

Phytochemical properties of some Iranian medicinal plants

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ABSTRACT

There is renewal efforts toward the use of herbal medicines and preparation of natural new drugs. Phenolic compounds are a group of secondary plant compounds with significant antioxidant activity. Herbal based drugs with antioxidant activity are used against radical induced disorders like atherosclerosis. In the present study, antioxidant properties and the contents of phenolics and flavonoid components of hydro-alcoholic extract of eight medicinal plants (*Allium hirtifolium*, *Stachys lavandulifolia*, *Nigella sativa*, *Zataria multiflora*, *Tripleurospermum disciforme*, *Punica granatum*, *Echinophora platyloba* and *Portulaca oleracea*) were measured. The correlation between antioxidant activities and phenolic or flavonoid components were also evaluated. The results showed that there was a correlation between the amounts of total phenolics in hydro-alcoholic extract and antioxidant activities ($R^2=0.640$). Furthermore, the results revealed that there was not a linear correlation between antioxidant properties and flavonoids or flavonol contents of the examined plants. The antioxidant properties of the examined plants may show the potential property of these plants in prevention or treatment of free radical related disorders. The lack of a linear correlation between antioxidant properties and flavonoids or flavonol contents of the examined plants might result from existence of other compounds with antioxidant properties in these plants.

KEY WORDS: Phytochemical property, Antioxidant; phenolic compounds; flavonoid compounds; medicinal plants

1. INTRODUCTION

There are renewal efforts toward the use of herbal medicines and preparation of natural new drugs. Phenolic compounds are a group of secondary plant compounds with significant antioxidant activity (Nasri & Rafieian-Kopaei, 2014a; Rafieian-Kopaei, Baradaran, & Rafieian, 2013b). Herbal based drugs with antioxidant activity are used against radical contributed disorders including atherosclerosis (Rafieian-kopaei, Keshvari, Asgary, Salimi, & Heidarian, 2013; Rafieian-kopaei, Shahinfard, Rouhi-Boroujeni, Gharipour, & Darvishzadeh-Boroujeni, 2014), diabetes mellitus (Bahmani, Zargaran, Rafieian-Kopaei, & Saki, 2014; Mirhoseini, Baradaran, & Rafieian-Kopaei, 2013), neuronal diseases (Rabiei, Rafieian-kopaei, Heidarian, Saghaei, & Mokhtari, 2014; Rahnama, 2015), cardiovascular diseases (Karamati, Hassanzadazar, Bahmani, & Rafieian-Kopaei, 2014; Shayganni, Bahmani, Asgary, & Rafieian-Kopaei, 2015), infections (Bahmani, Rafieian-Kopaei, Jeloudari, 2014; Moghim, 2014; Rahimian, 2013) and wound healing (Asadi, 2013; Delfan, Bahmani, Eftekhari, 2014; Parsaei, Karimi, Asadi, & Rafieian-kopaei, 2013). Experimental and clinical investigations have also gained good results from the medicinal plants in prevention and treatment of a wide variety of complications (Bahmani, 2015; Bahmani, Mirhosseini, Fasihzadeh, Karimian, & Rafieian-Kopaei; Rahimi-Madiseh, Bahmani, Karimian, & Rafieian-kopaei; Taherikalani, Hassanzadazar, Bahmani, Baharvand-Ahmadi, & Rafieian-kopaei). Phenolic compounds are abundantly distributed in plants (CHALESHTORI, CHALESHTORI, & Rafieian, 2011; Karimi & Moradi, 2015; Sedighi, Gholami, & Rafieian-kopaei, 2014). They belong to several groups of compounds including anthraquinones, flavonoids, stilbenoids, and their derivatives. The antioxidant activity of them has been attributed to donation of electrons and scavenging free radicals which cause formation of stable phenoxyl groups (Azar Baradaran & Rafieian-kopaei, 2014; Rafieian-Kopaei, Baradaran, & Rafieian, 2013a). Many trials have revealed that overproduction of ROS (reactive oxygen species), which cause production of superoxide anions, hydroxyl radicals and hydrogen peroxide, might contribute to DNA damage, protein oxidation and lipid peroxidation in living tissues and cells (Nasri & Rafieian-Kopaei, 2013a). ROS may cause a broad spectrum of damage to biological systems, and oxidative stress plays a crucial role in many chronic and degenerative complications, including cardiovascular diseases, cancer, diabetes mellitus and ageing (Bahmani, Sarrafchi, Shirzad, & Rafieian-Kopaei, 2016; Madihi, 2013; Nasri & Rafieian-Kopaei, 2014b; Nasri, Shirzad, Baradaran, & Rafieian-kopaei, 2015; Sarrafchi, Bahmani, Shirzad, & Rafieian-Kopaei, 2016). Dietary supplements with antioxidant activity have become popular enhancing the antioxidant defense of the body for prevention and treatment of diseases (A Baradaran, Nasri, Nematbakhsh, & Rafieian-Kopaei, 2013; Nasri & Rafieian-Kopaei, 2013b, 2014b).

As important group of phytochemicals, phenolics universally are present in plants and most of them have antioxidant activity (Baharvand-Ahmadi, & Bahmani, 2015; Azar Baradaran & Rafieian-kopaei, 2014; Rafieian-Kopaei, Baradaran, 2013b). However, they are not the only components which have antioxidant propert. This study was aimed to measure the total phenolic, flavonols and flavonoid compounds of eight medicinal plants, and to evaluate the correlation between the amounts of these compounds and the possess antioxidant activities. The plants investigated were *Allium hirtifolium* Boiss, *Stachys lavandulifolia* Vahl, *Nigella sativa* L, *Zataria multiflora* Boiss, *Tripleurospermum disciforme*, *Punica granatum* L, *Echinophora platyloba* and *Portulaca oleracea* L.

2. MATERIALS AND METHODS

Chemicals: Gallic acid, Rutin and Linoleic acid were purchased from Sigma Chemical Co. Folin-Ciocalteu, sodium carbonate, aluminium chloride and potassium acetate were purchased through Merck chemical supplies. All other chemicals used, including the solvents, were of analytical grade.

Collection of Plant Materials: The dried plants were purchased from a local grocery. The plants were identified by a botanist in Shahrekord University of Medical Sciences, in Shahrekord, Iran. Voucher specimens have been deposited in the Herbarium of the University.

Preparation of Methanolic Extracts: Fifty gram of powder from plant was extracted with 80% methanol using a Soxhlet apparatus.

The methanolic extracts were filtered and evaporated to dryness under reduced pressure in a rotary evaporator. The extracts then transferred to vials, kept at 4°C until use, and examined for antioxidant activity.

Total Phenolic Determination: The extract total phenolic content was evaluated using the Folin–Ciocalteu method (Rahimi-Madiseh) Briefly, 0.1 mL of the diluted extracts (0.01 gr in 10 ml of 60% methanol) was added to 0.5 mL of 1:10 diluted Folin–Ciocalteu reagent and 0.4 mL solution of sodium carbonate (at 7.5%) was added. The mixture absorbance was determined at 765 nm after incubation for 0.5 h at room temperature. The samples were tested in triplicate and a calibration curve for gallic acid was obtained. The results were determined using gallic acid calibration curve and the contents of total phenolics of the extract were expressed as mg of gallic acid equivalents (GAE) per gram of dry extract.

Total Flavonoid Determination: The method of aluminum chloride colorimetric was employed (Taherikalani,). Rutin was employed for preparation of calibration curve. Rutin (10 mg) was dissolved in ethanol (80%) and diluted to three concentrations of 25, 50 and 100 µg/mL.

The standard solution (0.5 mL) was mixed with 1.5 mL ethanol (95%), 0.1 mL aluminum chloride (10%), potassium acetate (0.1 mL, 1M) and distilled water (2.8 mL). After 30 min incubation at room temperature, the mixture absorbance was measured at 415 nm. The same amount of distilled water in blank was used to substitute the amount of aluminum chloride. Similarly, 500 µL of ethanol extract or solution of standard flavonoid was reacted with aluminum chloride to determine flavonoid content the same as described above.

Total Flavonol Determination: Ten milligrams of rutin was dissolved in ethanol (80%) and diluted to three concentrations of 25, 50 and 100 µg/mL. The diluted standard (0.5 mL) was mixed with 1.5 mL of ethanol (95%), 0.1 mL aluminum chloride (10%), sodium acetate (0.1 mL of 1M) and distilled water (2.8 mL). After 30 min incubation at about 25 °C, the absorbance of the mixture was determined at 415 nm. Aluminum chloride was substituted by the same amount of distilled water in blank. Similar to the above, 500 µL of ethanol extract or standard solution of flavonoid was reacted with aluminum chloride to determine flavonoid content the same as described above (CHALESHTORI, 2011).

Antioxidant Assay Using B-Carotene Linoleate Model: To an emulsion of β-carotene (0.2 mg) in 0.2 mL of chloroform, 20 mg linoleic acid, and 200 mg Tween-40, forty mL of oxygenated water was added, which initiated this assay as an oxidant. Then, to test samples 40 milliliters aliquot of this emulsion was added.

The antioxidant activities (AA) of the extracts were determined in terms of bleaching β-carotene, measuring the absorbance at 470 nm, during $t = 180$ min at an interval of 15 min: $AA = 100 [1 - (A_0 - A_t) / (A^0 - A^0t)]$, where A_0 and A^0 were considered as absorbance values which were determined at zero time and A_t and A^0t were the absorbance values which were measured in the test sample and control, respectively, after 180 min incubation (Karimi & Moradi, 2015).

Statistical Analysis: The experimental results were expressed as the Mean±Standard Deviation. The correlation between phenolic components and antioxidant activities was performed by SPSS ver16. All assays were performed in triplicates.

3. RESULTS

Total phenolic contents: The content of extractable phenolic components in ethanolic extracts was determined by the Folin-Ciocalteu method.

The results, given in Table 1, show that the total phenolic components of *Punica granatum* L (flower) was 480.67 ± 14.18 mg equivalent gallic acid/g dry weight, which was markedly higher than that of other plants.

Total flavonoid contents: The total flavonoid contents of the eight extracts were varied considerably and ranged from 36 to 210 mg Rutin equivalent/g dry extract. The presented data in table 1 reveals that the highest content of total flavonoids was present in the extract obtained from *Tripleurospermum disciforme* (210.67 ± 21.0 mg Rutin equivalent/g), followed by *S. lavandulifolia* (176.67 ± 2.88 mg Rutin equivalent/g).

Table.1.Total phenolic content and antioxidant activity in different extracts.

Plant extracts	Flavonoid mg/g	Flavnlol mg/g	Phenol mg/g	Antioxidant activity %
<i>Allium hirtifolium</i> Boiss	39.0±4.0	35.66±4.04	139.67±4.50	57.33±3.40
<i>Stachys lavandulifolia</i> Vahl	176.67±2.88	132.67±13.01	44.33±8.14	44.66±5.50
<i>Nigella sativa</i> L	36.56±3.51	27.66±4.50	57.33±3.05	48.0±5.56
<i>Zataria multiflora</i> Boiss	131.33±4.50	92.0±5.67	283.33±11.06	71.0±4.0
<i>Tripleurospermum disciforme</i>	210.67±21.0	237.33±4.72	186.67±6.11	69.33±2.51
<i>Punica granatum</i> L	55.66±7.02	52.0±3.60	480.67±14.18	73.0±4.58
<i>Echinophora platyloba</i>	144.33±4.50	144.67±6.42	152.67±5.13	69.0±2.0
<i>Portulaca oleracea</i> L	36.66±4.72	30.66±3.51	119.33±5.85	54.66±5.13

Total flavonol contents: The total flavonol contents were varied considerably and ranged from 27 to 237 mg Rutin equivalent /g dry extract. The presented data in Table 1 reveals that the highest content of total flavonols were present in extract obtained from *Tripleurospermum disciforme* (237.33 ± 4.72 mg Rutin equivalent/g).

Antioxidant assay: The antioxidant property of the ethanolic extracts of studied plants on β -Carotene Linoleate Model is presented in Table 1. The results showed that antioxidant property of eight medicinal plants varied from 44.66 ± 5.50 % (*S. lavandulifolia*) to 73.0 ± 4.58 % (*P. granatum* (flower)). The antioxidant activities of other extracts were as follows: *Allium hirtifolium* Boiss 57.33 ± 3.40, *Nigella sativa* L 48.0±5.56, *Zataria multiflora* Boiss 71.0 ± 4.0, *Tripleurospermum disciforme* 69.33 ± 2.51, *Echinophora platyloba* 69.0±2.0, *Portulaca oleracea* L 54.66 ± 5.13% .

Correlation between the antioxidant property and the phenolic, flavonoid & flavonol contents of the extract: There was a linear correlation between the amounts of total phenolic compounds and antioxidant their activities (Figure 1). However, there was not any correlation between the amounts of flavonoid or flavonol and antioxidant property in the plants extracts (Figures 2 and 3).

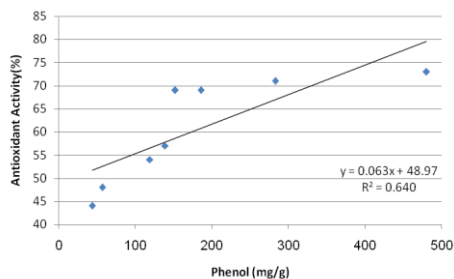


Figure.1. Correlation between the antioxidant activity and total phenolic content of the selected plants.

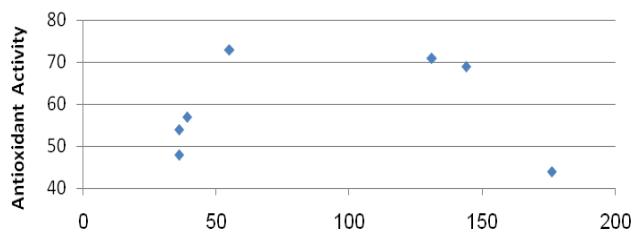


Figure.2. Correlation between the antioxidant activity and Flavonoid content of the selected plants.

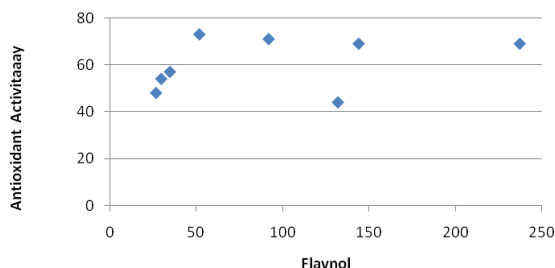


Figure.3. Correlation between the antioxidant capacity and Flavonol content of the selected plants.

DISCUSSION

In the present study, 8 medicinal plants used for various diseases were evaluated for their total flavonol, flavonoid and phenolic components, as well for antioxidant activities. Phenolic components are largely contributed to the antioxidant properties of these plants and play important roles in their beneficial effects (Bahmani, Saki, 2014; Bahmani, Zargarani, 2014; Delfan, Bahmani, Hassanzadazar, Saki, & Rafieian-Kopaei, 2014; Delfan, Bahmani, Rafieian-Kopaei, Delfan, & Saki, 2014; Karamati, 2014). All extracts studied in present study had moderate to high level of flavonoids and phenolic components with reasonable antioxidant activities.

Plants with high antioxidant activity are usually useful in prevention or treatment of a wide variety of diseases including cardiovascular disease, diabetes, hypertension, atherosclerosis, infective, and inflammatory diseases (Asadbeigi, 2014; Asadi-Samani, Bahmani, & Rafieian-Kopaei, 2014; Bahmani, Rafieian-Kopaei, Hassanzadazar, 2014; Bahmani, Rafieian-Kopaei, Jeloudari, 2014; Karagiorgou, Grigorakis, Lalas, & Makris, 2016;

Mohammadian, 2016; Saki, Bahmani, & Rafieian-Kopaei, 2014). Oxidative stress is largely involved in induction of these diseases. With increasing recognition of medicinal plants which can be used as alternative medicine, the screening of herbals for active biological compounds has become an important source of medications (Bahmani, Rafieian-Kopaei, Jeloudari, 2014; Bahmani, Shirzad, Majlesi, Shahinfard, & Rafieian-Kopaei, 2014; Karamati, 2014). Hence, for choosing plant extracts with useful contents, *in vitro* and *in vivo* screening methods have been employed for further chemical elucidation and pharmacological investigations. Some studies conducted to evaluate antioxidant capacity in medicinal plant or fruits and vegetables, such as an investigation of antioxidant capacity of vegetables and fruits (Leong & Shui, 2002).

The results of present study showed that there was a positive correlation between the amounts of phenolic components in extracts and antioxidant activities. The results are in accordance with some other researches, such as survey on phenolic components and antioxidant properties of medicinal plants (Song, 2010). This correlation may confirm the hypothesis that antioxidant activities of plants are largely related to their phenolic compounds. However, there was no correlation between the amounts of flavonoid or flavonol and antioxidant activity in these medicinal plants.

This may result from the presence of high level of other antioxidant compounds in some of these plants. Plants usually contain a mixture of various kinds of active ingredients, and the components other than phenolic compounds should not be neglected.

Many synthetic antioxidants are currently in use; nevertheless, there is a growing evidence of consumer preference for natural antioxidants due to potentially lower toxicity (Nasri & Shirzad, 2013). Plants are a good source of natural antioxidants. They contain phenolic components including phenolic diterpenes, phenolic acids, flavonoids and tannins. The presence of hydroxyl groups; various phenolic compounds have the potential to act as antioxidant by stabilizing or scavenging free radicals which involved in oxidative processes through complexing with oxidizing compounds which are much stronger than those of vitamins C and E (Nasri & Rafieian-Kopaei, 2014b).

4. CONCLUSION

The study demonstrated different antioxidant activities and phenolic compounds of the medicinal plants examined. Furthermore, the results should that there was not any correlation between antioxidant property and flavonoids or flavonol contents of these plants which might be due to existence of other compounds with antioxidant properties in these plants.

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Conflict of Interests: We declare that there was no conflict of interest.

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