ANTIOXIDANT PROPERTIES AND PHENOLIC COMPOSITION OF *SALVIA VIRGATA* JACQ.

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RUNNING TITLE HEAD: Antioxidant activity of *Salvia virgata*

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Antioxidant Properties and Phenolic Composition of *Salvia virgata* Jacq.

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Methanol and water extracts of Salvia virgata Jacq. aerial parts were examined for its phenolic contents and antioxidant activities. Both extracts were investigated for their total phenolic contents, total flavonoids and flavonols, qualitative-quantitative compositions (by HPLC-PDA analysis). Free radical scavenging activities (using DPPH$^{\bullet}$ and ABTS$^{\bullet+}$), iron(II) chelation activity and also the peroxidation level was determined by TBA method. The antioxidant activity of extracts were given as IC_{50} values and compared with standards; butylated hydoxyanisole, butylated hydoxytoluene, rosmarinic acid, ascorbic acid, and gallic acid. Methanol extract was found to be active for DPPH$^{\bullet}$ radical scavenging activity where as water extract was more active for ABTS$^{\bullet+}$ radical scavenging. The methanol extract enriched in phenolic compound and rosmarinic acid as the main component in both extracts has been identified.

Key Words: *Salvia virgata*, Antioxidant Activity, HPLC

Salvia virgata Jacq.'ın antioksidan özellikleri ve fenolik içeriği.

Salvia virgata Jacq.'ın toprak üstü kısımlarının metanol ve su ekstresinin fenolik içeriği ve antioksidan özellikleri belirlenmiştir. Her iki ekstrenin de toplam fenolik, toplam flavonoit ve flavonol içeriği, kalitatif-kantitatif kompozisyonları (YBSK ile), serbest radikal süpürücü aktiviteleri (DPPH$^{\bullet}$ ve ABTS$^{\bullet+}$), demir(II) ile şelat oluşumu ve TBA yöntemi ile peroksidasyon seviyeleri incelenmiştir. IC_{50} değeri olarak verilen antioksidan aktiviteler butil hidroksianisol, butil hidroksitoluen, rozmarinik asit, askorbik asit ve gallik asit standartları ile karşılaştırılmıştır. Metanol ekstresi DPPH$^{\bullet}$ radikalini süpürücü etkide aktif bulunurken, su ekstresi ABTS$^{\bullet+}$ radikalini süpürücü etkide aktif bulunmuştur. Metanol ektresinin fenolik bileşiminin daha zengin olduğu ve her iki ekstrede de rozmarinik asitin ana bileşen olduğu tespit edilmiştir.

Anahtar Kelimeler: *Salvia virgata*, Antioksidan Aktivite, YBSK

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INTRODUCTION

Numerous lines of evidence have indicated that free radicals play a critical role in a variety of pathological conditions such as brain dysfunction, cancer, heart diseases and inflammation by damaging cellular components of DNA, proteins and lipids. Therefore antioxidants present in human diet may play an important role in disease prevention (1-3). Recently intense addition has been devoted to natural sources of phenolic antioxidants, not only for the prevention and treatment of diseases but also for protecting food quality by preventing oxidative deterioration of lipids (4, 5). Syntethic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluen (BHT) and tert-butyl hydroquinone have been used to protect food products against lipid peroxidation. Many researchers have focused on the search for natural compounds with antioxidant properties because of the harmful effects of synthetic antioxidants in the human body (6, 7).

The genus *Salvia* with about 900 species, is one of the most widespread members of the *Lamiaceae* family (2, 8). *Salvia* have been used since ancient times in folk medicine and have been subjected to extensive researches and it features prominently in the pharmacopoeias of many countries throughout the world (7-9). *Salvia officinalis* serves as standard sage is well known with its antioxidant activity and used as by this purpose. Hydroxycinnamic acid derivatives (caffeic and rosmarinic acids), flavonoids (luteolin, apigenin and glycosides) and diterpenoids (carnosol, carnosic acid and methyl carnosol) are responsible by the antioxidant effects of *Salvia* species (10).

Salvia genus is represented by totally 95 species in Turkey (11). In Anatolian folk medicine the infusions of *Salvia* species generally have been used as carminative, antiperspirant, diüretic and for wound healing (12). In Turkey, *S.virgata* Jacq. is known as ‘yılancık’ and it’s leaves used for externally wound healing. The decoction of *S.virgata* is also used for leukemia (13). The objective of this work was to evaluate the antioxidant activity of *S.virgata*. Therefore water extract and methanol extract were investigated in different *in vitro* antioxidants test systems. Furthermore the total phenols, total flavonoids, total flavonols were also analyzed. The phenolic compositions of the extracts were identified by HPLC-PDA analysis.

EXPERIMENTAL

Plant material and reagents

Air-dried aerial plant material (*S. virgata* Jacq., Lamiaceae) was collected from University of Uludağ /Bursa on 22/06/2009. A voucher specimen has been deposited at the Uludağ University Herbarium of Faculty of Arts and Sciences (BULU 29050). Chromatographic standards *p*-OH benzoic acid (65-85-0), caffeic acid (331-39-5), *o*- coumaric acid (614-60-8), rosmarinic acid (20283-92-5), luteolin (491-70-3) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Ultra-pure water was used throughout and was prepared using a Millipore Milli-RO 12 plus system (Millipore Corp., Billerica, MA, USA). All remaining reagents were of the highest purity available and obtained from the Sigma Chemical Company (St. Louis, MO, USA).

Preparation of the extracts

Air-dried *S. virgata* was cut into small pieces and sequentially extracted with sufficient amount of 70% methanol and water for 24 h at 40 °C in a water bath with shaking and was filtered. This procedure was repeated three times using the same batch of starting material and the resultant filtrates were combined and the solvent was removed in vacuo (40 °C). All extracts stored at -20 °C until the analysis time. Prior to the analysis, an aliquot of each extract was dissolved and filtered through 0.45µm membrane (Whatman, Maidstone, UK) and used in all subsequent experiments.

Determination of Total phenolics, flavonoids, and flavonols

Total phenols were estimated as gallic acid equivalents (GAE), per gram of extract (14). To ca. 6.0 mL H_2O, 100 µL sample (conc. 2-6 mg/mL) were transferred into a 10.0 mL volumetric flask, to which 500 µL undiluted Folin–Ciocalteu reagent were subsequently added. After 1 min, 1.5 mL 20% (w/v) Na_2CO_3 were added and the volume was made up to 10.0 mL with H_2O. After 2 h incubation at 25 °C, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve. The data are presented as the mean value of triplicate analyses.

Total flavonoids and total flavonols were estimated as rutin equivalents (RE), expressed as mg rutin eq./g extract (15). One milliliter of plant extract in methanol (2.5 mg/mL) was mixed with 1 mL aluminum trichloride in ethanol (20 g/L) and diluted with ethanol to 25 mL. The absorbance at 415 nm was read after 40 min at 20 °C. Blank samples were prepared from 1 mL plant extract (conc. 2.5 mg/mL) and 1 drop of acetic acid, and diluted to 25 mL. The rutin calibration curve was prepared in ethanolic solutions using the same procedure. All determinations were carried out in quadruplicate and the mean values were calculated.

For total flavonols, the rutin calibration curve was prepared by mixing 2 mL of 0.5–0.015 mg/mL rutin in ethanol with 2 mL (20 g/L) aluminum trichloride and 6 mL (50 g/L) sodium acetate. The absorbance at 440 nm was read after 2.5 h at 20 °C. The same procedure was carried out with 2 mL of plant extract (2.5 mg/mL) instead of the rutin solution. All determinations were carried out in quadruplicate and the mean values were calculated.

Qualitative–quantitative chromatographic analysis

The liquid chromatographic apparatus (Shimadzu LC 10Avp, Kyoto, Japan) consisted of an in-line degasser, pump and controller coupled to a SPD-M10 Avp photodiode array detector equipped with an automatic injector interfaced to Class VP chromatography manager software (Shimadzu, Kyoto, Japan). Separations were performed on a 250 x 4.6 mm i.d., 5 µm particle size, reverse-phase Discovery-C18 analytical column (Supelco, Bellefonte, PA, USA) operating at room temperature (22 °C) at a flow rate of 1 mL min^{-1}. Detection was carried out between the wavelengths of 200 and 550 nm. Elution was carried out using a ternary non-linear gradient of the solvent mixture MeOH/H_2O/CH_3COOH (10:88:2, v/v/v) (solvent A), MeOH/H_2O/CH_3COOH (90:8:2, v/v/v) (solvent B) and MeOH (solvent C).

Table 1: Gradient flow of HPLC

time(min)	Solvent A (%)	Solvent B (%)	Solvent C (%)
0	85	15	0
15	70	30	0
18	60	40	0
30	60	40	0
35	100	0	0
37	85	0	15
48	70	0	30
50	85	15	0

Components were identified by comparison of their retention times to those of authentic standards under identical analysis conditions and UV spectra using our in house PDA-library. A 10 min equilibrium time was allowed between injections. All the standard and sample solutions were injected triplicate.

1,1-Diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging activity

The ability of the extracts to scavenge $DPPH^{\bullet}$ was determined by the method of Gyamfi et al. (1999) (16). A 50 µL aliquot of each extract, in 50 mM Tris-HCl buffer (pH 7.4), was mixed with 450 µL of Tris-HCl buffer and 1.0 mL of 0.1 mM $DPPH^{\bullet}$ in MeOH. After 30 min incubation in darkness and at ambient temperature, the resultant absorbance was recorded at 517

nm. The percentage inhibition was calculated using eq. 1. Estimated IC_{50} values are presented as the mean value of triplicate analyses.

$$\% \text{ inhibition} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100 \qquad \text{(Eq. 1)}$$

$ABTS^{\bullet+}$ radical scavenging activity

The determination of $ABTS^{\bullet+}$ radical scavenging was carried out as described by Re et al. (17). The $ABTS^{\bullet+}$ radical was generated by reacting an (7 mmol/L) $ABTS^{\bullet+}$ aqueous solution with $K_2S_2O_8$ (2.45 mmol/L, final concentration) in the dark for 12–16 h, at ambient temperature, and adjusting the Abs734nm to 0.700 (70.020) with ethanol. The samples were diluted, such that, a 15 mL sample when added to 1.485 mL $ABTS^{\bullet+}$ resulted in a 20–80% inhibition of the blank absorbance. After 1.485 mL $ABTS^{\bullet+}$ solution was added to 15 mL sample, the absorbance at 734 nm was recorded 1 min after initial mixing and subsequently (for 15 min in total). The results are expressed as the Trolox equivalent antioxidant capacity (TEAC, mmol/L Trolox) at 1, 5, 10 and 15 min times and as the relative antioxidant activity (AUC), as described by Re et al. (17).

Iron(II) chelation

The ability of the extract to chelate iron(II) was carried out as described by Carter (1971) (18). To 200 µL of extract dissolved in an appropriate solvent was added 100 µL (2.0 mmol/L) $FeCl_2$ -$4H_2O$ and 900 µL methanol. After 5 min incubation, the reaction was initiated by the addition of 400 µL (5.0 mmol/L) ferrozine. After a further 10 min incubation period, the absorbance at 562 nm was recorded. EDTA was used as a positive control. The $Fe2^{+}$ chelating activity was calculated using eq. 1. The values are presented as the mean of three measurements.

Ascorbate-Iron(III)-Catalyzed Phospholipid Peroxidation

The ability of the extracts to scavenge hydroxyl radicals was determined by the method of Aruoma et al.(19). Bovine brain extract (Folch type VII) was mixed with 10 mM phosphate-buffered saline (PBS, pH 7.4) and sonicated in an ice bath until an opalescent suspension was obtained, containing 5 mg/mL phospholipid liposomes. The liposomes (0.2 mL) were combined with 0.5 mL of PBS buffer, 0.1 mL of 1 mM $FeC1_3$, and 0.1 mL of extract dissolved in PBS. Peroxidation was initiated by adding 0.1 mL of 1 mM ascorbic acid. The mixture was incubated at 37 °C for 60 min, after which, 50 μL of 2.0% butylated hydroxytoluene (BHT) was added to each tube, followed by 1 mL of 2.8% trichloroacetic acid (TCA) and 1 mL of 1.0% 2-thiobarbituric acid (TBA) in 0.05 M NaOH. The samples were vortexed and heated in a water bath at 100 °C for 20 min. The reaction was stopped by a 5 min ice H_2O bath. To each tube was

added 2 mL of *n*-butanol, and the mixture was vigorously vortexed. After centrifugation, the extent of oxidation was estimated from the absorbance of the organic layer at 532 nm. The percentage inhibition was calculated using eq 1, where the controls contain all the reaction reagents except the extract or positive control substance, and the IC_{50} values were estimated using a nonlinear regression algorithm (Sigma Plot 2001 version 7.0). The values are presented as the mean values of five measurements.

Statistical analysis

Data are presented as mean values ±95% confidence interval. Analysis of variance was performed using ANOVA procedures. Significant differences between means were determined by Tukey's pairwise comparison test at a level of $p < 0.05$. IC_{50} values were estimated using a nonlinear regression algorithm unless stated otherwise.

RESULTS

Extract yields, total phenolic, flavonoid and flavonol contents

S.virgata herb was extracted with 70% methanol and water. The results of extract yields, total phenols, total flavonoids and total flavonols are presented in Table 2. Aqueous methanol extract contained the highest amount of total phenol, flavonoids, flavonols. According to the Table 2, the yields of both extracts were also found to be almost same.

Table 2. Extract Yields, Total Phenols, Flavonoids and Flavonols

Extracts*	Yield[a] [%]	Total Phenols[b] [$mg_{GAE}/g_{extract}$]	Total Flavonoids[c] [$mg_{RE}/g_{extract}$]	Total Flavonols[d] [$mg_{RE}/g_{extract}$]
SME	25.94	195.22 ± 0.25	62.20 ± 0.57	15.24 ± 1.12
SWE	24.99	120.14 ± 2.27	14.17 ± 0.83	9.62 ± 0.60

[a] Extract yields expressed as milligrams of extract per gram (dry weight) of root material. [b] Total phenolics expressed as mg gallic acid per gram (dry weight) of extract. [c] Total flavonoids expressed as mg rutin per gram (dry weight) of extract. [d] Total flavonols expressed as mg rutin per gram (dry weight) of extract. SME 70% methanol extract; SWE water extract.

Qualitative–quantitative chromatographic analysis

The qualitative–quantitative analyses of the extracts, carried out using an HPLC apparatus coupled to a PDA detector, are presented in Table 3. Phenolic compounds were identified and quantified at 280, 320, and 360 nm as benzoates, hydroxycinnamates, and flavonoids, respectively. Gallic, *p*-OH-benzoic, caffeic, *o*-coumaric, rosmarinic acids, luteolin-7-*O*-glycoside, and luteolin were identified by comparison with the retention times and UV spectra of authentic standards, while quantitative data were calculated from their calibration curves. For *S. virgata*, the aqueous methanol extract was found to contain phenolics in high amount as measured by both UV spectrophotometry and HPLC. Rosmarinic acid was the main compound in both extracts. The rosmarinic acid concentration of methanolic extract was found as 66.94 ± 0.47 mg/g (w/w) and water extract 26.81 ± 0.05 mg/g (w/w). *o*-Coumaric acid and caffeic acid were found in the *S. virgata* extracts as the other hydroxycinnamic acids. Also it was found that the extracts contain more flavonoid glycosides than aglycones. The luteolin concentrations of the extracts were 0.97 ± 0.03 mg/g for methanolic extract and 0.22 ± 0.00 mg/g for water extract. As well known from the literature, rosmarinic acid was found as the principal component (5, 20-25).

Table 3. HPLC Results of *S. virgata* extracts

Compounds	**Equation and r^2 of standards**	**Extracts (mg/g)**	
		SWE mean ± SD (n=3)	**SME** mean ± SD (n=3)
p-OH-benzoic acid	y=13918x + 7,95 r^2=0,998	0.83 ± 0.013	0.69 ± 0.040
caffeic acid	y=47251x+191,94 r^2=0,996	0.77 ± 0.001	0.97 ± 0.004
o-coumaric acid	y=25443x -12,69 r^2=0,998	0.90 ± 0.002	3.25 ± 0.240
rosmarinic acid	y=23903x -29,76 r^2=0,998	26.81 ± 0.047	66.94 ± 0.471
luteolin	y=42001x -84,34 r^2=0,997	0.22 ± 0.003	0.97 ± 0.031

SWE: water extracts; SME: 70% methanol extracts

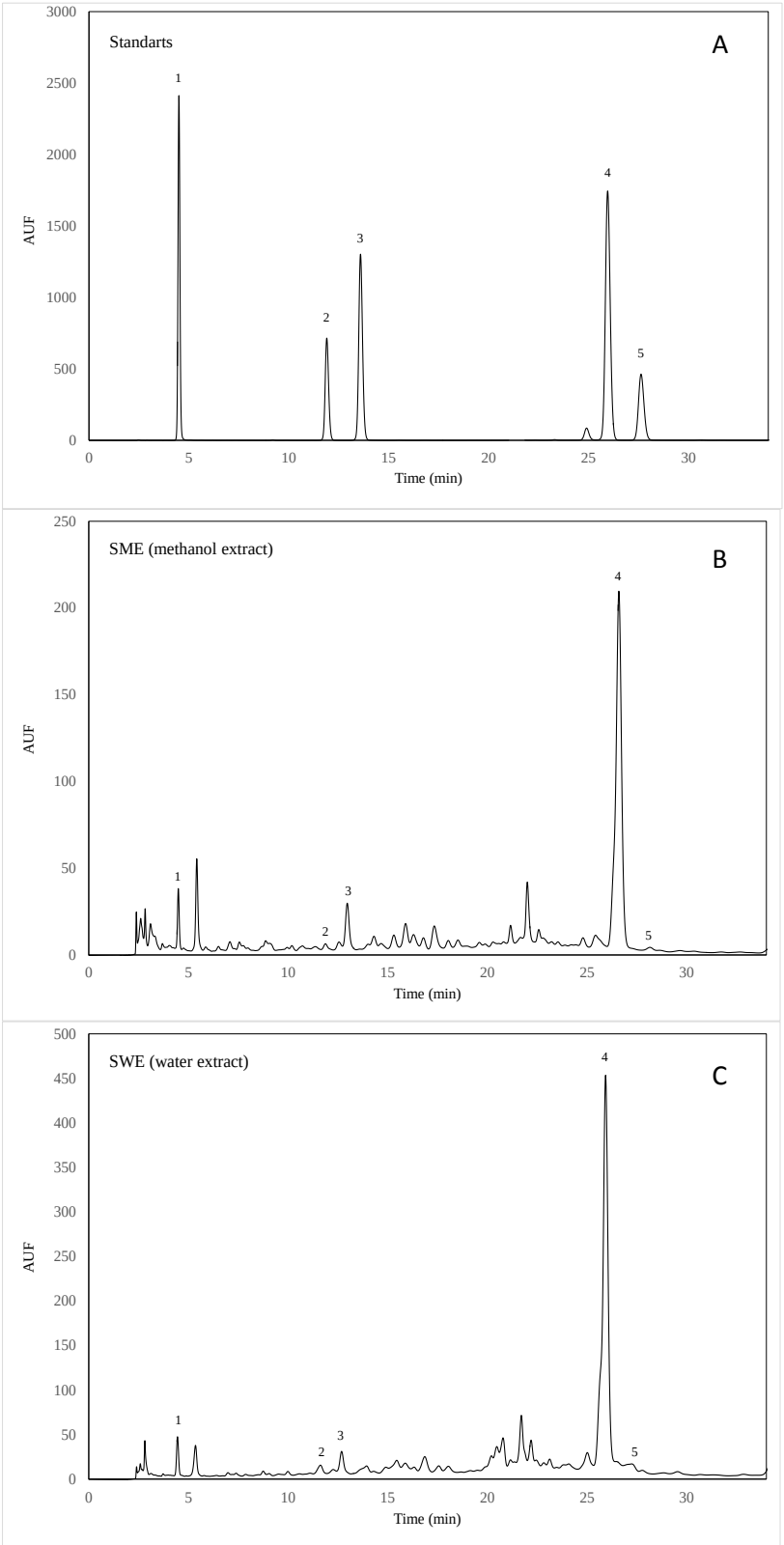

Figure 1. HPLC chromatogram of *Salvia virgata* extracts and standards. A; Standards, B; Methanol extract, C; Water extract, 1; *p*-OH-benzoic acid, 2; caffeic acid, 3; *o*-coumaric acid, 4; rosmarinic acid, 5; luteolin.

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH•) is a kind of stable organic radical. The DPPH• oxidative assay is used worldwide in the quantification of radical-scavenging capacity. The DPPH• alcohol solution is deep purple and possesses a characteristic absorption maximum between 515-517 nm. When reduced to hydrazine derivative by an antioxidant *via* electron or hydrogen atom transfer reactions, this absorption maximum decreases (25-27). The reactive rate and the ability of the antioxidant depend on the rate and the peak value of disappearance of the DPPH•. All sage extracts managed to scavenge the DPPH• radicals at physiological pH and did so in a concentration-dependent fashion. IC_{50} values defined as the concentration required to scavenge 50% of the available free radicals, estimated by non linear regression for all the extracts are presented in Figure 2. According to Figure 2, the aqueous methanol extract was more active than (IC_{50}: 0.16 mg/mL) the water extract (IC_{50}: 0.20 mg/mL). Also aqueous methanol extract was as active as ascorbic acid. The IC_{50} value of rosmarinic acid was found 0.20 mg/mL. The radical scavenging of rosmarinic acid was higher than ascorbic acid, BHT and BHA. It is estimated that the concentration of rosmarinic acid as main component is related to DPPH• radical scavenging activity. The strong superoxide scavenging activities of the rosmarinic acid and its derivatives like their DPPH• radical- scavenging activities may also be attributed to the presence of the caffeic acid moiety which is contained in sage fractions (28).

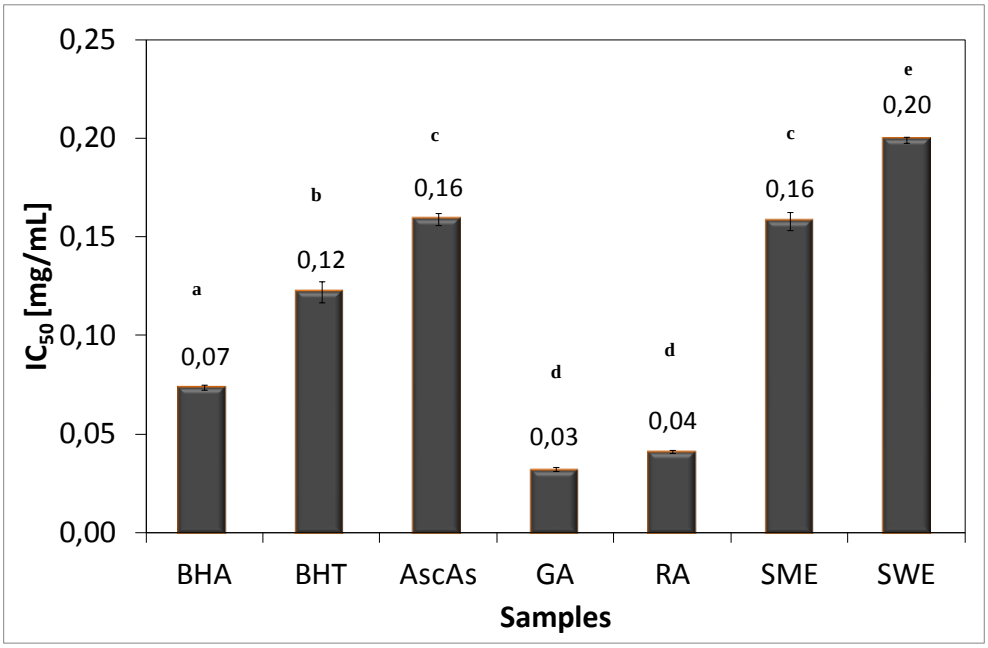

Figure 2. Effect of the extracts of *S.virgata* and positive controls on DPPH• radical scavenging. AscAs, ascorbic acid; BHT, Butylated hydroxytoluene; BHA, Butylated hydroxyanisole; GA, gallic acid; RA,

rosmarinic acid; SME 70% methanol extract; SWE water extract.Values are presented as means ± standard error. Bars with the same letter (a-e) are not significiantly ($p< 0.05$) different.

ABTS$^{\bullet+}$ radical scavenging

The DPPH radical is frequently used to determine the free radical scavenging activity of natural products (29). Occasionally, however, it is inappropriate to use this particular free radical due to sample solubility or spectral interference (30). An alternative synthetic radical is ABTS$^{\bullet+}$ a moderately stable nitrogen-centered radical species. Although the principle underpinning the use of ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ free radicals is essentially identical, ABTS$^{\bullet+}$ based models are more versatile as both non polar and polar samples can be assessed and spectral interference is minimized as the absorption maximum used is 760 nm, a wavelength not normally encountered with natural products (31). The extracts analyzed at two different concentrations and the percentage inhibition was plotted as function of concentration and the total antioxidant activity was calculated using Trolox calibration curve. As can be seen in Figure 3, all extracts and positive controls manage to inhibit the ABTS^{+} radical in a concentration-depended fashion. Also it is found that aqueous methanol extract and water extract were more active than ascorbic acid. Water extract inhibit the ABTS^{+} radical both two concentrations (0.25 mg/mL and 0.5 mg/mL). Rosmarinic acid (TEAC 1.23-2.26 mg/mL) was found to be more active than BHT (TEAC 0.57-1.17 mg/mL).

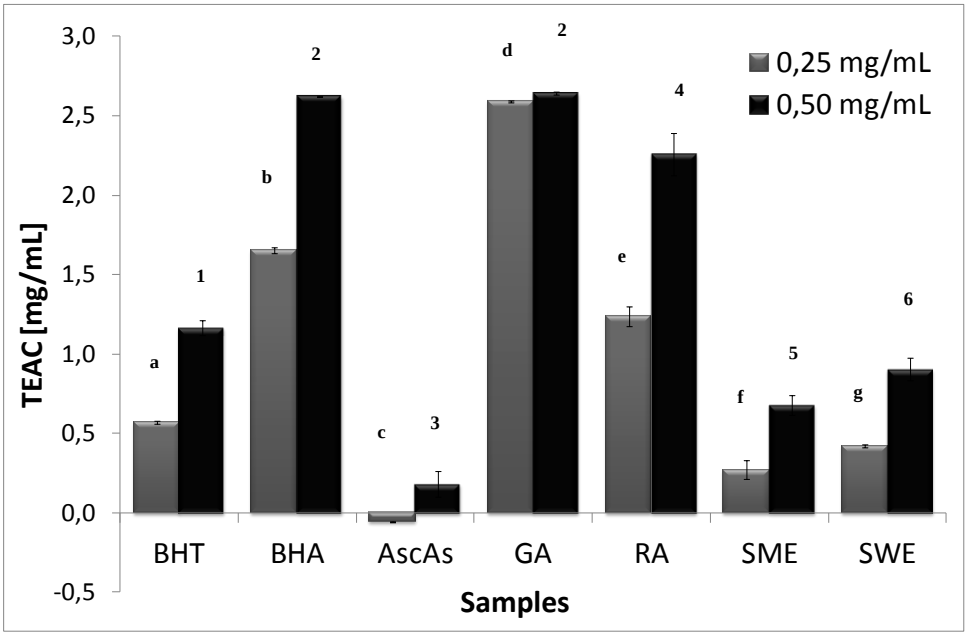

Figure 3. Effect of the extracts of *S.virgata* and positive controls on ABTS$^{\bullet+}$ radical scavenging. AscAs, ascorbic acid; BHT, Butylated hydroxytoluene; BHA, Butylated hydroxyanisole; GA, gallic acid; RA,

rosmarinic acid; SME 70% methanol extract; SWE water extract. Values are presented as means ± standard error. Bars with the same letter (a-g) and number (1-6) are not significantly ($p< 0.05$) different.

Iron(II) chelation activity

Foods are often contaminated with transition metal ions which may be introduced by processing methods. Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions *via* Fenton chemistry processes can be delayed by iron chelation and deactivation (32). Therefore, the ability of the extracts to chelate iron(II) ions was evaluated and expressed as Na_2EDTA equivalents ($mg_{Na2EDTA}/g_{extract}$). The results are presented in Figure 4.The aqueous methanol extract and water extract showed an activity on Iron (II) chelation. Flavonoids that are presented in these extracts are responsible for this activity. The structural characteristics of flavonoids are efficient on binding the Iron (II) (17). None of the extracts were not as active as EDTA.

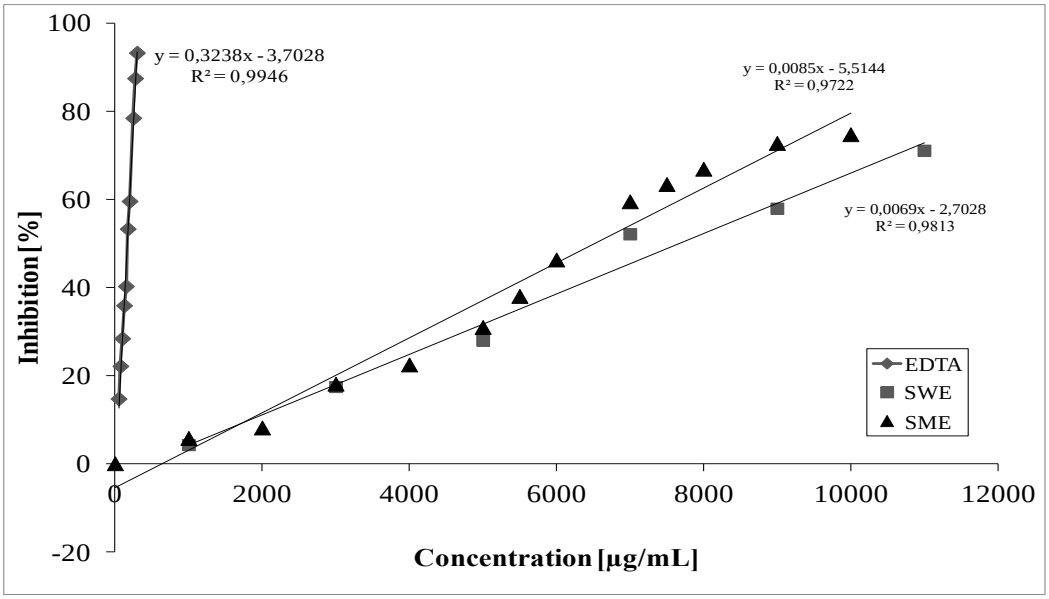

Figure 4. Effect of the extracts of *S.virgata* and positive control on Iron(II) chelation. EDTA ethylenediaminetetraacetic acid; SME 70% methanol extract; SWE water extract. Data are presented as means ± standard error.

Ascorbate-Iron(III)-Catalyzed Phospholipid Peroxidation

Polyunsaturated fatty acid rich phospholipids are important biologically relevant substrates and are susceptible to hydroxyl radical-mediated degradation. Furthermore, it is considered that the oxidative deterioration of lipids is the principal cause of the development of rancidity in lipid-rich products (33, 34).Therefore, it was considered important to assess the ability of the *S.virgata* extracts to protect phospholipids against hydroxyl radical mediated oxidative

degradation. In the ascorbate-iron(III)-catalyzed hydroxyl radical-mediated degradation of bovine brain-derived phospholipid liposomes, a sample capable of inhibiting the formation of TBARS can be described as an antioxidant capable of protecting phospholipids at physiological pH from oxidative degradation via a mechanism of hydroxyl radical scavenging. As seen in Figure 5, none of the *S.virgata* extracts were not as active as positive controls. Aqueous methanol extract was found to be more active than water extract.

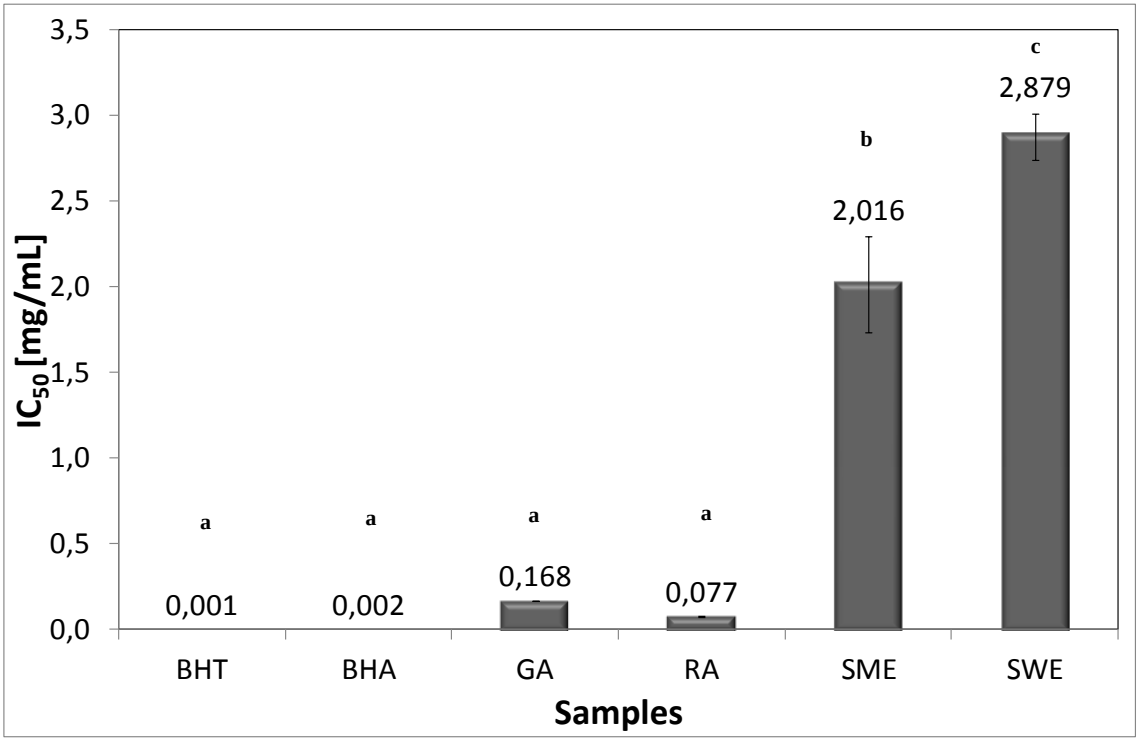

Figure 5. Effect of the extracts of *S.virgata* and positive controls on Hydroxyl radical-mediated phospholipid degradation. BHT, butylated hydroxytoluene; BHA, butylated hydroxyanilin; GA, gallic acid; RA, rosmarinic acid; SME 70% methanol extract; SWE water extract. Data are presented as means ± standard error. Bars with the same letter (a-c) are not significantly ($p< 0.05$) different.

DISCUSSION

As well known from the literature, rosmarinic acid was found as the principal component. Also rosmarinic acid, carnosic acid, carnosol and their derivatives in sage species are main components and these compounds are responsible for their antioxidant activities (5, 20, 21, 23, 24, 35, 36).

In current study the aqueous methanol extract is determined to have the higher phenolic composition and higher rosmarinic acid level. Therefore it’s found to be the most effective

extract. Evaluation of relationship between antioxidant activity of plant products and their phenolic content has been discussed in several studies. Some authors found a correlation while others found no relationship (35) between total phenolic content and antioxidant activity. Göger et al. also demonstrated that the 50% methanol extract of *S.virgata* was found to have significantly higher activity than the other extracts on reducing the iron (III) to iron (II) (12). Phenolic acids and flavonoids are dissolved in polar solvents so they show strong activity in the polar systems like, iron(III) to iron(II) reduction activity and $DPPH^{\bullet}$ scavenging activity. Therefore the fractions which are rich by the phenolic acids and flavonoids found to be efficient in these assays.

It is estimated that the concentration of main component rosmarinic acid is related to the scavenging activity against $DPPH^{\bullet}$ radical. The strong superoxide scavenging activities of the rosmarinic acid and its derivatives may also be attributed to the presence of the caffeic acid moiety like their $DPPH^{\bullet}$ radical-scavenging activities (28). The results showed that phenolic acids (rosmarinic acid derivatives, sagecoumarin, sagerinic acid, salvianolic acid) has potent activity while the flavonoid glycosides (luteolin and apigenin glycosides) all display weak to moderate activities, though they are still better than trolox (28). Also Kosar et al. reported that carnosic acid and rosmarinic acid were found to be the principal radical scavengers in sage by the HPLC- post column $DPPH^{\bullet}$ method (5).

The ABTS assay is based on the generation of a blue/green $ABTS^{\bullet+}$, which is applicable to both hydrophilic and lipophilic antioxidant systems; whereas DPPH assay uses a radical dissolved in organic media and is, therefore, applicable to hydrophobic systems (36). The difference between antioxidant capacities of the extracts determined by the two assays is agreed with the principles of the assays.

Plant extracts are rich in phenolic compunds and therefore these phenolics complex with transition metal ions, rendering them unable to participate in metal- catalyzed initiation (37). From the represented data *S.virgata* extracts may be able to afford protection against oxidative damage by complexing free iron(II) ions.

Free radical mediated degradation of phospholipids is resulted with cell death and also considered to be responsible for the oxidative deterioration and off flavor development of foods. Non polar antioxidants are concentrated at the lipid-air interface and demonstrate high protection in emulsions against the polar antioxidants presented in aqueous phase (28, 30) used the same method for *Sideritis* extracts and compared this method with some other antioxidant tests. According to the data presented the extracts were able to protect phospholipids by scavenging hydroxyl radicals before the reaction of susceptible components within the lipid

matrix. Therefore the extracts especially the methanol extract have a potent protection ability of biological membrane from the actions of reactive hydroxyl radical.

CONCLUSION

Overall, the data suggest that the *S.virgata* used in this study possess promising antioxidant properties *in vitro*. This plants also can be used to developing ingredient for the new antioxidant agents for different materials such as cosmetics and dietary foods. Further work should be carried out to determine whether extracts possess *in vivo* activities; otherwise, their potential use in the functionalization of cosmetics and foods cannot be confidently assumed.

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