Total phenolic and Flavonoid Contents, Antimicrobial and Antioxidant Properties of *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* Boiss & Bal from Turkish Flora

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ABSTRACT

Objective: Some *Salvia* species have been used in folk medicine for the last centuries. The purpose of this study is to screen the possible antimicrobial and antioxidant properties as well as total phenolic and flavonoid contents of *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia*.

Methods: In order to chromatographically analyse resveratrol and flavonoid content of methanolic extracts of *Salvia* species HPLC was used. Total phenolic constituents of methanolic extracts of these plants were performed. The DPPH and ABTS.+ scavenging ability of methanolic extracts of *Salvia* species aerial parts were determined. Antimicrobial activities of extracts were determinated by the disc diffusion method against test microorganisms.

Results: The total phenolic content was higher in *Salvia verticillata* L. var. *amasiaca* with value of 119.45 mg GAE/g extre than *Salvia microstegia* (118.08 mg GAE /g extre). Resveratrol content was also higher in *Salvia verticillata* L. var. *amasiaca* (3.65 µg/ml) than *Salvia microstegia* (0.5 µg/ml). Just as kaempferol was a major flavonoid content for *Salvia verticillata* L. var. *amasiaca* (350.45 µg/ml), katesin was a major flavonoid content for *Salvia microstegia* (315.5 µg/ml). Additionally it was determined that *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* had antimicrobial and antioxidant properties at different rates. However, these plants did not exhibit any antimicrobial activity against *Escherichia coli* ATCC 11229, *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae*.

Conclusions: *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* possess antibacterial and antioxidant properties. Moreover, these plants have high total phenolic contents and flavonoid contents. Accordingly, these plants can be used for food and in industries

KEY WORDS: ***Salvia verticillata*** ***Salvia microstegia*** **Antimicrobial** **Antioxidant**

Introduction

The genus *Salvia*, with about 900 species throughout the world, as one of the most widespread members of the Lamiaceae family. Although *Salvia* species are abundant in the Turkish flora, as being represented by 88 species and 93 taxa and 45 of which are endemic [1], only biological activities and bioactive properties of a few are available in the literature while most have yet to be tapped into. Among them, the species evaluated here are aromatic plants widely distributed in Anatolia and Mediterranean region where they are commonly known as "adacayi", "salba" and/or "dadirak" by the locals. They are commonly used in local folk medical practices [2]. Plants evaluated here together with the other members of the genus *Salvia* used in diarrhea, gonorrhea and hemorrhoids, eye diseases and as an antiseptic, antispasmodic and stomachic[3]. Additionally, many *Salvia* species are used in herbal tea and for food flavouring, as well as in cosmetics, perfumery and the pharmaceutical industries throughout the world [4].

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Antioxidant activities of the many members of the genus *Salvia* were reported in earlier studies. Additionally, previous reports concerning the biological activities of *Salvia* species native to the Turkish flora confirm that this genus has great potential, especially in the antioxidant system, and for the food and cosmetic industries [5, 6, 7, 8]. Additionally, we also aimed to investigate the biological activities of two *Salvia* species in this study.

Materials and methods

Plant samples

Plant samples were obtained from the Ardahan region (eastern part of Turkey) in June 2011 (Table 1). Plant samples were identified by Flora of Turkey and The East Aegean Island (by Dr. Ahmet Ilcim) [9] and voucher specimens were deposited in the herbarium of the Department of Biology, Faculty of Art and Science, Kahramanmaraş Sutcu Imam University, Turkey. Different parts of plant such as the root, leaf, stalk, flower, and aerial parts were cleaned from debris, dried in the shate at room temperature and powdered.

Preparation of extracts

The extraction method used is a modified version of the method developed by Ulukanlı and Akkaya [10]. Powdered plant materials such as root, leaf, stalk, flower, and aerial parts (10 g) were loaded to a soxhlet apparatus. The extraction was carried out using five solvents; purified chlorophorm (polarity index: 4.1), hexane (pi: 0), acetone (pi: 5.1), ethanol (pi: 5.2) and methanol (pi: 5.1) (300 ml) for 6 h. The resulting mixture was then filtered and concentrated under vacuum at 40 °C (Buchi, Rotavapor R-210, Labortechnik, AG, Flavil, Switzerland). Filter-sterilized and concentrated extracts were refrigerated (-18 °C) until use.

Resveratrol and flavonoid contents

The HPLC method used is a modified version of the method developed by Tsao and Yang [11]. In order to analyse the flavonoid content of *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* (methanolic extracts of aerial parts) , ALTIMA C18 (15x4.6 mm, GRACE, USA) HPLC colon was used. Methanol/water/acetonitrile mix (46/46/8, v/v/v) which include 1% acetic acid was also used as mobile phase and this mobile phase was filtrated by 0.45 µm membrane filter. 280 nm (for catechin and naringin), 254 nm (for rutin, myricetin and quercetin), 306 nm (for resveratrol) and 265 nm (for kaemoferol) were used as wave lengths and were HPLC seperated. After these processes, flavonoids were measured by DAD (Diode-arrey Detector). All chromatographic processes were done at 25 °C.

Total phenolic contents

Total phenolic constituents of *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* methanolic extracts (aerial parts) were performed employing methods from literature involving Folin-Ciocalteu reagent and used gallic acid as a standard [12].

Antioxidant activity

Test of DPPH free radical scavenging activity

The scavenging of DPPH radical was carried out according to a modified version of the method described by Blois [13]. 1 mM solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was used as free radical. 10, 20, 40, 80, and 200 µl methanolic extracts of aerial parts of *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* were tested against DPPH. The reaction mixtures were incubated at room temperature and in darkness for 30 min. The reduction of DPPH was followed by monitoring the decrease in absorbance at 517 nm. The percentage of free radical scavenging effect was calculated as follows: $SC\%=[(A_{control}-A_{test})/A_{control}]$ x 100, where $A_{control}$ is the absorbance of the control (DPPH solution without test sample) and A_{test} is the absorbance of the test sample (DPPH solution plus extracts). All tests were performed in triplicate and means were centred.

Test of ABTS free radical scavenging activity

The $ABTS^{\bullet+}$ scavenging ability of methanolic extracts of *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* aerial parts were determined according to a method described by Re et al. [14]. $ABTS^{\bullet+}$ was generated by reacting ABTS solution (7 mM) with $K_2S_2O_8$ (2.45 Mm) in the dark and at room temperature for 16 h and adjusting to 734 nm. 10, 20, 40, 80, and 200 µl extracts were added to 4.0 ml $ABTS^{\bullet+}$ solution and absorbances were measured at 734 nm after 2 h. The percentage of free radical scavenging effect was calculated as follows: $SC\%=[(A_{control}-A_{test})/A_{control}]$ x 100, where $A_{control}$ is the

absorbance of the control (ABTS solution without test sample) and A_{test} is the absorbance of the test sample (ABTS solution plus extracts). All tests were performed in triplicate and means were centred.

Antimicrobial activity

The antimicrobial activities of extracts were determinated by the disc diffusion method [15]. 100 µl of each extract was absorbed onto a sterile disc 12 mm in diameter.

To inoculate the media for assay, 1% rate of each microorganism from 10^6-10^7 cfu/ml suspension was added to 15 ml steril media (for bacteria Muller-Hintone agar, for yeast Sabourand 2% Glucose agar). Each of these inoculated mediums was poured into petri dishes (9 cm) and left at +4 °C for 1 h. Subsequently discs prepared from *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* extracts were added on these inoculated medias and left again at +4 °C for 1 h.

Seven standard antibiotic discs were used as positive controls. Sensitivity was deduced by comparing the inhibition zone diameter produced by the Erythromycin (E-15), Gentamicin (CN-10), Chloramphenicol (C-30), Penicillin (P-10), Cefoperazone (CEP-75), Ceptazidime (CAZ-30) ve Ampicillin (AM-10).

The petri dishes were incubated at 35 °C for 18-24 h, except for *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* which were incubated at 27 °C. Inhibition zones were measured using a caliper and recorded as the mean diameter of 3 replications in mm. All tests were performed in triplicate and means were centred.

Statistical analysis

One-Way ANOVA test (SPSS 16.0) was used to analyse data obtained from the zone of inhibition produced by different extracts.

Results

Resveratrol and flavonoid contents

Methanolic extracts of two *Salvia* species were screened for their resveratrol, and flavonoid contents, the results are shown in Table 2. According to these results quercetin (122.95 µg/ml), kaempferol (350.45 µg/ml) and katesin (292.3 µg/ml) were major flavonoid contents for *Salvia verticillata* L. var. *amasiaca*, while katesin (315.5 µg/ml) and naringenin (227.2 µg/ml) were mojor flavonoid contents for *Salvia microstegia*. *Salvia verticillata* L. var. *amasiaca* was superior to *Salvia microstegia* with resveratrol value of 3.65 µg/ml.

Table 1. Locality data of *Salvia* species

Plant samples	Locality	Coordinate	Harvest time	Altitude
Salvia verticillata L. var. *amasiaca*	Ardahan District/ Around Kuru River (Turkey)	N41° 07.13' E042° 41.55'	23.06.2011	1900
Salvia microstegia Boiss & Bal.	Ardahan District (Turkey)	N41° 13,49' E042° 43.01'	25.06.2011	1960

Table 2. Resveratrol and flavonoid contents of *Salvia* species

Plant samples	Rutin (µg/ml)	Myricetin (µg/ml)	Quercetin (µg/ml)	Kaempferol (µg/ml)	Katesin (µg/ml)	Naringin (µg/ml)	Naringenin (µg/ml)	Resveratrol (µg/ml)
Salvia verticillata L. var. *amasiaca*	0.5	75.6	122.95	350.45	292.3	0.7	36.25	3.65
Salvia microstegia	0.5	11.9	43.6	0.45	315.5	0.5	227.2	0.5

Table 3. DPPH and ABTS free radical scavenging activi of *Salvia* species

Plant samles	DPPH (%)					ABTS (%)				
	10 µl	20 µl	40 µl	80 µl	200 µl	10 µl	20 µl	40 µl	80 µl	200 µl
Salvia verticillata L. var. *amasiaca*	90.49	86.50	78.71	69.39	27.38	98.37	98.57	99.18	99.38	84.37
Salvia microstegia	99.81	99.81	91.64	57.41	14.18	98.78	98.57	98.78	99.39	97.55

Total phenolic contents

Based on the absorbance values of the various extract solutions, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents as described by Singleton et al. [12]. The total phenolic content was higher in *Salvia verticillata* L. var. *amasiaca* with an extra value of 119.45 mg GAE/g than *Salvia microstegia* (118.08 mg GAE /g extre).

Antioxidant activity

Methanolic extracts of *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* were screened for their possible antioxidant activity by two test systems, namely DPPH and ABTS free radical scavenging systems. As listed in Table 3, the results showed that DPPH and ABTS free radical scavenging activity values of methanolic extracts of *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* dwindled from 10 µl to 200 µl concentration.

Table 4. Antimicrobial activity of *Salvia verticillata* L. var. *amasiaca*

Microor-ganisms	Root					Stalk					Leaf					Flower					AP
	Antimicrobial activity (mm)																				
	C	H	A	E	M	C	H	A	E	M	C	H	A	E	M	C	H	A	E	M	M
B. subtilis ATCC 6633	-[1]	-	-	17[2] ±0.33	33 ±0.57	-	-	-	-	24 ±0.0	23 ±0.57	-	18 ±0.88	16 ±0.33	39 ±0.57	22 ±0.57	15 ±0.33	24 ±0.0	18 ±0.33	32 ±0.0	41 ±0.57
E. aerogenes ATCC 13048	-	-	-	15 ±0.33	29 ±0.57	-	-	-	-	25 ±1.73	23 ±0.0	-	15 ±0.57	16 ±0.57	38 ±0.33	26 ±0.33	-	21 ±0.0	19 ±0.57	22 ±0.88	44 ±0.33
E. cloacae ATCC 13047D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14 ±0.33	-	-	-	13 ±0.33	14 ±0.33
M. luteus NRLL B-4375	-	-	-	-	22 ±2.88	-	-	-	-	17 ±0.33	-	-	15 ±0.00	-	24 ±0.88	18 ±0.57	-	21 ±0.33	-	31 ±0.57	38 ±0.88
S. aureus ATCC 25923	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14 ±0.57	16 ±0.57	-	-	-	18 ±0.33	18 ±0.33
E. coli ATCC 11229	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans ATCC 10231	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. cerevisiae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1]: No inhibtition zone, [2]: İnhibition zone (mm), AP: Aerial part, C: Chlorophorm, H: Hexane, A: Acetone, E: Ethanol, M: Methan

Table 5. Antimicrobial activity of *Salvia microstegia*

Microor-ganisms	Root					Stalk					Leaf					Flower					AP
	Antimicrobial activity (mm)																				
	C	H	A	E	M	C	H	A	E	M	C	H	A	E	M	C	H	A	E	M	M
B. subtilis ATCC 6633	14[2] ±0.33	-[1]	15 ±0.33	-	26 ±1.15	-	-	-	-	16 ±1.15	-	-	-	-	14 ±0.0	-	-	-	-	18 ±1.15	24 ±0.88
E. aerogenes ATCC 13048	-	-	-	-	21 ±0.88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20 ±3.46	19 ±0.57
E. cloacae ATCC 13047D	19 ±0.00	-	18 ±0.00	15 ±0.0	16 ±0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M. luteus NRLL B-4375	17 ±0.57	16 ±0.33	16 ±0.00	-	25 ±0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17 ±0.00	22 ±0.57
S. aureus ATCC 25923	16 ±0.33	15 ±0	-	-	20 ±0.00	-	-	-	-	12 ±0.00	-	-	-	-	12 ±0.0	-	-	-	-	14 ±0.00	-
E. coli ATCC 11229	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans ATCC 10231	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. cerevisiae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1]: No inhibtition zone, [2]: İnhibition zone (mm), AP: Aerial part, C: Chlorophorm, H: Hexane, A: Acetone, E: Ethanol, M: Methanol

While the first concentration (10 µl) of *Salvia microstegia* exhibited higher antioxidant activity against DPPH, the latest concentration (200 µl) of *Salvia verticillata* L. var. *amasiaca* showed higher antioxidant activity against DPPH. In addition *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* exhibited similar free radical scavenging activities against ABTS at 10 µl concentration, but *Salvia microstegia* showed higher ABTS free radical scavenging activity at 200 µl concentration than *Salvia verticillata* L. var. *amasiaca* (97.55 and 84.37 respectively). Consequently ABTS free radical scavenging activity of plant extracts is higher than DPPH free radical scavenging activity, but all plant extracts have got free radical scavenging activity against DPPH and ABTS.

Antimicrobial activity

Antimicrobial activities of different extracts of root, stalk, leaf, flower and aerial parts of *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* were tested against test microorganisms. Antimicrobial activity results were demonstrated in Tables 4 and 5.

Antimicrobial activity results of *Salvia verticillata* L. var. *amasiaca* extracts are given in Table 4 and these results showed that methanolic extracts of root, stalk, leaf, flower and aerial part of this plant exhibited more effective antibacterial effects than other extracts. In addition, it was determined that the most sensitive bacteria were *Bacillus subtilis* ATCC 6633 and *Enterobacter aerogenes* ATCC 13048, but none of extracts exhibited antimicrobial activity against *Escherichia coli* ATCC 11229, *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae*.

The results of *Salvia migrostegia* extracts showed in Table 5. According to these results, root extracts were more effective than extracts of other parts against test microorganisms. Additionally, chloroform, hexane, acetone and ethanol extracts of stalk, leaf and flower parts did not exhibite antimicrobial activity against any test microorganisms. The extracts of this plant did not showed any effects on *Escherichia coli* ATCC 11229, *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae*. It is observed that *Salvia verticillata* L. var. *amasiaca* has

more effective antimicrobial activities than *Salvia migrostegia* on test microorganisms.

Discussion

Plant-derived compounds are mostly secondary metabolites, most of which are phenols or their oxygen-substituted derivates. These secondary metabolites have various benefits including antimicrobial activities against pathogenic and spoilace microorganisms [16]. Major groups of compounds include phenolics, phenolic acids, quinones, saponins, flavonoids, tannins, coumarins, terpenoids, and alkaloids [17, 18]. Many studies regarding phenolic compounds and their effects were made by researchers. The study of Tosun et al. [19] demonstrated that the amount of the total phenolics was higher in *Salvia verticillata* (167.1 mgGAE/g DW) than *Salvia migrostegia* (50.3 mgGAE/g DW). These results are similar to results of this study. Many *Salvia* species and their components possess antioxidant properties in enzyme-dependent and enzyme-independent systems [20, 21, 22, 23, 24]. The results of this study showed that methanolic extracts of *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* exhibited antioxidant effects against DPPH and ABTS free radical agents. It is also reported elsewhere that many members of the genus *Salvia* possess antioxidant properties. For instance, some previous studies regarding the biological activities of *Salvia* species support that the members of this genus have great potential, especially in antioxidant systems for food and cosmetic industries [5, 6, 7]. Additionally, the antioxidant activities of *Salvia verticillata* and *Salvia microstegia* were assessed by both chemical and enzymatic methods against DPPH and XO system. The ethyle acetate and methanol extracts of these plants were observed to be highly active against both DPPH and XO [25]. Moreover, it is showed that *Salvia verticillata* has higher antioxidant activity than *Salvia microstegia* [19]. Most of the previous studies related to plant-derived antimicrobials found in scientific literature involve the antimicrobial or antioxidant properties of herbs and their compounds [26, 27, 28]. It was demostrated that different extracts of *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* exhibited antimicrobial effects on test microorganisms at different rates in this study. Antimicrobial activities of some *Salvia* species also investigated in a previous study. Similar activity pattern was obtained (both in disc diffusion and MIC tests). Antimicrobial activity of *S. aramiensis* was followed by *S. aucheri* and *S. pilifera*, respectively [2]. Additionally, Nikolic et al. [29] demonstrated that essential oil of *Salvia lavandulifolia* exhibited significant antimicrobial activity against test microorganisms. In conclusion, antimicrobial effects of plants are of great interest because resistance to antimicrobial agents has become an increasingly important global problem. Additionally, antioxidant properties and phenolic contents of plants are also the center of attention. It is demostrated in this study that *Salvia microstegia* and especially *Salvia verticillata* L. var. *amasiaca* have antibacterial properties against used test bacteria, but both *Salvia microstegia* and *Salvia verticillata* L. var. *amasiaca* do not have antifungal effects on *Candida albicans* and *Saccharomyces cerevisiae*. In addition, not only *Salvia verticillata* L. var. *amasiaca* but also *Salvia microstegia* have antioxidant effects and flavonoid contents at different rate. These properties of *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* are of great importance to academia, food, pharmaceutical industries and folk medicine.

Conflict of Interest

We declare that we have no conflict of interest.

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