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Original Article

Biochemical parameters and antioxidant property of three *Salvia* L. taxa endemic in Turkey

Parâmetros bioquímicos e propriedade antioxidante de três espécies de *Salvia* L. endêmicos da Turquia

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Abstract

The aim of the present study was to determine and compare the fatty acids, lipid soluble vitamins, sterols, phenolics, and antioxidant capacities of three endemic *Salvia* L. taxa (*S. euphratica* var. Montbret & Aucher ex Bentham *leiocalycina* (Rech. Fil.) Hedge, *S. euphratica* var. Montbret & Aucher ex Bentham *euphratica* (Rech. Fil.) Hedge and *S. pseudoeuphratica* Rech.Fil.) and to evaluate these results systematically. The fatty acid compositions were determined by using gas chromatography, while the lipid soluble vitamins, sterols, and phenolics were determined by using HPLC. Also, the antioxidant capacities of three *Salvia* taxa were measured *in vitro*. Palmitic acid was found as major saturated fatty acid while oleic acid, linoleic acid, α-linolenic acid, and erucic acid were found as major unsaturated fatty acids in the present study. It was found that *S. euphratica* var. *euphratica* had lower palmitic acid (8.94 ± 0.71%), total saturated fatty acid (19.16 ± 0.15%), and higher unsaturated fatty acid content (82.08 ± 0.52%) than other studied taxa. Furthermore, it was shown that *S. euphratica* var. *euphratica* had different 18.3/18:2 (0.36) unsaturated/saturated fatty acid (4.28) ratios. However, this study demonstrated that *Salvia* taxa had low lipid soluble vitamins, sterol contents. On the other hand, it was shown that *Salvia* taxa had similar catechin (509.2 ± 4.21 µg/g and 552.2 ± 9.21 µg/g) and vanillic acid amounts (351.2 ± 2.17 µg/g and 396.8 ± 4.1 µg/g) in this study. And also, it was found that *Salvia* taxa had high rosmarinic acid content while *S. euphratica* var. *leiocalycina* had the highest rosmarinic acid content (1480 ± 7.57 µg/g). On the other hand, it was shown that the two ferulic acid contents of *S. euphratica* varieties were higher (1175 ±/5.21 µgmg-1740.2 ± 4.82 µg/mg) than the ferulic acid content of *S. pseudoeuphratica* of which was the lowest (19.2 ± 0.97 µg/mg). The present results suggested that the biochemical results guided the morphological studies, and *Salvia* taxa have a potent antioxidant capacity.

Keywords: antioxidant property, biochemical parameters, GC-MS, HPLC, *Lamiaceae*.

Resumo

O objetivo do presente estudo foi determinar e comparar os ácidos graxos, vitaminas lipossolúveis, esteróis, fenóis e capacidades antioxidantes de três espécies endêmicas de *Salvia* L. (*S. euphratica* var. Montbret & Aucher ex Bentham *leiocalycina* (Rech. Fil.) Hedge, *S. euphratica* var. Montbret & Aucher ex Bentham *euphratica* (Rech. Fil.) Hedge e S. *pseudoeuphratica* Rech.Fil.) e avaliar esses resultados sistematicamente. As composições de ácidos graxos foram determinadas por cromatografia gasosa, enquanto as vitaminas lipossolúveis, esteróis e fenóis foram determinadas por HPLC. Além disso, as capacidades antioxidantes das três espécies de *Salvia* foram medidas *in vitro*. O ácido palmítico foi encontrado como ácido graxo saturado principal, enquanto o ácido oleico, ácido linoleico, ácido α-linolênico e ácido erúcico foram encontrados como principais ácidos graxos insaturados no presente estudo. Verificou-se que *S. euphratica* var. *euphratica* tem menor teor de ácido palmítico (8.94 ± 0.71%) e ácido graxo saturado total (19.16 ± 0.15%) e maior teor de ácidos graxos insaturados (82.08 ± 0.52%) do que as outras espécies estudadas. Além disso, foi demonstrado que a *S. euphratica* var. *euphratica* apresentou diferentes proporções 18:3/18:2 (0.36) de ácidos graxos insaturados/saturados (4.28). No entanto, este estudo demonstrou que o gênero *Salvia* tinha baixo teor de vitaminas lipossolúveis e baixo conteúdo de esteróis. Por outro lado, foi demonstrado que as espécies do gênero *Salvia* contém quantidades de catequinas (509.2 ± 4,21 µg/mg-552.2 ± 9.21 µg/mg) e ácido vanílico semelhantes (351.2 ± 2.17 µg/mg 396,8 ± 4,1 µg/mg). Descobriu-se também que o gênero *Salvia* tinha alto conteúdo de ácido rosmarínico enquanto a espécie *S. euphratica* var. *leiocalycina* apresentou o maior teor desse ácido (1.480 ± 7.57 µg/g). Por outro lado, foi demonstrado que os teores de ácido ferúlico da espécie *S. eupratica* foram maiores (1.175 ± 5.21 µg/mg-1740.2 ± 4.82 µg/mg) do que o conteúdo de ácido ferúlico da espécie *S. pseudoeuphratica* dos quais foi o mais baixo (19.2 ± 0.97 µg/mg). Os resultados atuais sugerem que os resultados bioquímicos orientaram os estudos morfológicos e as espécies de *Salvia* têm uma potente capacidade antioxidante.

Palavras-chave: propriedade antioxidante, parâmetros bioquímicos, GC-MS, HPLC, *Lamiaceae*.

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1. Introduction

The Lamiaceae, is one of the largest families in the plant kingdom, and includes more than 240 genera that are aromatic (Sharafzadeh and Zare, 2011). Sage, Thymus, Rosemary, Marjoram and Basil are some of the popular aromatic plants belonging to Lamiaceae growing in both the Mediterranean and Asia (Naghibi et al., 2005; Hossain et al., 2010; Khled Khoudja et al., 2014). Sage (*Salvia* L.), from the subfamily Nepetoideae of the Mentha tribe in the *Lamiaceae,* is spread throughout different regions of the world and has more than 1000 taxa (Kalaycioğlu et al., 2018). Some species of *Salvia* L. have the significant pharmaceutical properties and have been used in traditional medicine since ancient times (Asadi et al., 2010; Loizzo et al., 2010). Also, the members of *Salvia* L. are consumed as herbal tea and also used in cosmetics, flavouring agents, and perfumery industries (Loizzo et al., 2010; Akbulut and Bayramoglu, 2013). The genus is represented by 100 taxa of which 57 are endemic, with Anatolia being a remarkable center for the genus (Celep et al., 2015; Bardakci et al., 2019).

The studies demonstrated that *Salvia* L. species have antidiabetic, antiviral, antioxidant, anticancer, and anti-inflammatory effects due to possessing the most important biological active compounds including sterols, terpenoids, essential oils, and plyphenolics (Asadi et al., 2010; Ben Farhat et al., 2015a; El Euch et al., 2019). There are several studies related to determining the biochemical characteristics and antioxidant capacity of different *Salvia* species (Tepe et al., 2006; Kelen and Tepe, 2008; Tosun et al., 2009; Gezek et al., 2019). However, to the best of our knowledge, there is no biochemical and antioxidant report concerning three endemic taxa (*S. euphratica* var. *leiocalycina, S. euphratica* var. *euphratica* and *S. pseudoeuphratica*). Therefore, the aim of this study is to determine and compare the fatty acid compositions, lipid-soluble vitamins, sterols, flavonoids, phenolic acids, radical scavenging activities, and total phenolics of *S. euphratica* var. Montbret & Aucher ex Bentham *leiocalycina* (Rech. Fil.) Hedge, *S. euphratica* var. Montbret & Aucher ex Bentham *euphratica* (Rech. Fil.) Hedge and *S. pseudoeuphratica* Rech. Fil. Another purpose is to compare biochemical results with morphological results. Systematically, Hedge (1982a, b) changed the taxonomic position of *S. euphratica* after it had been identified by Bentham (1836) from Turkey (Dizkirici et al., 2015). However, *S. leiocalycina* has been evaluated under a variety of the *S. euphratica* based on the results of morphological and ecological studies, and in the most recent studies, *S. pseudoeuphratica* is accepted as being a different species by Kahraman et al. (2010).

2. Material and Methods

2.1. Chemical agents

All the chemicals were provided by Sigma-Aldrich.

2.2. Plant materials

S. euphratica var. Montbret & Aucher ex Bentham *leiocalycina* (Rech. Fil.) Hedge (B7, Baskil district, marble factory around, railway near, 1330 m, 38° 34'56 °N, 38° 50'23 °E, M. KURŞAT 1610), *S. euphratica* var. Montbret & Aucher ex Bentham *euphratica* (Rech. Fil.) Hedge (B7, Elazig-Malatya road, Komurhan district,755 m, 38° 27'10 °N, 38 48'28 °E, M. KURŞAT 1611) and *S. pseudoeuphratica* Rech. Fil. (B7, Elazığ, Keban-Elazığ road, 3rd-4th km, 900 m, 38° 44'46 °N, 38° 47'59 °E, M. KURŞAT 1622) were collected that grow as native plants in Turkey in 2011, which were deposited in FUH (Firat University Herbarium). The identification of collected materials were done by Murat KURSAT. After the collection and identification, the plant materials were immediately extracted, and HPLC and GC-MS analyses were completed.

2.3. Extraction of plant materials

2.3.1. The analysis of fatty acid, lipid soluble, vitamins and sterol

After collection, each plant material was dried at room temperature, grounded and then, extracted with isopropanol/hexane (2:3) as suggested by Hara and Radin, (1978). The lipid samples were centrifuged at 10.000 *g*, for 5 min. The solvent was evaporated by using a rotary evaporator at 40 °C, and the samples were kept at -25 °C.

2.3.1.1. Preparation of fatty acid methyl esters

In order to determine fatty acids analyses in lipids using gas chromatography, they must be converted into methyl esters derivatives. The FAME analysis was conducted based on the method by Christie (1990). Fatty Acid Methyl Esters (FAME) are produced from vegetable oils by transesterification. In this process, a glyceride reacts with an alcohol in the presence of a catalyst, forming a mixture of fatty acids esters and an alcohol (Schuchardta et al., 1998). Hexane was used to extract FAME. To prepare methyl ester, the lipid extract in the hexane/isopropanol phase (3:2, v/v) was taken into 30 mL non-leakage test tubes. 5 mL of 2% methanolic sulfuric acid was added to the extraction and mixed using a vortex. This mixture was left to be methylated in a 50 °C oven for 15 hours, and then the tubes were cooled to room temperature and mixed well by adding 5 mL of 5% NaCl. The fatty acid methyl esters formed were extracted with 5 mL hexane, and the hexane phase was taken, and treated with 5 mL of 2% $KHCO_3$. The extracts were left for four hours to separate phases and the solvent in the mixture including the methyl esters was evaporated at 45ºC and under nitrogen flow. Then, the samples were dissolved with 1 mL of hexane and taken into 2 mL otosampler vials to analyze with gas chromatography (Bahsi, 2008).

2.3.1.2. Fatty acid methyl esters in the gas chromatography

After obtaining the methyl esters from fatty acids, they were analyzed by SHIMADZU (Kyoto, Japan) GC 17 Ver. 3 Gas Chromatography. GC Capillary Column Permabond CW 20 M-DEG ID: 0.25 mm, film thickness: 0.25 µm length: 25 (Macherey-Nagel® Germany) was used for the analysis. The column temperature was adjusted between

120-220 °C, the injection temperature was kept at 240 °C, and the detector temperature was at 280 °C during analysis. Prior to the analysis of the fatty acid methyl esters, the retention times of each fatty acid were determined by injecting mixtures of standard fatty acid methyl esters, and an analysis of fatty acid methyl esters mixtures was performed. Each of the total fatty acids were calculated as a percentage amount. Results were calculated by using the GC Solution 2.3 program.

2.3.1.3. Chromatographic analysis and quantification of lipid soluble vitamins and sterols

The Shimadzu SPD UV detector VP series HPLC apparatus equipped with a DAD detector was used to determine the sterols (ergosterol, sitosterol and beta-sitosterol) and to perform a lipid-soluble vitamins analysis according to the Sánchez-Machado et al. (2002)' method. The samples were homogenized in a 2 mL acetonitrile/methanol (3/1, v/v) mixture for one minute. The extracts were centrifuged at 6000 *g* for 10 min. at 4 °C, and 1 mL supernatant was taken into vials and analyzed on HPLC. The extracts were treated with acetonitrile/methanol (75/25 v/v). The flow rate of the mobile phase was determined to be 1 mL/min., and the temperature of the analytical column was kept at 40 °C. Supelcosil LC 18 DB (250 × 4.6 mm, 5 µm; Sigma, USA) colon was used as a reverse phase column. Class Vp 6 1 Software supplied by the Shimadzu Corporation was used to determine the amount of each compound in the samples, and results were given as µg/mL. The detection of retinol and retinol acetate were done at 320 nm, while α-tocopherol, vitamin D, a-tocopherol, α-tocopherol acetate was detected at 215 nm, and phytosterols were detected at 202 nm, and vitamin K1 and K2 were detected at 265 nm (López-Cervantes et al., 2006). Class Vp 6.1 software was used to calculate the amounts in the study.

2.4. The extraction procedure

2 g seed was ground and treated with 5 mL 80% methanol to homogenization for flavonoids and phenolic acids analyses. The homogenization was performed at 5000 rpm at +4º C. And rotary evaporation was used to obtain a supernatant. Lastly, extracts were suspended by 2 mL dimethyl sulphoxide (DMSO) (Kursat et al., 2011).

2.4.1. Chromatographic conditions for phenolics

The flavonoids and phenolic acids were determined based on the method by Zu et al. (2006). Two g of plant materials were homogenized with a 5 mL 80% methanol solution, and extracts were centrifuged at 5000 rpm at +4 °C, and dimethyl sulphoxide (DMSO) was used to supply a stock solution. The column was a PREVAIL C18 reversed-phase column (15 × 4.6 mm, 5 µm, USA), and the mobile phase was methanol/water/acetonitrile (46/46/8, v/v/v) comprised of 1.0% acetic acid in the chromatographic analysis (Zu et al., 2006). The flow ratio was 1.05 mL/min., and the injection volume was 10 µL. The chromatographic peaks were confirmed by determining the retention times with those of the standards. Rutin, myricetin, morin, quercetin, and vanillic acid at 254 nm; kaempferol at 264 nm; catechin, naringin and cinnamic acid at 280 nm; naringenin at 285 nm; resveratrol at 306 nm; and caffeic acid, ferulic acid and rosmarinic acid at 330 nm were determined by DAD following the RP-HPLC (Shimadzu SPD UV detector VP series HPLC). The chromatographic studies were done at 25 °C.

2.5. Antioxidant activity

2.5.1. DPPH Radical scavenging capacity

The DPPH radical scavenging capacity was measured based on the method by Liyana-Pathirana and Shahidi (2005). 4.0 mL DPPH solution was mixed with 25, 50, 100, 150, and 250 µL of extract. The complex was kept in darkness for 30 minutes at room temperature (Liyana-Pathirana and Shahidi, 2005). The absorbances were measured at 517 nm, using Shimadzu UVmini-1240 (UV-VIS Spectrophometer). Quercetin (1 µM) was used as reference standard. The results were determined by using Formula 1:

$$\text{DPPH radical scavenging capacity } (\%) = \left[(\text{Abs\_control} - \text{Abs\_sample})\right] / \ (\text{Abs control})] \text{ x } 100 \quad (1)$$

Abs_control is the absorbance of the DPPH radical + methanol; Abs_sample is the absorbance of DPPH radical + sample extract/standard.

2.5.2. ABTS assay

The *2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt* (ABTS) method was used in accordance to Re et al. (1999). The ABTS (7 mM) was mixed with 2.45 mM potassium persulphate and used to obtain ABTS radical cation ($ABTS^{\bullet+}$). The solution was stored for 12-16 h at room temperature. The ($ABTS^{\bullet+}$) solution was dissolved with water to measure an absorbance of 0.700 ± 0.020 at 734 nm. The 3 mL ABTS solution was treated with 25, 50, 100, 150 and 250 µL extracts, and the absorption was detected at 6 min. The absorbance of the control (3.0 mL ($ABTS^{\bullet+}$) solution with 30 L water) was written as Acontrol (Skotti et al., 2014) (Formula 2).

$$\text{ABTS radical cation scavenging capacity } (\%) = \left[(\text{Abs\_control} - \text{Abs\_sample})\right] / \ (\text{Abs\_control})] \text{ x } 100 \quad (2)$$

2.5.3. Metal chelating activity

The chelating capacity of the plant materials were assessed based on the Dinis et al.'s (1994) method. According to this method, 50 µL of 2 mM $FeCl_2$ was added to several concentrations including 50, 100, 250, and 500 µg/mL of extracts. 0,2 mL ferrozine was added to the mixture to start the reaction. The solution was kept at room temperature for 10 minutes after the solution was firmLy mixed. 562 nm was used as absorbance (Dinis et al., 1994). The percentage inhibiting of the ferrozine–Fe^{2+} complex was found as the following Formula 3:

$$\text{The Ferrous ion chelating activity } (\%) = \left[1 - (\text{As} \ / \text{Ac})\right] \times 100 \quad (3)$$

where A_c is the absorbance of the control, and As is the absorbance of the extract/ standard (Kizilpinar-Temizer et al., 2017). Positive control is Na_2EDTA.

2.5.4. Determination of antioxidant activity by MDA/ TBARS formation

The antioxidant activity of materials was done using the method proposed by Shimoi et al. (1994). The extracts were prepared by using DMSO (dimethyl sulfoxide). Oleic acid (3.35 mM), linoleic acid (9.01 mM), linolenic acid (2.30 mM) dissolving in the DMSO, Fe^{2+} ($FeCl_2.2H_2O$) and hydrogen peroxide are used in the experiment. The Control, Fenton reagent group, and the group of sage extracts were formed. The control group contained 0.5 mL of fatty acid and a buffer solution (0.2% Tween 20/0.05 M Tris HCl/0.15 M KCl, pH=7.4), whilst the fenton group contained 0.5 mL of fatty acid, a buffer solution, $FeCl_2.2H_2O$ (50 µM), and hydrogen peroxide (0.01 mM). Also, the sample extracts contained 0.5 mL of fatty acid, a buffer, $FeCl_2$ (50 µM), hydrogen peroxide (0.01 mM), and 0.25 mL sage extract. All the groups were stored at 37 °C for 24 h for incubation by adding 0.1 mL of a 4% (w/v) BHT to protect the greater oxidation. After that, 1 mL was taken from the samples in all the three groups and 1 mL of 0.6% TBA was added to the reaction mixture and samples incubated at 90 °C for 30 min. Finally, 4 mL butan-1-ol was added to the tubes, mixed, and centrifuged at 4250 rpm for 10 min. The absorbance of the supernatant was determined at 532 nm using a spectrophotometer Shimadzu UV mini–1240. MDA standard curves were formed by 1,1,3,3-tetramethoxypropane, and thiobarbituric acid-reactive substances (TBARS) were expressed as mg MDA/kg dry matter (Keser et al., 2014).

3. Results and Discussion

This study showed that the main saturated fatty acids in *Salvia* taxa were palmitic acid (16:0) and stearic acid (18:0) (Table1). The palmitic acid contents of the studied *Salvia* taxa were between 8.94 ± 0.71% (*S. euphratica* var. *euphratica*) and 32.57 ± 1.29% (*S. euphratica* var. *leiocalycina*). The stearic acid was the second major saturated fatty acid in the studied *Salvia* taxa (3.04 ± 0.22%-7.89 ± 0.86%). The other saturated fatty acids were myristic acid (14:0) and arachidic acid (20:0). Caprylic acid (8:0), capric acid (10:0), undecylic acid (11:0), behenic acid (22:0) and lignoceric acid (24:0) were either absent or in low amounts (Table 1). Azcan et al. (2004) showed that palmitic acid and stearic acid were major saturated fatty acids in *Salvia.* Also, different studies demonstrated that *Salvia* species have palmitic acid and stearic acid as saturated fatty acids (Bagci et al., 2004; Kilic et al., 2005; Alipour-Gough and Asgarpanah, 2015; Moazzami Farida et al., 2016). However, the results of Habibvash et al. (2007) conflict with the present study because they found that palmitic acid and stearic acid content of *Salvia* taxa were lower than in this study (Habibvash et al., 2007). Moreover, they determined that some *Salvia* taxa had a high arachidic acid content (14.82-26.91%) (Habibvash et al., 2007). Also, Delange et al. (2012) determined that *Salvia* had a higher stearic acid and arachidic acid content than palmitic acid content. Furthermore, Bakoglu et al. (2016) determined that *Salvia* had higher stearic acid (2.88-22.54%) and arachidic acid (4.32-13.9%) content than the present study. Furthermore, Kilic (2018) found that the stearic acid (36.33%) content of *S. euphratica* var. *leiocalycina* were higher than in the present study while he found that it has a similar palmitic acid content with current study (Kilic, 2018). On the other hand, α-linolenic acid (18:3 n3), linoleic acid (18:2 n6), oleic acid (18:1 n9) were determined as a major unsaturated fatty acids in this study (Table 1). *S. pseudoeuphratica* had the highest α-linolenic acid (29.45 ± 1.32%), is ω-3 fatty acid, while *S. euphratica* var. e*uphratica* had the lowest α-linolenic acid (11.53 ± 1.19%) in this study. The current study also showed that the α-linolenic acid content of *S. euphratica* var. *leiocalycina* was 16.23 ± 1.01%. Also, it was found that *S. euphratica* var. *euphratica* had the highest linoleic acid content (31.58 ± 1.16%), ω-6 fatty acids, and its ω-6 fatty acid content was different from *S. euphratica* var. *leiocalycina* (12.06 ± 0.98%). Also, it was found that the linoleic acid content of *S. pseudoeuphratica* was 7.48 ± 0.63%. Essential fatty acids (EFAs) are polyunsaturated fatty acids and cannot be synthesized by humans but must be taken from food, and they are divided into two groups, omega-6 and omega-3 (Turan et al., 2013). Similarly, previous studies showed that linoleic acid, oleic acid and linolenic acid were primary unsaturated fatty acids in *Salvia* (Azcan et al., 2004; Kilic et al., 2005; Alipour-Gough and Asgarpanah, 2015; Ben Farhat et al., 2015b). Furthermore, it was indicated that an 18:3/18:2 ratio should be used as a taxonomic tool in Lamiaceae (Azcan et al., 2004; Goren et al., 2006) and it was found that the 18:3/18:2 ratio of *S. euphratica* var. *euphratica* (0.36) was different from *S. euphratica* var. *leiocalycina* (1.34) and *S. pseudoeuphratica* (3.93) in the present study. Besides, *S. euphratica* var. *leiocalycina* has higher oleic acid content (11.82 ± 1.24%) than *S. euphratica* var. *euphratica* (9.07 ± 1.13%) and *S. pseudoeuphratica* (4.71 ± 0.49%). Palmitoleic acid (16:1 n7) and erucic acid (22:1) were the other dominant unsaturated fatty acids in the three *Salvia* taxa. *S. euphratica* var. *euphratica* had higher erucic acid (22:1; 20.13 ± 1.47%) than *S. euphratica* var. *leioclaycina* (2.42 ± 0.3%) and *S. pseudoeuphratica* (5.87 ± 0.59%), but it had the lowest palmitoleic acid (16:1 n7) content in the study. Moreover, myristoleic acid (14:1), pentadecanoic acid (15:1), heptadecanoic acid (17:1), eicosadienoic acid (20:2 n6) and eicosapentaenoic (20:5 n3) contents were absent or low in the present study. Also, this study showed that the ratio of unsaturated fatty acids (61.38 ± 0.67%-82.08 ± 0.52%) were more than the ratio of saturated fatty acids and it was determined that the proportion of unsaturated fatty acids were larger than the saturated fatty acids in Lamiaceae (Azcan et al., 2004). And it was found that the ratio of unsaturated fatty acid to saturated fatty acid (U/S) of *S. euphratica* var. *euphratica* (4.28) was different from *S. euphratica* var. *leiocalycina* (1.32) and *S. pseudoeuphratica* (1.67) (Table 1).

The current study showed the studied three *Salvia* taxa low lipid soluble vitamins and sterol content (Table 1). It was determined that *S. euphratica* var. *leiocalycina* and *S. pseudoeuphratica* had higher α-tocopherol (25.05 ± 1.14 µg/g, 19.5 ± 0.97 µg/g, respectively) than *S. euphratica* var. e*uphratica* (6.14 ± 0.37 µg/g). Also, it was found that K1 vitamin contents of three *Salvia* taxa were determined as 4.05 ± 0.15 µg/g (*S. euphratica* var. *leiocalycina*), 7.87 ± 0.14 µg/g (*S. euphratica* var. e*uphratica*) and 2.7 ± 0.2 µg/g (*S. pseudoeuphratica*). Similarly, Sari et al. (2009)

Table 1: Fatty acid, lipid soluble vitamins, sterols content (μg/mg) in polar extracts from three Turkish *Salvia* taxa

Chemical composition	*S. euphratica* var. *leiocalycina*	*S. euphratica* var. e*uphratica*	*S. pseudoeuphratica*
Saturated fatty acids*			
(8:0)	-	0.39 ± 0.01	-
10:0	-	-	1.01 ± 0.45
11:0	-	0.42 ± 0.01	-
14:0	1.37±0.14	0.69 ± 0.01	1.25 ± 0.27
16:0	32.57±1.29	8.94 ± 0.71	24.16 ± 1.42
18:0	7.12±0.57	3.04 ± 0.22	7.89 ± 0.86
20:0	1.7±0.32	1.07±0.1	2.24 ± 0.27
22:0	-	2.00±0.11	
24:0	-	2.61±0.1	
Total	42.76± 0.58	19,16± 0.15	36.55 ± 0.65
Unsaturated fatty acids*			
14:1	1.3±0.1	-	-
15:1	-	1.26±0.1	4.28±0.37
16:1 n7	12.9±1.21	2.9±0.1	9.59±0.65
17:1	-	2.27±0.12	-
18:1 n9	11.82±1.24	9.07±1.13	4.71±0.49
18:2 n6	12.06±0.98	31.58±1.16	7.48±0.63
18:3 n3	16.23 ±1.01	11.53±1.19	29.45±1.32
18:3 n6	-	0.7±0.01	-
20:2 n6	-	0.39±0.1	-
20:5 n3	-	1.67±0.24	
22:1	2.42±0.3	20.13±1.47	5.87±0.59
Total	56.73 ± 0.8	82.08 ± 0.52	61.38 ± 0.67
18:3/18:2	**1.34**	**0.36**	**3.93**
Unsaturated/Saturated(U/S)	**1.32**	**4.28**	**1.67**
Lipid soluble vitamin and sterol**			
K1	4.05±0.15	7.87 ±0.14	2.7±0.2
K2	0.25±0.01	0.68±0.01	0.65±0.01
R-tocopherol	0.22±0.01	1.04±0.01	0.2±0.01
D2	1.2±0.12	1.34±0.01	1.5±0.35
D3	0.32±0.01	0.35±0.01	1.15±0.32
a-tocopherol	25.05±1.14	6.14±0.37	19.5±0.97
Retinol	-	-	0.25±0.01
Retinol-acetate	1.1±0.1	2.1±0.32	1.35±0.14
Stigmasterol	44.21±1.22	80.14±1.34	66.4±1.27
B-sitosterol	42.45±1.41	102.17±1.24	87.15±1.53

*By Gas chromatography. **By High pressure liquid chromatography.

found that *Salvia* taxa had a low lipid soluble vitamin content and they determined that D2, D3, α-tocopherol acetate and K1 content of *S. euphratica* were 15.0 μg/g, 18.0 μg/g, 7.4 μg/g and 7.8 μg/g, respectively. Sterols are structural elements of the cell membrane and play a significant role in the regulation of membrane fluidity and permeability (Trautwein and Demonty, 2007). Also, they are used as therapeutic agents to reduce plasma cholesterol concentration (St-Onge and Foo, 2003). This study determined that the ß-sitosterol content of the studied *Salvia* taxa were between 42.45 ± 1.41 μg/g (*S. euphratica* var. *leiocalycina*) and 102.17 ± 1.24 μg/g (*S. euphratica* var. e*uphratica*) and their stigmasterol content were between 44.21 ± 1.22 μg/g (*S. euphratica*

var. *leiocalycina*) and 80.14 ± 1.34 µg/g (*S. euphratica* var. *euphratica*; Table 1).

Recent studies have shown that phenolic compounds from medicinal plants are important because of the various benefits, especially their antioxidant effect, and these studies supported significant steps for the discovery of new drugs (Tungmunnithum et al., 2018; Najjaa et al., 2020). It was found that *Salvia* taxa had high catechin content (509.2 ± 1.21 µg/mg-552.2 ± 9.21 µg/mg; Table 2) in the present study. It was suggested that catechin has free radical scavenging effects, inhibit the extracellular matrix degradation caused by UV, and have anti allergenic and anti-inflammatory effects (Bae et al., 2020). Also, catechin plays a significant role against cancer, diabetes, obesity, cardiovascular diseases, infections, and neurodegenerative diseases (Isemura, 2019).

Also, it was determined that *S. euphratica* var. *euphratica* and *S. pseudoeuphratica* had high rutin content (328.4 ± 4.27 µg/mg and 308.6 ± 5.01 µg/mg, respectively). However, it was seen that the rutin amount of *S. euphratica* var. *leiocalycina* was the lowest (32 ± 1.12 µg/mg). On the other hand, it was showed that the naringenin amount of *S. euphratica* var. *leiocalycina* was higher (92.8 ± 2.17 µg/mg) than the other studied taxa (Table 2). The myricetin, morin, quercetin, kaempferol, and naringin amounts of the studied taxa were either absent or in low amounts in the present study (Table 2). In a study conducted by Kivrak et al. (2019), it was reported that *Salvia* had kaempferol, naringenin, rutin, vanillic acid, caffeic acid, and ferulic acid content. However, they found that *Salvia* did not have myricetin, resveratrol and quercetin (Kivrak et al., 2019). Also, Zengin et al. (2018) reported that in *Salvia* species including *S. euphratica* var. *leiocalycina* possess rutin, apigenin, kaempferol, luteolin, protocatechuic acid, rosmarinic acid, caffeic acid, and 3- O-caffeoylquinic acid. Conversely, the present study showed that the vanillic acid contents of *Salvia* taxa were between 351.2 ± 2.17 µg/mg and 396.8 ± 4.1 µg/mg. The results obtained from this study found that *S. euphratica* var. *leiocalycina* and *S. euphratica* var. *euphratica* had a higher rosmarinic acid content (1480 ± 7.57 µg/mg, 989 ± 4.92 µg/mg, respectively) than *S. pseudoeuphratica* (546.2 ± 7.61 µg/mg; Table 2). It was reported that rosmarinic acid is the most prominent compound in *Salvia* species, and it is chiefly responsible for the antioxidant activity in *Salvia* (Lu and Yeap, 2002). Meanwhile, it was reported that vanillic acid has pharmacological effects against cancer, cardiovascular disease, inflammation, oxidative stress, and is used as a food additive (Dandekar and Wasewar, 2020). In addition, two *S. euphratica* taxa had the highest ferulic acid content (1740.2 ± 4.82 µg/mg and 1175 ± 5.21 µg/mg) whereas it was found that *S. pseudoeuphratica* had the lowest ferulic acid (19.2 ± 0.97 µg/mg). It was reported that ferulic acid contributed to the reduction in oxidative stress in the ß-cells and stimulated insulin secretion, and it has anticarcinogenic activity by stimulating cytoprotective enzymes against free radical damage (Kumar and Pruthi, 2014). However, *S. pseudoeuphratica* had higher caffeic acid content (153.2 ± 2.13 µg/mg) than *S. euphratica* taxa, while the cinnamic acid content of the studied *Salvia* taxa had the lowest or had trace amounts (Table 2). A study conducted by Yumrutas et al. (2012), found that rosmarinic acid and caffeic acid were dominant phenolics in two varieties of *S. euphratica,* whereas the present study showed that *S. euphratica* var. *leiocalycina* does not have caffeic acid. Adimcilar et al. (2019) determined that the rosmarinic acid content of *S. euphratica* var. *leiocalycina* was 9.81 ± 0.23 mg/g. Similarly, some studies showed that *Salvia* taxa had high rosmarinic acid content (Tepe, 2008; Erdogan-Orhan et al., 2012; Kocak et al., 2016).

Table 2: Flavonoids and phenolic acid content (µg/mg) in polar extracts from three Turkish *Salvia* taxa

Compound	*S. euphratica* var. *leiocalycina*	*S. euphratica* var. *euphratica*	*S. pseudoeuphratica*
Flavonoids*			
Rutin	32 ± 1.12	328.4 ± 4.27	308.6 ± 5.01
Myricetin	-	8.9±0.47	1.8±0.2
Morin	3.2±0.21	6.2±0.1	14±0.32
Querecetin	0.2±0.01	-	0.6±0.01
Kaempherol	-	-	-
Catechin	552.2±9.21	509.2±4.21	539±7.62
Naringenin	92.8±2.17	-	7.01±0.34
Resveratrol	2.2±0.01	2.5±0.11	10.8±0.37
Phenolic acid*			
Vanilic acid	382.1± 3.41	351.2 ± 2.17	396.8 ± 4.1
Cinnamic acid	-	0.6±0.1	1.6±0.15
Caffeic acid	16.8±1.12	10.4±0.17	153.2±2.13
Ferulic acid	1740.2±4.82	1175±5.21	19.2±0.97
Rosmarinic acid	1480±7.57	989±4.92	546.2±7.61

*By High pressure liquid chromatography.

Furthermore, this study demonstrated that *Salvia* taxa had high DPPH and ABTS radical scavenging activities (Figures 1 and 2). Tepe et al. (2006) indicated that *S. euphratica* var. *euphratica* had more active plants based on the DPPH radical scavenging activity. Similarly, Senol et al. (2010) found that methanol extracts of *Salvia* taxa including *S. euphratica* var. *leiocalycina* (89.02 ± 0.80%-91.28 ± 0.44%) and *S. euphratica* var. *euphratica* (88.85 ± 0.22%-92.39 ± 0.22%) had high DPPH radical scavenging capacity. Also, Senol et al. (2010) indicated that the ferrous ion chelating activity of *S. euphratica* var. *leiocalycina* (23.23 ± 0.84%) and *S. euphratica* var. *euphratica* (12.9 ± 1.32%) were higher than the present results. Furthermore, Yumrutas et al. (2012) showed that the DPPH radical scavenging capacity of *S. euphratica* var. *leiocalycina was* higher than in *S. euphratica* var. *euphratica.* The literatures reported that phenolic compounds were responsible for the antioxidant capacities in Lamiaceae including *Salvia* (Tosun et al., 2009; Roby et al., 2013; Kocak et al., 2016).

This study demonstrated that *S. euphratica* var. *leiocalycina* and *S. euphratica* var. *euphratica* had high metal chelating activity (82.22 ± 1.29%-84.4 ± 1.1%, respectively)

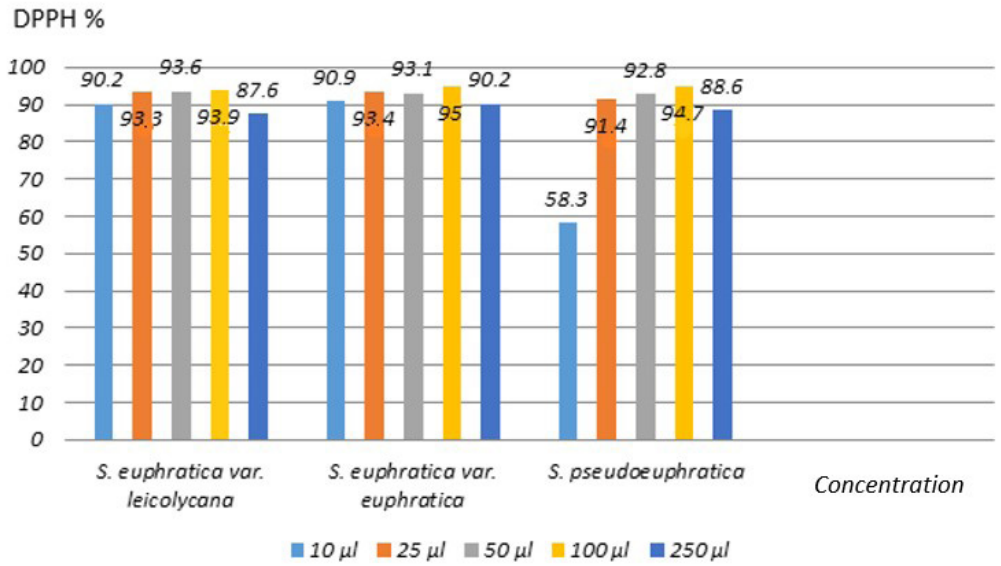

Figure 1. DPPH % Radical Scavenging Activities of three Salvia taxa.

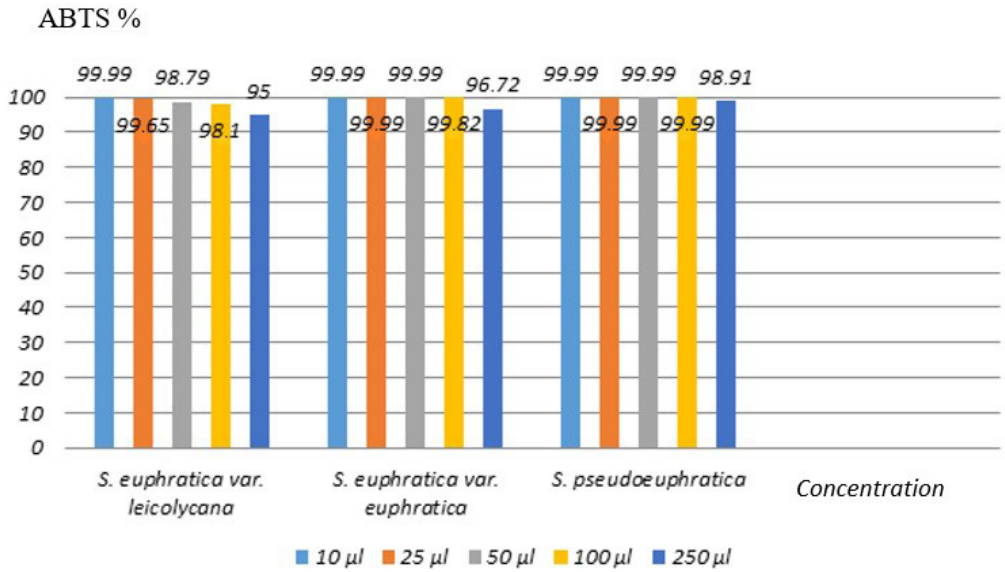

Figure 2. ABTS % Radical Scavenging Activities of three Salvia taxa.

while it was found that *S. pseudoeuphratica* had low metal chelating activity (30.33 ± 0.39%). Some studies reported that *Salvia* taxa comprising of *S. euphratica* var. *leiocalycina* had strong metal chelating capacity (Topcu et al., 2007; Zengin et al., 2018). In contrast, a study done by Senol et al. (2010) suggested that *S. euphratica* var. *euphratica,* and *S. euphratica* var. *leiocalycina* had low metal chelating activity. Additionally, it was observed that lipid peroxidation inhibitions of *S. euphratica* var. *leiocalycina* (2.1 ± 0.1 mg/kg) and *S. euphratica* var. *euphratica* ((3.47 ± 0.2 mg/kg) are quite similar. But the lipid peroxidation inhibition of *S. pseudoeuphratica* calculated as 15.32 ± 0.41 mg/kg lipid peroxidation. Sari et al. (2012) found that lipid peroxidation inhibition of *Salvia* taxa was between 0.58mg/l and 0.92 mg/l. Also, Khlifi et al. (2006) indicated that *Salvia* had significant inhibition of oxygen consumption. Furthermore, Giamperi et al. (2012) suggested that *Salvia* taxa had significant antioxidant activity against lipid peroxidation.

Furthermore, it was demonstrated that the biochemical results in the current study supported the morphological studies. In the flora of Turkey, *S. pseudoeuphratica* formerly has taken its place as a synonym of *S. euphratica* var. *euphratica* (Hedge, 1982a, b). However, it was shown that *S. pseudoeuphratica* apparently diverged from *S. euphratica* according to its many morphological characters, and it was demonstrated that former was clustered differently from latter in the dendrogram (Kahraman et al., 2010). In particular, it was found that some biochemical parameters (such as palmitic acid, linoleic acid, α-linolenic acid, 18:3/18:2, rutin, naringenin, caffeic acid, ferulic acid and metal chelating) of *S. pseudoeuphratica were* different from the those of *S. euphratica* var. *euphratica* in the present study. Moreover, Kahraman et al. (2010) found that the populations of *S. euphratica* var. *euphratica* was clustered in a different position than the populations of *S. euphratica* var. *leiocalycina* in the dendrogram constructed using morphological characters. Also, Yilmaz et al. (2019) indicated that two varieties of *S. euphratica* had difference hair cover on the stem, bracts, calyx and pedicel, and Bagherpour (2010) reported that they had differences in glabrous inflorescence, the bracts, and the calyx. Similarly, it was reported that the current biochemical results (palmitic acid, oleic acid, linoleic acid, α-linolenic acid, γ-linolenic acid, 18:3/18:2, U/S, α-tocopherol, rutin, quercetin, naringenin, resveratrol, vanillic acid, caffeic acid, ferulic acid, and rosmarinic acid) of both variety were different from each other in the current study. Although it was stated that the difference in biochemical content is due to environmental conditions, soil structure, variation in sunlight hour, altitude, temperature and exposure to UV-B (Çetinkaya et al., 2017; Çoklar, 2017), the difference in the biochemical content of these two varieties was thought to be caused not only by environmental conditions but also by genetic differences.

4. Conclusion

The current study showed that palmitic acid was found as a major saturated fatty acid, and *S. euphratica* var. *euphratica* had lower palmitic acid (8.94 ± 0.71%) and total saturated fatty acid (19.16 ± 0.15%) content than the two studied taxa. In addition, *S. euphratica* var. *euphratica* had the highest unsaturated fatty acid content (82.08 ± 0.52%), and oleic acid, linoleic acid, α-linolenic acid, and erucic acid were found as major unsaturated fatty acids. Furthermore, it was found that 18.3/18:2 (0.36) and the unsaturated/saturated fatty acid (4.28) ratios of *S. euphratica* var. *euphratica* were different from the two other studied taxa. It can be concluded that the fatty acids could be used as systematical tool, and the fatty acid content of *S. euphratica* var. *euphratica* diverged from the other two taxa. However, this study demonstrated that *Salvia* taxa had low lipid soluble vitamins and sterol contents. On the other hand, the present study demonstrated that the three studied *Salvia* taxa were found to have similar catechin (509.2 ± 4.21 µg/g and 552.2 ± 9.21 µg/g) and vanillic acid amounts (351.2 ± 2.17 µg/g and 396.8 ± 4.1 µg/g). Also, the studied *Salvia* taxa had high rosmarinic acid content; in particular, *S. euphratica* var. *leiocalycina* had the highest level of rosmarinic acid (1480 ± 7.57 µg/g). On the other hand, two *S. euphratica* varieties had the highest ferulic acid content (1175 ± 5.21 µg/mg-1740.2 ± 4.82 µg/mg) while it was found the ferulic acid content of *S. pseudoeuphratica* was the lowest (19.2 ± 0.97 µg/mg). Moreover, this study showed that *Salvia* taxa have a potent antioxidant capacity, and it was shown that the biochemical results were supported in the morphological studies.

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