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Comparative Study of the Antioxidant Activities of Eleven *Salvia* Species

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The lipid peroxidation-inhibiting activities of aqueous methanolic extracts of eleven *Salvia* species (Fam. Lamiaceae) were evaluated in an enzyme-independent biological system. The total polyphenol contents and the amounts of the most abundant phenoloids of the genus, caffeic and rosmarinic acids, were also determined. The EC_{50} values of the extracts displayed substantial differences. All of the investigated species except *S. jurisicii* (EC_{50} 191.2 µg/mL) exhibited higher activities than that of ascorbic acid (EC_{50} 123.8 µg/mL), the reference compound. Among the studied species, *S. scabiosifolia* (EC_{50} 5.4 µg/mL) demonstrated the highest effect, followed in sequence by *S. dumetorum*, *S. transsylvanica*, *S. officinalis* l *albiflora*, *S. nemorosa* l *albiflora* and *S. recognita* (EC_{50} 6.5 – 10.2 µg/mL). The close correlation was confirmed between the antioxidant activities and the total phenol contents of the extracts. For caffeic and rosmarinic acids, the correlation was much weaker, indicating the important role of other polyphenols in the antioxidant activity.

Keywords: Antioxidant, *Salvia*, Polyphenol, Rosmarinic acid, Caffeic acid.

During the past decade, the importance of antioxidants has been discussed in many scientific reports [1a,1b]. Such compounds help protect living organisms against oxidative stress either by scavenging free radicals (a direct effect) or by inducing antioxidant enzymes (an indirect effect). It is well known that highly reactive oxidative fragments of either endogenous or exogenous origin can damage cellular macromolecules. It has been proven in recent years that, as a consequence of the destruction of components sensitive to oxidation, harmful processes are initiated that can lead to the development of such conditions as inflammation, hypertension, cancer, diabetes, and Alzheimer's disease [2a-2c]. Herbal-based products with antioxidant action can, therefore, be suggested as potential preventive or complementary medicines for these disorders. A growing body of evidence supports the development of such preparations [2d].

Besides synthetic materials (for example, butylated hydroxyanisole and butylated hydroxytoluene), natural compounds, including extracts of Lamiaceae species, have been introduced in the food and cosmetics industries, because of their low toxicity and marked reducing ability [2e].

Analysis of plant extracts with free radical-neutralizing ability has revealed that various phenoloids (hydroxycinnamic acid derivatives, diterpenes and flavonoids) are responsible for the beneficial effects [3a,3b]. Many of the non-volatile ingredients described from *Salvia* species belong to this group of phenoloids [3c]. Thus, plants rich in such compounds may be taken into account as potential antioxidants; this is confirmed by the results of many studies of different *Salvia* taxa [3d-3f]. Our previous study on 11 European *Salvia* species indicated a positive correlation between the total phenol content and the scavenger capacity [4]. A thorough phytochemical investigation of *S. candelabrum*, the most active species of this screen, resulted in the identification of polyphenolic abietane diterpenes [5] with higher antioxidant activities than that of rosmarinic acid, widely regarded as one of the strongest scavengers [6]. As a continuation of our earlier studies on the antioxidant activities of *Salvia* species, the present work involved an *in vitro* evaluation of the protective effects of 11 subsequent *Salvia* species against enzyme-independent lipid peroxidation (LPO) in a biological matrix. Activities were quantified in order to examine the correlation between antioxidant capacity and chemical composition. Such results can contribute

to the selection of the most active species worthy of detailed phytochemical studies.

Fifty percent aqueous methanolic extracts were prepared from the leaves of *S. argentea, S. dumetorum*, *S. hispanica, S. jurisicii, S. nemorosa* l. *albiflora*, *S. officinalis* l. *albiflora*, *S. recognita, S. scabiosifolia, S. sclarea, S. transsylvanica* and *S. viscosa,* concentrated and subjected to anti-LPO assay on a standard rat-brain homogenate.

All 11 *Salvia* extracts exhibited concentration-dependent LPO-inhibitory activities when tested against the auto-oxidation of the rat-brain homogenate (Figure 1). With the only exception of *S. jurisicii* (EC_{50} 191.2 µg/mL), the tested extracts were found to be more effective than the positive control ascorbic acid (EC_{50} 123.8 µg/ml). The most pronounced activities were demonstrated by *S. dumetorum*, *S. officinalis* l. *albiflora*, *S. scabiosifolia* and *S. transsylvanica* (EC_{50} 6.5 – 7.0 µg/mL). The antioxidant capacities of *S. nemorosa* l. *albiflora* (EC_{50} 9.6 µg/mL) and *S. recognita* (EC_{50} 10.2 µg/mL) were only slightly lower. For the two white-flowered varieties, the EC_{50} values obtained on the rat-brain homogenate were practically the same as those found in an earlier experiment with *S. officinalis* and *S. nemorosa* on ox-brain homogenate [4].

In order to establish a possible relationship between the LPO-inhibitory activities and the chemical compositions of the extracts, quantitative determinations were carried out on some compounds. Similar to the LPO-inhibitory activities, the total phenol contents of the extracts, determined with the Folin-Chiocalteus method, varied in a wide range (between 1.7% and 9.1%) (Table 1). As for the EC_{50} values, the lowest and highest total phenol contents were those of *S. jurisicii* and *S. scabiosifolia*, respectively. The rosmarinic acid contents of the extracts were also investigated because this compound is the most abundant caffeic acid derivative in *Salvia* species, and their free radical-scavenger activities are often primarily attributed to this compound [7,8]. Rosmarinic acid levels were found to be from 0.15 to 3.37%. Its monomer, caffeic acid, exhibited a lower concentration (0.005-0.20%) than that of rosmarinic acid in all the investigated extracts, which is characteristic for the genus *Salvia* [9].

The antioxidant activities of the extracts of the eleven *Salvia* species correlated closely with the total phenol contents (Figure 2), as indicated by the R value of 0.892. This corresponds with the results of our former *Salvia* study [4]. However, for the rosmarinic and caffeic acid contents, the correlations were much weaker, with R values of 0.627 and 0.500, respectively. These data indicate that the presence of rosmarinic acid is an important, but not dominant condition for strong antioxidant activity. There have been several reports of the good scavenging potential of other polyphenolic substances, for example, diterpenes and flavonoids. Abietane diterpenoids containing an aromatic ring have been described among others from *S. candelabrum,* with LPO-inhibiting potentials exceeding that of rosmarinic acid [5]. Flavonoids in sage extracts have antioxidant potentials lower than those of hydroxycinnamic acid derivatives and diterpenes, but they may be involved in synergism, resulting in enhancement of the free radical-neutralizing activity [3b,10].

Table 1: Rosmarinic acid, caffeic acid and total phenol contents of the investigated *Salvia* extracts.

Salvia species	Rosmarinic acid (%)	Caffeic acid (%)	Total phenol (%)
S. argentea	0.19	0.03	4.98
S. dumetorum	2.09	0.08	6.54
S. hispanica	0.15	0.02	2.97
S. jurisicii	0.87	0.005	1.74
S. nemorosa l. *albiflora*	0.93	0.06	4.47
S. officinalis l. *albiflora*	0.85	0.05	6.44
S. recognita	3.37	0.20	6.62
S. scabiosifolia	2.59	0.08	9.10
S. sclarea	0.87	0.05	3.07
S. transsylvanica	3.09	0.11	8.04
S. viscosa	0.20	0.03	2.62

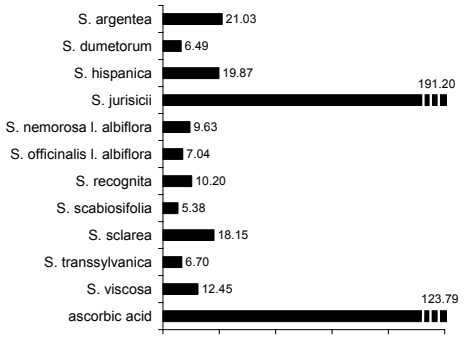

Figure 1: Antioxidant activities of the investigated *Salvia* extracts.

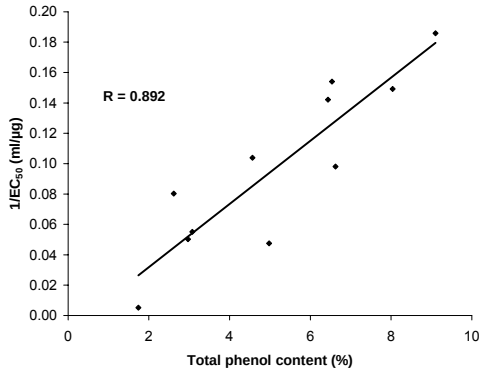

Figure 2: Relationship between the antioxidant potencies ($1/EC_{50}$) of the investigated *Salvia* extracts and their total phenol contents in the rat-brain assay.

Our literature survey did not reveal any references to previous work on the antioxidant activities of the herbs of *S. dumetorum*, *S. hispanica, S. jurisicii*, *S. recognita, S. scabiosifolia*, *S. transsylvanica,* and *S. viscosa*. The methanolic extract of *S. argentea* has been reported to display antioxidant potency in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) test systems [11]. The antioxidant potential of the methanolic extract of *S. sclarea* was studied by Turkish research groups in the DPPH and β-carotene/linolenic acid systems and via the xanthine/xanthine oxidase enzymatic method, and the extract was observed to display high activity in all tests [3d,12]. Comparison of the results published in the various papers is rather difficult because the same plant extract showed diverse activities in different test systems, indicating the complexity of the oxidation process. In general, combined plant extracts are advantageous in overcoming oxidative stress as the complex phenoloid content allows them to intervene at diffent levels of the chain process and inhibit the deleterious effects.

In conclusion, the antioxidant activities of selected *Salvia* species make them a valuable subject for the pharmaceutical and food industries. Some species, such as *S. dumetorum*, *S. recognita*, *S. scabiosifolia*, *S. transsylvanica* and *S. viscosa*, have been poorly investigated earlier, and they are therefore of interest, primarily in the search for promising new sources of natural antioxidants.

Experimental

Materials: The leaves of the 11 *Salvia* species were harvested, at the flowering stage, in 2004 (*S. viscosa* in 2005), mainly in the experimental field of the Institute of Ecology and Botany of the Hungarian Academy of Sciences, Vácrátót, Hungary. In the case of *S. scabiosifolia*, the plant material (herb with green fruits) was collected in the Shumensko Plato National Park, Bulgaria. Voucher specimens (10824/04 *S. argentea* L., 8001/04 *S. dumetorum* Andrz., 11241/04 *S. hispanica* L., 8945/04 *S. jurisicii* Kosanin, T040/04 *S. nemorosa* L. l. *albiflora*, 1805/04 *S. officinalis* L. l. *albiflora*, L1567/04 *S. recognita* Fisch & Meyer, SSB/04 *S. scabiosifolia* Lam., 11144/04 *S. sclarea* L., 8068/04 *S. transsylvanica* (Schur) Schur, and 11237/05 *S. viscosa* Jacq.) have been deposited in the Herbarium of the Institute of Ecology and Botany in Vácrátót. The collected plant materials were dried at room temperature. Rosmarinic acid was purchased from ICN Pharmaceuticals, Inc. (Costa Mesa, USA); caffeic acid and ascorbic acid were from Sigma (St Louis, USA).

Extraction: Air-dried and powdered leaves of the *Salvia* species (0.5 g) were extracted with 50% aqueous methanol (8.00 mL) for 10 min. by means of an ultrasonic extractor (Tesla, Czechoslovakia). This procedure was repeated three times, and the filtered extracts were combined. The solvent was then removed under reduced pressure at 65°C, to yield a residue of 26.0% for *S. argentea*, 34.6% for *S. dumetorum*, 30.4% for *S. hispanica*, 34.7% for *S. jurisicii*, 31.7% for *S. nemorosa* l. *albiflora*, 24.3% for *S. officinalis* l. *albiflora*, 17.3% for *S. recognita*, 26.3% for *S. scabiosifolia*, 37.3% for *S. sclarea*, 26.3% for *S. transsylvanica*, and 27.5% for *S. viscosa*.

Measurements of antioxidant activity: The antioxidant effects of the extracts were measured via inhibition of the auto-oxidation of unsaturated fatty acids present in rat-brain tissue [13a]. Briefly, a lipid-rich fraction was prepared from the brains of male Sprague-Dawley rats (250-300 g) by homogenization and centrifugation. The fatty acids in this fraction are spontaneously oxidized during incubation for 1 h at 37°C, a process which can be inhibited by antioxidants. The oxidized products were determined by spectrophotometry after reaction with thiobarbituric acid. Experiments were carried out in duplicate and sigmoid curves were fitted to the results [13b]. EC_{50} values were calculated by means of GraphPad Prism 4 (GraphPad Software, San Diego, CA, USA). All experimental animal protocols fully conformed to the Guidelines for Animal Experimentation approved by the Animal Experimentation Committee at the University of Szeged.

Determination of rosmarinic and caffeic acid contents: The TLC densitometric method for the determination of rosmarinic acid and caffeic acid contents is described by Janicsák and Máthé [13c].

Determination of total phenol content: The total phenol content was determined by the colorimetric method adapted from European Pharmacopoeia edition 3, using the Folin-Chiocalteus reagent. Results were expressed in g of caffeic acid per 100 g of dry matter. Each sample was analysed in duplicate, and a calibration graph with 3 datapoints for caffeic acid was used. Correlations between antioxidant potencies and total phenol contents were computed with the Microsoft Excel 2003 program.

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