

## Screening of antioxidant activity and phenolic content of 24 medicinal plant extracts

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### ABSTRACT

**Background:** Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical-induced oxidative stress. A variety of free radical scavenging antioxidants are found in dietary sources like fruits, vegetables and tea. The purpose of this study was to evaluate the antioxidant activity of methanolic extracts of 24 selected plant materials (seeds or fruits), which are used by Iranian people as folk remedies and/or food supplements.

**Methods:** The antioxidant activity was evaluated against linoleic acid peroxidation using 1,3-diethyl-2-thiobarbituric acid as reagent. At the same time the phenolic content of the extracts was determined using Folin-Ciocalteu reagent to evaluate their contribution to total antioxidant activity.

**Results:** The antioxidant activity expressed as IC<sub>50</sub> ranged from 1.25 µg/ml in cucumber to 167.29 µg/ml in cardamom. Phenolic contents, expressed as gallic acid equivalents, varied from 21.76 mg/100g of the dried weight in linseed to 919.12 mg/100 g of the dried weight in Bishop's weed. No significant correlation was observed between antioxidant activity and phenolic content in the studied plant materials.

**Conclusion:** The results of this study showed that there is no significant correlation between antioxidant activity and phenolic content of the studied plant materials and phenolic content could not be a good indicator of antioxidant capacity.

**Keywords:** Antioxidant, Free radical; Linoleic acid; Medicinal plant; Phenolic content

### INTRODUCTION

It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, diabetes mellitus, cancer, Parkinson's disease and immune dysfunction and is involved in aging (1-3). Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in prevention of the free radical formation by scavenging or promotion of their decomposition and suppression of such disorders (1, 4). There is growing interest toward natural antioxidants from herbal sources (5-7). Epidemiological and *in vitro* studies on medicinal plants and vegetables strongly have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (8-10). Phenolic compounds with antioxidant activity, which are widely distributed in many fruits, vegetables, and tea are believed to account mainly for the antioxidant capacity of many

plants (11-13).

On continuation of our work on the antioxidant activity of popular medicinal plants of Iran (14-15), the antioxidant activity of some selected dried fruits and seeds, obtained from local herbal markets which traditionally used by Iranian people as medicine and/or food supplement, was measured. At the same time, phenolic content of the same plant materials was determined to evaluate their probable contribution to the total antioxidant capacity.

### MATERIALS AND METHODS

#### *Plant material*

Twenty four medicinal plant materials (13 seeds and 11 fruits) were purchased from the local herbal market in Tehran. Voucher specimens from all plant materials were deposited at the Herbal Museum, Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences for identification. The plant materials were cleaned, washed, dried and carefully

powdered. All samples were kept in tightened light-protected containers.

#### *Chemicals*

Linoleic acid, gallic acid and Folin-Ciocalteu reagent were obtained from Merck (Darmstadt, Germany). 1,3-Diethyl-2-thiobarbituric acid (DETBA) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Alpha-tocopherol, sodium dodecyl sulfate (SDS) and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents were analytical grades and obtained from Merck (Darmstadt, Germany).

#### *Extraction*

A quantity (50 g) of each powdered plant material was soaked in 150 mL of methanol at room temperature overnight. The solvents were decanted and residues macerated two more days with the same solvent. The pooled solvents were combined and filtered. The filtrates were concentrated under reduced pressure and yields of extract were calculated.

#### *Determination of antioxidant activity*

The antioxidant activity of plant extracts against peroxidation of linoleic acid was determined by the reported method (16). Alpha-tocopherol was used as reference compound. For a typical assay an aliquot of 20  $\mu$ l of three dilutions of each extract in ethanol (0.002, 0.02 and 0.2 mg/ml) and 20  $\mu$ l of 2 mg/ml linoleic acid in ethanol were used. A spectrofluorimeter (Model RF-5000, Shimadzu, Kyoto, Japan) at an excitation wavelength of 515 nm and an emission wavelength of 555 nm was used for measurements and the antioxidant activity was calculated as the percent of peroxidation inhibition.

All extracts and reference substance were assayed in triplicates and averages of results were calculated. A percent inhibition versus log concentration curve was plotted and the concentration of sample required for 50 % inhibition was determined and expressed as IC<sub>50</sub> value.

#### *Determination of phenolic content*

The phenolic contents were determined according to the described method (17), using the Folin-Ciocalteu reagent and a Shimadzu spectrophotometer (Model UV-160A, Kyoto, Japan) at 725 nm. Aliquots of 100  $\mu$ l of each diluted extract (20 mg/ml in ethanol) were used

for measurements. Phenolic contents of the samples were calculated on the basis of the standard curve for gallic acid. The results were expressed as milligrams of gallic acid equivalents per 100 g of the dry weight of the plant materials.

## RESULTS

#### *Antioxidant activity*

The characteristics of the used medicinal plants and the inhibitory effects of their methanolic extracts on linoleic acid peroxidation, expressed as IC<sub>50</sub>, are presented in Table 1. Considering the large variation of IC<sub>50</sub> values, ranging from 1.25  $\mu$ g/ml in cucumber to 167.29  $\mu$ g/ml in cardamom, the potential of antioxidant activity of plant materials of this study was divided into 3 groups: high (IC<sub>50</sub><20  $\mu$ g/ml), moderate (20  $\mu$ g/ml <IC<sub>50</sub><75  $\mu$ g/ml) and low (IC<sub>50</sub>>75  $\mu$ g/ml). Eight plant materials out of 24 samples showed IC<sub>50</sub> values comparable to IC<sub>50</sub> of  $\alpha$ -tocopherol (IC<sub>50</sub>=15.00  $\mu$ g/ml). These samples were cucumber, cumin, fennel, lettuce, nutmeg, great plantain, common purslane and Bishop's weed. Six samples including celery, coriander, flixweed, basil, opium poppy were in moderate range and the remained plant materials showed low antioxidant activity.

#### *Phenolic content*

The phenolic content of the studied fruits and seeds are also given in Table 1. Phenolic content of plant materials, calculated as gallic acid equivalent, varied from 21.76 mg/100 g of the dry weight in linseed to 919.12 mg/100g of the dry weight in Bishop's weed. Considering the broad range of variation of the results, the phenolic contents were also categorized into three groups: high (> 300 mg), moderate (100-300 mg) and low (< 100 mg).

#### *Relationship between phenolic content and antioxidant activity*

Attempts to correlate the level of phenolic content of these medicinal plants with their antioxidant activity were not successful. No significant correlation ( $R^2=0.04$ ) was observed between phenolic content and IC<sub>50</sub> values when all plant materials were included in the calculation.

## DISCUSSION

In this study 24 medicinal plant fruits or seeds, which are used traditionally in Iran for various disorders were studied for their antioxidant activity and phenolic content. Seven plant

**Table 1.** Botanical and English common name, traditional or folk uses, extract yield (% of the dried weight), antioxidant activity (IC<sub>50</sub>) against peroxidation of linoleic acid (2 mg/ml) and phenolic content of 24 plant species

No	Scientific name and family	English common name	Part used	Tradinational and folk indications	Extract Yield (%)	IC <sub>50</sub> (µg/ml) (mean±SD)	Phenol content (mg/100g dry)
1	<i>Allysum homolocarpum</i> (F&M.) Boiss (Cruciferae)	Alyssum	Seed	Coughs <sup>a</sup> ; Demulcent <sup>b</sup>	4.12	94.25 ± 4.01	165.68 ± 3.22
2	<i>Anethum graveolens</i> L (Umbelliferae)	Dill	Fruit	Carminative <sup>a</sup> ; Diuretic <sup>b</sup>	7.62	146.75 ± 1.04	263.59 ± 3.37
3	<i>Apium graveolens</i> L. (Umbelliferae)	Celery	Fruit	Carminative, Chest inflammation <sup>a</sup> ; diuretic <sup>b</sup>	18.04	34.75 ± 0.50	436.05 ± 8.62
4	<i>Bunium persicum</i> (Bioss) B. Fedtsch (Umbelliferae)	Wild caraway	Fruit	Carminative <sup>b</sup>	6.24	82.25 ± 1.25	214.03 ± 4.10
5	<i>Coriandrum sativum</i> L. (Umbelliferae)	Coriander	Fruit	Toothache, Headache <sup>a</sup>	8.32	41.25 ± 2.08	227.69 ± 5.97
6	<i>Cordia myxa</i> L. (Borraginaceae)	Sebestan plums	Fruit	Coughs, Chest complaints <sup>a</sup>	24.08	132.53 ± 5.75	373.91 ± 13.93
7	<i>Cucumis sativus</i> L. (Cucurbitaceae)	Cucumber	Seed	Demulcent, Typhoid <sup>a</sup>	4.54	1.25 ± 0.03	27.79 ± 0.89
8	<i>Cuminum cyminum</i> L. (Umbelliferae)	Cumin	Fruit	Carminative <sup>a</sup>	11.76	5.76 ± 0.24	241.41 ± 2.39
9	<i>Descureania Sophia</i> (L.) Webb & Berth. (Cruciferae)	Flix – weed	Seed	Aphrodisiac, Purifying the blood <sup>a</sup> ; Heat- stroke <sup>b</sup>	8.42	23.27 ± 0.76	265.22 ± 2.67
10	<i>Elettaria cardamomum</i> (L.)Maton (Zingiberaceae)	Cardamom	Fruit	Flavoring <sup>a</sup> ; Carminative <sup>b</sup> ; Diarrhea <sup>c</sup>	9.28	167.29 ± 1.72	84.19 ± 4.64
11	<i>Foeniculum vulgare</i> Mill (Umbelliferae)	Fennel	Fruit	Dysentery, Colds <sup>a</sup> ; Carminative <sup>b</sup>	9.06	8.01 ± 0.51	165.07 ± 11.43
12	<i>Lactuca sativa</i> L. (Compositae)	Lettuce	Seed	Fevers <sup>a</sup>	6.02	14.28± 1.32	168.56 ± 1.21
13	<i>Lepidium perfoliatum</i> L.(Cruciferae)	Alyssum	Seed	Coughs <sup>a</sup> ; Demulcent <sup>b</sup>	8.25	78.54 ± 5.21	276.19 ± 24.64
14	<i>Linum usitatissimum</i> L. (Linaceae)	Linseed	Seed	Boils <sup>a</sup> ; Demulcent <sup>b</sup>	3.26	53.52 ± 1.56	21.76 ± 0.12
15	<i>Myristica fragrans</i> Houtz. (Myristicaceae)	Nutmeg	Fruit	Tonic <sup>b</sup>	17.08	7.29 ± 0.03	543.70 ± 17.67
16	<i>Nigella sativa</i> L. (Ranunculaceae)	Black cumin	Seed	Carminative <sup>b</sup>	12.34	146.84 ± 1.73	122.67 ± 3.03
17	<i>Ocimum basilicum</i> L. (Labiatae)	Basil	Seed	Influenza <sup>a</sup> ; Demulcent <sup>b</sup>	3.82	4.78 ± 1.77	106.52 ± 3.27
18	<i>Papaver somniferum</i> L. (Papaveraceae)	Opium poppy	Seed	Epistaxis <sup>a,c</sup> Boils <sup>a</sup> ; Analgesic <sup>b</sup>	8.42	49.75 ± 1.04	44.42 ± 2.99
19	<i>Pimpinella anisum</i> L. (Umbelliferae)	Aniseed	Fruit	Coughs <sup>a</sup> ; Carminative <sup>b</sup>	11.06	101.26 ± 0.52	353.92 ± 1.64
20	<i>Plantago major</i> L. (Plantaginaceae)	Great plantain	Seed	Affection of bowels, Dysentery, Demulcent <sup>b</sup>	6.28	16.77 ± 1.56	672.79 ± 11.62
21	<i>Plantago ovata</i> Forsk. (Plantaginacea)	Blond plantain	Seed	Gonorrhea, Dysentery <sup>a</sup>	5.36	126.56 ± 3.23	249.40 ± 2.74
22	<i>Portulaca oleracea</i> L. (Portulacaceae)	Common purslane	Seed	Coughs, Inflammation <sup>b</sup>	4.22	11.74 ± 1.61	33.66 ± 0.81
23	<i>Trachyspermum copticum</i> (L.) (Umbelliferae)	Bishop's weed	Fruit	Carminative <sup>a,b</sup>	8.48	14.36 ± 1.25	919.12 ± 34.67
24	<i>Trigonella foenum-graecum</i> L. (Leguminosae)	Fenogreek	Seed	Stomach Pains, Menorrhagia <sup>a</sup>	11.12	91.66 ± 3.05	194.63 ± 7.32

References <sup>a</sup> (18); <sup>b</sup> (19) and <sup>c</sup> (20)

materials (cumin, fennel, lettuce, nutmeg, great plantain, common purslane and Bishop's weed) showed high antioxidant activities which is in close agreement with other studies for some species (21-23). While low antioxidant activity is reported for cucumber (8), the cucumber seed showed an exceptional antioxidant activity ( $IC_{50}=1.25 \mu\text{g/ml}$ ), which was about ten times higher than  $\alpha$ -tocopherol ( $IC_{50}=15.00 \mu\text{g/ml}$ ). The seed of this plant has been used as a favorite nutritive, emollient and as infusion for typhoid in folk remedies in Iran due to its cold temperament (18). Positive relationship was found between high antioxidant activity and phenolic content just for a few species like cumin, nutmeg and Bishop's weed. Findings of this study showed that no reasonable relationship could be found between antioxidant activity and phenolic content. The exceptional high antioxidant activity of some specimens like cucumber with low phenolic content may be attributed to some individual phenolic units with special high antioxidant

activity or some other constituents. Nonphenolic compounds of the plants such as trace elements may also decrease the antioxidant activity of the phenolic compounds (12). Thus the measurement of phenolic content could not be a good indicator of the antioxidant capacity.

In conclusion, the findings of this study support this view that some medicinal plants are promising sources of potential antioxidants and may be efficient as preventive agents in the pathogenesis of some diseases. However, the strength of the existing data is not enough to suggest a reasonable mode of action for antioxidant effects. The data of this study may just enrich the existing comprehensive data of antioxidant activity of plant materials.

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