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Comparison of Antioxidant Capacities of the Leaves and Flowers of *Salvia fruticosa* Grown in Turkey

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Total phenolic contents, hydroxyl (•OH) and 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radicals scavenging, reducing powers and total antioxidant capacities in the leaves and flowers extracts of *Salvia fruticosa* (Lamiaceae) from Turkey were investigated *in vitro*. Total phenolic contents of leaves and flowers extracts were determined to be 1.154 ± 0.060 and 0.384 ± 0.015 µg gallic acid/µg, respectively. The highest. OH scavenging capacity was 74 % in presence of 0.174 mg/mL leaves extract. The leaves extracts of *S. fruticosa* showed higher DPPH• scavenging with 96.220 % at 2.780 mg/mL as compared with flowers extracts. The reducing powers, •OH and DPPH• scavenging capacities of leaves and flowers extracts of *S. fruticosa* were concentration dependent and increased with increasing amount of extract sample. Vitamin C equivalences of reducing power values of leaves and flowers extracts in presence of 5.6 µg/mL extracts were determined as 5.937 ± 0.440 and 1.851 ± 0.060 µg/mL, respectively. The reducing effectiveness' on the formation of linoleic acid peroxides were higher in leaves extracts than flowers extracts.

Key Words: *Salvia fruticosa* (Lamiaceae), Total antioxidant capacity, reducing power, 2,2-Diphenyl-1-picrylhy-drazyl radical, Hydroxyl radical, Total phenolic content.

INTRODUCTION

In healthy aerobic organisms, production of reactive oxygen species (ROS) is approximately balanced by antioxidant defense systems. The situation of a serious imbalance between production of ROS and antioxidant defense system is refers to the oxidative stress. ROS is a collective term often used to include not only the oxygen radicals (superoxide anion radical, hydroxyl radical, *etc.*) but also some non-radical derivatives of oxygen (hydrogen peroxide, *etc.*)[1]. ROS are capable of damaging many targets *in vivo*, including lipids, proteins and DNA[2]. Antioxidant defense systems consist of flavanoids, carotenoids, phenolic compounds, vitamins and antioxidant enzymes, *etc.*[3,4].

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Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which commonly used in processed foods, are very effective but they may possess mutagenic activity and toxicological effect[5]. The dietary intake of antioxidant compounds is important for health[6]. For this reason, recently increasing attention has been directed toward natural antioxidants. Because they can protect the human body from free radicals and retard the progression of many chronic diseases[7]. Natural antioxidants occur in all higher plants and in all parts of the plant such as leaves, fruit, flowers and seeds[8].

The genus *Salvia* L. with over 900 species is probably the largest member of the family Lamiaceae and is found in both subtropical and temperate parts of the world. *Salvia* (Labiatae) is represented in Turkey by 94 taxa belonging to 89 species with 50 % endemism[9]. *Salvia* taxa of Turkey were classified by according to main components[10,11].

Most *Salvia* species (69 %) are moderately rich in oil (0.1-1.0 %), while nine taxa (14 %), which comprise commercial species, contains > 1 % oil[12,13]. Essential oil, which gives off a fragrance, is a characteristic feature of many species of *Salvia*. For this reason, it is widely used in perfumery and as a sweetener in the food industry[14].

Moreover, some scientists have studied flavonoids (from *Salvia palaestina*) effects on *Staphylococcus aureus, S. epidermis, E. coli, P. aeruginosa, Proteus vulgaris* and *Klepsiella* as well as their minimum inhibition concentrations (MIC) and minimum antibacterial concentration (MBC) rates[15]. *Salvia fruticosa* is used as antiseptic and tea for cold, cough and stomachache. This plant species are important in the diagnosis and treatment of some diseases[16,17].

The purpose of this study was to investigate the total phenolic contents, hydroxyl ($OH^{\bullet}$) and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radicals scavenging, reducing powers and total antioxidant capacities in extracts of *Salvia fruticosa* leaves and flowers.

EXPERIMENTAL

All solvents/chemicals used were of analytical grade and obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Plant samples used in this study were leaves and flowers of *S. fruticosa*. This plant is shrub species growing in western provinces of Turkey. The parts of *S. fruticosa* were collected from the B1 Izmir-Odemis, Bozdag, Mermeroluk (1250 m) when flowering. Taxonomical description of the species has been made according to Davis[9]. This plant was identified by Ass. Prof. Dr. M.Nakiboglu and voucher specimen was deposited in the herbarium of Ege University of Botanic Garden (EGE 31949).

Extraction: Leaves and flowers of *S. fruticosa* were collected from Izmir, Turkey. Dried plant parts were crushed in a coffee grinder for 2 min,

but at 15 s intervals the process was stopped for 15 s to avoid heating of the sample. Powdered plant samples (0.26 g) were infused in boiled water (20 mL) by agitating in magnetic stirrer for 5 min. Then, the extracts were filtered and centrifuged at 5000 rpm for 10 min. The obtained extracts were expressed as leaves extract and flowers extract.

Determination of total phenolic contents: Total phenolic contents in the extracts were measured by using the prussian blue assay, based on oxidation and reduction of iron[18]. Gallic acid (0.0-1.7 µg/mL) was used as the standard and results were expressed as gallic acid equivalents (GAE) in (µg GAE/µg extract) dry material.

The extract (0.10 mL), 50.0 mL distilled water and 3.0 mL 0.10 M $FeNH_4(SO_4)_2$ (in 0.10 M HCI) were mixed. Exactly 20 min after the addition of the ferric ammonium sulphate, 3.0 mL 0.008 M $K_3Fe(CN)_6$ were added and mixed. 20 min after the addition of ferricyanide, absorbance was read at 720 nm against to blank.

Determination of hydroxyl radical ($OH^{\bullet}$) scavenging capacity: The deoxyribose method was used for. OH scavenging capacity[19]. Reaction mixture contained in a final volume of 1.0 mL, following reagents at the final concentrations stated: deoxyribose (2.8 mM), $FeCl_3$ (0.1 mM), EDTA (0.104 mM), H_2O_2 (1 mM), ascorbate (0.1 mM) and extracts or BHA, as a positive control. If a Fe^{2+}-EDTA chelate is incubated with deoxyribose in 20 mM phosphate buffer at pH 7.4, $^{\bullet}OH$ radicals formed. Reaction mixture was incubated at 37 ºC for 1 h and colour developed with thiobarbituric acid (TBA). The absorbance at 532 nm was measured as a pink malondialdehyde-TBA chromagen.

Determination of 1,1-diphenyl-2-picryl-hydrazil ($DPPH^{\bullet}$) radical scavenging capacity: The $DPPH^{\bullet}$ radical scavenging capacities in leaves and flower extracts of *S. fruticosa* was measured by 2,2-diphenyl-1-picryl-hydrazil ($DPPH^{\bullet}$) using the method of Shimada *et al.*[20]. The $DPPH^{\bullet}$ solution (1 mM) was prepared in methanol. Half of millitres of various dilutions of the extracts or ascorbic acid as a positive control was mixed with 1.5 mL of $DPPH^{\bullet}$ solution. The samples were incubated for 0.5 h at 25 ºC and the decreases in the absorbance values were measured at 517 nm.

Determination of reducing power: The reducing power of the extract was quantified by the method of Oyaizu[21]. The method is based on reducing of Fe^{3+} to Fe^{2+}. Samples at different concentrations, phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1 %, w/v) were mixed and incubated for 20 min at 50 ºC. The reaction was terminated by adding trichloroacetic acid solution (10 %, w/v) and the mixture was centrifuged at 5000 rpm for 10 min. The supernatant was mixed with distilled water and ferric chloride (0.1 %, w/v) solution and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicated the reducing power of the samples.

Determination of total antioxidant capacity: Total antioxidant capacities of the extracts were determined according to thiocyanate method[22]. Linoleic acid emulsion (0.02 M) in phosphate buffer (0.02 M, pH 7.0) was prepared by mixing linoleic acid with Tween 20 (0.02 M). Each extract was mixed with linoleic acid emulsion and incubated in dark at 37 ºC. The amount of peroxide was determined, by reading the absorbance at 500 nm after colouring with $FeCl_2$ and thiocyanate, at intervals during incubation.

Statistical analysis: Tukey test, one of the multiple comparisons, was used for statistical significance analyses. The values are the mean of three separate experiments. Also comparisons between antioxidant capacities in the extracts were made with Pearson correlation.

RESULTS AND DISCUSSION

Total phenolic contents: The total phenolic contents in leaves and flower extracts of *S.* fruticosa were determined to be 1.154 ± 0.060 and 0.384 ± 0.015 µg gallic acid/µg dry biomass, respectively. These content values were higher than those of *Polygonum cognatum* Meissn, *Salvia officinalis*, *Thymus vulgaris, Rosmarinus officinalis* and *Origanum vulgaris*[23,24]. It was reported that major phenolic compounds identified in the *Salvia* species were rosmarinic acid, salvianolic acid, carnosol and carnosic acid[25,26]. The ability of the phenolics to act as antioxidants depends on the redox properties of their phenolic hydroxyl groups that allow them to act as reducing agents, hydrogen-donating antioxidants[27]. *S. fruticosa* belonging to the Lamiaceae family are very rich in polyphenolic compounds.

Hydroxyl radical ($^{\bullet}$OH) scavenging: Hydroxyl radical can be generated in biologically relevant systems by multiple reactions. $^{\bullet}$OH reacts quickly with almost every type of molecule found in living cells: sugars, amino acids, phospholipids, DNA bases and organic acids. Indeed, this radical is the most reactive oxygen radical known[1,2]. $^{\bullet}$OH scavenging potentials of the obtained both extracts were shown in Fig. 1. While the $^{\bullet}$OH scavenging capacity of leaves extract was determined as 74 % in presence of 0.174 mg/mL of extract, that of flower extract was 72.080 % in presence of 0.337 mg/mL of extract. The scavenging effect increased with increasing both sample concentrations up to a certain extent ($p < 0.01$).

Hydroxyl radical scavenging capacities (%) in leaves extract of *S. fruticosa* were higher than extracts of *Origanum vulgaris, Rosmarinus officinalis, Salvia officinalis* and *Thymus vulgaris*[24]. IC_{50} is the amount of extract providing 50 % inhibition of $^{\bullet}$OH. Lower IC_{50} value reflects better protective action of the extracts. IC_{50} values in leaves and flower extract of *S. fruticosa* were determined as 0.065 and 0.140 mg/mL, respectively. IC_{50} values for BHA, *O. vulgaris*, *R. officinalis, S. officinalis* and *T. vulgaris* were 2.2 µg/mL, 3.375, 3.764, 2.159 and 3.747 mg/mL, respectively.

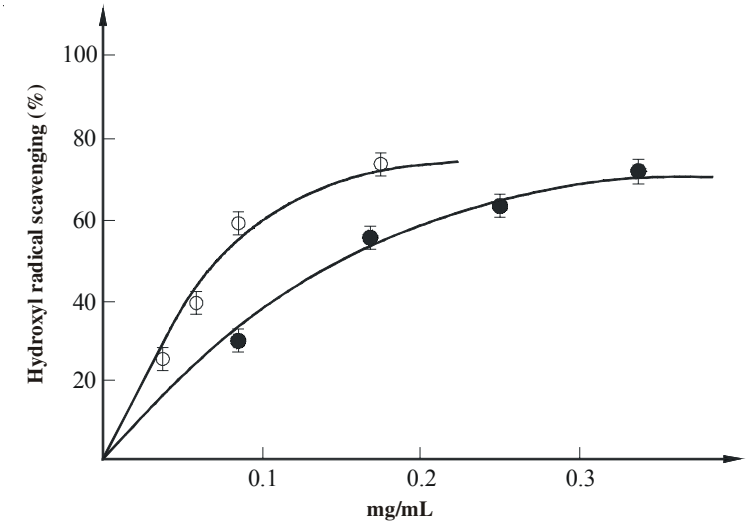

Fig. 1. Hydroxyl radical scavenging activities (%) of the leaves (Φ) and flowers (●) of *S. fruticosa* depending extract concentration

Similarly, Ozcan[13] reported that antioxidant effect of sage (*Salvia fruticosa* L) was low compared with that of BHT. The result would support that *S. fruticosa* extracts have effective •OH scavenging potential compared with these other studied plants.

DPPH Radical scavenging capacity: The synthetic nitrogen-centered DPPH. is not biologically relevant, but is often used as indicator compound in testing hydrogen-donation capacity and thus antioxidant capacity. The leaves and flower extracts of *S. fruticosa* showed a concentration dependent antiradical capacity by scavenging DPPH• (%) (Fig. 2). The leaves extracts of *S. fruticosa* showed higher radical scavenging activity with 96.220 % at 2.780 mg/mL as compared with that's of flower extracts. The highest scavenging capacity (80.730 %) for flower extracts was accompanied in presence of 4.010 mg/mL extract amount and this amount was approximately 1.5-fold higher than leaves extracts. The DPPH• scavenging capacity of the extracts may be mostly related to their phenolic hydroxyl group[28]. DPPH• scavenging capacity of leaves extracts was higher than that of *Salvia palaestina*[29]. IC_{50} values for leaves and flower extracts were determined as 0.700 and 1.550 mg/mL, respectively. In addition, ascorbic acid concentration providing IC_{50} value was found to be 4.500 μg/mL.

It appears that the obtained extracts possess hydrogen donating capabilities and act as an antioxidant. However, the antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of radical chain initiation, binding of transition metal ions catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging[30].

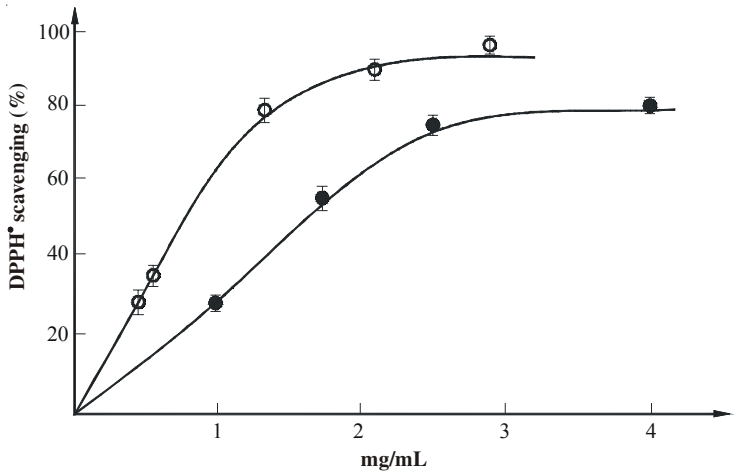

Fig. 2. DPPH radical scavenging activities (%) of the leaves (Φ) and flowers (●) of *S. fruticosa* depending extract concentration

Reducing rower: Table-1 shows the reductive capabilities (as indicated by the absorbance at 700 nm) of the extracts compared to a known reducing reagent, vitamin C. In presence of 5.600 µg/mL extract amount the absorbances at 700 nm for leaves and flower extracts of *S. fruticosa* were determined as 0.937 ± 0.070 and 0.292 ± 0.010, respectively. The reducing powers of both extracts were concentration dependent and increased with increasing amount of sample ($r = 0.780$; 0.702, $p < 0.01$). High absorbance indicates high reducing power. Since phenolic compounds present in the extracts are good electron donors, they show the reducing power. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[31]. The data from the iron reduction assay suggest that the extracts are able to donate electrons to reactive radicals, converting them into more stable and unreactive species. The reducing properties are generally associated with the presence of reductones[32]. Reductones are reported to react with certain precursors of peroxide, thus preventing peroxide formation.

Vitamin C equivalence of leaves extracts has more than flower extracts ($p < 0.01$). Vitamin C equivalences of leaves and flower extracts, at the same extract amount (5.6 µg/mL), were determined as 5.937 ± 0.440 and 1.851 ± 0.060 µg/mL, respectively. In addition, the reducing powers of both extracts were *ca.* 100-fold higher than extracts of *Origanum vulgaris, Rosmarinus officinalis, Salvia officinalis* and *Thymus vulgaris* and also, 45-fold higher than that's of fennel ($p < 0.01$)[24,33].

Total antioxidant capacity: Total antioxidant capacities in leaves and flower extracts of *S. fruticosa* were determined by the thiocyanite method, in which the amount of peroxides formed in the emulsion during incubation. The presence of the obtained extracts in the linoleic acid emulsion

TABLE-1
REDUCING POWERS OF LEAVES AND FLOWERS OF *S. fruticosa* DEPENDENT EXTRACT AMOUNT

S. fruticosa leaves			*S. fruticosa* flowers		
µg/mL	Abs-700 nm	Vit. C eqv. (µg/mL)	µg/mL	Abs-700 nm	Vit. C eqv. (µg/mL)
0.96	0.095 ± 0.008	0.605 ± 0.051	5.60	0.292 ± 0.010	1.851 ± 0.060
2.79	0.476 ± 0.030	3.017 ± 0.190	10.81	0.555 ± 0.035	3.518 ± 0.220
3.61	0.628 ± 0.040	3.981 ± 0.254	13.52	0.711 ± 0.046	4.511 ± 0.292
5.59	0.937 ± 0.070	5.937 ± 0.440	15.71	0.812 ± 0.060	5.189 ± 0.383

was able to reduce the formation of peroxides (Fig. 3). There were statistically differences between the control, in which there was no extract and these extracts ($p < 0.01$). It was determined that total antioxidant capacity of LE was significantly higher than its FE ($p < 0.01$).

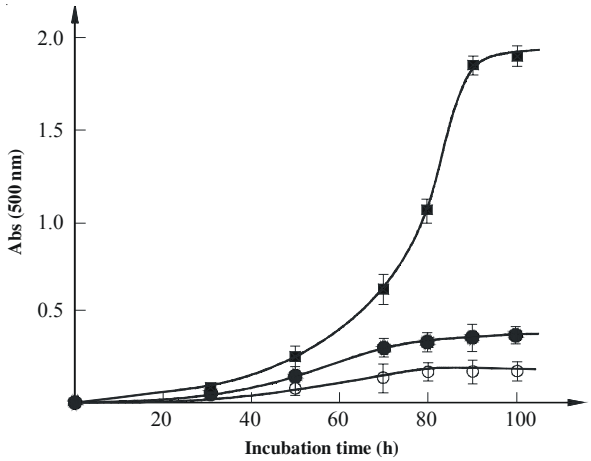

Fig. 3. Total antioxidant capacities of control (■), the water extracts of leaves (Φ) and flowers (●) of *S. fruticosa* in presence of 0.25 mg/mL extract concentration

Conclusion

The total phenolic contents, •OH, DPPH• radicals scavenging, reducing power and total antioxidant capacities in leaves extract of *S. fructicosa* were significantly higher than those of flower extract ($p < 0.01$). The antioxidative capacities of both extracts are mainly due to the phenolic components. The result of this study suggests that the *S. fructicosa* can be used as a potential source of natural antioxidants in food and pharmaceutical industries as well as medicine. Although the extracts were found to be effective natural antioxidants, their potential exploitable beneficial effects and safety need to be proven in further trials.

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