ORIGINAL ARTICLE

IN VITRO FREE RADICAL SCAVENGING ACTIVITY OF FIVE *SALVIA* SPECIES

BAHMAN NICKAVAR*, MOHAMMAD KAMALINEJAD AND HAMIDREZA IZADPANAH

Department of Pharmacognosy, School of Pharmacy,
Shaheed Beheshti Medical University, P. O. BOX: 14155-6153, Tehran, Iran
**Pharmaceutical Sciences Research Center, Shaheed Beheshti Medical University,*
P.O. Box 14155-3817, Tehran, Iran

ABSTRACT

The radical scavenging activity of ethanolic extracts from five *Salvia* species including *S. hypoleuca* Benth., *S. reuterana* Boiss., *S. verticillata* L., *S. virgata* Jacq. and *S. officinalis* L. (as the reference plant with well documented free radical scavenging and antioxidant properties) was evaluated in vitro with the spectrophotometric method based on the reduction of the stable DPPH free radical. All the extracts showed radical scavenging activity, especially *S. verticillata* [IC_{50} = 23.53 (20.56-26.93) µg ml^{-1}] and *S. virgata* [IC_{50} = 27.01 (24.08-30.29) µg ml^{-1}] were found to be the most active species. Furthermore, the extracts were investigated regarding their total flavonoid content (TFC) by $AlCl_3$ reagent. The extracts *S. hypoleuca* (TFC = 53.16 ± 1.95 µg mg^{-1}) and *S. reuterana* (TFC = 46.97 ± 4.43 µg mg^{-1}) had the highest content of flavonoid. However, a favourable correlation was not found between the radical scavenging potency and the total flavonoid content. This study suggests that *S. verticillata* and *S. virgata* are the possible sources of natural radical scavengers.

Keywords: *Salvia* species, DPPH assay, free radical scavenging activity.

INTRODUCTION

The large generation of free radicals, particularly reactive oxygen species and their high activity plays an important role in the progression of a great number of pathological disturbances like inflammation, atherosclerosis, stroke, heart disease, diabetes mellitus, multiple sclerosis, cancer, Parkinson's disease, Alzheimer's disease, etc (Mensor *et al*., 2001; Parejo *et al*., 2002; Hou *et al*., 2003; Orhan *et al*., 2003; Tepe *et al*., 2005; Ozgen *et al*., 2006). Therefore, the great interest has been recently focused on the natural foods, medicinal plants and phytocostituents due to their well-known abilities to scavenge free radicals (i.e. antioxidant power) (Hou *et al*., 2003; Galvez *et al*., 2005; Kukic *et al*., 2006).

Plants constitute an important source of active natural products which differ widely in terms of structures, biological properties and mechanisms of actions. Various phytochemical components, especially polyphenols (such as flavonoids, phyenyl propanoids, phenolic acids, tannins, etc) are known to be responsible for the free radical scavenging and antioxidant activities of plants. Polyphenols possess many biological effects. These effects are mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidantion and chelating transition metals. In generally, polyphenols all share the same chemical patterns, one or more phenolic groups for which they react as hydrogen donors and in that way neutralize free radicals (Heinonen *et al*., 1998; Parejo *et al*., 2002; Lee *et al*., 2003; Miliauskas *et al*., 2004; Atoui *et al*., 2005; Capecka *et al*., 2005; Galvez *et al*., 2005; Melo *et al*., 2005).

In recent years, the extracts of many plants have been screened for their antioxidant activities. Among these, sage (*Salvia officinalis* L.) is well-known for their antioxidant properties and most of its active components have been identified. It has been established that the antioxidant effects are mainly due to the phenolic compounds of the plant (Bandoniene *et al*., 2002; Miura *et al*., 2002; Tepe *et al*., 2006). However, these studies concerning the other *Salvia* species are very limited (Tepe *et al*., 2006; Tepe *et al*., 2007).

In generally, the genus *Salvia* (Lamiaceae) includes about 700 species spread throughout the world. In the Flora of Iran, the genus is represented by nearly 58 specie (Mozaffarian, 1996). Many *Salvia* species are commonly used in the food, drug, cosmetic and perfumery industries. They are well known among people and widely used as flavorings or fragrances and for the medicinal purposes in the several regions of the world (Miliauskas *et al*., 2004; Tepe *et al*. 2006). The Infusion and decoction of the aerial

**Corresponding author:* E-mail: bnickavar@yahoo.com, Phone: +98-21-88773521, Fax: +98-21-88795008

parts of the genus have been used as tonic, carminative, digestive, antispasmodic and anti-inflammatory in Iranian traditional medicine.

In this study (i) the radical scavenging activity of four *Salvia* species from Iran (*S. hypoleuca* Benth., *S. reuterana* Boiss., *S. verticillata* L. and *S. virgata* Jacq.) was evaluated against the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) radical and the activity of the plants was compared with *S. officinalis* L. (ii) the total flavonoid content of each species was determined by $AlCl_3$ and then (iii) the possible composition-activity relationship was investigated. A literature survey did not show any reference to a previous work on the free radical scavenging and/or antioxidant properties *S. hypoleuca*, *S. reuterana*, *S. virgata*.

MATERIALS AND METHODS

Chemicals

All of the chemicals used in this work were purchased from Merck (Germany), with the exception of DPPH, which was purchased from Sigma (USA). The chemicals were analytical grade.

Plant material

Aerial flowering parts of the plants (*S. hypoleuca*, *S. officinalis*, *S. reuterana*, *S. verticillata* and *S. virgata*) were collected from natural populations in Tehran province in summer 2004. Voucher specimens were deposited in the Herbarium of Pharmacognosy Department, Shaheed Beheshti Medical University, Tehran, Iran.

Preparation of extracts and solutions

Plant materials were air dried at room temperature and finely grounded. Each sample (100 g) was macerated with ethanol 90% (500 ml) three times. Solvent was evaporated under reduced pressure at approximately 40 ° C. The dried extracts were dissolved in ethanol 90% to a final concentration of 1000 µg ml^{-1} (sample stock solution), then the different concentrations of each sample (500, 250, 100, 50, 25, 10, 5 µg ml^{-1}) were prepared.

Antiradical activity test

The antiradical activity of the extracts was estimated according to the procedure described by Nickavar *et al*. (2006). Briefly, a 0.3mM solution of DPPH radical solution in ethanol 90% was prepared and then 1 ml of this solution was mixed with 2.5 ml of different concentrations of each extract (sample). After 30 min incubation in dark and at room temperature, absorbance (A) was measured at 518 nm in a SHIMADZU Multispect-1501 spectrophotometer. The percentage of the radical scavenging activity (RSA) was calculated by the following equation:

$$RSA\% = [A_{control} - (A_{sample} - A_{blank})] / A_{control} \times 100$$

Ethanol 90% (1 ml) plus each sample solution (2.5 ml) was used as a blank. DPPH solution (1 ml) plus ethanol 90% (2.5 ml) was used as a negative control. Rutin solution (at the concentrations of 100, 50, 25, 10, 5, 2.5 µg ml^{-1}) was used as a positive control.

The IC_{50} value for each sample, defined as the concentration of the test sample leading to 50% reduction of the initial DPPH concentration, was calculated from the non linear regression curve of Log concentration of the test extract (µg ml^{-1}) against the mean percentage of the radical scavenging activity.

Amount of total flavonoid content

The determination of the total flavonoid content (TFC) was carried out as described by Nickavar *et al*. (2006). Briefly, 2.5 ml of each extract solution was mixed with 2.5 ml $AlCl_3$ reagent in ethanol 90% and allowed to stand for 40 min at room temperature. After that, the absorbance of the mixture at 415 nm was measured with a SHIMADZU Multispect-1501 spectrophotometer. Ethanol 90% (2.5 ml) plus sample solution (2.5 ml) was used as a blank. Rutin was used as a reference compound. The TFC for each extarct [as µg rutin equivalents (RE) / mg of extract] was determined on the basis of the linear calibration curve of rutin (absorbance versus rutin concentration).

Statistical analysis

All of the experiments were carried out in triplicate. The IC_{50} (µg ml^{-1}) values were presented by their respective 95% confidence limits. The TFCs (µg mg^{-1}) were shown as mean ± SEM. One-way analysis of variance (ANOVA) followed by Tuckey's post test was used to assess significant differences ($p < 0.05$) between extracts. All of the statistical analyses were accomplished using the computer software GraphPad Prism 3.02 for Windows (GraphPad Software, USA).

RESULTS AND DISCUSSION

Antioxidant tests could be based on the evaluation of lipid peroxidation or on the measurement of free radical scavenging potency (hydrogen-donating ability). The radical scavengers donate hydrogen to free radicals, leading to non toxic species and therefore to inhibition of the propagation phase of lipid oxidation. The use of DPPH radical provides an easy, rapid and convenient method to evaluate the antioxidants and radical scavengers (Soler-Rivas *et al*., 2000; Kansci *et al*., 2003; Argolo *et al*., 2004; Roginsky and Lissi, 2005). Therefore, in this study, the selected *Salvia* extracts were screened for their possible antioxidant and radical scavenging activity by DPPH technique and their IC_{50} values were calculated for further comparisons. All of the extracts

exhibited concentration dependent radical scavenging activity (fig. 1) and the IC_{50} values ranged from 23.53 – 125.1 µg ml^{-1} (Table 1). When the values were compared for five extracts analyzed, the most effective RSA was shown by *S. verticillata* [23.53 (20.56 – 26.93) µg ml^{-1}] while the least effective was the extract of *S. reuterana* [125.1 (129.6-142.7) µg ml^{-1}]. Anyway, *S. verticillata* and *S. virgata*, *S. virgata* and *S. officinalis*, *S. officinalis* and *S. hypoleuca* showed the similar activity with no significant differences between them ($p > 0.05$).

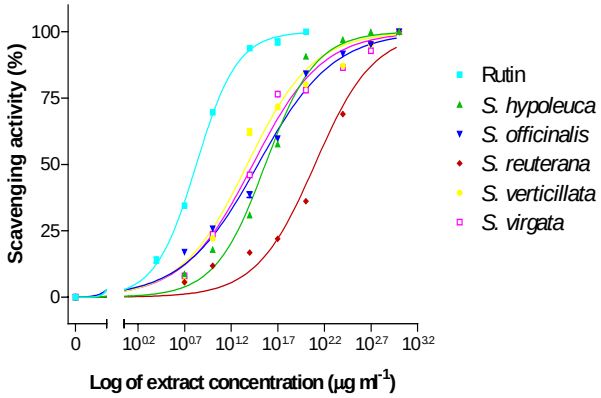

Fig. 1: Radical scavenging activity of the studied *Salvia* species extracts using DPPH. Each point represents the mean of three experiments and the vertical bars represent the SEM.

Phenolic compounds, especially flavonoids, constitute one of the most divers and widespread group of natural compounds. These compounds possess a broad spectrum of biological activities including antioxidant and radical scavenging properties (Parejo *et al*., 2002; Galvez *et al*., 2005; Melo *et al*., 2005), therefore the TFC in the extracts was determined (Table 1). The TFC, in rutin equivalent, varied from 8.54 ± 0.99 to 53.16 ± 1.95 µg RE mg^{-1} of extract. The highest amount of the total flavonoid was found in the extract of *S. hypoleuca* (53.16 ± 1.95 µg mg^{-1}), while *S. virgata* (8.54 ± 0.99 µg mg^{-1}) contained remarkably lower amount of these compounds. Any way, *S. verticillata* and *S. virgata*, *S. officinalis* and *S. virgata*, *S. officinalis* and *S. verticillata*, *S. hypoleuca* and *S. reuterana* exhibited the similar flavonoid content with no significant differences between them ($p > 0.05$).

After these analyses, the relationship between the antioxidant potency and TFC was studied. However, among the analyzed extracts of *Salvia*, a favorable correlation between the two parameters was not observed. This lack of relationship is in agreement with other literature (Heinonen *et al*., 1998). It is known that only flavonoids with a certain structure and particularly hydroxyl position in the molecule can act as proton-donating and show radical scavenging activity (Mensor *et al*., 2001; Hou *et al*., 2003). Furthermore, the extracts are very complex mixtures of many different compounds with distinct activities (Mensor *et al*., 2001; Hou *et al*., 2003; Galvez *et al*., 2005).

CONCLUSSION

This study indicate that some *Salvia* species (*S. hypoleuca*, *S. virgata*, *S. verticillata*) have a similar activity to or even higher than *Salvia officinalis* which is one of the most effective plants in terms of antioxidant properties and can serve as natural sources to develop the free radical scavengers and antioxidant agents. However, the components responsible for the radical scavenging activities of the extracts are known. Therefore, further research is needed for the isolation and identification of the active components in the extracts.

REFERENCES

Argolo ACC, Sant'Ana AEG, Pletsch M and Coelho LCB (2004). Antioxidant activity of leaf extracts from *Bauhinia monandra*. *Bioresource Technol*., **95**: 229-

Table 1: IC_{50} values of DPPH scavenging activity and total flavonoid content (TFC) of the studied *Salvia* extracts.

Sample	IC_{50} (µg ml^{-1})[1,3]	TFC (µg mg^{-1})[2,3]
S. hypoleuca	36.81 (33.63-40.29)[a]	53.16 ± 1.95[a]
S. officinalis	30.67 (27.88-33.73)[a,b]	17.24 ± 1.31[b,c]
S. reuterana	125.1 (129.6-142.7)[c]	46.97 ± 4.43[a]
S. verticillata	23.53 (20.56-26.93)[d]	10.81 ± 1.98[b,d]
S. virgata	27.01 (24.08-30.29)[b,d]	8.54 ± 0.99[c,d]

*Note: The IC_{50} value of the positive control, rutin, was measured as 6.69 (6.48-6.91) µg ml^{-1}.
[1]The IC_{50} values are presented with their respective 95% confidence limits.
[2]The TFC values are mean ± SEM of three determinations.
[3]Letters (a-d) devote homogenous subsets at $p < 0.05$ (one way ANOVA followed by Tuckey's post test).

233.

Atoui AK, Mansouri A, Boskou G and Kefalas P (2005). Tea and herbal infusions: their antioxidant activity and phenolic profile. *Food Chem*., **89**: 27-36.

Bandoniene D, Murkovic M, Pfannhauser W, Venskutonis PR and Gruzdiene D (2002). Detection and activity evaluation of radical scavenging compounds by using DPPH free radical and on-line HPLC-DPPH methods. *Eur. Food Res. Technol*., **214**: 143-147.

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

Galvez M, Martin-Cordero C, Houghton PJ and Ayuso MJ (2005). Antioxidant activity of methanol extracts obtained from *Plantago* species. *J. Agric. Food Chem*., **53**: 1927-1933.

Heinonen IM, Lehtonen PJ and Hopia AI (1998). Antioxidant activity of berry and fruit wines and liquors. *J. Agric. Food Chem*., **46**: 25-31.

Hou WC, Lin RD, Cheng KT, Hung YT, Cho CH, Chen CH, Hwang SY and Lee MH (2003). Free radical scavenging activity of Taiwanese native plants. *Phytomedicine*, **10**: 170-175.

Kansci G, Dongo E and Genot C (2003). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) test demonstrates antiradical activity of *Dorstenia psilurus* and *Dorstenia ciliata* plant extracts. *Nahrug/Food*, **47**: 434-437.

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

Lee KW, Kim YJ, Lee HJ and Lee CY (2003). Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. *J. Agric. Food Chem*., **51**: 7292-7295.

Melo EA, Filho JM and Guerra NB (2005). Characterization of antioxidant compounds in aqueous coriander extract (*Coriander sativum* L.). *Lebensm-Wiss. Technol*., **38**: 15-19.

Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS and Leitao SG (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res*., **15**: 127-130.

Miliauskas G, Venskutonis PR and van Beek TA (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem*., **85**: 231-237.

Miura K, Kikuzaki H and Nakatani N (2002). Antioxidant activity of chemical components from Sage (*Salvia officinalis* L.) and Thyme (*Thymus vulgaris* L.) measured by the oil stability index method. *J. Agric. Food Chem*., **50**: 1845-1851.

Mozaffarian V (1996). A Dictionary of Iranian Plant Names. Farhang Moaser Publishers, Tehran.

Nickavar B, Kamalinejad M, Haj-Yahya M and Shafaghi B (2006). Comparison of the free radical scavenging activity of six Iranian *Achillea* species. *Pharm. Biol*., **44**: 208-212.

Orhan I, Aydin A, Colkesen A, Sener B and Isimer AI (2003). Free radical scavenging activities of some edible fruit seeds. *Pharm. Biol*., **41**: 163-165.

Ozgen U, Mavi A, Terzi Z, Yildirim A, Coskun M and Houghton PJ (2006). Antioxidant properties of some medicinal Lamiaceae species. *Pharm. Biol*., **44**: 107-112.

Parejo I, Viladomat F, Bastida J, Rosas-Romero A, Flerlage N, Burillo J and Codina C (2002). Comparison between the radical scavenging activities and antioxidant activity of six distilled and nondistilled maditerranean herbs and aromatic plants. *J. Agric. Food. Chem*., **50**: 6882-6890.

Roginsky V and Lissi EA (2005). Review of methods to determine chain-breaking antioxidant activity in food. *Food Chem*., **92**: 235-254.

Soler-Rivas C, Espin JC and Wichers HJ (2000). An easy and fast test to compare total free radical scavenger capacity of foodstuffs. *Phytochem. Analysis*, **11**: 1-9.

Tepe B, Sokmen M, Akpulat HA and Sokmen A (2005). *In vitro* antioxidant activities of the methanol extracts of four *Helichrysum* species from Turkey. *Food Chem*., **90**: 685-689.

Tepe B, Sokmen M, Akpulat HA and Sokmen A (2006). Screening of the antioxidant potentials of six *Salvia* species from Turkey. *Food Chem*., **95**: 200-204.

Tepe B, Eminagaoglu O, Akpulat HA and Aydin E (2007). Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of *Salvia verticillata* (L.) subsp. *verticillata* and *S. verticillata* (L.) subsp. *amasiaca* (Freyn & Bornm.) Bornm. *Food Chem*., **100**: 985-989.