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Original Article

Screening of Thirteen Medicinal Plant Extracts for Antioxidant Activity

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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. In this study the antioxidant activity and radical scavenging activity of methanolic extracts of selected plant materials, traditionally used by Iranian population as folk remedies was evaluated against linoleic acid peroxidation and 2,2-diphenyl-1-picrylhydrazyl radical. The antioxidant activity expressed as IC_{50} ranged from1.28 ng/ml in *Biebresteinia multifida* to 63.48 ng/ml in *Polypodium vulgare.* Radical scavenging activities expressed as IC_{50} varied from 1.83 µg/ml in *Salix sp*. to 187.88 µg/ml in *Allium hirtifolium*.

Keywords: Antioxidant; Free radical; Linoleic acid; Medicinal plant; Radical scavenging; DPPH.

Introduction

It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others (1-6). The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants, both exogenous or endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders (3, 7-9). Currently, there is a growing interest toward natural antioxidants of herbal resources (10-12). Epidemiological and in vitro studies on medicinal plants and vegetables strongly supported this idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (13-16).

On continuation of our screening project for the search of antioxidant activity of popular medicinal plants in Iran, we studied 13 plant extracts. The antioxidant activity of these plant extracts against linoleic acid peroxidation and radical scavenging activity against 2, 2-diphenyl-1-picrylhdydrazyl (DPPH) were studied in this report. Trolox and quercetin were used as antioxidant reference compounds.

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Experimental

Plant material

Thirteen medicinal plant materials were purchased from the local herbal market of 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 and powdered. The botanical name, family name, English name, part used, and traditional use (17) are presented in Table 1.

Chemicals

Linoleic acid was obtained from Merck (Darmstadt, Germany). 1, 3-diethy-2-thiobarbituric acid (DETBA) and Trolox, a vitamin E analog (6-hydroxy, 2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Sodium dodecyl sulfate (SDS) and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and quercetin dehydrate were purchased from Fluka (Buchs SG. Switzerland). All other chemicals and solvents were of analytical grade from Merck.

Extraction

A quantity (100 g) of each powdered plant material was soaked in 300 ml of methanol after 1h stirring at room temperature overnight. The solvent was decanted and the residue was macerated two more days with the same solvent. The pooled solvents were combined and filtered. The filtrates were concentrated under reduced pressure and their extract yields were calculated. The extraction yields (% dry weight basis) are presented in Table 1.

Antioxidant activity on linoleic acid peroxidation

The antioxidant activity of plant extracts against peroxidation of linoleic acid was

Table 1. Characteristics of the used medicinal plants.

Extract yield (%)	Traditional indications	Part used	English name	Family name	Scientific name
13.0	Stomach pain, Disinfectant	Flower	Yarrow	Compositae	*Achillea tenuifolia* Lam.
6.1	Rheumatoid, pain, Expectorant	Corm	Wild garlic	Alliaceae	*Allium hirtifolium* Bioss.
13.7	Analgesic, Carminative, Tonic	Fruit	Milk vetch	Papilionaceae	*Astragalus hamosus* L.
16.0	Rheumatoid pain	Root	Bieberstein	Berberidaceae	*Biebresteinia multifida* DC.
15.8	Hemorrhoid, Rheumatoid pain, Laxative	Fruit	Bitter apple	Cucurbitaceae	*Citrulus colocynthis* (L.) Schrad.
9.1	Analgesic	Root stock	Colchicum corm	Colchicaceae	*Colchicum speciosum* Stev.
15.6	Inflammation, Tonic, Flavoring	Rhizom	Zedoary	Zingiberaceae	*Curcuma zedoaria* Rosc.
17.3	Carminative, Stomach pain	Leaf	Mint	Labiatae	*Mentha spicata* L.
24.7	Rheumatoid pain	Rhizom	Common polypody	Polypodiaceae	*Polypodium vulgare* L.
6.8	Fever, Rheumatoid pain	Stem bark	Willow bark	Salicaceae	*Salix sp*.
7.4	Gum hemorrage and complaints	Stem	Miswak	Salvadoraceae	*Salvadora persica* L.
17.2	Carminative, Rheumatoid pain	Aerial part	Mountain sage	Labiatae	*Salvia hydrangea* DC.
18.4	Stomach pain, Analgesic, Colds	Leaf and twig	Saatar	Labiatae	*Zataria multiflora* Boiss.

determined based on a method reported by Furuta et al (18). Trolox and quercetin were used as reference compounds. Three dilutions of each extract in methanol (0.002, 0.02 and 0.2 mg/ml) were prepared. For a typical assay an aliquot of 20 µl of each dilution was mixed with 20 µl of 2 mg/ml linoleic acid in ethanol and incubated at 80 °C for 60 min. Incubated samples were cooled in an ice bath, followed by 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. After mixing, the tubes were heated at 95 °C for 15 min, and cooled in an ice bath. Then 4 ml of ethyl acetate was added to each tube, vortexed to extract the pink adduct from the aqueous phase, and centrifuged at 700 g 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 fluorescence intensities of ethyl acetate layer of sample (F_1) and control solution (F_2) were measured at an excitation wavelength of 515 nm and an emission wavelength of 555 nm in a spectrofluorimeter (Model RF-5000, Shimadzu, Kyoto, Japan) against their blanks (F_3 and F_4 respectively) prepared as described above without linoleic acid. The antioxidant activity was calculated as the percent of peroxidation inhibition using the following equation (19):

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

All extracts and reference substances were assayed at least in triplicate, and the results were averaged. By using XLfit 4 software, a percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined by linear interpolation and expressed as IC_{50} value.

DPPH free radical scavenging activity

The DPPH free radical scavenging activity was assessed according to Okada & Okada method (19). An ethanolic solution of DPPH (0.05 mM) (300 µl) was added to 40 µl of extract solution with different concentrations (0.02-2 mg/ml). The DPPH solution was freshly prepared and kept in the dark at 4 °C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and the absorbance was measured using a spectrophotometer at 517 nm. Ethanol was used to zero the spectrophotometer. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation (20):

$$\text{Percent of DPPH inhibition} = [(A_B-A_A)/A_B]\times 100$$

Where A_A and A_B are the absorbance values of the test and of the blank sample, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value.

Results

Antioxidant activity

The inhibiting effect of methanolic extracts of studied plants on linoleic acid peroxidation, expressed as IC_{50}, are presented in Table 2. Large variation in IC_{50} was observed ranging from 1.28 ng/ml in *Biebresteinia multifida* to 63.48 ng/ml in *Polypodium vulgare*. Seven plant materials showed IC_{50} values lower (2-30 times) than quercetin and all plant materials showed IC_{50} values smaller than Trolox.

Radical scavenging activity

The change in absorbance produced by reduced DPPH was used to evaluate the ability of test compounds to act as free radical scavengers. The results of DPPH test showed that the extract of *Salix sp*. was the most active with an IC_{50} value of 1.03 µg/ml followed by *Mentha spicata*, *Salvia hydrangea*, *Zataria multiflora* and *Achillea tenuifolia* with IC_{50} values of 1.14, 1.55, 1.80 and 1.89 µg/ml, respectively. These plant extracts showed higher radical scavenging activity compared to Trolox (IC_{50}, 8.64 µg/ml) and quercetin (IC_{50}, 5.22 µg/ml).

Discussion

In this study 13 medicinal plants, traditionally used in Iran for various disorders were studied for their antioxidant activity against linoleic peroxidation and free radical scavenging activity on DPPH. *Biebresteinia multifida* was the most active antioxidant against linoleic peroxidation with an IC_{50} value of 1.28 ng/ml. The antioxidant activity of this plant material has not been reported before. The anti-inflammatory and analgesic activities of this plant have been studied (21) and there may be a relationship between these two effects.

Salix sp. was the most active radical scavenging plant material in our study with IC_{50} value of 1.03 µg/ml. The antioxidant activity of *Salix caprea* flowers has been reported before (22).

Literature survey indicated that antioxidant activity and radical scavenging activity of some other potent plant materials such as *Mentha spicata* (23-26) and *Curcuma zedoaria* (27) were also studied by others, which were in close agreement with the results of this study. The antinociceptive and anti-inflammatory effects of *Zataria multiflora* (28-30) and anti-inflammatory effects of sesquiterpens isolated from *Curcuma zedoaria* (31, 32) have been studied before.

Some of studied species in this report showed low radical scavenging activity compared to positive controls but moderate antioxidant activity against linoleic acid peroxidation or vice versa. The same results using different tests on plant materials were observed in some other studies (33-36). Different antioxidant and radical scavenging activity may partly be due to wide variety of antioxidant constituents such as phenolics, ascorbate and carotenoids. Also two types of antioxidants, inhibitors of free radicals which initiate oxidation and inhibitors of free radical chain propagation reactions, are known. Different mechanism of action and kinetics of the inhibitory effect of these antioxidants using different procedures resulted in the discrepancy of these findings (8, 12, 33). Owing to the complexity of the antioxidant materials and their mechanism of actions, it is obvious that no single testing method is capable of providing a comprehensive picture of the antioxidant profile of a studied samples and a combination of different methods is necessary. Despite such limitations, DPPH or linoleic acid peroxidantion methods can be helpful for primary screening and finding of novel antioxidants (37).

In conclusion, the results of the present

Table 2. Results of antioxidant and free radical scavenging activities of the plant extracts.

$IC_{50\ (DPPH)}$ (µg/ml)[a]	IC_{50} (Linoleic acid peroxidation) (ng/ml)[a]	Scientific name
8.64±0.03	97.34±0.66	Trolox
5.22±0.13	40.83±3.39	Quercetin
1.89±0.05	39.71±1.72	*Achillea tenuifolia* Lam.
187.88±60.40	30.67±4.35	*Allium hirtifolium* Boiss.
32.36±4.43	7.65±1.07	*Astragalus hamosus* L.
37.14±3.23	1.28±0.14	*Biebresteinia multifida* DC.
74.41±12.67	24.85±2.15	*Citrulus colocynthis* (L.) Schrad.
24.63±1.02	39.77±4.67	*Colchicum speciosum* Stev.
10.60±1.53	3.77±0.51	*Curcuma zedoaria* Rosc.
1.14±0.10	4.16±0.11	*Mentha spicata* L.
2.14±0.08	63.48±6.89	*Polypodium vulgare* L.
1.03±0.05	21.57±3.11	*Salix sp.*
37.19±6.00	52.35±1.28	*Salvadora persica* L.
1.55±0.09	2.50±0.42	*Salvia hydrangea* DC.
1.80±0.17	3.23±0.12	*Zataria multiflora* Boiss.

[a] IC_{50} values are represented as means±SD (n=3).

study suggest that tested plant materials have moderate to potent antioxidant activity and/or free radical scavenging activity. However, we do not know what components in the plant extracts show these activities. More detailed studies on chemical composition of the plant extracts, as well as other in vivo assays are essential to characterize them as biological antioxidants which are beyond the scope of this study. It should also be kept in mind that antioxidant activity measured by in vitro methods may not reflect in vivo effects of antioxidants (38). Many other factors such as absorption/metabolism are also important. The findings of this study support this view that some medicinal plants are promising sources of potential antioxidant and may be efficient as preventive agents in some diseases. The providing data can just enrich the existing comprehensive data of antioxidant activity of plant materials.

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