samples. (Apak, Güçlü, Özyürek, & Karademir, 2004).

DPPH radical scavenging activity

The ability of free radical scavenging of the four different extracts was tested using the DPPH technique. To summarize, 240 mL of DPPH solution (0.1 mM) was combined with 10 mL of extracts (5 mg/mL-0.5 mg/mL) at varied concentrations. The combination was then held at room temperature for another 30 minutes before being used. Using a microplate reader set at 517 nm, the absorbance of the mixture was measured in comparison to a standard (Wei et al., 2010).

Reducing power capacity (FRAP technique)

The FRAP technique was used to test the capacity of the samples (5 mg/mL-0.5 mg/mL concentrations) to reduce ferric ion. In brief, FRAP reagent (3.8 mL) was combined with samples (0.2 mL) and the absorbance of the combination was measured at 593 nm 4 minutes later. The standard curve was made using $FeSO_4.7 \times H_2O$, and the FRAP values of the samples were expressed in mM Fe^{2+}/mg extract (Benzie & Strain, 1996).

Antimicrobial activities of the samples

Determination of minimum inhibitory concentrations (MICs)

Staphylococcus aureus ATCC 29213, *S. epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *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 were used in this study for *in vitro* antimicrobial activities of various extracts obtained from three different *Stachys* species. The antimicrobial activity assay of the samples against these strains was determined using the broth microdilution technique as described by the Clinical and Laboratory Standards (CLSI, 1997; CLSI, 2020). The MIC was defined as the lowest concentration of antibiotics giving complete inhibition of visible growth. The following standard antibacterial and antifungal agents were used as standard compounds: Cefuroxime-sodium, cefuroxime, ceftazidime, amikacin, amphotericin B, and clotrimazole. RPMI-1640 medium for the yeast strain and Mueller-Hinton broth for bacteria were used as negative controls.

Statistical analysis

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

RESULTS AND DISCUSSION

The yield and total phenolic contents of the samples

Aerial parts of *Stachys cretica* subsp. *bulgarica, S. byzantina* and *S. thirkei* were extracted successively using several solvents to obtain crude extracts and the results of fractions yielded and also their total phenolic content are demonstrated in Table 2.

Total phenolic compounds contained in plants were determined by the FCR method. Compared with other extracts, infusion and methanol extracts prepared from plants were found to have higher quantities of phenolic compounds. The total amount of phenolics contained in the methanol extracts prepared from three plants is as follows: SC methanol (0.101 mg GAE/mg extract)> SB methanol (0.090 mg GAE/mg extract)>ST methanol (0.058 mg GAE/g extract)

In this investigation, it was discovered that methanol and infusion extracts prepared from aerial parts of the plants had significant amounts of phenolic compounds and, as a result, showed substantial biological activity, which is in line with the literature.

Antioxidant capacity of the samples

The antioxidant capacity of the samples was measured by CUPRAC activity, DPPH• free radical scavenging, and FRAP methods. The results of the three different methods aredemonstrated in Table 3.

Table 2. The yield and total phenolic content of the extracts (beginning with 10 g of material).

Samples	Extracts	Yield	Total phenolics
Stachys cretica subsp. *bulgarica* (SC)	*n*-hexane	0.1177	0.00237±0.00180
	chloroform	0.194	0.0496±0.0014
	methanol	1.6308	**0.1010±0.0117**
	infusion	0.224	**0.0684±0.0058**
Stachys byzantina (SB)	*n*-hexane	0.1157	0.0173±0.0109
	chloroform	0.1903	0.0386±0.0062
	methanol	2.3717	**0.0901±0.098**
	infusion	0.3784	**0.0630±0.0057**
Stachys thirkei (ST)	*n*-hexane	0.1462	0.0032±0.0056
	chloroform	0.1278	0.0134±0.0030
	methanol	1.236	**0.0578±0.0104**
	infusion	0.197	**0.0523±0.0027**

Table 3. Antioxidant activity of the various extracts from three *Stachys* species.

Samples	DPPH (mg AaE/mg extract)	FRAP assay (mM Fe^{2+} /mg extract	CUPRAC (mM trolox/mg extract)
SC-H	0.00496±0.004288	0.122 ± 0.01^{9}*	0.055 ± 0.00^{3}*
SC-C	0.013456±0.000626	0.337 ± 0.02^{7}*	0.097 ± 0.00^{2}*
SC-M	0.062819±0.000371	0.454 ± 0.00^{7}*	0.102 ± 0.00^{3}*
SC-I	0.049691±0.01142	0.459 ± 0.00^{2}*	0.097 ± 0.00^{1}*
SB-H	0.00231± 0.001782	0.197 ± 0.03^{0}*	0.052 ± 0.00^{4}*
SB-C	0.004962±0.001384	0.339 ± 0.01^{2}*	0.072 ± 0.00^{5}*
SB-M	0.0627±0.000412	0.476 ± 0.00^{2}*	0.098 ± 0.00^{1}*
SB-I	0.055988±0.000713	0.434 ± 0.00^{5}*	0.095 ± 0.00^{4}*
ST-H	0.00258±0.000879	0.038 ± 0.00^{6}*	0.051 ± 0.00^{2}*
ST-C	0.00609±0.000713	0.331 ± 0.03^{5}*	0.079 ± 0.00^{2}*
ST-M	0.039118±0.005585	0.363 ± 0.03^{3}*	0.089 ± 0.00^{6}*
ST-I	0.05076±0.001029	0.451 ± 0.00^{5}*	0.092 ± 0.00^{4}*
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
H: *n*-hexane extracts, C: Chloroform extracts; M: Methanol extracts; I: Infusion; BHA: butylated hydroxyanisole; BHT: butylated hydroxytoluene; AaE: ascorbic acid equivalent

The cupric reducing antioxidant capacity of infusion and methanol extracts of three plants were found to be quite similar in this investigation. The methanol extracts of the SB (0.098 mM trolox/mg extract) and SC (0.102 mM trolox/mg extract) plants, as well as the infusion of ST (0.092 mM trolox/mg extract) plant, were found to have the greatest CUPRAC value.

The methanol extract produced from SC (0.102 mM trolox/mg extract) had a higher cupric reducing antioxidant capacity than the other extracts, according to the cupric reducing antioxidant capacity of the plants. When the cuprac values of all extracts were compared to the standard substance (1.622 mM trolox/mg extract), it was discovered that they had a reduced activity potential.

As a result of DPPH radical scavenging activities of the methanol extracts from SB (**0.063** mg AaE/mg extract) and SC (**0.062** mg AaE/mg extract) plants, as well as the infusion extract from the ST (0.051 mg AaE/mg extract) plant, were shown to have a greater radical scavenging capability than the other extracts in the present investigation. When the plants utilized in this study were examined, it was discovered that the radical scavenging

activity of the SB and SC was quite similar. In comparison to the two other species, ST exhibited to have low radical scavenging activity.

A general association between reductive capacity and the presence of antioxidant agents is that they break free radical chains by donating hydrogen atoms. Reducing power assays are commonly used to determine whether an antioxidant is capable of transforming a Fe^{+3} to Fe^{+2} (El Atki, et al., 2020). Hence, the FRAP assays were employed to determine the ferric reducing power of the samples. The FRAP values of infusion and methanol extracts produced from SB and SC were found to be extremely near to each other in the FRAP experiment. Infusion of SC (0.459 mM Fe^{2+} /mg extract) and ST (0.451 mM Fe^{2+} /mg extract), as well as methanol extract of the SB (0.476 mM Fe^{2+} /mg extract), showed to have greater iron (III) ion reducing power activity than other extracts. When the plants' iron-reducing activities were evaluated, the methanol extract of SB was found to have the greatest FRAP value. All of the extracts obtained from the plants showed to have a lower iron reduction capacity than the positive control, BHT compound (1.1 mM Fe^{2+} /mg extract).

Numerous investigations confirmed not only the antioxidant potential of many *Stachys* species but also the rich diversity of their chemical composition many of which reduce free radical damage (Jassbi, Miri, Asadollahi, Javanmardi, & Firuzi, 2014; Sarikurkcu, Kocak, Uren, Calapoglu, & Tepe, 2016; Bahadori, Zengin, Dinparast, & Eskandani, 2020). This is the first study to identify and compare the antioxidant capacity of different solvent extracts of these three species. It is well-known that the antioxidant capacities of extracts are influenced by many factors including extraction procedure, polarities of used solvents, and also polymorphic properties and diversity of the species (BilušićVundać, 2019; Tomou, et al., 2020). Thus, different types of complementary methods are recommended to evaluate the antioxidant potential of the extracts. Generally, the highest antioxidant activity is observed in polar extracts of plants such as methanol, infusion, and decoction due to containing high amounts of phenolic compounds (Ertas, &Yener, 2020). As a result of the evaluation of the various solvents' extract from three *Stachys* species in several assays, infusion and methanol extracts showed the highest antioxidant capacity with the highest total phenolic content compared with the other extracts.

Antimicrobial activity of the samples

To provide comparable data for samples, the samples obtained from three *Stachys* species and prepared with various types of solvents were tested against a panel of three Gram-positive bacteria and four Gram-negative bacteria by using the broth micro dilutions technique according to the Clinical Laboratory Standards Institute (CLSI) recommendations (CLSI, 1997; CLSI, 2020). Well-known commercial antibiotics were used as the standard drugs and the minimal inhibitory concentrations (MIC) values compared with the standard drugs are presented in Table 4. The extracts exhibited moderate to mild antimicrobial activities compared with the standards. Nevertheless, infusion of all species was not shown to have any antimicrobial activity. Depending on the antibacterial results of all compounds, it was observed that all of the tested *Stachys'* samples displayed no inhibitory activity against any Gram-negative bacteria except methanol extract of SC for *E. coli*. *In addition to this, n-hexane extracts of SCB also showed antimicrobial activity against tested Gram-positive bacteria with the MIC values ranging from 312.5 to 625 mg/L.*

Antifungal activity of each extract was tested against three pathogenic yeasts, namely *C. albicans*, C. *parapsilosis*, and *C. tropicalis*. Among all the extracts studied for antifungal potency, *n*-hexane extracts of each species showed strong activity against *C. parapsilosis* and *C. tropicalis*. The most resistant yeast against the samples was determined as *C. albicans*. The *n*-hexane extracts of SC and ST exhibited strong anticandidal activity

Table 4. The antimicrobial and antifungal activity of the various extracts from three *Stachys* species (MIC, µg/mL).

Strains	SC-H	SC-C	SC-M	SB-H	SB-C	SB-M	ST-H	ST-C	ST-M
P. aeruginosa ATCC 27853	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500
E. coli ATCC 25922	>2500	>2500	**312.5**	>2500	>2500	>2500	>2500	>2500	>2500
K. pneumoniae ATCC 4352	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500
P. mirabilis ATCC 14153	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500
E. faecalis ATCC 29212	**312.5**	>2500	>2500	>2500	**156.2**	>2500	>2500	>2500	>2500
S. epidermidis ATCC 12228	625	>2500	>2500	>2500	>2500	1250	>2500	625	625
S. aureus ATCC 29213	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500
C. albicans ATCC 10231	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500
C. parapsilosis ATCC 22019	**78.12**	>2500	>2500	**156.2**	>2500	>2500	**78.12**	>2500	>2500
C. tropicalis ATCC 750	**156.2**	>2500	>2500	**312.5**	>2500	>2500	>2500	>2500	>2500

H: *n*-hexane extracts, C: Chloroform 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.*

against *C. parapsilosis* with 78.12 mg/L MIC values whereas the *n*-hexane extracts of SB and SC exhibited moderate anticandidal activity against *C. tropicalis* with MIC values ranging from 312.5-156.2 mg/L. As to the antibacterial activity of chloroform extracts, *E. faecalis* and *S. epidermis* were found to be susceptible to the SB and ST samples.

According to the literature, the essential oil and extracts of *Stachys* species possess remarkable antimicrobial activity related to the polarities of extracts, composition of samples, and species' growing conditions (İşcan et al., 2012; Leblebici, Kaygusuz, Korkmaz, & Darcan, 2016). Previously, the antimicrobial activity of essential oil from several *Stachys* species were investigated using the disc diffusion method and among them, considerable antifungal activity from the essential oil of *Stachys cretica* subsp. *Bulgarica* was observed against *C. albicans* (Goren et al., 2011). In another study, the *n*-butanolic and light petroleum extracts of *Stachys cretica* subsp. *lesbiaca* and *S. cretica* subsp. *trapezuntica* showed antifungal activity whereas no antibacterial activity was observed in either ethanol, dichloromethane, or ethyl acetate extracts (Şerbetçi et al., 2010). The methanol extracts of *S. byzantina*, *S. inflata*, *S. lavandulifolia,* and *S. taxa* were tested against Gram-positive bacteria and yeast, in contrast, in the other studies no antifungal activities were determined in the extracts whereas remarkable concentration-dependent antibacterial activity was determined in both disc diffusion and MIC methods (Saeedi, Morteza-Semnani, Mahdavi, & Rahimi, 2008).

CONCLUSION

Interest in ethnopharmacology has recently increased due to the recent development of phytochemistry methodologies (Bremner & Heinrich, 2002). Aromatic plants are of great potential for both antioxidant and antimicrobial activities owing to the rich synergy of different types of secondary metabolites (Tomou et al., 2020). In the present study, the antimicrobial and antioxidant activities of four type of extracts obtained from *Stachys cretica* subsp. *bulgarica*, *S. byzantina* and *S. thirkei* were carried out and their total phenolic contents were determined.

In several investigations was noticed that many progressive illnesses are related to the imbalance of oxidative stress in the body. Thus, finding alternative extracts or compounds in the plant kingdom becomes one of the most important targets in the scientific area. The present study results indicated that the polar extracts of all investigated *Stachys* species showed considerable antioxidant effects and also had high amounts of phenolic contents. Furthermore, the increased bacterial resistance is one of the significant global health concerns all around the world. As to the MIC values, the extracts of *Stachys cretica* subsp. *bulgarica*, and *S. byzantina* exhibited strong to moderate antimicrobial activity while the extracts of *Stachys thirkei* exhibited moderate to poor antimicrobial activity. Consequently, these findings highlight the value of these three *Stachys* species. But still more research into the chemical compositions, molecular mechanisms, and adverse impacts of extracts is needed to determine their potential.

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