Echophytochemical, Antioxidant and Ethnopharmacological Properties of *Stachys inflata* Benth.Extract from Chahar Bagh Mountain

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ABSTRACT

BACKGROUND AND OBJECTIVE: *Stachys inflata* Benth. is used as an anti-inflammatory and antiseptic agent in traditional medicine in most mountain villages of Golestan province. Therefore, this study aimed to investigate the antioxidant, ethnopharmacological and phytochemical properties of extract from different parts of *S. inflata*, collected from Chahar Bagh Mountain.

METHODS: Flowering branches and root of the plant were collected from Chahar Bagh Mountain (2100 m) in July 2013. At the same time, the most important information about traditional uses of the plant (ethnopharmacology) was recorded by questioning local people. Phytochemical evaluation (total phenolic, flavonoid and anthocyanins content) of ethanolic extract of plant organs was done using spectrophotometry and folin-ciocalteu. The antioxidant activity of the extract was evaluated by DPPH test. $P \leq 0.05$ was considered as statistically significant.

RESULTS: The amount of chemical compounds in the extract of flowering branches and root extract was significantly different. The total phenolic (129.96 ± 5.6 mgGAE/g), flavonoid (29.62 ± 1.4 mgQUE/g) and anthocyanin (0.021 ± 0.001 µg/g) content in the extract of aerial parts of the plant was approximately 1.5 to 3 times higher than those in the root. Due to higher production of active compounds, the antioxidant activity of the aerial parts' extract showed a greater potential in free radical scavenging (IC_{50}= 76.33 ± 4.2 µg/ml) compared to the root extract.

CONCLUSION: Phytochemical findings and antioxidant activity of the extract of aerial parts of the plant in free radical scavenging, confirm the traditional applications of this plant as analgesic, anti-inflammatory and antiseptic agent in treatment of rheumatism, wounds, burns and diarrhea. It is recommended that further evaluation of the plant's traditional applications be conducted in vivo and in vitro.

KEYWORDS: Antioxidants, Anthocyanins,*Stachys inflata*, Root, Branch, Flavonoids, Phenols, Golestan Province.

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INTRODUCTION

Medicinal plants have a long history for use in treatment. They are currently considered as the main treatment strategy in many countries (1). At the same time, approximately 30% of pharmaceutical products are of plant origin (2).

The distribution, diversity of vegetation and quality of medicinally active compounds in plants in every habitat are affected by environmental factors and ecological stresses in that area. Therefore, the approach of the World HealthOrganizationis is toward the identification of native medicinal plants,ethnopharmacology, extraction of active compounds and most importantly, assessing their antioxidant properties with the aim of producing natural, safe and effective medicine for treatment and prevention of diseases(3).

Medicinal plants are potential source of natural antioxidant compounds such as flavonoids and phenolic acids that are capable of eliminating superoxide radicals. These compounds are used as effective antioxidant and anti-inflammatory agents for reducing the risk of cancer, cardiovascular disease, diabetes, hyperlipidemia and hypertension (4, 5). *Stachys inflata* Benth. is a species of the genus *Stachys* (family *Lamiaceae*) that grows wild in the Mediterranean and Irano-Turanian regions. It is often found in hot and dry to cold and dry mountainous areas (3, 6). Several therapeutic effects have been attributed to various species of this genus (*Stachys*) in textbooks of traditional medicine in Iran and other countries. These include hypnotic, sedative, hypoglycemic, antitussive, analgesic and wound healing effects. It can also stop bleeding, increase bile secretion and treat pyelonephritis (6). In addition, various studies have mentioned the antioxidative, antibacterial and anti-inflammatory effects of this plant that are often attributed to the quality and quantity of active phenolic and flavonoid compounds in the *Stachys* species (7-9). Due to different ecological conditions, Golestan province has diverse habitats for *S. Inflata*, which has potential importance in traditional medicine. Thus, this study aimed to investigate the ethnopharmacological, phytochemical and antioxidant activities of the plant's extract from various parts, collected from Chahar Bagh Mountain (Golestan province, altitude of 2100 m).

MATERIAL AND METHODS

Identification of the plant and useable plant parts was done, and information about different methods of using medicinal plants and their local names were collected and recorded during several visits to the mountain village of Chahar Bagh in Southeast of Gorgan. The flowering branches and roots of *S. Inflata* were collected in June 2013 (from altitude of 2100 meters) for laboratory testing. Using reliable sources, herbarium code 5240 (*S. inflata*Benth.) was identified at Islamic Azad University of Gorgan. For extraction, the collected samples were dried in the laboratory and powdered by an electric mill.

Preparation of ethanolic extract

First, 50 g of each sample (flowering aerial parts and root) was weighed and soaked separately in 1000 ml of 70% ethanol and then placed on a shaker for 24 hours at 250 rpm. The extracts were filtered, and the solvent was evaporated at temperature of less than 50 °C by rotary to obtain dried extract. The samples' container was covered with aluminum foil and placed in a refrigerator at 4 °C until the start of experiments (10). In order to measure anthocyanins, one gram of the samples from aerial parts and root was triturated with 10 ml of acidic ethanol, and then placed in the dark at 4 °C for 24 hours. The extract was then centrifuged for 10 minutes at 4000g, and the supernatant's absorbance at wavelength of 520 nm was read using a spectrophotometer (Heidolph Instruments GmbH & Co., Model KG 91126 Schwabach.Walpersdorfer Str.12,Germany). The amount of anthocyanin was measured using the following formula:

$A = \varepsilon bc$

ε (extinction coefficient)=3300 mMm^{-1}

A= absorbance, b= width of the cuvette (1cm), c= amount of anthocyanin (mol/gram of plant's fresh weight).

Finally, the amount of anthocyanin was expressed as μmol/gof fresh weight (11).

For measuring total phenolic content, 2 ml of sodium carbonate (2%), 8.2 ml of distilled water and 100 μl Folin-Ciocalteu's reagent (50%) were added to 100 μl of the aerial parts' extract. After 30 minutes, the absorbance at wavelength of 720 nm was measured against a control in triplicate. The control contained all the aforementioned compounds, but equal volume of 70% ethanol was added instead of the extract. Gallic acid with different

concentrations (25, 50, 100, 150, 200, 250) was used as the standard for plotting the standard curve. The total phenolic content was reported in milligrams of gallic acid equivalent per gram dry weight (12). In order to measure total flavonoid content, 1.5 ml ethanol (70%), 100 µl aluminum chloridesolution (10%), 100 µl potassium acetate solution (1M) and 2.8 ml distilled water were added to 500 µl of the extracts. After 40 minutes, the mixture's absorbance was measured at 415 nm against a control. The control contained all the aforementioned compounds, but equal volume of 70% ethanol was added instead of the extract. Quercetin with different concentrations (25, 50, 100, 150, 200, and 250) was used for plotting the standard curve. The total flavonoid content of the extracts was expressed as milligrams of quercetin equivalent per gram dry weight (13). The ability of extract to combine with hydrogen or donate electrons was evaluated by discoloration assay using pink colored DPPH ethanol solution. In this spectrophotometric measurement, 2, 2-diphenyl-1-picrylhydrazyl was used as reagent (14, 15). Next, 0.01 g of the dry extract was brought to volume of 100 ml using ethanol, and then concentrations of 50, 100, 150, 200, 500 and 1000 µg/ml were prepared in triplicate. Fifty µl of each concentration of ethanolic samples was added to 5 ml of 0.004% ethanol solution of DPPH. After 30 minutes of exposure to laboratory temperature, absorbance at 517 nm was read against a control. Free radical scavenging activity of DPPH was calculated using the following formula:

$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$

A_{blank} = absorbance of blank or the control (containing all the ingredients except the compounds tested)

A_{sample} = absorbance of the test sample

Half maximal inhibitory concentration (IC50) was calculated based on the graph that showed the percentage of inhibition against the concentration of the extract. This test was repeated three times for each sample (16).

Statistical analysis was done using ANOVA and SPSS (version 21). Comparison of the mean values was carried out using the Duncan test with 99% confidence interval (P <0/1). The graphs were plotted using Excel software (version 2007). The results were expressed as mean ± standard deviation (SD).

RESULTS

Ethnopharmacological survey of medicinal plants in this area showed that the brewed root of *S. inflata* Benth. and *Menthalongifolia L.* is used in wound healing and treatment of fungal nail infections. Decoction of the plant's flowering branches with *Perovskiaabrotanoides* and *Artemisia sieberi* are used as anti-inflammatory and analgesic agents to relieve rheumatoid arthritis pains. Brewedaerial parts of the plant with *Nigella sativa* and barberry are used in treatment of hypertension and sinusitis.

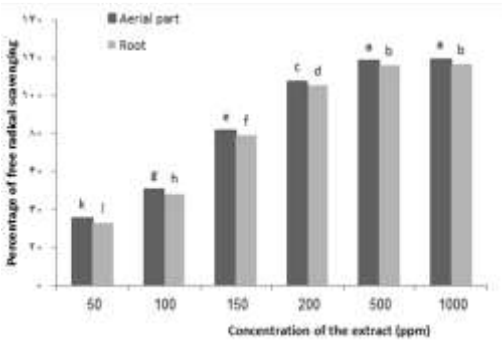
The laboratory studies also showed that the total phenolic, flavonoid and anthocyanin contents (Tables 1 and 2) were significantly higher in the flowering aerial parts of the plant when compared with the root. The total phenolic, flavonoid, anthocyanin content in the flowering aerial part was 1.5-, 1.7- and 3-fold higher than the root, respectively.

Table 1- Total phenolic, flavonoid and anthocyanin contents in aerial flowering branches and root

Total (m g^{-1} WWµ) anthocyanins	Total (mg QUE g^{-1} DW)flavonoid	Total (mg GAE g^{-1} DW) Phenol	est
0.021±0.001	29.62±1.4	129.96±5.6	erial parts
±0.0003	17.05±0.9	84.99±2.3	
0.007			oot

The results are shown as mean ± SD (3 replicates)

Figure 1-Comparison of percentage of DPPH free radical in different concentrations of the extract



DISCUSSION

Nowadays, value of natural products and antioxidants found in native plants is on the rise. *S. inflata* is among the plants that have anti-inflammatory, antimicrobial and analgesic effects due to presence of secondary medicinal compounds (polyphenols, flavonoids, tannins, saponins and terpenoids). Because of their anti-inflammatory and antioxidant role in prevention, control and treatment of common diseases, they are highly regarded by specialists for controlling many common and chronic diseases such as cancer, cardiovascular disease and diabetes (17- 19). Due to higher synthesis of phenolic, flavonoid and anthocyanin metabolites, the aerial branches of *S. inflata* have the highest antioxidant activity when compared with the root of the plant. This confirms the ethnopharmacological findings of this study that indicates the aerial parts of the plantare used in traditional medicine in Chahar Bagh region as anti-inflammatory, antibacterial, antiseptic and analgesic agents in treatment of rheumatic diseases, hypertension and wound infection. Due to their antioxidant and anti-inflammatory activity, the importance of active polyphenolic and flavonoid compounds in nutrition and human health has been recognized. They are also involved in free radical scavenging because of their chelating and eliminating potential (8). Consistent with the findings of the present study, some studies reported a direct relationship between antioxidant activity and total phenolic and flavonoid content in extracts of medicinal plants (20-22). In line with ethnopharmacological findings of this study, researchers showed that flavonoids (apigenin, luteolin and hesperidin) and phenolic compounds (rosmarinic acid) in methanolic extract, and terpenoids (sabinene, pathulenol, and germacrene D) in essential oil of the plant of different species (*S. cretica subsp*., *smyrnaea Rech Fil.* and *S. cretica L.*) have a high antimicrobial activity, especially against *Mycobacterium* (12). Anthocyanins are also among the most important phenolic and flavonoid antioxidant compounds. Based on laboratory and clinical evidence, these compounds can be used as anti-cancer, anti-inflammatory and antiseptic agents in prevention and treatment of cardiovascular disease, diabetes and hypertension (23-26).

CONCLUSION

Extract of the aerial parts of *S. inflata* has higher inhibitory activity compared to the root extract in blocking DPPH free radicals. The increase of the extract from 50 to 1000 µg/ml raises the antioxidant activity. This trend could be because of the increased number of hydroxyl groups in the reaction medium at higher concentrations of phenolic compounds. Therefore, the possibility of donating hydrogen to free radicals and subsequently inhibitory activity of the extract will increase. In other words, there is a direct relationship between the plant part used, active constituents and antioxidant activity of different plant organs.

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CONFLICT OF INTEREST

All contributing authors declare no conflicts of interest.

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