Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 99 (2008) 1584–1588
BIORESOURCE TECHNOLOGY

Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of *Salvia virgata* (Jacq), *Salvia staminea* (Montbret & Aucher ex Bentham) and *Salvia verbenaca* (L.) from Turkey

Bektas Tepe *

Department of Molecular Biology and Genetics, Faculty of Science and Literature, Cumhuriyet University, Sivas 58140, Turkey

Received 10 November 2006; received in revised form 12 March 2007; accepted 9 April 2007
Available online 24 May 2007

Abstract

This study was designed to examine the *in vitro* antioxidant activities and rosmarinic acid levels of the methanol extracts of *Salvia virgata*, *Salvia staminea* and *Salvia verbenaca*. The extracts were screened for their possible antioxidant activity by two complementary test systems, namely DPPH free radical scavenging and β-carotene/linoleic acid systems. In the first case, the most active plant was *S. verbenaca* (14.30 ± 1.42 μg mg^{-1}), followed by *S. virgata* (65.70 ± 2.12 μg mg^{-1}). *S. staminae* exhibited the weakest antioxidant activity in this test system of which IC_{50} value is 75.40 ± 0.57 μg mg^{-1}. In β-carotene/linoleic acid test system, *S. verbenaca* extract was superior to the other extracts studied (inhibition value is $77.03\% \pm 0.42$). Antioxidant activities of BHT, ascorbic acid, curcumin and α-tocopherol were determined in parallel experiments. Activity of rosmarinic acid was also screened for better establishing the relationship between rosmarinic acid level and antioxidant activity for the plant extracts. According to the results obtained by spectrophotometric analysis and further supported by HPLC, *S. verbenaca* has the highest rosmarinic acid level with a value of 29.30 ± 0.24 μg mg^{-1}. Our results showed that the rosmarinic acid and its derivatives are more likely to be responsible for most of the observed antioxidant activities of *Salvia* species.

Keywords: *Salvia virgata*; *Salvia staminea*; *Salvia verbenaca*; Antioxidant activity; Rosmarinic acid

1. Introduction

The genus *Salvia*, with about 700 species, is one of the most widespread members of the *Lamiaceae* family. An unusually large number of useful secondary metabolites, belonging to various chemical groups, such as essential oils, terpenoid compounds and phenolic derivatives, have been isolated from the genus, which features prominently in the pharmacopoeias of many countries throughout the world (Gibbs, 1974; Banthorpe et al., 1989; Luis et al., 1992; Ulubelen and Topcu, 1992).

Many *Salvia* species and their isolated constituents possess significant antioxidant activity in enzyme-dependent and enzyme-independent systems (Dorman et al., 1995; Hohmann et al., 1999; Lu and Foo, 2001; Malencic et al., 2000; Zupko et al., 2001). *Salvia lavandulaefolia* ethanolic extracts (both the water soluble and chloroform soluble fractions), individual constituents of the essential oil (the monoterpenoids, 1,8-cineole, linalool, α- and β-pinene) and herb (the phenolic monoterpenoid carvacrol, the flavone luteolin and the phenolic rosmarinic acid) have been reported to be antioxidant (Adam et al., 1998; Dorman et al., 1995; Lu and Foo, 2001; Malencic et al., 2000; Perry et al., 2001; Zupko et al., 2001) while camphor (20–30% of essential oil) has demonstrated pro-oxidant effects in a liposome peroxidation preparation (Perry et al., 2001).

Rosmarinic acid (α-*O*-caffeoyl-3,4-dihydroxyphenillactic acid) is mainly found in the species of *Boraginaceae* and the subfamily *Nepetoidae* of the *Lamiaceae* (Peterson and Simmonds, 2003). A multitude of biological activities have been described for rosmarinic acid. The main activities are

* Tel.: +90 346 219 1010x2907; fax: +90 346 219 1186.
E-mail address: bektastepe@yahoo.com

0960-8524/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2007.04.008

adstringent, anti-inflammatory, antimutagen, antibacterial and antiviral (e.g. Parnham and Kesselring, 1985). The latter activity is used in the therapy of *Herpes simplex* infections with rosmarinic acid-containing extracts of *Melissa officinalis*. The anti-inflammatory properties are thought to be based on the inhibition of lipoxygenases and cyclooxygenases and the interference of rosmarinic acid with the complement cascade (Parnham and Kesselring, 1985). Rosmarinic acid is rapidly eliminated from the blood circulation after intravenous administration ($t^{1/2} = 9$ min) and shows a very low toxicity with a LD_{50} in mice of 561 mg kg^{-1} after intravenous application (Parnham and Kesselring, 1985). Phenolic compounds like rosmarinic acid can provide protection against cancer. It is also one of the efficient natural antioxidants, hence its application in the food industry (Shahidi et al., 1992). The herb constituent rosmarinic acid displays more potential radical scavenging activity than trolox (a derivative of α-tocopherol) (Lu and Foo, 2002) and the inhibition of liposome peroxidation by the monoterpenoids is considered weak, though significant compared to the standard antioxidant propyl gallate (Perry et al., 2001).

Antioxidant activities of the many members of the genus *Salvia* were reported elsewhere. Additionally, our previous papers concerning the biological activities of *Salvia* species (Tepe et al., 2004, 2005a,b) confirms that this genus has a great potential, especially in the antioxidant systems, for the food and cosmetic industries.

In this paper, antioxidant potentials of the methanolic extracts of *Salvia virgata*, *Salvia staminea* and *Salvia verbenaca* were determined in relation to their rosmarinic acid levels.

2. Methods

2.1. Plant material

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

1. *S. virgata*: Gardaslar Hill (1350 m), Sivas–Turkey; 16th July 2004.
2. *S. staminea*: Kilickaya district, Yusufeli, Artvin–Turkey; 06th September 2004.
3. *S. verbenaca*: Kilickaya district, Yusufeli, Artvin–Turkey; 06th September 2004.

The voucher specimens have been deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas–Turkey (CUFH-Voucher No: 1-AA 3427; 2-AA 3485 and 3-AA 3486, respectively).

2.2. Preparation of the methanolic extracts

The air-dried and finely ground samples were extracted by using the method as described elsewhere (Sokmen et al., 1999). Briefly, the sample weighing about 100 g was extracted in a Soxhlet with methanol (MeOH) at 60 °C for 6 h (11.28%, 14.20% and 17.54%, 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 atoms or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple coloured methanol solution of DPPH. This spectrophotometric assay uses stable radical 2,2′-diphenylpicrylhydrazyl (DPPH) as a reagent (Cuendet et al., 1997). Fifty microliters of various concentrations of the extracts in methanol was 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 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 plotted 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 et al., 1998). A stock solution of β-carotene–linoleic acid mixture was prepared as following: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade), 25 μl linoleic acid, and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml distilled water saturated with oxygen (30 min, 100 ml/min) was added with a vigorous shaking. 2.5 ml of this reaction mixture was dispersed to test tubes and 350 μl portions of the extracts prepared at 2 g l^{-1} concentrations were added and emulsion system was incubated up to 48 h at room temperature. The same procedure was repeated with synthetic antioxidant, buthylated hydroxytoluene (BHT) as positive control, and a blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Antioxidative capacities of the extracts were compared with those of BHT and blank.

2.4. Determination of the rosmarinic acid levels

The rosmarinic acid was isolated from the dried methanolic extracts of the plants studied. Isolation was made with 50% (v/v) ethanol (for 1 h) at 70 °C. The extract was evaporated to dryness, the dry residue was dissolved in

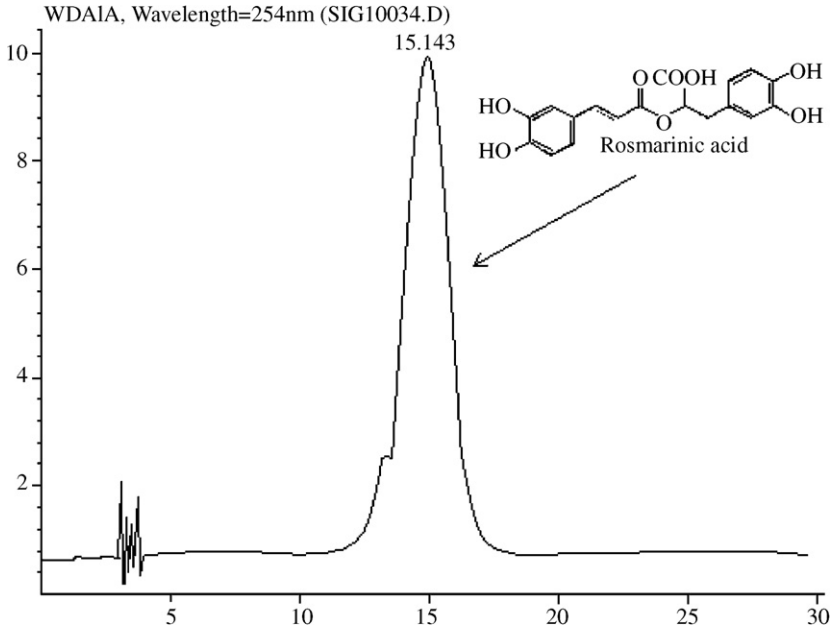

Fig. 1. HPLC chromatogram of rosmarinic acid obtained by using standard solution (50 mg/ml).

70% (v/v) ethanol and then it was stored for 24 h at −10 °C. The precipitate was filtered off and the filtrate was used to determinate rosmarinic acid from its absorbancy at 327 nm (Lopez-Arnaldos et al., 1995). The data presented are the average from three independent experiments.

The rosmarinic acid content in the extracts was also determined by HPLC analysis (Waters 2690 Alliance, Waters Corporation, Milford, MA, USA) on a Perkin–Elmer (series 4) 250 × 4.6 mm reversed phase column (C18, 10 μm) with a 996 photodiot array dedector, an online degasser and an automatic sampler. The solvent system was 2% acetic acid (A) and 2% acetic acid/acetonitrile 7:3 (B), and a linear gradient from 70% to 30% A in 40 min was applied. The flow rate was 1 ml/min and column effluent was monitored by UV detection at 320 nm (Ilieva and Pavlov, 1997). The data presented are the average from three independent experiments.

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. Free radical scavenging capacities of the corresponding extracts measured by DPPH assay are shown in Table 1. According to the findings presented in the table, the most active plant was *S. verbenaca* (14.30 ± 1.42 μg mg^{-1}), followed by *S. virgata* (65.70 ± 2.12 μg mg^{-1}). *S. staminae* exhibited the weakest antioxidant activity in this test system of which IC_{50} value is 75.40 ± 0.57 μg mg^{-1}.

In β-carotene/linoleic acid system (Table 1), *S. verbenaca* extract was superior to the other extracts studied (inhibition value is $77.03\% \pm 0.42$). As can be seen from the table, the results obtained from the both test systems confirmed to each other. Additionally, antioxidant activities of BHT, ascorbic acid, curcumin and α-tocopherol were determined in parallel experiments. Antioxidant activity of rosmarinic acid was also screened for better establishing the relationship between the rosmarinic acid content and antioxidant activity for the plant extracts.

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 (μg mg^{-1})	Results in β-carotene–linoleic acid system (inhibition%)
Salvia virgata	65.70 ± 2.12	54.42 ± 2.44
Salvia staminea	75.40 ± 0.57	51.35 ± 1.78
Salvia verbenaceae	14.30 ± 1.42	77.03 ± 0.42
BHT	18.80 ± 1.21	96.00 ± 0.23
Ascorbic acid	3.80 ± 0.10	94.50 ± 2.14
Curcumin	7.80 ± 0.30	89.30 ± 1.86
α-Tocopherol	6.50 ± 0.70	96.65 ± 1.72
Rosmarinic acid	2.90 ± 0.30	100.00 ± 0.27

[a] Results are means of three different experiments.

As can be seen from Table 2, rosmarinic acid levels were determined by using two different chromatographic test systems (spectrophotometric and HPLC) (Fig. 1). According to the results presented in the table, the results are highly consistent with each other. There is a strong correlation between the rosmarinic acid level and antioxidant activity potential. According to the table, *S. verbenaca* has the highest rosmarinic acid level with a value of 29.30 ± 0.24 μg mg^{-1}.

Biotechnological techniques have been recently reported to significantly facilitate the production of some important

Table 2
Amounts of rosmarinic acid in the methanol extracts of *Salvia* species[a]

Plants	Amount of rosmarinic acid (μg mg^{-1})	
	Spectrophotometric results	HPLC results
Salvia virgata	7.42 ± 1.36	6.19 ± 0.39
Salvia staminea	5.29 ± 1.27	4.82 ± 0.27
Salvia verbenaceae	29.30 ± 0.24	26.12 ± 0.73

[a] Results are means of three different experiments.

bioactive compounds from the genus *Salvia*. By the development of these techniques, numerous *Salvia* species were used for the production of various secondary metabolites, such as rosmarinic acid, cryptotanshinone, camphor, ferruginol and sclareol (Funk et al., 1992; Hippolyte et al., 1992; Tawfic et al., 1992; Morimoto et al., 1994; Luis et al., 1992).

Rosmarinic acid is a natural phenolic compound extracted from *Rosemarinus officinalis* L. The presence of this phytochemical in the members of the genus *Salvia* is well known. It contains two phenolic rings of which both have the *ortho*-position hydroxyl groups. There is a carbonyl, an unsaturated double bond and a carboxylic acid between the two phenolic rings. Its structure is different from the flavonoids, which have been studied extensively. It has many biological activities such as inhibiting the HIV-1, antitumor, antihepatitis and protecting the liver, inhibiting the blood clots and anti-inflammation. Some experiments have reported the strong capacity of rosmarinic acid scavenging the free radicals, which showed that the antioxidant activity is over three times than trolox that rosmarinic acid can inhibit the activity of Xanhine Oxidase, and it is used to scavenge the surplus free radicals in the body. In addition, rosmarinic acid can reduce Mo(VI) to Mo(V), preventing the product of free radicals caused by the metal (Peterson and Simmonds, 2003). Today, rosmarinic acid is an important candidate for the food industries.

Our results presented both in Tables 1 and 2 showed that, the rosmarinic acid and its derivatives are more likely to be responsible for most of the observed antioxidant activity of the non-diterpenoid components in *Salvia* species evaluated here.

As far as our literature survey could ascertain, we could reach several reports concerning the phytochemical compositions of the plants given here. Moreover, there is no detailed study dealing with the biological activities of them except for Ahmed et al. (2005).

In earlier studies, sage and rosemary were shown to have similar patterns of phenolic compounds and the antioxidative activity had been attributed mainly to carnosic acid and rosmarinic acid (Brieskorn and Domling, 1969; Cuvelier et al., 1996). Studies on the antioxidant activity of sage had also been limited to the diterpenoid compounds (Cuvelier et al., 1994; Zhang et al., 1990). It was only recently that Wang et al. (1998, 1999) reported two sage phenolic glycosides that showed moderate antioxidant activities. Lu and Foo (1999, 2000) and Lu et al. (1999) previously characterized a number of flavonoids and phenolic acids, including the novel rosmarinic acid derivatives, sagecoumarin and sagerinic acid as the new potential antioxidant substances.

As a result, the potency of these compounds could provide a chemical basis for some of the health benefits claimed for sage in folk medicine and warrant further studies to assess their potential as effective natural remedies.

References

Adam, J.N., Sivropoulou, A., Kokkini, S., Lanaras, T., Arsenakis, M., 1998. Antifungal activities of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. Journal of Agricultural and Food Chemistry 46, 1739–1745.

Ahmed, B., Al-Howiriny, A.A., Mossa, J.S., El Tahir, K.E.H., 2005. Isolation, antihypertensive activity and structure activity relationship of flavonoids from three medicinal plants. Indian Journal of Chemistry Section B: Organic Chemistry Including Medicinal Chemistry 44, 400–404.

Banthorpe, D.V., Bilyard, H.J., Brown, G.D., 1989. Enol esters of caffeic acid in several genera of the *Lamiaceae*. Phytochemistry 28, 2109–2113.

Brieskorn, C., Domling, H.J., 1969. Carnosic acid as an antioxidant in rosemary and sage leaves. Zeitschrift für Lebensmittel-Wissenschaft und Technologie 28, 25–30.

Cuendet, M., Hostettmann, K., Potterat, O., 1997. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. Helvetica Chimica Acta 80, 1144–1152.

Cuvelier, M.E., Berset, C., Richard, H., 1994. Antioxidant constituents in sage (*Salvia officinalis*). Journal of Agricultural and Food Chemistry 42, 665–669.

Cuvelier, M.E., Richard, H., Berset, C., 1996. Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. Journal of the American Oil Chemists' Society 73, 645–652.

Dapkevicius, A., Venskutonis, R., Van Beek, T.A., Linssen, P.H., 1998. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. Journal of the Science of Food and Agriculture 77, 140–146.

Dorman, H.J.D., Deans, S.G., Noble, R.C., 1995. Evaluation in vitro of plants essential oils as natural antioxidants. Journal of Essential Oil Research 7, 645–651.

Funk, C., Koepp, A.E., Croteau, R., 1992. Induction and characterization of a cytochrome P-450-dependent camphor hydroxylase in tissue cultures of common sage (*Salvia officinalis*). Archives of Biochemistry and Biophysics 294, 306–313.

Gibbs, R.D., 1974. In: Chemotaxonomy of Flowering Plants, vol. III. McGill-Queen's University Press, Montreal (pp. 1405–1406).

Hippolyte, I., Marin, B., Baccou, J.C., Jonard, R., 1992. Growth and rosmarinic acid production in cell suspension cultures of *Salvia officinalis* L. Plant Cell Reports 11, 109–112.

Hohmann, J., Zupko, I., Redei, D., Csanyi, M., Falkay, G., Mathe, I., 1999. Protective effects of the aerial parts of *Salvia officinalis*, *Melissa officinalis*, and *Lavandula angustifolia* and their constituents against enzyme-dependent and enzyme-independent lipid peroxidation. Planta Medica 65, 576–578.

Ilieva, M., Pavlov, A., 1997. Rosmarinic acid production by *Lavandula vera* MM cell-suspension culture. Applied Microbiological Technology 47, 683–688.

Lopez-Arnaldos, T., Lopez-Serrano, M., Ros Barcelo, A., Calderon, A.A., Zapata, J.M., 1995. Spectrofotometric determination of rosmarinic acid in plant cell cultures by complexation with Fe^{2+} ions. Fresenius Journal of Analytical Chemistry 351, 311–314.

Lu, Y., Foo, L.Y., 1999. Rosmarinic acid derivatives from *Salvia officinalis*. Phytochemistry 51, 91–94.

Lu, Y., Foo, L.Y., 2000. Antioxidant and radical scavenging activities of polyphenols from apple pomace. Food Chemistry 68, 81–85.

Lu, Y., Foo, L.Y., 2001. Antioxidant activities of polyphenols from sage (*Salvia officinalis*). Food Chemistry 75, 197–202.

Lu, Y., Foo, L.Y., 2002. Polyphenolics of *Salvia*—a review. Phytochemistry 75, 197–202.

Lu, Y., Foo, L.Y., Wong, H., 1999. Sagecoumarin, a novel caffeic acid trimer from *Salvia officinalis*. Phytochemistry 52, 1149–1152.

Luis, J.G., Gonzalez, A.G., Andrews, L.S., Mederos, S., 1992. Diterpenes from in vitro-grown *Salvia canariensis*. Phytochemistry 59, 140–177.

Malencic, D.J., Gasic, O., Popovic, M., Boza, P., 2000. Screening for antioxidant properties of *Salvia reflexa* Hornem. Phytotherapy Research 14, 546–548.

Morimoto, S., Goto, Y., Shoyama, Y., 1994. Production of lithospermic acid B and rosmarinic acid in callus tissue and regenerated plantlets of *Salvia miltiorhiza*. Journal of Natural Products 57, 817–823.

Parnham, M.J., Kesselring, K., 1985. Rosmarinic acid. Drugs Future 10, 756–757.

Perry, N.S.L., Houghton, P.J., Sampson, J., Theobald, A.E., Hart, S., Lis-Balchin, M., 2001. In vitro activity of *Salvia lavandulaefolia* (Spanish sage) relevant to treatment of Alzheimer's disease. Journal of Pharmacy and Pharmacology 53, 1347–1356.

Peterson, M., Simmonds, M.S.J., 2003. Rosmarinic acid. Phytochemistry 62, 121–125.

Shahidi, F., Yanita, P.K., Nanosudra, P.D., 1992. Phenolic antioxidants. Critical Reviews in Food Science and Nutrition 32, 67–103.

Sokmen, A., Jones, B.M., Erturk, M., 1999. The *in vitro* antibacterial activity of Turkish plants. Journal of Ethnopharmacology 67, 79–86.

Tawfic, A.A., Read, P.E., Cuppert, S.L., 1992. Stimulation of growth and monoterpene production of sage (*Salvia officinalis*) by benzyladenine in vitro. Plant Growth Regulation 20, 200–206.

Tepe, B., Donmez, E., Unlu, M., Candan, F., Daferera, D., Vardar-Unlu, G., Polissiou, M., Sokmen, A., 2004. Antimicrobial and antioxidative activity of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex. Benth.) and *Salvia multicaulis* (Vahl). Food Chemistry 84, 519–525.

Tepe, B., Daferera, D., Sokmen, A., Sokmen, M., Polissiou, M., 2005a. Antimicrobial and Antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). Food Chemistry 90, 333–340.

Tepe, B., Sokmen, M., Akpulat, H.A., Sokmen, A., 2005b. Screening of the antioxidant potentials of six *Salvia* species from Turkey. Food Chemistry 95, 200–204.

Ulubelen, A., Topcu, G., 1992. Abietane diterpenoids from *Salvia pomifera*. Phytochemistry 31, 3949–3951.

Wang, M., Li, J., Rangarajan, M., Shao, Y., La Voie, E.J., Huang, T.C., Ho, C.T., 1998. Antioxidative phenolic compounds from sage (*Salvia officinalis*). Journal of Agricultural and Food Chemistry 46, 4869–4873.

Wang, M., Shao, Y., Li, J., Zhu, N., Rangarajan, M., La Voie, E.J., Ho, C.T., 1999. Antioxidant phenolic glycosides from sage (*Salvia officinalis*). Journal of Natural Products 62, 454–456.

Zhang, K.Q., Bao, Y., Wu, P., Rosen, R.T., Ho, C.T., 1990. Antioxidative components of Tanshen (*Salvia miltiorrhiza* Bung). Journal of Agricultural and Food Chemistry 38, 1194–1197.

Zupko, I., Hohmann, J., Redei, D., Falkay, G., Janicsak, G., Mathe, I., 2001. Antioxidant activity of leaves of *Salvia* species in enzyme-dependent and enzyme-independent systems of lipid peroxidation and their phenolic constituents. Planta Medica 67, 366–368.