

LC-MS/MS Analysis of Phenolic Compounds and *In Vitro* Antioxidant potential of *Stachys lavandulifolia* Vahl. var. *brachydon* Boiss.

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Abstract. The identification and quantification of phenolic compounds of *Stachys lavandulifolia* Vahl. var. *brachydon* Boiss. by LC-MS/MS (Liquid Chromatography- tandem Mass Spectrometry) technique is the main purpose of the current study. The high concentrations of quinic acid (2534±12 ppb) and chlorogenic acid (1882±92 ppb) were detected by LC-MS/MS.. Another goal of the study is to evaluate the antioxidant activities of both ethanol and aqueous extracts of the plant material. The antioxidant potentials of extracts were determined by using five different *in vitro* methods including; ABTS (2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), DPPH(1,1-diphenyl-2-picryl-hydrazyl), FRAP (Ferric ions Reducing Antioxidant Power), CUPRAC (Cupric ions Reducing Antioxidant Power), and ferric thiocyanate methods. The results revealed that the aqueous and ethanol extracts of *S. lavandulifolia* leaves have good antioxidant potential with high phenolic content.

Introduction

The *Stachys* is the largest genera of the Lamiaceae with its nearly 300 species. They mostly exist in the warm regions such as Mediterranean, South-West Asia, America and Southern Africa [1]. In the Flora of Turkey, *Stachys* is represented by 86 species (109 taxa), 37 of which are endemic and divided into fourteen sections [2]. *Stachys lavandulifolia* has three varieties (*brachydon*, *glabrescens*, and *lavandulifolia*). *Stachys lavandulifolia* Vahl. var. *brachydon* Boiss. separated from other varieties by different structure of leaves and length of calyxes [3].

Some previous phytochemical identification studies of *Stachys* species were demonstrated the phenolic and flavonoid glycosides [4], terpenoids and steroids [5], flavonoid constituents [6]. Also, phytochemical investigations on different *Stachys* taxa have shown that they have various pharmacological effects, such as anti-inflammatory, antitoxic, hypoazotemic, antihepatitis, antibacterial and antioxidant [7, 8]. Besides, some *Stachys* taxa have been reported in folk medicine to treat genital tumors, sclerosis, inflammatory tumors, cancer, and ulcers. Furthermore, some of them are used in phytotherapy, possessing sedative, antispasmodic, diuretic and emmenagogue activities as a tea preparations [9]. *Stachys* genera is classified in Lamiaceae family which has been reported widely for their phenolic and flavonoid contents as well as some biological properties [10-13]. The major compounds of *S. lavandulifolia* were determined by some previous reports [14, 15]. Best of our knowledge there is no comprehensive research on the literature about phenolic content and antioxidant activity of *Stachys lavandulifolia* Vahl. var. *brachydon* Boiss.

We investigated antioxidant potential of *Stachys lavandulifolia* Vahl. var. *brachydon* Boiss. (*S. lavandulifolia*) by five *in vitro* common methods; ABTS, DPPH, CUPRAC (Cupric ions Reducing Antioxidant Power), FRAP, and ferric thiocyanate techniques, separately. According to the data that obtained from the mentioned methods, *S. lavandulifolia* extracts showed a great deal of antioxidant activity on scavenging of radicals and reducing ions properties. Additionally, the data of LC-MS/MS method displayed the organic compounds of *S. lavandulifolia*. Quinic acid and chlorogenic acid were the most abundant phenolic compounds with respect to the results.

Theory

Antioxidant potential is mostly related with phenolic content. Owing to the high biological activity and phenolic contents, detailed further studies about of *S. lavandulifolia* might be beneficial on food industry and pharmacology.

Materials and Methods

The plant material

Stachys lavandulifolia Vahl var. brachydon Boiss. leaves were collected from Bingol, a South Eastern city of Turkey, on July 2016. The samples were identified by Dr. Ömer Kılıç based on the Flora of Turkey and East Aegean Islands, volume VII [3].

Chemicals

The chemicals that used for antioxidant assays as well as standard organic compounds that used for LC-MS/MS analyses were purchased from Fluka (Germany) and Sigma-Aldrich (Germany) companies.

Preparation of aqueous extract

The air dried leaves of *S. lavandulifolia* (20 g) were powdered and mixed with 200 mL distilled water (w/v:1/10). The mixture was homogenized by a magnetic mixer about 12 h, at room conditions. The mixture was filtered with filter paper. The elution was frozen and lyophilized in a lyophilizer (Labconco, Freezone 1L) at 5 mm Hg pressure and at -50 °C. Then, the lyophilized sample was keep in reserve at -30 °C until used.

Preparation of ethanol extract

The air dried leaves of *S. lavandulifolia* (20 g) were powdered and mixed with 200 mL ethanol (w/v:1/10). The mixture was homogenized by a magnetic mixer about 12 h, at room conditions. The mixture was filtered with filter paper. The elution was evaporated at 50 °C with a rotary evaporator (Heidolph 94200, Bioblock Scientific) and sample was keep in reserve at -30 °C until used.

LC-MS/MS studies for phenolic content

LC-MS/MS technique was used for identification of phenolic compounds quantitatively. Phenolic content of *S. lavandulifolia* was compared to standard mix of twenty-seven organic compounds.

The diluted ethanol extract (1 mg/mL) was used for LC-MS/MS analyses. LC-MS triple quadrupole mass spectrometer (Shimadzu, 8040) was the main part of the LC-MS/MS system. The ionizations were detected by ESI. The binary pumps (LC-30AD), column oven (CTO-10ASvp) degasser (DGU-20A3R), and auto sampler (SIL-30AC) were integrated to the LC system. The chromatographic separation was performed on a C18 (150 mm × 4.6 mm, 3 µm) reversed phase analytical column (Inertsil ODS-4). The mobile phase A consisted of 5 mM ammonium formate, water and 0.1 % formic acid. The mobile phase B consisted of 5 mM ammonium formate, 0.1 % formic acid and methanol. The injection volume of sample was 4 µL. HPLC was runned at 0.5 mL/min flow. The multiple reaction monitoring (MRM) mode was used to quantify the analyzes. 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. The analyses of samples were carried out after three transitions for the sample. First transition was for quantitative aim and second and third transitions were for verification [16].

***In vitro* antioxidant activity assays**

Ferric thiocyanate method

The ferric thiocyanate method was used to evaluate the effects of WS (aqueous extract of *S. lavandulifolia*) and ES (ethanol extract of *S. lavandulifolia*) on the prevention of peroxidation of linoleic acid emulsion [17]. The samples of WS and ES (20 µg/mL) were prepared by diluting in 2.5 mL of sodium phosphate buffer (0.04 M, pH 7.0) and these were added to 2.5 mL of linoleic acid emulsion in sodium phosphate buffer (0.04 M, pH 7.0). The linoleic acid emulsion was prepared by homogenizing 15.5 µL of linoleic acid, 17.5 mg of Tween-20 as emulsifier, and 5 mL phosphate buffer (pH 7.0). The control without sample was composed of 2.5 mL of linoleic acid emulsion and 2.5 mL 0.04 M sodium phosphate buffer (pH 7.0). The reaction mixtures (5 mL) were incubated at 37 °C. The peroxide levels were determined by reading the absorbance at 500 nm in a spectrophotometer (Shimadzu, UV-1208 UV-VIS Spectrophotometer, Japan) after reactions with FeCl₂ and thiocyanate at intervals during incubation. The peroxides formed during linoleic acid peroxidation oxidize Fe²⁺ to Fe³⁺, and Fe³⁺ forms a complex with thiocyanate that has a maximum absorbance at 500 nm. The assay steps were repeated every 10 h until the maximum was reached. The percentage of inhibition was calculated at this point (100 h). The linoleic acid mixture without the addition of sample was used as control.

FRAP reducing method

Transforming ferric ions to ferrous ones indicate antioxidant reducing potential of a substance. Antioxidants do this like a proton donor or single electron transfer. The extracts (WS and ES) and standard antioxidants were prepared in different concentrations (10-30 µg/mL), respectively. The mixtures were incubated for 20 min (50 °C) after adding 1 mL potassium ferricyanide (1 %) and 1 mL buffer solution (sodium phosphate 0.2 M; pH 6.6). One milliliter TCA solution (10 %) was added to complete the reaction. Finally, 0.25 mL FeCl₃ (0.1 %) was put in test tubes. The absorbances at 700 nm were reported by using a UV spectrophotometer [18].

CUPRAC reducing method

Transforming cupric ions (Cu²⁺) to cuprous ones (Cu⁺) mostly related with antioxidant reducing potential of a substance. In this technique, 0.5 mL neocuproine (7.5×10⁻³ M), 0.5 mL CuCl₂ (0.01 M), and 0.5 mL buffer solution (acetate, 1 M; pH 5.0) were added to each tubes, respectively. Then, different concentrations (10-30 µg/mL) of extracts were added. The volumes were completed to 4 mL with purified water. The samples were waited at room conditions for 30 min and their absorbances at 450 nm were reported. Increasing absorbance indicates reducing capacity of a sample [19].

ABTS radical scavenging method

The ABTS⁺ radical scavenging activity of *S. lavandulifolia* extracts (WS, ES) were evaluated according to the method that previously reported [20]. ABTS⁺ is blue-green in colour with a characteristic absorbance at 734 nm. ABTS⁺ cation radical was produced by reacting ABTS (2mM) in H₂O and potassium persulphate (2.45 mM) at room temperature for 12 h. The ABTS⁺ solution was diluted with phosphate buffer (0.1 M, pH 7.4) to achieve an absorbance of 0.750±0.025 at 734 nm. Then, 1 mL of ABTS⁺ solution was added to 3 mL of extract solution in methanol at different concentrations (10–30 µg/mL) of the samples and standards. These samples were vortexed and incubated in the dark for 30 min. Later, absorbances at 734 nm were measured relative to a blank. Decreased absorbance of the samples indicates ABTS⁺ cation radical scavenging activity.

DPPH radical scavenging method

The DPPH radical scavenging activity of *S. lavandulifolia* extracts (WS, ES) were evaluated according to the method that previously reported [21]. The DPPH method is based on the reducing absorbance of DPPH radicalic solution because of radical scavenging effects of antioxidants. For this purpose, different concentrations (10–30 µg/mL) of samples and standard compounds were

prepared in 2 mL ethanol. Then, 1 mL DPPH radical solution (0.1 mM) was added to each sample and left in the dark for 30 min. The absorbances were measured at 517 nm using a spectrophotometer.

Results and Discussion

Phenolic compounds contain hydroxyl group on their chemical structure. Phenolic compounds exist on natural plants are showing an extensive range of physiological feature. Also, they have many biological activities such as antioxidants and antiradical [22].

In this study, phenolic content of *S. lavandulifolia* was determined by UHPLC-ESI-MS/MS method. The most abundant organic compounds were detected as quinic acid (2534±12 ppb) and chlorogenic acid (1882±92 ppb) among the twenty-seven standard compounds. Moreover, lower amounts of another compounds were identified and were given on Table 1. Also, the chromatograms of standards (Fig. 1) and *S. lavandulifolia* were presented (Fig. 2).

Table 1. Organic compounds of *S. lavandulifolia* and LC-MS/MS parameters.

No	Analytes	^a RT	^b Parent ion (m/z)	Ionization Mode	^c R ²	^d RSD%	Linearity Range (µg/L)	^e LOD/LOQ (µg/L)	Recovery (%)	^f U	^g Amount
1	Quinic acid	3.32	190.95	Neg	0.9927	0.0388	250-10000	22.3 / 74.5	103.3	4.8	2534±122
2	Malic acid	3.54	133.05	Neg	0.9975	0.1214	250-10000	19.2 / 64.1	101.4	5.3	
3	tr-Aconitic acid	4.13	172.85	Neg	0.9933	0.3908	250-10000	15.6 / 51.9	102.8	4.9	
4	Gallic acid	4.29	169.05	Neg	0.9901	0.4734	25-1000	4.8 / 15.9	102.3	5.1	
5	Chlorogenic acid	5.43	353	Neg	0.9932	0.1882	250-10000	7.3 / 24.3	99.7	4.9	1882±92
6	Protocatechuic acid	5.63	152.95	Neg	0.9991	0.5958	100-4000	25.8 / 85.9	100.2	5.1	117±6
7	Tannic acid	6.46	182.95	Neg	0.9955	0.9075	100-4000	10.2 / 34.2	97.8	5.1	
8	tr-caffeic acid	7.37	178.95	Neg	0.9942	1.0080	25-1000	4.4 / 14.7	98.6	5.2	32±2
9	Vanillin	8.77	151.05	Neg	0.9995	0.4094	250-10000	10.1 / 33.7	99.2	4.9	
10	p-Coumaric acid	9.53	162.95	Neg	0.9909	1.1358	100-4000	15.2 / 50.8	98.4	5.1	112±6
11	Rosmarinic acid	9.57	358.9	Neg	0.9992	0.5220	250-10000	10.4 / 34.8	101.7	4.9	
12	Rutin	10.18	609.1	Neg	0.9971	0.8146	250-10000	17.0 / 56.6	102.2	5.0	23±1
13	Hesperidin	9.69	611.1	Poz	0.9973	0.1363	250-10000	21.6 / 71.9	100.2	4.9	
14	Hyperoside	10.43	463.1	Neg	0.9549	0.2135	100-4000	12.4 / 41.4	98.5	4.9	170±8
15	4-OH Benzoic acid	11.72	136.95	Neg	0.9925	1.4013	25-1000	3.0 / 10.0	106.2	5.2	17±1
16	Salicylic acid	11.72	136.95	Neg	0.9904	0.6619	25-1000	4 / 13.3	106.2	5.0	16±1
17	Myricetin	11.94	317	Neg	0.9991	2.8247	100-4000	9.9 / 32.9	106.0	5.9	
18	Fisetin	12.61	284.95	Neg	0.9988	2.4262	100-4000	10.7 / 35.6	96.9	5.5	
19	Coumarin	12.52	146.95	Poz	0.9924	0.4203	100-4000	9.1 / 30.4	104.4	4.9	
20	Quercetin	14.48	300.9	Neg	0.9995	4.3149	25-1000	2.0 / 6.8	98.9	7.1	
21	Naringenin	14.66	270.95	Neg	0.9956	2.0200	25-1000	2.6 / 8.8	97.0	5.5	22±1
22	Hesperetin	15.29	300.95	Neg	0.9961	1.0164	25-1000	3.3 / 11.0	102.4	5.3	
23	Luteolin	15.43	284.95	Neg	0.9992	3.9487	25-1000	5.8 / 19.4	105.4	6.9	
24	Kaempferol	15.43	284.95	Neg	0.9917	0.5885	25-1000	2.0 / 6.6	99.1	5.2	49±3
25	Apigenin	17.31	268.95	Neg	0.9954	0.6782	25-1000	0.1 / 0.3	98.9	5.3	36±2
26	Rhamnetin	18.94	314.95	Neg	0.9994	2.5678	25-1000	0.2 / 0.7	100.8	6.1	
27	Chrysin	21.18	253	Neg	0.9965	1.5530	25-1000	0.05 / 0.17	102.2	5.3	

^aRT: Retention time

^bParent ion (m/z): Molecular ions of the standard compounds (mass to charge ratio)

^cR²: coefficient of determination

^dRSD: relative standard deviation

^eLOD/LOQ (µg/L): Limit of detection/Limit of quantification

^fU (%): Percent relative uncertainty at 95% confidence level (k=2).

^gAmount: Quantitative phenolic acid composition of *S. lavandulifolia* (ppb; µg analyte/kg extract)

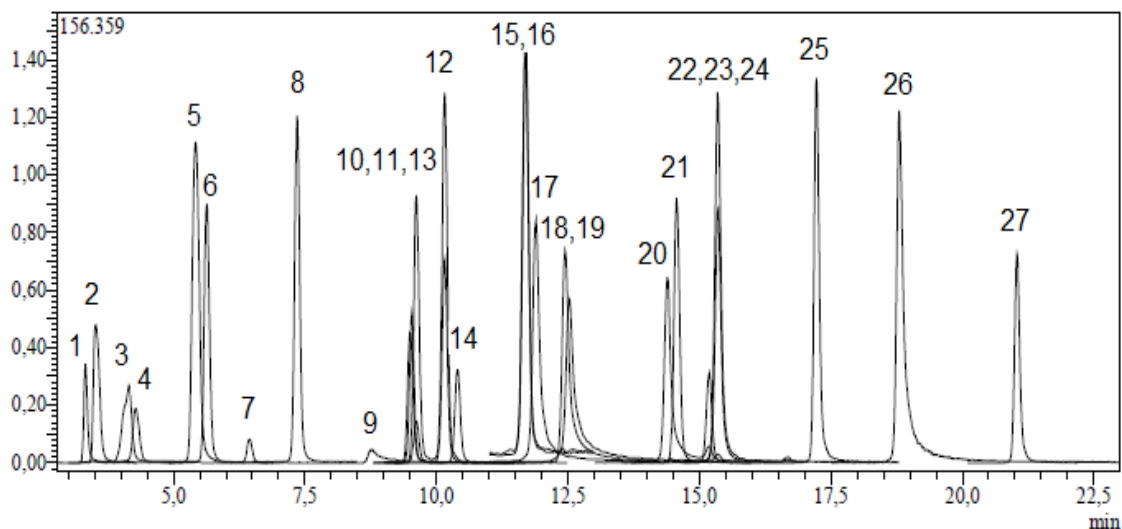


Figure 1. UHPLC-ESI-MS/MS chromatograms of 250 ppb standard mix

(1: quinic acid, 2: malic acid, 3: tr-aconitic acid, 4: gallic acid, 5: chlorogenic acid, 6: protocatechuic acid, 7: tannic acid, 8: tr-caffeic acid, 9: vanillin, 10: p-coumaric acid, 11: rosmarinic acid, 12: rutin, 13: hesperidin, 14: hyperoside, 15: 4-OH benzoic acid, 16: salicylic acid, 17: myricetin, 18: fisetin, 19: coumarin, 20: quercetin, 21: naringenin, 22: hesperetin, 23: luteolin, 24: kaempferol, 25: apigenin, 26: rhamnetin, 27: chrysin).

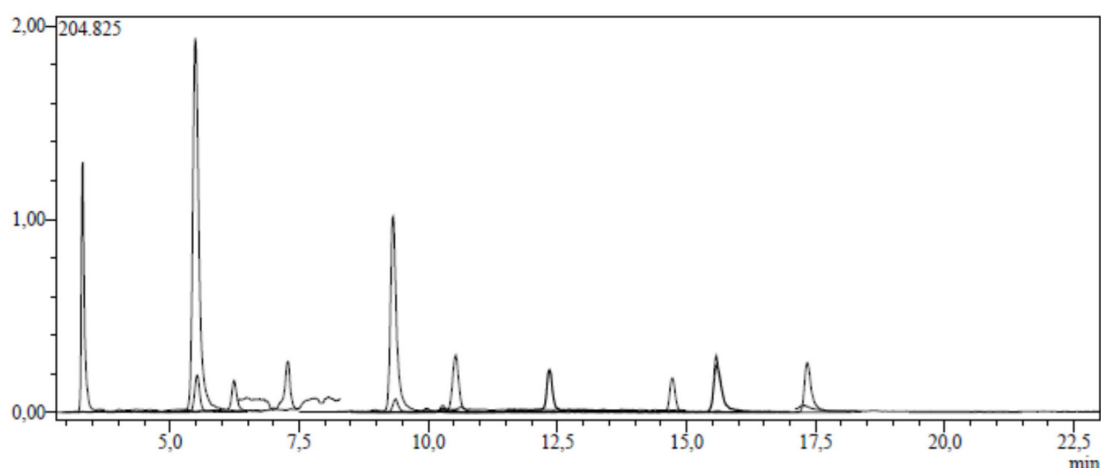


Figure 2. UHPLC-ESI-MS/MS chromatograms of *S. lavandulifolia*.

Antioxidants prevent negative effects of radical species due to their radical scavenging properties. Antioxidant compounds donate hydrogen atoms or transfer single electrons to the radical species to produce nonradical forms [23]. Most of plants have plenty amount of antioxidant potentials and consuming them on diet can prevent some degenerative diseases such as cardiovascular problems, cancer, diabetes, arthritis, and atherosclerosis. Investigation of natural antioxidant sources became very popular research topic in last decades [24].

The ferric thiocyanate method was the one of *in vitro* antioxidant method that we tried to analyze antioxidant potential of *S. lavandulifolia*. The method measures the amount of peroxide produced during the initial stages of oxidation which are the primary products of oxidation. The samples (WS and ES) exhibited effective antioxidant activity in the linoleic acid emulsion system. Peroxidation of linoleic acid emulsion without samples or standard compounds was accompanied by a rapid increase in peroxides. Consequently, these results clearly indicate that WS and ES had effective and potent antioxidant activity in the ferric thiocyanate assays. The effect of 20 $\mu\text{g/mL}$ extracts and standards on lipid peroxidation of a linoleic acid emulsion is shown in Fig. 3.

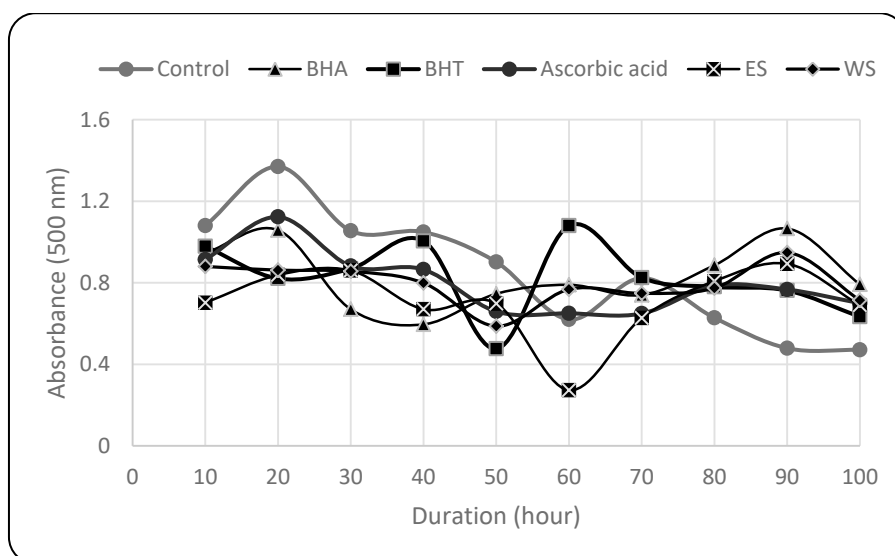


Figure 3. Antioxidant activity of *S. lavandulifolia* by ferric thiocyanate method.

The antioxidant potentials of the samples were determined by reducing power assays. According to the data of FRAP method, samples and standard antioxidants reduce the ferric ions to the ferrous ions. The reducing amounts of samples and standards antioxidants in same concentration (30 $\mu\text{g/mL}$) ordered as BHA > ascorbic acid > BHT > aqueous extract (WS) > ethanol extract (ES), respectively. Fig. 4 demonstrated lower reducing activity of *S. lavandulifolia* extracts than standards.

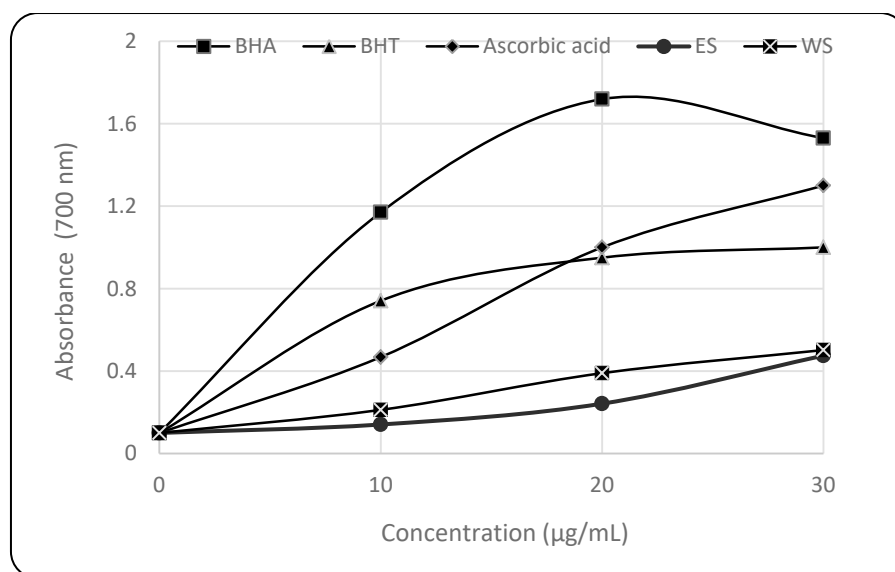


Figure 4. Ferric reducing antioxidant potential of *S. lavandulifolia* by FRAP method.

The cupric reducing potentials of *S. lavandulifolia* extracts were measured by *in vitro* CUPRAC method. The extracts of *S. lavandulifolia* (WS, ES) and standard antioxidants showed high reducing antioxidant capacities (Fig. 5). Cupric reducing amounts of samples and standard antioxidants in the same concentration (30 $\mu\text{g/mL}$) ordered as BHA > BHT > ascorbic acid > ethanol extract (ES) > aqueous extract (WS).

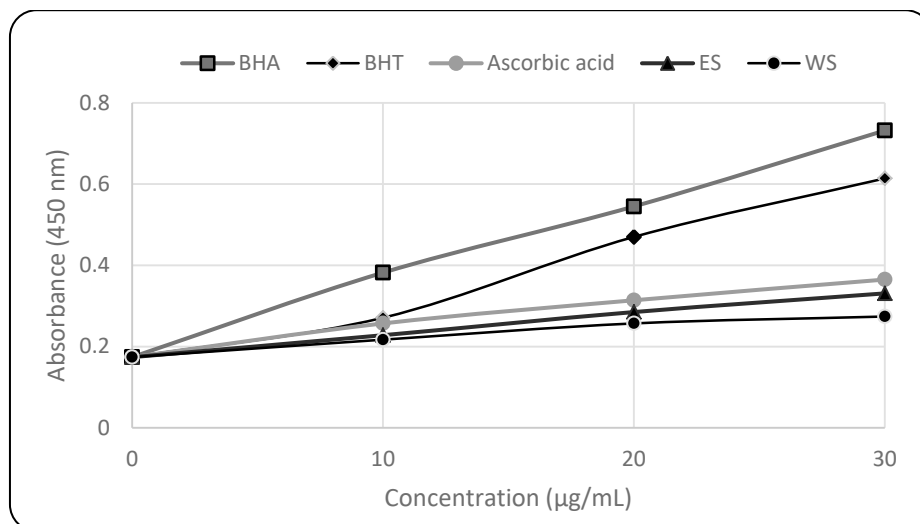


Figure 5. Cupric reducing antioxidant potential of *S. lavandulifolia* by CUPRAC method.

ABTS cation radical scavenging level related with antioxidant capacity. ABTS method based on the reduction of cation radicals by antioxidant substances. According to the data of ABTS radical scavenging, decrease on graph indicated scavenging of cation radicals as shown on Fig. 6. The extracts showed effective ABTS radical scavenging activity like standard antioxidants. Also, the ethanol extract (ES) demonstrated higher cation radicals scavenging activity than aqueous extract (WS).

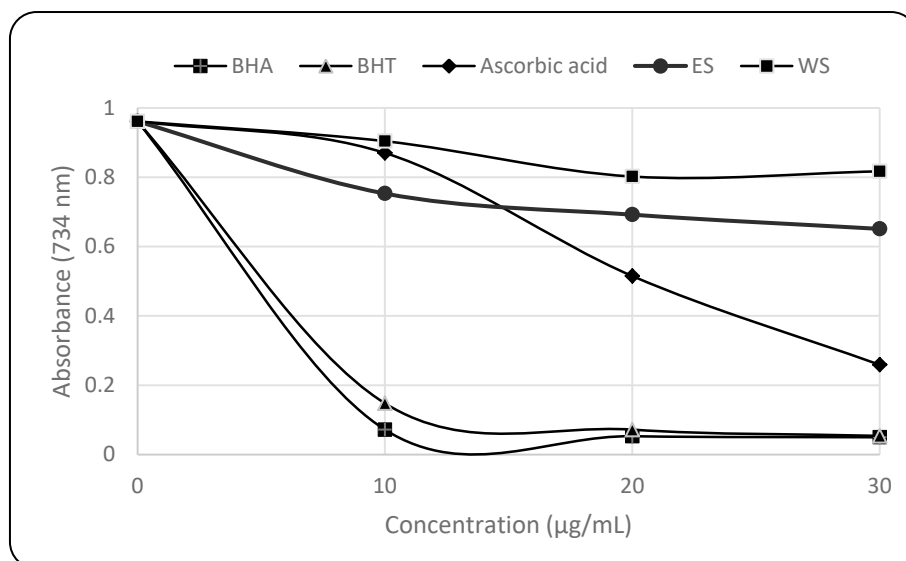


Figure 6. Cation radical scavenging activity of *S. lavandulifolia* by ABTS method.

The DPPH method is a common technique for determination of antioxidant capacity that scavenged free radicals. A noteworthy correlation between concentration and antioxidant potential was detected. *S. lavandulifolia* ethanol extract showed effective DPPH radical scavenging activity close to BHT, a standard antioxidant. Also, the aqueous extract showed low-level free radical scavenging effect (Fig. 7).

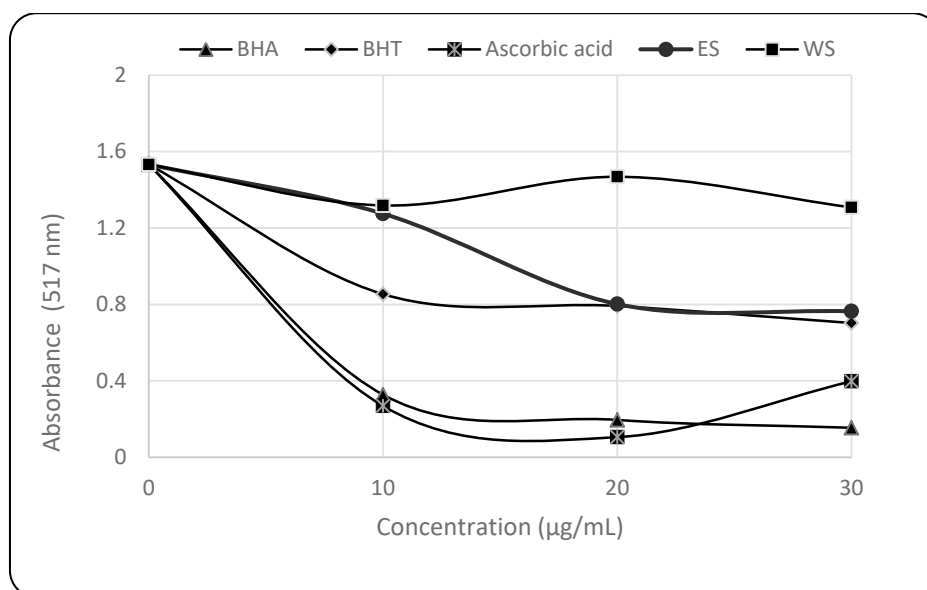


Figure 7. Free radical scavenging activity of *S. lavandulifolia* by DPPH method.

Conclusions

We investigated *in vitro* antioxidant activities and some organic compounds of *S. lavandulifolia* in this study. The results showed that the extracts of *S. lavandulifolia* have notable antioxidant potential on radical scavenging and reducing capability methods. Furthermore, quinic acid and chlorogenic acid were detected as the main phenolic compounds by LC-MS/MS. correlated with. The rich phenolic content of *S. lavandulifolia* might contributed its high antioxidant and antiradical potential. This study provide an important step for further studies to perform the features of *S. lavandulifolia* on food industry or pharmacology.

Conflict of Interest

No potential conflict of interest was reported.

References

- [1] B. Bhattacharjee, Taxonomic studies in Stachys: 2. A new infrageneric classification of Stachys L, Notes Roy. Bot. Gard. Edinburgh. 38(1) (1980) 65-96.
- [2] N. Özhatay, Ş. Kültür, Check-list of additional taxa to the Supplement Flora of Turkey III, Turkish Journal of Botany. 30(4) (2006) 281-316.
- [3] P. Davis, Flora of Turkey-VII, Edinburg University Press, 1982.
- [4] T. Miyase, R. Yamamoto, A. Ueno, Phenylethanoid glycosides from Stachys officinalis, Phytochemistry. 43(2) (1996) 475-479.
- [5] R. Yamamoto, T. Miyase, A. Ueno, Stachyssaponins I-VIII, new oleanane-type triterpene saponins from Stachys riederi Chamisso, Chemical and Pharmaceutical Bulletin. 42(6) (1994) 1291-1296.
- [6] M. El-Ansari et al., Flavonoid constituents of Stachys aegyptiaca, Phytochemistry. 30(4) (1991) 1169-1173.
- [7] H. Skaltsa et al., Inhibition of prostaglandin E2 and leukotriene C4 in mouse peritoneal macrophages and thromboxane B2 production in human platelets by flavonoids from Stachys chrysantha and Stachys candida, Biological and Pharmaceutical Bulletin. 23(1) (2000) 47-53.

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- [8] N. Maleki et al., Potent anti-inflammatory activities of hydroalcoholic extract from aerial parts of *Stachys inflata* on rats, *Journal of Ethnopharmacology*. 75(2-3) (2001) 213-218.
- [9] M. Couladis et al., Screening of some Greek aromatic plants for antioxidant activity, *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*. 17(2) (2003) 194-195.
- [10] O. Kilic, E. Bagci, Essential Oils of Three *Ziziphora L. Taxa* from Turkey and Their Chemotaxonomy, *Asian Journal of Chemistry*. 25(13) (2013).
- [11] E. Köksal et al., Assessment of antimicrobial and antioxidant activities of *Nepeta trachonitica*: analysis of its phenolic compounds using HPLC-MS/MS, *Scientia Pharmaceutica*. 85(2) (2017) 24.
- [12] A. Aras, E. Bursal, M. Dogru, UHPLC-ESI-MS/MS analyses for quantification of phenolic compounds of *Nepeta nuda* subsp. *Lydiae*, *Journal of Applied Pharmaceutical Science*. 6(11) (2016) 009-013.
- [13] A. Aras, M. Dogru, E. Bursal, Determination of antioxidant potential of *Nepeta nuda* subsp. *lydiae*, *Analytical Chemistry Letters*. 6(6) (2016) 758-765.
- [14] M. Ramezani, M. Hassanzadeh, D. Safdarabadi, Volatile constituents of *Stachys lavandulifolia* Vahl growing in Iran, *ACGC Chem Res Commun*. 15(15) (2002) 20-23.
- [15] K. Javidnia, F. Mojab, S. Mojahedi, Chemical constituents of the essential oil of *Stachys lavandulifolia* Vahl from Iran, *Journal of Essential Oil Bearing Plants*. 6(3) (2003) 174-178.
- [16] A. Aras et al., Identification of bioactive polyphenolic compounds and assessment of antioxidant activity of *Origanum acutidens*, *International Letters of Natural Sciences*. 66 (2017) 1-8.
- [17] İ. Gülçin, Antioxidant properties of resveratrol: a structure–activity insight, *Innovative Food Science and Emerging Technologies*. 11(1) (2010) 210-218.
- [18] E. Bursal, R. Boğa, Polyphenols analysed by UHPLC-ESI-MS/MS and antioxidant activities of molasses, acorn and leaves of oak (*Quercus robur* subsp. *pedunculiflora*), *Progress in Nutrition*. 20(1-S) (2018) 167-175.
- [19] M. Bener, F.B. Şen, R. Apak, Heparin-stabilized gold nanoparticles-based CUPRAC colorimetric sensor for antioxidant capacity measurement, *Talanta* 187 (2018) 148-155.
- [20] N. Turan et al., Spectroscopic, thermal and antioxidant properties of novel mixed ligand-metal complexes obtained from saccharinate complexes and azo dye ligand (mnppa), *International Journal of Pharmacology*. 12(2) (2016) 92-100.
- [21] N. Turan et al., Investigation of synthesis, structural characterization, antioxidant activities and thermal properties of Zn (II), Fe (II) and Mn (II) complexes with thiophene-carboxylate ligand, *Journal of Chemistry and Biochemistry*. 3(2) (2015) 13-29.
- [22] E. Bursal, Kinetic properties of peroxidase enzyme from chard (*Beta vulgaris* Subspecies *cicla*) leaves, *International Journal of Food Properties*. 16(6) (2013) 1293-1303.
- [23] E. Köksal et al., Antioxidant activity and polyphenol content of Turkish thyme (*Thymus vulgaris*) monitored by liquid chromatography and tandem mass spectrometry, *International Journal of Food Properties*. 20(3) (2017) 514-525.
- [24] E. Bursal, A. Aras, Ö. Kılıç, Evaluation of antioxidant capacity of endemic plant *Marrubium astracanicum* subsp. *macrodon*: Identification of its phenolic contents by using HPLC-MS/MS, *Natural Product Research*. (2018) 1-5.