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Antioxidant activities of *Eryngium caucasicum* inflorescence

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Abstract. – OBJECTIVE: Medicinal plants are good sources for discovery of safe and new antioxidants. The aim of present study was to evaluate the efficiencies of three methods for evaluated for antioxidants from *Eryngium caucasicum*.

MATERIALS AND METHODS: Ultrasonically assisted extraction, Soxhlet extraction and percolation method were evaluated for the extraction of antioxidants from *E. caucasicum* inflorescence. Antioxidant activities of extracts were evaluated with four different *in vitro* tests.

RESULTS: Soxhlet extract had higher total phenolic and flavonoid contents than other extracts. Soxhlet extract showed the highest activity in DPPH radical scavenging (IC_{50} = 83.1 ± 2.1 μg ml^{-1}). Soxhlet extract showed the best activity in iron chelatory (IC_{50} = 272 ± 6.3 μg ml^{-1}) followed by ultrasonic extract. Percolation extract showed higher NO radical scavenging (IC_{50} = 390 ± 11.4 μg ml^{-1}) than other extracts. Extracts showed good reducing power ($p > 0.05$).

CONCLUSIONS: The results obtained indicated that all three extraction methods especially Soxhlet method could effectively extract antioxidants from this plant.

Key Words:
Apiaceae, Extraction methods, Soxhlet, Total phenols.

Introduction

The roles of oxidants have been known in pathogenesis of many diseases such as cancer and cardiovascular diseases. Plants contain antioxidants and have protective roles against these diseases. There is an interest in the utilization of natural antioxidants for the prevention or treatment of pathological disorders[1]. *Eryngium* species (Apiaceae) have numerous medicinal uses as diuretic, stone inhibitor, expectorant, anti-inflammatory and antinociceptive properties[2]. *E. caucasicum* has been reported recently[2]. Its leaf is used for tasting and preparation of native and regional foods. Antioxidant activities of leaf of this plant have been reported recently[3,4]. Its good antihypoxic activity has been reported recently[5]. The development of new techniques for extraction of biologically active compounds from plants sources, especially by industries, has led to an increased demand for an efficient extraction procedure that can achieve the highest yield of the biologically active constituents in the shortest processing time at the lowest charge. In this study, the efficiencies of three methods (ultrasonically assisted extraction, Soxhlet extraction and percolation method) were evaluated for extract antioxidants from *E. caucasicum* inflorescence. The antioxidant capacities of the extracts were measured by 1,1-diphenyl picrylhydrazyl (DPPH) and nitric oxide (NO) radical scavenging activities, iron chelating capacity and reducing power.

Materials and Methods

Plant Materials and Preparation of Extracts

E. caucasicum inflorescence were collected in Sari, Iran in June 2012 and authenticated by Dr B. Eslami. A voucher specimen (Number 1242) has been deposited at the Sari Faculty of Pharmacy Herbarium. Inflorescences were dried under dark conditions at room temperature and then grounded. 50 g of dried powdered sample were macerated for 24h with 300 ml of methanol. Extraction was repeated three times and the resulting extracts were concentrated over a rotary evaporator until a solid sample was obtained (percolation extract). The sample was extracted exhaustively in a Soxh-

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let extractor with methanol for 24 h. The crude solid extracts were freeze-dried for complete solvents removal and used as Soxhlet extract. Sample was extracted with methanol in an ultrasonic bath at a frequency of 100 kHz for one hour (3 × 20 min) to yield ultrasonic extracts[6].

Determination of Total Phenol and Flavonoid Contents

Total phenolic contents were determined by Folin Ciocalteau reagent[6]. The standard curve was drawn by 0-250 μg ml^{-1} solutions of gallic acid. The total phenolic contents are expressed in terms of gallic acid equivalents (GAE). The total flavonoid content was measured by colorimetric method[6]. The total flavonoids contents were determined as quercetin equivalent from a standard curve which was prepared by quercetin at concentrations 12.5 to 100 μg ml^{-1}. The total flavonoid contents are expressed in terms of quercetin equivalent (QE).

DPPH Radical-Scavenging Activity

Two ml of extracts solutions were added to 2 ml of DPPH solution (100 μM). After 15 minutes, the absorbance was recorded at 517 nm. BHA was used as standard compounds[6].

Iron Chelating Capacity

Different concentrations of extracts were added to a solution of 0.05 ml of ferrous chloride (2 mM). 0.2 ml of ferrozine solution was added. After 10 minutes, the absorbance of the solution was recorded at 562 nm. EDTA was used as a standard[7].

Nitric Oxide-Scavenging Activity

A solution of sodium nitroprusside (10 mM) in PBS was mixed with different concentrations of extracts. The mixture was left for 2.5 hours. After the incubation time, 0.5 ml of Griess reagent was added and absorbance of the coloured mixture was recorded at 546 nm. Quercetin was used as control[8].

Reducing Power

The reducing powers of extracts were measured according to our recently published paper[7]. 2.5 ml of each extract was mixed with 2.5 ml of phosphate buffer and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid was added. 0.5 ml of the solution was mixed with distilled water (0.5 ml) and ferric chloride (100 μl). The absorbance was read at 700 nm. Vitamin C was used as control.

Statistical Analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($p < 0.05$) and the means separated by Duncan's multiple range tests. The IC_{50} values were calculated from linear regression analysis.

Results

Total phenols were measured using Folin Ciocalteu method, and reported as GAE by reference to standard curve. Total phenolic contents ranged from 58.8 to 105.5 mg GAE g of extract. Total phenolic contents were in order of: Soxhlet extract > percolation method > ultrasonic extract, respectively. The total flavonoid content ranged from 11.9 to 18.7 QE g of extract. Soxhlet extract had higher flavonoids contents than other extracts, too. The capacities of extracts to scavenge DPPH are shown in Table I. Soxhlet extract showed the highest activity (IC_{50} = 83.1 ± 2.1 μg ml^{-1}) followed by percolation extract with IC_{50} = 177.3 ± 5.9 μg ml^{-1}. IC_{50} of BHA was 53.8 ± 3.7. The absorbance of Fe^{2+}-fer-

Table I. Phenol and flavonoids contents and antioxidant activities of *E. caucasicum* inflorescence,

Fe^{2+} chelating ability, IC_{50} (μg/ml)[c]	NO scavenging activity, IC_{50} (μg/ml)[b]	DPPH radical scavenging, IC_{50} (μg/ml)[a]	Total flavonoid contents (QE mg/g)	Total phenolic contents (GAE mg/g)	Extraction method
286 ± 9.2	2416 ± 69.6	188.7 ± 7.2	18.2 ± 0.7	58.8 ± 1.5	Ultrasonic
421 ± 13.6	390 ± 11.4	177.3 ± 5.9	11.9 ± 0.5	60.1 ± 2.3	Percolation
272 ± 6.3	583 ± 9.8	83.1 ± 2.1	18.7 ± 0.9	105.5 ± 2.8	Soxhlet

[a]IC_{50} of BHA was 53.8 ± 3.7 μg/ml.b IC_{50} for quercetin was 155.0 ± 6.4 μg/ml.c EDTA used as control (IC_{50} = 17.4 ± 0.4 μg/ml).

rozine complex was decreased dose-dependently. Soxhlet extract showed the best activity (IC_{50} = 272 ± 6.3 μg ml^{-1}) followed by ultrasonic extract with IC_{50} = 286 ± 9.2 μg ml^{-1}. Soxhlet extract had the highest amount of phenol and flavonoid contents. EDTA showed strong activity (IC_{50} = 17.4 ± 0.4 μg ml^{-1}). Extracts exhibited weak nitric oxide-scavenging activity. Percolation extract showed higher NO radical scavenging (IC_{50} = 390 ± 11.4 mg ml^{-1}) than other extracts. Quercetin which was used as standard was more potent (IC_{50} = 155.0 ± 6.4 μg ml^{-1}) ($p < 0.01$). Figure 1 shows the dose-response curves for the reducing powers of extracts. All extracts showed the same reducing power ($p > 0.05$). Their activities were not comparable to that of vitamin C ($p < 0.001$).

Discussion

Medicinal plants have been used traditionally for the prophylaxis or treatment of different problems. This protection is because of their antioxidant constituents such as flavonoids or phenolic acids[9]. Polyphenols are important constituents in fruits. They are thought to be instrumental in combating oxidative stress and can prevent many oxidative stress disorders such as cardiovascular, atherosclerosis, neurodegenerative diseases, cancer and aging. The Folin Ciocalteu is a reagent used commonly for determination of total phenolic contents of different extracts. Soxhlet extract had higher phenolic content than other extracts. This plant was a good source of phenols and contains very high amount of total phenolics.

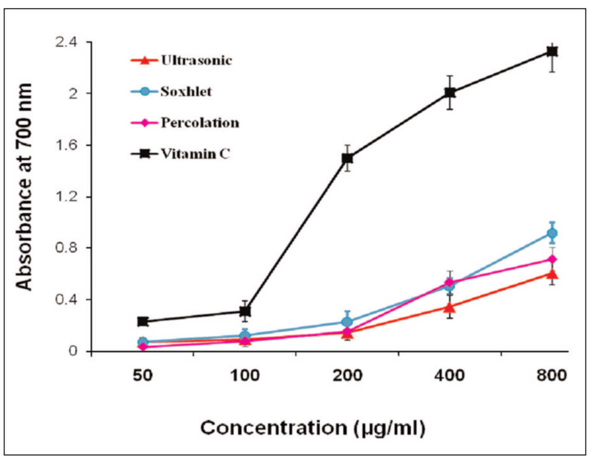

Figure 1. Reducing power of *E. caucasicum* inflorescence. Vitamin C used as positive control.

Flavonoids are widely found in the plant kingdom. They are very important in pharmaceutical industries because they have antioxidant activities. Flavonoids may slow the pathogenesis of over 60 diseases by their ROS scavenging effects. Soxhlet extract had higher flavonoid contents than other extracts, too.

Extracts showed a concentration-dependent antiradical activity by inhibiting DPPH radical. Soxhlet extract showed the highest activity followed by percolation extract. Soxhlet extract with higher phenol and flavonoids contents showed the best activity and ultrasonic extract with lowest phenol and flavonoid contents were the least active.

Ferrozine can quantitatively form complexes with Fe^{2+}. The absorbance of Fe^{2+}-ferrozine complex was decreased dose-dependently. Soxhlet extract showed the best activity followed by ultrasonic extract. Soxhlet extract had the highest amount of phenol and flavonoid contents. It is known that flavonoids with a certain structure and particularly hydroxyl position in the molecule can act as proton donating and show radical scavenging activity.

NO has been associated with a variety of physiologic processes. NO plays very important role in several vital physiologic functions such as immune and respiratory systems[8]. In the nervous system, it works as a neural modulator, excitability of neurons, learning and memory. NO also has an important role in some pathogenic disorders such as inflammatation, multiple sclerosis, and stroke[10]. There are some evidences that strongly suggest involvement of NO signaling pathway in CNS disorders[11]. Extracts exhibited weak nitric oxide-scavenging activity. Percolation extract showed higher NO radical scavenging than other extracts.

Reducing power has been used as an antioxidant capability indicator of medicinal herbs. Increasing the absorbance at this wave length indicates an increase in the reducing power. All extracts showed the same reducing power ($p > 0.05$). Their activities were not comparable to that of vitamin C ($p < 0.001$).

Conclusions

The extraction efficiencies of the three methods for the extraction of antioxidants from *E. caucasicum* inflorescence were evaluated. These extraction methods especially Soxhlet method could effectively extract antioxidants from this plant. Further investigation of individual compounds is needed.

Acknowledgements

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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