

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*, *Pteropryum aucheri*, *Rhus coriaria*, *Rosa canina*, *Scutellaria pinnatifida*, *Thymus pubescens*, *Verbascum alceoides* and *Ziziphora clinopodioides* subsp. *rigida* showed antioxidant activity ($0.41 < IC_{50} < 1.64 \mu\text{g}$) comparable to α -tocopherol ($IC_{50} = 0.60 \mu\text{g}$), 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.

Measurement 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 μ L of each dilution (equal to 0.4, 4 and 40 μ g of extract) was mixed with 20 μ L 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 μ L of 20 mM BHT, 200 μ L of 8% SDS and 400 μ L 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, Shimadzu, 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

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*, *Pteropryum 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. IC₅₀ values of the studied plants showed considerable differences with each other in the range of 0.41-45.76 µg. IC₅₀ 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 than α-tocopherol (IC₅₀= 0.60 µg). The IC₅₀ values of *Berberis crataegina* (0.81 µg), *Buxus hyrcana* (1.15 µg), *Hyoscyamus niger* (0.64 µg), *Pteropryum 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 (IC₅₀= 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 spectrophotometrically, 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.

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