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Scientific paper

Phytochemical Profile, Antioxidant and Antimicrobial Potency of Aerial Parts of *Salvia Tomentosa* Miller

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Abstract

Antioxidant activity, antimicrobial potency and components of the aerial parts (leaf, stem, flower and mixture) of *Salvia tomentosa* Miller were determined qualitatively and quantitatively in this study. Aqueous extracts of *Salvia tomentosa* (ST) were prepared by using the flower, leaf and stem parts and all the above-ground parts of the plant (flower-leaf-stem mixture) for this purpose. The radical scavenging activity, total antioxidant/oxidant status, antimicrobial potential, phenolic substances and qualitative/quantitative analyzes of the components in the extracts were determined. ST-stem phenolic acid amount (599 ± 34 mg gallic acid equivalent (GAE)/g extract) was found to be close to the standard substance caffeic acid (651 ±3 1 mg GAE/g extract). Total antioxidant status of ST-mix (3.4 ± 0.1 mmol Trolox Equiv./L) and ST-stem (3.4 ± 0.1 mmol Trolox Equiv./L) and natural antioxidant Vitamin C (3.6 ± 0.1 mmol Trolox Equiv./L) were not statistically different. The extract produced by using *S. tomentosa* aerial parts (flower-stem-leaf) showed stronger antioxidant and antimicrobial activity than the aqueous extracts obtained separately from the flower, stem and leaf of the plant. However, it was determined that the components of the separately prepared flower, stem and leaf extracts and the extract components obtained from the aerial parts were largely similar. At the same time, it was observed that there were significant differences in the presence of these components.

Keywords: *Salvia tomentosa* Miller, antimicrobial, antioxidant, LC-MS/MS

1. Introduction

Salvia tomentosa is a perennial semi-shrub with white or lilac-purple flowers up to 55 cm tall. It blooms between April and September. It grows in forests of *Pinus brutia*, *Pinus nigra*, *Quercus pubescens* and on limestone and volcanic slopes. The leaves are used as herbal tea.[1–3] *Salvia* species contain many bioactive compounds that can be classified as monoterpenes, diterpenes, triterpenes and phenolic compounds. The most common monoterpenes are; α-thujone and β-thujone, 1,8-cineole and camphor, diterpenes; carnosol, carnosic acid, rosmadial and manool, triterpenes; oleanolic and ursolic acids. Phenolic components are phenolic acids such as caffeic, vanillic, ferulic and rosmarinic acids, and flavonoids such as luteolin, apigenin and quercetin.[4–6] Sage taxa grown in Turkey were classified by Başer[7] (2002) according to the main components of essential oil. According to this classification, *Salvia tomentosa* belongs to the pinene group; the plant which contains 0.6–1.3% essential oil, contains 6–29% α-pinene and 5–33% β-pinene.[7–9]

The use of herbal medicines for therapeutic purposes, the fact that fragrant plants constitute the main raw material of perfumery, food and cosmetics industry, and the emergence of new areas of use increases the demand for medicinal and aromatic plants day by day and causes the industrial sector to consume these plants as raw materials intensively.[10, 11] Like many other medicinal plants, *S. tomentosa* was extensively collected from its natural habitat, and this careless collection has caused some plants to become extinct. Therefore, these plants are grown in order to promote sustainable and standard agricultural production.[12] It is stated that the essential oil of *Salvia tomentosa* aerial parts significantly inhibits the growth of Gram-positive and Gram-negative bacteria tested except *Pseudomonas aeruginosa* in the literature.[13] In another study, it is stated that the plant has antioxidative effects.[14] In this study, the biological activity and phytochemical content of the parts of *Salvia tomentosa* consumed as tea are presented in detail.

2. Materials and Methods

2. 1. Plant Material and Extraction Protocol

Salvia tomentosa is a perennial plant. The stem of the plant consists of upright, four-sided, usually branched, hairy and sessile glands extending up to 1 m. Leaves simple, narrowly oblong to ovate, 2–11 x 0.8–5 cm, rounded to cordate at base, entire to oblong-cubic. The petiole is 1.7–5.5 cm. Verticillasters 4–10 flowered, distant or dense at the top. The crowns are lilac, purple or white. They usually grow in the forests of *P. brutia* and *P. nigra*, on limestone or magmatic slopes, at an altitude of 90–2000 m. *Salvia tomentosa* was collected in 2020 from Ardıçlı village, Keçiborlu, Isparta, Turkey (37° 48´ 8.9928" and 30° 12´ 13.4676"). Separate aqueous extracts were prepared by using the flower (ST-flower), leaf (ST-leaf) and stem (ST-stem) parts of the plant and the total aerial parts of the plant (flower-leaf-stem mixture, ST-mix). The plant, which was allowed to dry well in the shade. Each separated part was crushed into powder with the help of a special blender (Waring 32BL80, Connecticut, USA). The water extract from the samples was obtained using the maceration method. Water was placed in glass bottles and heated in a water bath until boiling. The powder sample was added to glass bottles. The plant-water suspension in glass bottles was incubated in the dark at room temperature for 24 hours. At the end of the incubation, the mixture in the glass bottle was passed through filter paper (Whatman, Grade 589/1) and the plant particles were separated from the filtrate. In order to remove the solvent in the filtrate, the filtrate was placed in the balloon of the rotary evaporator (Heidolph, 562-00000-00-0, Germany) under vacuum. Solutions containing dense extract and poured into glass petri dishes were kept in dark at room temperature until the solvent evaporated completely (2–3 days). Extracts obtained in dry form were stored at +4 °C to be used in qualitative, quantitative and biological activity analyses.[15]

2. 2. Qualitative and Quantitative Analysis of Phytochemicals in Extracts

Determination of Total Phenolic Acid amount

Total phenolic acid content was measured by the modified Folin-Ciocalteu method. The reference material caffeic acid was used in order to compare the amounts of phenolic substances in the extracts in the analysis. A standard curve of gallic acid was created for this purpose. The standard solutions of gallic acid at different concentrations (100, 200, 400, 600, 800 µg/mL) were prepared. Folin-Ciocalteu reagent was added to the prepared solutions of plant extract, caffeic acid and standards in the analyses. Sodium carbonate was added and incubated for 2 hours at room temperature. The absorbance of the mixture was measured spectrophotometrically at 760 nm against water. The total amount of phenolic acid in 1 mg of the extracts was calculated as gallic acid equivalent (µg GAE/mg extract) using the absorbance obtained as a result of the analysis of the plant extracts and the straight equation obtained from the gallic acid standard curve.[16]

Determination of Components in Extracts and Concentrations

Qualitative and quantitative determination of the components in the extracts were made by LC-MS/MS system in the laboratories of Dicle University Science and Technology Application and Research Center. The reverse phase UHPLC system used in the LC-MS/MS system preferred in the analysis; It consisted of an autosampler (SIL-30AC), a column furnace (CTO-10ASvp), a gradient pump system (LC-30AD) and a degaser (DGU-20A3R). Chromatographic separation was performed using a column (Agilent Poroshell 120 EC-C18) (150 mm×2.1mm, 2.7 µm). The column temperature was set to 40 °C. The elution gradient was composed of mobile phase A (ultra pure water+5 mM ammonium formate+0.1% formic acid) and mobile phase B (ultrapure water +5 mM ammonium formate+0.1% formic acid).[15]

The gradient elution profile used was as follows: 20–100% B (0–25 min), 100% B (25–35 min), 20% B (35–45 min). In addition, the mobile phase flow rate and injection volume were determined as 0.5mL/min and 5 µL, respectively. For mass spectrometry detection of the LC-MS/MS system used, a Shimadzu LCMS-8040 model sequential mass spectrometer equipped with an electrospray ionization source operating in both positive and negative modes was used. LC-ESI-MS/MS data was acquired and processed with LabSolutions software (Shimadzu). MRM (multiple reaction monitoring) mode was used for the quantification of phytochemicals. The MRM method has been optimized for the selective detection and quantification of phytochemicals based on the screening of specific major ion-fragmentation ion transitions. Collision energies (CE) are optimized to achieve optimum phytochemical fragmentation and maximal migration of desired fragmentation ions. Applied MS operating conditions: drying gas (N_2) flow, 15 L/min; nebulizer gas (N_2) flow, 3 L/min; DL temperature, 250 °C; The heat block temperature was determined as 400 °C and the interface temperature as 350 °C. The amounts of phenolic acid species whose amounts were determined in the extracts were expressed as mg-analyte/g-extract.[17]

2. 3. Determination of Biological Activity of Extracts

Free Radical Scavenging Activity

Free radical scavenging activity of *S. tomentosa* and standard antioxidant substances (Butylated Hydroxy Toluene-BHT and Vitamin C) used in the study were determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. A calibration curve was created using different concentrations of DPPH solution. Using the curve equation obtained from the calibration chart and sample absorbances, it was determined how much DPPH radical each sample inhibited. Extracts and standards were added to the solution containing DPPH radical and their absorbance was measured at 517 nm. Concentrations from the measured absorbances were determined by the calibration curve. % inhibitions were determined using the formula below.[15]

$$\text{Inhibition rate (\%)} = [(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}) / \text{Absorbance}_{\text{control}})] \times 100$$

Total Antioxidant Status (TAS), Total Oxidant Status (TOS) Levels

Total Antioxidant Status (TAS) levels were measured using spectrophotometric commercial kits (Rel Assay, Gaziantep, Turkey). In order to determine TAS levels, 0.5–2 mmol/L Trolox was used as a standard. TAS levels were determined as mmol Trolox Equivalent/L according to the calibration graph taken from the ELISA reader using three standards (0.5 mmol/L Trolox, 1mmol/L Trolox and 2 mmol/L Trolox) according to the kit protocol.

TOS levels were determined at 540 nm by spectrophotometric method using commercial kits (Rel Assay, Gaziantep, Turkey). To determine TOS levels, a calibration curve was created using three H_2O_2 standards (5 μmol/L, 10 μmol/L and 20 μmol/L) according to the kit protocol. The results were determined as μmol H_2O_2 Equiv./L. Oxidative stress index (OSI) levels were determined by dividing the TOS level of each sample by the TAS level.[18]

Evaluation of Antimicrobial Efficacy of Extracts

Microdilution method was used to determine the antimicrobial activity of extracts of *Salvia tomentosa*. Stock solutions were prepared from all extracts at a concentration of 60 mg/mL. 1/2, 1/4, 1/8, 1/16, 1/32 and 1/64 dilutions were prepared from these stock solutions, respectively. 10^7 CFU/mL of 1% (v/v) bacterial solutions were added to the same volume of extract solutions from the dilutions and incubated at 37°C for 24 hours. Minimum inhibitory concentration (MIC) is inhibited value after incubation; The lowest extract concentrations at which bacterial growth was inhibited were evaluated by both measuring at 450 nm and inoculation into the medium.[19]

2. 4. Statistical analysis

The extracts used in the study were prepared in triplicate, and the measured results were expressed as mean ± standard deviation (mean ± SD). SPSS 20 package program was used in the statistical analysis of the data in this study. Differences between groups were determined by one-way analysis of variance (one-way ANOVA). The difference between which groups was determined at $p < 0.05$ significance value according to Duncan's multiple range test.

3. Results

Plants have many activities such as antioxidant, antimicrobial and anticarcinogenic effects due to the secondary metabolites they synthesize and possess.[20] The amount of phenolic acid, which is one of the secondary metabolites in *S. tomentosa*, was determined by modifying the method.[16] The amounts of phenolic substances expressed as mg gallic acid equivalent (GAE)/g-extract in the samples were interpreted by comparing the amount of phenolic substance in the extracts with the amount of phenolic substance in the caffeic acid. The data obtained are presented in Figure 1. It was determined that the total amount of phenolic substance was the least in the aqueous extract obtained from the flower part of the plant. The extracts of the stem (ST-stem), leaf (ST-leaf), flower (ST-flower) and aerial parts of the plant (stem-leaf-flower; ST-mix) have high total phenolic content close to caffeic acid were determined. These data show that especially the stem and leaf of the plant are rich in phenolic content. The amount of phenolic acid in ST-flower was found to be statistically significantly lower ($p < 0.05$) than the others.

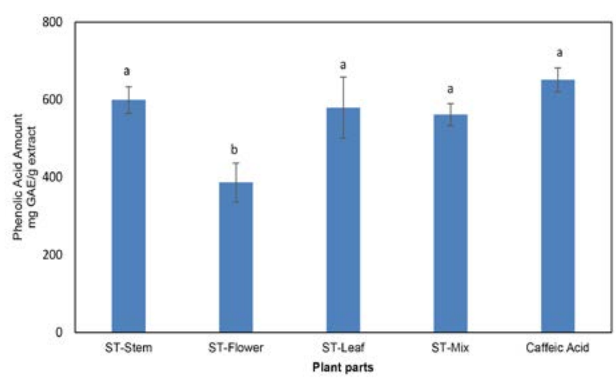

Figure 1. The total amount of phenolic substances detected in the extracts and the standard. ST-Stem; Aqueous extract of stem of *Salvia tomentosa*, ST-Flower; Aqueous extract of flower of *Salvia tomentosa*, ST-Leaf; Aqueous extract of leaf of *Salvia tomentosa*, ST-Mix; Aqueous extract of a mixture of stem, leaf and flower of *Salvia tomentosa*. (a, b) The difference between the means labelled with different letters is statistically significant ($p < 0.05$).

LC-MS/MS system was used to determine the phytochemicals contained in *Salvia tomentosa*. The components of the aerial parts of the plant are shown separately in Table 1. When the amount of components in the extracts is examined in general, it can be said that especially three components are more intense than *S. tomentosa* extracts compared to other components. These components are rosmarinic acid, quinic acid and fumaric acid, respectively. When the extracts were analyzed separately, the presence and concentrations of 16 components in ST-stem extract, 15 components in ST-flower extract, and 17 components in ST-leaf and ST-mix extracts were determined. Hesperidin, quinic acid, fumaric acid, aconitic acid, protocatechuic acid, gentisic acid, protocatechuic aldehyde, chlorogenic acid, caffeic acid, salicylic acid, apigenin, o-coumaric acid, rosmeric acid, cosmosiin, luteolin are chemicals found in all parts of the plant.

Table 1. Types and amounts of some phytochemicals in *Salvia tomentosa* extract

	Concentration (mg analyte/g extract)			
Plant parts	**ST-Stem**	**ST-Flower**	**ST-Leaf**	**ST-Mix**
Hesperidin	5.507	0.081	0.718	2.060
Quinic acid	25.628	29.217	32.247	37.750
Fumaric aid	10.888	0.923	7.592	11.155
Aconitic acid	0.525	0.677	0.373	0.391
Protocatechuic acid	0.851	1.829	0.694	0.568
Gentisic acid	0.231	0.546	0.209	0.168
Protocatechuic aldehyde	0.883	0.712	1.046	0.844
Chlorogenic acid	1.632	1.242	1.429	1.757
Caffeic acid	2.247	0.560	2.245	2.339
Salicylic acid	1.596	2.102	1.473	0.799
Apigenin	0.003	0.006	0.057	0.035
o-Coumaric acid	0.042	0.037	0.056	0.032
Rosmarinic acid	50.235	61.590	89.892	83.625
Cosmosiin	0.077	0.931	0.793	0.560
Luteolin	0.017	0.035	0.348	0.210
Hesperetin	0.071	ND	0.025	0.077
Naringenin	ND	ND	0.011	0.011

ND: Not detected. ST-Stem; Aqueous extract of stem of *Salvia tomentosa*, ST-Flower; Aqueous extract of flower of *Salvia tomentosa*, ST-Leaf; Aqueous extract of leaf of *Salvia tomentosa*, ST-Mix; Aqueous extract of a mixture of stem, leaf and flower of *Salvia tomentosa*

It was determined that the major component determined in all of the extracts obtained from *S. tomentosa* was rosmarinic acid. The amount of rosmarinic acid is quite high when compared to other ingredients.

One of the commonly used methods to determine the antiradical activity of a sample is the method using the DPPH radical. In this method, sample (extract) at certain concentrations is added to the DPPH radical dissolved at a certain concentration in a suitable solvent. The percentages of inhibition of DPPH radical by using the solutions of extracts and standards (BHT and Vitamin C) of *S. tomentosa* obtained at the same concentrations are presented in Figure 2. When the data are examined, it is seen that the ST-leaf extract is statistically significantly ($p < 0.05$*) lower than all other extracts. It can be mentioned that the* other extracts (ST-stem, ST-flower and ST-mix) have close radical scavenging effects, such as BHT and Vitamin C, which are antioxidants used for comparison.

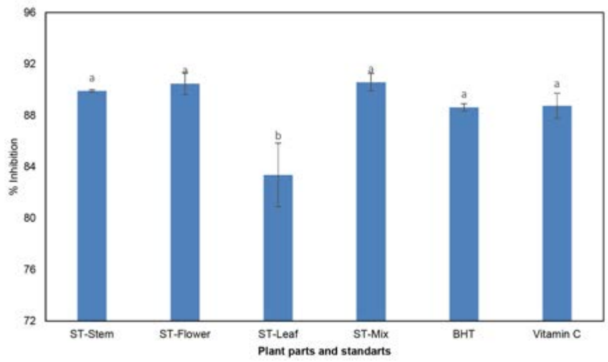

Figure 2. DPPH radical inhibition ratios of the extracts. ST-Stem; Aqueous extract of stem of *Salvia tomentosa*, ST-Flower; Aqueous extract of flower of *Salvia tomentosa*, ST-Leaf; Aqueous extract of leaf of *Salvia tomentosa*, ST-Mix; Aqueous extract of a mixture of stem, leaf and flower of *Salvia tomentosa*. BHT; Butylated Hydroxy Toluene. (a,b) The difference between the means labelled with different letters is statistically significant ($p < 0.05$).

Spectrophotometric method were used to determine the antioxidant and oxidant capacity of extracts of *S. tomentosa*. Oxidative stress indexes of the samples were determined using these antioxidant and oxidative capacity datas. It was evaluated that the antioxidant activity of the extracts with low oxidative stress index data was higher. Antioxidant and oxidant capacity, oxidative stress indexes values were shown in Figure 3.

As a result of the analysis, it was determined that the extract with the highest antioxidant capacity among the extracts of *S. tomentosa* belonged to ST-stem and ST-mix. TAS levels of ST-flower and ST-leaf extract were found to be statistically significantly ($p < 0.05$*) lower than other extracts and Vitamin C.* When TOS levels were examined, it was determined that the TOS level of ST-stem extract was the lowest, while the TOS level of ST-flower extract was the highest. OSI values obtained by dividing the TOS levels of the extracts of *S. tomentosa* by the TAS levels, express the capacity of a plant to create oxidative stress in the organism from which it is taken. The OSI values of the ST-stem extract, which were determined to have the highest TAS levels and the lowest TOS levels, were found to be the lowest. It was observed that the extract with the highest OSI value was ST-leaf.

When the analyzes made on behalf of the antioxidant activity of the extracts were examined in general, the phenolic substance amounts, DPPH scavenging effects and TAS levels of the ST-stem and ST-mix extracts of the

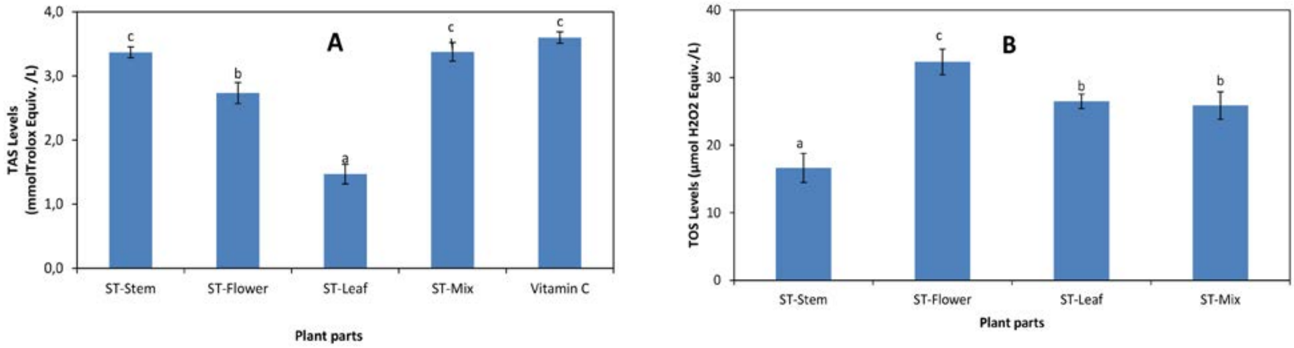

Figure 3. A: Total antioxidant capacities of extracts of *Salvia tomentosa*. B: Total oxidant capacities of extracts of *Salvia tomentosa* (a, b, c) The difference between the means labelled with different letters is statistically significant ($p < 0.05$)

plant were found to be higher than the other leaf and flower extracts.

The antimicrobial activity of extracts of *S. tomentosa* was determined by microdilution method and given in Table 2. When the data were examined, it was found that the lowest 1/16 dilution of ST-flower extract was effective on *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Candida albicans*; The lowest 1/8 dilution of ST-leaf extracts was effective on *Listeria monocytogenes*, *Salmonella typhimurium* and *Candida albicans*; The lowest 1/16 dilution of ST-Stem extracts was effective on all agents (*L. monocytogenes*, *S. typhimurium*, *S. aureus*, *C. albicans*) except *E. coli* O157:H7; All dilutions of ST-mix extract were found to be effective on *L. monocytogene* and *C. albicans*.

Based on the data obtained from Table 2, considering the doses corresponding to each dilution ratio, the MIC values of the extracts are presented in Table 3. It was determined that ST-mix was the most effective inhibitory extract for all microorganisms used. This data shows that

Table 2. Antimicrobial activity of *Salvia tomentosa* extracts

Extract	Dilution Rate	*E. coli*	*L. monocytogenes*	*S typhimurium*	*S. aureus*	*C. albicans*
ST-Flower	1/2	+	+	+	+	+
	1/4	+	+	+	+	+
	1/8	+	+	+	+	+
	1/16	+	–	–	+	+
	1/32	–	–	–	–	–
	1/64	–	–	–	–	–
ST-Leaf	1/2	+	+	+	–	+
	1/4	+	+	+	–	+
	1/8	–	+	+	–	+
	1/16	–	–	–	–	+
	1/32	–	–	–	–	–
	1/64	–	–	–	–	–
ST-Stem	1/2	+	+	+	+	+
	1/4	+	+	+	+	+
	1/8	+	+	+	+	+
	1/16	–	+	+	+	+
	1/32	–	–	–	–	–
	1/64	–	–	–	–	–
ST-Mix	1/2	+	+	+	+	+
	1/4	+	+	+	+	+
	1/8	+	+	+	+	+
	1/16	+	+	+	–	+
	1/32	–	+	–	–	+
	1/64	–	+	–	–	+

ST-Stem; Aqueous extract of stem of *Salvia tomentosa*, ST-Flower; Aqueous extract of flower of *Salvia tomentosa*, ST-Leaf; Aqueous extract of leaf of *Salvia tomentosa*, ST-Mix; Aqueous extract of a mixture of stem, leaf and flower of *Salvia tomentosa* the use of the aerial part of the plant together is more beneficial in terms of providing antimicrobial activity.

Table 3. MIC values of extracts

Microorganisms	ST-Flower	ST-Leaf	ST-Stem	ST-Mix
E. coli	3.75	15	7.5	1.875
L. monocytogenes	7.5	7.5	3.75	0.9375
S. typhimurium	7.5	7.5	3.75	1.875
S. aureus	3.75	–	3.75	7.5
C. albicans	3.75	3.75	3.75	0.9375

ST-Stem; Aqueous extract of stem of *Salvia tomentosa*, ST-Flower; Aqueous extract of flower of *Salvia tomentosa*, ST-Leaf; Aqueous extract of leaf of *Salvia tomentosa*, ST-Mix; Aqueous extract of a mixture of stem, leaf and flower of *Salvia tomentosa*. MIC; Minimum concentration at which microorganism growth is inhibited

4. Discussion

Metabolites consist of intermediate products formed by the effect of metabolic activities. Metabolites have functions such as energy generation, building blocks, stimulating enzymes and additionally inhibiting them, being under the influence of catalyst, defense and other organisms, giving odor. Primary metabolites are directly involved in processes involved in normal growth, development and reproduction. Although secondary metabolite species are not directly related to these processes, they contribute to the maintenance of these processes by the plant. Secondary metabolites may contribute to the adaptation of the species to its specific conditions. These compounds, which enable plants to have their unique color, taste, aroma and texture, also play an important role in many metabolic events that occur in the plant.[21, 22] In addition to contributing to the resistance mechanism in the body at the time of disease in plants, they are produced as a defense mechanism for plants and increases as stress increases. Phenolic compounds are synthesized during the normal development of plants and when the plant is sick and injured. In addition, phenolic production is dependent on environmental conditions and are synthesized when exposed to UV rays, at low temperatures and during periods when nitrogen, phosphate and iron content are low.[21, 22]

In a study, the phenolic composition and antioxidant properties of wild and cultured *S. tomentosa* were investigated. Total phenolics of *S. tomentosa* were found between 49.27 and 66.15 mg GAE/dw. The total phenolic content of the grown samples was determined to be higher than that of the wild samples. 17 different phenolic compounds containing 7 phenolic acids and 10 flavonoids were identified and quantified in *S. tomentosa*. Rosmarinic acid was measured as the main component of *S. tomentosa*. It is followed by caffeic acid, morin, p-coumaric acid and myricetin.[23] In the present study, the phenolic acid amounts of plant parts ranged from 386.48 to 599.24 mg GAE/g extract. Identified and quantified phenolic substances are respectively, rosmarinic acid (50.235 mg/g extract), quinic acid (25.628 mg/g extract), fumaric acid (10.888 mg/g extract), hesperidin (5.507 mg/g extract), caffeic acid (2.247 mg/g extract), chlorogenic acid (1.632 mg/g extract) and salicylic acid (1.596 mg/g extract).

Rosmarinic acid is one of the most abundant phenolic substances in all *S. tomentosa* extracts. In many studies, it is stated that rosmarinic acid has antiviral, antibacterial, anti-inflammatory and antioxidant effects.[24] In a study, biotechnological production of rosmarinic acid, which is found in high amounts in *Salvia officinalis*, has been suggested.[25] A high amount of rosmarinic acid is also expected in *S. tomentosa*, another species from the *Salvia* family. It can be said that rosmarinic acid, the major component of *S. tomentosa*, has an important role in the formation of antioxidant and antimicrobial activity of the extracts.

Fumaric acid and its derivatives are among the well-known antioxidants that provide various health benefits due to their potent free radical scavenging properties, anti-inflammatory and immunomodulatory effects.[26,27] These data in the literature may be related to the wound healing activity of *Salvia tomentosa* due to its high fumaric acid content.

When the analysis made on behalf of the antioxidant activity of the extracts are examined in general, the phenolic substance amounts, DPPH scavenging effects and TAS levels of the ST-stem and ST-mix extracts of the plant were found to be higher than the other ST-leaf and ST-flower extracts. It is seen that the antioxidant active substance levels of hesperidin and fumaric acid in ST-stem extract, and hesperidin, fumaric acid and rosmarinic acid in ST-mix extract are higher than other extracts. Among these components, besides the antioxidant activities of fumaric acid and hesperidin, antimicrobial activities are prominent in the literatüre.[28–30]

In addition, the antioxidative properties of ST-flower extract (except for DPPH radical scavenging activity) were found to be lower than other extracts, while TOS levels were found to be high. This may be due to the fact that the levels of hesperidin, fumaric acid and caffeic acid in the flower extract are lower than in other extracts. While the antioxidative property of ST-flower extract was low, DPPH radical scavenging activity was found to be high. Some antioxidants act by preventing the formation of free radicals, while other antioxidants act by scavenging existing radicals. Therefore, there may be more components in the flower extract that increase the radical scavenging activity of the extracts. It is seen that the amounts of some components (aconitic acid, protocatechuic acid, gentisic acid, salicylic acid and cosmosiin) are higher in the flower extract.

The second component, which was determined to be higher in ST-stem and ST-mix extracts compared to other extracts, is hesperidin. Hesperidin, which has a flavonoid structure, protects the organism against oxidants with its strong antioxidant property in biological systems[31], and also protects the body against infections with its antiviral and antibacterial activity.[32] Recent studies on wound healing show that hesperidin is very effective in healing wounds that develop due to various diseases or that may occur in daily life[25]. For thousands of years, extract of St. John's Wort (*Hypericum perforatum*) flour obtained with olive oil have been used traditionally in the treatment of burns and open wounds and in the removal of their scars. Considering that one of the major components of St. John's wort is hesperidin[33], the effectiveness of hesperidin can be better understood in terms of wound healing activity. From this point, it is expected that the extract of *Salvia tomentosa*, which contain plenty of hesperidin, prepared with water, have antimicrobial effects.

It was determined that the ST-mix extract showed stronger antioxidant and antimicrobial activity than those obtained from parts of the plant. However, it was determined that the components of the ST-flower, ST-stem and ST-leaf extracts and the components of the ST-mix extract were largely similar. On the other hand, it was observed that there were significant differences in the proportion of the same type of components. This probably caused the different antimicrobial and antioxidant activities of each extract in the components they contained in different proportions.

5. Conclusions

The phenolic and flavonoid contents of the aqueous extracts of the ST-flower, ST-stem, ST-leaf and ST-mix parts of *S. tomentosa* were determined qualitatively and quantitatively. The phenolic content of the fractions was found to be quite high except for ST-flower. Rosmarinic acid, quinic acid and fumaric acid are phenolic substances found in high amounts in all parts of the plant. Total antioxidant levels of ST-stem and ST-mix are quite high. It has been determined that plant parts are very effective radical scavengers expect for ST-leaf. In addition, it was determined that the most effective extracts from *S. tomentosa* in terms of antibacterial activities were ST-stem and ST-mix. It has been interpreted that the antimicrobial and antioxidant activities between the extracts may be due to the component differences in their contents. It is thought that the obtained data will contribute to the literature in order to explain the phytotherapeutic activity of *Salvia tomentosa Miller*.

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6. References

1. V. Papageorgiou, C. Gardeli, A. Mallouchos, M. Papaioannou, M. Komaitis, *J. Agr. Food Chem.* **2008**, *56*, 7254–7264. **DOI:**10.1021/jf800802t
2. M.Z. Haznedaroglu, N.U. Karabay, U. *Fitoterapia* **2001**, *72*, 829–831. **DOI:**10.1016/S0367-326X(01)00335-5
3. Y. Lu, L. Foo, *Phytochemistry* **2002,** *59*, 117–140. **DOI:**10.1016/S0031-9422(01)00415-0
4. N. Tan, S.T. Yazıcı, Y.C. Yeşil, B. Demirci, Tan E. *Rec. Nat. Prod.* **2017**, *11*, 456–461. **DOI:**10.25135/rnp.57.16.12.086
5. M. Jakovljević, S. Jokić, M. Molnar, M. Jašić, J. Babić, H. Jukić, I. Banjari, *Plants* **2019**, *8*, 1–30. **DOI:**10.3390/plants8030055
6. E. Hanlidou, R. Karousou, D. Lazari, *Chem. Biodivers.* **2014**, *11*, 1205–1215. **DOI:**10.1002/cbdv.201300408
7. K.H.C. Başer, *Pure Appl. Chem.* **2002**, *74*, 527–545. **DOI:**10.1351/pac200274040527
8. T. Aşkun, K.H.C. Başer, G. Tümen, M. Kürkçüoğlu, *Turk. J. Biol.* **2010**, *34*, 89–95. **DOI:**10.3906/biy-0809-2
9. G. Ozek, T. Ozek, K.H.C. Baser, E. Hamzaoglu, A. Duran, *Chem. Nat. Compd.* **2007**. *43*, 667–671. **DOI:**10.1007/s10600-007-0224-9
10. Z. Dumanoğlu, S. Mokhtarzadeh. *TURKJANS* **2020**, *7*, 596–602. **DOI:**10.30910/turkjans.663891
11. S. Mokhtarzadeh, B. Demirci, H.G. Ağalar, K.M. Khawar, N. Kırımer, *Rec. Nat. Prod.* **2019,** *13*, 121–128. **DOI:**10.25135/rnp.86.18.04.105
12. I. Erdogan-Orhan, E. Baki, S. Senol, G. Yilmaz, *J. Serb. Chem. Soc.* **2010**, *75*, 1491–1501. **DOI:**10.2298/JSC100322115E
13. M.Z. Haznedaroglu, N.U. Karabay, U. Zeybek. *Fitoterapia* **2001**, *72*, 829–831. **DOI:**10.1016/S0367-326X(01)00335-5
14. B. Tepe, D. Daferera, A. Sokmen, M. Sokmen, M. Polissiou, *Food Chem.* **2005**, *90*, 333–340. **DOI:**10.1016/j.foodchem.2003.09.013
15. Ö. Hazman, L. Aksoy, A. Büyükben, R. Kara, M. Kargıoğlu, İ.H. Cigerci, M.A. Yılmaz, *Indian J. Exp. Biol.* **2022**, *60,* 743–752. **DOI:**10.56042/ijeb.v60i10.55070
16. K. Slinkard, V.L. Singleton, *Am. J. Enol. Vitic.* **1977**, *28*, 49–55. **DOI:**10.5344/ajev.1974.28.1.49
17. M.A. Yılmaz, *Ind. Crops Prod.* **2020,** *149*, 112347. **DOI:**10.1016/j.indcrop.2020.112347
18. L. Aksoy, İ. Güzey, M. Düz, *Turk. J. Pharm. Sci.* **2022**, *19*, 76–83. **DOI:**10.4274/tjps.galenos.2021.86422
19. S.S. Shaikh, A.S. Bawazir, B.A. Yahya, *Turk. J. Pharm. Sci.* **2022**, *19*, 145–152. **DOI:**10.4274/tjps.galenos.2021.02058
20. B. Tepe, D. Daferera, A. Sokmen, M. Sokmen, M. Polissiou, *Food Chem.* **2005,** *90,*333–340. **DOI:**10.1016/j.foodchem.2003.09.013
21. S. Neugart, S. Baldermann, F.S. Hanschen, R. Klopsch, M. Wiesner-Reinhold, M. Schreiner, *Scient. Hortic.* **2018,** *233*, 460–478. **DOI:**10.1016/j.scienta.2017.12.038
22. M. Wink, *Nat. Prod. Commun.* **2008**, *3*, 1205–1216. **DOI:**10.1177/1934578X0800300801
23. C. Dinçer, I. Tontul, I. B. Çam, K.S. Özdemir, A. Topuz, H.Ş. Nadeem, S.T. Ay, R. S. Göktürk. *Turk. J. Agric. For.* **2013**, *37*, 561–567. **DOI:**10.3906/tar-1211-72

24. H. Guan, W. Luo, B. Bao, Y. Cao, F. Cheng, S. Yu, Q. Fan, L. Zhang, Q. Wu, M. Shan. *Molecules* **2022**, *27*, 3292. **DOI:**10.3390/molecules27103292
25. M. Petersen, M.S. Simmonds, *Phytochemistry* **2003**, *62*, 121–125. **DOI:**10.1016/S0031-9422(02)00513-7
26. S.A. Scuderi, A. Ardizzone, I. Paterniti, E. Esposito, M. Campolo. *Antioxidants* **2020**, *9*, 630. **DOI:**10.3390/antiox9070630
27. A. Shakya, G.K. Singh, S.S. Chatterjee, V. Kumar, *J. Intercult. Ethnopharmacol.* **2014**, *3*, 173–178. **DOI:**10.5455/jice.20140912021115
28. U. Wollina, *Indian Dermatol. Online J.* **2011**, *2*, 111. **DOI:**10.4103/2229-5178.86007
29. M. Vabeiryureilai, K. Lalrinzuali, G.C. Jagetia, *Burns.* **2022**, *48*, 132–145. **DOI:**10.1016/j.burns.2021.04.016
30. W. Li, A.D. Kandhare, A.A. Mukherjee, S.L. Bodhankar, *Excli. J.* **2018,** *17*, 399–419. **DOI:** 10.17179/excli2018-1036
31. P.K. Wilmsen, D.S. Spada, M. Salvador, *J. Agric. Food Chem.* **2005**, 53, 4757–4761. **DOI:**10.1021/jf0502000
32. H.W. Kim, H.J. Woo, J.Y. Yang, J.B. Kim, S.H. Kim, *Int. J. Mol. Sci.* **2021**, *17*, 10035. **DOI:**10.3390/ijms221810035
33. C. Sarikurkcu, M. Locatelli, A. Tartaglia, V. Ferrone, A.M. Juszczak, M.S. Ozer, B. Tepe, M. Tomczyk, *Molecules* **2020**, *6*, 1202. **DOI:**10.3390/molecules25051202

Povzetek

V tej študiji smo kvalitativno in kvantitativno določili antioksidativno aktivnost, protimikrobno učinkovitost in sestavine nadzemnih delov (listov, stebla, cvetov in mešanice) rastline *Salvia tomentosa* Miller. Vodne izvlečke *S. tomentosa* (ST) smo pripravili z uporabo cvetov, listov in stebel ter vseh nadzemnih delov rastline (mešanica cvetov, listov in steblo). Določili smo aktivnost odstranjevanja radikalov, skupni antioksidantivni/oksidativni status, protimikrobni potencial, fenolne snovi in kvalitativne/kvantitativne analize sestavin v ekstraktih. Ugotovljeno je bilo, da je količina fenolnih kislin v ST-steblu (599 ± 34 mg ekvivalenta galne kisline (GAE)/g ekstrakta) blizu količini standardne snovi kofeinske kisline (651 ± 31 mg GAE/g ekstrakta). Skupni antioksidativni statusi mešanice ST (3,4 ± 0,1 mmol ekvivalenta troloxa /L), stebla ST (3,4 ± 0,1 mmol ekvivalenta troloxa /L) in naravnega antioksidanta vitamina C (3,6 ± 0,1 mmol ekvivalenta troloxa /L) se statistično niso razlikovali. Izvleček, pridobljen z uporabo nadzemnih delov *S. tomentosa* (cvet-steblo-listi), je pokazal močnejše antioksidativno in protimikrobno delovanje kot vodni izvlečki, pridobljeni ločeno iz cvetov, stebla in listov rastline. Ugotovljeno je bilo, da so si sestavine ločeno pripravljenih izvlečkov cvetov, stebla in listov ter sestavine izvlečka, pridobljenega iz nadzemnih delov, v veliki meri podobne. Hkrati pa je bilo ugotovljeno, da obstajajo pomembne razlike v prisotnosti teh sestavin.

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