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Investigation of phenolic profiles and antioxidant activities of some *Salvia* species commonly grown in Southwest Anatolia using UPLC-ESI-MS/MS

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Abstract

Aerial parts of *Salvia albimaculata Hedge & Hub.-Mor., Salvia potentillifolia Boiss & Heldr. ex Bentham.* and *Salvia nydeggeri Hub.-Mor.* from Soutwest Anatolia, Turkey were evaluated to determine their phenolic compounds and antioxidant properties. According to the ultra performance liquid chromatography with electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS) analysis results, caffeic acid (3582.8 ± 2.5 µg/g, 2956.5 ± 4.6 µg/g and 2457.7 ± 3.1 µg/g) and 3,4-dihydroxy benzoic acid (1846.2 ± 3.1 µg/g, 2019.1 ± 2.2 µg/g and 1901.3 ± 1.5 µg/g) were found to be in the highest concentrations in *S. potentillifolia, S. albimaculata* and *S. nydeggeri*, respectively. Total amounts of phenolics and flavonoids were determined highest in ethyl acetate extracts of samples and varied from 62.4 ± 0.1 to 55.4 ± 0.0 µg PEs/mg and from 296.8 ± 1.4 to 198.4 ± 1.5 µg QEs/mg, respectively. Antioxidant activity of *S. potentillifolia* was found to be higher than the others for $ABTS^{\bullet+}$ and β-carotene linoleic acid assays (SC_{50} = 49.8 ± 0.9 and IC_{50} = 26.1 ± 0.6 µg /mL, respectively) while *S. albimaculata* was found to be higher for $DPPH^{\bullet}$ assay (SC_{50} = 227.4 ± 1.1 µg/mL).

Keywords: antioxidant activity; phenolics; *Salvia albimaculata*; *Salvia nydeggeri*; UPLC-ESI-MS/MS.

Practical Application: The results of this study indicated that *Salvia* species possess a potential source of phenolics, antioxidants, nutrients and those may be used in food, pharmaceutical and cosmetic industries as a natural antioxidant. To the best of our knowledge, this is the first report on the determination of individual phenolic profiles of *S. potentillifolia, S. albimaculata and S. nydeggeri* with high accuracy and precision owing to high throughput instrument UPLC-ESI-MS/MS and sample analysis technique. Natural antioxidants are used as preservatives in many industries, especially in food and cosmetic industries. Therefore, the need for new and safer antioxidant sources is still maintained and may be compensated by the studied *Salvia* species. Thus scientists and manufacturers may benefit from the valuable properties of *S. potentillifolia, S. albimaculata and S. nydeggeri*.

1 Introduction

The genus *Salvia* is the largest member of the Lamiaceae family with nearly 1000 species spread throughout the various regions of the World mainly central and south America, western Asia (especially Turkey, Iran, Russia) and eastern Asia. Recently, 99 species of the genus *Salvia* have been identified in Turkey and 52 (52%) of them are endemic to Turkey (Alziar, 1988; Celep et al., 2014). Some members of the *Salvia* genus are commercially important and used for flavouring agents in foods as well as cosmetics, perfumery and the pharmaceutical industries with its biological activities (Chalchat et al., 1998; Villa et al., 2009).

The traditional medical practices of the *Salvia* species have been studied all over the world (Martínez-Francés et al., 2017; Li et al., 2013). It is known that *Salvia* species have been used as infusions against simple diseases in Anatolian traditional medicine applications (Baytop, 1999). Many *Salvia* species have been reported for use in the treatment of diseases such as epilepsy, colds, bronchitis and tuberculosis (Dweck, 2000) as well as biological activities such as antioxidant, antimicrobial (Kelen & Tepe, 2008), anti-inflammatory (González-Chávez et al., 2017), antidiabetic (Eidi & Eidi, 2009), antitumor (Fiore et al., 2012), anti cancer (Jiang et al., 2017) ve antiviral activities (Šmidling et al., 2008). Additionally, Scholey et al. (2008) examined the administration of a standardised *Salvia* extract to improve cognitive function in healthy older individuals and reported enhancement in cognative performance. Recently, Lopresti (2017) published a useful review about potential cognitive-enhancing and protective effects of *Salvia* and Miroddi et al. (2014) has reviewed clinical trials assessing pharmacological properties of *Salvia* species on memory, cognitive impairment and alzheimer's disease.

Today, it is well known that free radicals cause many diseases. Antioxidants have great importance in the fight against free radicals, which can damage biological molecules with different mechanisms of action (Young & Woodside, 2001) and the interest in the usage of antioxidants in the food, pharmaceutical and cosmetic industries is constantly increasing. Nowadays, synthetic antioxidants such as butylated hydroxyanisole (BHA),

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Butylated hydroxytoluene (BHT) and natural antioxidants are used as preservatives in many industries, especially in food industry. However, the concerns about the safety and toxicity of synthetic antioxidants have not been overcome yet (Kahl, 1984; Pokorný, 2007; Ito et al., 1986). Therefore, the need for new and safer antioxidant sources is still maintained.

As one of the plants used as natural antioxidant source is the genus *Salvia* and the antioxidant activities of *Salvia* extracts have been associated mainly with their total phenolic contents (Farhat et al., 2013; Dudonné et al., 2009). Plants with phenolic content are used especially in oily food because of their significant functions such as dealing with undesirable fragrances, prolonging thier shelf life, delaying the formation of toxic oxidation products, increasing nutritional value and preventing microbial growth. (Tepe et al., 2006; Karpinska et al., 2001; Rota et al., 2004). Phenolic compounds are known to be extremely beneficial in terms of human nutrition (Ou & Kwok, 2004), cosmetic (Magnani et al., 2014) and pharmacological (Galati & O'Brien, 2004).

A wide variety of *Salvia* species have been studied as novel phenolic compound sources and qualitative and quantitative analyzes of phenolic compounds have been carried out using various techniques. (Orhan et al., 2012; Kamatou et al., 2010; Lu & Foo, 2002). It is known that the phenolic compositions and antioxidant activities of *Salvia* species vary depend on species. For example, Şenol et al. (2010) have examined the radical-scavenging activities of 55 *Salvia* species include *S. nydeggeri* using DPPH$^{\bullet}$ and FRAP assays and found *S. fruticosa* and *S. cilicica* as most active species while Asadi et al. (2010) reported *S. hydrange* had higher FRAP (ferric reducing antioxidant power) activity than *S. lachnocalyx*. A number of studies have been done to determine antioxidant activities and phenolic contents of *Salvia* species (Erdemoglu et al., 2006; Tosun et al., 2009; Akkol et al., 2008; Alimpić et al., 2017). To the best of our knowledge, there is no any study on determination of phenolic profile of *S. nydeggeri, S. albimaculata* and *S. potentilifolia* using UPLC-MS/MS.

In this study, phenolic profiles of three edible and commercially valuable *Salvia* species were analyzed using UPLC-ESI-MS/MS with new extractions techniques. Quantitative determinations were made using calibration curves. Total phenolic and flavonoid concentrations were determined for each plant. The antioxidant activity of plant extracts was determined using three complimentary methods (β-carotene-linoleic acid bleaching, DPPH$^{\bullet}$ free radical scavenging and ABTS$^{\bullet+}$ cation radical scavenging). Correlations between phenolic and flavonoid content and antioxidant activity results were determined.

2 Materials and methods

2.1 Chemicals and reagents

All Phenolic standards purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Quercetin, pyrocatechol, β-carotene, linoleic acid, polyoxyethylene sorbitan monopalmitate (Tween-40), Folin-Ciocalteu's reagent (FCR), potassium acetate, butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Steinheim, Germany). 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt was obtained from Fluka Chemie (Fluka Chemie GmbH, Steinheim, Germany). All solvents and other chemicals were of analytical grade purity and were supplied from Merck KGaA (Darmstadt, Germany). HPLC-grade water (18.2 mΩ) was purified using a Milipore Elix Advantage 10 and Milli-Q Advantage A10 (Molsheim, France) system that comprise reverse osmosis, ion exchange, and filtration steps.

2.2 Plant materials

Salvia albimaculata Hedge & Hub.-Mor. samples were collected in the region between Burdur, 15. km from Tefenni to Söğüt, Turkey. *Salvia potentillifolia Boiss. & Heldr. ex Bentham.* samples were collected in the region between Burdur, 15. km from Gölhisar to Altınyayla, Turkey and *Salvia nydeggeri Hub.-Mor.* samples were collected in the region between Muğla-Fethiye, 8. and 9. km from Fethiye to Antalya, Turkey. All *Salvia* species were endemic to their region and collected in August, 2015. Samples were dried under shadow in ambient temperature (25 °C) for the extraction procedures. Authentication of the plant materials were performed by Dr. Ergun Kaya from Department of Molecular Biology and Genetics, Faculty of Science, Muğla Sıtkı Koçman University, Muğla (Turkey).

2.3 Extraction of Salvia species

In the determination of total phenolic content, total flavonoid content and antioxidant activities of *Salvia* species, methanol, hexane, ethyl acetate and water extracts were used. The *Salvia* samples were extracted five times for 24 h at room temperature with methanol, filtered through Whatman no 4 and solvents were evaporated (Heidolph, Hei-VAP Precision). Then dry plant extracts were dissolved in distilled water and subjected to liquid-liquid extraction with hexane and ethly acetate, respectively. For each plant, hexane and ethyl acetate extracts were evaporated to dryness under vacuum. The plants remaining after extraction with methanol were used for the water extraction. For this purpose, the plants remaining were extracted with water at 80 °C, filtered through Whatman no 4 and the water extracts were lyophilized (Christ Freeze Dryer, Alpha 1–4 LD plus, Germany). All extracts were stored in deepfreeze (-18 °C) for further analysis.

2.4 Determination of total phenolic and flavonoid concentration

The total concentrations of phenolic content of extracts were determined using the Folin-Ciocalteu Reagent (FCR) according to the method described by (Slinkard & Singleton, 1977; Singleton et al., 1999) and results expressed as microgrammes of pyrocatechol equivalents (PEs). Briefly, the sample solution (1 mL) dissolved in methanol was added to distilled water (46 mL) and mixed with FCR (1 mL). After 3 min, 3 ml of Na_2CO_3 (2%) was added to the mixture and this mixture was kept in room temperature for 2 h by shaken intermittently. The absorbance was

read at 760 nm. The concentration of total phenolic compounds was calculated according to the following equation (Equation 1) that was obtained from the standard pyrocatechol graph:

$$Absorbance = 0.0073.x\ (\mu g)\ Pyrocatechol - 0.1665; r^2 = 0.9976 \quad (1)$$

Quantification of total flavonoid concentrations of the extracts were determined as the aluminum nitrate method described by Moreno et al. (2000) as quercetin equivalents (QEs). 1 mL of solution containin 1 mg of sample in ethanol was mixed with 10% aluminum nitrate (100 µL) followed by 1 M potassium acetate (100µL), and 80% ethanol (3.8 mL) in test tubes. Mixtures were kept in room temperature for 40 min and then absorbance was measured at 415 nm. The total concentrations of flavonoid contents were calculated according to the following equation (Equation 2) that was obtained from the standard quercetin graph:

$$Absorbance = 0.0082.x\ (\mu g)\ Quercetin + 0.0073; r^2 = 0.9998 \quad (2)$$

2.5 Antioxidant activity of the extracts

β-Carotene-Linoleic Acid Bleaching Assay

The total antioxidant activity was determined using β-carotene-linoleic acid assay based on the detection of inhibition of conjugated dien hydroperoxides due to oxidation of linoleic acid (Miller, 1971). This method uses the bleaching of β-carotene. Briefly, β-carotene (0.5 mg) was dissolved in 1 mL of chloroform. Tween 40 (200 mg) and linoleic acid (20 µL) were added to this mixure. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 mL of distilled water, saturated with oxygen, was added by vigorous shaking. 4 mL of this emulsion was mixed with 1 mL extract solutions at different concentrations ranging 500 µg to 4000 µg. Zero time absorbances were measured at 470 nm just after emulsions were transfered to each tube. 1 mL of methanol was used as control. The emulsion systems were incubated at 50 °C untill the color of β-carotene was disappeared after 120 min. The results were given as 50% inhibition concentration (IC_{50}).

DPPH• Free Radical Scavenging Assay

The free radical scavenging activities of plant extracts were determined using DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical assay (Blois, 1958) with slight modifications. Briefly, 4 mL of 0.004% DPPH• solution in ethanol was added to the extract solutions (1 mL) at concentrations ranging from 500 µg to 4000 µg. 1 mL of ethanol was used as a control. After 30 min of incubation at room temperature, the absorbance was measured at 517 nm. Absorbance values of the samples were evaluated against the control. The free radical scavenging activity (RSA) was calculated using the following equation (Equation 3):

$$DPPH\ RSA\ (\%\ inhibition) = \left[\left(A_{Control} - A_{Sample}\right) / A_{Control}\right] \times 100 \quad (3)$$

Where A_{Sample} is the absorbance of the solution containing the sample and $A_{Control}$ is the absorbance of the DPPH• solution.

$ABTS^{•+}$ Cation Radical Decolorization Assay

The cation radical scavenging activities of the extracts were determined using ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) (Re et al., 1999). Briefly, 7 mM ABTS in water and 2.45 mM K_2SO_8 reacted to form 5 mL of $ABTS^{•+}$ radical. The mixture was stored in the dark at room temperature for 16 h to allow cation radical formation. 1 mL of this radical solution was adjusted by diluting with ethanol to give an absorbance about 0.700. Then 4 mL of the ethanol-prepared $ABTS^{•+}$ solution was added onto 1 mL of the sample at concentrations ranging from 500 µg to 4000 µg. Ethanol (1 mL) was used as control. After incubation at room temperature for 10 min, the absorbance was measured at 734 nm. Absorbance values of the samples were evaluated against the control. $ABTS^{•+}$ cation radical removal activity was calculated using the following equation (Equation 4):

$$ABTS^{•+}RSA\ \% = \left[\left(A_{Control} - A_{Sample}\right) / A_{Control}\right] \times 100 \quad (4)$$

Where A_{Sample} is the absorbance of remaining concentration of $ABTS^{•+}$ in the presence of sample and $A_{Control}$ is the initial concentration of the $ABTS^{•+}$.

2.6 Determination of phenolic compounds using UPLC-ESI-MS/MS

Phenolic compounds of plant samples were analyzed using high-throughput instrument, a Waters UPLC-ESI-MS/MS and C18 column (Acquity UPLC BEH C18 100 mm × 2.1 mm, 1.7-µm particle size) and the separation of phenolic compounds was performed by gradient elution at 40 °C. The mobile phases were composed of solvent A (0.5% acetic acid in water) and solvent B (0.5% acetic acid in methanol), and the flow rate was 0.650 mL/min.

Approximately 20 g of each plant sample was frozen with 200 mL of liquid nitrogen and then lyophlized. A mixture of 30 mL of acetone:water (80:20) was added to lyophlized powder and the mixture was allowed to extraction for 6 h at -86 °C. Then, ultrasonic extraction was applied for 15 min., the extract was centrifuged at 4000 rpm for 10 min at 20 °C and filtered using Whatman No 4. The residue was extracted twice more with 30 mL of acetone:water mixtures (Kıvrak et al., 2013, 2017; Kıvrak & Kıvrak, 2016; Kıvrak, 2015), extracts were combined, the solvents in the combined extracts was evaporated at 40 °C (Rotary Evaporator Heidolph Basis Hei-VAP ML). The aqueous phase was washed 3 times with 30 mL of n-hexane and 3 times with 30 mL of ethyl acetate for liquid-liquid extraction. The organic phases were combined and evaporated to dryness at 40 °C. The residue was redissolved in a mixture of water:methanol (80:20). The solution (2 µL) was passed through Macherey-Nagel Chromafil Xtra PTFE-20/25 0.20 µm filters and injected to UPLC-ESI-MS/MS (Waters Acquity Ultra Performance LC, Xevo TQ-S MS-MS). In this present study, method parameters of UPLC-ESI-MS/MS for the phenolic compounds analysis were applied according to our previous literatures (Kıvrak et al., 2013, 2017; Kıvrak & Kıvrak, 2016; Kıvrak, 2015) (Table 1). All extractions techniques and UPLC-ESI-MS/MS analysis methods used in the analysis of phenolic compounds are original.

Table 1. Method parameters for the phenolic compounds analysis using UPLC-ESI-MS/MS.

No	Compounds	Quantification > confirmatory transition (m/z)	Cone voltage (V)	Collision energy (V)	Retention time (min)
1	Pyrogallol	125.01 > 69.10, 79.04, 81.02	20	17, 17, 14	0.97
2	Gallic acid	168.95 > 125.02, 107.02, 97.02	20	25, 20, 14	1.10
3	Homogentisic acid	167.03 > 123.03, 122.08, 108.00	10	20, 20, 10	1.47
4	3,4-dihydroxy benzoic acid	153.06 > 108.00, 81.01, 91.01	10	20, 25, 20	1.86
5	Gentisic acid	153.05 > 109.04, 108.03, 81.00	10	20, 20, 12	1.86
6	Pyrocatechol	153.06 > 81.01, 108.00, 109.04	8	20, 25, 20	2.38
7	Galantamin	288.10 > 198.00, 213.09, 230.95	20	32, 23, 17	2.68
8	4-Hydroxy benzoic acid	136.98 > 93.03, 65.10	10	25, 14	2.75
9	3,4-Dihydroxybenzaldehyde	137.00 > 91.93, 107.94, 136.00	8	21, 20, 18	2.76
10	Catechin hydrate	288.88 > 109.15, 124.99, 245.26	30	25, 20, 15	3.45
11	Vanillic acid	166.98 > 151.97, 108.03, 123.03	20	18, 12, 14	3.61
12	Caffeic acid	179.10 > 135.14, 107.10, 133.9	32	23, 23, 24	3.67
13	Syringic acid	197.20 > 123.00, 167.00, 182.00	15	22, 18, 14	4.11
14	Vanillin	150.95 > 135.94, 91.90, 107.97	30	20, 20, 14	4.51
15	*p*-Coumaric acid	163.01 > 119.04, 93.00, 117.01	5	27, 27, 15	4.65
16	Ferulic acid	193.03 > 134.06, 178.00, 149.02	20	16, 12, 13	5.36
17	Epicatechin	189.18 > 151.00, 203.00, 205.00	20	20, 20, 20	5.50
18	Chlorogenic acid	353.02 > 191.01, 179.09, 161.02	30	30, 28, 24	5.52
19	Catechin gallate	441.00 > 168.98, 288.97	30	20, 20	5.91
20	Rutin	609.00 > 254.99, 270.93, 299.90	17	55, 55, 40	5.95
21	*trans*-2-Hydroxycinnamic acid	163.04 > 119.04, 117.01, 93.07	10	25, 22, 13	6.32
22	Myricetin	316.90 > 107.07, 137.01, 150.97	30	30, 25, 25	6.83
23	Resveratrol	227.01 > 143.01, 159.05, 185.03	30	25, 18, 18	7.13
24	*trans*-Cinnamic acid	146.98 > 103.03, 62.18	30	10, 10	8.19
25	Luteolin	284.91 > 107.01, 133.05, 151.02	20	30, 33, 30	8.32
26	Quercetin	303.00 > 137.00, 153.00, 229.00	20	30, 32, 30	8.29
27	Naringenin	270.98 > 107.00, 119.04, 150.97	20	25, 25, 20	9.07
28	Genistein	271.00 > 153.00, 215.00, 243.00	20	27, 25, 24	9.22
29	Apigenin	269.10 > 107.00, 117.00, 149.00	20	30, 30, 25	9.35
30	Kaempferol	284.90 > 158.97, 117.10, 227.14	10	34, 40, 30	9.50
31	Hesperetin	301.02 > 108.01, 136.00, 163.99	20	36, 30, 24	9.71
32	Chrysin	252.99 > 63.05, 107.05, 142.99	20	30, 25, 25	11.06

3 Results and discussion

3.1 Total phenolic and flavonoid concentrations

Methanol, hexane, ethyl acetate and water extracts of *Salvia* species were examined for the determination of total phenolic and flavonoid contents. The results expressed as pyrocatechol and quercetin equivalents, respectively (Table 2). According to the study, ethyl acetate extract of *S. albimaculata* showed the highest amount of total flavonoid content (296.8 ± 1.4 µg QEs/mg) and the lowest content (29.1 ± 0.7 µg QEs/mg) of total flavonoid were found belonging to hexane extract of *S. nydeggeri*.

On the other hand, total phenolic content analysis of *Salvia* species revealed that ethyl acetate extract of *S. potentillifolia* had the highest concentration (62.4 ± 0.1 µg PEs/mg) of total phenolics among all extracts and species while the water extract of *S. nydeggeri* had the lowest amount (36.3 ± 0.8 µg PEs/mg) of total phenolic content. For all species, the results indicated that ethyl acetate extracts had the highest total phenolic and

Table 2. Total phenolic and flavonoid concentrations of *Salvia* samples.

Extracts		Total Phenolic Content (µg PEs /mg of extact)	Total Flavonoid Content (µg QEs /mg of extact)
S. albimaculata	Methanol	52.3 ± 0.5	158.4 ± 1.0
	Hexane	41.3 ± 0.6	32.3 ± 0.7
	Ethyl acetate	56.3 ± 0.0	296.8 ± 1.4
	Water	36.4 ± 0.5	88.1 ± 1.0
S. potentillifolia	Methanol	53.2 ± 0.4	168.5 ± 1.0
	Hexane	49.2 ± 1.1	35.1 ± 0.7
	Ethyl acetate	62.4 ± 0.1	292.2 ± 1.1
	Water	46.0 ± 1.3	118.5 ± 0.9
S. nydeggeri	Methanol	46.1 ± 0.5	136.0 ± 1.2
	Hexane	41.1 ± 1.0	29.1 ± 0.7
	Ethyl acetate	55.4 ± 0.0	198.4 ± 1.5
	Water	36.3 ± 0.8	99.1 ± 0.9

The values were given as averages of 3 parallel measurements $p<0.05$.

flavonoid content while the lowest amounts of total phenolics and flavonoids determined in water extracts and hexane extracts, respectively.

3.2 Individual phenolic compounds

In this study, individual phenolic compounds of three *Salvia* species were determined using UPLC-ESI-MS/MS. Thirty two of phenolic compounds were identified and 21 of them were detected and quantitated in all *Salvia* samples. Pyrogallol, galantamin, catechin hydrate, epicatechin, catechin gallate, *trans*-2-hydroxycinnamic acid, myricetin, resveratrol, quercetin, chlorogenic acid and gallic acid were not detected. All results were summarized in Table 3 and total ion chromatograms (TIC) of most abundant phenolic compunds of *Salvia* species were given in Figure 1.

The major phenolic compounds revealed for all *Salvia* species as caffeic acid and 3,4-dihydroxy benzoic acid. On the other hand, gentisic acid, syringic acid and 4-hydroxy benzoic acid were also quite high. A significant component, caffeic acid, was indicated highest amount in *S. potentillifolia* (3582.8 ± 2.5 µg/g) compared to the other *Salvia* species *S. albimaculata* (2956.5 ± 4.6 µg/g) and *S. nydeggeri* (2457.7 ± 3.1 µg/g) evaluated in this study. Caffeic acid contents of various *Salvia* species were previously investigated using different methods. For example, Farhat et al. (2013) reported that caffeic acid was detected in methanolic extracts of *S. officinalis*, *S. verbenaca*, *S. argentea* and *S. aegyptica* as 236.5 ± 4.8, 90.5 ± 0.5, 62.5 ± 0.2 and 117.6 ± 2.7 µg/g of dry plant material weight, respectively. In other report, caffeic acid values were determined as 15.6 ± 0.9 and 4.8 ± 0.4 mg/kg for ethyl acetate extracts of *S. halophila* and *S. virgata*, respectively (Akkol et al., 2008). These results indicates that phenolic content of *Salvia* species may change depends on location, season, origin of plant or investigation method as previously reported by other researchers too (Alimpić et al., 2017). The second major phenolic compound 3,4-dihydroxybenzoic acid was found to be highest in *S. albimaculata* (2019.1 ± 2.2 µg/g) followed by *S. nydeggeri* (1901.3 ± 1.5 µg/g) and *S. potentilifolia* (1846.2 ± 3.1 µg/g).

Table 3. Phenolic content (µg/g dry weight ± standard deviation) of *Salvia* species.

No	Compounds	Retention time (min)	*S. potentillifolia*	*S. nydeggeri*	*S. albimaculata*
1	Pyrogallol	0.97	nd	nd	nd
2	Gallic acid	1.10	nd	nd	nd
3	Homogentisic acid	1.47	690.1 ± 1.2	100.2 ± 0.9	550.3 ± 1.7
4	3,4-dihydroxy benzoic acid	1.86	1846.4 ± 3.1	1901.3 ± 1.5	2019.1 ± 2.2
5	Gentisic acid	1.86	1240.2 ± 1.3	1236.7 ± 2.9	1201.1 ± 1.5
6	Pyrocatechol	2.38	294.8 ± 1.1	251.4 ± 0.7	198.5 ± 0.8
7	Galantamin	2.68	nd	nd	nd
8	4-Hydroxy benzoic acid	2.75	1126.9 ± 2.3	998.5 ± 1.3	1236.5 ± 2.5
9	3,4-Dihydroxybenzaldehyde	2.76	996.9 ± 0.7	1047.6 ± 1.5	1006.1 ± 3.3
10	Catechin hydrate	3.45	nd	nd	nd
11	Vanillic acid	3.61	927.5 ± 1.1	1021.5 ± 2.2	999.5 ± 1.2
12	Caffeic acid	3.67	3582.8 ± 2.5	2457.7 ± 3.1	2956.5 ± 4.6
13	Syringic acid	4.11	1322.1 ± 3.3	963.6 ± 2.8	1635.7 ± 3.1
14	Vanillin	4.51	1223.0 ± 3.1	734.6 ± 1.0	1146.1 ± 2.1
15	*p*-Coumaric acid	4.65	312.4 ± 1.0	214.5 ± 1.0	424.3 ± 3.7
16	Ferulic acid	5.36	981.0 ± 2.1	1002.5 ± 1.9	1057.1 ± 2.3
17	Epicatechin	5.50	nd	nd	nd
18	Chlorogenic acid	5.52	nd	nd	nd
19	Catechin gallate	5.91	nd	nd	nd
20	Rutin	5.95	266.8 ± 2.3	254.4 ± 1.6	199.1 ± 0.9
21	*trans*-2-Hydroxycinnamic acid	6.32	nd	nd	nd
22	Myricetin	6.83	nd	nd	nd
23	Resveratrol	7.23	nd	nd	nd
24	*trans*-Cinnamic acid	8.19	10.3 ± 0.9	12.9 ± 0.7	14.7 ± 1.0
25	Luteolin	8.32	857.0 ± 3.6	1101.5 ± 2.0	953.0 ± 2.2
26	Quercetin	8.29	nd	nd	nd
27	Naringenin	9.17	11.1 ± 1.0	15.4 ± 1.3	12.1 ± 0.9
28	Genistein	9.22	160.1 ± 0.1	99.1 ± 0.1	178.1 ± 0.2
29	Apigenin	9.35	171.1 ± 1.0	151.3 ± 0.9	182.0 ± 1.2
30	Kaempferol	9.50	941.3 ± 2.5	1121.9 ± 4.4	854.6 ± 2.1
31	Hesperetin	9.71	71.1 ± 1.2	112.3 ± 2.6	82.1 ± 2.5
32	Chrysin	11.06	8.1 ± 0.5	9.3 ± 1.0	7.1 ± 0.5

nd: not detected.

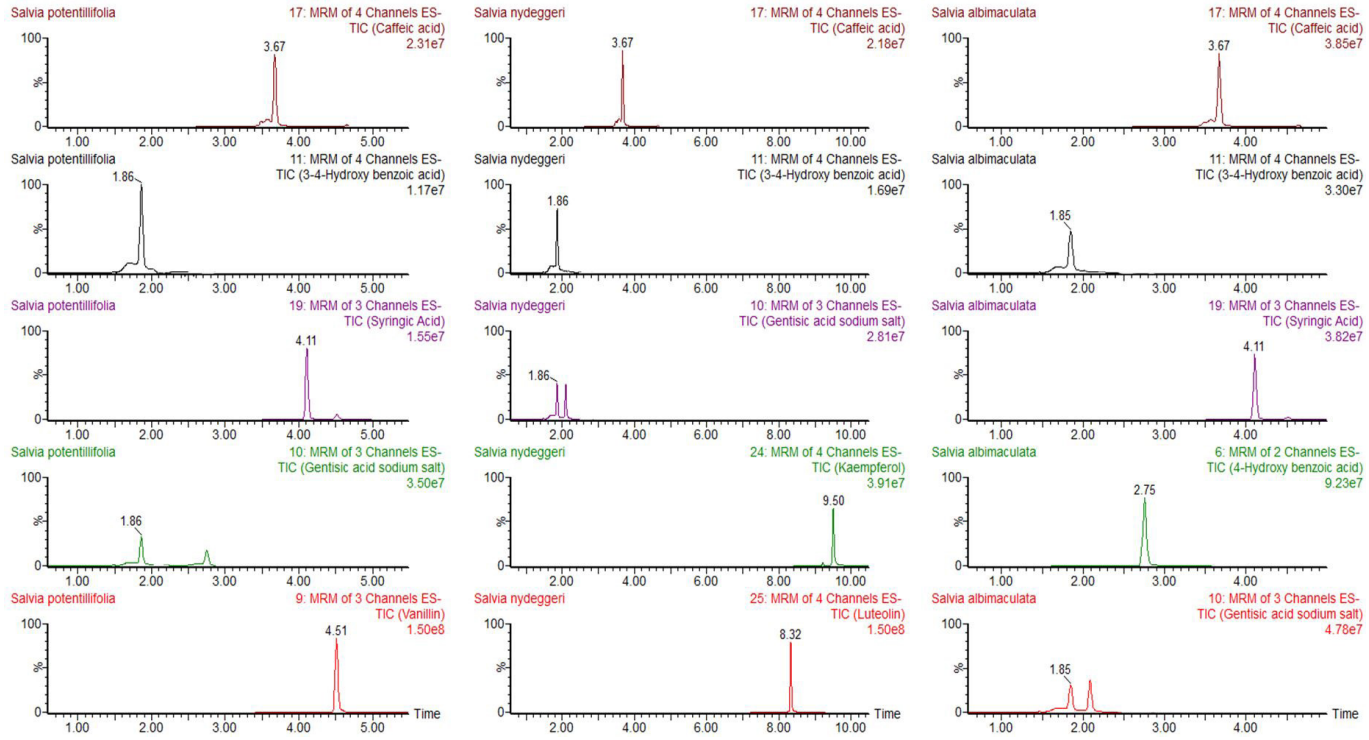

Figure 1. Total ion chromatograms of major phenolic compounds determined in *Salvia* species.

In the comparision of *Salvia* species, *S. albimaculata* was determined richest in syringic acid. The amount of syringic acid determined for *S. albimaculata* (1635.7 ± 3.1 µg/g) and *S. potentillifolia* (1322.1 ± 3.3 µg/g) was distincly higher than *S. nydeggeri* (963.6 ± 2.8 µg/g).

3.3 Antioxidant activity

The antioxidant activity of plant extracts was determined using three complimentary methods (β-carotene-linoleic acid bleaching, DPPH$^{\bullet}$ free radical scavenging and ABTS$^{\bullet+}$ cation radical scavenging). According to the total phenolic and flavonoid determination results, the ethyl acetate extracts were presented higher total phenolic and flavonoid content in all species. With this reason, ethyl acetate fractions of *Salvia* extracts were choosen for comparision of antioxidant activities instead of methanol, water and hexane extracts. Antioxidant activity values were examined and the 50% inhibition concentration (IC_{50}) of ethyl acetate extract was found to be highest as 26.1 ± 0.6 µg/mL for *S. potentillifolia* in the β-carotene-linoleic acid method. The antioxidant activities of the *Salvia* species and standartds were decreased in the following order: *S. potentillifolia* > *S. albimaculata* > BHA > *S. nydeggeri* > BHT > α-Tocopherol. The total antioxidant activity results consistent with total phenolic content results (*S. potentillifolia* > *S. albimaculata* > *S. nydeggeri*). Increased concentrations in all samples also indicate increased inhibition.

In DPPH$^{\bullet}$ assay, none of the species showed better antioxidant activity than standards BHA, BHT or α-Tocopherol but highest antioxidant activity was observed in the *S. albimaculata* extract (227.4 ± 1.1 µg/mL) and a lower antioxidant activity values were found in *S. potentillifolia* (248.4 ± 2.2 µg/mL) and *S. nydeggeri* (312.6 ± 0.7 µg/mL). ABTS$^{\bullet+}$ cation radical scavenging SC_{50} value of ethyl acetate extract of *S. potantillifolia* was found to be 49.8±0.9 µg/mL as highest, whereas butylated hydroxyanisole (BHA) SC_{50} value, used as standard, was 20.6 ± 0.9 µg/mL (Table 4).

Table 4. Antioxidant activities of ethyl acetate extracts of *Salvia* samples.

Extracts/ Standards	β-Carotene-linoleic acid IC_{50} (µg/mL)	DPPH$^{\bullet}$ SC_{50} (µg/mL)	ABTS$^{\bullet+}$ SC_{50} (µg/mL)
S. albimaculata	29.3 ± 1.2	227.4 ± 1.1	50.2 ± 1.0
S. potentillifolia	26.1 ± 0.6	248.4 ± 2.2	49.8 ± 0.9
S. nydeggeri	32.6 ± 1.4	312.6 ± 0.7	52.3 ± 1.0
BHA	32.3 ± 1.2	168.5 ± 0.8	20.6 ± 0.9
BHT	34.3 ± 1.0	155.4 ± 0.6	28.6 ± 0.7
α-Tocopherol	38.1 ± 0.7	179.5 ± 1.3	32.1 ± 0.6

IC_{50} and SC_{50} values represent the means ± SD of three parallel measurements; BHA; Butylated hydroxyanisole, BHT; Butylated hydroxytoluene.

According to the β-Carotene-linoleic acid and ABTS$^{\bullet+}$ assays, increasing in total phenolic content and antioxidant activity was in positive correlation. These results suggest that the major part of the antioxidant activities in *Salvia* species selected in this study is a result of the phenolic compounds. This observation has been in accordance with previous literatures, exhibited similar correlations between total phenolic content and antioxidant activity of various plants (Kıvrak et al., 2017; Kumar et al., 2014; Mammadov et al., 2012; Piluzza & Bullitta, 2011).

In general, the differences between the results obtained in this study and in previous reports is considered to be related by the conditions of experiments, the instrument used and the growing areas and collection times.

4 Conclusion

Investigation of phenolic compositions and antioxidant properties of plants or their different extracts revealed remarkable data since the interest on the use of natural sources for food, pharmaceutical and cosmetic industries increased tremendouly in the last decade. In this study, individual phenolic compounds of *S. albimaculata*, *S. nydeggeri* and *S. potentillifolia* were analyzed and quantitated using UPLC for the first time. The studied three *Salvia* species presented rich phenolic content. In addition, up to now the lack of information about antioxidant activity studies using three complemantry method on *S. albimaculata*, *S. nydeggeri* and *S. potentillifolia* makes this present study unique and important. Considering the results obtained in this study and the large number of researches on *Salvia* species previously reported, all selected plants have great potential for use in pharmaceutical, cosmetics and many other industrial fields, especially in the food industry. In the light of findings of the present study, particularly remarkable antioxidant activities and rich phenolic contents of plants could trigger the scientists. We also think that the results obtained in this study provide useful of information for researchers who want to study various biological activities of these three *Salvia* species and encourage entrepreneurs to use them for commercial purposes with the respect of biodiversity conversation.

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