Botanica SERBICA

(2021) 45 (2): 223-231

DOI: https://doi.org/10.2298/BOTSERB2102223G
journal homepage: botanicaserbica.bio.bg.ac.rs

Original Scientific Paper

Phytochemical analysis, antioxidant and antimicrobial activities of *Salvia virgata* mericarps

Sevda Güzel Kara[1*], Mahmut Ülger[2] and Ahmet Kahraman[3]

1 Mersin University, Faculty of Pharmacy, Department of Pharmacognosy, 33169, Mersin, Turkey
2 Mersin University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 33169, Mersin, Turkey
3 Uşak University, Faculty of Arts and Science, Department of Biology, 64200, Usak, Turkey
* Correspondence: guzelsevda@mersin.edu.tr

ABSTRACT:
The phytochemical contents of *Salvia virgata* mericarps and the antioxidant and antimicrobial activities of its ethanol extract were studied for the first time. *S. virgata* mericarps were collected in the province of Trabzon, Turkey. Chemical analyses were performed using chromatographic methods. The total phenolic and flavonoid contents and antioxidant potential were measured using Folin-Ciocalteu, $Al(NO_3)_3$, and DPPH radical scavenging activity methods, respectively. The antimicrobial activity was evaluated using the microdilution method for all the tested bacterial and fungal strains, with the exception of *Mycobacterium tuberculosis* for which the resazurin microtiter plate method was applied. The mericarps were rich in glutamic acid (3934 mg/100 g), potassium (12578.8 µg/g), calcium (12092.0 µg/g), and dietary fibers (35.565 g/100 g). The total phenolic and flavonoid contents were 2.50 µg GAE/mg extract and 0.34 µg QE/mg extract, respectively. The most effective DPPH free radical scavenging activity determined for the highest applied concentration was 92.44%. The ethanol extract obtained from the mericarps was found to be as effective as the reference drug ampicillin (MIC value = 125 µg/mL) against the nosocomial pathogen *Acinetobacter baumannii.* In conclusion, *S. virgata* mericarps provide good nutritional value with low amounts of carbohydrates and high dietary fibers, amino acids, minerals and total phenolic and flavonoid contents and medicinal properties.

Keywords:
Salvia virgata, mericarps, proximate analysis, amino acid, mineral, antioxidant and antimicrobial activities

UDC: 581.192:582.929.4

Received: 04 April 2021
Revision accepted: 24 June 2021

INTRODUCTION

The intake of dietary phytochemicals plays an important role in the prevention of various illnesses including cancer, and inflammatory and cardiovascular diseases. Due to their medicinal properties, plants and their isolated metabolites are also used in different fields of the pharmaceutical industry (Jaradat *et al.* 2016). *Salvia* is one of the largest genera in the family of Lamiaceae with approximately 1000 species, which are distributed extensively in the tropical and temperate areas of the New and Old World (Büyükkartal *et al.* 2011). One of the main diversity centers of the genus is South-West Asia where Turkey counts the largest number of species of 100, including 53 endemics (Hatipoglu *et al.* 2016).

Certain species of the genus have been widely used in folk medicine for the treatment of cancer, microbial infections, memory loss, the common cold, tuberculosis, wounds, malaria, inflammation, hemorrhaging and hepatitis since ancient times (Güzel *et al.* 2018), while some species have been used for their nutritional properties (Falco *et al.* 2017). Flavonoids, phenolic acids, terpenoids, essential oils, fatty acids, tocopherols, phytosterols, and minerals have been reported as the secondary metabolites of the *Salvia* species (Azcan *et al.* 2004; Bagci *et al.* 2004; Gören *et al.* 2006; Kamatou *et al.* 2008; Kirmizibekmez

et al. 2012; Temel *et al.* 2016; Güzel 2020; Güzel *et al.* 2020a). Antibacterial, antifungal, wound-healing, antioxidant, antiproliferative and cytotoxic activities of *Salvia* species were previously shown (Ulubelen *et al.* 2001; Güzel *et al.* 2019a, b).

The fruit of the genus *Salvia*, as in other genera of Lamiaceae, is a schizocarp. The schizocarp fruit, which consists of indehiscent locules separating to form four fruitlets, is called as mericarp and/or nutlet. However, these fruitlets are commercially known as seeds since a seed is contained within each fruitlet. Each mericarp possesses a stratified pericarp including the cuticle, epicarp (exocarp), mesocarp, a layer of bone cells, and the endocarp. Moreover, each mericarp is in contact with the true seed (Segura-Campos *et al.* 2013).

Salvia virgata Jacq. is a perennial herbaceous plant which is distributed through the Balkans, Crimea, the Caucasus, Italy, Northern Iraq, Afghanistan, Iran, and Turkey (Coşge Şenkal *et al.* 2019). The plant is known as "yılancık" or "fatmanaotu" in Turkey and is used for the treatment of wounds and skin diseases (Küpeli-Akkol *et al*. 2008; Coşge Şenkal *et al.* 2019). The decoction of the aerial parts is traditionally used for leukemia in Turkey (Koşar *et al.* 2008) and the plant is consumed as a strong tea in Iran. Several biological activities of the plant, including antimicrobial, anti-inflammatory, antioxidant, antinociceptive, and the inhibition of alpha-amylase, GSH-Px, peroxidase, acetylcholinesterase, and polyphenol oxidase, were reported (Coşge Şenkal *et al.* 2019). According to the literature data, fatty acids (Azcan *et al.* 2004; Bagci *et al*. 2004; Gören *et al*. 2006), and the tocochromanol (Gören *et al*. 2006), phytosterol (Farida & Radjabian 2019), and vitamin (Sari *et al*. 2009) contents of *S. virgata* mericarps have been investigated. However, no study has been carried out on the other chemical constituents and biological activities of the mericarps. Therefore, the aim of this study was to evaluate the proximate composition; amino acid, mineral, total phenolic and flavonoid contents; and the *in vitro* antioxidant and antimicrobial activities of *S. virgata* mericarps from Turkey.

MATERIAL AND METHODS

Chemicals. Isoniazid, fluconazole, ethambutol, RPMI 1640 Medium, 3-(N-morpholino)-propanesulfonic acid, resazurin sodium salt powder, Folin-Ciocalteu reagent, gallic acid, and quercetin were purchased from Sigma-Aldrich (St. Louis, MO, USA); ethanol, dimethyl sulfoxide (DMSO), acetonitrile, 2,-2-diphenyl-1-picrylhydrazyl (DPPH), and butylated hydroxyanisol (BHA) were purchased from Merck (Darmstadt, Germany); and Middlebrook 7H9 broth, casitone, glycerol, oleic acid, albumin, dextrose, and catalase were purchased from Becton Dickinson (Sparks, MD, USA). All the solutions were prepared with distilled water and only freshly prepared solutions were used.

Plant material. *Salvia virgata* was collected in the Trabzon province of Turkey (Çaykara/Uzungöl, situated at an altitude of 1500 m, roadsides) in the year 2018. The plant was identified and confirmed by Dr. A. Kahraman (Department of Biology, Faculty of Arts and Science, Usak University, Usak, Turkey). The dried voucher specimens were deposited in the Plant Systematics and Phylogenetics Research Laboratory, Usak University (voucher reference: A. Kahraman 1895B).

Preparation of plant extract. Powdered *S. virgata* mericarps were extracted with ethanol [1:20 (w/v); ×2; 96%] by stirring overnight at room temperature then filtered using Whatman Grade No.1 filter paper. The solvent was evaporated via a vacuum evaporator (Heidolph Instruments GmbH & CO. KG, Schwabach, Germany) and the extract was kept in the dark at 4°C for further analyses on the total phenolic and flavonoid contents as well as biological activity tests. The yield of the ethanol extract was 234.65 mg/g dry mericarp.

All determinations were conducted in triplicate and the results were represented as the mean ± standard deviation (SD) except for the antimicrobial activity results where the tests were carried out in duplicate.

Phytochemical contents

Proximate analysis. Standard methods from the Association of Official Analytical Chemists (AOAC) were used to determine the moisture (method no. 930.04), protein (method no. 960.52), ash (method no. 900.02) and dietary fiber (method no. 991.43) contents of the *S. virgata* mericarps (AOAC 2000). The Soxhlet extraction procedure was used for the oil content. The carbohydrate content was calculated as the difference between 100 and the sum of the moisture, protein, oil and ash contents. The energy content was calculated from the energy-yielding nutrients (i.e. protein, oil and carbohydrate) (Merrill & Watt 1973). All the results were expressed as g/100 g, while the energy result was expressed as kcal/100 g.

Amino acid content. The acid hydrolysis of proteins for determining 16 amino acids including aspartic acid, serine, glutamic acid, glycine, histidine, arginine, alanine, threonine, lysine, leucine, proline, tyrosine, isoleucine, valin, phenylalanine, and methionine and the derivatization of the *S. virgata* mericarps were performed according to the method reported by Dimova (2003) and Gheshlaghi *et al*. (2008) with a slight modification. The powdered mericarps (0.1-1 g) were dissolved in HCl (6 M, 20 mL) in hydrolysis tubes and hydrolyzed under a nitrogen atmosphere in an oven at 110°C for 24 h. After hydrolysis the mixture was allowed to cool at room temperature. Following the protein hydrolysis, the pre-column derivatization method with phenylisothiocyanate was used. The dry samples were dissolved in 20 µL of ethanol:water:triethylamine (2:2:1) and dried again under vacuum. Finally, derivatization was

performed using 20 µL of derivatizating reagent [ethanol: water:triethylamine:phenylisothiocyanate (7:1:1:1)] for 20 min at room temperature following which the reagent was removed under vacuum at 45°C. The derivatized samples were dissolved in 0.1 mL of 0.14 M sodium acetate and pH adjusted to 6.4 with dilute acetic acid. The alkaline hydrolysis for determining tryptophan was carried out according to the method reported by Yust *et al.* (2004) and Zhang *et al.* (2009). A standard stock solution of tryptophan (100 µg/mL) was prepared in a brown volumetric flask with water (pH 6.3) and stored at 4°C in darkness for 1 month. Individual working standard solutions of six concentrations (range: 0.0625-5.0 µg/mL) were freshly prepared from the stock solution and analyzed for calibration curves. The powdered mericarps (0.1-1 g) were dissolved in NaOH (5 N, 20 mL) in hydrolysis tubes under a nitrogen atmosphere. After hydrolysing in an oven at 120°C for 12 h, the mixture was cooled down to room temperature and pH adjusted to 6.3 using the diluted HCl. Prominence ultrafast liquid chromatography system (Shimadzu, Tokyo, Japan) equipped with a binary pump, and UV/Vis detectors were used for analysis. For the separation and detection a reversed phase analytical column [Shim-pact XR-ODS (75 mm × 3.0 mm i.d.)] with a fluorescence detector was used. The analysis conditions were as follows; mobile phase A: 10 mmol/L (potassium phosphate buffer (pH: 7.0), mobile phase B: acetonitrile [5% (0 to 0.3 min), 5% to 40% (0.3 to 3.4 min)], 40°C column temperature, 1.2 mL/min flow rate, 1 µL injection volume. Amount of amino acid was presented as mg amino acid/100 g of dry sample.

Mineral content. Mineral analysis was performed according to the method proposed by Başgel & Erdemoğlu (2006). A CEM MARS 240/50 (CEM Co., NC, USA) oven model with a timer and variable temperature settings was used for the microwave-assisted digestion of the *S. virgata* mericarps. Mineral analysis was performed using a Thermo Scientific™ iCAP Q ICP-MS (Thermo Scientific, Waltham, USA). The analysis conditions were as follows; torch: Quartz, auto sampler: Cetac ASX-520, Rf power: 1548.6 W, auxiliary flow: 0.79 L/min, nebulizer flow: 0.94 L/min, spray chamber temperature: 2.55°C, read time: 0.01 sec, detector voltage: 1189 V, delay time: 30 sec, wash time: 30 sec. The results were expressed as µg of mineral/g of dry sample.

Total phenolic content. The total phenolic content was studied using the Folin-Ciocalteu method as described in Güzel *et al.* (2019b). 40 µL of extract dissolved in ethanol (10 mg/mL) and distilled water (1160 µL) were mixed with the Folin-Ciocalteu reagent (200 µL, 2.0 N). After 5 min (25°C), 20% Na_2CO_3 solution (600 µL) was added and mixed (40°C, 30 min, dark). The absorbance was measured at 765 nm using a spectrophotometer (Cary 60 UV-Vis, Agilent, USA). Quantification was done on the basis of the calibration curve obtained from the measurement of the standard solutions of gallic acid (GA) (500–50 µg/mL). The total phenolic content was calculated by means of the following equation which was obtained using the standard GA curve ($R^2 = 0.999$): $A_{765\,nm}=0.0029 \times [GAE]$ and the result was expressed as µg GA equivalent (GAE)/mg extract.

Total flavonoid content. The total flavonoid content was measured according to the method used by Moreno *et al.* (2000) with some modifications (Güzel *et al.* 2019b). Quercetin (Q) was used for the standard calibration curve. 40 µL of extract dissolved in ethanol (10 mg/mL) or 40 µL of various concentrations (5-100 µg/mL) of quercetin were added into tubes containing $Al(NO_3)_3$ (40 µL; 10%), CH_3COOK (40 µL; 1.0 M), and ethanol (1520 µL). The mixture was vigorously shaken then kept at room temperature. After 40 min, the absorbance was measured using a spectrophotometer at 415 nm. The total flavonoid content was calculated using the following equation obtained using the standard Q curve ($R^2 = 0.999$): $A_{415\,nm} = 0.0102 \times [Q]$ and the results were expressed as µg Q equivalent (QE)/mg extract.

Evaluation of antioxidant activity using DPPH assay. The DPPH free radical scavenging activity assay was performed according to the method used by Blois (1958) with some modifications (Güzel *et al.* 2019b). BHA was used as a reference standard. Various concentrations (10.0-1.0 mg/mL) of the extract (1 mL) dissolved in ethanol were mixed with 1 mL of ethanolic solution of DPPH (0.1 mM). The mixture was shaken vigorously then incubated in the dark for 30 min at room temperature. The absorbance of the reaction mixtures was read using a spectrophotometer at 517 nm. The DPPH radical scavenging activity (%) was calculated using the following equation:

DPPH radical scavenging activity (%) = $100 ((A_0-A_1) / A_0)$ where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Evaluation of antimicrobial activity

Microbial strains. Gram-negative bacterial strains [*Acinetobacter baumannii* (ATCC 02026), *Escherichia coli* (ATCC 25923), *Aeromonas hydrophila* (ATCC 95080)]; gram-positive bacterial strains [*Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25925)]; *Mycobacterium tuberculosis* H37Rv; and fungal strains [*Candida albicans* (ATCC 14053), *Candida tropicalis* (ATCC 1369), *Candida glabrata* (ATCC 15126)] were procured from the Refik Saydam Hıfzıssıhha Institute, Ankara, Turkey.

Antibacterial activity. *In vitro* antibacterial activity against *A. baumannii*, *E. coli*, *A. hydrophila, B. subtilis*, and *S. aureus* was studied using the broth microdilution method (Güzel *et al.* 2019b). Ampicillin was used as the reference drug. The sample was dissolved in DMSO to prepare the initial concentration (2000 µg/mL). The mixture was used for the preparation of the stock solution which was

diluted in Mueller-Hinton broth. Further dilutions of the reference drug and extract were prepared (1000-0.9 µg/mL). The working suspensions of the standard strains were made in sterile tubes. Turbidity was adjusted to match McFarland standard No. 0.5. Further dilutions (1:20) of the suspensions were prepared in distilled water and added to each plate (10 µL). Thus, each plate's bacterial concentration was adjusted to 5×10^5 CFU/mL. The effect of DMSO was also tested. The minimal inhibitory concentration (MIC) values were visually detected. *In vitro* antibacterial activity against *M. tuberculosis* H37Rv was studied using the resazurin microtiter assay (Güzel *et al*. 2019b). Isoniazid and Ethambutol were used as the reference drugs. The resazurin reagent was prepared using resazurin sodium salt powder. Middlebrook 7H9 broth containing 0.1% casitone, 0.5% glycerol, and 10% oleic acid-albumin-dextrose-catalase and a 7H9-S medium were used for preparing the culture medium. A resazurin working solution made in distilled water (0.01%, w/v) and stock solutions (1000 µg/mL) of the extract and reference drugs prepared in DMSO were filtered through a 0.22 µm membrane filter (Ministar, Goettingen, Germany). A two-fold serial dilution was performed using a 7H9-S medium (100 µL) in a 96-well microtiter plate. A concentration range of 0.12-250 µg/mL was detected. A growth control and a sterility control were added to each plate. The bacterial inoculum was prepared in a tube containing a 7H9-S medium (5 mL) by resuspending a loopful of Lowenstein-Jensen culture medium. The contents of the tube were mixed for 2 min then left to allow the sediment to settle. After the supernatant was added to a sterile tube, the turbidity was adjusted to match McFarland standard No. 1.0. A 7H9-S medium was used to prepare the dilutions (1:20) of the suspensions. The plates were inoculated with the diluted suspension (100 µL) and incubated (37°C, 7 days). The resazurin working solution (30 µL) was added and incubated (37°C, 24 h) again. The results were visually recorded. The lowest concentration which prevents the complete colour change of resazurin from blue to pink was determined as the MIC value.

Antifungal activity. *In vitro* antifungal activity was studied using a broth microdilution method according to the NCCLS M27-A2 standard document (2002) with minor modifications (Güzel *et al*. 2019b). The RPMI 1640 medium which buffered to pH 7.0 with 0.165 M 3-(N-morpholino) propanesulfonic acid was used. Fluconazole was used as the reference drug. Working suspensions of standard strains were made as a 1:100 dilution followed by a 1:20 dilution of the stock suspensions using the RPMI 1640 medium. The stock solutions (1000 µg/mL) of the extract and reference drug dissolved in DMSO were filtered through membrane filters. A two-fold serial dilution was added to a 96-well microtiter plate using 100 µL of RPMI 1640 medium. The concentration range from 250-0.12 µg/mL was tested. A growth control and sterility control were added to each plate. 100 µL of the working inoculum suspension was added to each plate and the plates were incubated (48 h, 35°C) then the MIC values were visually determined.

RESULTS AND DISCUSSION

Phytochemical contents

Proximate analysis. The proximate analysis results are given in Table 1. According to the results, the *S. virgata* mericarps are rich in dietary fibers, oil, and protein (35.57 ± 0.11 g/100 g, 24.02 ± 0.03 g/100 g, and 22.10 ± 0.09 g/100 g, respectively). Dietary fibers have attracted a great deal of attention due to their health benefits such as reducing the risk of heart attack, obesity, colon cancer, appendicitis, blood pressure, and various other diseases. In Britain, the National Advisory Committee recommends a fiber intake of 25-30 g/day per person (Naidu *et al.* 2011). The dietary fiber values of the *S. virgata* mericarps are similar to the *Salvia hispanica* L. mericarps which are also known as Chia seeds (35%), with higher levels than other cereals, such as amaranth (7.3%), quinoa (7.0%), and corn (8.3%) (Grancieri *et al.* 2019). The literature reports that Chia seeds contain a low amount of carbohydrates (3.4%) and high protein (18.9%) and lipid (31.2%) contents (Grancieri *et al.* 2019), and our results indicate that the *S. virgata* mericarps have good nutritional value with low amounts of carbohydrates (6.37 g/100 g) and high protein (22.095 g/100 g) and oil (24.02 g/100 g) contents.

Amino acid content. The amino acid content of the *S. virgata* mericarps is shown in Table 2. The mericarps contain both essential (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, and arginine) and non-essential (alanine, aspartic acid, glycine, glutamic acid, proline, serine, tyrosine, and tryptophan) amino acids. Glutamic acid was the major amino acid with a value of 3934 ± 14.52 mg/100 g. A multifunctional amino acid, glutamic acid is involved in excitatory neurotransmission, taste perception, and intermediary metabolism. Moreover, it plays an essential role in gastric phase of digestion with multiplicity effects in the gastrointestinal tract when consumed with foods by increasing gastric exocrine secretion (Zareian *et al.* 2012). In the literature, there is no study on the amino acid content of the mericarps of *Salvia* species with the exception of Chia seeds. Our results indicate that the glutamic acid value of the *S. virgata* mericarps

Table 1. Proximate analysis of *S. virgata* mericarps.

Parameters	*S. virgata*
Energy (kcal/100 g)	401 ± 0.00
Moisture (g/100 g)	5.69 ± 0.02
Ash (g/100 g)	6.31 ± 0.01
Protein (g/100 g)	22.10 ± 0.09
Carbonhydrate (g/100 g)	6.37 ± 0.01
Dietary fiber (g/100 g)	35.57 ± 0.11
Oil content (g/100 g)	24.02 ± 0.03

The data are presented as mean ± SD; n=3

Table 2. Total amino acid content of *S. virgata* mericarps.

Amino acid content					
Essential amino acids	**Symbol**	**Amount (mg/100 g)**	**Non-essential amino acids**	**Symbol**	**Amount (mg/100 g)**
Histidine	HIS	505 ± 2.11	Alanine	ALA	1249 ± 8.01
Isoleucine	ILE	977 ± 6.20	Aspartic acid	ASP	1670 ± 0.00
Leucine	LEU	1781 ± 4.83	Glycine	GLY	1678 ± 11.31
Lysine	LYS	849 ± 0.01	Glutamic acid	GLU	3934 ± 14.52
Methionine	MET	470 ± 0.20	Proline	PRO	996 ± 0.00
Phenylalanine	PHE	1336 ± 1.00	Serine	SER	1373 ± 9.80
Threonine	THR	724 ± 1.14	Tyrosine	TYR	792 ± 1.51
Valine	VAL	1312 ± 5.13	Tryptophan	TRP	141 ± 1.00
Arginine	ARG	1377 ± 0.10			

The data are presented as mean ± SD; n=3

was higher than that of Chia seeds (3.500 g/100 g seed) (Muňoz *et al.* 2013).

Mineral content. The mineral content of the *S. virgata* mericarps is presented in Table 3. The mericarps contain eleven different minerals including K, Na, P, Mg, Fe, Mn, Ca, Cu, Al, Zn, and Sr. Among the macro minerals, K (12578.8 ± 0.15 μg/g), Ca (12092.0 ± 0.25 μg/g), P (3356.8 ± 0.01 μg/g), and Mg (2881.2 ± 0.01 μg/g) were determined as the major ones. In terms of the essential trace minerals, the amount of Fe (13.36 ± 0.05 μg/g) was the highest. According to the literature, the minerals reported so far in the mericarps of some *Salvia* species are K, Ca, Mg, Na, Zn, Fe, Cu, Mn, Ni, Se, Co, Al, and P (Ullah *et al.* 2016; Ding *et al.* 2018; Güzel *et al.* 2019a, 2020a; Güzel 2020), with K, Mg, Ca, and P determined as the major ones (Ding *et al.* 2018; Güzel *et al.* 2019a, 2020a; Güzel 2020). The results of our study are consistent with the literature. The mineral content of the aerial parts of *S. virgata* was investigated in previous studies (Coşge Şenkal *et al.* 2019; Gezek *et al.* 2019); however, no study had been carried out on the mineral content of the mericarps of *S. virgata*. Adequate mineral intake is important for the prevention of several diseases and health promotion (Verkaik-Kloosterman *et al.* 2012); therefore, *S. virgata* mericarps might be preferable as a source of dietary minerals.

Table 3. Mineral content of *S. virgata* mericarps.

Minerals	**Symbol**	**Amount (μg/g)**
Macro minerals		
Sodium	Na	884 ± 0.01
Magnesium	Mg	2881.20 ± 0.01
Phosphorus	P	3356.80 ± 0.01
Potassium	K	12578.80 ± 0.15
Calcium	Ca	12092 ± 0.25
Essential trace minerals		
Manganese	Mn	10.68 ± 0.03
Iron	Fe	13.36 ± 0.05
Zinc	Zn	6.64 ± 0.06
Copper	Cu	0.79 ± 0.23
Other minerals		
Aluminium	Al	4.53 ± 0.06
Strontium	Sr	3.93 ± 0.07

The data are presented as mean ± SD; n=3

Total phenolic content. The total phenolic content of the ethanol extract of the *S. virgata* mericarps was determined as 2.50 ± 0.06 μg GAE/mg extract. In our previous studies, for the ethanol extracts of *S. hispanica* and *S. longipedicellata* Hedge mericarps the total phenolic contents were determined as 0.93 μg GAE/mg extract (Güzel 2020) and 1.04 μg GAE/mg extract (Güzel *et al.* 2020a), respectively. Hence, in the current study, the total phenolic content result obtained for the ethanol extract of the *S. virgata* mericarps was found to be higher than that of the *S. hispanica* and *S. longipedicellata* mericarps. According to the literature review, the total phenolic content of the aerial parts of Turkish *S. virgata* was studied previously and the results of different samples of 80% methanol extract were reported as between 125.11-68.71 mg GAE/g sample (İnan *et al.* 2020); the results of *n*-hexane, ethyl acetate, methanol, and 50% aqueous methanol extracts were reported as between 212.3-28.3 mg GAE/g extract (Koşar *et al.* 2008; Küpeli-Akkol *et al.* 2008); and those of 70% methanol and water extracts were reported as 195.22 mg GAE/g extract and 120.14 mg GAE/g extract (Karatoprak *et al.* 2016), respectively. These results indicated that the total phenolic contents of the aerial parts were higher than the mericarps.

Total flavonoid content. The total flavonoid content of the ethanol extract of the *S. virgata* mericarps was determined as 0.34 ± 0.02 μg QE/mg extract. In our previous studies, for the ethanol extracts of *S. hispanica* and *S. longipedicellata* the total flavonoid contents of the mericarps were determined as 0.17 μg QE/mg extract (Güzel 2020) and 0.32 μg QE/mg extract (Güzel *et al.* 2020a), respectively. Thus, in this study, the total flavonoid content result obtained for the ethanol extract of the *S. virgata* mericarps was found to be higher than that of the *S. hispanica* and *S. longipedicellata* mericarps. In previous studies, the total flavonoid content of the aerial parts of *S. virgata* from Turkey was studied and the results of different samples of 80% metha-

Table 4. MIC values of the ethanol extract of *S. virgata* mericarps and the reference drugs against the tested bacterial strains.

Bacterial strains	Code	MIC values of tested materials (μg/mL)			
		S. virgata	Ampicillin	Isoniazid	Ethambutol
Bacillus subtilis	ATCC 6633	250	0.90	-	-
Staphylococcus aureus	ATCC 25925	250	31.25	-	-
Escherichia coli	ATCC 25923	250	15.62	-	-
Aeromonas hydrophila	ATCC 95080	125	31.25	-	-
Acinetobacter baumannii	ATCC 02026	125	125	-	-
Mycobacterium tuberculosis	H37Rv	62.50	-	0.97	1.95

-: Not tested.

Table 5. MIC values of the ethanol extract of *S. virgata* mericarps and the reference drug against the tested fungal strains.

Fungal strains	Code	MIC values of tested materials (μg/mL)	
		S. virgata	Fluconazol
Candida albicans	ATCC 14053	62.50	31.25
Candida glabrata	ATCC 15126	62.50	3.90
Candida tropicalis	ATCC 1369	62.50	15.62

nol extract were reported as between 28.03-20.62 mg QE/g sample (İnan *et al.* 2020); the results of different extracts (*n*-hexane, ethyl acetate, methanol, and 50% aqueous methanol) were reported as between 6.5-0.1 mg RE/g extract (Koşar *et al.* 2008; Küpeli-Akkol *et al.* 2008); and those of 70% methanol and water extracts were reported as 62.2 mg RE/g extract and 14.17 mg RE/g extract (Karatoprak *et al.* 2016), respectively. These results indicated that the total flavonoid contents of the aerial parts were higher than the mericarps.

Antioxidant activity. The DPPH radical scavenging activity results presented in Fig. 1 show that the tested extract neutralized the DPPH radicals in a dose-dependent manner. As expected, the highest DPPH radical scavenging activity was determined at a concentration of 10 mg/mL with a value of 92.44 ± 0.69%. In our previous studies, for the ethanol extracts of *S. hispanica* and *S. longipedicellata* mericarps the highest DPPH radical scavenging activity was determined at a concentration of 10 mg/mL with values of 74.54% (Güzel 2020) and 53.34% (Güzel *et al.* 2020a), respectively. In our study, the DPPH radical scavenging activity results obtained for the ethanol extract of the *S. virgata* mericarps were found to be higher than those of *S. hispanica* and *S. longipedicellata* mericarps. The antioxidant activity of the aerial parts of Turkish *S. virgata* was studied previously and the results of different samples obtained from an 80% methanol extract of the aerial parts were reported as EC_{50} values between 0.79-0.57 μg/mL (İnan *et al.* 2020) and the results of 70% methanol and water extracts of the aerial parts were reported as IC_{50} values of 0.16 mg/mL and 0.2 mg/mL, respectively (Karatoprak *et al.* 2016).

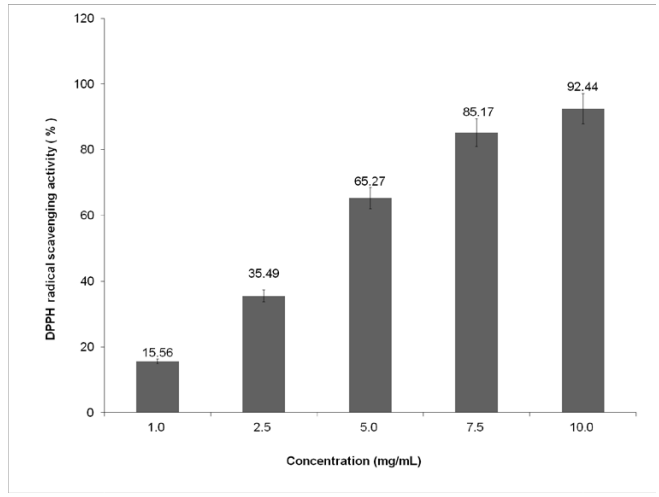

Fig. 1. Free radical scavenging activity of *S. virgata* mericarps by the DPPH assay. (The values are presented as mean ± SD of the data; n=3).

Although the total phenolic and flavonoid contents and antioxidant activity of the aerial parts of *S. virgata* from Turkey were examined in previous studies (Koşar *et al.* 2008; Küpeli-Akkol *et al.* 2008; Karatoprak *et al.* 2016; İnan *et al.* 2020), this is the first study on the mericarps of *S. virgata*. According to the literature, free radicals play an important role in several pathological conditions including cancer, brain dysfunction, heart disease, and inflammation by damaging the cellular components of proteins, DNA and lipids. In addition to the treatment and/or prevention of diseases, natural sources of phenolic antioxidants are often the preferred choice for preserving food quality by preventing the oxidative deterioration of lipids (Karatoprak *et al.* 2016). Because of food safety, ethanol and water are the most suitable solvents for the extraction of phenolics (Alcântara *et al.* 2019). Therefore, in this study ethanol extract was used to investigate the total phenolic and flavonoid contents and the DPPH radical scavenging activity.

Antimicrobial activity. The antibacterial and antifungal activity results are shown in Tables 4 and 5, respectively. Although the ethanol extract of the *S. virgata* mericarps showed antibacterial (MIC values: between 250-62.5 μg/mL) and antifungal (MIC value: 62.5 μg/mL) activities

against all the tested microorganisms, the efficiency of the extract was not found to be as strong as the tested reference drugs (MIC values: ranging from 31.25-0.9 µg/mL) with the exception of nosocomial pathogen *A. baumannii*. The tested extract was found to be as effective against *A. baumannii* as the reference drug Ampicillin (Both MIC values: 125 µg/mL).

Ethanol extracts of some *Salvia* mericarps including *S. pilifera* (Güzel *et al.* 2019a), *S. hispanica* (Kobus-Cisowska *et al.* 2019; Güzel *et al.* 2020b) and *S. longipedicellata* (Güzel *et al.* 2020a) were studied for their antimicrobial activity in our previous research, while this is the first report on the antimicrobial activity of *S. virgata* mericarps. In the literature, the antimicrobial activity of essential oils obtained from the aerial parts of *S. virgata* from Iran was examined using the disc diffusion method against *C. albicans* (ATCC 10231) and *S. aureus* (ATCC 6538), showing moderate antimicrobial activity against both the tested microorganisms (Alizadeh 2013). In this study, the mericarps exhibited antibacterial activity against all the tested Gram-negative and Gram-positive bacterial strains. Due to their complex cell wall structure Gram-negative bacteria are more resistant to natural compounds than Gram-positive bacteria (Bajpai 2016). In the current study, the *S. virgata* mericarps were found to be as strong as the reference drug against Gram-negative microorganism *A. baumannii*, which is one of the most problematic pathogens for health care institutions globally (Peleg *et al.* 2008). Over the last 15 years in particular, clinical attention to *A. baumannii* has increased because of its extraordinary ability to up-regulate and/or acquire resistance determinants, making it one of the microorganisms threatening the current antibiotic era (Peleg *et al.* 2008). Due to their antimicrobial efficiency, *S. virgata* mericarps might be used as a natural source in the discovery of new antimicrobial agents against infections caused by *A. baumannii*.

CONCLUSIONS

This is the first report describing the proximate, amino acid, mineral, total phenolic and flavonoid contents, as well as the antioxidant and antimicrobial activities of *S. virgata* mericarps. According to the results, the mericarps of *S. virgata* have good nutritional value with low amounts of carbohydrates and high dietary fibers, protein, oil, amino acids, minerals and total phenolic and flavonoid contents with antioxidant and antimicrobial activities. The increasing uses of natural compounds as antioxidants and antimicrobial agents and food stabilizers mean that the mericarps of *S. virgata*, which is abundant in nature, might be a promising source in the development of novel therapeutic agents and food supplements for various industries.

Acknowledgements – The authors would like to express their gratitude to the TUBITAK Marmara Research Center (Kocaeli, Turkey).

REFERENCES

Alcântara MA, Polari ILB, Meireles BRLA, Lima AEA, Junior JCS, Vieira EA, Santos NA & Cordeiro AMTM. 2019. Effect of the solvent composition on the profile of phenolic compounds extracted from chia seeds. *Food Chemistry* **275**: 489–496.

Alizadeh A. 2013. Essential oil constituents, antioxidant and antimicrobial activities of *Salvia virgata* Jacq. from Iran. *Journal of Essential Oil Bearing Plants* **16**(2): 172–182.

AOAC. 2000. *Official methods of analysis of the association of official analytical chemists.* Association of official analytical chemists.

Azcan N, Ertan A, Demirci B & Baser KHC. 2004. Fatty acid composition of seed oils of twelve *Salvia* species growing in Turkey. *Chemistry of Natural Compounds* **40**(3): 218–221.

Bagci E, Vural M, Dirmenci T, Bruehl L & Aitzetmüllerd K. 2004. Fatty acid and tocochromanol patterns of some *Salvia* L. species. *Zeitschrift für Naturforschung C* **59**(5–6): 305–309.

Bajpai VK. 2016. Antimicrobial bioactive compounds from marine algae: A mini review. *Indian Journal of Geo-Marine Sciences* **45**(9): 1076–1085.

Başgel S & Erdemoğlu SB. 2006. Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey. *Science of the Total Environment* **359**(1–3): 82–89.

Blois MS. 1958. Antioxidant determinations by the use of a stable free radical. *Nature* **181**: 1199–1200.

Büyükkartal HN, Kahraman A, Çölgeçen H, Doğan M & Karabacak E. 2011. Mericarp micromorphology and anatomy of *Salvia hedgeana* Dönmez, *S. huberi* Hedge and *S. rosifolia* Sm. (section *Salvia* Hedge, Lamiaceae). *Acta Botanica Croatica* **70**(1): 65–80.

Coşge Şenkal B, Uskutoğlu T, Cesur C, Özavci V & Doğan H. 2019. Determination of essential oil components, mineral matter, and heavy metal content of *Salvia virgata* Jacq. grown in culture conditions. *Turkish Journal of Agriculture and Forestry* **43**: 395–404.

Dimova N. 2003. RP-HPLC analysis of amino acids with UV-detection. *Bulgarian Academy of Science* **56**(12): 75–78.

Ding Y, Lin H, Lin Y, Yang D, Yu Y, Chen J, Wang S & Chen Y. 2018. Nutritional composition in the chia seed and its processing properties on restructured ham-like products. *Journal of Food and Drug Analysis* **26**(1): 124–134.

Falco B, Amato M & Lanzotti V. 2017. Chia seeds products: an overview. *Phytochemistry Reviews* **16**: 745–760.

Farida SHM & Radjabian T. 2019. Phytosterols in *Salvia* seeds: content and composition and Ccorrelation with environmental parameters. *Iranian Journal of Science and Technology, Transactions A: Science* **43**: 2129–2140.

Gezek G, Hashemi P, Kalaycioğlu Z, Kaygusuz H, Sarioğlu G, Döker S, Dirmenci T & Erim FB. 2019. Evaluation of some Turkish *Salvia* species by principal component analysis based on their vitamin B2, mineral composition, and antioxidant properties. *Journal of Food Science and Technology* **100**: 287–293.

Gheshlaghi R, Scharer JM, Moo-Young M & Douglas PL. 2008. Application of statistical design for the optimization of amino acids separation by reverse-phase HPLC. *Analytical Biochemistry* **383**: 93–102.

Gören AC, Kiliç T, Dirmenci T & Bilsel G. 2006. Chemotaxonomic evaluation of Turkish species of *Salvia*: Fatty acid compositions of seed oils. *Biochemical Systematics and Ecology* **34**(2): 160–164.

Grancieri M, Martino HSD & Mejia EG. 2019. Chia seed (*Salvia hispanica* L.) as a source of proteins and bioactive peptides with health benefits: A review. *Comprehensive Reviews in Food Science and Food Safety* **18**(2): 480–499.

Güzel S. 2020. Bolivya'da yetişen Chia (*Salvia hispanica* L.) tohumlarının yağ asidi, sterol, tokoferol ve mineral kompozisyonları; toplam fenol ve flavonoit miktarları ve antioksidan aktivitesi. *Mersin* Üniversitesi *Sağlık Bilimleri Dergisi* **13**(3): 428–436.

Güzel S, Kahraman A, Ülger M, Özay Y, Bozgeyik İ & Sarikaya Ö. 2019a. Morphology, myxocarpy, mineral content and *in vitro* antimicrobial and antiproliferative activities of mericarps of the vulnerable Turkish endemic *Salvia pilifera*. *Journal of Research in Pharmacy* **23**(4): 729–739.

Güzel S, Özay Y, Kumaş M, Uzun C, Gökalp-Özkorkmaz E, Yildirim Z, Ülger M, Güler G, Çelik A, Çamlica Y & Kahraman A. 2019b. Wound healing properties, antimicrobial and antioxidant activities of *Salvia kronenburgii* Rech. f. and *Salvia euphratica* Montbret, Aucher & Rech. f. var. *euphratica* on excision and incision wound models in diabetic rats. *Biomedicine & Pharmacotherapy* **111**: 1260–1276.

Güzel S, Özay Y, Ülger M, Kahraman A & Aslan G. 2018. Evaluation of antimicrobial activity of three endemic *Salvia* species growing in Turkey. *Hacettepe University Journal of the Faculty of Pharmacy* **38**(1): 4–10.

Güzel S, Ülger M & Kahraman A. 2020a. Chemical composition and some biological activities of *Salvia longipedicellata* Hedge mericarps. *Chemistry of Natural Compounds* **56**(5): 788–792.

Güzel S, Ülger M & Özay Y. 2020b. Antimicrobial and antiproliferative activities of Chia (*Salvia hispanica* L.) seeds. *International Journal of Secondary Metabolite* **7**(3): 174–180.

Hatipoglu SD, Zorlu N, Dirmenci T, Goren AC, Ozturk T & Topcu G. 2016. Determination of volatile organic compounds in fourty five *Salvia* species by thermal desorption-GC-MS technique. *Records of Natural Products* **10**(6): 659–700.

İnan Y, Kurt-Celep I, Akyüz S, Barak TH, Celep E & Yeşilada E. 2020. An investigation on the enzyme inhibitory activities, phenolic profile and antioxidant potentials of *Salvia virgata* Jacq. *South African Journal of Botany*, 1–9.

Jaradat NA, Shawahna R, Hussein F & Al-Lahham S. 2016. Analysis of the antioxidant potential in aerial parts of *Trigonella arabica* and *Trigonella berythea* grown widely in Palestine: A comparative study. *European Journal of Integrative Medicine* **8**(5): 623–630.

Kamatou GPP, Makunga NP, Ramogola WPN & Viljoen AM. 2008. South African *Salvia* species: A review of biological activities and phytochemistry. *Journal of Ethnopharmacology* **119**(3): 664–672.

Karatoprak GS, Ilgün S & Koşar M. 2016. Antioxidant properties and phenolic composition of *Salvia virgata* Jacq. *Turkish Journal of Pharmaceutical Sciences* **13**(2): 201–212.

Kirmizibekmez H, Bardakci-Altan H, Liktor-Busa E, Zana A, Yesilada E & Hohmann J. 2012. Chemical constituents of *Salvia dichroantha*. *Biochemical Systematics and Ecology* **42**: 18–20.

Kobus-Cisowska J, Szymanowska D, Maciejewska P, Kmiecik D, Gramza-Michalowska A, Kulczynski B & Cielecka-Piontek J. 2019. *In vitro* screening for acetylcholinesterase and butyrylcholinesterase inhibition and antimicrobial activity of chia seeds (*Salvia hispanica*). *Electronic Journal of Biotechnology* **37**: 1–10.

Koşar M, Göger F & Başer KHC. 2008. *In vitro* antioxidant properties and phenolic composition of *Salvia virgata* Jacq. from Turkey. *Journal of Agricultural and Food Chemistry* **56**: 2369–2374.

Küpeli-Akkol E, Gören F, Koşar M & Başer KHC. 2008. Phenolic composition and biological activities of *Salvia halophila* and *Salvia virgata* from Turkey. *Food Chemistry* **108**(3): 942–949.

Merrill AL & Watt BK. 1973. *Energy value of foods*. Agricultural Research Service. United States Department of Agriculture Handbook.

Moreno MIN, Isla MI, Sampietro AR & Vattuone MA. 2000. Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. *Journal of Ethnopharmacology* **71**(1–2): 109–114.

Muñoz LA, Cobos A, Diaz O & Aguilera JM. 2013. Chia seed (*Salvia hispanica*): an ancient grain and a new functional food. *Food Reviews International* **29**(4): 394–408.

Naidu M., Shyamala BN, Naik JP, Sulochanamma G & Srinivas P. 2011. Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds. *LWT - Food Science and Technology* **44**(2): 451–456.

NCCLS. 2002. *Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard*. NCCLS document M27-A2.

Peleg AY, Seifert H & Paterson DL. 2008. *Acinetobacter baumannii*: Emergence of a successful pathogen. *Clinical Microbiology Reviews* **21**(3): 538–582.

Sari A, Kusat M, Civelek S & Emre I. 2009. Vitamin contents of some *Salvia* L. Taxa growing in Turkey. *Chemistry of Natural Compounds* **45**(6): 944–946.

Segura-Campos MR, Salazar-Vega IM, Chel-Guerrero LA & Betancur-Ancona DA. 2013. Biological potential of chia (*Salvia hispanica* L.) protein hydrolysates and their incorporation into functional foods. *LWT-Food Science and Technology* **50**: 723–731.

Temel HE, Demirci B, Denirci F, Celep F, Kahraman A, Doğan M & Başer KHC. 2016. Chemical characterization and anticholinesterase effects of essential oils derived from *Salvia* species. *Journal of Essential Oil Research* **28**(4): 322–331.

Ullah R, Nadeem M, Khalique A, Imran M, Mehmood S, Javid A & Hussain. 2016. Nutritional and therapeutic perspectives of Chia (*Salvia hispanica* L.): a review. *Journal of Food Science and Technology* **53**: 1750–1758.

Ulubelen A, Öküz S, Topcu G, Gören AC & Voelter W. 2001. Antibacterial diterpenes from the roots of *Salvia blepharochlaena*. *Journal of Natural Products* **64**(4): 549–551.

Verkaik-Kloosterman J, McCann MT, Hoekstra J & Verhagen H. 2012. Vitamins and minerals: issues associated with too low and too high population intakes. *Food & Nutrition Research* **56**: 5728–5736.

Yust MM, Pedroche J, Girón-Calle J, Vioque J, Millán F & Alaiz M. 2004. Determination of tryptophan by high-performance liquid chromatography of alkaline hydrolysates with spectrophotometric detection. Analytical, nutritional and clinical methods section. *Food Chemistry* **85**(2): 317–320.

Zareian M, Ebrahimpour A, Bakar FA, Mohamed AKS, Forghani B, Ab-Kadir MSB & Saari N. 2012. A glutamic acid-producing lactic acid bacteria isolated from Malaysian fermented foods. *International Journal of Molecular Sciences* **13**(5): 5482–5497.

Zhang J, Xue X, Zhou J, Chen F, Wu L, Li Y & Zhao J. 2009. Determination of tryptophan in bee pollen and royal jelly by high-performance liquid chromatography with fluorescence detection. *Biomedical Chromatography* **23**(9): 994–998.

REZIME

Botanica SERBICA

Fitohemijske analize, antioksidantna i antimikrobna aktivnost merikarpa *Salvia virgata*

Sevda Güzel Kara, Mahmut Ülger i Ahmet Kahraman

Fitohemijski sastav merikarpa *Salvia virgata* i antioksidantna i antimikrobna aktivnost njegovog etanolnog rastvora su proučavani po prvi put. Merikarpi su sakupljeni u provinciji Trabzon u Turskoj. Hemijske analize su urađene hromatografijom. Ukupni sastav fenola i flavonoida, kao i antioksidativni potencijal su izmereni pomoću Folin-Ciocalteu, $Al(NO_3)_3$ i DPPH metode uklanjanja radikala, respektivno. Antimikrobna aktivnost je procenjena pomoću metode dilucije za sve testirane sojeve bakterija i gljiva, izuzev *Mycobacterium tuberculosis* za koji je korišćen metod resazurin mikrotitarskih ploča. Merikarpi su bogati glutaminskom kiselinom (3934 mg/100 g), kalijumom (12578.8 µg/g), kalcijumom (12092.0 µg/g), i vlaknima (35.565 g/100 g). Ukupni sadržaji fenola i flavonoida su bili 2.50 µg GAE/mg ekstrakta i 0.34 µg QE/mg ekstrakta, respektivno. Najefikasnije uklanjanje slobodnih radikala DPPH utvrđeno za najvišu primenjenu koncentraciju bilo je 92,44%. Protiv bolničkog patogena *Acinetobacter baumannii*, etalnolni rastvor merikarpa se pokazao efikasnim kao i referentni lek ampicilin (MIC value = 125 µg/mL). Kao zaključak, merikarpi *S. virgata* imaju dobru hranljivu vrednost sa malom količinom ugljenih hidrata i visokim sadržajem vlakana, aminokiselina, mineralnim i ukupnim sadržajem fenola i flavonoida i lekovitim svojstvima.

Ključne reči: *Salvia virgata*, merikarpi, proksimativna analiza, aminokiselina, mineral, antioksidantne i antimikrobne aktivnosti