

Antioxidant, antimicrobial activity and therapeutic profile of *Satureja hortensis* from Erzincan province

Erzincan bölgesinde toplanan *Satureja hortensis* bitkisinin antioksidan, antimikrobiyal aktivite ve terapotik profilinin belirlenmesi

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SUMMARY

In this study, ethanol (EtOH), methanol (MeOH) and dichloromethane (DCM) extracts of the plant samples were obtained. The total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) and antimicrobial activity of the different extracts of *Satureja hortensis* L. collected from Erzincan (Turkey) province were determined. Rel Assay Diagnostics kits are used to determine TAS, TOS and OSI values. Antimicrobial activity was determined with 9 different bacteria and fungi strain (*Staphylococcus aureus*, *Staphylococcus aureus* MRSA, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Candida albicans*, *Candida krusei* and *Candida glabrata*) using modified agar dilution method. The plant extracts in this study were antimicrobially effective between 25-800 µg/mL concentrations level on 9 different microorganisms strain. Also the plant extracts showed the highest activity against *A. baumannii*. As a result, *S. hortensis* plant from Erzincan province could be a good natural antioxidant and antimicrobial source with its high antioxidant and low oxidant capacity

Keywords: Antimicrobial, antioxidant, medicinal plants, oxidant, *Satureja hortensis*

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ÖZET

Bu çalışmada Erzincan (Türkiye) ilinden toplanan *Satureja hortensis* L. bitkisinin etanol, metanol ve diklorometan özütleri elde edilmiştir. Daha sonra bitkinin tüm özütlerinin toplam antioksidan seviyesi (TAS), toplam oksidan seviyesi (TOS), oksidatif stres indeksi (OSI) ve antimikrobiyal aktivitesi belirlenmiştir. Bitkinin TAS, TOS ve OSI değerlerini tespit etmek için Rel Assay Diagnostics kitler kullanılmıştır. Bitkinin antimikrobiyal aktivitesi 9 farklı bakteri ve mantar suşu (*Staphylococcus aureus*, *Staphylococcus aureus* MRSA, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Candida albicans*, *Candida krusei* and *Candida glabrata*) üzerinde, modifiye edilmiş agar dilüsyon metodu kullanılarak belirlenmiştir. Bu çalışmadaki bitki özütleri 9 farklı bakteri ve mantar suşu üzerinde 25-800 µg/mL arasında değişen konsantrasyon seviyelerinde antimikrobiyal olarak etkili bulunmuştur. Ayrıca bitki ekstraktları en yüksek aktiviteyi *A. baumannii*'ye karşı sergilemiştir. Sonuç olarak, Erzincan ilinden toplanan *S.*

hortensis bitkisinin yüksek antioksidan ve düşük oksidan kapasitesi ile iyi bir doğal antioksidan ve antimikrobiyal kaynak olabileceği düşünülmektedir.

Anahtar sözcükler: Antimikrobiyal, antioksidan, oksidan, *Satureja hortensis*, tıbbi bitki

INTRODUCTION

People have become dependent on nature because of they need drug, shelter, food, perfume, clothes, spices, fertilizer and transportation vehicles. This is the case in countries where long-term use of herbal medicines is constantly being developed. The medical and financial development and recognition of these plants is increasing in both developed and developing countries^{1,2}. Today, millions of people around the world consume plant-based medicines as part of the traditional medicine for a range of medical disorders. The use of alternative medicine in developing countries directly contributes to the socio-economic situation and the welfare of rural communities³. Herbal drugs, especially in recent years in Europe and North America, has an annual market share of % 10-20 percent. In addition, there are many herbal products sold for health food, food supplements, herbal teas, various other health and personal care related purposes⁴.

S. hortensis naturally appearing in Turkey, it is an annual plant of the Lamiaceae members. This plant is also cultivated in Spain, Germany, and England⁵. *S. hortensis* is used in the treatment of some microbial illness and cancer. The phenolic compounds in volatile oil of this plant are especially very effective to treat this kind of illness. It also has stimulant, degassing, antipyretic and aphrodisiac properties^{6,7}. In this context, to determine the TAS, TOS, OSI capacities and antimicrobial activities of different extracts of *S. hortensis* plant from Erzincan province were aimed.

MATERIAL AND METHODS

The identification of *S. hortensis* (B8 Erzincan, Tercan, 1450 m, 18.07.2018. M. Sevindik 1559) was made by benefiting from the 7th volume of Flora of Turkey⁸. The samples transported to the laboratory environment under suitable conditions were extracted with ethanol (EtOH), methanol (MeOH) and dichloromethane (DCM) in the extractor (Gerhardt EV 14). The extracts were concentrated by rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator).

Determination of TAS, TOS and OSI Values

TAS, TOS and OSI values of *S. hortensis* were determined by using commercial Rel Assay

diagnostics kits. Trolox was used as calibrator in the TAS tests and hydrogen peroxide was used in the TOS tests^{9,10}. OSI (Arbitrary Unit = AU) value was determined according to the following formula¹⁰.

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

Antibacterial and Antifungal Activity Tests

The antibacterial and antifungal activity capacities of EtOH, MeOH and DCM extracts of the plant samples were determined using the agar dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). For each extract used, minimal inhibitor concentrations (MIC) were tested against standard bacteria and fungus strains. *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC 19606 were used as bacteria strains. *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135 ATCC 13803 ve *C. glabrata* ATCC 90030 were used as fungus strains. Pre-culturing of bacterial strains was carried out on Muller Hinton Broth medium. Fungus strains were made on RPMI 1640 Broth medium. To obtain a standard inoculum, the blur of bacteria and fungi was prepared according to McFarland 0.5. All extracts were tested at 12.5-800 µg/mL concentrations and all dilutions were made with distilled water. Fluconazole and amphotericin B for fungi, Amikacin, Ampicillin and Ciprofloxacin for bacteria were used as reference drugs. The lowest concentration which prevents the growth of bacteria and fungi was determined as MIC¹¹⁻¹⁶.

RESULTS and DISCUSSION

Antioxidant Activity Results

TAS, TOS and OSI values of the different extracts of *S. hortensis* plant from Erzincan province were determined by using the commercial kits with the 99,9 % reliability for the first time. The values obtained are shown in Table 1.

Table 1: TAS, TOS and OSI values of *S. hortensis*

	TAS mmol/L	TOS μmol/L	OSI
<i>S. hortensis</i>	5.403±0.102	3.537±0.076	0.065±0.003

Values are presented as mean±SD; number samples n=6, Experiments were made in 5 parallels

In previous studies on different plant species, TAS value of *Mentha longifolia* subsp. *longifolia* was reported 3.628 mmol/L, TOS value was 4.046 μmol/L and OSI value was 0.112². In a different study, TAS value of *Salvia multicaulis* was reported 6.434 mmol/L, TOS value was 22.441 μmol/L and OSI value was 0.349¹⁷. In addition, TAS values of *Thermopsis turcica*, *Brassica rapa* and *Calendula officinalis* have been reported as 2.06 mmol/L, 1.25 mmol/L and 5.55 mmol/L, respectively¹⁸⁻²⁰. In our study, TAS value of *S. hortensis* was found to be 5.403±0.102 mmol/L, TOS value was 3.537±0.076 μmol/L and OSI value was 0.065±0.003. Compared to these studies, TAS values of *S. hortensis* was found higher than *M. longifolia* subsp. *longifolia*, *T. turcica* and *B. rapa*. TAS values of *S. hortensis* was found lower than *S. multicaulis* and *C. officinalis* plants. This difference in TAS values is due to the capacity to produce endogenous antioxidants of plants. Plant extracts, foodstuffs and some herbal drinks are considered to be important sources of dietary antioxidants, which have positive effects on human health and aging process²¹. In previous studies, different extracts of *S. hortensis* collected another places were used by different researchers and antioxidant potential was reported with generally

ABTS cation reducing methods etc.²²⁻²⁶. In our study, it was determined that *S. hortensis* was high antioxidant potential and this data was showed with the more reliable determining methods with the rel assay diagnostics kits.

Furthermore, TOS and OSI values of *S. hortensis* was found lower than *M. longifolia* subsp. *longifolia* and *S. multicaulis*. In this context, it is seen that *S. hortensis* produces and accumulates less reactive oxygen species as a result of environmental and metabolic activities. When OSI values are considered, it is seen that *S. hortensis* appears to suppress the oxidant compounds by producing endogenous antioxidants. As a result, it was determined that *S. hortensis* can be used as a good natural antioxidant source due to its low TOS and OSI value as well as high TAS value. So, it can be used as a well antioxidant source of diet.

Antimicrobial Activity Results

EtOH, MeOH and DCM extracts of *S. hortensis* were used and the lowest extract concentrations were determined to prevent proliferation against *S. aureus*, *S. aureus* MRSA, *E. faecalis*, *E. coli*, *P. aeruginosa*, *A. baumannii*, *C. albicans*, *C. krusei* and *C. glabrata*. The results are shown in Table 2.

Table 2: Antibacterial and Antifungal Activity of different extracts from *S. hortensis*.

	<i>S. aureus</i>	<i>S. aureus</i> MRSA	<i>E. faecalis</i>	<i>E. coli</i>	<i>P.</i> <i>aeruginosa</i>	<i>A.</i> <i>baumannii</i>	<i>C.</i> <i>albicans</i>	<i>C.</i> <i>glabrata</i>	<i>C.</i> <i>krusei</i>
EtOH	25	25	200	100	200	25	100	100	100
MeOH	50	50	200	100	200	25	400	400	400
DCM	50	50	200	200	200	25	400	400	800
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

*The MIC values are presented in units of μg/mL.

EtOH, MeOH and DCM extracts of *S. hortensis* showed the highest activity against *A. baumannii*. The EtOH extract of the plant showed higher activity than the other extracts. In previous studies, essential oil of *S. hortensis* has been reported to be effective on *Erwinia amylovora*²⁷. In a different study, MeOH and hexane extracts of *S. hortensis* have been reported to be effective against *Bacillus amyloliquefaciens*, *B. atrophaeus*, *B. licheniformis*, *B. macerans*, *B. megaterium*, *B. pumilus*, *B. sphaericus*, *B. substilis*, *Escherichia coli*, *Kocuria varians*, *Micrococcus luteus*, *Pantoea agglomerans* and *Candida albicans* at different concentrations²⁸. In another study, it was reported that essential oil of *S. hortensis* was effective against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* at different concentrations²⁸. In this study, the plant extracts were antimicrobially effective between 25-800 µg/mL concentrations level on 9 different microorganisms strain. The antimicrobial activity values were significant when they were 100 µg/mL concentrations or lower. Also they were reported to be moderately effective in the range of 100 µg/mL < MIC ≤ 625 µg/mL and weakly effective when the MIC value was more than 625 µg/mL^{29,30}. According to the results, EtOH extract was generally found to be strong antimicrobial activities on all microorganisms except for *E. faecalis* and *P. aeruginosa*. MeOH and DCM extracts had strong antimicrobial activities on 2 *S. aureus* strain and *A. baumannii* microorganisms. In addition, MeOH and DCM extracts were almost moderately effective on the other bacteria and fungi strains. Finally, *S. hortensis* can be a natural antimicrobial agent against microorganisms that we tested.

CONCLUSION

In this study, antioxidant and antimicrobial activities of *S. hortensis* were determined. In this regards, plant extracts have been found high antioxidant and low oxidant activity. Also, plant extracts showed the highest antimicrobial activities against almost all microorganisms. But the best antimicrobial activity was obtained on *A. baumannii*. In conclusion, *S. hortensis* can be a natural antioxidant and antimicrobial agent that can be taken by diet.

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