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Screening of the antioxidant potentials of six *Salvia* species from Turkey

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Abstract

This study was designed to examine the in vitro antioxidant activities of the methanol extracts of six *Salvia* species [*Salvia caespitosa* Montbret & Aucher ex Bentham (ENDEMIC), *Salvia hypargeia* Fisch. & Mey. (ENDEMIC), *Salvia euphratica* subsp. *euphratica* Montbret & Aucher ex Bentham (ENDEMIC), *Salvia sclarea* L., *Salvia candidissima* subsp. *candidissima* Montbret & Aucher ex Bentham and *Salvia aethiopis* L.] from Turkey. The extracts were screened for their possible antioxidant activities by two complementary test systems, namely DPPH free radical-scavenging and β-carotene/linoleic acid systems. Non-polar subfractions of the methanol extracts of *Salvia* species studied did not show any antioxidant activity in both test systems. In the first case, the most active plant was *S. euphratica* subsp. *euphratica*, an endemic species, with an IC_{50} value of 20.7 ± 1.22 μg/ml, followed by *S. sclarea* (IC_{50} = 23.4 ± 0.97 μg/ml) among the polar subfractions. In the β-carotene/linoleic acid test system, polar extract of *S. hypargeia* was superior to the polar extracts of other *Salvia* species studied (69.2% ± 1.90%). This activity was followed by *S. sclarea* with 63.5% ± 4.24% inhibition rate. The inhibition rate of the synthetic antioxidant, buthylated hydroxytoluene (BHT), was also determined to be 96%. Since the polar extracts of *Salvia* species dealt with here exhibited excellent antioxidant activities when compared to BHT, it seems possible to keep perishable fat-containing food longer by direct addition of an extract of sage.

Keywords: *Salvia*; Antioxidant activity; DPPH; β-Carotene/linoleic acid test

1. Introduction

Preservation of the lipidic fraction of foods from oxidative deterioration represents an important aim from the point of view of quality and shelf-life. It is indeed well known that the products derived from lipid oxidation reduce organoleptic quality and safety, besides reducing nutritional properties because of destruction of oxygen-sensitive vitamins. In order to limit these undesirable effects, various methods have been proposed. Besides physical processes, such as oxygen removal and refrigeration, the use of substances that decrease the rates of these oxidation processes plays a very important role (Vichi, Zitterl-Eglseer, Jugl, & Franz, 2001). Synthetic antioxidants, such as BHA and buthylated hydroxytoluene (BHT), are very effective but they may possess mutagenic activity (Namiki, 1990). For this reason the desire for natural food has led to the search for naturally-occurring antioxidants.

Antioxidants can act by the following mechanisms in lipid peroxidation: (i) decreasing localized oxygen concentrations; (ii) preventing chain initiation by scavenging initiating radicals; (iii) binding catalysts, such as metal ions, to prevent initiating radical generation; (iv)

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decomposing peroxides so they can not be reconverted to initiating radicals; and (v) chain-breaking to prevent continued hydrogen abstraction by active radicals (Dorman, Peltoketo, Hiltunen, & Tikkanen, 2003).

In recent years, spice extracts have appeared on the market as antioxidants for food industry use. The antioxidant capacity of some of these compounds has been proved to be comparable to, and sometimes higher than, that of synthetic antioxidants (Cuvelier, Berset, & Richard, 1990; Pokorny, 1991). In particular, the Lamiaceae family includes a large number of plants that are well known for their antioxidant properties. Among these, rosemary and sage have been widely used and most of their antioxidant components have been identified. It has been established that the antioxidant effects are mainly due to phenolic compounds (Das & Pereira, 1990; Pokorny, 1991; Schwarz & Ternes, 1992).

The genus *Salvia* (Laminaceae) includes nearly 900 species spread throughout the world. This genus is represented in Turkey by 89 species and, althogether, 94 taxa, 45 of which are endemic in Turkey. The rate of endemism in the genus *Salvia* in Turkey is ca. 45% (Davis, 1982; Davis, Mill, & Tan, 1988; Guner, Ozhatay, Ekim, & Baser, 2000). Many *Salvia* spp. are used as herbal tea and for food flavouring, as well as in cosmetics, perfumery and the pharmaceutical industries (Chalchat, Michet, & Pasquier, 1998).

The aim of present study was to examine the in vitro antioxidant activities of the methanolic extracts of six *Salvia* species [*Salvia caespitosa* Montbret & Aucher ex Bentham (ENDEMIC), *Salvia hypargeia* Fisch. & Mey. (ENDEMIC), *Salvia euphratica* subsp. *euphratica* Montbret & Aucher ex Bentham (ENDEMIC), *Salvia sclarea* L., *Salvia candidissima* subsp. *candidissima* Montbret & Aucher ex Bentham and *Salvia aethiopis* L.] from Turkey as well as the antioxidant potential of rosmarinic acid (RA), a major phenolic compound of many *Salvia* species. A literature survey did not reveal any reference to a previous work on the antioxidant activities of *S. euphratica*, *S. hypargeia*, *S. caespitosa*, *S. aethiopis* and *S. candidissima*. On the other hand; Gulcin, Uguz, Oktay, Beydemir, and Kufrevioglu (2004) have examined the radical-scavenging activities of the chloroform and acetone extracts of *S. sclarea*. Most of the studies are focussed on *S. officinalis* (garden sage), since it was used as a reference plant due to its well-known and widely documented antioxidant properties. Therefore, we think that the results presented here will bring a great novelty for the species studied.

Numerous techniques are available to evaluate the antioxidant activities of compounds and complex mixtures, such as plant extracts. Despite the various methods, just one procedure cannot identify all possible mechanisms characterising an antioxidant. Therefore, the extracts obtained by Soxhlet extraction were screened for their possible antioxidant activity by two complementary test systems, namely DPPH free radical-scavenging and β-carotene/linoleic acid systems.

2. Materials and methods

2.1. Plant material

Herbarium information of the six plant species which are individually numbered are listed below:

1. *S. caespitosa*: Topcuyenikoy, Hafik, Sivas-Turkey; 05th July, 2004.
2. *S. hypargeia*: Gurun-Kangal turnoff, Sivas-Turkey; 07th July, 2004.
3. *S. euphratica* subsp. *euphratica*: between Bolucan-Sincan, Sivas-Turkey; 24th June, 2004.
4. *S. sclarea*: Taslidere, Sivas-Turkey; 16th July, 2004.
5. *S. candidissima* subsp. *candidissima*: Gurun-Kangal turnoff, Sivas-Turkey; 07th July, 2004.
6. *S. aethiopis*: Taslidere, Domuzlukici district, Sivas-Turkey; 16th July, 2004.

The voucher specimens have been deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas-Turkey (CUFH-Voucher nos.: 1-AA 3393; 2-AA 3410; 3-AA 3390; 4-AA 3441; 5-AA 3406 and 6-AA 3433, respectively).

2.2. Preparation of the methanolic extracts

The air-dried and finely ground samples were extracted by using the method described elsewhere (Sokmen, Jones, & Erturk, 1999). Briefly, the sample (weighing about 100 g) was extracted in a Soxhlet with methanol (MeOH) at 60 °C for 6 h (15.06%, 13.05%, 14.85%, 16.58%, 17.01% and 13.09%, w/w, respectively). The resulting extracts were suspended in water and partitioned with chloroform ($CHCI_3$) to obtain water-soluble (polar) (11.79%, 8.88%, 10.11%, 11.50%, 12.11% and 8.68%, w/w, respectively) and water-insoluble (non-polar, chloroformic) subfractions (3.27%, 4.15%, 4.74%, 5.06%, 4.90% and 4.41%, w/w, respectively), which were then lyophilised and kept in the dark at +4 °C until tested.

2.3. Antioxidant activity

2.3.1. DPPH assay

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-coloured methanol solution of DPPH. This spectrophotometric assay uses the stable radical, 2,2′-diphenylpicrylhydrazyl (DPPH·), as a reagent (Burits & Bucar, 2000; Cuendet,

Hostettmann, & Potterat, 1997). 50 μl of various concentrations of the extracts in methanol were added to 5 ml of a 0.004% methanol solution of DPPH·. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical DPPH in percent ($I\%$) was calculated in following way:

$$I\% = (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100,$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate.

2.3.2. β-Carotene/linoleic acid assay

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius, Venskutonis, Beek, & Linssen, 1998). A stock solution of β-carotene/linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade), and 25 μl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml distilled water, saturated with oxygen (30 min 100 ml/min), were added with vigorous shaking; 2.5 ml of this reaction mixture were dispersed into test tubes and 350 μl portions of the extracts prepared at 2 g l^{-1} concentrations, were added and the emulsion system was incubated for up to 48 h at room temperature. The same procedure was repeated with the synthetic antioxidant, BHT as positive control, and a blank. After this incubation period, absorbances of the mixtures were measured at 490 nm. Antioxidative capacities of the extracts were compared with those of BHT and blank.

3. Results and discussion

The extracts obtained by Soxhlet extraction were screened for their possible antioxidant activity by two complementary test systems, namely DPPH free radical-scavenging and β-carotene/linoleic acid systems. The overall activity test results from our experiments indicated the superiority of the polar extracts to the non-polar extracts studied. Therefore, antioxidant activity potential of the polar extracts produced from the herbal parts of the *Salvia* species studied are now considered. Free radical-scavenging capacities of the corresponding extracts measured by DPPH assay are shown in Table 1. Among the polar subfractions of the extracts, the most active one was *S. euphratica* subsp. *euphratica*, an endemic species, with an IC_{50} value of 20.7 ± 1.22 μg/ml, followed by *S. sclarea* (IC_{50} = 23.4 ± 0.97 μg/ml). Polar extracts of *S. aethiopis* did not show any radical-scavenging activity in this test system. The IC_{50} value of the synthetic antioxidant BHT was measured as 18.8 ± 1.21 μg/ml.

Table 1
Free radical-scavenging capacities and the inhibition ratio of linoleic acid oxidation by extracts measured in DPPH and β-carotene/linoleic acid assays[a]

Plants	Results in DPPH system (%)	Results in β-carotene/linoleic acid system (%)
Salvia caespitosa	41.3 ± 2.14	55.9 ± 2.40
S. hypargeia	34.6 ± 1.36	69.2 ± 1.90
S. euphratica subsp. *euphratica*	20.7 ± 1.22	59.1 ± 1.76
S. sclarea	23.4 ± 0.97	63.5 ± 4.24
S. candidissima subsp. *candidissima*	49.7 ± 1.72	62.3 ± 4.66
S. aethiopis	0	29.0 ± 2.05
BHT	18.8 ± 1.21	96.0 ± 0.23
Rosmarinic acid	2.90 ± 0.30	100 ± 0.27

[a] Results are means of three different experiments.

In the β-carotene/linoleic acid system, the polar extract of *S. hypargeia* was superior to the polar extracts of other *Salvia* species studied (69.2% ± 1.90%). This activity was followed by *S. sclarea* with a 63.5 % ± 4.24% inhibition rate. As confirmed by the first test system (DPPH), the weakest antioxidant activity was exhibited by the polar extract of *S. aethiopis* (Table 1).

It is very important to evaluate the molecular mechanisms of the radical-scavenging activities of polar extracts for better understanding of the mode of action. The capability of different phenolic substances to scavenge various types of oxidation-initiating radicals has been reported in the polar phase (Bors, Heller, Michel, & Saran, 1990; Rive-Evans, Miller, & Paganga, 1996; Yen & Duh, 1994). If there is an electron donation group, especially a hydroxyl group located on *o*- or *p* - positions of the phenolic compounds, it makes the compound polar and therefore antioxidant activities are increased (Duan et al., 1998; Weng, 1993). Also, this is one of the the main reasons why rosmanol, a major constituent of many *Salvia* species, has such strong antioxidant activities because these groups make the phenols more easily donate hydrogen atoms to activate free radicals to interrupt the chain reaction of autoxidation (Weng & Wang, 2000). This idea was also supported in a report by Gu and Weng (2001). These workers reported that the antioxidant activity of *S. plebeia* was concentrated in the acidic, weakly acidic and phenolic fractions of each extract while the activities of neutral fractions were negligible.

Plants belonging to the Laminaceae family are very rich in polyphenolic compounds. Polyphenolic compounds have been shown to have antioxidant activity and it is likely that the activity of the examined plants

is due to these compounds (Okuda, Yoshida, & Hatano, 1994). Major phenolic compounds identified in the extracts of sage are rosmarinic acid, carnosic acid, carnosic acid, salvianolic acid and its derivatives carnosol, rosmanol, epirosmanol, rosmadial and methyl carnosate (Lu & Fo, 2001; Madsen & Bertelsen, 1995; Wu, Lee, Ho, & Chang, 1982). Additionally, some other compounds were described by several scientists for many *Salvia* species: ferruginol, salvipisone, microstegiol, candidissol, 2,3-dehydrosalvipisone, aethiopinone, 1-oxoaethiopinone, salvinolone, crytojaponol, acetylsalvipisone and sclareapinone for *S. sclarea* (Ulubelen, Sonmez, & Topcu, 1997), aethiopinone for *S. aethiopis* (Hernandez-Perez, Rabanal, Arias, de La Torre, & Rodriguez, 1999), 6β-hydroxyisopimaric acid and 3-acetylvergatic acid for *S. caespitosa* (Ulubelen et al., 2001), 11β-hydroxymanoyl oxide, 8,13-diepimanoyl oxide, spathulenol, salvigenin crysoeriol, diosmetin, *o*,*p*-dimethoxybenzoic acid for *S. candidissima* (Topcu, Tan, Ulubelen, Sun, & Watson, 1995).

Carnosol and carnosic acid, which have ortho-dihydroxyl groups on the aromatic ring, possess good peroxyl and hydroxyl radicals scavenging activities. They inhibit the formation of hydroxyl radicals and chelate metals, while only carnosic acid appears to scavenge H_2O_2 (Aruoma, Halliwell, Aeschbach, & Loligers, 1992). According to Lu and Fo (2001), salvianolic acid L has the capacity to scavenge DPPH$^{\cdot}$ and superoxide anion radicals. RA also has a distinct antioxidative effect, which makes it a valuable product for the pharmaceutical and food industries (Petersen, Hausler, Meinhard, Karwatzki, & Gertlowski, 1994).

It can be concluded that the antioxidant activities of the selected plants have been poorly investigated; therefore testing of their anti radical properties is of interest, primarily in order to find new promising sources for natural antioxidants, functional foods and pharmaceuticals. In conclusion, we think that it might be possible to keep perishable fat-containing food longer by a direct addition of an extract of sage due to the measured antioxidative effects of that extract.

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