Polyphenolic Content, Antioxidant Potential and Antimicrobial Activity of *Satureja boissieri*

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ABSTRACT: *Antioxidant activities of Satureja boissieri extracts were detected by using specific in vitro techniques. Standard antioxidant compounds such as ascorbic acid, BHA (Butylated Hydroxyanisole) and BHT (Butylated Hydroxytoluene) were used to compare with the results obtained from ethanol and water extracts of the plant samples. Both extracts presented high antioxidant* actions *on ABTS cation radical and DPPH free radical scavenging methods. Furthermore, the antimicrobial activity of S. boissieri was defined by using three fungi species, three Gram-positive and four Gram-negative bacteria species. The reducing power antioxidant activities of samples were measured by CUPRAC and FRAP techniques. Also, phenolic compounds of S. boissieri leaves were identified by UHPLC-ESI-MS/MS. The high concentration of hesperidin (5051 ± 247 ppb) and rosmarinic acid (4364 ± 214 ppb) was characterized quantitatively. According to the results, high phenolic content can be thought of as one of the responsible parameters for effective biological activity. Also, its flavonoid and phenolic contents are good natural sources for using in the food industry and pharmacological process.*

KEYWORDS: *Antioxidant; Antimicrobial; LC-MS/MS; Phenolic compound; Satureja boissieri.*

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INTRODUCTION

Satureja L. consists of around two hundred kinds of herb bushes which spread widely in the northern regions of America, Asia and the Mediterranean [1]. Due to the rich essential oil contents, *Satureja* species have economic and medical significance. Moreover, these species have been consumed as a spice and herbal tea in different countries because of their pleasant fragrance. Some of the other *Satureja* species such as *S. hortensis* L., *S. cuneifolia* and *S. thymbra* L. have been consumed as diuretics and digestives in different parts of the world [2]. *Lamiaceae* taxa mostly have great economic importance and so that their structures have been investigated by many researchers. The genus *Satureja* is one of the member of the Lamiaceae family. The contents of some *Satureja* species have anti-inflammatory, antispasmodic, antidiarrheal, antioxidant, antifungal, antiviral, and antibacterial activities [3-4]. The phenolic compounds, antimicrobial and antioxidant activities of some *Satureja* taxa were well investigated but still, there is little information available about *S. boissieri*. The active components of essential oils, antibacterial, and antifungal bioassays of *S. boissieri* were identified in some previous studies [5-6]. It is clear that most of the fruits, vegetables, and green plants are known as main sources of natural antioxidants. Consuming these antioxidants in the human diet is a reasonable way to reduce the risk of cancers, cardiovascular diseases, cataracts, brain dysfunction [7].

In this research, we investigated antioxidant properties of *S. boissieri* leaves by four *in vitro* techniques; ABTS cation radical scavenging, DPPH free radical scavenging, Ferric ions Reducing Antioxidant Power (FRAP), and cupric ions reducing power (CUPRAC) techniques, separately. According to the mentioned methods, leaves of *S. boissieri* showed good antioxidant potential and reducing radical activity.

Phenolic acids are secondary metabolites that widely existed throughout the plants. Polyphenols have some important attributes to absorb free radicals and decompose the peroxides [8]. HPLC analytical techniques have been used for identification and quantification of different components [9]. Thus, we used UHPLC-ESI-MS/MS technique to determine the phenolic contents of *S. boissieri* on the present study.

The purpose of this article is to review of antioxidant and antimicrobial action of *S. boissieri* as well as test the capacity and efficacy of antioxidants and antimicrobials by different in vitro methods.

EXPERIMENTAL SECTION

Plant material

S. boissieri samples were collected on 20/08/2014 from north of Haserek mountain, stony areas (1950 m) Bingol/Turkey by Dr. Omer Kilic. The voucher specimens are deposited at the herbarium of the Yıldırımlı (Ankara) and at the Department of Park and Garden Plants of Bingol University, Turkey. Dr. Kilic identified the plant samples according to the Flora of Turkey [10]. The leaves were dried in air and powdered to small pieces before extraction.

Chemicals

Standard phenolic compounds of LC-MS/MS were purchased from Fluka and Sigma-Aldrich (Germany). The other chemicals were purchased from Sigma-Aldrich (Germany).

Extraction

For the preparation of water extract, 20 g of air dried leaves of *S. boissieri* were powdered by a blender and added to 200 mL distilled water (1/10 : w/v). The mixture was stirred at room temperature for 12 h by a magnetic stirrer. The mixture was filtered with filter paper. The filtrate sample was frozen and lyophilized in a lyophilizer (Labconco, Freezone 1 L) at 5 mm Hg at -50 °C. The lyophilized sample was stored at -30 °C until used for the experiments.

For the preparation of ethanol extract, 20 g of air-dried leaves of *S. boissieri* were powdered by a blender and added to 200 mL ethanol (1/10 : w/v). The mixture was stirred at room temperature for 12 h by a magnetic stirrer. The mixture was filtered with filter paper. Sample was evaporated with a rotary evaporator (Heidolph 94200, Bioblock Scientific). The evaporated sample was stored at -30 °C until using the experiments.

Antioxidant activity studies

DPPH• scavenging assay

The radical scavenging capacity of samples was evaluated by using DPPH assay [11]. This method is based on the removal of stable DPPH free radicals by reaction with antioxidants. According to this assay,

different concentrations (10-30 μg/mL) of extracts and standard antioxidants (ascorbic acid, BHA, BHT) were prepared and adjusted to 3 mL with ethanol. Then, 1 mL of DPPH radical solution (0.1 mM) was added to each sample. The mixtures were left in the dark at room temperature for half an hour. The absorbances of extracts were calculated at 517 nm by using a spectrophotometer (Shimadzu, UV-1800, Japan). The percentages of DPPH free radicals' consumption were calculated by comparing with different concentrations of extracts or standards. The percentages of scavenging capabilities of samples on DPPH free radicals were measured by using this equation:

$$\text{Radical scavenging capability}\ (\%) = \left(1 - \frac{A_{\text{Sample}}}{A_{\text{Control}}}\right) \times 100$$

Also, IC_{50} values of samples and standard compounds were determined by measuring the concentration of a sample to scavenge 50 % of radicals.

$ABTS^{\bullet+}$ scavenging assay

The cation free radical scavenging potential of extracts were evaluated by ABTS scavenging technique [12]. First of all, the $ABTS^{\bullet+}$ was provided by reaction of ABTS (2 mM) in pure water with potassium persulphate (2.45 mM) solution during 12 h at room temperature. The ABTS cation with a characteristic absorption at 734 nm has a dark blue-green color. The solution of ABTS cation was diluted with phosphate buffer concentration (0.1 M, pH 7.4) to get absorbance of 0.9 ± 0.1 at 734 nm. Then, 1 mL of $ABTS^{\bullet+}$ solution was added to 3 mL of extract solution in methanol at different concentrations (10-30 mg/mL) of extracts and standard compounds (ascorbic acid, BHA, BHT) separately. Samples were vortexed and left in the dark for half an hour. Then absorbance at 734 nm was measured for samples. The decrease in sample absorption indicates $ABTS^{\bullet+}$ radical scavenging activity.

FRAP assay

Antioxidant capacity of plant sample was analyzed by FRAP assay [13]. According to this method, ferric ion reducing antioxidant power of extracts were assessed by measuring the reduction of ferric ions to ferrous ions. This technique is explained by the reduction of stoichiometric excess ferricyanide compared to antioxidants. For this reason, 0.75 mL of extracts or standard antioxidants (ascorbic acid, BHA, BHT) on different concentrations (10, 20 and 30 μg/mL) were prepared separately. Then, 1 mL of sodium phosphate buffer (0.2 M; pH 6.6) and 1 mL of potassium ferricyanide (1 %) was added to the samples. The mixtures were incubated at 50 °C for 20 min. The reaction was completed by adding 1 mL of TCA solution (10 %). Then, 0.25 mL of $FeCl_3$ (0.1 %) was added to each tubes. Distilled water was used as a blank and for control instead of a sample. Finally, the absorbance evaluations were acquired at 700 nm by using a UV spectrophotometer.

CUPRAC assay

In the CUPRAC technique, reactive copper (II) neocuproine was used as a chromogenic oxidizing agent for the determination of the antioxidant capability of polyphenols. Firstly, 1 mL neocuproine solution (7.5×10^{-3} M), 1 mL $CuCl_2$ solution (0.01 M) and 1 mL CH_3COONH_4 buffer (1 M) were added to test tubes, respectively. After that, different concentrations (10-30 μg/mL) of extracts were added to those mixtures. Volumes of the mixtures were completed to 4.1 mL with purified water. The samples were left at room temperature for half an hour to fully reveal their reducing capacity. Finally, absorbance at 450 nm was reported. Increasing absorbance of a sample was commented as increasing reducing capacity [14].

Antimicrobial activity

For this purpose, three fungi species (*Candida albicans* ATCC 10231, *Saccharomyces cerevisiae*, *Yarrowia lipolytica*), four Gram-negative bacteria (*Escherichia coli* ATCC 11229, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeroginosa* ATCC 9027, *Enterobacter aerogenes* ATCC 13048) and three Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Bacillus megaterium* DSM 32, *Staphylococcus aureus* ATCC 25923) were used as test microorganisms. Also, amikacin (AK-30 μg), ampicillin/sulbactam (SAM-20 μg), erythromycin (E-15 μg) and rifampicin (RD-5 μg) were utilized as positive controls.

The antimicrobial potentials of the plant samples were measured by the disc diffusion technique. Initially, 30 μL, 60 μL and 90 μL of each extract were absorbed to sterile disc (8 mm diameter). For inoculating, 1 % rate of each microorganism from 10^6-10^7 CFU/mL suspension was added to 15 mL sterile media (Muller-Hinton agar) for

bacteria and Sabourand 2 % Glucose agar for yeast). The inoculated medias were poured into Petri dishes (9 cm) and left to 4 °C for 1 h. Four different standard antibiotics were used for positive controls. Susceptibility was sensed by comparing the inhibition zone caliber made up by erythromycin, ampicillin/sulbactam, amikacin, and rifampicin. The Petri dishes were incubated at 35 °C for 18-24 h, except for *Candida albicans*, *Yarrowia lipolytica*, and *Saccharomyces cerevisiae* which were incubated at 27 °C. Inhibition zones were calculated a caliber and recorded as the mean diameter of 3 replications in mm [15].

Determination of phenolics by using LC-MS-MS

First of all, extraction for LC-MS/MS system was prepared; the air-dried and powdered plant material (100 g) was extracted three times with 300 mL of ethanol for 24 h at room temperature. The ethanol solvent was removed under vacuum at 30 °C with a rotary evaporator (Yield: 15.6 %). Dry filtrate sample was diluted to 1000 mg/L and filtrated with 0.2 µm microfiber filter before LC-MS/MS analysis.

Spectrophotometric measurements of phenolics were carried out with LC-MS/MS by using a UHPLC (Nexera/Shimadzu) coupled to a tandem MS instrument. This system was used for separation and identification of polyphenols. LC-MS system consisting of DGU-20A3R degasser, LC-30AD binary pumps, CTO-10ASvp column oven, and SIL-30AC autosampler. C18 reversed-phase Inertsil ODS-4 (150 mm × 4.6 mm, 3 µm) analytical column was performed for phenolic content definition. The temperature was set at 40 °C. The mobile phase A consisted of water, 5 mM ammonium formate, and 0.1 % formic acid. The mobile phase B consisted of methanol, 5 mM ammonium formate, and 0.1 % formic acid. The solvent flow rate was kept at 0.5 mL/min and the injection volume was adjusted as 4 µL.

MS system consisting of a triple quadrupole mass spectrometer integrated with an ESI source was used to detect for both positive and negative ionization modes. LC-MS/MS software was used for data acquisition and processing performed by Lab Solutions software (Shimadzu, Kyoto, Japan). MRM mode (Multiple reaction monitoring) was used for quantification. The analysis was carried out by two or three transitions for per sample. The first transition was for quantitation, the second and/or the third transition was made up for verification. The optimum ESI conditions were set as 350 °C for interface temperature, 250 °C for DL temperature, and 400 °C for heat block temperature, 3 L/min for nebulizing gas flow and 15 L/min for drying gas flow [16].

RESULTS AND DISCUSSION

Antioxidant activity

Reactive oxygen species are thought to be responsible for many cell disorders through their chemical attacks on biostructures. Reactive oxygen species exist both endogenous and exogenous for living organisms. These structures are constantly made up of human metabolism due to oxidation [17]. It has been reported that 1-3 % of the oxygen molecules reduced in mitochondria may be the reason of the superoxide radical formation [18]. Presence of the free radicals *in vivo* may trigger many diseases in the human body by affecting DNA, lipids and proteins in the mitochondria [19].

Antioxidant compounds block the oxidation chain reactions by scavenging free radicals in the metabolism. In the past few decades, investigation about new natural sources of antioxidants became more popular. Various plants have been screened and reported to possess antioxidant potential [20]. The antioxidant potential of ethanol and water extracts of *S. boissieri* were measured by four *in vitro* spectrophotometric technique including DPPH, ABTS, FRAP and CUPRAC assays.

DPPH technique has been extensively used for determination of the radical scavenging activities of different samples by many researchers [21]. Radical scavenging level of a sample shows its antioxidant potential that prevents the oxidation chain initiation. DPPH radical scavenging capacity of *S. boissieri* extracts and standard antioxidants were compared. The decrease in absorbance of the control (in our study, it was 1.532 at 517 nm) indicates radical scavenging activity of a sample.

According to the results, ethanol extract of *S. boissieri* has effective DPPH radical scavenging activity. Likewise, water extracts of *S. boissieri* showed effective free radicals scavenging activity. It was monitored that the free radical scavenging potential of the standards and plant extracts increased with increasing their concentration (Fig. 1A).

The DPPH free radical inhibition percentages of extracts and standard antioxidants at the same concentration

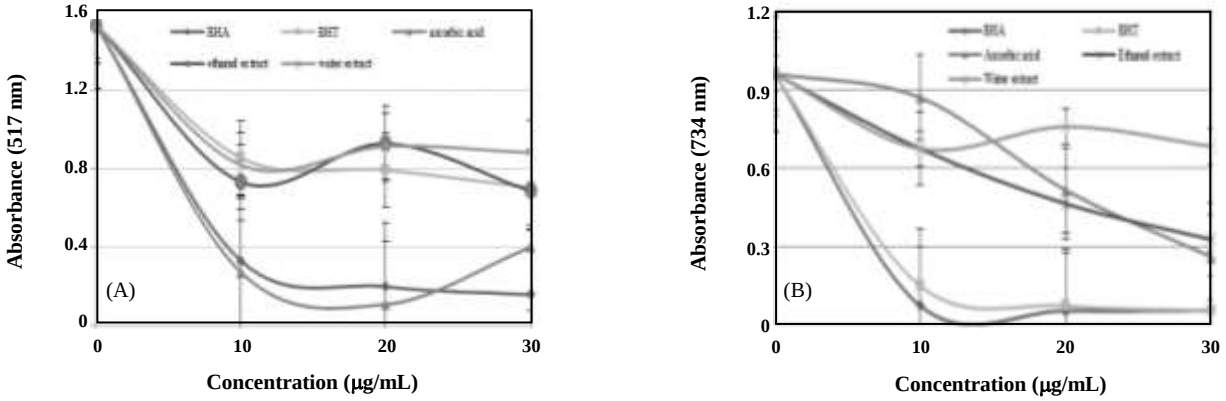

Fig. 1: Radical scavenging activity of S. boissieri by DPPH method (A) and ABTS method (B).

(30 µg/mL) decreased in the order of BHA (89.9 ± 5.9 %) > ascorbic acid (74.0 ± 9.6 %) > ethanol extract of *S. boissieri* (55.3 ± 8.4 %) > BHT (54.1 ± 4.9 %) > water extract of *S. boissieri* (42.5 ± 3.2 %). The high inhibition percentages of *S. boissieri* extracts show that it might be useful for preventing some diseases and protecting our health. Furthermore, radical scavenging amounts with IC_{50} values (concentration of a sample to scavenge 50 % of free radicals) were determined as 27.1 ± 1.3 µg/mL and 35.3 ± 1.8 µg/mL for ethanolic extract and water extract, respectively. In a previous study, IC_{50} value (116.36 µg/mL) of *S. montana* ssp. *kitaibelii* on DPPH free radicals was reported [22]. Compared with this study, performed on *S. montana* ssp. *kitaibelii*, the IC_{50} values of *S. boissieri* water and ethanol extracts were lower and DPPH free radical scavenging activities were higher.

The ABTS cation radical inhibition percentage of extracts and standard antioxidants at the same concentration (30 µg/mL) decreased in the order of BHA (94.8 ± 1.1 %) ≥ BHT (94.4 ± 5.2 %) > ascorbic acid (73.0 ± 1.9 %) > ethanol extract of *S. boissieri* (66.1 ± 8.2 %) > water extract of *S. boissieri* (28.9 ± 4.8 %). The decrease in absorbance of the control (in our study, it was 0.961 at 734 nm) indicates radical scavenging activity
of a sample. The result indicates that ethanol extract of *S. boissieri* has effective ABTS radical scavenging activities similar to standard antioxidants (Fig. 1B). There is a remarkable point as clearly shown in Fig. 1A-B, the absorbances sharply decreased until 20 µg/mL and then absorbances remain almost constant. The absorbances decreased at increasing concentration of standard antioxidant compounds at the beginning, the active sites and amounts of radicals might be occupied with antioxidant compounds as the reaction is proceeding. When the reaction is complete, increasing the concentration of substrate will not decrease the absorbance.

Also, ABTS and DPPH radical scavenging amounts with IC_{50} values were determined and shown in Table 1. IC_{50} values were determined by calculating the concentration of a sample to scavenge 50 % of radicals.

The reduction capability of a compound may relate to its possible antioxidant capacity. Antioxidant compounds have the ability to transfer electrons into reactive radicals. Thus reduce them into more stable and unreactive species. [23]. Plants have the potential to stabilize or prevent radical chain reactions due to certain specific reducing agents in their structure [24]. Reduction of ferric ion (Fe^{3+}) and cupric ion (Cu^{2+}) are generally used as an electron donor and it is important for phenolic compounds action mechanism [25]. The increasing of the reducing power of a sample is proportional to the increasing of it is antioxidant potential. The reducing power antioxidant activities of samples of *S. boissieri* were evaluated by both FRAP and CUPRAC methods.

FRAP method is one of the easy ways to measure the total antioxidant power evaluated as the reducing capacity. Antioxidants cause the reduction of ferric (Fe^{3+}) ions to the ferrous (Fe^{2+}) form due to their reducing capacities. In the present study, ferric reducing powers increased with increasing concentration of the extracts and standard antioxidants. However, increasing levels of extracts were lower than increasing of the standards.

Ferric ions reducing antioxidant capacities of the extracts

Table 1: Radical scavenging IC_{50} values of standard antioxidants and S. boissieri.

	DPPH radical scavenging	ABTS radical scavenging
BHA	16.8 ± 0.8 µg/mL	16.1 ± 0.7 µg/mL
BHT	28.5 ± 1.4 µg/mL	16.3 ± 0.8 µg/mL
Ascorbic acid	16.5 ± 1.0 µg/mL	20.4 ± 1.2 µg/mL
Ethanol extract	27.1 ± 1.3 µg/mL	22.7 ± 1.1 µg/mL
Water extract	35.3 ± 1.8 µg/mL	51.9 ± 2.3 µg/mL

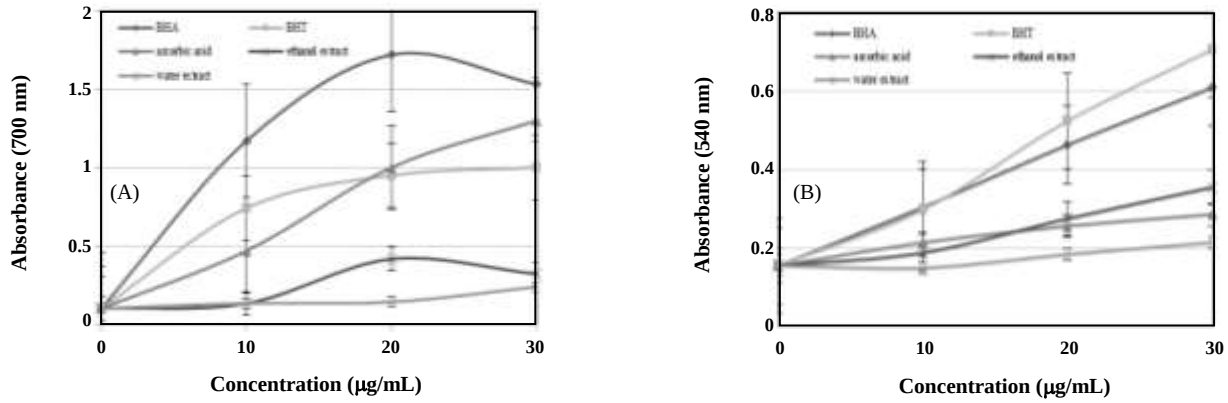

Fig. 2: Reducing power potential of S. boissieri extracts by FRAP method (A) and CUPRAC method (B).

and standard antioxidants at the same concentration (30 µg/mL) decreased in the order of BHA > ascorbic acid > BHT > ethanol extract of *S. boissieri* > water extract of *S. boissieri* (Fig. 2A). The results demonstrated that the extracts of *S. boissieri* are less efficient than some antioxidants (ascorbic acid, BHA and BHT).

Cupric reducing potential of *S. boissieri* extracts were experimentally measured and compared to BHA, BHT, and ascorbic acid. The extracts of *S. boissieri* and standards demonstrated high cupric ions reducing capacities in the CUPRAC method. Cupric ions reducing capacities of extracts and standard antioxidants at the same concentration (30 µg/mL) decreased in the order of BHT > BHA > ethanol extract of *S. boissieri* > ascorbic acid > water extract of *S. boissieri* (Fig. 2B). According to the CUPRAC results, the ethanol extract of *S. boissieri* has stronger reducing power activity than ascorbic acid which is one of the well-known standard antioxidants. However, water extract has the lowest scavenging activity in both FRAP and CUPRAC reducing methods.

Antimicrobial activity

The importance of antimicrobial activity has been increased due to the increasing of infectious diseases. Special substances of plants have effects on pathogens, either preventing spread or scavenge them with very less toxicity to host [26]. This is widely known that plant originated phenolic compounds have some antibacterial properties [27].

Antimicrobial activity of *S. boissieri* was investigated against three Gram-positive (*B. subtilis, S. aureus*, and *B. megaterium*) and four different Gram-negative (*E. aerogenes, E. coli, K. pneumoniae*, and *P. aeruginosa*) bacteria. We also tested whether the sample is effective against three fungus species (*Y. lipolytica, C. albicans*, and *S. cerevisiae*). To determine the inhibition of bacteria growth, the inhibition zones were calculated for each sample concentration (Table 2). To evaluate the value of the sample, the reference antibiotics were also analyzed for comparison.

The ethanolic extract of *S. boissieri* showed a strong effect on *B. megaterium* (13.0 ± 1.24 inhibition zone). Additionally, the highest antimicrobial activity against *B. megaterium* was observed when 90 mL sample extract was used. *S. aureus* and *B. megaterium* were also sensitive to the sample extract and the effect was increased

Table 2: Antibacterial and antifungal activities of 20 mg/mL concentration of S. boissieri extract and antibiotic discs (mm zone).

Microorganisms		Antimicrobial Activity (mm)			Antibiotic Discs			
		S. boissieri 30 µL (0.6 mg)	*S. boissieri* 60 µL (1.2 mg)	*S. boissieri* 90 µL (1.8 mg)	Erythro-mycin (E-15 µg)	Ampicillin / sulbactam (SAM- 20 µg)	Amikacin (AK-30 µg)	Rifampicin (RD-5 µg)
Gram (+)	*B. subtilis*	-	9 ± 0.00	10 ± 0.00	20 ± 1.24	14 ± 0.47	11 ± 1.24	21 ± 1.24
	S. aureus	9 ± 0.00[2]	10 ± 0.47	12 ± 1.24	21 ± 0.00	10 ± 0.81	9 ± 0.00	18 ± 1.69
	B. megaterium	10 ± 0.47	11 ± 1.24	13 ± 1.24	25 ± 1.69	-	10 ± 0.81	16 ± 1.24
Gram (-)	*E. aerogenes*	-[1]	10 ± 0.47	12 ± 0.00	27 ± 1.24	10 ± 0.47	9 ± 0.00	16 ± 0.47
	E. coli	-	9 ± 0.00	10 ± 0.47	19 ± 0.00	13 ± 1.24	13 ± 0.81	18 ± 1.24
	P. aeroginosa	-	9 ± 0.47	10 ± 0.00	19 ± 1.69	-	14 ± 0.00	8 ± 0.00
	K. pneumoniae	-	9 ± 0.00	10 ± 0.47	19 ± 0.47	16 ± 1.69	10 ± 0.47	19 ± 1.69
Fungus	*Y. lipolytica*	-	-	-	-	-	-	-
	C. albicans	-	-	-	-	-	-	-
	S. cereviciae	-	-	-	-	-	-	-

with increasing sample amount. The extract of *S. boissieri* showed a weak and similar effect on *B. subtilis*, *E. aerogenes*, *E. coli*, and *K. pneumoniae*. However, the extract did not show antifungal activity against *C. albicans*, *Y. lipolytica* and *S. cerevisiae* (Table 2).

The degree of antibacterial activities of the samples also changed with increasing concentration of the samples. Roughly 1–2 mm increases were observed when the concentrations of the samples were increased from 30 µL, 60 µL, and 90 µL. The antibacterial activity of *S. boisseri* was also compared with some reference antibiotics (Table 2). According to the results, the extracts showed very weak antibacterial activities against *B. subtilis*, *P. aeroginosa*, *K. pneumoniae*, and *E. coli*.

Phenolic compounds

Polyphenols have many biological properties such as antioxidant potential, radical scavenging, anddisease prevention effects [28]. Phenolic compounds are containing at least one hydroxyl group and they exist in various species of plants. The antioxidant potential of polyphenols is mostly attributed to their redox features which let them show actions as hydrogen donors, reducing agents, and quenchers of singlet oxygen [29]. These compounds can block the chain reactions by the way of chelating metals and donating a hydrogen atom. Also, the structure of the aromatic ring and the number of hydroxyl groups of phenolic compounds are important factors on antioxidant abilities. The plants that contain polyphenols have efficient antioxidant activity. Many enzymatic and spectrophotometric assays have been used for identification of the phenolic acids in plants or foods. Various liquid chromatography methods have been used to identify organic acids due to their reproducibilities. Also, UV-vis spectrophotometry assay has been generally used for the detection of organic acids. Because of the high sensitivity and selectivity, liquid chromatography coupled to ESI-MS has attracted notable attention for the identification of organic acids [30].

Phenolic compounds which contain more than two hydroxyl groups have significant roles on antioxidant properties. The antioxidant action of polyphenols is proportional to the redox properties of the phenolic groups. It was reported that compounds which contain functional hydroxyl groups have the ability to show metal chelating activity [31].

In this study, the identification of phenolic acids of *S. boissieri* was analyzed by UHPLC-ESI-MS/MS. The major phenols were detected such as hesperidin (5051 ± 247 ppb), rosmarinic acid (4364 ± 214 ppb), naringenin (1703 ± 94 ppb), and quinic acid (935 ± 45 ppb). Moreover, small amounts of kaempferol, apigenin, luteolin, p-coumaric acid, tr-caffeic acid, vanillin, rutin, hyperoside, and hesperetin were identified, quantitatively (Table 3).

The chromatograms of standards and *S. boissieri* are presented in Fig. 3A-B, respectively.

Hesperidin, rosmarinic acid, naringenin, and quinic acid which are the main phenolic compounds of *S. boissieri*, contain at least two hydroxyl groups which have significant roles on antioxidant properties. Hesperidin is a natural dietary flavonoid has been identified in different species of plants. Hesperidin also

Table 3: LC-MS/MS parameters of S. boissieri's selected compounds.

Analytes	RT[a]	Parent ion (m/z)[b]	Ioniza-tion Mode	R^{2c}	RSD %[d]	Linearity Range (µg/L)	LOD/LOQ (µg/L)[e]	Reco-very (%)	U[f]	MS^2 (CE)[g]	*S. boissieri*[h]
Quinic acid	3.32	190.95	Neg	0.9927	0.0388	250-10000	22.3 / 74.5	103.3	4.8	85 (22), 93 (22)	935 ± 45
Malic acid	3.54	133.05	Neg	0.9975	0.1214	250-10000	19.2 / 64.1	101.4	5.3	115 (14), 71 (17)	
tr-Aconitic acid	4.13	172.85	Neg	0.9933	0.3908	250-10000	15.6 / 51.9	102.8	4.9	85 (12), 129 (9)	
Gallic acid	4.29	169.05	Neg	0.9901	0.4734	25-1000	4.8 / 15.9	102.3	5.1	125 (14), 79 (25)	
Chlorogenic acid	5.43	353	Neg	0.9932	0.1882	250-10000	7.3 / 24.3	99.7	4.9	191 (17)	
Protocatechuic acid	5.63	152.95	Neg	0.9991	0.5958	100-4000	25.8 / 85.9	100.2	5.1	109 (16), 108 (26)	
Tannic acid	6.46	182.95	Neg	0.9955	0.9075	100-4000	10.2 / 34.2	97.8	5.1	124 (22), 78 (34)	
tr- caffeic acid	7.37	178.95	Neg	0.9942	1.0080	25-1000	4.4 / 14.7	98.6	5.2	135 (15), 134 (24)	52 ± 3
Vanillin	8.77	151.05	Neg	0.9995	0.4094	250-10000	10.1 / 33.7	99.2	4.9	136 (17), 92 (21)	34 ± 2
p-Coumaric acid	9.53	162.95	Neg	0.9909	1.1358	100-4000	15.2 / 50.8	98.4	5.1	119 (15), 93 (31)	58 ± 3
Rosmarinic acid	9.57	358.9	Neg	0.9992	0.5220	250-10000	10.4 / 34.8	101.7	4.9	161 (17), 133 (42)	4364 ± 214
Rutin	10.18	609.1	Neg	0.9971	0.8146	250-10000	17.0 / 56.6	102.2	5.0	300 (37), 271 (51)	31 ± 2
Hesperidin	9.69	611.1	Poz	0.9973	0.1363	250-10000	21.6 / 71.9	100.2	4.9	303, 465	5051 ± 247
Hyperoside	10.43	463.1	Neg	0.9549	0.2135	100-4000	12.4 / 41.4	98.5	4.9	300, 301	19 ± 1
4-OH Benzoic acid	11.72	136.95	Neg	0.9925	1.4013	25-1000	3.0 / 10.0	106.2	5.2	93, 65	
Salicylic acid	11.72	136.95	Neg	0.9904	0.6619	25-1000	4 / 13.3	106.2	5.0	93, 65, 75	
Myricetin	11.94	317	Neg	0.9991	2.8247	100-4000	9.9 / 32.9	106.0	5.9	179, 151, 137	
Fisetin	12.61	284.95	Neg	0.9988	2.4262	100-4000	10.7 / 35.6	96.9	5.5	135, 121	
Coumarin	12.52	146.95	Poz	0.9924	0.4203	100-4000	9.1 / 30.4	104.4	4.9	103, 91, 77	
Quercetin	14.48	300.9	Neg	0.9995	4.3149	25-1000	2.0 / 6.8	98.9	7.1	179, 151, 121	
Naringenin	14.66	270.95	Neg	0.9956	2.0200	25-1000	2.6 / 8.8	97.0	5.5	151, 119, 107	1703 ± 94
Hesperetin	15.29	300.95	Neg	0.9961	1.0164	25-1000	3.3/ 11.0	102.4	5.3	164, 136, 108	12 ± 1
Luteolin	15.43	284.95	Neg	0.9992	3.9487	25-1000	5.8 / 19.4	105.4	6.9	175, 151, 133	70 ± 5
Kaempferol	15.43	284.95	Neg	0.9917	0.5885	25-1000	2.0 / 6.6	99.1	5.2	217, 133, 151	374 ± 19
Apigenin	17.31	268.95	Neg	0.9954	0.6782	25-1000	0.1 / 0.3	98.9	5.3	151, 117	137 ± 7
Rhamnetin	18.94	314.95	Neg	0.9994	2.5678	25-1000	0.2 / 0.7	100.8	6.1	165, 121, 300	
Chrysin	21.18	253	Neg	0.9965	1.5530	25-1000	0.05 / 0.17	102.2	5.3	143, 119, 107	

a: Retention time; b: Molecular ions of the standard compounds (mass to charge ratio); c: coefficient of determination; d: relative standard deviation e: Limit of detection/Limit of quantification; f: Percent relative uncertainty at 95% confidence level (k=2).; g: MRM fragments for the related molecular ions and collision energies of the fragment ions); h: Quantitative phenolic acid composition of S. boissieri (ppb; µg analyte/kg extract)

known as a potent anti-inflammatory and anticarcinogenic agent. Previous studies demonstrated that hesperidin improves the antioxidant defense mechanism by increasing the level of enzymatic and non-enzymatic antioxidants [32]. Rosmarinic acid is a natural polyphenolic acid that an ester of caffeic and 3,4-dihydroxyphenyl lactic acids. Rosmarinic acid is present as a secondary metabolite in medicinal and food plants. It was reported that rosmarinic acid has several biological activities, namely antioxidant, anti-inflammatory, antimutagenic, antibacterial, antiviral, antitumor, hepatoprotective and cardioprotective properties [33].

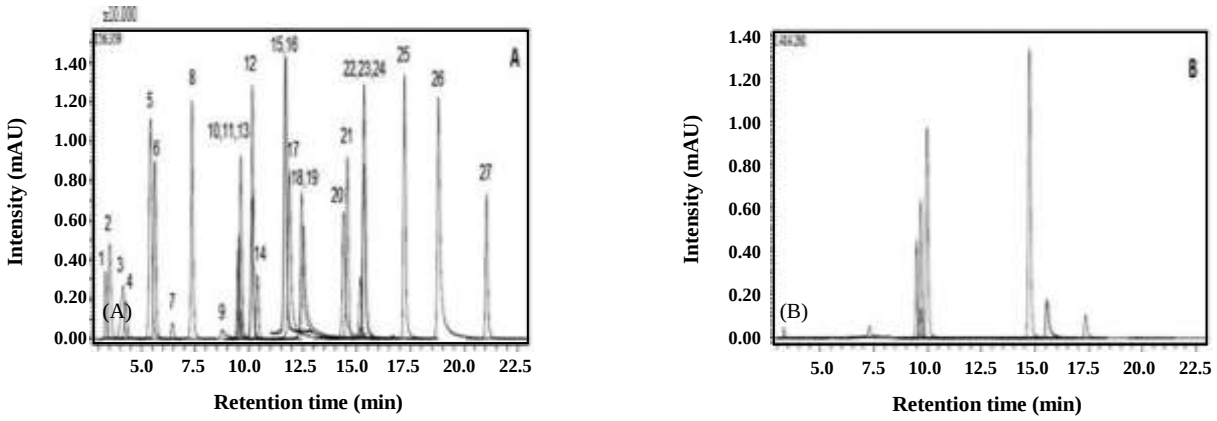

Fig. 3: UHPLC-ESI-MS/MS chromatograms of 250 ppb standard mix (A) and S. boissieri (B).

Naringenin is a well-known flavonoid, richly found in different species of plants. It was reported that naringenin exhibits antioxidant activity and exerts anti-inflammatory effects in the liver [34]. Quinic acid is obtained from cinchona bark, coffee beans, and other plant products. Quinic acid is a constituent of tannins. It was reported that quinic acid detected in the extract of *Caesalpina spinosa* [35].

CONCLUSIONS

UHPLC-ESI-MS/MS analysis of phenolic content, antioxidant and antimicrobial activities of *S. boissieri* were investigated and reported in this study. The results revealed that extracts have high antioxidant capabilities on radical scavenging activity and reducing antioxidant power. Also, the plant sample showed some antibacterial activity which can be used as an alternative for antibiotics. In addition, hesperidin and rosmarinic acid were detected as the main phenolic compounds. The high antiradical and antioxidant activity of plant extracts may because of its rich amounts of phenols. Thus, *S. boissieri* have potential in the food industry as a food ingredient to produce functional food products. As a result, some other studies can be performed in order to improve the food industrial and pharmacological features of plant material of this study.

Conflict of Interest

The authors declare that there is no conflict of interest.

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REFERENCES

[1] Rechinger K.H., Satureja. "Flora Desiranischen Hoclandes and der Umrahmenden Gebirge", vol. 150, Akademische Druku Verlags Antalt Graz, Austria (1982).

[2] Kilic O., Bagci E., Essential Oil Composition of Wiedemannia Fisch. and C.A.Mey. Genus from Turkey, a Chemotaxonomic Approach, *J Essent Oil Bear Pl*, **17**(5): 741–746 (2014).

[3] Hajhashemi V., Sadraei H., Ghannadi A.R., Mohseni M., Antispasmodicanti-diarrhoeal Effect of Satureja Hortensis L. Essential Oil, *J Ethnopharmacol*, **71**: 187–192 (2000).

[4] Kurkcuoglu M., Tumen G., Baser K.H.C., Essential Oil Constituents of Satureja Boissieri from Turkey, *Chem Nat Compd*, **37**(4): 327–331 (2001).

[5] Azaz D., Demirci F., Satil F., Kurkcuoglu M., Baser K.H.C., Antimicrobial Activity of Some Satureja Essential Oils, *Z Naturforsch C*, **57**(9-10): 817–821 (2002).

[6] Alizadeh A., Essential Oil Composition, Phenolic Content, Antioxidant and Antimicrobial Activity of Cultivated Satureja Rechingeri Jamzad at Different Phenological Stages, *Z Naturforsch C*, **70**(3-4): 51–58 (2015).

[7] Aras A., Bursal E., Dogru M., UHPLC-ESI-MS/MS Analyses for Quantification of Phenolic Compounds of Nepeta Nuda Subsp. Lydiae, *J. Ap. Pharma. Sci.*, **6**(11): 9–13 (2016).

[8] Sisecioglu M., Cankaya M., Gulcin I., Ozdemir H., Interactions of Melatonin and Serotonin to Lactoperoxidase Enzyme, *J. Enzyme Inhib. Med. Chem.*, **25**: 779–783 (2010).

[9] Yang B., Feng X., Xu J., Lei H., Zhang L., Multi-Component HPLC Analysis and Antioxidant Activity Characterization of Extracts from Dipsacus Sativus (Linn.) Honck, *Int. J. Food. Prop.*, **19**(5): 1000–1006 (2016).

[10] Davis P.H., "Flora of Turkey and the East Aegean Islands", Un. Press, Edinburgh 7: Pp 321 (1982).

[11] Aras A., Doğru M., Bursal E., Determination of Antioxidant Potential of Nepeta Nuda Subsp. Lydiae, *Ana. Chem. Lett.*, **6**(6): 758–765 (2016).

[12] Koksal E., Tohma H., Kilic O, Alan Y., Aras A., Gulcin I, Bursal E., Assessment of Antioxidant and Antimicrobial Activities of Nepeta Trachonitica - Analysis of its Phenolic Compounds Using HPLC-MS/MS. *Sci .Pharm.*, **85**: 24 (2017).

[13] Oyaizu M., Studies on Product of Browning Reaction Prepared from Glucose Amine, *Japan. J. Nutr.*, **44**: 307–315 (1986).

[14] Apak R., Guclu K., Ozyurek M., Karademir SE., Novel Total Antioxidant Capacity Index for Dietary Polyphenols and Vitamins C and E, Using Their Cupric Ion Reducing Capability in the Presence of Neocuproine: CUPRAC Method, *J. Agric. Food Chem.*, **52**: 7970–7981 (2004).

[15] Tohma H., Koksal E., Kilic O., Alan Y., Yilmaz MA., Gulcin I., Bursal E., Alwasel S., RP-HPLC/MS/MS Analysis of the Phenolic Compounds, Antioxidant and Antimicrobial Activities of Salvia L. Species. *Antioxidants*, **5**(4): 38 (2016).

[16] Ertas A., Boga M., Yılmaz M.A., Yesil Y., Tel G., Temel H., Hasimi N., Gazioglu I., Ozturk M., Ugurlu P., A Detailed Study on the Chemical and Biological Profiles of Essential Oil and Methanol Extract of Thymus Nummularius (Anzer tea): Rosmarinic Acid, *Ind. Crop. Prod.*, **67**: 336–345 (2015).

[17] Bursal E., Kinetic Properties of Peroxidase Enzyme from Chard (Beta Vulgaris Subspecies Cicla), *Int. J. Food. Prop.*, **16**(6): 1293-1303 (2013).

[18] Turrens JF., Superoxide Production by the Mitochondrial Respiratory Chain, *Bioscience Rep.*, **17**: 3–8 (1997).

[19] Li W., Beta B., Evaluation of Antioxidant Capacity and Aroma Quality of Anthograin Liqueur, *Food Chem.*, **127**: 968–975 (2011).

[20] Abideen Z., Qasim M., Rasheed A., Adnan M.Y., Gul B., Khan M.A., Antioxidant Activity and Polyphenolic Content of Phragmites Karka Under Saline Conditions, *Pakistan Pak. J. Bot.*, **47**: 813-818 (2015).

[21] Jacob JK, Hakimuddin F, Paliyath G and Fisher H., Antioxidant and Antiproliferative Activity of Polyphenols in Novel High-Polyphenol Grape Lines, *Food Res. Int.*, **41**: 419–428 (2008).

[22] Lopez-Cobo A., Gomez-Caravaca AM., Svarc-Gajic J., Segura-Carretero A., Fernandez-Gutierrez A., Determination of Phenolic Compounds and Antioxidant Activity of a Mediterranean Plant: The Case of Satureja Montana subsp. Kitaibelii, *J. Funct. Foods*, **18**: 1167–1178 (2015).

[23] Turan N., Adıgüzel R., Buldurun K., Bursal E., Spectroscopic, Thermal and Antioxidant Properties of Novel Mixed Ligand-Metal Complexes Obtained from Saccharinate Complexes and azo Dye Ligand (mnppa), *Int. J. Pharm.*, **12**(2): 92–100 (2016).

[24] Hu W., Yu L., Wang MH., Antioxidant and Antiproliferative Properties of Water Extract from Mahonia Bealei (Fort.) Carr. Leaves, *Food Chem. Toxicol.*, **49**:799–806 (2011).

[25] Dorman HJD., Peltoketo A, Hiltunen R, Tikkanen MJ., Characterization of the Antioxidant Properties of De-odourised Aqueous Extracts from Selected Lamiaceae Herbs, *Food. Chem.*, **83**: 255–262 (2003).

[26] Ahmad I., Beg AZ., Antimicrobial and phytochemical Studies on 45 Indian Medicinal Plants Against Multi-drug Resistant Human Pathogens, *J. Ethnopharmacol*, **74**(2): 113–123 (2001).

[27] Del Monte D., De Martino L., Marandino A., Fratianni F., Nazzaro F., De Feo V., Phenolic Content, Antimicrobial and Antioxidant Activities of Hypericum Perfoliatum L, *Ind. Crop. Prod.*, **74**: 342–347 (2015).

[28] Al-Juhaimi F., Ghafoor K., Total Phenols and Antioxidant Activities of Leaf and Stem Extracts from Coriander, Mint and Parsley Grown in Saudi Arabia, *Pak. J. Bot.*, **43**(4): 2235-2237 (2011).

[29] Isik M., Korkmaz M., Bursal E., Gulcin I., Koksal E., Tohma H., Determination of Antioxidant Properties of Gypsophila Bitlisensis Bark, *Int. J. Pharm.*, **11**(4): 366–371 (2015).

[30] Sandin-Espana P., Mateo-Miranda M., Lopez-Goti C., De Cal A., Alonso-Prados JL., Development of a Rapid and Direct Method for the Determination of Organic Acids in Peach Fruit Using LC–ESI-MS. *Food Chem.*, **192**: 268–273 (2016).

[31] Fiorucci SB., Golebiowski J., Cabrol-Bass D., Antonczak S., DFT Study of Quercetin Activated forms Involved in Antiradical, Antioxidant and Prooxidant Biological Processes, *J. Agric. Food Chem*, **55**, 903–911 (2007).

[32] Devi KP., Rajavel T., Nabavi SF., Setzer WN., Ahmadi A., Mansouri K., Nabavi SM., Hesperidin: A Promising Anticancer Agent from Nature, *Ind. Crop. Prod.*, **76**: 582–589 (2015).

[33] Gulcin I., Scozzafava A., Supuran C.T., Koksal Z., Turkan F., Cetinkaya S., Bingol Z., Huyut Z., Alwasel S., Rosmarinic Acid Inhibits Some Metabolic Enzymes Including Glutathione S-Transferase, Lactoperoxidase, Acetylcholinesterase, Butyrylcholinesterase and Carbonic Anhydrase Isoenzymes, *J. Enzyme Inhib. Med. Chem.*, **31**(6): 1698–1702 (2016).

[34] Chtourou Y., Slima A.B., Makni M., Gdoura R., Fetoui H., Naringenin Protects Cardiac Hypercholesterolemia-Induced Oxidative Stress and Subsequent Necroptosis in Rats, *Pharmacol. Rep.*, **67**: 1090–1097 (2015).

[35] Garro Galvez., JM, Ried B., Conner A.H., Analytical Studies on Tara Tannins, *Holzforschung*, **51**: 235–243 (1997).