



Antioxidant and Antimicrobial Activities of *Salvia multicaulis*

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ABSTRACT

The present study aimed to determine antioxidant and antimicrobial activities of *Salvia multicaulis* Vahl plant collected in Gaziantep province, Turkey. Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) were determined using Rel Assay kits. Antimicrobial activity was determined with modified agar dilution method. The findings demonstrated that *S. multicaulis* had high antioxidant activity. However, it is recommended to avoid excessive consumption of the plant due to high OSI. It was also determined that plant extracts possessed antimicrobial potential. As a result, it was determined that the plant can be used as a natural antioxidant and antimicrobial resource.

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Salvia multicaulis'in Antioksidan ve Antimikrobiyal Aktiviteleri

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ÖZET

Bu çalışmada, Gaziantep (Turkey) ilinden toplanan *Salvia multicaulis* Vahl bitkisinin antioksidan ve antimikrobiyal aktivitelerinin belirlenmesi amaçlanmıştır. Bu kapsamda toplam antioksidan durumu (TAS), toplam oksidan durumu (TOS) ve oksidatif stres indeksi (OSI) Rel Assay kitleri kullanılarak tespit edilmiştir. Antimikrobiyal aktivite modifiye agar dilüsyon metodu ile belirlenmiştir. Yapılan çalışmalar sonucunda *S. multicaulis*'in yüksek antioksidan aktiviteye sahip olduğu belirlenmiştir. Fakat OSI değerinin de yüksek çıkması nedeniyle bitkinin aşırı kullanımına dikkat edilmesi önerilmektedir. Ayrıca bitki özütlerinin antimikrobiyal potansiyellerinin olduğu tespit edilmiştir. Sonuç olarak bitkinin doğal antioksidan ve antimikrobiyal kaynak olarak kullanılabilceği belirlenmiştir.

Introduction

Since prehistoric times, humankind developed medicines to treat diseases. To concoct these medicines, they used natural products such as plants, fungi, animals, microorganisms and marine organisms (Yuan et al., 2016; Selamoglu et al., 2016). Secondary metabolites that plants usually produce under stress are non-nutritious compounds, however they are quite important in medicinal uses. It was reported that these herbaceous compounds possess several biological activities as antioxidants and antimicrobials (Orangi et al., 2016; Pasdaran et al., 2017; Kılıç et al., 2017).

Antioxidants play a crucial role in the protection against oxidative stress and free radical damage induced by diabetes, cardiologic disorders and cancer (Hamidpour et al., 2014; Daglia et al., 2014). Thus, determination of new natural antioxidant sources is very important for the treatment of several diseases.

Despite the technological developments experienced in recent years, the ultimate control of the spread of infectious diseases was not possible. The resistance of bacteria to synthetic antibiotics became a global problem. Thus, researchers considered the use of natural products such as medicinal plants in the production of new antibiotic drugs (Abdallah 2011). Identification of new antimicrobial sources is very important in the fight against harmful microorganisms.

Thus, the present study aimed to determine the antimicrobial activities, total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) of *Salvia multicaulis* Vahl plant collected in Gaziantep province (Turkey). It is considered that the study findings would contribute to the production of new antioxidant and antimicrobial agents.

Material and Method

S. multicaulis samples were collected in Şahinbey region in Gaziantep (Turkey) province. Herbarium specimens are stored at Gaziantep University, Department of Biology Herbarium. The plant samples were dried in an incubator at 40°C. The extraction process was conducted with ethanol (EtOH), methanol (MeOH) and dichloromethane (DCM) in a Soxhlet device (Gerhardt EV 14). The extracts were then concentrated in a rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator) and then stored at + 4°C until the experiments were conducted.

TAS, TOS and OSI Tests

TAS, TOS and OSI of the plant EtOH extracts were measured with Rel Assay kits (Rel Assay Kit Diagnostics, Turkey). TAS value was calculated as mmol Trolox equiv./L and Trolox was used as the calibrator (Erel, 2004). The TOS value was calculated as µmol H₂O₂ equiv./L and hydrogen peroxide was used as the calibrator (Erel, 2005). The OSI (arbitrary unit: AU) value was calculated with the formula below and the value is expressed as percentage (Erel, 2005).

$$OSI = \frac{TOS, \mu\text{mol H}_2\text{O}_2\text{equiv./L}}{TAS, \text{mmol Trolox equiv./L} \times 10}$$

Antimicrobial Activity Tests

Antimicrobial activity tests were conducted on the plant EtOH, MeOH and DCM extracts using the agar dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Minimal inhibitor concentrations (MIC) for each extract were determined against standard bacterial and fungal strains. The following microorganisms were used for this purpose: *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606, *Candida albicans* ATCC 10231, *Candida krusei* ATCC 34135 ATCC 13803 and *Candida glabrata* ATCC 90030. Bacteria strains were pre-cultured in Muller Hinton Broth medium and fungal strains were pre-cultured in RPMI 1640 Broth medium. To obtain standard inoculum, the turbidity of the bacteria and fungi was set based on the McFarland 0.5 scale. All extracts were tested at 800-12.5 µg/mL concentrations and distilled water was used in all dilutions. The solvents used in extracts were also individually tested for antimicrobial activity. Fluconazole and Amphotericin B were used as reference drugs for the fungi. Amikacin, Ampicillin and Ciprofloxacin were used as reference drugs for the bacteria (CLSI, 2002; 2003). The lowest concentration that prevented the proliferation of bacteria and fungi was determined as the minimal inhibitor concentration (MIC) (Bauer et al., 1966; Hindler et al., 1992; CLSI, 2012; EUCAST, 2014; EUCAST, 2015; Matuschek et al., 2014).

Results and Discussion

TAS, TOS and OSI

It is known that several diseases are caused by oxidative stress, which consists of the extreme production of reactive oxygen species and the imbalance between antioxidant compounds and their neutralization (Hazra et al., 2008). Antioxidants are actively involved in the suppression of the excessive oxidant compounds. When the endogenous antioxidants produced in living organisms are at insufficient levels, these oxidant compounds can be suppressed by external antioxidant supplements. Thus, the identification of natural antioxidant sources is very important (Selamoglu et al., 2017). In the present, it was determined that the TAS value of *S. multicaulis* was 6.434 ± 0.113, the TOS value was 22.441 ± 0.231 and the OSI value was 0.349 ± 0.004. There are no previous oxidative stress studies on *S. multicaulis*. However, there are oxidative stress studies on plants. In these studies, it was determined that the TAS value of *Mentha longifolia* subsp. *longifolia* was 3.628 ± 0.234, the TOS value was 4.046 ± 0.615 and the OSI value was 0.112 ± 0.025 (Sevindik et al., 2017). When compared to these findings, higher TAS, TOS and OSI values were determined in the present study. This could be due to the differences in the capacity of plants to produce antioxidant compounds and the impact of environmental factors. In another study, it was determined that the TAS value of *Muscari aucheri* MeOH extracts was 1.61 ± 0.03, the TAS value of *Tulipa armena* var. *lycica* was 1.34 ± 0.07 and the TAS value of

Bellevalia gracilis was 1.66 ± 0.04 (Yıldırım et al., 2013). Furthermore, in different studies, it was observed that the TAS value of *Thermopsis turcica* was 2.06 ± 0.09 $\mu\text{mol/g}$ (Aksoy et al., 2013), the TAS value of *Brassica rapa* was 1.25 mmol/L (Gul et al., 2013), and the TAS value of the ethanol extract of *Calendula officinalis* was 5.55 ± 0.41 (Verma et al., 2016). It was determined that the *S. multicaulis* used in the present study had a higher TAS value when compared to the findings of the abovementioned studies. Thus, it was determined that *S. multicaulis* has high antioxidant potential. However, the fact that the plant exhibited high oxidative stress levels suggested that it also contained high levels of oxidant compounds. Thus, it is recommended to avoid excessive consumption of this plant. In conclusion, *S. multicaulis* has antioxidant potential and it can be used as a natural antioxidant source.

Antimicrobial Activity

Today, several researchers have focused on natural resources such as plants, animals, and fungi to identify new antimicrobial sources. Plants are rich sources of secondary metabolites that have antimicrobial effects. Essential oils and extracts have a wide range of biological activities, especially antimicrobial effects on different groups of pathogenic organisms (Shams-Ghahfarokhi et al., 2006; Bakkali et al., 2008; Tolouee et al., 2010; Nabavi et al., 2015). In the present study, the effects of

the EtOH, MeOH and DCM extracts of *S. multicaulis* were examined on the test microorganisms. Study findings are presented in Table 1.

The study findings demonstrated that highest activity was observed in the EtOH extracts of *S. multicaulis* and the lowest activity was identified in the DCM extracts. It was also determined that plant extracts were more effective on fungal strains. Previous studies reported that *S. multicaulis* essential oils were effective against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Streptococcus mutans* (Paknejadi et al., 2012). In another study, it was determined that *S. multicaulis* essential oils were effective on *Bacillus subtilis*, *B. pumilus*, *Enterococcus faecalis*, *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans* and *Saccharomyces cerevisiae* (Yousefzadi et al., 2007). Furthermore, another study reported that the essential oil, ethyl acetate and ether extracts of *S. multicaulis* were effective at different levels on *Enterococcus faecalis*, *Staphylococcus aureus*, *S. epidermidis*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Candida albicans* and *Saccharomyces cerevisiae* (Mojtaba et al. 2011). In contrast to the findings of previous studies, it was found that the plant extracts were effective on *P. aeruginosa*, *A. baumannii*, *C. glabrata* and *C. krusei*. In conclusion, it was determined that *S. multicaulis* was a natural antimicrobial agent against the tested microorganisms.

Table 1 Antimicrobial Activities of *S. multicaulis*

Activities	<i>S. aureus</i>	<i>S. aureus</i> MRSA	<i>E. faecalis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. baumannii</i>	<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. krusei</i>
EtOH	25	50	25	200	200	400	25	25	50
MeOH	25	50	25	200	400	400	50	50	50
DCM	400	400	400	400	800	800	200	200	400
Ampicillin	1.56	3.12	1.56	3.12	3.12	-	-	-	-
Amikacin	-	-	-	1.56	3.12	3.12	-	-	-
Ciprofloksasin	1.56	3.12	1.56	1.56	3.12	3.12	-	-	-
Flukanazol	-	-	-	-	-	-	3.12	3.12	-
Amfoterisin B	-	-	-	-	-	-	3.12	3.12	3.12

Conclusion

In the present study, total antioxidant status, total oxidant status, oxidative stress index and antimicrobial activities of *S. multicaulis* were determined. Study findings demonstrated that the plant had a strong antioxidant potential. However, it is suggested to avoid excessive consumption due to the presence of excessive oxidant compounds in the plant. It was determined that the plant could be considered as a natural antimicrobial source against the tested microorganisms, in addition to its antioxidant potential.

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