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Original article

Comparative study of the total phenol content and antioxidant activity of some medicinal herbal extracts

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Abstract

Herbal medicines can be used as the potential sources of anti-oxidative compounds to help the treatment of diseases associated to oxidative stress. In this paper, the Ferric Reducing Antioxidant Power (FRAP) activity of four Lamiaceae herbal extracts, which traditionally applied in oxidative stress related diseases, has been evaluated and total phenolics contents of these extracts determined by using Folin-Ciocalteu reagent. The aqueous methanol extracts were prepared by percolation method and investigated for antioxidant properties and total phenolics content evaluation. All the extracts showed antioxidant effect from 123.6±4.6 mmol of $FeSO_4.7H_2O$ equivalent/100 g dried extract in *Scutellaria tornefortii* to 551.5±16.0 mmol of $FeSO_4.7H_2O$ equivalent/100 g dried extract in *Satureja sahendica*. Interestingly, although *Satureja sahendica* exhibited the most antioxidant activity, the highest content of polyphenolics belonged to *Stachys byzantina.* Taking together, antioxidant activity of the mentioned medicinal plants is not necessarily associated with polyphenolic compounds and might be partially due to the presence of other polar constituents like terpenoid-glycosides in aqueous extracts that traditionally used as decoction.

Keywords*:* antioxidant activity, FRAP, *Satureja sahendica*, *Stachys byzantina*, total phenolics

Introduction

Polyphenols and flavonoids are the common antioxidant natural products found in medicinal plants. The Ferric Reducing Antioxidant Power, well-known as FRAP assay, is a valuable method for evaluation of total antioxidant power of the herbal extracts [1]. This is a sensitive method for measuring of antioxidant activity of the plant homogenates and phytopharmaceuticals and acts on the basis of reduction of ferric-tripyridyltriazine complex to ferrous form which produces an intensive blue color with absorption in 593 nm [1,2]. Free radicals associate to more than one hundred diseases in human such as gastritis, atherosclerosis, arthritis, neurodegenerative disorders (Alzheimer), and many kinds of cancers. Free radicals have the potential of depletion in the immune system antioxidants and may cause changes in the gene

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expression to induce production of abnormal proteins. The oxidation process is one of the most important ways to produce free radicals in living systems [3-5].

Literature review shows that herbal medicines (especially from large families, Asteraceae and Lamiaceae) have been used from ancient times as remedies for treatment of diseases because they contain pharmacological and biological active ingredients [6,7]. Furthermore, the usage of herbal extracts or active compounds (such as rosmarinic acid) in food, cosmetic and pharmaceutical industries have being increased, so that biological and phytochemical study of medicinal plants is of essential and interesting areas [8,9].

Based on a systematic bibliography on one of the most important medicinal herbal family, Lamiaceae, we focused on the genus, *Salvia, Satureja, Scutellaria* and *Stachys* and selected more frequently used species of them in order to evaluate their antioxidant activity. Previously, we reported the brine shrimp cytotoxicity of *Salvia macrosiphon*, *Scutellaria tornefortii* and *Stachus byzantina* (different extracts) and also the main components of *Satureja sahendica* and *Salvia macrosiphon* [10-12]. In the present paper, we aimed to evaluate both antioxidant and total phenolics content of these species in order to compare whether the usage of them in treatment of oxidative stress related diseases could be related to the high contents of phenolic compounds or not.

Experimental

Plant material

The plant materials (flowered aerial parts) were collected from wild growing areas of Iran (Tehran, Azerbaijan and Mazandaran provinces) in June-July (2005-2008), during full flowering stage. The plants were identified by Dr. A.R. Naghinejad and Mr. Y. Ajani. The voucher herbarium specimens were deposited at the Herbarium of Institute of Medicinal Plants, ACECR.

Extraction

Aerial parts of the plants were dried at shade and milled to powder, washed with ethyl acetate and then extracted with aqueous methanol (50%), respectively, employing percolation (48 h) at room temperature. The methanol 50% extracts were concentrated under reduced pressure and were dried by freeze dryer.

FRAP assay

The key solutions for performing FRAP assay were prepared as followed: a) Acetate buffer 300 mM pH 3.6; b) TPTZ (2,4,6-tripyridyl-s-triazine): 10 mM in 40 mM HCl; c) $FeCl_3$. $6H_2O$: 20 mM.

The FRAP solution were prepared by mixing a, b and c in the ratio of 10:1:1 just before testing. Standard was $FeSO_4.7H_2O$: 0.1 - 1.5 mM in methanol. FRAP solution (3.6 mL) was incubated at 37 ˚C for 5 min. Then the solution was mixed with 0.4 mL distilled water and certain concentration of the plant extract (80 µL) and incubated at 37 ˚C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For plotting of the calibration curve, five concentrations of $FeSO_4.7H_2O$ (0.1, 0.4, 0.8, 1, 1.5 mM) were used and the absorbance values were measured as for sample solutions [13]. Butylated Hydroxy Toluene (BHT) was used as positive control.

Determination of total phenolics content

The total phenolics contents of the extracts were determined spectrophotometrically according to the Folin-Ciocalteu method [14] using chlorogenic acid (concentrations, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL) as the standard. The reaction mixture was prepared by mixing the methanolic solution (1 mL) of the extract, distilled water (9 mL), Folin-Ciocalteu's reagent (1 mL) and sodium carbonate (10 mL, 7%). Incubation at room temperature was down for 90 min and the absorbance was determined at 765 nm. The total phenolics content was expressed as chlorogenic acid equivalent in milligram per 100 gram dried extract.

Results and Discussion

The results of FRAP assay and total phenolics evaluation of the aqueous methanol extracts of four species (Lamiaceae family), *Salvia macrosiphon, Scutellaria tornefortii, Satureja sahendica* and *Stachys byzantina*, have been summarized in Table 1. The data were obtained according to calibration curves of $FeSO_4.7H_2O$ ($y=1.0436x-0.0183$, $r^2= 0.9972$) and chlorogenic acid ($y=2.0912x-0.0227$, $r^2= 0.9989$). All the extracts showed antioxidant activity from 123.6±4.6 mmol of $FeSO_4.7H_2O$ equivalent/100 g dried extract in *Scutellaria tornefortii* to 551.5±16.0 mmol of $FeSO_4.7H_2O$ equivalent /100 g dried extract in *Satureja Sahendica* which were considerable compared to BHT (265.2±0.4 mmol of $FeSO_4.7H_2O$ equivalent /100 g). The results revealed that the total phenolics content of *Stachys byzanthina* was higher than the others (46.0±1.2 mg ChAE/100 g EXT). *Salvia macrosiphon* and *Satureja sahendica* approximately contain equal amounts of phenolic compounds, 35.4±0.4 and 33.2±0.6 mg ChAE/100 g EXT, respectively; while, *Scutellaria tornefortii* showed the lowest amount of phenolic compounds. Antioxidant activity of these species might be partially due to the presence of phenolic compounds and somewhat in part related to other polar constituents. Bibliography revealed that the above mentioned plants have been employed for treatments of various diseases, which some of them are now categorized as oxidative stress related diseases (Table 2). So that, the antioxidant activity of these herbal extracts would be of the most considerable effects of them. Interestingly, although *Satureja sahendica* exhibited the most antioxidant activity, the highest content of polyphenolics belonged to *Stachys byzantina*, suggesting that antioxidant activity of the mentioned medicinal plants is not necessarily associated with polyphenolic compounds and

Table 1. Antioxidant activity and total phenolics content of the aqueous methanol extracts of *Salvia macrosiphon, Scutellaria tornefortii, Satureja sahendica* and *Stachys byzanthina*

Name	*Origin*	*Antioxidant Activity**	*Total Phenolics Content***
Salvia macrosiphon	Tehran	309.3±15.1	35.4±0.4
Scutellaria tornefortii	Mazandaran	123.6±4.6	12.8±0.1
Satureja sahendica	Azerbaijan	551.5±16.0	33.2±0.6
Stachys byzantina	Mazandaran	186.8±10.6	46.0±1.2
BHT	-	265.2±0.4	-

* FRAP values are indicated as mmol of $FeSO_4.7H_2O$ equivalent in 100 g of the dried extract.
**Total phenolics content was calculated on the basis of the milligram of chlorogenic acid per 100 gram of the dried extract (ChAE/100 g EXT).

Table 2. Usage and main active constituents of four Lamiaceae species, *Salvia macrosiphon, Scutellaria tornefortii, Satureja sahendica* and *Stachys byzanthina*

Plant Name	Evidence Based Usage	Chemical constituents	Ref.
Salvia macrosiphon	Protective against Alzheimer, diuretic, tonic, anti-rheumatoid and chronic pains, flavor and spices as well	β-sitosterol, salvigenin, apigenin-7-*O*-glucoside and luteolin-7-*O*-golucoside	11,5
Scutellaria tornefortii	Sedative and sleep promoter, anti-cancer, anti-inflammation and analgesic	baicalin, baicalein and wogonin	16-18
Satureja sahendica	Antimicrobial, antiviral, spasmolytic, anti-cancer, flavor, anti-HIV and antifungal	luteolin, oleanolic acid, beta-sitosterol and diosmetin	19
Stachys byzanthina	Trypanocidal and antimicrobial activity, effective in genital tumors, sclerosis of the spleen, inflammatory tumors and cancerous ulcers	flavonoids, diterpenes, phenyl ethanoid, glycosides and saponins	20,21

might be partially due to the presence of other polar constituents like terpenoid-glycosides in aqueous extracts that traditionally used as decoction or infusions.

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Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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