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Araştırma Makalesi/Research Article

Determination of The Antimicrobial and Antioxidant Activities of *Satureja hortensis* Ingredients

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Abstract: The antimicrobial activity of essential oil and different extracts of *Satureja hortensis* against some microorganisms were evaluated in the present research work by agar well diffusion method. The strongest antibacterial activity was observed in the essential oil of *S. hortensis* against the *Bacillus subtilis* ATCC 6633 and *Staphylococc<us aureus* ATCC 25923. Essential oils showed a wider spectrum of activity but less strong inhibition as compared to the investigated commercial antibiotic. The extracts of *S. hortensis* (chloroform and hexane) showed the similar rates of activity with ampicillin and amikacin. Also, the essential oil of *S. hortensis* showed the similar rates of activity with erythromycin and rifampicin. The antioxidant properties of the *S. hortensis* were evaluated by using different antioxidant assays such as 1, 1-diphenyl-2-picryl-hydrazyl free radical ($DPPH^{\cdot}$) scavenging, total antioxidant activity, reducing power and ABTS radical scavenging. Total antioxidant activity was measured according to ferric thiocyanate method. butylated hydroxyanisole (BHA), butylhydroxytoluene (BHT) and α-tocopherol were used as the reference antioxidant compounds. At the concentrations of 100 µg/mL essential oil of *S. hortensis* showed 87.2%, water extract of *S. hortensis* (SS) 80.4%, chloroform extract of *S. hortensis* (SK) 77.4%, hexane extract of *S. hortensis* (SH) 74.0% and methanol extract of *S. hortensis* (SM) 73.6% inhibition on lipid peroxidation of linoleic acid emulsion, respectively. The activities of samples were greater than that of α -tocopherol (57.9%), BHA (72.3%) and similar to BHT (72.7 %) at this concentration. In addition, *S. hortensis* had an effective 1,1-diphenyl-2- picrylhydrazil ($DPPH^{\cdot}$) scavenging, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging and total reducing power activities.

Keywords: Antimicrobial activity, Antioxidant activities, *Satureja hortensis*, Antibiotic.

Satureja hortensis'İN BİLEŞENLERİNİN ANTİMİKROBİYAL VE ANTİOKSİDAN AKTİVİTELERİNİN BELİRLENMESİ

Özet: *Satureja hortensis*'in uçucu yağı ve farklı ekstraktları bazı mikroorganizmalara karşı antimikrobiyal aktiviteleri uyuk agar difüzyon metodu ile değerlendirildi. En güçlü antimikrobiyal aktiviteyi *S. hortensis*'in uçucu yağı *Bacillus subtilis* ATCC 6633 ve *Staphylococcus aureus* ATCC 25923 karşı gösterdiği belirlendi. Uçucu yağlar ticari antibiyotiklere karşı daha geniş aktivite gösterdiği gözlemlendi. *S. hortensis*'in ekstraktları (kloroform ve hekzan) ampisilin ve amikasin'e benzer oranda aktivite gösterdi. Ayrıca *S. hortensis*'in uçucu yağı ise eritromisin ve rifampisin'e benzer oranda aktivite gösterdiği belirlendi. *S. hortensis*'in antioksidan özellikleri, 1,1-difenil-2-pikril-hidrazil serbest radikal (DPPH) temizleme, toplam antioksidan aktivite, total indirgenme ve ABTS radikal süpürme gibi farklı antioksidan yöntemler kullanılarak değerlendirildi. Toplam antioksidan aktivite ferrik tiyosiyanat yöntemine göre ölçüldü. Referans antioksidan bileşikler olarak bütillenmiş hidroksianisol (BHA), butilhidroksitoluen (BHT) ve α-tokoferol kullanıldı. *S. hortensis*'in 100 µg/mL konsantrasyonunda linoleik asit emülsiyonunun lipid peroksidasyonu üzerindeki inhibisyonu sırasıyla şu şekilde belirlendi: uçucu yağ (SY) % 87.2, *S. hortensis*'in su ekstraktı (SS) % 80.4, *S. hortensis*'in kloroform ekstraktı (SK) % 77.4, *S. hortensis*'in heksan ekstraktı (SH)% 74.0 ve *S. hortensis*'in metanol ekstraktı (SM) % 73.6. Bu konsantrasyonda örneklerin aktiviteleri α-tokoferol'den (% 57.9) daha yüksek, BHA (% 72.3) ve BHT'ye (% 72.7) yakın değerde ölçüldü. Buna ek olarak, 1,1-difenil-2-pikrilhidrazil (DPPH) 2,2'-azino-bis-3-etilbenzotiazolin-6-sülfonik asit (ABTS) radikal süpürme ve total indirgeme gücü sonuçlarına bakıldığında *S. hortensis*'in güçlü antioksidan etkiye sahip olduğu belirlendi.

Anahtar Kelimeler: Antimikrobiyal aktivite, Antioksidan aktiviteler, *Satureja hortensis*, Antibiyotik.

Introduction

Aromatic plants have been known about for a very long time and owing to their aromatic and antiseptic properties they are used as spices and natural food preservatives, in the perfume industry, for aromatherapy and for different medical purposes. Among the aromatic plant species, together with *Origanum* and *Thymus*, the genus *Satureja L*. occupies a special position. The genus *Satureja* L. (savory, saturei) includes more than 30 species belonging to the family Lamiaceae, subfamily Nepetoideae, tribe Mentheae (Ball and Getliffe 1973).

The Lamiaceae comprises about 40 species distributed in Europe, Asia and Africa (Ersöz et al. 2007). The genus Satureja belongs to the Lamiaceae family, and comprises over 30 species whose centre of distribution is located in the eastern part of the Mediterranean area (Šilic´, 1984). *Satureja* is a genus of common aromatic plants belonging to the family Lamiaceae. *Satureja* is represented by 15 species in Turkey, which is an important biodiversity hotspot for the Lamiaceae family (Davis, 1982; Tumen et al. 2000).

S. hortensis has been also used as folk remedies to treat various ailments such as cramps, muscle pains, nausea, indigestion, diarrhea, and infectious diseases. It has showed antispasmodic, anti-diarrhoeal, antioxidant, sedative as well as antimicrobial and antifungal properties (Leung and Foster, 1996; Hajhashemi et al. 2000). *S. hortensis has* antimicrobial activity against a wide range of clinic and plant-associated microorganisms such as bacteria, yeast, and fungi species (Gormez et al. 2015). In recent years, multiple drug/chemical resistance in both human and plant pathogenic microorganisms have been developed due to indiscriminate use of commercial antimicrobial drugs/chemical commonly used in the treatment of infectious diseases (Davies, 1994; Service, 1995).

In this paper, the antibacterial effects of leaves extracts and essential oil against different bacterial strains, fungi species and antioxidant activities of *S. hortensis* is presented.

MATERIALS AND METHODS

Plants Materials

Plants materials were collected from Bingol city of the Turkey at flowering stage. The samples were dried in a canopy room. The identification of plant materials was confirmed by a plant taxonomist, Assoc. Prof. Dr. Ozkan Aksakal in the Department of Biology, Ataturk University, Erzurum, Turkey.

Preparation of Extracts and Essential oil

About 20 g of the airdried and powdered samples were extracted with 500 mL of Chloroform, Methanol, Hexane and Distilled Water by using the Soxhlet extractor for 4 h at room temperature. The extracts were concentrated in vacuum at 40°-50° C using a rotary evaporator yielding a waxy material. Then, the extracts were kept in the dark at +4°C until tested.

The aerial parts of the plant were powdered with blender and then subjected to water distillation for 2-3 h in a Clevenger-type apparatus (Thermal Laboratory Equipment, TURKEY). The EO was stored at +4 ºC for further studies (Bozarı,2012).

Microorganisms

In study, three Gram positive bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Bacillus megaterium* DSM 32), four Gram negative bacteria (*Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 11229, *Pseudomonas aeroginosa* ATCC 9027, *Klebsiella pneumoniae* ATCC 13883) and three fungi species (*Candida albicans* ATCC 10231, *Yarrowia lipolytica* and *Saccharomyces cerevisiae*) were used as test microorganisms. Additionally, Erythromycin (E-15), Ampicillin (AM- 10), Rifampicin (RD-5) Amikacin (AK-30) and Fluconazole (25 µg) were also used as positive control.

Antimicrobial Activity Assays

Well diffusion method was used for the determination of antimicrobial activity of the samples (Collins et al. 1989). For this purpose, bacterial and fungal strains were cultured overnight at 37 ºC in Nutrient agar and 30 ºC in Sabouraud dextrose agar medium, respectively. 100 µL of suspension of test microorganisms, containing $1x10^8$ colony-forming units CFU/mL of bacteria cells and $1x10^4$ CFU/mL spores of fungal strains spreaded on Nutrient agar and Sabouraud dextrose agar medium, respectively. Then, the bacterial suspensions were added into sterilized agar medium at 45 ºC and the mixture suspension was solidified on Petri dishes. The medium was poured into Petri dishes on a horizontally leveled surface. After the medium was solidified, 10 mm diameter wells per dish were made in the agar medium. The extracts and essential oil were dispersed in DMSO at 5% wt /V concentration and effects of concentration on their antibacterial and antifungal activities were investigated. For this purpose, the extracts (30 µL, 60 µL and 90 µL) and essential oil (20 µL, 40 µL and 60 µL) were taken separately and loaded into the wells. The Petri dishes loaded were incubated at 37 ºC for 24 h for bacteria and at 27 ºC for 48 h for fungi. Then, the average inhibition zone diameters of samples were measured after repeating the experiment for at least three times. Also, antimicrobial activities of the reference antibiotics were tested against same microorganisms and results obtained were compared with that's of the samples.

Minimum inhibition concentration (MIC)

The MIC was determined by micro-broth dilution methods (NCCLS, 2000). The reconstituted extract was serially diluted 2- fold in Mueller-Hinton broth medium. Duplicate tubes of each dilution (0.391, 0.780, 1.563, 3.125, 6.25, 12.5, 25.0 and 50.0 mg/mL) were inoculated with 5 x 105 cells (cfu) of the test bacterial strain and cultures incubated at 37 °C for 18 h. MIC was taken as the highest dilution (least concentration) of extract or drug showing no detectable growth.

Antioxidant Activity Assays

Total reduction capability

The reducing power of different extracts and essential oil of *S. hortensis* was determined by the method of (Oyaizu, 1986). Different concentrations of samples (25–100 mg/mL) in 1mL of distilled water were mixed with phosphate

buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50^0C for 20 min. Aliquots (2.5 mL) of trichloroacetic acid (10%) were added to the mixture. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates an increase in reduction capability.

Total antioxidant activity determination by ferric thiocyanate method

The antioxidant activity of *S. hortensis* and standards was determined according to the ferric thiocyanate method (Mitsuda et al. 1996). The solution which contains the same concentration of stock extracts and essential oil of *S. hortensis* or standard samples (30 µg/mL) in 2.5 mL of potassium phosphate buffer (0.04 M, pH 7.0) was added to 2.5 mL of linoleic acid emulsion in potassium phosphate buffer (0.04 M, pH 7.0). Therefore, 5 mL of linoleic acid emulsion contained 17.5µg Tween-20, 15.5 µL linoleic acid, and 0.04 M potassium phosphate buffer (pH 7.0). On the other hand, 5 mL control was composed of 2.5 mL of linoleic acid emulsion and 2.5 mL, 0.04 M potassium phosphate buffer (pH 7.0). The mixed solution (5 mL) was incubated at 37 ^{o}C in glass flask. The peroxide level was determined by reading the absorbance at 500 nm in spectrophotometer after reaction with $FeCl_2$ and thiocyanate at intervals during incubation. During the linoleic acid oxidation, peroxides are formed and that leads to oxidation of Fe^{+2} to Fe^{+3}. The latter ions form a complex with thiocyanate and this complex has a maximum absorbance at 500 nm. This step was repeated every 10 h until the control reached its maximum absorbance value. Therefore, high absorbance indicates high linoleic acid emulsion oxidation. The solutions without samples were used as blank samples. All data on total antioxidant activity are the average of duplicate experiments. The inhibition percentage of lipid peroxidation in linoleic acid emulsion was calculated by following equation:

Inhibition of lipid peroxidation (%) = 100 – [(A_1/Ao)] × 100

where Ao is the absorbance of control reaction and A_1 is the absorbance in the presence of sample *S. hortensis* or standard compounds (Gülçin et al. 2004a).

1, 1-Diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging activity

The free radical scavenging activity of different extracts and essential oil of *S. hortensis* was determined by the 1,1-diphenyl-2-picryl-hydrazil ($DPPH^{\cdot}$). This activity was measured by following the methodology described by (Blois, 1958) wherein the bleaching rate of a stable free radical, DPPH is monitored at a characteristic wavelength in the presence of the sample. In its radical form, $DPPH^{\cdot}$ absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.1 mM solution of DPPH in ethanol was prepared and 1 mL of this solution was added 3 mL of samples in water at different concentrations (25–100 mg /mL). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

ABTS radical scavenging activity

The spectrophotometric analysis of $ABTS^{\cdot+}$ radical scavenging activity was determined according to the method of

(Re et al. 1999) described previously by (Gülçin et al. 2004b). The $ABTS^{\cdot+}$ was produced by reacting 2mM ABTS in H_2O with 2.45 mM potassium persulfate ($K_2S_2O_8$), in the dark at room temperature for 4 h. Before usage, the $ABTS^{\cdot+}$ solution was diluted to get an absorbance of 0.750 ± 0.025 at 734 nm with phosphate buffer (0.1 M, pH 7.4) before use. Then, 1mL of $ABTS^{\cdot+}$ solution was added to 3 mL of *S. hortensis* extracts and essential oil and standards in ethanol at different concentrations (50–100 mg/mL). After 30 min, the percentage inhibition at 734 nm was calculated for each concentration relative to a blank absorbance. Solvent blanks were run in each assay.

RESULT AND DISCUSSION

Antimicrobial Activity

The antimicrobial activity is increasing due alarming increase in infectious diseases across the world. Plants produce these special substances that can effect on pathogens either inhibiting their growth or kill them with least toxicity to host (Ahmad and Beg. 2001).

The antimicrobial activities of *S. hortensis* extracts and essential oil were evaluated against 7 pathogenic bacterial strains (*B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *B. megaterium* DSM 32, *E.aerogenes* ATCC 13048, *E. coli* ATCC 11229, *P. aeroginosa* ATCC 9027 and *K. pneumoniae* ATCC 13883) and 3 fungi species (*C. albicans* ATCC 10231, *Y. lipolytica* and *S. cerevisiae*). These strains have been selected for the basis of its application purpose of further formulation study. Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antimicrobial activities are presented in Table.1-2.

In this study, the strongest antibacterial activity was observed in the essential oil of *S. hortensis* against the *B. subtilis* and *S. aureus* (27 mm inhibition zone) (Table 1). However the extract of chloroform showed a very weak antibacterial activity against *B. subtilis* (12 mm inhibition zone). The essential oil of *S. hortensis* showed the highest antifungal activity against *S. cerevisiae* (20 mm inhibition zone) but the hexane extract of *S. hortensis* showed at least antifungal activity against *C. albicans* (14 mm inhibition zone). However the other extracts (Chloroform, methanol and distilled Water) did not show any antifungal activity. (Table 2).

It was observed that the antimicrobial effect of plant extract varies from one plant to another in different researches carried out in different regions of the world. This may be due to many factors such as, the effect of climate, soil composition, age and vegetation cycle stage, on the quality, quantity and composition of extracted product, different bacterial strains (Angioni, et al. 2006).The degree of antimicrobial activity of the samples also changed with the increase in concentration of the samples. Roughly 2-3 mm increases were observed when the concentrations of samples were increased. Antimicrobial activities of *S. hortensis* were also compared with the reference antibiotics. As shown in Table 2, the extracts of *S. hortensis* (chloroform and hexane) showed the similar rates of activity with ampicillin and amikacin but the essential oil of *S. hortensis* showed the similar rates of activity with erythromycin and rifampicin

Table 1. Antimicrobial activity of *S. hortensis* extracts and essential oil using well diffusion (inhibition zone) method.

Microorganisms		Concentration of the extract and essential oil 5% DMSO														
		Chloroform			**Methanol**			**Hexane**			**Distilled Water**			**Essential oil**		
		30 µL	**60 µL**	**90 µL**	**30 µL**	**60 µL**	**90 µL**	**30 µL**	**60 µL**	**90 µL**	**30 µL**	**60 µL**	**90 µL**	**20 µL**	**40 µL**	**60 µL**
Gram-positive	***Bacillus subtilis* ATCC 6633**	-[a]	-	12[b]	-	-	-	-	-	-	-	-	-	18	24	27
	***Staphylococcus aureus* ATCC 25923**	-	12	14	-	-	-	-	13	16	-	-	-	18	24	27
	***Bacillus megaterium* DSM 32**	-	14	16	-	-	13	-	14	16	-	-	-	14	23	26
Gram-negative	***E. aerogenes* ATCC 13048**	-	12	14	-	-	-	-	13	15	-	-	13	13	17	20
	***Eshericha coli* ATCC 11229**	-	12	18	-	-	-	-	-	-	-	-	-	-	15	19
	***Pseudomonas aeroginosa* ATCC 9027**	-	13	15	-	-	-	-	13	15	-	-	13	18	23	25
	***Klebsiella pneumonia* ATCC 13883**	13	15	18	-	13	15	-	13	15	-	-	-	15	20	23
Fungus	***Yarrowia lipolytica***	-	-	-	-	-	-	-	14	16	-	-	-	-	16	19
	***Candida albicans* ATCC 10231**	-	-	-	-	-	-	-	-	14	-	-	-	-	18	19
	Saccharomyces cereviciae	-	-	-	-	-	-	-	-	-	-	-	-	14	16	20

Similar findings could be found in the literature. For example *S. hortensis* containing some substances with antibacterial properties (Leung and Foster 1996; Zargari, 1990). Since only the essential oils from *S. hortensis* have been evaluated in terms of antimicrobial activity against a limited number of microorganisms up to now (Deans and Svoboda, 1989). The essential oil of *S. hortensis* L. showed antifungal activity against phytopathogenic fungi (Boyraz and Ozcan 2006) and against food spoilage fungi (Adiguzel et al. 2007). *S. hortensis* L. essential oil the higher susceptibility showed Gram (+) bacteria: *B. subtilis* (18-23-45 mm), *S. lutea* (19-23-38 mm), *M. flavus* (17-22-33 mm), *S. aureus* ATCC 8538 (18-20-30 mm), *C. perfringens* (16-18-27 mm) and *S. aureus*

ATCC 25923 (15-15- 20 mm, respectively) Gram (-) bacteria were slightly more resistant, where smaller, but similar inhibition zones were measured: *S. enteritidis* (18-19-19 mm), *E. coli* ATCC 25922 (16- 16-20 mm), *E. coli* 8739 (18-17-18 mm) and *P. aeruginosa* (15-16-15 mm, respectively). All tested fungal strains were highly sensitive to the activity of *S. hortensis* L. essential oil: *A. niger* (18- 34-41 mm), *S. cerevisiae* (18-27-31 mm) and *C. albicans* (18-19-26 mm) (Mihajilov-Krstev et al. 2010).

Table 2. Antimicrobial activity of standard antibiotic discs using disc diffusion (inhibition zone) method.

Microorganisms		Antibiotics Disk				
		Erythromycin	**Ampicillin**	**Rifampicin**	**Amikacin**	**Fluconazole**
Gram-positive	***Bacillus subtilis* ATCC 6633**	20[b]	10	21	11	-[a]
	***Staphylococcus aureus* ATCC 25923**	21	8	18	9	-
	***Bacillus megaterium* DSM 32**	25	10	16	10	-
Gram-negative	***E. aerogenes* ATCC 13048**	27	10	16	9	-
	***Eshericha coli* ATCC 11229**	19	-	18	13	-
	***Pseudomonas aeroginosa* ATCC 9027**	19	8	8	14	-
	***Klebsiella pneumonia* ATCC 13883**	19	12	19	10	-
Fungus	***Yarrowia lipolytica***	-	-	-	-	21
	***Candida albicans* ATCC 10231**	-	-	-	-	23
	Saccharomyces cereviciae	-	-	-	-	-

[a] No antimicrobial effect, [b]mm inhibition zone

Antioxidant Activity

Total reductive capability using the potassium ferricyanide reduction method

Antioxidants can be considered as reductants, and inactivation of oxidants by reductants are redox reactions where one reaction species is reduced at the expense of the oxidation of the other. The presence of reductants such as antioxidant substances in the antioxidant samples causes reduction of the Fe^{3+}/-ferricyanide complex to Fe^{2+} which can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. (Chandrika et al. 2006, Gülçin and Daştan 2007).

Antioxidant activities of different extracts and essential oil of *S. hortensis* and standards (BHA, BHT, α-tocopherol) shown according to the FRAP (Ferric Reducing Antioxidant Power) method in Figure 1. Reduction potential of the samples was determined by measuring absorbance at 700 nm of the solution in different concentrations (25 µL, 50 µL ve 100 µL). Power reduction activity of samples with standard antioxidants in 100 µL was determined respectively as BHA > BHT > α- tocopherol > SS > SY> SK > SM >SH.

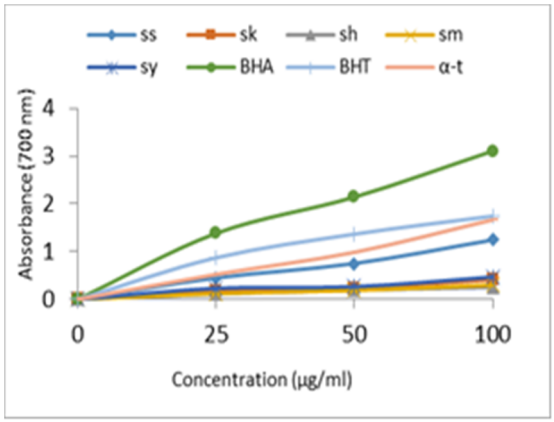

Figure 1. Total reductive potential of different concentrations (25-100 mg/mL) of *Satureja hortensis* extracts and essential oil and standards

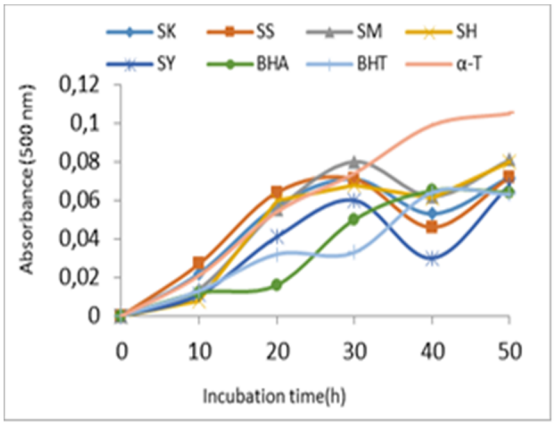

Figure 2. Total antioxidant activities of SK, SS, SM, SH, SY, BHA, BHT and a-tocopherol at the same concentration (100 mg/mL) in the linoleic acid emulsion system by the ferric thiocyanate method.

Total antioxidant activity determination in a linoleic acid emulsion by the ferric thiocyanate method

Lipid peroxidation involves a series of free radical mediated chain reactions and is also associated with several types of biological damage. The role of free radicals and ROS is becoming increasingly recognized in the pathogenesis of many human diseases, including cancer, aging, and atherosclerosis (Gülçin, 2006; Perry et al. 2000). Total antioxidant activity of samples and standards was determined by the ferric thiocyanate method in the linoleic acid system; extracts of *S. hortensis* (SK-SS-SM-SH) and its essential oil (SY) and standard compounds exhibited effective antioxidant activity. The effects of the same concentration (100 mg/mL) of samples and standards on lipid peroxidation of linoleic acid emulsion are shown in Figure 2 and were found to be 77.4 (SK), 80.4 (SS), 73.6 (SM), 74.0 (SH) and 87.2 % (SY), respectively, and their activities were greater than that of a-tocopherol (57.9%), BHA (72.3%) and similar to BHT (72.7 %) at this concentration.

DPPH Free Radical Scavenging Activity

DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances (Cotelle et al. 1996; Özçelik et al. 2000). The method is based on the reduction of an alcoholic DPPH solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction. DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants (Oyaizu, 1986). The DPPH radical due to the scavenging ability of different extracts and essential oil of *S. hortensis* and standards (Figure 3) in 100 µL which decreased in the order of BHA ≥ α-tocopherol ≥ SS ≥BHT> SK> SM≥ SY> SH. And were 88.9, 88.4, 3.88, 17.26, 55.31, 62.92% (for KN6) and -2.53, 3.73, 26.65, 55.31, 60.31, 62.92% at a concentration of 100 mg/mL, respectively.

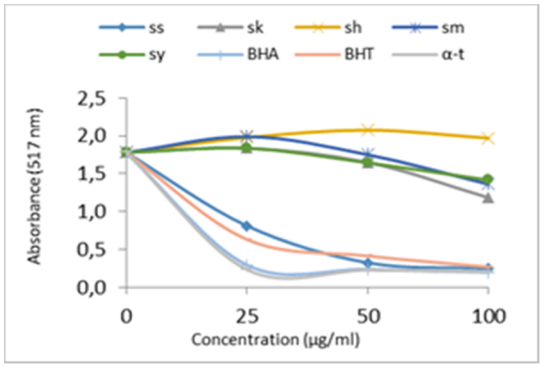

Figure 3. DPPH· free radical scavenging activity of SK, SS, SM, SH, SY, BHA, BHT and a-tocopherol at the same concentration (100 mg/mL).

ABTS$^{\cdot+}$ Free Radical Scavenging Activity

All the tested samples of *S. hortensis* exhibited effective radical scavenging activity. As can seen from Figure 4, essential oil of *Satureja hortensis* had highest ABTS radical scavenging activity also all extracts had effective ABTS$^{\cdot+}$ radical scavenging activity in a concentration-dependent manner (50–100 mg/mL) which was decreased in the order: BHA = α-tocopherol = BHT = SY> SM ≥ SK ≥ SS > SH. which were 96.1, 96.1, 96.1, 96.1, 95.9, 95.6, 95.4 and 71.2% at a concentration of 45 mg/mL, respectively.

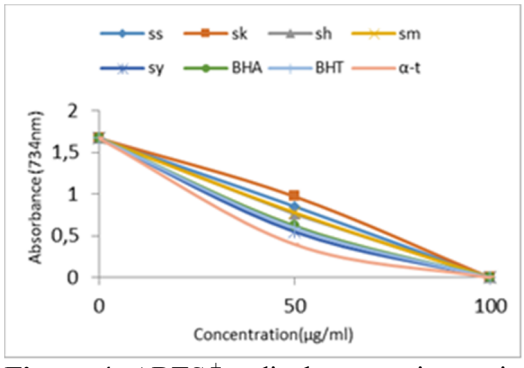

Figure 4. ABTS$^{\cdot+}$ radical scavenging activity of SK, SS, SM, SH, SY, BHA, BHT and a-tocopherol at the same concentration(100 mg/mL).

CONCLUSION

We investigated antioxidant, antimicrobial activity of *S. hortensis* from Turkey. This study is the compound extracted and essential oil from *S.* hortensis exhibited a broad spectrum of antimicrobial activity, which can be used as an alternative source for antibiotics. According to data of the present study, *S. hortensis* was found to be an effective antioxidant in different in vitro assay including reducing power, DPPH radical and ABTS radical scavenging and total antioxidant activities when it is compared to standard antioxidant compounds such as α-tocopherol, a natural antioxidant, BHA and BHT. However, pharmacological test is necessary to isolate and characterize their active compounds. These plant extracts and essential oil should be investigated *in vivo* to better understand their safety, efficacy and properties. Overall these species appears to be promising sources of various bioactive compounds that could be specifically used to treat certain diseases.

REFERENCES

Adiguzel, A., Ozer, H., Kilic, H., and B. Cetin., 2007. Screening of Antimicrobial Activity of Essential Oil and Methanol Extract of *Satureja hortensis* on Foodborne Bacteria and Fungi. Chez Journal of Food Science. 25;2: 81-89.

Ahmad, I., Beg, A.Z., 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human

pathogens. J. Ethnopharmacol. 74;2: 113-123.

Angioni, A.; Barra, A.; Coroneo, V.; Dessi, S.; Cabras, P., 2006. Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. stoechas essential oils from stem/leaves and flowers. J. Agric. Food Chem. 54: 4364-4370.

Ball, P. W., and F. M. Getliffe., 1973. *Satureja* L. *In*: Tutin, T.G., J.R., Heywood, V.H., Moore, Valentine, S.M., Webb, D.A. Flora Europaea 3. 163-165. The University Press, Cambridge.

Blois MS., 1958. Antioxidant determinations by the use of a stable free radical. Nature. 26: 1199–1200.

Boyraz, N., M. Ozcan., 2006. Inhibition of phytopathogenic fungi by essential oil, hydrosol, ground material and extract of summer savory (*Satureja hortensis* L.) growing wild in Turkey. International Journal of Food Microbiology. 107: 238–242.

Bozari, S., 2012. Determination of the Genotoxic Effects of the Potential Allelopathic Essential Oils Obtained from Various Species of Lamiaceae Family pHD, Biology, Atatürk University, Erzurum Turkey.

Chandrika M, Pathirana L, Shahidi F., 2006. Antioxidant properties of commercial soft and hard winter wheats (Triticum aestivum L.) and their milling fractions. J Sci Food Agric. 86:477–485.

Collins, C.H., Lyne, P.M., Grange, J.M., 1989. Microbiologycal Methods. Butterworths & Co. (Publishers) Ltd. 410s. London.

Cotelle N, Bemier JL, Catteau JP, Pommery J, Wallet JC, Gaydou EM., 1996. Antioxidant properties of hydroxyl-flavones. Free Radical Bio Med. 20:35–43.

Davis P. H., 1982. Flora of Turkey and the East Aegean Islands. Vol. **7**, Edinburgh University Press. 319 p. Edinburgh.

Davies, J., 1994. Inactivation of antibiotics and the dissemination of resistance. Sciences 264:375-382.

Deans, S.G., Svoboda, K.P., 1989. Antibacterial activity of summer savory (*Satureja hortensis* L.) essential oil and its constituents. Journal of Horticulturel Science 64: 205–210.

Service, R.F., 1995. Antibiotics that resist resistance. Science. 270, 724– 727.

Gülçin İ., 2006. Antioxidant and antiradical activities of L-Carnitin. Life Sci. 78:803–811.

Gülçin İ, Beydemir Ş,, Alici HA, Elmastas¸ M., 2004b. In vitro antioxidant properties of morphine. Pharmacol Res. 49:59–66.

Gülçin İ, Daştan A., 2007. Synthesis of dimeric phenol derivatives and determination of in vitro antioxidant and radical scavenging activities. Journal of Enzyme Inhibition and Medicinal Chemistry. 22;6: 685–695.

Gülçin, I˙., Şat, İ.G., Beydemir, Ş¸ ., Elmastas¸, M., Küfrevioğlu, Ö .İ., 2004a. Comparison of antioxidant activity of clove (Eugenia caryophylata Thunb) buds and lavender (Lavandula stoechas L.). Food Chemistry. 87: 393–400.

Gormez, A., S. Bozari, D. Yanmis, M. Gulluce, F. Sahin., G. Agar., 2015. Chemical composition and antibacterial activity of essential oils of two species of Lamiaceae against phytopathogenic bacteria. Polish journal of microbiology. 64 ;2: 121-127.

Hajhashemi, V., Sadraei, H., Ghannadi, A.R., Mohseni, M., 2000. Antispasmodic and anti-diarrhoel effect of *Satureja hortensis* L.

essential oil. Journal of Ethnopharmacology 71: 187–192.

Leung, A.Y., Foster, S., 1996. Encyclopaedia of Common Natural Ingredients used in Foods, Drugs, and Cosmetics, 2nd ed. Wiley, New York, pp. 465–466.

Leung, A.Y., Foster, S., 1996. Encyclopaedia of Common Natural Ingredients used in Foods, Drugs, and Cosmetics, 2nd ed. Wiley, New York, pp. 465–466.

Mitsuda, H., Yuasumoto, K., Iwami, K., 1996. Antioxidation action of indole compounds during the autoxidation of linoleic acid. Eiyo to Shokuryo 19: 210– 214.

Mihajilov-Krstev, T., Radnovic, D., Kitic, D., Stojanovic-Radic, Z., Zlatkovic, B., 2010. Antimicrobial activity of *Satureja hortensis* L. essential oil against pathogenic microbial strains. Archives of Biological Sciences, 62;1: 159-166.

Mkaddem, M., Bouajıla, J., Ennajar, M., Lebrıhı, A., Mathıeu, F., Romdhane, M., 2009. Chemical Composition and Antimicrobial and Antioxidant Activities of *Mentha* (*longifolia* L. and *viridis*) Essential Oils, Journal of Food Scıence. 74;7: 358- 363.

Nccls., 2000. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Approved Standard Fifth Edition, NCCLS document M7- A5, NCCLS: Wayne, PA, USA.

Oyaizu, M., 1986. Synthesis, characterization, antibacterial, and thermal studies of unsymmetrical Schiff-base complexes of cobalt (II). Jpn. J. Nutr. 44: 307-315.

Özcelik B, Lee JH, Min DB., 2003. Effects of light, oxygen and pH on the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method to evaluate antioxidants. J Food Sci; 68: 487–490.

Perry G, Raina AK, Nonomura A,Wataya T, Sayre LM, Smith MA., 2000. How important is oxidative damage? Lessons from Alzheimer's disease. Free Radical Bio Med. 28: 831–834.

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 Bio Med. 26: 1231–1237.

Šilić, Č., 1984. Endemične biljke [Endemic plants]. "Svjetlost", OOUR Zavod za udžbenike i nastavna sredstva, Sarajevo, Zavod za udžbenike i nastavna sredstva. 227 pp. Beograd, Sarajevo.

T. Ersöz, D. Kaya, F.N. Yalçin, C. Kazaz, E. Palaska, C.H. Gotfredsen, S.R. Jensen., I. Çalis., 2007. Iridoid glucosides from Lamium garganicum subsp laevigatum Turk. J. Chem., 31, 155-162.

Tumen G, Satýl F, Duman H, Baser KHC., 2000. Two New Records for the Flora of Turkey: Satureja icarica P.H. Davis, S. pilosa Velen. Turk. J. Bot., 24;1: 211-214.

Zargari, A., 1990. Medicinal Plants, 4th ed. Tehran University Publications. pp. 42–45. Tehran.