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A screening to determine total phenol and flavonoid content of some Iran's medicinal plants grown in Chaharmahal va Bakhtyari province

Majid Asadi-Samani[1], Mahmoud Rafieian-Kopaei[2], Zahra Lorigooini[2] and Hedayatollah Shirzad[3*]

[1]Student Research Committee, [2]Medical Plants Research Center, Basic Health Sciences Institute, [3]Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran

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The study of phytochemical compounds of plants grown in different regions can reveal their biological potentials for the identification and discovery of effective drugs. This study was conducted to investigate the total phenolic and flavonoid content of twenty Iranian medicinal plants. The plants were gathered from Chaharmahal va Bakhtyari and their aerial parts were used for extraction by maceration using ethanol 70 %. To determine total phenolic and flavonoid content of the extracts, the Folin-Ciocalteu method and the aluminum chloride colorimetric method were used respectively. *Stachys inflate and Euphorbia szovitsii* Fisch. & C.A. Mey. with 87.458 and 72.9644 mg GAE/g had the highest total phenolic content. Also, *Urtica dioica* (81.4945 mg RUT/g) and *Satureja bachtiarica* (41.5445 mg GAE /g) had the highest flavonoid content among the investigated plants. According to the result, all plants have different values of phenolic compounds. However, *S. inflate, E. szovitsii, U. dioica* and *S. bachtiarica* due to high values of phenolic and flavonoid contents can be considered as a potent resource of natural products for discovering antioxidant and anti-inflammatory drugs.

Keywords: Anti-inflammatory, Antioxidant, Flavonoid, Traditional medicine, Phenolic compounds.

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Introduction

Nowadays, it is essential to discover new drugs due to the side effects of synthetic chemical drugs, drug resistance resulting from these drugs, the high cost of many of them, and the lack of their optimal efficiency in the prevention and treatment of various diseases. In this regard, medicinal plants, as valuable sources of natural resources and reservoirs of phytochemical compounds that are agreeable to the physiological system of the body, they can be considered an appropriate alternative for the production of new drugs with comparatively higher efficiency and fewer side effects[1-7].

Phenolic compounds are one of the most widely occurring groups of phytochemicals. These compounds are responsible for colour, taste, and quality of plant-based foods. Phenolic compounds including a diversity of structures such as phenolic acids, flavonoids, and polymeric compounds. Flavonoids are the largest group of natural phenolics. They as plant pigments that are synthesized from phenylalanine, generally display marvellous colours known from petals of a flower. Flavonoids can be classified into flavonols (3-hydroxy-2-phenylchromen -4-one), flavanones (2, 3-dihydro-2-phenylchromen-4-one), flavones (2-phenylchromen-4-one), and isoflavones (3-phenylchromen-4-one).

Phenolic and flavonoids compounds are able to scavenge excess radicals and maintain the balance of reactive oxygen species (ROS) in the human body. They exert many favorable effects such as antioxidant, anti-inflammatory, anti-allergenic, anti-microbial, and anti-cancer and are effective for the treatment of psychotic disorders, hyperlipidemia, hyperglycemia and related diseases. Therefore, they are interest among scientists, consumers, and food manufactures[8-12].

Many plant species have been investigated in the search for novel antioxidants but generally, there is still a demand to find more information concerning the antioxidant potential of plant species[13].

On the other hand, the plants are grown in different regions can have different amounts of active compounds with various characteristics depending on climatic conditions and characteristics. Researchers have tried to evaluate the relationships between the chemical composition of a sample and its geographical origin. Since Iran, especially the Chaharmahal and Bakhtiari province, is considered rich in medicinal plants, and

*Correspondent author
Email: shirzadeh@yahoo.com
Mob: 09838333330061

many medicinal plants are found only in these regions and are native to these regions, and the climatic conditions of these areas have caused these plants to have high amounts of phytochemical compounds with various therapeutic effects, this study was conducted to investigate the total phenolic and flavonoid content of less known Iranian medicinal plants gathered from Chaharmahal va Bakhtyari province.

Material and Methods

Plant material

The plants were collected locally from the different points of Chaharmahal va Bakhtiari province in Iran in May-Sep 2015 and botanically authenticated by Dr. Shirmardi (Research Center for Agricultural & Natural Resources, Shahrekord, Iran) and Miss S. Khademian (Department of Pharmacognosy, Faculty of Pharmacy, Shiraz University of Medical Sciences).

Preparation of extracts

The herbal samples were cleaned and dried in shade. The aerial parts of the plants ware pulverized in a mechanical grinder and macerated in ethanol (70 %) at room temperature for 72 hours. The extracts were then concentrated under reduced pressure using a rotary evaporator.

Determining total phenolic and flavonoid content

Total phenolic content was measured by Folin-Ciocalteu colourimetric assay and expressed in terms of gallic acid equivalent (GAE) and total flavonoid content by aluminium chloride colourimetric method and in terms of rutin equivalent (RE)[14-19].

For total phenolic content, 0.1 mL of extract was transferred into a test tube, and 0.5 mL of Folin-Ciocalteu reagent was added and mixed gently. After 5 minutes incubation, 0.4 mL of 7.5% (w/v) sodium carbonate was added to the mixture. The mixture was allowed to stand at room template for 30 minutes. UV–Vis absorption measurements were carried out at 765 nm using a spectrophotometer (UNICO 2100: USA). The standard curve was plotted using 12.5-100 mg/mL solutions of gallic acid in methanol and distilled water (60:40, v/v) (Fig. 1). The total phenolic content was expressed as gallic acid equivalents (mg GAE/g dry weight of the extract).

For flavonoid content, the extracts were dissolved in methanol 60% (1 mg/mL). Exactly 0.5 mL of extract separately was mixed with 1.5 mL of methanol. Then, 0.5 mL of 2% aluminium chloride ($AlCl_3$) and 3 mL of 5% potassium acetate were added to the extract. After 40 minutes incubation, UV–Vis absorption measurements were carried out at 415 nm using a spectrophotometer (UNICO 2100, USA). The standard calibration curve was plotted using 12.5-100 mg/mL of rutin in methanol and distilled water (60:40, v/v). The total flavonoid content was expressed as mg RE/g dry weight of the extract (Fig. 2).

Results

In this study, 20 species of Iranian medicinal plants were investigated (Fig. 3).

Bakhtiari province, Iran

A: Euphorbia szovitsii Fisch. & C.A.Mey (Skums-935)*; B: Urtica dioica* (Skums-303)*; C: Medicago sativa* (Skums-742)*; D: Parietaria judaica* (Skums-617)*; E: Plantago lanceolata* (Skums-252)*; F: Stachys inflate* (Skums-260)*; G: Haplophyllum perforatum* (Skums-150)*; H: Phlomis persica* (Skums-700)*; I: Onosma sericeum* (Skums-841)*; J: Hertia angustifolia* (Skums-701)*; K: Euphorbia microsciadia* Boiss. (Skums-659)*; L: Acanthophyllum glandulosum* Bung. ex Boiss (Skums-896); M: *Achillea wilhelmsii* (Skums-207)*; N: Sophora alopecuroides* (Skums-258)*; O: Teucrium orientale* L. subsp. taylori. (Boiss.)

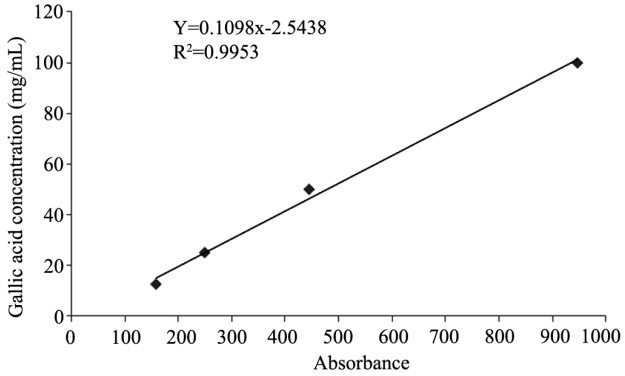

Fig. 1 — Standard curve of gallic acid

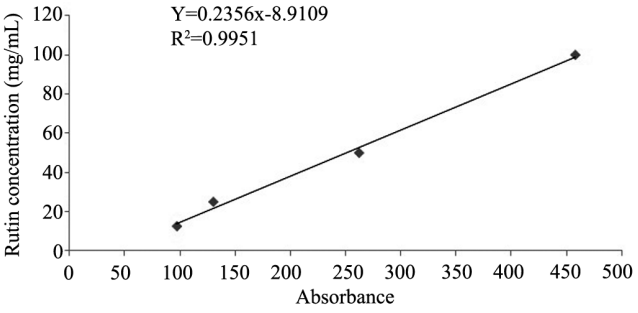

Fig. 2 — Standard curve of rutin

Fig. 3 — Pictures of plants gathered from Chaharmahal va Bakhtiari province

(Skums-522); P: *Ziziphora clinopodioides* (Skums-253); *Q: Satureja bachtiarica* (Skums-208); *R: Moriera spinosa* Boiss. (Skums-623); *S: Salvia multicaulis* Vahl (Skums-301); *T: Echinophora platyloba* D.C (Skums-249).

In this study, with the aim of evaluating the phytochemical properties of 20 species of medicinal plants grown in Chaharmahal and Bakhtiari province, all the plants were found to contain some amounts of phenolic and flavonoid compounds.

S. inflate and *E. szovitsii* with 87.458 and 72.9644 mg GAE/g DW had the highest total phenolic content. In addition, *U. dioica* (81.4945 mg RE/g DW) and *S. bachtiarica* (41.5445 mg RE/g DW) had the highest flavonoid content among the investigated plants (Table 1).

Discussion

The pharmacological activities of any plant sample are due to the presence of secondary metabolites and secretory products in it. On the other hand, the quantitative evaluating of the phenolic compounds found in plants grown in different regions is very important for establishing its efficacy. In this regards, we evaluated the total phenolic and flavonoid content of less known Iranian medicinal plants gathered from Chaharmahal va Bakhtyari province. The present study indicated that *S. inflate* and *E. szovitsii* have the

Table 1 — Total phenolic and flavonoid contents of Iranian medicinal plants investigated in this study

No.	Scientific names	Total phenolic content (mg gallic acid equivalent/g dry weight of extract))	Flavonoid content (mg rutin equivalent/g dry weight of extract))
1	*Stachys inflate*	87.458	15.6945
2	*Salvia multicaulis* Vahl	63.9608	26.7395
3	*Hertia angustifolia*	57.263	11.2295
4	*Sophora alopecuroides*	65.8274	18.0445
5	*Haplophyllum perforatum*	48.0398	10.2895
6	*Moriera spinosa* Boiss.	54.8474	31.6745
7	*Teucrium orientale* L. subsp. taylori. (Boiss.)	64.6196	27.6795
8	*Achillea wilhelmsii*	49.3574	39.6645
9	*Urtica dioica*	39.2558	81.4945
10	*Plantago lanceolata*	50.8946	18.2795
11	*Euphorbia microsciadia* Boiss.	69.5606	24.3895
12	*Medicago sativa*	49.7966	16.8695
13	*Satureja bachtiarica*	64.5098	41.5445
14	*Acanthophyllum glandulosum* Bung. ex Boiss	63.851	34.0245
15	*Onosma sericeum*	54.518	13.8145
16	*Parietaria judaica*	67.5842	35.1995
17	*Phlomis persica*	55.5062	24.3895
18	*Ziziphora clinopodioides*	51.3338	14.5195
19	*Echinophora platyloba* D.C	43.9772	6.5295
20	*Euphorbia szovitsii* Fisch. & C.A.Mey.	72.9644	32.8495

GAE: gallic acid equivalent

highest total phenolic content with 87.458 and 72.9644 mg GAE/g DW respectively. Moreover, *U. dioica* (81.4945 mg RE/g DW) and *S. bachtiarica* (41.5445 mg RE/g DW) have the highest flavonoid content among the investigated plants. In the other studies from different regions, the total phenolic content and flavonoid content of these plants have been evaluated. A phytochemical study of selected Iranian medicinal plant species extracts (*Mellilotus officinalis*, *Equisetum maximum*, *P. major*, *Adiantum capillus-veneris* and *U. dioica*) indicated that the total phenolic content was between 24.1 to 289.5 mg/g and flavonoid contents varied from 25.15 and 78.3 mg/g in the extracts[13]. In another study, total phenolic content and flavonoid content of methanol extracts of *S. lavandulifolia*, *Dracocephalum multicaule* Montbr & Auch, *S. bachtiarica* and *Thymus daenensis* Celak. was evaluated. The total phenolic and flavonoid contents were between 99 to 208 mg tannic acid equivalent (TAE)/g DW extract and 10.1 to 22.2 mg RE/g DW, respectively. *T. daenensis* Celak. exhibited the highest phenolic content and *Dracocephalum multicaule* exhibited the highest flavonoid content[20].

S. inflate has the highest total phenolic content between the plants studied in our study. In a study in 2017, Ahmadvand *et al.* reported that the total phenol content of ethanolic extract of *S. inflata* leaves grown wildly in Lorestan province was 830.334±26.41 mg GAE/g DW and flavonoid content of the ethanolic extract was 17.09±0.154 mg quercetin equivalent (QE)/g DW[21]. In the other study by Khanavi *et al.*, evaluating total phenolic contents of the methanolic extracts of the aerial parts of nine *Stachys* species from Golestan national park (province of Golestan, Iran) indicated that *S. persica* Gmel. and *S. fruticulosa* M.B. had the highest total phenolic content (3294.96 and 4450.36 mg GAE/100 g DW) between the studied species. Total phenolic content of the *S. inflata* had been 1478.808±44.3195 mg GAE/100 g DW[22]. In Iranian traditional medicine, *S. inflata* Benth. is used for inflammatory disorders, infection diseases, and asthma which can be due to phenolic and flavonoid contents of this plant[23].

In the present study, *E. szovitsii* after *S. inflate* has the highest total phenolic content between the studied plants. *Euphorbia* genus includes about 2000 species and spreads in Iran, India, Pakistan and some other

countries. A variety of species of *Euphorbia* have been found in Iran. Up to now, phytochemical screening of some species of *Euphorbia* genus such as *E. hirta* and *E. helioscopia* has been reported. Phytochemical evaluating of the methanol extracts of *E. hirta* L. indicated that total phenolic contents in different parts of the plant were between 65 to 206 mg GAE/g DW and total flavonoids contents were between 24 to 37 mg CEQ/g DW. The leaves had the highest contents[24]. In another species of *Euphorbia* genus, aerial parts of *E. helioscopia* was evaluated for their phenolic and flavonoid contents. Methanolic extracts of flowers had the highest and ethanol extracts of the stem had the lowest contents. The range of phenolic and flavonoid contents was between 4.8 to 51.49 mg GAE/g DW and 1.69 to 11.38 mg QE/g DW, respectively[25]. Another study reported that in the ethanolic extract of *E. hirta*, the total phenol content was 285 mg GAE/g DW while the total flavonoid content was 118.46 mg QE/g DW[26]. *In vitro* and *in vivo* experimental on *E. hirta* L. showed that powerful antioxidant activity of the plant may be responsible for its traditional uses[27]. In the study of Kefayati *et al.* on another species of the *Euphorbia* (*E. splendida* Mobayen), it was reported that the total methanol extract of *E. splendida* had total phenolic and flavonoid contents with 270.74 mg GAE/g DW and 208.23 mg QE/g DW, respectively[28]. In addition, the total phenolic content in the *E. lathyris* L. 290.46 mg GAE/g DW has been reported. The root of *E. lathyris* indicated the highest total flavonoid contents (215.68 mg RE/g DW) compared to other parts of the plant[29]. The studies on Euphorbia species or their active compounds showed that different biological effects of them such as antitumor, antifungal, antioxidant, antibacterial, anti-inflammatory, antispasmodic, antidiabetic and antinociceptive activities can be due to their phenolic and flavonoids.

U. dioica that had the highest flavonoid content among the investigated plants in the present study, widely used in folk medicine to treat a variety of diseases including hypertension, diabetes, and cancer. The leaves and roots both used. The hydroalcoholic extract from roots of *U. dioica* has been used for the treatment of prostatic hyperplasia[30]. Three classes of phenolic compounds were characterized in the wild and cultivated *U. dioica* that are including flavonoids, anthocyanins, and hydroxycinnamic acid derivatives[31]. In a study by Pourmorad *et al*., *U. dioica* indicated phenolic and flavonoid contents with 24 mg GAE/g DW and 43 mg RE/ g DW, respectively[13].

In the present study, *S. bachtiarica* had also high flavonoid content between the plants. *S. bachtiarica* is a perennial aromatic herb distributed in Zagros mountains in Iran. In a study by Ghasemi Pirbalouti *et al.*, *S. bachtiarica* had 103+1.78 (mg TAE/g extract) and 10.05+0.13 (mg RE/g extract) for its phenolic and flavonoid content, respectively[20]. The infusions and decoctions of aerial parts of *S. bachtiarica* are used to treat colds and also as an antiseptic and analgesic by the Chaharmahal and Bakhtiari tribes in Iran[32]. It reported that the antibacterial activities of the volatile oil of *S. bachtiarica* may be mainly due to their phenolic compounds. However, the importance of this endemic *Satureja* species could be considered regarding its effectiveness in Alzheimer's disease[23,35,36]. The major constituents of this plant are polyphenolic and flavonoid compounds such as luteolin, naringenin, and rosmarinic acid that possessed antioxidant activities and may play a main role in neuroprotection via plant[33].

Our study and the other studies indicated that the presence of phenolic and flavonoid compounds in plants can be associated with their antioxidant and anti-inflammatory effects. These compounds can counteract free radicals and donate electron or hydrogen due to hydroxyl groups. Studies have confirmed the effect of phenolic compound-containing plants in preventing and treating diseases including cancer. However, they have been used more often as agents to prevent diseases because of their antioxidant effects[34-36.] High amounts of flavonoids, particularly anthocyanins and flavonols, exist in the human diet, which potentially contributes to preventing cancer. Flavonoids can modulate many biological processes in cancer patients such as vascularization, apoptosis, and cell differentiation and proliferation[37-39].

Conclusion

The present study showed that all plants had some concentrations of *phenolic* compounds. But, *S. inflate, E. szovitsii, U. dioica* and *S. bachtiarica* can be considered as good sources of natural compounds to discover antioxidant and anti-inflammatory drugs due to having comparatively higher concentrations of phenolic and flavonoid compounds.

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Conflict of interest

The authors declare no conflict of interest.

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