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Research Article

Antioxidative effects of liquid and organic extracts from Iranian nettle (*Urtica dioica* L.)

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Abstract

The aim of the present study was to evaluate antioxidant capacities of the liquid and organic extracts of Iranian nettle (*Urtica dioica L.*) in pre-flowering and post-flowering stages. Antioxidant and radical-scavenging properties were tested by means of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay and β-carotene/linoleic acid system. Values were compared between pre and post flowering stages and also between liquid and etheric extracts using t-student test. IC50 showed a remarkable decrease in response to both liquid and etheric extracts in pre-flowering (11.7±0.2 and 22.5±0.2 µg/ml, respectively) and post-flowering (8.4±0.1 and 12.4±0.1 µg/ml, respectively) stages. In this respect, β-carotene/linoleic acid assay showed 91.6, 68% and 93.4, 69.3% inhibition in pre-flowering and post flowering states, respectively. Values of DPPH and β- carotene/linoleic acid assays on butylated hydroxyl toluene were 5µg/ml and 95%, respectively. Pre-flowering extracts showed higher antioxidative properties compared to post-flowering ($p<0.05$). Meanwhile, etheric extract was more potent compared to the etheric extract. Liquid extract from pre-flowering Iranian nettle can be considered as a potential source of natural antioxidants for food and pharmaceutical industries.

Keywords: Antioxidant activity, extract, nettle

Introduction

Lipid oxidation is a highly deteriorative process in food, as it leads to unacceptable properties for customers and loss in nutritional value. In addition, oxidation leads to health disorders such as atherosclerosis and cancerogenesis, amongst others. Oxidation of lipids occurs during raw material storage, processing, heat treatment and further storage of final products [1, 2, 3]. To retard or minimize oxidative deterioration, effective antioxidants are added to food. Synthetic antioxidants have long been used, but their use has recently come into dispute due to a suspected carcinogenic potential and the general rejection of synthetic food additives by

consumers. There is, therefore, a growing interest in the identification of new, natural antioxidants that would serve as alternatives to the synthetic compounds [1, 2, 4, 5, 6]. Natural antioxidants, mainly in fruit and vegetables, have gained increasing interest among consumers because epidemiological studies have indicated that frequent consumption of vegetables and fruit is associated with a lower risk of cardiovascular disease and cancer [4, 5, 7].

Phenolic or polyphenolic compounds constitute one of the largest groups of plant secondary metabolites, which are therefore an integral part of the diet with significant amounts being reported in vegetables, fruit and certain beverages [8]. The antioxidant properties of polyphenols are mainly due to their redox properties. They act as free radical ferminators, hydrogen donors and metal chelators [9].

Nettle (*Urtica dioica L.*) is both an annual and perennial herb, distinguished with stinging hairs. Among *Urtica* species, *U. dioica* and *U. urens* have already been known and therefore consumed for a long time as medicinal plants in many parts of the world. The isolated major flavonoid glycosides have been determined to be immune stimulatory, anticarcinogenic, anti inflammatory, antioxidant and antiallergenic activities [10]. Young leaves are cooked as a pot herb and added to soups. Nettle is very valuable addition to the diet, easily digested and is high in minerals and vitamins. An aqueous extract of *Urtica dioica*, exhibited antioxidant activity towards iron-promoted oxidation of phospholipids, linoleic acid and deoxyribose [11].

The aim of the present study was to evaluate the in vitro antioxidant capacities of the liquid and organic extracts of Iranian nettle in both pre-flowering and post-flowering stages.

Materials and Methods

Plant material and extraction

Urtica dioica was collected from plants growing wild (Babol, Iran) in both pre-flowering and post flowering stages. The taxonomic identification of plant materials was confirmed by the Department of Plant Taxonomy, School of Sciences University of Tehran. Meanwhile, a voucher specimen of this plant was deposited at the Herharium of Department of Food Hygiene, School of Veterinary Medicine, University of Tehran. The aerial parts of the plant were dried in the shade, ground in a grinder and a portion (15g) of dried plant material was extracted with 100 ml of water and diethyl ether by using an electrical shaker for 6 hours. The extracts were filtered using Whatman filter paper (No.1) and then concentrated *in vacuo* at 40°C using a rotary evaporator extractor. Extracts were kept in the dark at 4°C until used.

Antioxidant activity

DPPH assay

The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple coloured methanol solution of DPPH. This spectrophotometer assay uses stable radical diphenyl picrylhydrazyl as a reagent [12, 13].

Aliquots (50 ml) of various concentrations of the extracts in methanol were added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percentage terms (1%) was calculated in the following way: $1\%=(A_{blank}-A_{sample}/A_{blank}) \times 100$.

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compounds) and A_{sample} is the absorbance of the test compounds. Extract concentration providing 50% inhibition (IC50) was calculated from the graph plotted inhibition percentage against extract.

β-carotene-linoleic acid assay

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [14]. A stock of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg of β-carotene was dissolved in 1ml of chloroform (HPLC grade); 25 µl linoleic acid and 200mg Tween 40 were added.

Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml distilled water, saturated with oxygen (30min 100 ml/min), were added with vigorous shaking. Two thousand five hundred micro-liters of this reaction mixture were dispensed to test tubes and 350 µl portions of the extracts prepared at 2g/l concentrations were added and emulsion system was incubated for 48h at room temperature. The same procedure was repeated with synthetic antioxidant, BHT, as positive control, and a blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Antioxidant capacities of the extracts were compared with those of BHT and blank.

Values were compared within and between stages (pre and post- flowering stages) using t-student test.

Results and Discussion

In this study, the ability to control free radicals and to inhibit lipid oxidation by Iranian nettle extracts in the laboratory was evaluated. The ability to control free radicals was investigated by DPPH test. In the test, radicals were more strongly controlled by increasing extract concentration. The concentration of extract which caused 50% inhibition (IC50) is shown in Table 1. The ability to control free radicals by liquid extract of nettle in pre and post-flowering stages, which are 11.7 and 8.4 µg/ml, respectively is weaker compared to BHT (5µg/ml). Moreover, the ability to control free radicals by nettle etheric extract before and after flowering stages which were 22.5 and 12.4 µg/ml, respectively are weaker compared to BHT. Based on the obtained data, the ability to control free radicals of liquid extract obtained after flowering stage are the highest. After that, liquid extract of nettle in pre-flowering stage, etheric after flowering and finally etheric extract of nettle before flowering stage are the highest. Respectively as it can be seen the differences between nettle extracts and BHT, especially liquid extract of nettle, are very variable.

The study undertaken by Gulcin *et al* [11, showed that nettle water extract had an appropriate antioxidant power so that the its inhibitor power of free radicals in DPPH test was almost equivalent to BHA, but lower than quercetine antioxidant activity of nettle has also been proven [15, 16]. In all these reports this fact has been mentioned that due to having hydroxyl groups, phenolic compounds are among the most important component of plants which have antioxidant activity. Based on recent reports, there is a very close relation between the amount of phenolic compounds and antioxidant activities in plants. Key role of phenolic compounds in scavenging free radicals has been mentioned in several reports. Polyphenolic components can have a controlling effect on mutagenic and carcinogenic properties of mutagen and carcinogen materials if more than one gram of them is received daily through fruit and vegetables. It shall be mentioned that free radicals cause oxidation of unsaturated fatty acids of cell walls and other lipids which from biological systems and also cause formation of

Table 1. Effect of *Urtica doioica* liquid and etheric extracts and BHT on *in vitro* free radical (DPPH) scavenging.Values were expressed as IC50.

Sample	IC50%(µg/ml)
Pre-flowering water Extract	11.7±0.2
Post-flowering water Extract	8.4±0.1
Pre-flowering Etheric Extract	22.5±0.2
Post-flowering Etheric Extract	12.4±0.1
Butylated hydroxytoluen	5±0.5

malondialdehyde which may have several pathological effects on organisms. Some researchers have shown that inhibitory power of free radicals may be more related to phenolic compounds of plants such as flavonoids and phenolic acid in polar extract of plants. Polar extracts have had higher controlling effect on free radicals compared to non-polar extracts. It seems that phenolic compounds such as phenolic acid and poly phenolics are responsible for making inhibitory power on free radicals [11, 17, 18, 19].

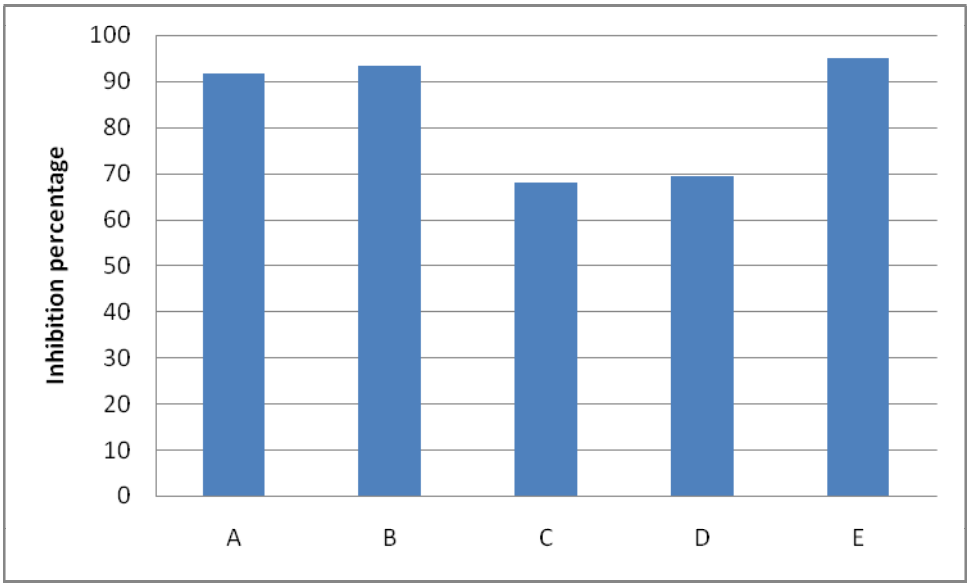

Figure 1. Antioxidant activities of *Urica dioica* extracts and BHT.
Values were expressed as inhibition percentage through β-carotene-linoleic acid assay. A, liquid extract (pre-flowering stage); B, liquid extract (post-flowering stage); C, etheric extract (pre-flowering stage); D, etheric extract (post flowering stage); E, BHT.

Based on available information, as shown in Figure 1, in the linoleic acid oxidation inhibition test in β-carotene – linoleic acid system, 91.6% and 93.4% controlling effects were obtained by liquid extract of nettle, respectively in a concentration of 2g/l in pre and post-flowering stages. Moreover, for BHT as a synthetic antioxidant, 95% inhibitory effect was obtained in β-carotene - linoleic acid test. In this test, inhibitory power of nettle etheric extract in pre and post-flowering stages were 68 and 93.3%, respectively. Also in this test, the inhibitory power of obtained liquid extract in flowering stage was the highest and after that, pre-flowering

water extract, post-flowering etheric extract and finally pre-flowering etheric extract were the highest, respectively. Based on our information, existing compounds in the extract cause more inhibition against formation of conjugated compounds than scavenging of free radicals, but polar section has high effects against both tests. In this study, polar section of extract had a higher inhibitory effect on linoleic oxidation. Antioxidants minimize oxidation amount of lipid compounds in cell walls or prevent from formation of volatile organic compounds and dien conjugated hydroproxides which are the result of lioneic acid oxidation and have carcinogenic effects. Here again, polar extracts have more activities compared to non-polar extracts which is probably due to presence of polyphenils or flavanone and flavonoids. Difference in antioxidation power of different extract may be related to amount and type of phenolic compounds. Moreover, presence of non-phenolic antioxidants such as vitamin C and A and β-Carotene will also be effective in antioxidant power [11, 14, 18, 19]. It should be noted that phenolic compounds act more effectively as hydrogen suppliers and therefore they act as an effective antioxidant [20, 21].

Conclusion

In summary, it seems that etheric extract of Iranian nettle is more potent than the liquid extract. However, pre-flowering stage has more antioxidant content than post-flowering stage. Etheric extract of this plant can be purified and used for food and pharmaceutical industries.

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