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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 (R^2 = 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).

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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., 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; Digřak 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

al., 2000). 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* C. 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 tripyridyl triazine (Fe (III)-TPTZ) complex to the ferrous tripyridyl 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-tripyridyl- 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 37^0C. 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 37^0C 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 colorimetrcially 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^oC for 5 min. A 1.5 ml sodium bicarbonate solution (60 g/L) was added to the mixture. After 90 min at 220C, 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.

Species	FRAP Value[a]	Phenol Content[b]
S. trinervis	9.1092 ± 0.6923*	430.584 ± 29.8511
S. byzantina	9.3283 ± 1.0254	638.304 ± 30.6108
S. setifera	11.3923 ± 0.5109	708.744 ± 83.9830
S. subaphylla	17.1142 ± 0.9799	1016.04 ± 76.6006
S. turcomanica	22.5698 ± 1.2646	1313.568 ± 78.9441
S. inflata	31.0787 ± 0.5319	1478.808 ± 44.3195
S. laxa	35.0629 ± 1.0583	2089.992 ± 157.1322
S. persica	61.4267 ± 4.3554	3294.96 ± 313.8671
S. fruticulosa	62.0945 ± 4.5272	4450.368 ± 280.0766

*Values are mean± SD.
a) In unit mmol Fe^{2+}/100 g dry weigh plant. Each plant was analyzed five times.
b) Expressed as mg gallic acid equivalent/100 g dry weigh 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 anti-oxidant effect from 9.11 mmol of FeSO4/100 g dry plant equivalents in *S. trinervis* to 62.0945 mmol of FeSO4/100 g dry plant in S. fruticulosa.

Total phenol estimation

The results of the Folin-Ciocalteu 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 pre-sence of various polyphenol compounds was reported. In methanol and ethanol extract of aerial parts of this genus, apigenin, chrysoeriol, forsithoside 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 theses 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) ($R^2 = 0.9446$, $p \leq 0.001$).

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.

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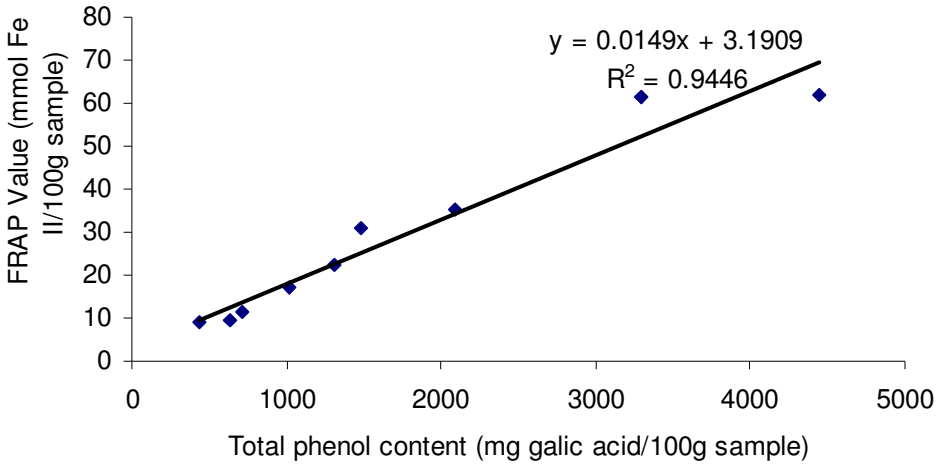

Figure 1. Relationship between total phenolic content (expressed as mg gallic acid equivalent/100 g dry weigh plant) and antioxidant activity (in units mmol $Fe2^{+}$/100 g dry weigh plant) of the arial parts of nine *Stachys* species ($R^2 = 0.9446$, $p \leq 0.001$).

REFERENCES

Al-Farsi M, Alasalvar C, Morris A, Baron M, Sahidi F (2005). Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (*Phoenix dactylfera* L.) varieties grown in Oman. J. Agric. Food Chem. 53: 7592-7599.

Aligianis N, Mitaku S, Tsitsa-Tsardis E, Harvala C, Tsaknis I, Lalas S, Haroutounian S (2003). Methanolic extract of *Verbascum macrurum* as a source of natural preservatives against oxidative rancidity. J. Agric. Food Chem. 51: 7308-7312.

Amirghofran Z, Bahmani M, Azadmehr A, Javidnia K (2006). Anticancer effects of various Iranian native medicinal plants on human tumor cell lines. Neoplasma. 53: 428-433.

Amirghofran Z, Bahmani M, Azadmehr A, Javidnia K (2007). Immunomodulatory and apoptotic effects of *Stachys obtusicrena* on proliferative lymphocytes. Med. Sci. Monit. 13: 145-150.

Aruoma OI (1998). Free radicals, oxidative stress, and antioxidants in human health and disease. J. Am. Oil Chem. Soc. 75: 199-212.

Aydin A, Sener B, Cakici I, Turan NN, Erdemoglu N (2006). Antioxidant activities of some Lamiaceae plant extracts. Phytother. Res. 20: 91-93.

Bonkova V, Koeva-Todorovska J, Stambolijska T, Ignatova-Groceva MD, Todorova D, Popov S (1999). Polyphenols in *Stachys* and *Betonica* species (Lamiaceae). Z. Naturforsch. 54c: 876-880.

Benzie IFF, Strain JJ (1999). The ferric reducing ability of plasma as a power: The FRAP assay. Anal. Bio. Chem. 239: 70-76.

Capeca E, Mareczek A, Leja M (2005). Antioxidant activity of fresh and dry herbs of some Lamiacese species. Food Chem. 93: 223-226.

Chen JH, HO CT (1997). Antioxidant activities of caffeic acid and its related hydroxy cinnamic asid compounds. J. Agric. Food Chem. 45: 2374-2378.

Digrak M, Hakki Alma M, Ilcjm A (2001). Antibacterial and antifungal activities of Turkish medicinal plants. Pharm. Biol. 39: 346-350.

El-Ansari MA, Nawwar MA, Saleh NAM (1995). Stachysetin, a diapigenine-7-glucoside-p-p_-dihydroxy-truxinate from *Stachys Aegyptiaca*. Phytochemistry. 40: 1543-1548.

Erdemoglu N, Turan NN, Cakc I, Sener B, Aydn A (2006). Antioxidant activities of some Lamiaceae plant extracts. Phytother. Res. 20: 9-13.

Gruenwald J, Brendler T, Jaenicke C (2000). PDR for Herbal Medicines. Medical Economics Company, Second Edition, Montvale, New Jersey, p 832.

Grujic-Jovanovic S, Skaltsa HD, Marin P, Sokovic M (2004). Composition and antibacterial activity of the essential oil of six *Stachys* Species from Serbia. Flavour Frag. J. 19: 139-144.

Hayashi K, Nagamatsu T, Ito M, Hattori T, Suzuki Y (1994). Acotoside, a component of *Stachys sieboldii* MIQ, may be a promising antinephritic agent. 1) Effects of acetoside on crescentic-type anti-GBM nephritis in rats. Jpn. J. Pharmacol. 65: 143-151.

Javidnia K, Miri R, Azarpira A, Tabaei SMH (2003). Composition of the essential oil of *Stachys setifera* C. A.Mey ssp. iranica growing in Iran. Flavour Fragr. J. 18: 299-300.

Kahkonen MP, Hopia AL, Vurrela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M (1999). Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 47: 3954-3962.

Kregel KC, Zhang HJ (2006). An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am. J. Physiol. Regul. Integr. Comp. Physiol. 292: 18-36.

Khanavi M, Hadjiakhoondi A, Amin G, Amanzadeh Y, Rustaiyan A, Shafiee A (2004). Comparison of the volatile composition of *Stachys persica* Gmel. and *Stachys byzantina* C. Koch. Oils obtained by hydrodistillation and steam distillation. Z. Naturforschung. 59 c: 463-467.

Khanavi M, Sharifzadeh M, Hadjiakhoondi A, Shafiee A (2005). Phytochemical investigation and anti-inflammatory activity of aerial parts of *Stachys byzanthina* C. Koch. J. Ethnopharmacol. 97: 463-468.

Klaunig JE, Kamendulis LM (2004). The role of oxidative stress in carcinogenesis. Annu. Rev. Pharmacol. Toxicol. 44: 239-267.

Kukic J, Dobric S, Petrovic S (2007). Influence of some *Stachys taxa* on carrageenan-induced paw edema in rats. Pharm. Biol. 45: 560-563.

Kukik J, Petrovic S, Niketic M (2006). Antioxidant activity of four endemic *Stachys taxa*. Biol. Pharm. Bull. 29: 725-729.

Lee JC, Kim J, Park JK, Chung GH, Jang YS (2003). The antioxidant, rather than prooxidant, activities of quercetin on normal cells: quercetin protects mouse thymocytes from glucose oxidase-mediated apoptosis. Exp. Cell Res. 291: 386-397.

Lenherr A, Mabry TJ (1987). Acetylated allose-containing flavonoid glucosides from *Stachys anisochila*. Phytochemistry. 26: 1185-1188.

Lenherr A, Meier B, Sticher O (1984). Modern HPLC as a tool for chemotoxonomical investigations: iridoid glucosides and acetylated flavonoids in the group of *Stachys recta*. Plant Med. 50: 403-409.

Maleki N, Garjani A, Nazemiyah H, Nilfouroushan N, Eftekhar Sadat AT, Allameh Z, Hasannia N (2001). Potent anti-inflammatory activities of hydroalcoholic extract from aerial parts of *Stachys* inflate on rats. J. Ethnopharmacol. 75: 213-218.

Marin PD, Grayer RJ, Grujic-Jovanovic S, Kite GC, Veitch NC (2004). Glycosides of tricentin methyl ethers as chemosystematic markers in *Stachys subgenus* Betonica. Phytochemistry. 65: 1247-1253.

Matkowski A, Piotrowska M (2006). Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae.

Fitoterapia. 77: 346-353.
Maxwell SRJ (2000). Coronary artery disease-free radical damage, antioxidant protection, and the role of homocysteine. Basic Res. Cardiol. 95: 65-71.
Mittler R (2002). Oxidative stress, antioxidants, and stress tolerance. Trends Plant Sci. 7: 405-410.
Miyase T, Yamamoto R, Ueno A (1996). Phenyl ethanoid glycosides from *Stachys officinalis*. Phytochemistry. 43: 475-479.
Morteza-Semnani K, Akbarzadeh M, Changizi S (2006a). Essential oils composition of *Stachys byzantina*, S. inflata, S. lavandulifolia and *S. laxa* from Iran. Flavour Fragr. J. 21: 300-303.
Morteza-Semnani K, Saeedi M, Shahani S (2006b). Antioxidant activity of the methanolic extracts of some spicies of *Phlomis* and *Stachys* on sunflower oil. Afr. J. Biotechnol. 5: 2428-2432.
Mozaffarian V (1996). A Dictionary of Iranian Plant Names. Farhang Moaser, Tehran, Iran, p. 522.
Nishimura H, Sasaki H, Inagaki N, Chin M, Mitsuhashi H (1991). Nine phenethyl alcohol glycosides from *Stachys seiboldii*. Phytochemistry. 30: 9659-9669.
Norouzi-Arasi H, Yavari I, Kia-Rostami V, Jabbari R, Ghasvari-Jahromi M (2006). Volatile constituents of *Stachys inflata* Benth. from Iran. Flavour Fragr. J. 21: 262-264.
Pellati F, Benvenuti S, Magro L, Melegari M, Soragni F (2004). Analysis of phenolic compounds and radical scavenging activity of *Echinacea* spp. J. Pharm. Biomed. Anal. 35: 289-301.
Rabbani M, Sajjadi SE, Zarei HR (2003). Anxiolytic effects of *Stachys lavandulifolia* Vahl on the elevated plus-maze model of anxiety in mice. J. Ethnopharmacol. 89: 271-276.
Rao AV, Balachandran B (2002). Role of oxidative stress and antioxidant in neurodegenerative diseases. Nutr. Neurosci. 5: 291-309.
Rechinger KH, Hedge IC (1982). Flora Iranica. Akademisch Druck-und Verlagsanstalt, Graz, Austria, 150: 360-361.
Riso P, Visioli F, Gardana C, Grande S (2005). Effect of blood orange juice intake on antioxidant bioavailability and on different markers related to oxidative stress. J. Agric. Food Chem. 53: 941-947.
Sajjadi SE, Somae M (2004). Chemical composition of the essential oil of *Stachys inflata* Benth. from Iran. Chem. Nat. Compd. 4: 378-380.
Salehi P, Sonboli A, Eftekhar F, Nejad-ebrahimi S, Yousefzadi M (2005). Essential Oil Composition, Antibacterial and Antioxidant Activity of the Oil and Various Extracts of Ziziphora clinopodioides subsp. rigida (BOISS.) RECH. F. from Iran. Biol. Pharm. Bull. 28: 1892-1896.
Sharifzadeh M, Sharifzadeh K, Khanavi M, Hadjiakhoondi A, Shafiee A (2005). Anti-inflammatory activity of aerial parts of *Stachys setifera* and *Stachys persica*. Int. J. Pharmacol. 1: 132-137.
Sies H (1997). Oxidative stress: oxidants and antioxidants. Exp. Physiol. 82: 291-295.
Skaltsa HD, Bermejo P, Lazari DM, Silvan AM, Skaltsounis AL, Sanz A, Abad MJ (2000). Inhibition of prostaglandin E2 and leukotriene C4 in mouse peritoneal macrophages and thromboxan B2 production in human platelets by flavonoids from *Stachys chrysantha* and *Stachys Candida*. Biol. Pharm. Bull. 23: 47-53.
Sonboli A, Salehi P, Nejad Ebrahimi S (2005). Essential oil composition and antibacterial activity of the leaves of *Stachys schtschegleevii* from Iran. Chem. Nat. Comp. 41: 171-174.
Stamatis G, Kyriazopoulos P, Golegou S, Basayiannis A, Skaltsas S, Skaltsa H (2003). *In vitro* anti-Helicobacter pylori activity of Greek herbal medicines. J. Ethnopharmacol. 88: 175-179.
Vundac VB, Brantner AH, Plazibat M (2007). Content of phenolic constituents and antioxidant activity of some *Stachys taxa*. Food Chem. 104: 1277-1281.
Vundac VB, Males Z, Plazibat M, Golja P, Cetina-Cizmek BC (2005). HPTLC determination of flavonoids and phenolic acids in some Croatian *stachys taxa*. J. Planar Chromatogr. -Mod. TLC. 18: 269-273.
Wang JS, Maldonado MA (2006). The ubiquitin-proteasome system and its role in inflammatory and autoimmune diseases. Cell Mol. Immunol. 3: 255-261.
Wei A, Shibamoto T (2007). Antioxidant activities and volatile constituents of various essential oils. J. Agric. Food Chem. 55: 1737-1742.