Full Length Research Paper

Comparison of the antioxidant activity and total phenolic contents in some Stachys species

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The methanolic extracts of the aerial parts of nine Stachys species: S. persica Gmel., S. fruticulosa M. B., S. laxa Boiss. & Buhse., S. inflata Benth., S. turcomanica Trautv., S. subaphylla Rech. F., S. setifera C. A. Mey., S. byzantina C. Koch and S. trinervis Aitch. & Hemsl. were investigated for their antioxidant activity and total phenolic content using FRAP and Folin-Ciocalteu assays respectively. S. persica Gmel. and S. fruticulosa M. B. had the highest antioxidant activity (61.42 and 62.02 mmol FeII/100g) and total phenolic content (3294.96 and 4450.36 mg gallic acid/100 g) among these nine species. There was a direct correlation between total phenol and antioxidant activity (R² = 0.9446, p ≤ 0.001) which indicates that polyphenols are the main antioxidants.

Key words: Stachys, antioxidant, total phenol.

INTRODUCTION

Free radicals are constantly generated in vivo for physiological purposes (Arouma, 1998). They can be overproduced in pathological conditions, causing oxidative stress (Sies, 1997). A large number of civilization-associated diseases such as autoimmune diseases, inflammation, cardiovascular-neurological diseases, cancer and aging are attributed to oxidative stress (Klaunig and Kamendulis, 2004; Kregel and Zhang, 2006; Maxwell, 2000; Rao and Balachandran, 2002; Wang and Maldonado, 2006). An adequate intake of natural antioxidants could protect macromolecules against oxidative damage in cells (Mittler, 2002; Riso et al., 2005). The term antioxidant refers to free radical scavengers, inhibitors of lipid peroxidation and chelating agent (Lee et al., 2003). Phenolic compounds possess a wide spectrum of biological effects including antioxidant and free radical scavenging (Kahkoneh et al., 1999; Pellati et al., 2004).

The genus Stachys (Lamiaceae) includes about 200 – 300 species in the world (Rechinger and Hedge, 1982). In Iran, this genus is represented by 34 species (Mozaffarian, 1996). Phytochemical studies in Stachys species have shown the presence of polyphenols including flavonoids (El-Ansari et al., 1995), tannins (Vundac et al., 2005), phenolic acids (Vundac et al., 2007), phenolic acids (Vundac et al., 2005), and phenyl ethanoid glycosides (Miyase et al., 1996; Nishimura et al., 1991).

Many studies have shown various activities in this genus such as anti-inflammatory (Khanavi et al., 2005; Kukik et al., 2007; Maleki et al., 2001; Sharifzadeh et al., 2005; Skaltsa et al., 2000), anti anxiety (Rabbani et al., 2003), antibacterial (Grujic-Jovanovic et al., 2004; Sonboli et al., 2005; Diğrak et al., 2001), anti-nephritic (Hayashi et al., 1994), anticancer (Amirghofran et al., 2006, 2007), anti-Helicobacter pylori (Stamatis et al., 2003), and antioxidant effects (Aydin et al., 2006; Kukik et al., 2006; Matkowski and Piotrowska, 2006). Some species of this genus are used in folk medicine, specially S. paalustris L. and S. sylvatica L. which are approved for healing wounds, treating abdominal pains and as disinfectant, anti-spasmodic and anti-fever (Gruenwald et

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In Iranian folk medicine, the aerial parts of *S. inflata* Benth. is used for infection, asthma, rheumatic and inflammatory disorders (Maleki et al., 2001). *S. recta* is used as an effective drug in treatment of wounds and *S. lovandulifolia* Vahl. is used for digestive disorders.

Most of species of this genus has been previously analyzed in numerous studies concerning their chemical composition, pharmacological properties and therapeutic uses. Nevertheless, the literature data on their antioxidant activities are scarce and little is known about chemical components with antioxidant activity. It seems that there is a significant relationship between the presence of total phenol and antioxidant activity in *Stachys* species.

In this research the antioxidant activity and total phenol contents of *Stachys* persica Gmel., *S. fruticulosa* M. B., *S. laxa* Boiss. & Buhse., *S. inflata* Benth., *S. turcomanica* Trautv., *S. subaphylla* Rech. F., *S. setifera* C. A. Mey., *S. byzantina* Koch and *S. trinervis* Aitch. & Hemsl. were investigated.

### MATERIALS AND METHODS

#### Plant material

Aerial parts of *S. setifera* C. A. Mey., *S. inflata* Benth., *S. persica* Gmel and *S. byzantina* were collected from Khalkhal, province of Ardabil, Iran. Others including, *S. laxa* Boiss., *S. turcomanica* Trautv., *S. subaphylla* Rech. F and *S. trinervis* were gathered from Golestan national park, province of Golestan, Iran. *S. fruticulosa* M. B was collected from Karaj, province of Tehran, Iran. All plants were cultivated in June 2006 during the flowering stage. Voucher specimens have been deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

#### Chemicals

All chemicals and reagents were analytical grade or purest quality purchased from Sigma, Merck, Aldrich and Fluka.

#### Apparatus

A UV visible Cintra 40 double-beam spectrophotometer equipped with a 1.0 cm path length glass cell and connected to an IBM compatible Pentium 100 computer was used.

#### Extraction methods

The extraction of antioxidant compounds and total phenolics from dried and finely powdered aerial parts were carried out using seven different solvents to compare the effect of extraction methods on antioxidant activity and content of total phenolic compounds. These solvents included water, ethanol, methanol, acetone/water (50:50, v/v), ethanol/water (50:50, v/v), methanol/water (50:50, v/v) and methanol/water/buthanol (40:40:20, v/v). 0.05 g of plant sample was extracted with 3×2 ml of methanol, using a shaker for 3×2 h.

The filtered extracts were applied freshly to measure antioxidant activity and total phenolics.

#### Evaluation of antioxidant activity using TPTZ

Several methods are known to measure the total antioxidant capacity of herbal samples, but we tried the FRAP assay, with depends upon the reduction of ferric tripiprydil triazine (Fe (III)-TPTZ) complex to the ferrous tripiprydil triazine (Fe (II)-TPTZ) by a reductant at low pH. (Fe (II)-TPTZ) has an intensive blue color and can be monitored at 593 nm (Benzie and Strain 1999). Briefly, the FRAP reagent contained 5 ml of a (10 mmol/L) TPTZ (2, 4, 6-tripiryldil-s-triazine) solution in 40 mmol/L HCl plus 5 ml of (20 mmol/L) FeCl3 and 50 ml of (0.3 mol/L) acetate buffer, pH 3.6 and was prepared freshly and warmed at 37°C. Aliquots of 100 μl sample were mixed with 3 ml FRAP reagent and the absorbance of reaction mixture at 539 nm was measured spectrophotometrically after incubation at 37°C for 10 min. For construction of calibration curve five concentrations of FeSO4.7H2O (1000, 750, 500, 250, 125 μg/L) were used and the absorbencies were measured as sample solutions. Antioxidant activity of nine *stachys* species were measured with this method and the values were expressed as the concentrations of antioxidants having a ferric reducing ability equivalent to that of 1 mmol/L FeSO4. Antioxidant activity of *stachys* species was measured five times for each species and the results are shown in Table 1.

#### Measurement of total phenolics

Total phenolics were determined colorimetrically using Folin-Ciocalteau reagent (Al-Farsi et al., 2005). The prepared extract (200 μl) was mixed with 1.5 ml of Folin-Ciocalteau reagent (previously diluted 10-fold with distilled water) and allowed to stand at 220°C for 5 min. A 1.5 ml sodium bicarbonate solution (60 g/L) was added to the mixture. After 90 min at 220°C, absorbance was measured at 725 nm using a UV-visible spectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the absorbance of a known concentration of gallic acid standard (25 - 150 μg/ml) in 50% methanol. The concentrations are expressed as milligrams of gallic acid equivalents (GAE) per 100 g of dry weight. The total phenolics assay of *Stachys* species was measured five times for each species and the results are shown in Table 1.

#### Statistical analyses

The values are reported as mean ± SD. One–way ANOVA and Tukey posthoc multi comparison tests were used for data analysis.

#### RESULTS

#### Extraction methods

In order to compare the effect of extraction methods on antioxidant activity and content of total phenolics in *Stachys* species, seven different solvents were used. Only one species was used as a representative of the plants to evaluate the solvent extraction process. Significant (p < 0.05) differences existed among different solvent used, with some exception. Extraction in to methanol gave the highest antioxidant activity and total phenolic content, whereas water afforded the lowest amount. These results showed that most of the potent antioxidant and phenolic compounds in *Stachys* species were soluble in methanol; so it was selected to extract the remaining species.
Table 1. Antioxidant activity and total phenolic content of the aerial parts of nine Stachys species.

<table>
<thead>
<tr>
<th>Species</th>
<th>FRAP Value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Phenol Content&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. trinervis</td>
<td>9.109 ± 0.6923*</td>
<td>430.584 ± 29.8511</td>
</tr>
<tr>
<td>S. byzantina</td>
<td>9.328 ± 1.0254</td>
<td>638.304 ± 30.6108</td>
</tr>
<tr>
<td>S. setifera</td>
<td>11.392 ± 0.5109</td>
<td>708.744 ± 83.9830</td>
</tr>
<tr>
<td>S. subaphylla</td>
<td>17.114 ± 0.9799</td>
<td>1016.04 ± 76.6006</td>
</tr>
<tr>
<td>S. turcomanica</td>
<td>22.569 ± 1.2646</td>
<td>1313.568 ± 78.9441</td>
</tr>
<tr>
<td>S. inflata</td>
<td>31.078 ± 0.5319</td>
<td>1478.808 ± 44.3195</td>
</tr>
<tr>
<td>S. laxa</td>
<td>35.062 ± 1.0583</td>
<td>2089.992 ± 157.1322</td>
</tr>
<tr>
<td>S. persica</td>
<td>61.426 ± 4.3554</td>
<td>3294.96 ± 313.8671</td>
</tr>
<tr>
<td>S. fruticulosa</td>
<td>62.094 ± 4.5272</td>
<td>4450.368 ± 280.0766</td>
</tr>
</tbody>
</table>

*Values are mean ± SD.

<sup>a</sup> In unit mmol Fe<sup>2+</sup>/100 g dry weight plant. Each plant was analyzed five times.

<sup>b</sup> Expressed as mg gallic acid equivalent/100 g dry weight plant. Each plant was analyzed five times.

FRAP assay was used for measuring total antioxidant capacity and Folin-Ciocalteau method for determination of total phenolic content.

Antioxidant estimation

The results of the FRAP assay are reported in Table 1. All extracts contained a considerable amount of antioxidant effect from 9.11 mmol of FeSO<sub>4</sub>/100 g dry plant equivalents in S. trinervis to 62.0945 mmol of FeSO<sub>4</sub>/100 g dry plant in S. fruticulosa.

Total phenol estimation

The results of the Folin-Ciocalteau total phenol assay are reported in Table 1. All extracts contained a considerable amount of phenolic metabolites from 430.584 mg of gallic acid/100 g dry plant equivalents in S. trinervis to 4450.368 mg of gallic acid/100 g dry plant in S. fruticulosa.

DISCUSSION

In previous investigations of Stachys species, the presence of various polyphenol compounds was reported. In methanol and ethanol extract of aerial parts of this genus, apigenin, chrysoeriol, forrsithoside B, caffeic, sinapic, protocatechuic, chlorogenic and rosmarinic acids were identified (Bonkova et al., 1999; Capeca et al., 2005; Kukic et al., 2006; Lenherr et al., 1984; 1987; Marin et al., 2004). Some of these compounds were assessed on their antioxidant activity earlier (Aligianis et al., 2003; Capeca et al., 2005; Chen and Ho, 1997; Kukic et al., 2006). Most of the major constituents of the essential oil of stachys species were piperitenone, hexadecanoic acid, germacrene D, α-pinene, 4-hydroxy-4-methyl-2-pentanone, beta caryophyllene, limonene, pulegone, bicyclogermacrene, β-pinene, spathulenol, carvacrol and eugenol (Javidnia et al., 2003; Khanavi et al., 2004; Morteza-Semnani et al., 2006a; Norouzi-Arasi et al., 2006; Sajjadi et al., 2004). Among them phenolic compounds had shown significant antioxidant activity (Vundac et al., 2007; Matkowski and Piotrowska, 2006; Salehi et al., 2005; Wei and Shibamoto, 2007).

Semnani et al. (2006) had investigated the stabilizing effect of methanolic extract of S. byzantina, S. inflata and S. laxa on sunflower oil as antioxidant agents (Morteza-Semnani et al., 2006b) and the results showed that S. laxa had a potential source of antioxidants. In our study S. persica and S. fruticulosa showed antioxidant effect about two times more than S. laxa. Also in some other studies the antioxidant effects of S. inflata, S. byzantina, S. setifera and S. laxa were investigated (Erdemoglu et al., 2006; Morteza-Semnani et al., 2006b), but different methods were used for the studies and the results were not directly comparable.

As it was shown in Table 1, all the studied species possessed antioxidant activity, while S. persica and S. fruticulosa showed the highest results and S. trinervis showed the lowest (p < 0.05). With respect to the table, there is a significant relationship between accumulation of high amount of phenolic compounds and antioxidant activity (Figure 1) (R<sup>2</sup> = 0.9446, p ≤ 0.001).

As mentioned earlier, Stachys species have major medicinal effects and used traditionally. Therefore the potency of these extracts could provide a chemical basis for some of the health benefits claimed for Stachys species in folk medicine. Further studies are necessary to assess their potential components as effective natural remedies.

ACKNOWLEDGMENT

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REFERENCES


