Iranian Journal of Pharmaceutical Research (2004) 3: 55-59
Received: December 2003
Accepted: February 2004

Original Article

Antioxidative Activity of Sixty Plants from Iran

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Abstract

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical-induced oxidative stress. A variety of free radical scavenging antioxidants exist within the body which many of them are derived from dietary sources like fruits, vegetables and teas.

This article describes a test method for screening the antioxidant activity of 60 Iranian plants of Iran by linoleic acid peroxidation test using 1, 3-diethyl-2-thiobarbituric acid as the reagent. Some plants including *Achillea wilhelmsii*, *Berberis crataegina*, *Buxus hyrcana*, *Chrysanthemum cinerariaefolium*, *Colutea persica*, *Hyoscyamus niger*, *Mentha pulegium*, *Nerium oleander*, *Pteropyrum aucheri*, *Rhus coriaria*, *Rosa canina*, *Scutellaria pinnatifida*, *Thymus pubescens*, *Verbascum alceoides* and *Ziziphora clinopodioides* subsp. *rigida* showed antioxidant activity ($0.41<IC_{50}<1.64$ μg) comparable to α-tocopherol ($IC_{50}=0.60$ μg), which was used as the positive control.

Keywords: Plant; Antioxidant; Linoleic acid; 1, 3-Diethyl-2-thiobarbituric acid.

Introduction

In recent years, it has been established that free radicals and oxidative stress are involved in the pathophysiology of a variety of disorders including atherosclerosis, chronic renal failure, diabetes mellitus, cancer, immune dysfunction and aging (1-6). In relation to these findings an extensive range of antioxidants both exogenous and endogenous, whether synthetic or natural have been presented for the treatment or prophylaxis of disorders attributed to free radical oxidative damages (3, 4, 7). Restriction on the use of synthetic antioxidants due to their probable side-effects has increased the contribution of natural antioxidants (8).

The antioxidant activity of several plant constituents, beyond the vitamins, in the form of crude extract or isolated compound has been put widely into consideration (8-10). Antioxidant activity of many phenolic compounds, including flavonoids, has attracted considerable attention and reported to be more powerful antioxidants than vitamins C, E and β-carotene which are largely in routine use (11). Consumption of the flavonoids and their potential significance as antagonists of oxidative stress has been the interesting subject

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of many investigations (8, 9, 11, 12). Vegetables and fruits are also reported to decrease the risk of degenerative diseases and could have a protective effect against oxidative stress (11). Antioxidants are also important for food protection against deterioration reactions caused by atmospheric oxygen (8). Considerable effort has been directed in search for safe antioxidants from natural sources. Naturally occurring antioxidants could be found in fruits, vegetables, nuts, seeds, leaves, flowers, roots and barks. Many investigators have found different types of antioxidants in various kinds of plants (8-12).

One of the best approaches for discovering new antioxidants is the screening of plant extracts. This study was carried out as part of a project to investigate the antioxidant activity of 60 selected plants growing in Iran, against linoleic acid peroxidation.

Materials and Methods

Plant material

The plants were collected from different regions of Iran. Information regarding the collection of plants is mentioned in table 1. Voucher specimens of all plants were deposited at the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences. The aerial parts were separated, air dried in the shade, powdered and kept in tightened light-protected containers.

Chemicals

Linoleic acid, 1, 3-diethyl-2-thiobarbituric acid (DETBA) and quercetin dihydrate were obtained from Merck (Darmstadt, Germany), Aldrich Chemical Co. (Milwaukee, WI, USA) and Fluka Chemical Co. (Buchs, Switzerland) respectively. α-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 of analytical grade from Merck.

Extraction

A quantity (50 g) of each powdered plant was extracted in a Soxhlet apparatus with 80% methanol. 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 and examined for antioxidant activity.

Measurment of antioxidant activity

The potential of plant extracts to inhibit peroxidation of linoleic acid was assessed based on a procedure described by Furuta et al. (13). α-Tocopherol was used as the reference compound. For a typical assay three dilutions of each extract (0.02, 0.2 and 2 mg/mL) were prepared. An aliquot of 20 µL of each dilution (equal to 0.4, 4 and 40 µg of extract) was mixed with 20 µL of linoleic acid (2 mg/mL in ethanol) and incubated at 80°C for 60 min. Incubated samples were cooled in an ice bath, followed by the addition of 200 µL of 20 mM BHT, 200 µL of 8% SDS and 400 µL of distilled water. After mixing, 3.2 mL of 12.5 mM DETBA in sodium phosphate buffer (0.125 M, pH 3.0) (warmed to 50°C) was added, mixed and heated at 95°C for 15 min. The mixture was cooled in an ice bath, 4 mL of ethyl acetate was added to each tube, vortexed to extract the pink adduct from the aqueous phase and centrifuged at 1500 rpm for 10 min (F_1). A control containing linoleic acid and other additives without antioxidants, representing 100% lipid peroxidation, was also prepared (F_2). The blank F_1 and F_2 solutions were prepared as described above but without linoleic acid. The fluorescence intensity of F_1 and F_2 samples was measured against their blanks (F_3 and F_4 respectively) at an excitation wavelength of 515 nm and an emission wavelength of 555 nm in a spectrofluorimeter (Model RF-5000, Schimadzu, Kyoto, Japan). The antioxidant activity was calculated as the percentage of peroxidation inhibition using the following equation (14):

$$\% \text{ of peroxidation inhibition} = [1-(F_1-F_3)/(F_2-F_4)] \times 100 \quad \text{(equation 1)}$$

All extracts and the reference substance were assayed at least at three concentrations in triplicates and the results were averaged. A percentage inhibition *vs* log concentration curve was drawn and the concentration of sample which is required for 50 % inhibition was

determined by linear interpolation and expressed as the IC_{50} value.

Results and Discussion

The botanical characteristics of studied plants and inhibitory effect of their methanolic extracts on linoleic acid peroxidation are provided in table 1 in alphabetical order. As displayed in table 1, most plant extracts (44 out of 60) showed more than 80% peroxidation inhibition, using 40 µg of plant extract in the reaction mixture. Plant extracts including *Achillea wilhelmsii*, *Buxux hyrcana*, *Chrysanthemum cinerariaefolium*, *Colutea persica*, *Hyoscyamus niger, Mentha pulegium*, *Myrtus communis*, *Nerium oleander*, *Paliurus spina-christi*, *Peganum harmala*, *Pterocarya fraxinifolia*, *Rhus coriaria*, *Rosa canina*, *Smilax excelsa*, *Thymus migricus*, *Thymus pubescens*,

Table 1. The botanical names and antioxidant activities of aerial parts of 60 plant extracts from Iran

No.	Location	Date*	Alt*	Voucher No.	Scientific name	Family name	Peroxidation inhibition(%) 0.4 µg	4 µg	40 µg	IC_{50} (µg)
1	N. of Karadj	99/6/6	1300	6570-TEH	*Achillea wilhelmsii* C. Koch	*Compositae*	27.37±2.66	78.93±0.65	75.27±4.05	1.41± 0.27
2	E. of Karadj	99/6/9	1200	6586-TEH	*Achillea tenuifolia* Lam.	*Compositae*	28.09±4.19	60.57±3.20	88.06±3.14	1.88± 0.37
3	E. of Karadj	99/6/9	1120	6585-TEH	*Acroptilon repens* (L.) DC. subsp. repens	*Compositae*	26.72±5.06	46.85±0.39	85.88±1.82	4.68± 0.13
4	Vaysar, S. of Chalus	99/6/4	600	6567-TEH	*Asperula stylosa* Trin	Rubiaceae	15.56±4.20	48.38±2.15	82.65±3.29	4.51± 0.57
5	E. of Tehran	99/7/21	1100	6587-TEH	*Astrodaucus orientalis* (L.) Drude	Umbelliferae	22.28±2.65	58.09±1.63	83.11±2.82	2.86± 0.27
6	Margun, W. of Shiraz	99/7/22	2000	6619-TEH	*Ballota aucheri* Boiss	Labiatae	2.65±1.63	47.92±1.86	90.54±4.57	5.09± 0.88
7	Ruin, Khorassan	99/6/12	1500	6590-TEH	*Berberis crataegina* DC.	Berberidaceae	49.95±4.65	52.02±3.07	90.29±2.18	0.81± 0.30
8	Talesh N. of Iran	99/6/8	930	6582-TEH	*Buxus hyrcana* Pojark	Buxaceae	35.23±6.83	71.92±8.31	86.94±2.79	1.15± 0.45
9	W. of Karadj	99/6/4	1100	6561-TEH	*Capparis spinosa* L.	Capparidaceae	20.61±0.69	49.29±6.78	64.58±6.83	5.84± 2.85
10	E. of Tehran	99/6/21	1100	6596-TEH	*Carthamus oxyacantha* M.B.	Compositae	27.05±0.15	28.35±2.18	71.27±2.50	9.11± 0.92
11	Ruin, Khorassan	99/6/12	1500	6616-TEH	*Centaurea bruguieriana* (DC.) Hand.-Mzt. subsp. *belangerana* (DC.) Bornm.	*Compositae*	25.63±7.44	62.98±0.37	91.00±5.65	1.74±0.24
12	E. of Tehran	99/6/21	1100	6598-TEH	*Chenopodium botrys* L.	*Chenopodiaceae*	31.72±1.05	36.26±4.07	51.59±2.57	45.76±14.4
13	Km.40 Lowshan – Rasht	99/6/8	1350	6583-TEH	*Chrysanthemum cinerariaefolium* (Trev) Vis.	*Compositae*	37.52±2.02	86.96±1.45	85.20±3.56	0.57±0.11
14	E. of Marzanabad	99/6/4	850	6566-TEH	*Colutea persica* Boiss.	Leguminosae	46.01±4.93	76.32±4.35	81.52±3.67	0.41±0.11
15	E. of Tehran	99/6/21	1200	6597-TEH	*Erodium oxyrrhynchum* M.B. subsp. *Oxyrrhynchum*	Geraniaceae	22.85±0.42	41.51±0.89	71.00±2.68	6.57±1.04
16	Near Shush	99/7/21	850	6618-TEH	*Eucalyptus camaldulensis* Dehn.	Myrtaceae	19.38±0.31	62.99±0.55	92.88±1.38	2.16±0.10
17	Hezarcham, Chalus-Karadj	99/6/22	2500	6613-TEH	*Ficus carica* L. subsp. *Carica*	Moraceae	27.12±6.14	52.53±0.99	97.05±1.62	3.18±0.33
18	Loshan, Ghazvin-Rasht	99/6/8	1030	6589-TEH	*Glaucium contortuplicatum* Boiss.	Papaveraceae	32.16±6.43	51.39±7.83	55.18±2.41	8.68±2.63
19	Aderan N. of Karadj	99/6/6	1300	6579-TEH	*Glaucium elegans* Fisch.& C.A.Mey.	Papaveraceae	7.79±1.25	38.46±0.27	61.76±1.23	14.20±0.61
20	Kandowan N. Karadj	99/6/6	2600	6578-TEH	*Glaucium fimbrilligerum* Boiss.	Papaveraceae	36.26±4.28	48.63±2.61	89.16±8.01	4.19±0.78
21	Ruin , Khorassan	99/6/12	1500	6593-TEH	*Glycyrrhiza glabra* L. var *glabra*	*Leguminosae*	0.86±1.29	62.19±1.59	96.14±0.71	2.86±0.70
22	Kandowan, N. of Karadj	99/6/22	2200	6612-TEH	*Hyoscyamus niger* L.	*Solanaceae*	22.96±7.64	71.86±1.03	91.19±5.08	1.64±0.32
23	Vaysar, S. of Chalus	99/6/4	600	6569-TEH	*Hypericum androsaemum* L.	*Hypericaceae*	22.84±2.43	55.37±4.55	93.37±2.34	2.60±1.13
24	Ruin, Khorassan	99/6/12	1500	6594-TEH	*Linaria pyramidata* (Lam.) Sprengl	Scrophullariaceae	18.78±1.68	39.60±2.42	79.31±3.08	5.57±0.93
25	Marzanabad, Chalus	99/6/4	650	6563-TEH	*Marrubium vulgare* L.	Labiatae	30.35±1.03	41.58±6.16	89.51±1.12	5.61±1.17
26	Rasht	99/6/9	350	6580-TEH	*Mentha pulegium* L.	Labiatae	45.64±6.95	81.86±0.93	81.44±2.51	0.57±0.29
27	Kandowan	99/6/22	2700	6610-TEH	*Minuartia lineata* Bornm.	Caryophyllaceae	38.99±3.38	50.48±5.91	96.16±1.50	3.34±1.55
28	Gachsaran- Shiraz	99/7/21	1200	6623-TEH	*Myrtus communis* L.	Myrtaceae	11.56±4.26	78.06±1.38	90.57±2.45	2.40±0.49
29	Polur, Haraz – Amol	99/6/21	1800	6604-TEH	*Nepeta glomerulosa* Boiss. subsp. *glomerulosa*	Labiatae	15.77±1.97	45.11±4.66	94.78±0.90	4.40±1.16
30	Tehran	99/6/17	1100	6615-TEH	*Nerium oleander* L.	*Apocyanaceae*	19.05±0.94	80.95±6.43	92.56±1.37	1.51±0.29
31	Yasouj – Esphahan	99/7/22	1200	6625-TEH	*Ononis spinosa* L.	*Leguminosae*	15.83±3.17	68.00±0.55	91.06±1.50	2.37±0.36
32	Marzanabad, Chalus	99/6/4	650	6564-TEH	*Paliurus spina – christi* Miller	*Rhamnaceae*	19.07±8.39	82.49±3.79	85.38±2.91	1.83±0.97
33	Polur, Haraz - Amol	99/6/21	1800	6603-TEH	*Papaver bracteatum* Lindl.	Papaveraceae	32.65±3.01	51.05±0.26	92.21±1.67	3.51±0.10
34	Aderan N. of Karadj	99/6/8	1300	6572-TEH	*Peganum harmala* L.	Zygophylaceae	10.20±3.16	71.51±3.38	70.15±5.07	1.98±0.13
35	Kandowan	99/6/22	2700	6608-TEH	*Phlomis anisodonta* Boiss.	Labiatae	33.03±1.43	48.73±4.53	88.34±8.91	3.14±1.16
36	Nowshahr	99/6/22	10	6627-TEH	*Phytolacca americana* L.	Phytolaccaceae	31.51±6.00	65.84±3.60	68.77±0.16	2.08±0.56
37	Gachsaran- Shiraz	99/7/21	1200	6624-TEH	*Pistacia atlantica* Desf. subsp.*mutica* (Fisch. & C.A. Mey.) Rech. f.	Anacardiaceae	28.75±7.13	50.86±3.93	56.46±2.79	7.51±6.14
38	E. of Tehran	99/6/21	1200	6599-TEH	*Prosopis stephaniana* (M.B)Kunth. ex spreng	Anacardiaceae	38.01±1.60	36.07±1.43	57.37±2.04	18.61±2.83
39	Asalem	99/7/14	150	6626-TEH	*Pterocarya fraxinifolia* (Poir.) Spach	*Juglandaceae*	5.94±4.39	73.87±1.58	93.99±2.68	2.60±0.11
40	Ghazvin	99/6/19	1200	6588-TEH	*Pteropyrum aucheri* Jaub.&. Spach	*Polygonaceae*	41.34±1.51	63.46±2.63	89.82±1.16	0.94±0.13
41	N. of Shiraz	99/7/20	2900	6621-TEH	*Rhamnus cornifolia* Boiss. & Hohen. subsp.*cornifolia*	*Rhamnaceae*	22.38±2.45	61.93±3.05	88.40±2.27	2.27±0.42
42	N. of Karadj	99/6/22	300	6614-TEH	*Rhus coriaria* L.	Anacardiaceae	33.45±1.72	77.82±1.40	93.81±2.13	0.91±0.04
43	Ruin, Khorassan	99/6/12	1500	6591-TEH	*Roemeria refracta* DC.	Papaveraceae	14.13±0.50	54.89±2.62	93.96±1.09	2.79±0.14
44	S. of Chalus	99/6/6	2100	6576-TEH	*Rosa canina* L.	Rosaceae	49.52±8.91	77.63±7.05	91.79±3.56	0.41±0.18
45	Tehran – Amol	99/6/21	2500	6601-TEH	*Salvia hypoleuca* Benth.	Labiatae	9.95±4.88	42.05±0.17	91.90±1.55	5.27±0.10
46	Ruin, Khorassan	99/6/12	1500	6592-TEH	*Salvia macrosiphon* Boiss.	Labiatae	24.20±9.90	58.50±6.59	91.52±0.20	2.96±0.91
47	Tehran – Amol	99/6/21	2000	6574-TEH	*Salvia verticillata* L.	Labiatae	38.67±3.14	42.70±2.20	76.01±1.84	6.38±0.55
48	E. of Tehran	99/6/21	1100	6595-TEH	*Scariola orientalis* (Boiss.) Sojak	*Compositae*	18.86±3.04	57.08±1.00	71.24±7.77	2.71±0.20
49	N. Shiraz	99/7/20	3200	6620-TEH	*Scutellaria multicaulis* Boiss. subsp. *multicaulis*	*Labiatae*	18.44±0.29	49.16±7.41	92.48±1.13	2.83±0.24
50	Kandowan	99/6/22	2700	6611-TEH	*Scutellaria pinnatifida* Art.et Hamilt.	*Labiatae*	47.60±5.88	64.06±7.91	94.87±2.19	0.76±0.27
51	E. of Assalem	99/6/12	250	6584-TEH	*Senecio cineraria* DC.	*Compositae*	36.31±3.63	37.55±2.07	83.26±6.14	7.29±1.41
52	E. of Tehran	99/6/21	1100	6633-TEH	*Silene coronaria* (L.) Clairv	Caryophyllaceae	22.65±2.59	64.92±6.82	89.11±1.54	2.04±0.42
53	Assalem	99/7/14	150	6628-TEH	*Smilax excelsa* L.	Asparaginaceae	13.19±2.17	78.10±5.12	94.21±0.70	2.00±0.36
54	NE. of Tehran	99/6/21	2100	6600-TEH	*Sophora alopecuroides* L.	Leguminosae	12.32±2.32	45.20±0.48	94.09±2.81	4.70±0.10
55	Kandowan	99/6/22	2700	6607-TEH	*Stachys lavandulifolia* Vahl.	Labiatae	23.76±0.42	52.25±5.74	91.56±2.16	3.11±1.15
56	Lahijan	99/6/21	30	6602-TEH	*Tamarix aralensis* Bge	Tamaricaceae	4.59±5.29	17.62±2.66	54.30±4.07	34.70±7.86
57	Kandowan	99/6/22	2700	6609-TEH	*Thymus migricus* klokov.& Desj.- Shost.	Labiatae	37.94±6.05	81.17±2.46	97.00±1.56	0.77±0.06
58	NE. of Tehran	99/6/21	2100	6605-TEH	*Thymus pubescens* Boiss. & Kotschy ex Celak.	Labiatae	38.86±1.49	72.68±0.55	97.42±1.12	0.84±0.03
59	Vaysar, S. of Chalus	99/6/4	600	6568-TEH	*Verbascum alceoides* Boiss. &. Hausskn.	Scrophulariaceae	37.82±0.83	72.79±6.97	75.99±3.30	0.87±0.021
60	Taleghan	99/7/21	1400	6617-TEH	*Ziziphora clinopodioides* Lam. subsp. *rigida* (Boiss.) Rech. f.	Labiatae	27.21±4.16	70.99±4.73	91.73±0.93	1.45±0.31

* Date=Date of collection; Alt=Altitude (m)

Verbascum alceoides and *Ziziphora clinopodioides* subsp. *rigida* showed more than 70% inhibition, using 4 μg of each plant extract. A limited number of plant extracts including *Berberis crataegina*, *Colutea persica*, *Mentha pulegium*, *Pteropyrum aucheri* and *Rosa canina* showed more than 40% inhibition, using 0.4 μg of plant extract in the reaction mixture. In all cases the antioxidant activity increased with increasing the concentration. IC_{50} values of the studied plants showed considerable differences with each other in the range of 0.41-45.76 μg. IC_{50} values of some plant extracts including *Chrysanthemum cinerariaefolium* (0.57 μg), *Colutea persica* (0.41 μg), *Mentha pulegium* (0.57 μg) and *Rosa canina* (0.41 μg) was lower then α-tocopherol (IC_{50}= 0.60 μg). The IC_{50} values of *Berberis crataegina* (0.81 μg), *Buxus hyrcana* (1.15 μg), , *Hyoscyamus niger* (0.64 μg), *Pteropyrum aucheri* (0.94μg), *Rhus coriaria* (0.91μg), *Scutellaria pinnatifida* (0.76 μg), *Thymus pubescens* (0.84 μg) and *Verbascum alceoides* (0.87 μg) were within the range of 0.64-1.15 μg, which is approximately in the range of α-tocopherol (IC_{50}= 0.60 μg).

Presence of unsaturated fatty acids in the lipid membranes, especially linoleic acid, makes them very susceptible to oxidative reactions. Inhibition of linoleic acid oxidation could be a good indication for antioxidant activity and has been widely used. In this study methanolic extracts of 60 plant species of Iran were evaluated for their antioxidant activity within at the range of 0.4 to 40 μg of the plant extracts against 4 μg of linoleic acid peroxidation in the reaction mixture. Linoleic acid peroxidation was determined spectrofuorimetrically, using 1, 3-diethyl-2-thiobarbituric acid as the reagent.

Natural antioxidants are usually phenolic and polyphenolic (including flavonoids) compounds (8, 15). The presence of these compounds in several plants examined in this study has already been reported as mentioned below:

Achillea wilhelmsii (16, 17), *Buxus hyrcana* (18), *Eucalyptus camaldulensis* (19, 20), *Mentha pulegium* (21), *Myrtus communis* (22-24), *Nerium oleander* (25), *Paliurus spina-christi* (26, 27), *Peganum harmala* (28), *Rhus coriaria* (29), *Rosa canina* (30, 31), *Senecio cineraria* (32), *Sophora alopecuroides* (33) and *Ziziphora clinopodioides* (34).

There are numerous of reports stating that the risk of degenerative diseases is diminished in people consuming large quantities of vegetables and fruits (11, 35, 36). At the same time it should be taken in to account that the antioxidant defense system of the human body is composed of different antioxidant compounds (12). The quality and antioxidant capacity of vegetables have also been recognized as effective supplement (11). Thus, the plants investigated in this study could provide protection against oxidative stress. However it is not known that whether components of the extracts are responsible. Further studies are in progress to elucidate identity of responsible compounds.

Acknowledgement

The authors would like to thank the research councils of Tehran University of Medical Sciences, and the Iranian Ministry of Health and Education for the financial support of this research project.

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