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(NO₃)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

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; Bagic et al. 2004; Gören et al. 2006; Kamatou et al. 2018; Kirmizibekmez...
oxidant, 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 La-
mieaeae, is a schizocarp. The schizocarp fruit, which con-
sists of indehiscent locules separating to form four fruit-
lets, 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 (exo-
carp), 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 (ÇOŞGE ŞENKAL et al. 2019). The plant is known
as “yilançik” or “fatmanaotu” in Turkey and is used for the
treatment of wounds and skin diseases (KÜPELİ-AKKOL et al. 2008; ÇOŞGE 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, includ-
ing antimicrobial, anti-inflammatory, antioxidant, anti-
ociceptive, and the inhibition of alpha-amylose, GSH-Px,
peroxidase, acetylcholinesterase, and polyphenol oxidase,
were reported (ÇOŞGE 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 con-
tents; and the in vitro antioxidant and antimicrobial activi-
ties 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 queretin were purchased from Sigma-
Aldrich (St. Louis, MO, USA); ethanol, dimethyl sulfox-
ide (DMSO), acetonitrile, 2,2-diphenyl-1-picrylhydrazyl
(DPPH), and butylated hydroxyanisol (BHA) were pur-
chased from Merck (Darmstadt, Germany); and Middle-
brook 7H9 broth, casitone, glycerol, oleic acid, albumin,
dextrose, and catalase were purchased from Becton Dick-
inson (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 Trab-
zon 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 (De-
partment 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 meri-
carps 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 evapo-
rated 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 de-
viation (SD) except for the antimicrobial activity results
where the tests were carried out in duplicate.

Phytochemical contents

Proximate analysis. Standard methods from the Associa-
tion 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 cal-
culated as the difference between 100 and the sum of the
moisture, protein, oil and ash contents. The energy con-
tent was calculated from the energy-yielding nutrients (i.e.
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, threo-
ine, 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 meri-
carps (0.1-1 g) were dissolved in HCl (6 M, 20 mL) in hy-
drolysis 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 derivatizing 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 Yüṣt 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 ultra-fast 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-pack 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% Na₂CO₃ 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 ($R^2 = 0.999$): $A_{765\text{nm}} = 0.0029 \times [\text{GAE}]$ 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(NO₃)₃ (40 µL; 10%), CH₃COOK (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 ($R^2 = 0.999$): $A_{415\text{nm}} = 0.0102 \times [Q]$ 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:

$$\text{DPPH radical scavenging activity (\%) = } 100 \left( \frac{A_0 - A_1}{A_0} \right)$$

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 Hizissıhha 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 (Minisart, 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 was 3934 ± 14.52 mg/100 g.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>S. virgata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/100 g)</td>
<td>401 ± 0.00</td>
</tr>
<tr>
<td>Moisture (g/100 g)</td>
<td>5.69 ± 0.02</td>
</tr>
<tr>
<td>Ash (g/100 g)</td>
<td>6.31 ± 0.01</td>
</tr>
<tr>
<td>Protein (g/100 g)</td>
<td>22.10 ± 0.09</td>
</tr>
<tr>
<td>Carbohydrate (g/100 g)</td>
<td>6.37 ± 0.01</td>
</tr>
<tr>
<td>Dietary fiber (g/100 g)</td>
<td>35.57 ± 0.11</td>
</tr>
<tr>
<td>Oil content (g/100 g)</td>
<td>24.02 ± 0.03</td>
</tr>
</tbody>
</table>

The data are presented as mean ± SD; n=3.
Table 2. Total amino acid content of *S. virgata* mericarps.

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

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

was higher than that of Chia seeds (3.500 g/100 g seed) (Muñoz 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.

**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-
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 *S. virgata* was studied previously and the results of different samples obtained from an 80% methanol extract of the aerial parts were reported as EC50 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 IC50 values of 0.16 mg/mL and 0.2 mg/mL, respectively (KARATOPRAK et al. 2016).

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

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Code</th>
<th>MIC values of tested materials (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. virgata</em></td>
<td>Ampicillin</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>ATCC 6633</td>
<td>250</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>ATCC 25925</td>
<td>250</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>ATCC 25923</td>
<td>250</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>ATCC 95080</td>
<td>125</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>ATCC 02026</td>
<td>125</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>H37Rv</td>
<td>62.50</td>
</tr>
</tbody>
</table>

- Not tested.

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

<table>
<thead>
<tr>
<th>Fungal strains</th>
<th>Code</th>
<th>MIC values of tested materials (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>ATCC 14053</td>
<td>62.50</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>ATCC 15126</td>
<td>62.50</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>ATCC 1369</td>
<td>62.50</td>
</tr>
</tbody>
</table>

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; KÜPELİ-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 (ALCÁNTARA 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 (BAJPAL 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.

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**REFERENCES**


**SEVDA GÜZEL KARA, MAHMUT ÜLGER I AHMET KAHRAMAN**

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

Fitochemijski 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(NO₃)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*, etanolni 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, aminokiselin, mineralnim i ukupnim sadržajem fenola i flavonoida i lekovitim svojstvima.

**Ključne reči:** Salvia virgata, merikarpi, proksimativena analiza, aminokiseline, mineral, antioksidantne i antimikrobne aktivnosti