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RESEARCH ARTICLE

Evaluation of Phytochemical Contents and Biological Activities of *Salvia officinalis* and *Salvia triloba* Grown with Organic Farming

Burçin ÖZÜPEK[*], Sultan PEKACAR[**], Didem DELİORMAN ORHAN[***°]

Evaluation of Phytochemical Contents and Biological Activities of Salvia officinalis and Salvia triloba Grown with Organic Farming

SUMMARY

Salvia officinalis L., known as medicinal sage, and Salvia triloba L., known as Anatolian sage, belong to the Lamiaceae family and are species that usually grow in the Mediterranean region. In this study, it was aimed to evaluate the in vitro antidiabetic, antiobesity and antioxidant potentials of the extracts prepared by infusion technique from S. officinalis and S. triloba has grown by organic farming methods. In addition, the effects of the extracts on the pancreatic cholesterol esterase enzyme were also investigated. The Reverse Phase-High Performance Liquid Chromatography (HPLC) technique was used to analyze the phytochemical contents of the extracts. At a concentration of 2 mg/mL, S. officinalis inhibited 64.69% ± 0.23, S. triloba 47.78 ± 2.11% on the α-glucosidase enzyme. Only S. triloba had an inhibitory effect on α-amylase and pancreatic lipase enzyme. On the pancreatic cholesterol esterase enzyme, inhibition values of S. triloba extract at all tested concentrations was found higher than S. officinalis extract. It was observed that the S. officinalis extract had the highest reducing power potential. The metal chelating capacity of both extracts at a concentration of 2 mg/mL was calculated as 100%. It was concluded that the ABTS radical scavenging activity of the extracts increased in a dose-dependently manner. The amounts of rosmarinic acid and hesperidin were found higher in S. officinalis extract than in S. triloba extract by Reverse Phase-HPLC technique. The presence of hesperidin in S. triloba was detected for the first time in this study. These findings considering it was concluded that activity-guided isolation and in vivo activity studies should be performed because these two species grown the organic farming methods have potent α-glucosidase enzyme inhibitory and antioxidant effects.

Keywords: *Antioxidant, enzyme inhibition, phytochemistry, Reverse phase-HPLC, Salvia officinalis, Salvia triloba*

Organik Tarım ile Yetiştirilen Salvia officinalis ve Salvia triloba'nın Fitokimyasal İçeriklerinin ve Biyolojik Aktivitelerinin Değerlendirilmesi

ÖZ

Tıbbi adaçayı olarak bilinen Salvia officinalis L. ve Anadolu adaçayı olarak bilinen Salvia triloba L., Lamiaceae familyasına ait olup, genellikle Akdeniz bölgesinde yetişen türlerdir. Bu çalışmada organik tarım yöntemleri ile yetiştirilmiş S. officinalis ve S. triloba'dan infüzyon tekniği ile hazırlanan ekstrelerinin in vitro antidiyabetik, antiobezite ve antioksidan potansiyellerinin değerlendirilmesi amaçlanmıştır. Bunların yanı sıra ekstrelerin pankreatik kolesterol esteraz enzimi üzerindeki etkileri de incelenmiştir. Ekstrelerin fitokimyasal içeriklerini analiz etmek için Ters Faz-Yüksek Performanslı Sıvı Kromatografisi (YPSK) tekniği kullanılmıştır. α-Glukozidaz enzimi üzerinde 2 mg/mL konsantrasyonda S. officinalis %64.69 ± 0.23, S. triloba ise %47.78 ± 2.11 inhibisyona neden olmuştur. α-Amilaz ve pankreatik lipaz enzimi üzerinde sadece S.triloba inhibitör etki oluşturmuştur. Pankreatik kolesterol esteraz enzimi üzerinde ise S. triloba ekstresinin test edilen tüm konsantrasyonlardaki inhibisyon değerlerinin S. officinalis ekstresinden daha yüksek olduğu tespit edilmiştir. S. officinalis ekstresinin en yüksek indirgeme gücü potansiyeline sahip olduğu gözlemlendi. Her iki ekstrenin 2 mg/mL konsantrasyonda metal bağlama kapasitesi %100 olarak hesaplanmıştır. Ekstrelerin ABTS radikal süpürücü aktivitesinin ise doz bağımlı olarak arttığı sonucuna varılmıştır. Ters Faz-YPSK tekniği ile S. officinalis ekstresinde rosmarinik asit ve hesperidin miktarları S. triloba ekstresine göre daha yüksek bulunmuştur. S. triloba'da hesperidinin varlığı ilk kez bu çalışmada tespit edilmiştir. Tüm bu bulgular göz önüne alındığında, organik tarım yöntemiyle yetiştirilen bu iki türün güçlü α-glukozidaz enzim inhibitör ve antioksidan etkilere sahip olmasından dolayı üzerlerinde aktivite yönlendirmeli izolasyon ve in vivo aktivite çalışmalarının yapılması gerektiği sonucuna varılmıştır.

Anahtar Kelimeler: *Antioksidan, enzim inhibisyonu, fitokimya, ters faz-YPSK, Salvia officinalis, Salvia triloba*

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[*] ORCID:0000-0003-2159-9860, Gazi Üniversitesi, Eczacılık Fakültesi, Farmakognozi Ana Bilim Dalı, 06510, Ankara, Türkiye,
[**] ORCID:0000-0002-7782-9832 , Gazi Üniversitesi, Eczacılık Fakültesi, Farmakognozi Ana Bilim Dalı, 06510, Ankara, Türkiye
[***] ORCID:0000-0003-3916-4048, Gazi Üniversitesi, Eczacılık Fakültesi, Farmakognozi Ana Bilim Dalı, 06510, Ankara, Türkiye

[°] Corresponding Author; Didem Deliorman Orhan
Tel. +90 3122023173, Fax: +90 3122023173, e.mail: didem@gazi.edu.tr, didemdeliorman@gmail.com

INTRODUCTION

The genus *Salvia* belongs to the Lamiaceae family, which consists of about 900 species (Lu, 2002). Salvia means "to be healed or to be safe and notharmed" in Latin, referring to the medicinal properties of some species (Kamatou, 2008). *Salvia* genus is widely distributed in three regions of Central-South America, Central Asia-Mediterranean and East Asia (Xu, 2018). Major phytochemical components in *Salvia* species include phenolic acids, diterpenoids, triterpenoids, flavonoids and saccharides. While flavonoids, triterpenoids and monoterpenes are primarily found in the aerial parts of the plant, especially in flowers and leaves; phenolic acids and diterpenoids are mainly found in roots (Xu, 2018). It is reported in the literature that *Salvia* species is used the treatment diabetes mellitus in folk medicine (Eidi, 2009). While the flower, leaf and root extracts of these species are used in wounds, pharyngitis, mouth ulcers and menstrual irregularities in Anatolia, teas prepared from the leaves are used for indigestion, insomnia and pain relief purposes (Sharma, 2019). Some species of *Salvia* genus are used as spices in various countries, while others are utilized in cosmetic formulations, aromatherapy and insecticides (Lu, 2002; Kamatou, 2008).

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia caused by defects in insulin secretion, insulin action, or both (American Diabetes Association, 2005). Globally, the prevalence of type 2 diabetes is increasing rapidly. The global prevalence of diabetes among adults was estimated at 150 million in 1995, projected to increase to 300 million by 2025 (Abubakari, 2008). DM and obesity are strongly and complexly related to each other. Obesity is defined as a risk factor for Type 2 Diabetes (Hussain, 2010). The organism is exposed to oxidative stress resulting in the attack of free radicals (reactive oxygen species (ROS)/ reactive nitrogen species (RNS) formed by the transfer of free unpaired electrons. combat the harmful effects of ROS, the body activates its endogenous antioxidant systems. As another option, the body takes advantage of dietary antioxidants that destroy or detoxify ROS and maintain homeostasis. Oxidative stress, which occurs as a result of increased blood glucose levels, is held responsible for the development of diabetes and its complications (Asmat, 2016). Therefore, in the fight against diabetes, which is a metabolic disorder, there is a need for new drugs of natural or synthetic origin that can both lower blood sugar, reduce oxidative stress caused by diabetes, and play a role in weight control.

The collection of medicinal and aromatic plants from wild sources has been a practice of humanity since ancient times and causes the decline of plant species. 13% of the plants unconsciously collected in the world are under threat, and 22-47% of this percentage has faced extinction (Pitman, 2002). The uncontrolled collection of medicinal and aromatic plants from nature reveals that natural resources will decrease in the future to treat diseases, considering that these plants are used for health purposes. The most significant disadvantage of medicinal plant production using organic farming techniques is the high cost. On the other hand, the benefits of organic agriculture cannot be ignored when the factors such as the healthier and higher quality of the plants grown in this way and the protection of biodiversity are taken into account. For these reasons, the cultivation of medicinal and aromatic plants using organic farming techniques should be encouraged and the phytochemical contents and biological activities of these plants should be investigated. to increase the sustainability of medicinal and aromatic plants, protecting natural resources is one of the most critical applications. For this purpose, it is necessary to cultivate the species or to produce them with organic farming techniques. In this way, it will be possible to protect biodiversity and make healthier and higher-quality products with higher yields.

In this study, which is considered to be a first, the effects of infusions prepared from organic farming-grown *Salvia officinalis* L. and *S. triloba* L. sam-

ples on some enzymes (α-amylase, α-glucosidase, pancreatic lipase, and pancreatic cholesterol esterase enzymes) that play a role in metabolic diseases and their antioxidant potential (2,2-diphenyl-1-picrylhydrazil (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6 sulfonate) cation ($ABTS^{\bullet+}$) radical scavenging activity, metal chelating, and ferric-reducing power) were evaluated. The total phenolic and total flavonoid contents of the extracts were determined by spectrophotometric methods. Additionally, qualitative and quantitative analysis of the phenolic compounds of the extracts were carried out with by Reverse Phase-HPLC (RP-HPLC) method.

MATERIAL AND METHODS

Plant material

S. officinalis and *S. triloba* has grown with organic farming were provided from Beyşehir Road, 3. km Akyokuş Mevki, Konya, in 2021 (Certificate no: TR-OT-014-İ-197/01, Temmuz Organik Çiftliği). These species were produced by the Organic Agriculture Law and Regulation of the Turkish Republic and have been certified by Nissert and authorized by the Ministry of Agriculture.

Extraction

200 mL of hot water was added to 10 g of powdered aerial parts for extraction. This extract was then filtered and this process was repeated three times. After the resulting filtrates were combined, they were freeze-dried. The yields (w/w %) of the extracts are given in Table 1.

Total phenol content

The extracts were incubated for 5 minutes at room temperature with 10% Folin-Ciocaltaeu reagent. Then sodium carbonate solution was added and the mixture was vortexed. After 30 minutes of incubation in the dark, the absorbance of the extracts was measured at 735 nm with a spectrophotometer (VersaMax ELISA Microplate Reader). Total phenol content was calculated as gallic acid equivalent (GAE) mg/g extract. The calibration equation was $y = 3.7855x + 0.1735$ and $r^2 = 0.9931$ (Zongo, 2010).

Total flavonoid content

Ethanol, sodium acetate and aluminum chloride solutions were added to the extracts, respectively, and the mixture was diluted to 1 mL with distilled water. After 30 minutes incubation at room temperature, the absorbance of the mixture was measured at 415 nm with the ELISA microtiter plate reader. The results were calculated as quercetin equivalent (QE) mg/g extract. Calibration curve equation; $y = 2.8193x - 0.0996$ and $r^2 = 0.9977$ (Kosalec, 2004).

Antioxidant activity

ABTS radical scavenging activity

ABTS (7 mM) was dissolved in distilled water and 2.45 mM potassium persulfate solution. The mixture was incubated for approximately 16 hours at 20°C in the dark. Phosphate buffer with pH 7.4 and ABTS solution was added to the extracts. After the samples were vortexed, their absorbance was read at 734 nm with an ELISA microtiter plate reader. Gallic acid was used as a reference compound (Orhan, 2017).

DPPH radical scavenging effect

After adding 1mM DPPH (2,2-diphenyl-1-picrylhydrazil) solution to 80 µL of extract, it was kept in the dark at room temperature for 30 minutes. The absorbance of the mixture at 520 nm was then measured. Ascorbic acid was used as reference material (Jung, 2011).

Metal chelating capacity

After adding 2 mM $FeCl_2$ solution to the extracts, the mixture was incubated for 5 minutes at room temperature. Then, 5 mM ferrozine solution was added to this mixture and kept at room temperature for 10 minutes, and absorbance was measured at 562 nm with a spectrophotometer. EDTA (Ethylene diaminetetraacetic acid) was used as reference material. Metal chelating capacity was calculated with the formula (%) = $[(A_{Control} - A_{Sample})/A_{Control}] \times 100$ (Dinis, 1994).

Ferric-reducing antioxidant power

After adding 0.1 mol/L sodium phosphate buffer (pH=7.2) to the extracts in various concentrations, 1% potassium ferricyanide solution was added to this

mixture and incubated in an oven at 37°C for 60 minutes. After the incubation period, 10% trichloroacetic acid solution was added and absorbance was measured at 700 nm. At the end of this process, 0.1% $FeCl_3$ solution was added, and the measurement was made again and the difference was taken. The percent ferric reducing power absorbance was calculated according to the following equation: ferric reducing power absorbance = $(B_2-B_1)-(A_2-A_1)$;what is stated here can be expressed : B_1: the first measurement value of the absorbance of the sample, B_2: the second measurement value of the absorbance of the example, A_1: first measurement value of the absorbance of the blank, A_2: the second measurement value of the absorbance of the blank. Quercetin compound was used as a reference substance (Orhan, 2017).

Enzyme assays

***α*-Glucosidase inhibitory activity**

The *α*-Glucosidase type IV enzyme (EC 3.2.1.20, Sigma) was dissolved in 0.5 M phosphate buffer (pH 6.5). Extracts were prepared at concentrations of 2, 1, and 0.5 mg/mL and tested in 3 replicates in 96-well microplates. p-nitrophenyl-*α*-D-glucopyranoside was used as the substrate. After adding the substrate, the plates were incubated at 37°C for 35 minutes. The absorbance of the mixture was then measured at 405 nm. Acarbose was used as a reference substance. The percent inhibition was calculated according to the following equation: Inhibition (%) = (1−(Y−y/X−x)) × 100; what is stated here can be expressed as: x is the negative control without inhibitor, X is the activity without inhibitor, y is the negative control with inhibitor and Y is the activity with inhibitor (Orhan, 2017).

***α*-Amylase enzyme inhibitory activity**

It was dissolved in α-Amylase type VI (EC 3.2.1.1. Sigma) buffer. Potato starch was used, and this substrate was prepared in a phosphate buffer with a pH of 6.9. After the samples were incubated with the enzyme solution at 37°C for 15 minutes, substrate solution was added. Then, incubation was continued with DNS (96 mM 3.5-dinitrosalicylic acid. 5.31 M sodium potassium tartrate) at 80°C for another 40 minutes. Then, distilled cold water was added and absorbance was measured at 540 nm. The amount of maltose produced was calculated using the standard maltose calibration chart (y = 0.6762x - 0.0404) and the net absorbance was obtained. Acarbose was used as a reference substance. The change in absorbance due to maltose formation was read at 540 nm and the calculations were made as follows: A_{Sample} or $_{Control} = A_{Sample} - A_{Blank.}$ Based on the amount of maltose formed, a calibration curve of maltose was formed and percent inhibition was determined. Inhibition (%) = (1−(average of maltose formed in test samples/mean of maltose formed in control)) × 100 (Orhan, 2017).

Pancreatic lipase inhibitory activity

Type II enzyme (EC 3.1.1.3, Sigma) obtained from pig pancreas was added to the extracts, and the pH 6.8 MOPS (morpholinepropanesulfonic acid) buffer and the pH 7.4 Tris HCl buffer wasadded, and the mixture was incubated at 37°C for 15 minutes. 10 mM p-nitrophenylbutyrate was used as substrate. Then the substrate was added and incubated at 37°C for 30 minutes. The absorbance of the samples at 405 nm was measured. Orlistat was used as a reference compound. The percent inhibition was calculated according to the following equation: Inhibition (%) = (1−(Y−y/X−x)) × 100; what is stated here can be expressed as: x is the negative control without inhibitor, X is the activity without inhibitor, y is the negative control with inhibitor and Y is the activity with inhibitor (Lee, 2010).

Cholesterol esterase enzyme inhibitory activity

Cholesterol esterase enzyme (EC3.1.1.13, Sigma) obtained from pork pancreas was dissolved in 100 mM phosphate buffer (pH=7) containing 100 mM NaCl. Phosphate buffer, 12 mM taurocholic acid and substrate solutions were added to the samples, respectively. As a substrate, p-nitrophenylbutyrate was used. After incubation at 25°C for 5 minutes, the enzyme was added and kinetic measurements were made at 405 nm for 15 minutes. Simvastatin was used as a reference compound. The percent inhibition was calculated as follows: Inhibition (%) = $[(A_{Control} - A_{Sample})/A_{Control}] \times 100$ (Ngamukote, 2011).

RP-HPLC analysis

HP Agilent 1260 series LC System and TC-(4.6 mm x 150 mm x 5 µm) column were used in the RP-HPLC system for analysis. The column temperature was kept constant at 25°C throughout the analysis. The following standard compound mixtures were used for the qualitative and quantitative analyzes of the phenolic compounds and flavonoids in the extract. Phenolic compound mixture: Gallic acid, protocatechuic acid, chlorogenic acid, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid, *trans*-cinnamic acid, rosmarinic acid, epicatechin, catechin. Flavonoid mixture: Umbelliferone, rutin, naringenin, hesperidin, quercetin-3-*O*-glucoside, apigenin-7-*O*-glucoside, myrcetin, quercetin, luteolin, apigenin. The gradient flow system was started with the mobile phase containing 5% solvent A (acetonitrile: water: formic acid, 50:50:0.5) and 95% solvent B (water: formic acid, 100:0.5). Total analysis time was 58 minutes and the injection volume was 20 µL. This process was carried out at 4 different wavelengths, 260, 280, 320 and 350 nm, using a DAD detector. The extracts were prepared in 25% acetonitrile solution at a concentration of 1 mg/mL. Sample solutions were prepared by filtration through a 0.45 µm membrane filter. A Calibration chart was prepared for hesperidin (Rt= 35.89) and rosmarinic acid (Rt= 37.149 min). from the stock solution of these two standard substances, dilution solutions were prepared at five different concentrations. The concentrations mentioned are 0.5, 10, 20, 50 and 100 ppm. Since the peak area values of these compounds in the extract are in the relevant concentration range, quantification was made by creating a calibration curve based on the ppm values (x) and peak areas (y) (Gök, 2020).

Statistical Analysis

All experiments were done in triplicate. Means of numerical values were calculated and presented in the tables as mean ± standard deviation (SD). Microsoft Excel and GraphPad Instat software programs were used in the calculations and the difference in $p<0.05$ values were evaluated as statistically significant in this study (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

RESULTS AND DISCUSSION

The yields of *S. officinalis* and *S. triloba* extracts prepared by the infusion technique were 27.13% and 16.82% (w/w), respectively. While the total phenol content in *S. officinalis* and *S. triloba* extracts was calculated as 193.50 ± 8.22 and 203.01 ± 7.85 GAE mg/g extract, respectively, the total flavonoid amounts of the extracts were determined as 71.51 ± 1.88 and 78.84 ± 8.76 QE mg/g extract, respectively (Table 1).

Table 1. Yield (w/w), total phenol and total flavonoid contents of *S. officinalis* and *S. triloba* extracts

Extracts	Yield (w/w%)	Total Phenolic Content[a] (Mean ± SD)	Total Flavonoid Content[b] (Mean ± SD)
S. officinalis	27.13	193.50 ± 8.22	71.51 ± 1.88
S. triloba	16.82	203.01 ± 7.85	78.84 ± 8.76

[a]mg GAE/g extract, [b]mg QE/g extract, SD: Standard Deviation

In experiments to evaluate the antioxidant potential of extracts; while the ABTS radical scavenging effect of the extracts of both species increased depending on the dose, on the contrary, the DPPH radical scavenging effect decreased. In the metal chelating activity assay, extracts of both species (100%) displayed higher activity at 2 mg/mL concentrations than EDTA (95.72 ± 0.63%) used as the reference compound. At the ferric reducing power, both species showed the highest absorbance at a concentration of 2 mg/mL, especially the *S. officinalis* extract (3.392 ± 0.01) gave absorbance values almost close to the quercetin (3.538 ±0.02) used as the reference compound (Table 2).

Table 2. ABTS, DPPH radical scavenging activity, metal chelating activity and the ferric-reducing power results of *S. officinalis* and *S. triloba* extracts

Extract	Concentration (mg/mL)	Antioxidant Activity			
		ABTS radical scavenging activity Inhibition% ± SD	DPPH radical scavenging activity Inhibition% ± SD	Metal Chelating Capacity % ± SD	Ferric Reducing Power Absorbance ± SD
S. officinalis	0.5	25.79 ± 0.90**	77.68 ± 0.94***	56.70 ± 3.76***	1.475 ± 0.09***
	1	46.91 ± 0.47***	74.56 ± 0.41***	86.70 ± 1.38***	2.531 ± 0.01***
	2	73.30 ± 0.60***	56.98 ± 6.25***	100***	3.392 ± 0.01***
S. triloba	0.5	31.22 ± 1.41**	79.04 ± 0.58***	-	1.293 ± 0.07**
	1	53.77 ± 0.52***	69.36 ± 2.47***	84.37 ± 5.13***	1.794 ± 0.38***
	2	87.41 ± 0.86***	60.05 ± 2.83***	100***	2.889 ± 0.61***
References	GA/AA/EDTA/QE 0.5	99.54 ± 1.04^{***a}	89.42 ± 3.07^{***b}	99.83 ± 0.39^{***c}	3.253 ± 0.38^{***d}
	GA/AA/EDTA/QE 1	98.94 ± 0.26^{***a}	90.71 ± 0.65^{***b}	99.78 ± 0.26^{***c}	3.560 ± 0.02^{***d}
	GA/AA/EDTA/QE 2	98.10 ± 0.80^{***a}	90.38 ± 0.86^{***b}	95.72 ± 0.63^{***c}	3.538 ±0.02^{***d}

-: No activity, SD: Standard Deviation, ns: Not statistically significant, *p<0.05 **p<0.01 ***p<0.001 GA: [a]Gallic acid, AA: [b]Ascorbic acid, EDTA: [c]Ethylenediamine tetraacetic acid, [d]QE: Quercetin

S. officinalis extract did not inhibit α-amylase and pancreatic lipase enzymes at the concentrations tested. *S. triloba* extract inhibited α-amylase enzyme at rates ranging from 11.33% to 19.48% at all doses tested, in contrast the same extract inhibited pancreatic lipase enzyme at 1 and 2 mg/ml concentrations, 8.84% and 22.79%, respectively. *S. officinalis* extract (64.69 %) inhibited the α-glucosidase enzyme much more potently than *S. triloba* extract (47.78 %) when compared with the reference compound acarbose (99.35 %). The extracts of both species (36.99 ± 2.00% and 38.03 ± 2.59%) at a concentration of 2 mg/mL showed a moderate inhibitory effect against the pancreatic cholesterol esterase enzyme when compared with simvastatin (53.18 ± 3.36%) (Table 3).

Table 3. Inhibitory effects of *S. officinalis* and *S. triloba* extracts on α-glucosidase, α-amylase, pancreatic lipase and pancreatic cholesterol esterase enzyme

Extract	Concentration (mg/mL)	Inhibition %± SD			
		α-Glucosidase	α-Amylase	Pancreatic Lipase	Pancreatic Cholesterol Esterase
S. officinalis	0.5	7.41 ± 0.35^{ns}	-	-	9.85 ± 5.97*
	1	24.28 ± 2.60**	-	-	23.95 ± 2.91**
	2	64.69 ± 0.23***	-	-	36.99 ± 2.00**
S. triloba	0.5	3.01 ± 0.29^{ns}	16.67 ± 1.99*	-	24.60 ± 2.26**
	1	23.73 ± 0.68*	11.33 ± 2.70*	8.84 ± 3.30^{ns}	31.21 ± 0.89**
	2	47.78 ± 2.11***	19.48 ± 2.26**	22.79 ± 4.31**	38.03 ± 2.59**
References	ACA/OR/SIM 0.5	98.88 ± 0.21^{***a}	94.85 ± 0.60^{***a}	52.16 ± 0.00^{***c}	47.88 ± 5.11^{***d}
	ACA/OR/SIM 1	99.47 ± 0.13^{***a}	98.38 ± 0.50^{***a}	69.54 ± 4.19^{***c}	52.21 ± 0.12^{***d}
	ACA/OR/SIM 2	99.35 ± 0.2^{2***}a	95.24 ± 2.60^{***a}	62.55 ± 1.76^{***c}	53.18 ± 3.3^{6***}d

-: No activity, SD: Standard Deviation, ns: Not statistically significant, *p<0.05 **p<0.01 ***p<0.001 ACA: [a] Acarbose, OR: [c]Orlistat, SIM: [d]Simvastatin

RP-HPLC technique was used for the qualitative and quantitative determination of 22 phenolic compounds (gallic acid, protocatechuic acid, chlorogenic acid, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid, *trans*-cinnamic acid, rosmarinic acid, epicatechin, catechin, umbelliferone, rutin, naringenin, hesperidin, quercetin-3-*O*-glucoside, apigenin-7-*O*-glucoside, myricetin, quercetin, luteolin, apigenin) in the extracts (Figure 1-3). The amount of rosmarinic acid determined as the main compound in the extracts was found to be approximately two times higher in *S. officinalis* (16.233 ± 0.034 g/100 g extract) than in *S. triloba* (6.975 ± 0.006 g/100 g extract) (Figure 4 and 5). According to the results of the quantitative analysis, the amount of hesperidin in the extracts was determined. The amount of hesperidin was calculated as 0.219 ± 0.002 g/100 g extract in *S. officinalis* and 0.187 ± 0.001 g/100 g extract in *S. triloba* (Figure 6-7).

Table 4. Results of rosmarinic acid and hesperidin (g/100g dry extract) determination of *S. officinalis* and *S. triloba* extracts by Reverse Phase-HPLC

Extract	Compounds	Rt (Minute)	g/100 g dry extract	Calibration Equations	Coefficient of Determination
S. officinalis	Rosmarinic acid	37.149	16.233 ± 0.034	y = 39.256x + 0.2194	r^2 = 1.000
	Hesperidin	35.890	0.219 ± 0.002	y = 46.366x – 5.3259	r^2 = 1.000
S. triloba	Rosmarinic acid	37.149	6.975 ± 0.006	y = 39.256x + 0.2194	r^2 = 1.000
	Hesperidin	35.890	0.187 ± 0.001	y = 46.366x – 5.3259	r^2 = 1.000

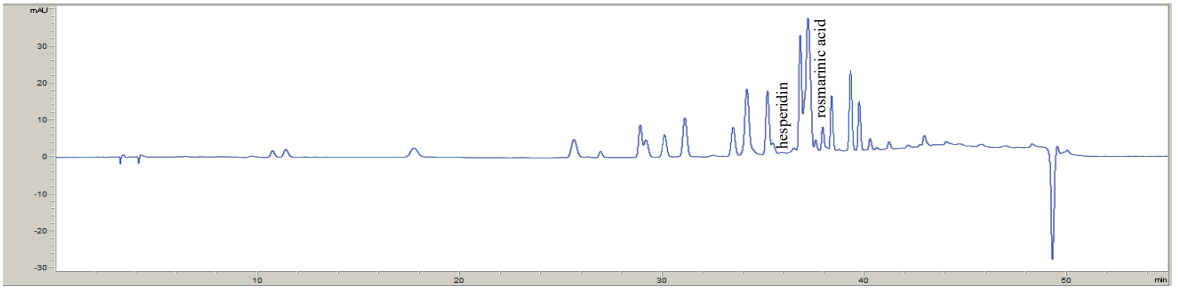

Figure 1. RP-HPLC chromatogram of the mixture standard containing phenolic and flavonoid substances at 280 nm

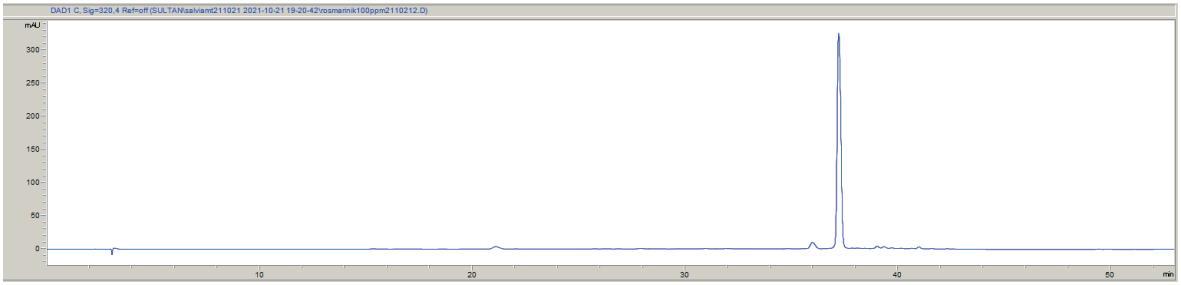

Figure 2. RP-HPLC chromatogram of the standard compound, rosmarinic acid

Figure 3. RP-HPLC chromatogram of the standard compound, hesperidin

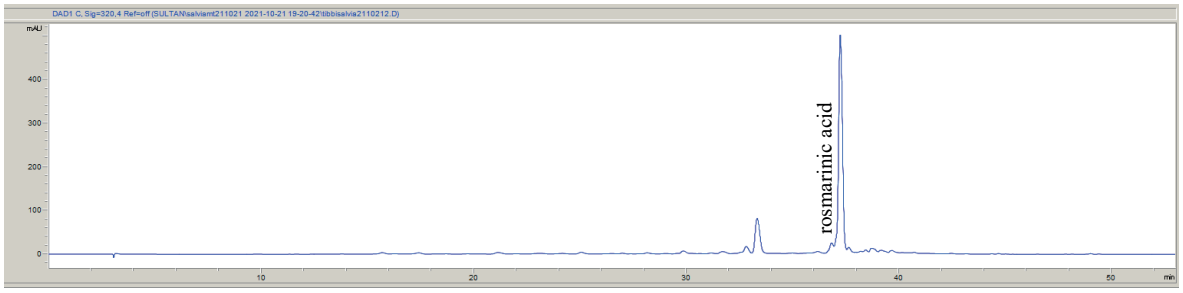

Figure 4. RP-HPLC chromatogram of *S. officinalis* at 320 nm

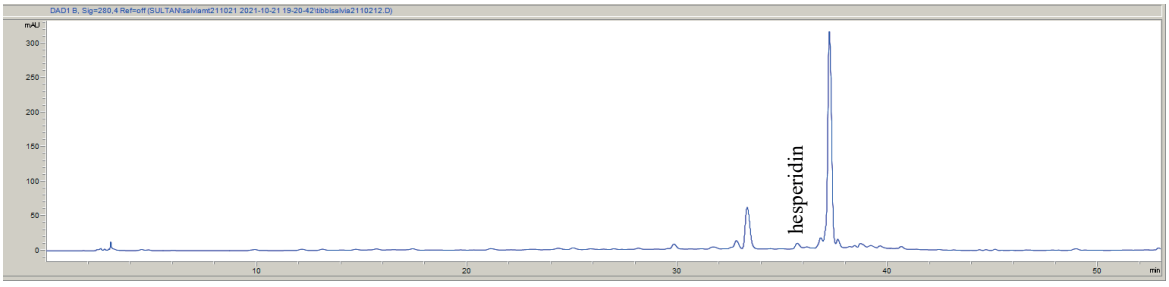

Figure 5. RP-HPLC chromatogram of *S. officinalis* at 280 nm

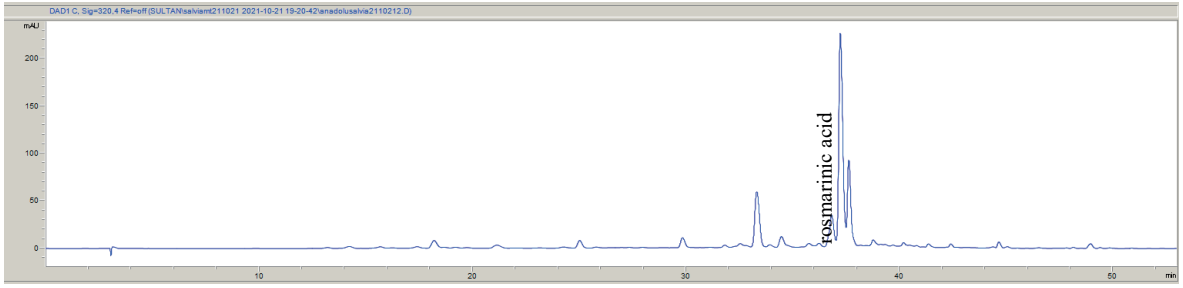

Figure 6. RP-HPLC chromatogram of *S. triloba* at 320 nm

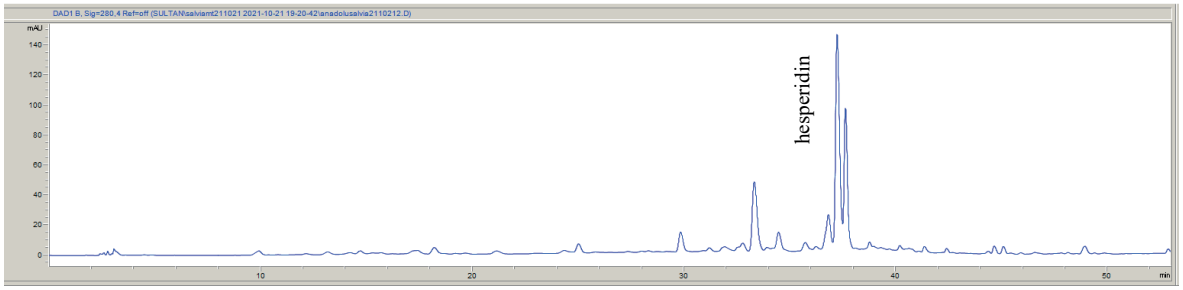

Figure 7. RP-HPLC chromatogram of *S. triloba* at 280 nm

When the activity (antioxidant and enzyme inhibitor activities) and phytochemical analysis studies performed on *S. triloba* and *S. officinalis* to date were examined, it was observed that the samples were generally wild and sometimes cultivated forms. For the first time in this study, activity studies and phytochemical analyzes were carried out on these species grown with the organic farming techniques.

Miliauskas et al. (2004) reported that the total flavonoid amount of *S. officinalis* was 3.5 mg QE/g dried material and the total phenol amount was 22.6 mg GAE/ g dried material (Miliauskas, 2004). Arıduru and Arabacı (2013) found the total phenol content between 11.58-43.55 mg GAE/g dried material of the extracts prepared with solvents of different polarities from *S. officinalis* samples collected from Sakarya (Arıduru, 2013). Hamrouni-Sellam et al. (2013) reported that the total phenol content of the aerial parts of *S. officinalis* samples collected from Tunisia ranged from 0.399 to 2.337 mg GAE/g dried material. Considering the previous studies, it was observed that the total phenol and total flavonoid content of *S. officinalis* samples grown with the organic farming techniques were generally high.

Dinçer et al. (2012) analyzed the total phenol and total flavonoid contents of wild and cultivated *S. triloba* samples in Antalya. The results showed that the total phenol content of the wild sample was 41.58±1.34 mg GAE/g dried material, the total flavonoid content was 35.58 ±0.85 mg QE/g dried material, the total phenol content of the cultivated sample was 44.60±1.21 mg GAE/g dried material and the total flavonoid content was 28.82± 2.53 mg QE/g dried material (Dincer, 2012). Boukhary et al. (2016) determined that the total phenol content of the methanolic extract of *S. triloba* samples collected from Lebanon was 122.67 ± 0.44 mg GAE/g extract (Boukhary, 2016). In a previous study, it was determined by spectrophotometric methods that the total phenol content of 20 *S. triloba* samples collected from the Marmara region varied between 8.47-13.45 mg GAE/g dried material and their total flavonoid contents varied between 5.52-7.63 mg QE/g dried material (Karık, 2018). In our study, it was observed that the total phenol content of *S. triloba* grown using organic farming techniques was higher than that of wild and cultivated samples. The total phenol content of the cultivated sample in the study of Dinçer et al. (2012) was higher than that of the sample grown with the organic farming technique in this study.

Yıldırım et al. (2000) reported that the extracts prepared using hot water from *S. triloba* samples purchased from the market had reducing solid activity. On the other hand, the DPPH radical scavenging effect of the extracts (extract type not specified) prepared from 20 *S. triloba* samples collected from the Marmara region was investigated using the HPLC technique. It was found that the total antioxidant activities of all samples varied between 820.00-876.79 µmol Trolox equivalent/100 g dried material (Karık, 2018). Arıduru and Arabacı (2013) investigated the DPPH radical scavenging effects of extracts prepared with solvents of different polarities (methanol, ethanol, ethyl acetate, and acetone) from *S. officina-*

lis samples collected from Sakarya. The most potent radical scavenging activity was observed in methanol (90.89%) and ethyl acetate (90.48%) extracts, followed by ethanol (86.31%) and acetone (84.78%) extracts. In this study, BHT and Trolox inhibited the DPPH radical by 91.64% and 90.32%, respectively. In a study examining the DPPH radical scavenging activity of extracts of *S. triloba* samples collected from Beirut (Lebanon) during the flowering period, prepared with solvents with different polarities, it was reported that the inhibition % values of the extracts varied between 25.43 and 42.15 (Boukhary, 2016). The EC_{50} values for DPPH radical scavenging activity and reducing power of the decoction prepared from the aerial parts of *S. officinalis* grown by organic farming techniques in Portugal were determined as 3.48±3.30 µg/mL and 40.0±11.2 µg/mL, respectively. Ascorbic acid was used for DPPH radical scavenging activity, BHT was used as a reference compound for reducing power, and their EC_{50} values were determined as 6.69 ±0.70 µg/mL and 16.30±1.50 µg/mL, respectively (Pereria, 2018). Jamous et al. (2018) evaluated the DPPH radical scavenging effect and reducing power of the ethanolic extract of *S. triloba* leaves grown in Palestine. While the IC_{50} value for the DPPH radical scavenging activity of the extract was 0.13±0.00 mg/mL, the EC_{50} value for its reducing power was calculated as 0.32±0.10 mg/mL. In all antioxidant methods tested in our study, both extracts were found to have a moderate to strong effect.

The decoction prepared from the aerial parts of *S. officinalis* grown by organic farming techniques in Portugal showed no inhibitory effect on the α-amylase enzyme but inhibited the α-glucosidase and pancreatic lipase enzymes with an EC_{50} value of 71.2±5.0 µg/mL and an inhibition rate of 4.6±3.6%, respectively. The reference compound acarbose inhibited the α-glucosidase enzyme with an EC_{50} value of 357.8 ±12.3 µg/mL and an inhibition rate of the pancreatic enzyme of 1.8±0.4 % (Pereira, 2018). In our study, the *S. officinalis* sample did not show any activity against pancreatic lipase and α-amylase enzymes. Jamous et al. (2018) reported that the ethanolic extract of the leaves of *S. triloba* grown in Palestine inhibited the pancreatic lipase enzyme by 65.8±2.1% at a concentration of 5 mg/mL (Jamous, 2018). In our study, 5 mg/ml concentration, which is known to be very high, was not used toevaluation of the *in vitro* enzyme inhibitory activities of the extracts. The phosphate-buffered extracts of dried *S. triloba* leaves purchased commercially in İzmir inhibited α-glucosidase, α-amylase and pancreatic lipase enzymes with IC_{50} values of 88.49 ±2.35, 107.65±12.64 and 6.20±0.63, respectively. No reference compound was used in this study to compare results (Ercan, 2018). It was found that the leaves of *S. triloba* and *S. officinalis* collected during the flowering period from different localities of Croatia between May and August 2018 inhibited the α-glucosidase enzyme with IC_{50} values of 5291.51 ± 335.08 µg/mL and 4451.85± 142.22 µg/mL, respectively. The IC_{50} value of acarbose used as the reference compound was calculated as 1104.76 ± 34.80 µg/mL. In fact, when the findings are evaluated, it would not be correct to talk about a strong antioxidant activity for the extracts (Mervić, 2022).

In our study, enzyme inhibitory effects are given as percent inhibition. In the literature presented above, results are expressed regarding IC_{50} and EC_{50} values. Therefore, isn't easy to compare them with our findings. No pancreatic cholesterol esterase enzyme inhibitory activity studies have been performed on either species . It was observed that *S. officinalis* was effective on both glucosidase and pancreatic cholesterol esterase enzyme, while *S. triloba* was effective on pancreatic cholesterol esterase enzyme. For this reason, these species may be the source of further studies for discovering new molecules to inhibit these enzymes. However, since oxidative stress, which occurs as a result of the increased blood sugar levels, is responsible for the development of diabetes and its complications; a relationship can be established between the antioxidant capacity of the extracts and the enzyme activities. In addition to these, it has been stated in the literature

that hesperidin and rosmarinic acid molecules, which were quantitatively analyzed in the extracts, have antidiabetic and obesity potentials, and this information makes us think that the efficacy may be due to these molecules (Ahmet, 2012; Ngo, 2018; Xiong, 2019; Vasileva, 2021)

Pizzale et al. (2002) determined that the methanolic extracts of *S. triloba* and *S. officinalis* samples collected from northern Italy contain rosmarinic acid at varying rates from 21.9 to 81.8 g/kg extract as the major compound. It has been reported that carnosol, carnosic acid and methyl carnosate are also present in the samples besides rosmarinic acid. In a previous study, different amounts of vanillic acid, gallic acid, (+)-catechin, (−)-epicatechin, hesperetin, chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, apigenin, rutin, morin, luteolin, myricetin, quercetin, and kaempferol were determined in aqueous-methanol extracts of wild and cultivated *S. triloba* samples by HPLC analysis depending on the parameters of storage time, growing conditions and harvest year (Dinçer, 2012). Boukhary et al. (2016) reported that the main components of the methanol extract of *S. triloba* collected from Lebanon were luteolin and rutin by HPLC technique. The amount of carnosic acid was determined as 3.76 ± 0.13 mg/g by HPLC technique in the phosphate-buffered extracts of dried *S. triloba* leaves commercially purchased in İzmir (Ercan, 2018). Başyiğit and Baydar (2017) conducted the qualitative and quantitative analyzes of chlorogenic acid, gallic acid, caffeic acid, ferulic acid, rosmarinic acid, *p*-coumaric acid, quercetin, rutin, naringin, and hesperidin in the methanolic extract of cultivated *S. officinalis* samples provided from Isparta using HPLC technique. Mervic et al. (2022) determined that 70% ethanolic extracts of *S. triloba* and *S. officinalis* samples collected from Serbia contained caffeic acid, chlorogenic acid, rosmarinic acid, *p*-coumaric acid, apigenin, luteolin and luteolin-7-*O*-glucoside by HPLC analysis. The analysis results indicated that rosmarinic acid was the main compound for both extracts. In addition to the compounds mentioned above, it has been reported that *S. triloba* extract contains ferulic acid, and quercetin, while *S. officinalis* extract contains rutin. HPLC analysis findings of our study showed that the amounts of rosmarinic acid were high for both species. In addition, the presence of hesperidin in *S. triloba* was detected for the first time in this study.

CONCLUSION

Phytochemical analyzes and activity studies of *S. triloba* and *S. officinalis* species grown with organic farming techniques were carried out for the first time in this study. As a result of the literature review, it has been seen that the phytochemical analysis and activity studies on *Salvia* species grown with organic agriculture are minimal. It has been determined that these two species, which were grown with organic farming techniques and are widely used as a tea in Anatolia, have high antioxidant activity and especially potential in terms of α-glucosidase inhibitor activity. In this context, the findings obtained from this study showed that these medicinal plants could also be evaluated as pharmaceutical raw materials.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept: BÖ, SP, DDO; Design: BÖ, SP, DDO; Control: BÖ, SP, DDO; Sources: BÖ, SP, DDO; Materials: BÖ, SP, DDO; Data Collection and/or processing: BÖ, SP, DDO; Analysis and/or interpretation: BÖ, SP, DDO; Literature review: BÖ, SP, DDO; Manuscript writing: BÖ, SP, DDO; Critical review: BÖ, SP, DDO; Other: BÖ, SP, DDO.

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