Antioxidant Activities of Some Lamiaceae Plant Extracts

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The antioxidant activities of four Lamiaceae plants, Salvia viridis L., Salvia multicaulis Vahl, Stachys byzantina C. Koch and Eremostachys laciniata (L.) Bunge have been determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as well as by flow injection analysis-luminol chemiluminescence (FIA-CL). All extracts were shown to possess a significant scavenger activity against DPPH free radical and an inhibitory effect on \( \text{H}_2\text{O}_2 \) or HOCl-luminol chemiluminescence. The extracts scavenged 50% of DPPH radical ranging in the following descending order: Salvia viridis > Stachys byzantina > Salvia multicaulis > Eremostachys laciniata. The most potent extract on \( \text{H}_2\text{O}_2 \)-induced peak chemiluminescence was that of Salvia viridis and on HOCl-induced peak chemiluminescence was that of Stachys byzantina. The results concluded that the extracts have a potential source of antioxidants of natural origin. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: antioxidant activity; Lamiaceae; plant extracts; radical scavenging activity; flow injection analysis; luminol chemiluminescence.

INTRODUCTION

Free radicals play a crucial role in the development of tissue damage in various human diseases such as cancer, aging, neurodegenerative disease, malaria and arteriosclerosis, and pathological events in living organisms (Gutteridge, 1994). Antioxidants may have an important role in the prevention of these diseases. There is an increasing interest in the antioxidant effects of compounds derived from plants, which could be relevant in relation to their nutritional incidence effects of compounds derived from plants, which could have an important role in the prevention of these diseases. Antioxidants.

Medicinal plants and herbs are promising and diverse sources of natural antioxidants. Therefore, a great number of different spices and aromatic herbs have been investigated for their antioxidant activity. Some, particularly those belonging to the family Lamiaceae, have been found to be very effective with regard to natural antioxidants. In various studies, rosemary, sage, oregano and thyme have shown strong antioxidant activity (Cuvelier et al., 1996; Hirasa and Takemasa, 1998; Frankel et al., 1996a; b; Frankel et al., 1997; Frankel, 1999; Huang and Frankel, 1997).

Salvia is one of the most diverse genera of plants in Turkey with 88 species, of which more than half are endemic (Davis, 1982). In Turkish folk medicine, an infusion of Salvia species herbs has been used to treat common colds, against abdominal pain and stomach disorders (Honda et al., 1996). On the other hand, Salvia officinalis (sage), a well-known antioxidant herbal plant, is used as a popular folk medicine for the treatment of various ailments, such as antispasmodic and antiseptic (Bruneton, 1995). The genus Stachys is also widespread in the flora of Turkey, and an infusion of these herbs has been used in Turkish folk medicine as a stomachic (Davis, 1982; Yesilada et al., 1995). Many reports are devoted to the chemical composition, as well as to the pharmacological activities of Salvia and Stachys species. The presence of essential oils (Baser et al., 1998; Skaltsa et al., 2003) and phenolic compounds (Lu and Foo, 2002; Meremeti et al., 2004) in these two genera has been determined. To our knowledge, there is no report on phytochemical and pharmacological studies on Eremostachys species in the available literature. The genus Salvia has been investigated particularly for antioxidant properties (Weng and Wang, 2000; Gu and Weng, 2001; Lu and Foo, 2001a, b; Choi et al., 2001; Bozan et al., 2002). The reported high antioxidant activity of sage prompted an investigation of Lamiaceae plants for which antioxidant potency has not been studied yet, to provide new potential sources of natural antioxidants.

The present study is aimed at the evaluation of the aqueous extracts of four Lamiaceae plants growing in Turkey, namely Salvia viridis, S. multicaulis, Stachys byzantina and Eremostachys laciniata by using two testing methods: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method and HOCl or \( \text{H}_2\text{O}_2 \)-luminol chemiluminescence (CL) by flow injection analysis (FIA).

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MATERIALS AND METHODS

Chemicals. All chemicals were of analytical reagent grade and obtained from the following sources: Luminol (5-amino-2,3-dihydro-phthalazinedione), ascorbic acid, sodium hydroxide, hexadecyltrimethylammonium bromide (HTAB), cobalt (II) chloride hexahydrate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), tert-butyl-1-hydroxytoluene (BHT) and quercetin (Sigma); hydrogen peroxide, sodium chloride, potassium dihydrogen phosphate, gallic acid and methanol (Merck); sodium hypochlorite (Aldrich Chemical Company).

A 10⁻³ m luminol stock solution was prepared by dissolving 0.0177 g of luminol in NaOH, and phosphate-buffered saline (PBS: 10 mm KH₂PO₄ and 150 mm NaCl, pH 7.4) was added up to 100.0 mL. PBS was used to control the acidity of the interacting system. HTAB (as a surfactant, final concentration was 10⁻³ m) was added into the working solution of 10⁻⁴ m luminol before adding PBS to maintain luminol in a basic environment (and 10⁻³ m in Co²⁺ when the oxidant in use was H₂O₂). It was stored at 4°C and the luminol solution was protected from light by a foil wrapper.

Hydrogen peroxide solutions were prepared daily by serial dilution of 100-volume hydrogen peroxide and protected from light by a foil wrapper. HOCl was prepared as described previously by Vissers et al. (1994). NaOCl was diluted with PBS and the pH of the solution readjusted to 7.4. At this pH, the solution contains approximately 1:1 HOCl and NaOCl.

Plant materials. Four Lamiaceae plants were collected from different regions of Turkey in their natural habitats. Authenticated voucher specimens (Herbarium number) were deposited in the Herbarium of Faculty of Pharmacy, Gazi University; Salvia multicaulis Vahl (GUE 2314) and Eremostachys laciniata (L.) Bunge (GUE 2315) were collected from the vicinity of Van, in June 1997; Stachys byzantina C. Koch (GUE 1852) was collected from Bolu-Abant in June 1997; Salvia viridis L. (GUE 2313) was collected from Denizli, Pamukkale in May 1998.

Preparation of plant extracts. The aerial parts of the plant material were air dried to dryness at room temperature and under shade, and then powdered to a fine grade by using a laboratory scale mill. Each 10 g plant material was extracted with distilled H₂O at room temperature twice (× 50 mL). The combined aqueous extract was lyophilized to give the crude dry extract. The extract yields (w/w) were Salvia multicaulis (21.8%), Eremostachys laciniata (22.7%), Stachys byzantina (23.6%) and Salvia viridis (19.6%).

Methods. In the present study, two different assays used to evaluate the antioxidant activity of the aqueous extracts of four Lamiaceae plants growing in Turkey, were those of DPPH (1,1-diphenyl-2-picrylhydrazyl) and HOCl or H₂O₂-luminol chemiluminescence (CL) by flow injection analysis (FIA). DPPH and chemiluminescence were often used to evaluate the free radical scavenging activity (antioxidant capacity) of various compounds and medicinal plants (Choi et al., 2000; Desmarchelier et al., 1997; Fletcher et al., 2001).

Flow injection analysis is a rapid and quantitative method which can be used coupled with chemiluminescence. Reactive oxygen species (H₂O₂, HOCl-, etc.) mediated oxidation of luminol produces a chemiluminescence peak and the inhibitory effect on the peak chemiluminescence is a useful tool for the detection and characterization of radical scavengers (Wheatley et al., 2003).

DPPH radical-scavenging activity. The free radical scavenging activities of the extracts on the stable radical DPPH were estimated by the method of Brand-Williams et al. (1995). 0.75 mL of a methanol solution of the extract at different concentrations was mixed with 1.5 mL of a DPPH methanol solution (20 mg/L). The absorbance was measured at 517 nm after 20 min of reaction. The percent DPPH decolorization of the sample was calculated according to the equation

\[
\% \text{ Decolorization} = \left[ 1 - \left( \frac{\text{ABS}_{\text{sample}}}{\text{ABS}_{\text{control}}} \right) \right] \times 100
\]

Ascorbic acid, BHT, gallic acid, quercetin and an extract of seeds of Vitis vinifera (Vitaceae) were used as controls.

HOCl or H₂O₂-luminol chemiluminescence. A schematic diagram of the FIA-CL system is shown in Fig. 1. The peristaltic pump was a Gilson Minipuls 3 and the injection valve was a Rheodyne RH-5020, obtained from Anachem (Luton, Bedfordshire, UK). The pump tubing had a suitable internal diameter to deliver the required flow-rate. The remainder of the flow-injection manifold was constructed from PTFE tubing joined with low-pressure fittings from Anachem (UK). CL detection was carried out using a lumino-meter (Model: Lumi-Flo, Chrono-log, USA). The results were recorded on a chart recorder (Model 706-707, Chrono-log, USA).

The oxidant stream was merged with a luminol/buffer reagent immediately before the luminometer. The total flow rate was 1 mL/min, shared equally between the luminol and the oxidant channel; the oxidant channel includes an injection valve in the middle allowing successive nominal 20 µL injections of the plant extracts to be made. Mixed flow of oxidant/antioxidant was matched with luminol/buffer before the entrance to the flow cell.

Statistics. The decolorization was plotted against the sample extract concentration, and a linear regression curve was established in order to calculate the IC⁵₀ (mg/mL) being the amount of sample necessary to decrease by 50% the absorbance of DPPH. All the analyses were done in triplicate, and mean values ± SE were calculated.
carried out in triplicate and the results were expressed as the mean ± SD.

The CL was measured as the photomultiplier output in mV; the effects of the antioxidants were measured by the depression of the signal from its uninhibited level and were expressed as a percentage attenuation of the maximum CL due to the antioxidant. The results were given as mean ± SEM, n referring to the number of experiments. IC₅₀ values of inhibitory effects of extracts and ascorbate were calculated by using probit regression analysis.

**RESULTS AND DISCUSSION**

**DPPH radical-scavenging activity**

The free radical scavenging activity of four Lamiaceae plants growing in Turkey, *Stachys byzantina*, *Salvia viridis*, *S. multicaulis* and *Eremostachys laciniata* was determined using a stable DPPH free radical. The DPPH scavenger capacity of the extracts was compared with known antioxidative substances (BHT, ascorbic acid, quercetin and gallic acid) and a known antioxidative plant extract, grape seed extract.

The DPPH radical-scavenging activities of the reference substances and the extracts studied in this study are shown in Table 1. All the extracts of Lamiaceae plants were shown to possess significant DPPH radical-scavenging activity. The most active plant was found to be *Salvia viridis* (IC₅₀ = 0.57). The effectiveness of antioxidants as DPPH radical scavengers ranged in the following descending order: *Salvia viridis* (0.57 mg/mL) > *Stachys byzantina* (0.64 mg/mL) > *Salvia multicaulis* (0.83 mg/mL) > *Eremostachys laciniata* (5.18 mg/mL) > gallic acid (40.0 mg/mL) > grape seed (66.6 mg/mL) > quercetin (85.0 mg/mL) > ascorbic acid (125.8 mg/mL) > BHT (194.9 mg/mL).

*Salvia* and *Stachys* (Lamiaceae) represent two of the most diverse genera of plants in Turkey. *Eremostachys* is represented by three species in Turkey (Davis, 1982). *Salvia* species have been investigated particularly as a source of natural antioxidants (Weng and Weng, 2000; Gu and Weng, 2001; Lu and Foo, 2001a, b; Choi et al., 2001; Bozan et al., 2002). Earlier studies on the antioxidative activity of *Salvia officinalis* was reported to be attributed mainly to the presence of phenolic compounds, such as carsonic acid, rosmarinic acid and salvianolic acid (Cuvelier et al., 1996; Lu and Foo, 2001b). Recently, in *Stachys* species, the *in vitro* antioxidant activity of *Stachys spruneri* was investigated and found to be as active as the reference drug, α-tocopherol (Couladis et al., 2003). To the best of our knowledge, the antioxidant activity of *Eremostachys* species has not been investigated. The ethanol extracts were applied to TLC. Spraying of TLC plates with a DPPH alcohol solution showed that all spots corresponding to phenolic compounds reacted with the DPPH reagent. Phenolic compounds are known as high-level antioxidants because of their ability to scavenge free radicals and active oxygen species such as singlet oxygen, superoxide anion radical and hydroxyl radicals (Hall and Cuppett, 1997). Plants of the *Salvia* genus mainly contain essential oil and phenolic compounds such as flavonoids, phenolic acids and phenolic diterpenes (Baser et al., 1998; Lu and Foo, 2002). The *Stachys* species contain essential oils, flavonoids and terpenoids (Skalska et al., 2003; Meremeti et al., 2004). Therefore, these phenolic compounds in the extracts could be responsible for the antioxidant activity found in the present study. To the best of our knowledge, this is the first report on the DPPH radical-scavenging activity of the four Lamiaceae plant extracts.

**Inhibitory effect on H₂O₂ or HOCl-luminol induced peak chemiluminescence**

This systematic study identified the direct antioxidant potential of some Lamiaceae plants growing in Turkey against a spectrum of oxidants (H₂O₂ or HOCl) by using FIA coupled to luminol chemiluminescence. By using flow-injection analysis-luminol chemiluminescence method, it was demonstrated that the aqueous extracts of *Salvia viridis*, *S. multicaulis*, *Stachys byzantina* and *Eremostachys laciniata* have significant inhibitory effects on the peak chemiluminescence signal produced by luminol–H₂O₂ or luminol–HOCl systems.

A continuous CL signal from H₂O₂ (10⁻⁴ m) (in the presence of 10⁻⁴ m luminol and 10⁻⁵ m Co²⁺ in PBS at pH 7.4) was obtained. The H₂O₂-dependent CL signal was inhibited by the aqueous extracts of *Salvia multicaulis* (10⁻⁴–10⁻¹ m), *Salvia viridis* (10⁻⁴–1 m), *Stachys byzantina* (10⁻³–1 m) and *Eremostachys laciniata* (10⁻³–10 m) (n = 6). Ascorbic acid (chain-breaking reference antioxidant) (10⁻⁶–10⁻³ m) (n = 6) also inhibited the CL signal in a concentration-dependent manner. The IC₅₀ values (mg/mL) were 0.53 ± 0.06, 0.18 ± 0.08, 0.36 ± 0.06, 1.74 ± 0.2, 1.4 × 10⁻⁴ ± 2.9 × 10⁻⁵ for *Salvia multicaulis*, *S. viridis*, *Stachys byzantina*, *Eremostachys laciniata* and ascorbic acid, respectively (Fig. 2). The potency order was: Ascorbic acid > *Salvia viridis* > *Eremostachys laciniata* > *Salvia multicaulis* > *Stachys byzantina*.

The continuous CL signal obtained from NaOCl (10⁻⁴ m) (in the presence of 10⁻⁴ m luminol in PBS at pH 7.4) was also inhibited by the aqueous extracts of *Salvia multicaulis* (10⁻⁴–10⁻¹ m), *Salvia viridis* (10⁻⁴–1 m), *Stachys byzantina* (10⁻³–1 m) and *Eremostachys laciniata* (10⁻³–10 m) (n = 6). Ascorbic acid (10⁻²–10⁻¹ m) (n = 6) also inhibited the CL signal in a concentration-dependent manner. The IC₅₀ values (mg/mL) were 3.3 × 10⁻⁴ ± 10⁻⁵, 0.09 ± 0.02, 0.13 ± 0.02, 2.1 × 10⁻³ ± 6 × 10⁻⁴, 1.8 × 10⁻⁵ ± 2.0 × 10⁻⁶ for *Stachys byzantina*, *Salvia multicaulis*, *S. viridis*, *Eremostachys laciniata* and ascorbic acid, respectively (Fig. 3). The potency

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**Table 1. DPPH radical-scavenging activity of the extracts**

<table>
<thead>
<tr>
<th>Extract/compound</th>
<th>DPPH (IC₅₀) (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHT</td>
<td>194.9 ± 2.1</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>125.8 ± 5.2</td>
</tr>
<tr>
<td>Quercetin</td>
<td>85.0 ± 6.2</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>40.0 ± 2.4</td>
</tr>
<tr>
<td>Grape seed</td>
<td>66.6 ± 1.7</td>
</tr>
<tr>
<td>Salvia viridis</td>
<td>0.57 ± 2.6</td>
</tr>
<tr>
<td>Salvia multicaulis</td>
<td>0.83 ± 1.5</td>
</tr>
<tr>
<td>Stachys byzantina</td>
<td>0.64 ± 2.1</td>
</tr>
<tr>
<td>Eremostachys laciniata</td>
<td>5.18 ± 1.4</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD.
order was: ascorbic acid > Stachys byzantina > Salvia multicaulis > Salvia viridis > Eremostachys laciniata.

CONCLUSION

It has been reported that reactive oxygen species contribute to various pathophysiological conditions and endogenous defense mechanisms have evolved to offer protection in these conditions. An increase in the antioxidant reserves of the organism can reduce oxidative stress and some of the plant-derived agents may help to reduce it (Reilly et al., 1991). Determination of the natural antioxidant compounds of plant extracts will help to develop new drug candidates for antioxidant therapy.

The present study showed that some Lamiaceae plants growing in Turkey, which are often present in Turkish folk medicine, are strong radical scavengers. These plants may be considered as good sources of natural antioxidants for medicinal uses such as against aging and other diseases related to radical mechanisms. In this context, Lamiaceae plants investigated in the frame of the present study seemed to have potential antioxidant compounds. It is reported that plants of the Salvia and Stachys genus contain various phenolic compounds (Lu and Foo, 2002; Meremeti et al., 2004). It is generally accepted that phenolic compounds inhibit the oxidation of lipids by donating hydrogen atoms to scavenge free radicals (Zhang, 1998). Flavonoid antioxidants have been studied intensively in recent years for their multiple health promoting properties. Several groups (Das and Dereira, 1990) have studied the relationship between structure and antioxidant activity of flavonoids. As a comparison, the antioxidant activities of aqueous extracts of four Lamiaceae plants were based on the radical scavenging effect. The potency of these extracts could provide a chemical basis for some of the health benefits claimed for Salvia and Stachys species in folk medicine and further studies are necessary to assess their potential as effective natural remedies.

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